Systems and Synthetic Biology

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Systems biology is a field of biology that was propelled into existence due to the development of methods to generate massively parallel experimental biological data, starting with genome sequencing projects approximately 30 years ago. Transcending the focus on single biological components, the goal of systems biology is to reverse-engineer mechanisms of biological systems to enhance our quantitative holistic understanding of biology and to translate this understanding to applications such as novel therapeutic approaches. As such, systems biology is the science counterpart to synthetic biology described below. A hallmark of biological systems is their sheer complexity, as evidenced by the existence of thousands of components and millions of interactions between them even in the simplest of living cells. While this degree of complexity is paralleled by some engineered systems, the other hallmark, large uncertainty, distinguishes biological systems from their human-made counterparts. In addition to complexity, limited observability, lower accuracy of measurements, and uncertain mechanisms make reverse-engineering in biology highly challenging.

This special issue brings together leading experts in the fields of systems and synthetic biology to provide a quantitative and analytical perspective of the state-of-the-art developments.

At least two reasons justify the excitement for the field: first, systems biology offers a new systems level perspective on biology—one that is grounded on analytical and quantitative methodologies that have proved wildly successful in unraveling complexity in other fields. This new perspective helps elucidate how complex biological function emerges from the interaction of individual parts, a task that is difficult to achieve by studying parts in isolation. For example, it is only in the context of a systems approach can recurrent design motifs be uncovered and explained. Second, a deeper quantitative understanding of biological systems offers tremendous opportunities for real-world applications. In addition to new designs and interventions described below, one major area of current development is in biomedicine, including rational patient stratification, therapy design based on biological network models, and systems pharmacology.

Synthetic biology is a relatively new field of biology that leverages our rapidly expanding scientific understanding of biology and combines it with powerful genetic manipulation techniques to engineer biosystems with new functionality. The goal of synthetic biology is to design and construct biological parts and components and use them to engineer novel synthetic biological circuits, devices, and systems with new functions. Simultaneously, synthetic biology is concerned with the redesign of existing natural systems to repair their aberrant function or to endow them with a modified function. As such, synthetic biology is first and foremost an engineering discipline—one that shares much in common with electrical engineering in both approach and philosophy, even if the biological substrate is very different from that used in electrical engineering. It is generally agreed that synthetic biology, as a discipline, started in the year 2000, when three groundbreaking articles appeared in the journal *Nature* almost simultaneously: one reported on a synthetic genetic toggle switch, another reported on a synthetic genetic oscillator, and the

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third reported on a synthetic feedback gene circuit. Since then, more sophisticated biological circuits were designed and tested, including complex logic gates, amplifiers, signal processing circuits, and circuits that implement dynamic genetic feedback control systems, such as proportional–integral feedback controllers.

As a new engineering discipline, synthetic biology shows tremendous promise, due to both its potential impact on basic science and its practical applicability. Its scientific raison d'être is that, by synthetically reconstituting natural systems or subsystems, one is better able to understand their underlying design principles. Thus, synthetic biology puts into practice Richard Feynman's credo: "what I cannot create, I do not understand." From this vantage point, synthetic biology offers a compelling path to a deeper understanding of biological complexity. The other reason for pursuing synthetic biology is more pragmatic but no less compelling. It derives from the field's tremendous potential to revolutionize industrial biotechnology and medical therapy. Indeed, synthetic biology is already catalyzing the development of new drugs, compounds, and therapeutic proteins. Synthetic biological processes are being used to develop more advanced biofuels, lightweight materials with novel properties, and novel environmentally friendly pesticides. Synthetic photosynthesis processes are being designed and tested for the reduction of CO_2, promising to contribute to a cleaner environment. At the same time, synthetic biology is driving the area of tissue engineering, whereby artificial tissues and organs are being created for drug development and ultimately patient transplants. Finally, synthetic cells are currently being tested for use as closed-loop feedback control systems for precision drug delivery and metabolic control in patients suffering from diseases that result from dysregulation, such as diabetes. Through transforming world industry in areas such as energy, health, agriculture, and the environment, synthetic biology is bound to become a wealth generator and a major economic driver.

