

Preface

Underlying every living cell are billions of molecules interacting in a beautifully concerted network of pathways such as metabolic, signalling, and regulatory pathways. The complexity of such biological systems has intrigued scientists from many disciplines and has given birth to the highly influential field of *systems biology* wherein a wide array of mathematical techniques, such as flux balance analysis, and technology platforms, such as next generation sequencing, is used to understand, elucidate, and predict the functions of complex biological systems. This field traces its roots to the general systems theory of Ludwig von Bertalanffy and effectively started in 1952 with a mathematical model of the neuronal action potential for which Alan Hodgkin and Andrew Huxley received the Nobel Prize in 1963. More recently, the field of *synthetic biology*, i.e., *de novo* engineering of biological systems, has emerged. Here, the phrase ‘biological system’ can assume a vast spectrum of meanings: DNA, protein, genome, cell, cell population, tissue, organ, ecosystem, and so on. Scientists from various fields are focusing on how to render this *de novo* engineering process more predictable, reliable, scalable, affordable, and easy. Systems biology and synthetic biology are essentially two facets of the same entity. As was the case with electronics research in the 1950s, a large part of synthetic biology research, such as the *BioFab* project, has focused on reusable macromolecular “parts” and their standardization so that composability can be guaranteed. Recent breakthroughs in DNA synthesis and sequencing combined with newly acquired means to synthesize plasmids and genomes have enabled major advances in science and engineering and marked the true beginning of the era of synthetic biology. Significant industrial investments are already underway. For example, in 2009, Exxon Mobil set up a collaboration worth \$600 million with Synthetic Genomics to develop next generation biofuels.

Recent advances in systems and synthetic biology clearly demonstrate the benefits of a rigorous and systematic approach rooted in the principles of systems and control theory—not only does it lead to exciting insights and discoveries but it also reduces the inordinately lengthy trial-and-error process of wet-lab experimentation, thereby facilitating significant savings in human and financial resources. So far, state-of-the-art systems and control-theory-inspired results in systems and synthetic biology have been scattered across various books and journals from various disciplines. Hence, we felt the need for an edited book that provides a

panoramic view and illustrates the potential of such systematic and rigorous mathematical methods in systems and synthetic biology.

Systems and control theory is a branch of engineering and applied sciences that rigorously deals with the complexities and uncertainties of interconnected systems with the objective of characterising fundamental systemic properties such as stability, robustness, communication capacity, and other performance metrics. Systems and control theory also strives to offer concepts and methods that facilitate the design of systems with rigorous guarantees on these fundamental properties. For more than 100 years, the insights and techniques provided by systems and control theory have enabled outstanding technological contributions in diverse fields such as aerospace, telecommunication, storage, automotive, power systems, and others. Notable examples include Lyapunov's theorems, Bellman's theory of dynamic programming, Kalman's filter, H^∞ control theory, Nyquist-Shannon sampling theorem, Pontryagin's minimum principle, and Bode's sensitivity integral. Can systems and control theory have, or evolve to have, a similar impact in biology? The chapters in this book demonstrate that, indeed, systems and control theoretic concepts and techniques can be useful in our quest to understand how biological systems function and/or how they can be (re-)designed from the bottom up to yield new biological systems that have rigorously characterized robustness and performance properties.

Several barriers must be overcome to contribute significantly in this exciting journey. One of these is the language barrier, e.g., what a systems theorist means by the word *sensitivity* is different from what a biologist means by it. Another one is the knowledge barrier as, traditionally, systems and control theorists and biologists are not well versed with each other's knowledge base (although that scenario is now fast changing for the better with the introduction of bioengineering courses in systems and control theory at the undergraduate and graduate levels). A third barrier is due to the sheer volume of *big data*: the European Bioinformatics Institute in Hixton, UK, which is one of the world's largest biological data repositories, currently stores 20 petabytes of data and backups about genes, proteins and small molecules, and this number is more than doubling every year. Finally, a fourth barrier comes from the effort required to produce timely contributions based on currently available models. As an example of this last barrier, the systems and control theory community could have played a greater role than it did in two of the most significant technological advances of the last 50 years: VLSI and Internet. In retrospect, besides the fact that the systems and control theorists caught on the Internet too late, by which time infrastructures based on TCP/IP were already in place, the main difficulty posed by the Internet for the systems and control theory community was a lack of *good* models of the underlying networked system. This lack-of-good-models barrier is even more daunting in biology since some of the currently available *big data* are not guaranteed to be reproducible. As Prof. M. Vidyasagar illustrates and observes in the September 2012 issue of IEEE Lifesciences, one of the major challenges to the application of systems and control

theory concepts in biology comes from “the fact that many biological experiments are not fully repeatable, and thus the resulting datasets are not readily amenable to the application of methods that people like us [i.e., systems and control theorists] take for granted.”

The chapters in this book serve to propose ways to overcome such barriers and to illustrate that biologists as well as systems and control theorists can make deep and timely contributions in life sciences by collaborating with each other to solve important questions such as how to devise experiments to obtain models of biological systems, how to obtain predictive models using information extracted from experimental data, how to choose components for (re-)engineering biological networks, how to adequately interconnect biological systems, and so on. Furthermore, and as Prof. Mustafa Khammash observes in his foreword, this research will fundamentally enrich systems and control theory as well by forcing it to investigate currently open questions that are specific to living biological systems, e.g., Why do biological systems naturally evolve the way they do? Can the evolvability of biological systems be consciously exploited for (re-)design and optimization purposes?

This book is intended for (1) systems and control theorists interested in molecular and cellular biology, and (2) biologists interested in rigorous modelling, analysis, and control of biological systems. We believe that research at the intersection of these disciplines will foster exciting discoveries and will stimulate mutually beneficial developments in systems and control theory and systems and synthetic biology.

