Synthetic Biology: A Control Engineering Perspective

Thomas P. Prescott Antonis Papachristodoulou

Abstract— **Synthetic Biology is a new, rapidly developing field at the interface of Engineering and Biology. It aims to design new, or redesign existing biological systems for a particular purpose. The early years have seen the design of simple devices and parts (such as switches and oscillators); Synthetic Biology is now entering a new phase of development as the successfully designed devices of recent years are exploited to create systems of increasing sophistication. Control theoretic techniques play an important part in the design of these networks, as well as for allowing increasing levels of complexity to be engineered into synthetic biological systems. At the same time, the implementation of feedback control in these networks will allow them to sense, process and actuate on environmental and internal cues.**

I. Introduction

Synthetic Biology aims to use engineering techniques to modify existing biological networks or to design *de novo* networks from biological components in order to perform specific tasks. It is a new field, with the potential to create new industries and technologies and advance economic growth, in application areas such as energy, the environment, healthcare and agriculture. It goes beyond simple understanding of how natural systems behave (the objective of Systems Biology) and considers biological networks as systems that can either be assembled together from parts, or ones that can be redesigned/synthesized. As such, Synthetic Biology is a discipline very close to Control Engineering and in fact Control Theory tools and ideas have had and will continue to have a significant impact in this new field.

In this brief tutorial review paper we outline some of the successes and future directions of Synthetic Biology from the perspective of Control Theory. We first describe how researchers have exploited techniques from the control theoretic toolkit in the design of small synthetic biomolecular devices. As the field progresses, the implementation of the 'second wave' [1] of 'next-generation' [2] synthetic biosystems will rely on feedback control for the design of synthetic biological systems of increasing functional sophistication. We therefore describe how applications of Synthetic Biology [3] will include the design and realisation of biologically-implemented sensors, controllers and actuators by which control systems can be implemented through biological systems.

Early successes in Synthetic Biology, such as the genetic toggle switch [4] and repressilator [5], heralded a great quantity of work in designing and implementing small synthetic biological circuits, termed *devices* or *modules* [6]. For example, logic gates [7], [8], [9], genetic switches [10], [11], [12], oscillators [13], [14], [15], and signalling pathways [16] have all been implemented in small synthetic molecular networks. As reviewed in [1] and [2], we must now progress to combining modules into larger systems in order to realise a much greater degree of functional sophistication. In the following section, we outline the application of the engineering design cycle in the optimisation of synthetic biological devices. We then proceed to outline the principles underlying module combination, and then explore possible functions of next-generation synthetic biological networks from the perspective of feedback control theory.

II. The Synthetic Biology design cycle

Many of the early successful devices, such as oscillators, switches, and so on, are designed to be tuned so that they can be optimised relative to design specifications. This principle, reviewed in [17], is an example of how the toolkit of Control Theory can be applied to inform design principles in Synthetic Biology.

The parameters to be tuned are identified by mathematical modelling techniques. There are often a large number of potential parameters, or 'dials', which can be tuned to optimise the performance of a device. Therefore it is important to identify the system parameters which can be easily and accurately tuned, and which give the greatest degree of control over the measured outputs of the device. This can be achieved through a detailed characterisation of the dials at the modelling stage, considering all of the temporal and spatial scales within the cell, especially genetic, post-transcription, and post-translation control.

Many of the potential dials may give a similar level of control over certain aspects of the modelled process. It is through the implementation of the tuning strategy that different types of uncertainties manifest themselves, and we see trade-offs between different types of dials. For example, controlling dials relating to the transcription process will have a delayed effect relative to tuning dials at the translation or post-translation levels. Moreover, different dials may produce the same desired effect but almost surely they will produce different side-effects as well as have varying degrees of implementation complexity.

The principle of device optimisation through tuning parameters in the synthetic biomolecular network is one aspect of the more general design cycle for Synthetic Biology [18]. This cycle requires extensive mathematical modelling, which informs the parameter tuning at the implementation stage, and also necessitates strategies for testing the success of the design in the presence of the inherent uncertainty in models of biological systems. In Section III-B we describe systems which are designed to enable more advanced tuning and testing methods through the transition from employing static dials to the dynamic control of system parameters.

III. Synthetic biomolecular plants

The many potential applications of Synthetic Biology [19], [20] are acknowledged to require designs with increasing levels of complexity, as simpler devices and modules are combined into layered systems. The dial-tuning approach above has demonstrated some success in matching levels between devices, enabling their integration into larger networks [21].

While tuning is a successful method for the *ad hoc* connection of devices, to achieve the vision of a true 'library of parts' we must ensure a more general approach to module integration. Feedback control theory provides important design principles for a 'top-down design, bottom-up construction' approach [6] to system design. In particular, the specific challenges of combining biomolecular modules into systems requires a strategy which ensures the robust behaviour of the modules subject to a large number of sources of environmental disturbance. We will consider the question of adding feedback around modules in order to improve their robustness and performance as part of large-scale systems [22].

A. Parts and modules

To build engineered biosystems from bottom-up, we cannot rely on the adaptive approach of evolution [23]. Other approaches instead focus on the composition of well-characterised parts into functional modules, and the composition of those modules into systems [6], [21], [24].