I. WHY THIS ISSUE IS OF INTEREST TO PROCEEDINGS OF THE IEEE READERS

The interest of readers of the Proceedings in systems biology stems, first, from the insights the field offers into how natural biological systems establish complex functions. Evolved biological systems need to deal with challenges such as resource constraints in fluctuating environments and limited or uncertain information. Despite that, they establish remarkably robust and efficient capabilities for the processing of information and materials. Principles such as evolutionary optimization and bio-inspired computing have already entered electrical and computer engineering disciplines, and new developments in systems biology could further foster such transfer. Examples include insights from the large-scale organizations of biological networks (e.g., scale-rich architectures), principles of resource allocation in cells, and mechanisms for robust information processing and control that go beyond commonly applied engineering principles. Second, systems biology offers unique opportunities to advance theory and methods in engineering and computer science, due to the field's many challenges. For example, analysis methods developed for coping with uncertainty and multiscale interactions in biology could transfer to and find new applications in engineering fields, such as formal approaches to learning in "simple" cells or complex neuronal networks. At the same time, systems biology heavily draws on concepts from dynamical systems theory and related disciplines to model and analyze biological objects. Transferring these concepts to biology has spurned new developments in uncertain dynamical systems, machine learning, and controllability of (biological) networks, with corresponding theories and algorithms.

Despite several key advances in the field of synthetic biology, there remain many challenges that the field must overcome to achieve its full potential. To begin with, biological systems are nonlinear, stochastic, and dynamic—properties that enable complex functions, but ones that also pose design challenges not unlike those faced when designing complex electrical circuits. In addition, novel genetic circuits consume energy and other resources that must be shared with the host cell, so energy and material supplies must match the demand of the endogenous and newly engineered circuits, much like the requirements of modern power systems. To sense and respond to their environment, living cells employ sophisticated signal processing pathways. Synthetic implementations of these signaling pathways should be designed to maximize information transmission capacity despite noise and crosstalk, all issues faced by communication and signal-processing engineers. Finally, achieving precise and robust set-point regulation of protein concentration despite the myriad disturbances and perturbations affecting the cell are goals facing synthetic biologists and control engineers alike.

These and other issues faced in systems and synthetic biology share much in common with similar problems faced in the various fields of electrical engineering. Readers of the Proceedings will resonate with many of these same issues, and will undoubtedly find many ways in which their existing tools and insights can be profitably applied to solve problems in systems and synthetic biology. At the same time, analysis and design in biology have unique challenges that are not usually faced by electrical engineers. Here also, there is an opportunity to adapt existing methods and develop new ones that are more suitable for dealing with the unique nature of biological circuits. Once the boundary between the fields is lowered, research at the interface between systems and synthetic biology and the various specialties of electrical engineering will be immensely beneficial for advancing this young and most promising field.

II. OVERVIEW OF THE SPECIAL ISSUE

We solicited contributions from experts in several areas of systems biology and synthetic biology. In selecting these contributors, we sought to find individuals who are highly accomplished, world-leading experts in their respective fields while having a quantitative and/or an analytical perspective that would enable them to convey their ideas in an engaging way to readers of the PROCEEDINGS. Thematically, we sought to cover a diversity of important areas of systems and synthetic biology that are likely to be of interest to the readers of the PROCEEDINGS.

