# Recent advances, opportunities and challenges in cybergenetic identification and control of biomolecular networks

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## 1 Abstract

Cybergenetics is a new area of research aimed at developing digital and biological controllers for living systems. Synthetic biologists have begun exploiting cybergenetic tools and platforms to both accelerate the development of mathematical models and develop control strategies for complex biological phenomena. Here we review the state of the art in cybergenetic identification and control. Our aim is to lower the entry barrier to this field and foster the adoption of methods and technologies that will accelerate the pace at which Synthetic Biology progresses towards applications.

## 2 Introduction

In the past two decades research in Synthetic Biology has witnessed tremendous progress towards the development of a robust engineering framework to reprogram living systems. Modelling and control of biomolecular networks has played a key role in this development. The advent of cybergenetics, the field concerned with the design and development of digital/biological feedback control systems, substantially accelerated the pace at which the Engineering Biology community was able to develop automated methods for efficient model extraction and *in vivo* control of biodynamics.

In this article we review the latest developments in the cybergenetic identification and control of biological systems. We examine the two topics separately following the same structure. After reviewing the state of the art in each field we discuss significant open challenges and then share our perspective on the most exciting opportunities in the respective areas. With this contribution we seek to offer interested readers a guide to navigate the *status quo* in cybergenetics.

# 3 Cybergenetic identification

The ability to mathematically formalise and predict the behaviour of biomolecular networks is crucial to the rational engineering of biological systems. Over the 20 years since the conception of Synthetic Biology, mathematical models have transitioned from post-hoc descriptors of data to key tools in the design and prototyping of biological parts and genetic circuits. Thermodynamic models have aided the design of promoters with user-defined transcriptional profile in *E. coli* [1], and *de novo* protein switches both *in vitro* and *in vivo* [2]. Following the commercial availability of low-cost DNA synthesis and standardised assembly services, computational tools have been proposed to expedite the physical implementation of synthetic devices, accelerating the design-build-test-learn cycle of gene circuits. For example, Halper and colleagues trained a machine learning model to quantify the risk of synthesis failure of a candidate DNA fragment and to identify which sequence determinants should be removed to reduce this risk [3].

Mathematical models of biological systems in literature can be broadly grouped into two categories: Differential Equation-based or Rule-based. Differential equations capture the dynamics of biological processes through relationship between functions, often associated to physical quantities, and their derivatives. The dimensionality of the domain of such functions, or the inclusion of random perturbations, determines the class of equations. Ordinary differential equations (ODEs) are used to describe single-variable, usually time-dependent, processes. These have constituted the bulk of published models. Currently, over 80% of the labelled entries on BioModels, the largest public repository of biological models [4], are formulated as ODE. The formalisation of multi-variable problems, such as those concerning transport phenomena, requires the inclusion of geometric considerations via Partial Differential Equations (PDEs). When random perturbations cannot be neglected, as is the case for Brownian motion of molecules within the cellular environment, Stochastic Differential Equations (SDEs) are commonly adopted.

Technological advances in our ability to decode cell dynamics, via single cell RNA-Seq or microscopy/microfluidics, have contributed to the recent surge in the use of statistical models in Systems and Synthetic Biology [5]. Chemical Master Equation (CME)-based models, where the time evolution of the probability of a cell state is calculated using the underlying chemical reaction

rates, have attracted considerable interest. Despite the high entry barrier for a non-technical audience and the computational effort of simulating such dynamical systems, recent contributions reported substantial progress towards making CME-based modelling a more accessible option. A Python package, Flips [6], now allows researchers to quickly set up CME models to study population-level stochasticity on top of intracellular noise. Gupta *et al.* [7] describe a new approach to CME simulation that combines Reinforcement Learning and Deep Neural Networks to effectively approximate infinite dimensional solutions and accelerate CME computations. Another algorithm, exploiting system decomposition and filtering to rapidly obtain more accurate solution approximations, has been reported in Fang *et al.* [8].