The book consists of 12 chapters contributed by leading researchers from the fields of systems and control theory, systems biology, synthetic biology, and computer science. Chapters 1–6 highlight some state-of-the-art methods used to address currently open questions in systems biology. Chapters 7–12 discuss frameworks and methods required to enable a bottom-up design of synthetic biology systems of increasing complexity. These chapters are organized into two main parts as follows.

- **Part I—Systems Biology:** Chapters 1–6 focus on specific problems in modelling biological systems. Examples of such problems include: characterization and synthesis of memory, understanding how homeostasis is maintained in the face of shocks and relatively gradual perturbations, understanding the functioning and robustness of biological clocks such as those at the core of circadian rhythms, and understanding how the cell cycles can be regulated, among others. A brief summary of each chapter is as follows.
 - Chapter 1: Today, several approaches used to identify biomarkers for a specific disease rely on genome-wide gene expression profiles without an explicit regard for how the genes are correlated. Wang and Chen present a network biomarker construction scheme that integrates microarray gene expression profiles and protein–protein interaction information so as to enable molecular investigation and diagnosis of lung cancer.

- Chapter 2: In this overview chapter, Lal and Seshashayee discuss next-generation sequencing techniques and illustrate how these have been used, at the scale of the whole bacterial genome, to investigate a variety of problems, from the analysis of gene expression and protein–DNA interactions to that of bacterial community function and evolution.
 - Chapter 3: Given a biological network of oscillators, such as circadian rhythm networks for instance, how do biological parameter variations affect the oscillation characteristics? Sacré and Sepulchre present a novel and scalable approach to characterize the parameter sensitivity of models of oscillators, and illustrate its use on a circadian rhythm network model.
 - Chapter 4: Osmosis facilitates the basic mechanism by which water is transported into and out of cells. Montefusco et al. demonstrate how a control theoretic analysis of the osmosis regulation system of *Saccharomyces cerevisiae* can be used to explain how cells maintain homeostasis in the face of osmotic perturbations.
 - Chapter 5: State synchronization is a recurring theme in neuronal networks and coupled networks of genetic clocks, among others. Hamadeh et al. explain how incremental dissipativity theory can be used to systematically analyse and/or synthesize feedback interconnections that ensure state synchronization in networks of identical oscillators and illustrate its use in the context of realising synchronization in a genetic repressilator network.
 - Chapter 6: Multistability is a key property of biological systems that characterizes salient phenotypes such as memory. Salerno et al. present a systematic approach to characterize bistability and explain its utility in characterizing the memory of the galactose regulatory system of *Saccharomyces cerevisiae*.
- **Part II—Synthetic Biology:** Chapters 7–12 focus on how biomacromolecules, platforms, and scalable architectures should be chosen and synthesized in order to build programmable *de novo* biological systems. For example, a standardization of the components used is a necessary step in the modular design of large scale systems and presents an opportunity to develop *in silico* design tools that optimize these systems with respect to a set of formal specifications. What are the types of constrained optimization problems encountered in this process and how can these be solved efficiently? Should DNA be used as the basic macromolecule in synthesising artificial biological networks or should it be used with other macromolecules to enable certain applications? This set of chapters aims at answering such questions. A brief summary of each chapter is as follows.
 - Chapter 7: Modern nucleic acid biochemistry extensively uses protein enzymes to manipulate nucleic acids. However, predictive modification of the behavior of protein enzymes remains a very difficult problem. Chandran et al. show how meta-biochemical systems offer the possible advantage of being far easier in terms of re-engineering and programming. They show how a biochemical system can be synthesized based entirely on strands of DNA as the

only component molecule. These *meta-DNAs* have the same pairing mechanism as DNA but have a much larger alphabet of bases, thereby providing an increased power of base addressing.

- Chapter 8: An open challenge today is to specify synthetic biological systems using high level languages. Chen and Cai choose a rule-based modelling framework that was originally developed for systems biology and extend it to synthetic biology. They introduce a new model-specification language that facilitates the swift generation of mathematical models that encode the phenotypic behaviors of biological systems.
- Chapter 9: Krishnan and Liu address how bistable and monostable switches give rise to irreversible transitions and decision making in cell cycles. They propose a modular framework to address such questions for binary signalling mechanisms, outlining some of the design principles of signalling networks, which can be exploited in synthetic biology.
- Chapter 10: In standardising the components for a scalable design, an important constrained optimization problem concerns the selection of kinetic parameters and protein abundances. Koepl et al. explain how this inverse problem can be solved more elegantly, by linearising the forward operator that maps parameter sets to specifications, and then inverting it locally, rather than relying on a brute force random sampling approach.
- Chapter 11: Marchisio and Stelling demonstrate how concepts and algorithms from electrical engineering can be exploited to set up a framework for the computation-based automated design of genetic Boolean gates and devices. They also explain how the Karnaugh algorithm used in the design of electrical circuits can be modified when it comes to the design of genetic circuits.
- Chapter 12: Kim and Franco focus on how to synthesize and couple transcriptional circuits by exploiting the modular architecture of nucleic acid templates as well as the catalytic power of natural enzymes. They illustrate the programmability of dynamic behaviors for elementary circuits such as adapters, bistable switches, and oscillators. They also present insulating and amplifying devices as a solution for the scaling-up of biomolecular networks.

The burgeoning fields of systems biology and synthetic biology have thrown up a very large number of interesting research problems. As the pre-eminent computer scientist Donald Knuth put it, “biology easily has 500 years of exciting problems to work on.” The chapters in this book address but a small fraction of these interesting challenges. Nevertheless, we believe this book can serve as a good introduction on some of the currently open problems and on some of the state-of-the-art concepts and techniques available to propose solutions to such problems.

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