A. Systems Biology

The Hallmarks of Mathematical Oncology

by J. A. Bull and H. M. Byrne

"The Hallmarks of Cancer" is a highly influential article that identifies key features distinguishing cancer cells from normal cells. The article by Bull and Byrne follows a similar spirit to introduce nonspecialists into how mathematical modeling has contributed, and can further contribute, to the understanding and treatment of cancer. It starts with classifying six mathematical hallmarks or decisions that relate to modeling frameworks. After introducing the fundamentals of cancer biology and radiotherapy, as well as a general roadmap for developing and exploiting mathematical models, the article discusses in detail three model classes of increasing complexity: ordinary differential equation, partial differential equation, and agent-based models. The emphasis is on qualitative insights that the models can generate and on their respective limitations, for example, in addressing the multiscale and spatially heterogeneous character of tumors. Key challenges are model parametrization and model (complexity) selection based on medical data; there, progress in new measurement and computational methods is encouraging. This justifies the article's positive conclusion: validated, clinically applicable cancer models are coming.

Metabolic Networks, Microbial Consortia, and Analogies to Smart Grids

by A. Theorell and J. Stelling

This article turns to a different aspect of biology, namely, the analysis of metabolic (chemical reaction) networks providing biological systems with building blocks for replication, energy, and other requirements. It introduces approaches to predict and determine fluxes in metabolic networks, many of which have close relations to the analysis of analog electrical circuits. The article starts by formally defining the steady-state analysis of metabolic networks. It then reviews computational methods and their associated challenges, reflecting the diversity of approaches used. These include convex analysis problems, complex optimization problems, sampling problems, and identification problems that are similar to, but also expanding on classical engineering problems. Lastly, it discusses the main current challenge of the field, namely, to extend formal analysis concepts to communities of interacting species, for example, in the human microbiome. Key questions on division of labor, decision-making, and communication bear strong analogies to (smart) electrical grids, and the article concludes by inviting cross-disciplinary collaborations.

Machine Learning Approaches to Single-Cell Data Integration and Translation

by C. Uhler and G. V. Shivashankar

Single biological cells display highly variable behaviors in a population, even for genetically identical cells. Experimental analysis of these behaviors is often destructive, resulting in partial observations. The article describes how machine learning can address the concomitant, translational and counterfactual problems, after motivating a new general categorization of such problems. It reviews three types of machine learning approaches in this context: generative models such as autoencoders that model a joint probability distribution, optimal transport between two distributions, and causal inference from a mix of observational and interventional data, which is a particularity of systems biology. The article illustrates the application of these machine learning approaches to single-cell biology with three examples that are motivated by the authors' own work. These deal with the problems of integrating different data modalities, tracing single cells in heterogeneous populations based on snapshot data, and inferring genetic regulatory networks as well as drug effects on their function. The article emphasizes how such biology problems inspired foundational developments in machine learning.

Spiking Control Systems

by R. Sepulchre

In contrast to digital signal processing, biological systems often communicate using spikes and rhythms. These have a mixed nature—they establish continuous-time but countable signals. This article reviews ongoing efforts toward developing a control theory of spiking systems by revisiting classical control theory. In introducing the circuit theory for spiking systems, the article emphasizes the theoretical challenges arising from interconnected active and passive components, and the central concept of (maximal) monotonicity. It then exposes conductance modeling of neuronal circuits, clarifying the notion of negative conductance. The central thesis of the article developed on this basis (with illustrations from neuronal and electrical circuits) is that a mixed feedback principle with a dynamic hierarchy of positive and negative feedback underlies spiking systems. Continuing with design problems, the article demonstrates, for example, a methodology for the physical realization of event-based control systems. It concludes by arguing for, and emphasizing the importance of, a unified theoretical framework for natural and engineered spiking systems, with mixed feedback at its core.

Learning Outside the Brain: Integrating Cognitive Science and Systems Biology
by J. Gunawardena
This thought-provoking article uses concepts ranging from cognitive science to control theory to investigate the question of if and how learning in biological systems is possible outside of, or even without, a brain. Here, learning is viewed through the lens of information processing, enabling rigorous theoretical formulations, for example, based on "surprises" associated with prediction errors. This view also links to concepts such as reinforcement learning in computation and internal model principles in control theory. The article then proceeds to three different contexts of learning outside the brain: physiology, organism development, and single cells. Examples from physiology help to clarify relations between internal models from learning and control theory. In development, nongenetic inheritance is a mechanism in complex constructions of multicellular organisms and their anticipation of the world. Because such mechanisms reside in single cells, the article continues with their capabilities for inference. This leads to two information-theoretic requirements for biological learning—and the conclusion that cells "... have minds of their own."