While ODE and statistical models have mostly been used to study the evolution over time of biosystems, open problems in developmental biology (e.g. segmentation) concern processes that vary over time, and crucially, space. These have traditionally been modelled via PDEs [9]. Recent developments in synthetic morphogenesis [10], the discipline studying how genetically engineered cells can generate desired shapes and structures, highlighted the potential of rule-based models to study emergent behaviours of cell populations [11]. Agent Based Models (ABM), where automata (cells) populate a 2- or 3D environment and execute internal "programs" in the form of algorithms or rules, are rapidly gaining traction in Synthetic Biology [12]. For example, the ABM simulation tool BSim [13] enabled the first *in silico* demonstration of real-time control of the relative abundance of cell phenotypes in microbial consortia. While the analytical toolbox available for ABM remains limited when compared to Differential Equations-based models, breakthroughs in scientific computing could overcome challenges in ABM identification where gradient-based methods cannot be used. Recent advances in differentiable programming [14], a computational technique to seamlessly evaluate the exact derivative of a function with respect to its arguments, could be used to differentiate programs with discrete randomness like ABM and speed up the process of parameterising them from data.

Having established a model formalism researchers must decide: (*i*) whether to cast model identification in a frequentist or Bayesian framework, (*ii*) how to define the structure and parameters of the model, and (*iii*) how to acquire the necessary experimental data. The approach taken to solving these problems has so far been opportunistic. Computationally cheaper frequentist problem formulations have been favoured over Bayesian ones, despite necessitating sometimes unrealistic assumptions such as the normality of measurement data. As for (*ii*), model discrimination and calibration techniques (i.e. systematic approaches to select a model and tune its parameters based on its objective distance from experimental data) are well established in Control Engineering, yet models of biomolecular networks often appeared as "given" in the literature and "manual search" of parameters is not uncommon. Crucially, and partly due to technical limitations, proper experimental design exercises have seldom been reported as a precursor to data acquisition campaigns.

Cybergenetic control and Optimal Experimental Design (OED), combined with the ability to dynamically modulate the microenvironments [15], have changed the *status quo*. Following the *in vitro* demonstration of how OED could inform the identification and control of a light-inducible gene circuit in *E. coli* [16], Bandiera *et al.* [17] showed through simulations that using optimally designed experiments, instead of intuition-driven schemes, could reduce by 80% the error of parameter estimates when modelling an inducible promoter in *S. cerevisiae*. Bandiera and colleagues went on to compare OED in the Bayesian and Frequentist frameworks for the discrimination of 3 candidate models of a genetic toggle switch in *E. coli* [18]: besides being able to select the model with the stronger support from data, the authors quantified benefits (accuracy) and cost (computational complexity) of Bayesian OED. The same group recently released a package that leverages the speed of Julia, a relatively new programming language, to enable experimentalists to reduce their experimental efforts using OED (see *BOMBS*; URL: https://juliahub.com/ui/Packages/BOMBs/MvNlh/0.2.3). Available python packages for OED include NLOed [19] and RED [20]; the latter exploiting a novel Reinforcement Learning-based approach to optimise experimental designs.

The surge in interest for OED methods catalysed the emergence of a field at the nexus of experimental biology, control engineering and optimisation which aims at the automated, experimentally efficient identification of mathematical models of biosystems. Inspired by the cybergenetics control literature [21], we refer to this nascent field as *cybergenetic identification*. Key to the success of this research area is the integration of microfluidics and microscopy in a cyberphysical system in which a computer periodically acquires and analyses microscopy images, estimates cellular states and determines which changes in the cellular microenvironment maximise the information obtained from the resulting experiment [22]. These cyberphysical platforms could unlock the potential of *on-line* optimal experimental design: the real-time identification of mathematical models of biomolecular networks using data acquired as the dynamically designed, optimal experiment progresses. This conceptually straightforward algorithm has hidden challenges (e.g. how to automatically segment cells) that have limited the adoption of cybergenetic identification. However, novel open source softwares are simplifying the establishment and widespread adoption of cybergenetic platforms, facilitating execution of reactive microscopy experiments [23] and deep learning-based image segmentation [24].

## 3.1 Open challenges

A number of outstanding challenges remain at the boundary of system identification and the life sciences. First, as multi-scale models encompassing

gene expression, protein/signalling networks and metabolism increase in size, the fraction of state variables we can directly measure decreases. This hampers accurate calibration of models, as structural and practical parameter identifiability are two key concerns for modellers. A number of methods, reviewed in Chis *et al.* [25], have been proposed to mitigate such issues. An additional challenge related to parameter identifiability is the general difficulty in mapping "arbitrary units" of fluorescence to actual biomolecule copies. While recent contributions have proposed experimental protocols and algorithms to convert fluorescence levels into protein mass [26], their scalability to models with hundreds of observables remains to be established. An additional challenge, again connected to the growth of model dimensionality, is computational cost. Mathematical models of multiscale biological systems often need to describe processes that operate at time scales of different orders of magnitude. "Stiffness" arises naturally in such models and forces researchers to use "stiff solvers" generally characterised by a higher computational complexity than their "non-stiff" counterparts. Depending on the research question, model reduction techniques can be used to project the original dynamics onto a smaller subspace.

### 3.2 Future opportunities

Scientific Machine Learning offers exciting opportunities for cybergenetic identification. As the availability of high-quality data acquired from fully automated, microfluidics [27] and macrofluidics [28] setups increase, we can expect our ability to leverage Machine Learning algorithms to expand. As a consequence the modelling of complex biophysical phenomena will benefit from Physics Informed Neural Networks (PINN) [29] and from Universal Differential Equations [30]. PINN are neural networks trained to learn the "dynamical behaviour" of a system under physical constraints, while systems of differential equations are called universal if the right-hand side is replaced by a universal approximator (e.g. Fourier expansion). Together with Dynamic Mode Decomposition [31], a dimensionality reduction algorithm that extracts the dominant dynamics from experimental data, and the application of Koopman Operator Theory [32] these methods will enable data-driven scientists and engineers to effectively capture the behaviour of complex biological systems. The interpretability of the models returned by these methods could be enhanced by searching for the closest functional to the inferred universal approximator (e.g. Neural Network). Akin to genetic programming, another class of Machine Learning algorithms, methods to solve this problem include the sparse identification of non linear systems using function dictionaries [33, 34].

## 4 Cybergenetic Control

We now turn to the state of the art in control strategies available for biological systems. We first illustrate the existing classes of control approaches, comparing their benefits and drawbacks. We then present the latest developments in *cybergenetic control*: the *in vivo* regulation of biomolecular networks (i.e. the *plant)* via feedback with engineered control systems [35]. This emerging research field is rapidly expanding the toolbox available to synthetic biologists for predictable cell reprogramming.

Cybergenetic *controllers*, like man-made feedback control systems, leverage the

difference between a reference signal and a real-time measure of the output of the biomolecular network ("the controlled variable") to compute and impose a steering action on the controlled system. All control strategies reported in literature can be classified into two categories: *embedded* and *external* controllers.

Embedded controllers are synthetic gene networks that implement a canonical controller. They are designed to "compare" a controlled biological quantity (e.g. protein abundance) and modulate the concentration of a biological species (e.g. transcription factor) to achieve a control objective. Embedded controllers can be further divided into two classes: "concentrated" designs, where the controller and controlled network reside in the same cell, and "distributed", where a microbial consortium of two cell types is formed in which one strain hosts the controller network and the other the controlled one. While embedded controllers do not require expensive measurements/computers, their design remains a complex task. Recent technological advances in gene editing tools, coupled with the ability to encode known control motifs in biomolecular networks, have proven successful at establishing high-performing genetic controllers. For example, Briat *et al.* [36] were the first to theorise the antithetic integral control motif: a 4 reaction network ensuring Robust Perfect Adaptation (RPA - return to pre-disturbance dynamics following response to an abrupt change in the input) and disturbance rejection in noisy networks. The motif owes its name to the molecular antagonism between a sensing and an actuating species, which engage in an irreversible hybridisation reaction leading to zero tracking error at steady-state. The same research group went on to physically implement the controller both in bacteria [37] and mammalian cells [38]. Notably, in Frei *et al.* [39] the motif was augmented with a proportional feedback action thereby improving control performance during the transient and reducing the steady-state variance of the response of the controlled network.

In external controllers the biological plant is interfaced with digital platform built on algorithms that measure a (fluorescent) readout of the controlled network, compute the control action, and manage actuators delivering chemical/physical inputs to the cellular environment. Following the seminal demonstration of the suitability of such strategy to control the dynamics of expression of a gene in yeast cells [15], the support offered by control theory notions and the relative ease of implementation of the control policies delivered numerous applications in synthetic biology, medicine and biotechnology [40–42]. These span from the rapid prototyping of synthetic networks [43, 44] to the design of therapies [45] and optimisation of bioproduction [46].