B. Synthetic Biology

Synthetic Gene Circuits: Design, Implement, and Apply
by A. Lezia, A. Miano, and J. Hasty
This article gives a balanced and accessible overview of the field of synthetic biology, focusing on the engineering of genetic circuits. It starts by reviewing some of the current genetic circuit design approaches for both analog and digital circuits. In the case of digital circuits, software packages for automated logic gates design are described; for analog circuits, complementary methods for modeling and design are described, ranging from the control theoretic to those based on Bayesian statistics and methods based on machine learning. The article then segues into a discussion of traditional and current approaches for implementing designed circuits using specific biological parts. Both mRNA and protein-based parts are described, culminating into the modern and versatile gene editing technology, CRISPR. Methods for the assembly of parts into genetic circuits are then explored, particularly Gibson assembly and Gold Gate assembly methods. The article then discusses several compelling applications of genetic circuits. These include synthetic bacterial and mammalian gene circuits for living therapeutics, such as cancer treatment and controlled drug release, as well as circuits for whole cell biosensors and biomanufacturing.

Cybergenetics: Theory and Applications of Genetic Control Systems
by M. H. Khammash
Regulation is a prevalent theme that runs across all of biology. With the advent of synthetic biology, it has become possible to engineer novel genetic control systems in living cells and to use them to regulate cellular processes with precision and robustness. *Cybergenetics* is the vibrant interdisciplinary field that has emerged at the interface of control theory and synthetic biology which deals with the theoretical and practical issues of designing genetic control systems. This tutorial article takes the reader into a journey through this exciting new field, and expounds on its fundamental tenets. Starting from the basics of genetic circuits and the way they are engineered in the biological chassis, the article explains the challenges of building synthetic genetic circuits and what makes them different from electronic ones. It then examines genetic control system design approaches, ranging from feedforward designs to negative feedback circuits, and culminating in the theory and implementation of synthetic integral feedback control systems in living cells. The control of cell consortia is discussed next, followed by a summary of methods for the *in silico* control of genetic systems. The article ends with a rundown of the applications of this nascent field along with an assessment of its outlook and many promising opportunities.

Advances in the Computational Design of Small-Molecule-Controlled Protein-Based Circuits for Synthetic Biology
by S. Kretschmer and T. Kortemme
In synthetic biology, small molecules often serve as sensor inputs to genetically engineered cellular devices. These molecules can be disease biomarkers, pollutants, or simply input signals that the biologist applies. Once in the cell, they bind partner proteins leading to their activation and triggering further action, such as gene expression. While synthetic biologists have repurposed natural proteins to carry out this sensing role, the repertoire of natural sensors is quite limited. At the same time, designing novel proteins to function as sensors has been very challenging, due to the difficulty of mapping DNA sequences to their expressed protein structure. This has all changed recently, as advances in computational protein design have facilitated the effective engineering of novel and modular proteins with customized functions. This article reviews these exciting developments in the field of computational protein design, focusing on the advances in the engineering of synthetic small-molecule-binding protein sensors as well as sensor-actuator proteins. The computational approaches described are grounded on the wildly successful Rosetta software suite framework, which has recently enabled remarkable scientific advances in computational biology, including the *de novo* design of a diversity of complex proteins with novel functions.

Bayesian and Algebraic Strategies to Design in Synthetic Biology
by R. P. Araujo, S. T. Vittadello, and M. P. H. Stumpf
This article aims to provide an overview of two complementary

approaches (algebraic and Bayesian) for rational design in synthetic biology and demonstrates how these approaches can be combined in order to more effectively explore the vast design space and identify the best designs. Taking the example of robust perfect adaptation (RPA) as the design criterion, the article explains how RPA systems can be algebraically characterized through a zero-determinant condition that can be translated into topological requirements for the network. Then, the problem of designing RPA networks is discussed from the Bayesian perspective, and it is explained how potential RPA models can be efficiently evaluated based on the approximate computation of their likelihood of exhibiting RPA. This approach is then illustrated through known examples of RPA networks and it highlights how algebraic characterization can be seamlessly integrated with Bayesian validation to select the best candidate models and their parameterizations. The article summarizes the strengths and weaknesses of algebraic and Bayesian approaches. It makes a strong case for using these approaches in conjunction with each other for more informed and effective rational design.

Synthetic Morphogenesis: Introducing IEEE Journal Readers to Programming Living Mammalian Cells to Make Structures
by J. A. Davies

The embryonic development of a living organism follows an exquisite master construction plan involving multiple steps that have to be executed in the correct order and at the right time. Developmental biologists have studied the emergence of shape and structure (morphogenesis) during this process for ages. With advances in our understanding of natural development, a new and exciting engineering discipline is now emerging whereby cells are designed and genetically engineered to make specific shapes and structures as intended by a designer. This new discipline, called synthetic morphogenesis, is the subject of this highly-accessible and masterfully written article. It shows how complex tissues can develop from a few elementary morphogenetic behaviors. Armed with this understanding, the focus shifts to ways in which these elementary behaviors can be synthetically manipulated using modern genetic techniques in combination with electronic/optical computer interfaces to yield synthetically designed shapes. The article covers the state-of-the-art of these methods, while making analogies with objects and devices familiar to electrical and electronic engineers. These novel synthetic biology techniques aim to bring about a deeper understanding of developmental processes and point to a future where tissues and organs can be rationally developed. ■

ABOUT THE GUEST EDITORS

Mustafa H. Khammash (Fellow, IEEE) received the Ph.D. degree in electrical engineering from Rice University, Houston, TX, USA, in 1990.

He joined the Electrical Engineering Department, Iowa State University (ISU), Ames, IA, USA. While at ISU, he created the Dynamics and Control Program and led that control group until 2002, when he joined the Faculty of the University of California at Santa Barbara, Santa Barbara, CA, USA. He has served as the Director of the Center for Control, Dynamical Systems and Computation, University of California at Santa Barbara, 2005 to 2011. In 2011, he joined the Department of Biosystems Science and Engineering at ETH Zürich, Zürich, Switzerland, where he served as the Department Chair from 2015 to 2017. He is currently a Professor of control theory and systems biology with the D-BSSE, ETH Zürich. He works at the interface of the areas of control theory, systems biology, and synthetic biology. He has pioneered the development of theoretical, computational, and experimental methods for the robust control of living cells through genetic and electronic control approaches (cybergenetics).

Prof. Khammash is a Fellow of the International Federation of Automatic Control (IFAC).

Jörg Stelling is Professor of Computational Systems Biology at the Department of Biosystems Science and Engineering at ETH Zurich. After receiving his Ph.D. degree in Control and Dynamic systems from the University of Stuttgart, Germany, in 2004, he joined faculty of the Computer Science Department at ETH Zurich. In 2008, he moved as one of the founding members of the newly created Department of Biosystems Science and Engineering (D-BSSE), ETH Zürich. At D-BSSE, he served as the Department Chair from 2013 to 2015 and continues to serve on the ETH-Wide Tenure Committee. He works at the interface of systems and synthetic biology, focusing on the analysis and synthesis of biological networks using—and further developing—methods from systems theory and computer science. His main aims of current research are to understand how interindividual variability impacts the functioning of complex networks in biological systems, and to provide concepts and methods for the rational design of biological systems despite uncertain and non-robust components and interactions.