While control inputs have traditionally been chemical (e.g. sugars) the useof light to regulate biological processes has recently attracted considerable interest. Indeed, its highly desirable spatio-temporal modulation, reversible induction, and low cost spurred a substantial interest in *optogenetic control* [47, 48]. A prime success story in this space is the optogenetic differentiation system in yeast proposed in [48]. Applying a model predictive control strategy, the platform allowed researchers to establish and regulate, in space and time, the composition of a microbial consortium. Similarly, a light-mediated control of the growth, and hence relative composition, of a two-strains microbial community was established in *E. coli* [21]. Informing the parameters of the optimal control strategy with a host-aware model, this work paves the way to dynamic compositional control —and hence optimal *division-of-labour* - in consortia for bioproduction.

## 4.1 Open challenges

The cybergenetics control community currently faces a number of open issues. First, the use of "concentrated controllers" comes at the cost of increased cellular burden and inevitable degradation of performance [49]. This limits the complexity of the policy that the controller can implement, which is a function of its topology. Strategies for the mitigation of cellular burden, largely based on establishing feedforward/feedback loops between a burden sensor and the network of interest [50], could be used in this context. However, computational studies —yet to be validated *in vitro*— indicate that a tightly regulated division of labour between cell populations of a multicellular controller would be a more promising solution [51, 52]. Another weakness of embedded controllers lies in their *genetic instability* i.e. the long-term loss of function due to random mutations and epigenetic silencing [49]. Novel strategies to preserve genetic stability remain to be developed to bridge the gap to medical applications. For external controller, the variety of techniques for real-time measurement of the biomolecular network status and actuation of the control policy remains limited and are generally unsuitable to industrial scale-up. For example, the use of optogenetic controllers in bioproduction remains constrained by the shallow penetration of light in large reaction vessels. Enhanced modelling of the light distribution, coupled with the development of optogenetics orthogonal tools, has been proposed to facilitate the industrial scale up and simplify the control of complex cultures [43].

## 4.2 Future opportunities

Cybergenetic controllers can be expected to provide transformative opportunities in biotechnology. As burden mitigation strategies for mammalian cell engineering become widespread [53], we expect that the synergistic application of control approaches will substantially change the medical therapies landscape.

In particular the rational design of smart biopharmaceutical, engineered cells locally delivering therapies tailored to the disease state they sense, and optimal treatment schedules, algorithm-based definition of drugs and dosages informed by the real-time characterisation of the disease phenotype [45], will disrupt the prevailing "one-size-fits-all" approach to therapies [54]. This can be expected to lead to more efficient, low-toxicity solutions. Encouraging proof-of-concepts for the treatment of diabetes and cancer in animal models [55, 56] seem to provide support for these predictions. Tissue engineering, with novel stem-cell derived constructs, will also benefit from cybergenetic controllers. These could provide means to identify the optimal sequence of differentiation stimuli [57] or ensure robustness to the detrimental action of undesired extracellular signals during the differentiation process [49]. Finally, cybergenetic controllers could provide a sustainable, yet competitive, edge to the industrial-scale production of food (e.g. vertical farming), bio-based products and bioenergy. Indeed, controllers could define operating conditions to optimally maximise yields at a reduced footprint. Initial successes in constraining cellular stress in favour of higher bioproduct yields [58, 59] indicate that this is a space ripe for disruption.

# 5 Conclusions

In this paper we reviewed the state of the art, opportunities and open challenges in the nascent field of cybergenetics from both the points of view of *in vivo* modelling and control.

Future progress in cybergenetics will likely rely on the integration of theories, specifically developed for living systems, and technological advances in our ability to investigate cell dynamics. Specifically, we envision that cyberphysical systems — balancing the trade-off between experimental costs and information content of the acquired data - will dominate an increasingly automated identification of mathematical models. At the frontiers of cybergenetic control we foresee the real-time, parallel operation of smart bioreactors and their digital counterpart (e.g. digital twins) as a game changer in the optimised productionof pharmaceutical and medical commodities.

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## Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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