The parts and modules that have been characterised up to now often behave differently in context of the rest of the cell, or one another if being composed, than they behave in isolation [25]. For example, when inserted into the context of a host organism such as *E. coli* or *S. cerevisiae*, synthetic processes may place a large degree of burden on that cell [26], [27]. Cells are inherently uncertain, and cell-cell variation [28] may counteract the careful tuning of synthetic devices described in Section II. When combining modules, retroactivity [29] and unintended crosstalk [30], [31], [32] resulting from interactions of shared biochemical resources may cause modules to behave unexpectedly in the context of the other modules or native biochemical processes.

A number of approaches to robustifying the behaviour of modules [33] have been taken. For example, mechanisms to interface modules using zinc finger transcription factors [34] and RNAs [35], [36] as information transmitters have been proposed. Other suggested methods to ensure insulation between modules include scaffolding [30], [37] and spatial compartmentalization [38]. Alternatively, modules can be designed to reduce burden and crosstalk by ensuring orthogonality to the host cell [39], [40].

A framework for analysing the interconnection of biomolecular subsystems, called layering [41], [42], has recently been introduced as an alternative to modular analysis. This approach defines subsystems so that they connect by overlaying dynamics, allowing retroactivity or crosstalk to be explicitly accounted for in the design process [43], and offers a new perspective for design.

Adapting modules to use feedback control has an important potential role in ensuring they are robust to interconnection. For example, as an alternative to inserting insulators into the network to reduce retroactivity [44], a feedback loop [45], [46] which monitors and corrects the deviation of a module from its isolated behaviour could instead be implemented for a similar robustifying effect.

Even neglecting the problems of interconnection, implementations of isolated modules and parts are subject to noise and fragile machinery, increasing uncertainty around the nominal designed behaviour. Designing feedback control around modules should help ensure their reliability, so that the desired behaviour is achieved [22], [6]. Indeed, evolved systems display classical feedback control architectures [47], [48], [49], [50] to improve their performance. Extending networks with feedback has been shown to be able to both enhance and attenuate noise [51] and ensure stability in the presence of cell-cell variation [52].

B. Systems

Following [6], Section III-A discusses the combination of modules into systems. The challenge is that what may be a system at one level of description may be a module at another level, as researchers combine it with other high-level systems to reach additional levels of functional sophistication. This nesting of specifications will give rise to a layered structure, as modules of increasing complexity are coordinated through higher-layer dynamics. A key problem is how to optimally distribute control strategies across this structure [53] to ensure a robust performance and subsequent adaptability to higher layers of abstraction.

Another consideration for the combination of modules into more complicated systems is that they are intended to interact with their environment. Therefore the architecture of a system needs to be designed to take into account and exploit environmental perturbations to the system. Thus the cellular environment can be used as an external input, which may or may not be influenced by the designer. In Section II we discussed the applications of Control Theory tools to the Synthetic Biology design cycle. Designing modules to interface between the researcher and the synthetic system will build upon the 'dial-tuning' approach by allowing the dynamic control of cells, possibly through implementing *in silico* feedback control. Examples of this approach include light-induced gene expression [54], [55], [56] and single-cell control through microfluidics [57]; further references can be found in [22].

Inputs can also be supplied to synthetic systems by chemical interactions with their environment. For example, chemotaxis modules can be extended and exploited [58], [59], and other chemical gradient sensing modules have also been adapted [60], [61] to induce cellular responses to environmental cues. Modularity, as discussed above, is an important feature of these subsystems; ideally the same sensing module can potentially be connected to more than one type of cellular response [62] in a 'plug-and-play' fashion. The cues which are sensed may arise from external

feedback controllers or from a given design-testing strategy, thereby enabling the rigorous dynamic control of synthetic biological networks.

IV. Synthetic biomolecular controllers

In the section above we discussed the use of feedback control to drive the implementation of Synthetic Biology designs through improving the reliability of their constituent parts and modules at various layers of organisation. In addition to the use of feedback control in the implementation of synthetic biological systems, an important application of these systems is to perform feedback control on plants (i.e. systems to be controlled) either within the cell, in its environment, or other neighbouring cells.

A. Intra-cell plant

Often, synthetic or engineered processes in a cell are controlled by inserting controller modules within the same cell. The inserted controllers are required to sense particular plant conditions and actuate to improve the performance or robustness to perturbations of the plant.

In [63], the authors proposed an implementation of basic block-diagram components such as integrators and summing junctions through a DNA implementation of idealised chemical reaction networks, which is an example application of the layered approach [42] to subsystem analysis. Implementation of synthetic control modules is challenging [64], but biomolecular applications of feedback control have been shown to work in principle [65].

An important application of feedback control of intracellular plants is in metabolic engineering [66]. As biosynthetic pathways become longer and more complex, feedback control becomes more important in ensuring the reliability and optimising yield. Synthetic feedback control systems have been advocated [67] as the next stage of development in metabolic engineering. By adapting upstream regulatory interactions in response to intermediate concentrations, these controllers can minimise wastage of resources and stabilise the system under environmental perturbations.

B. Extra-cell plant

In other situations, the plant to be controlled may be external to the cell. In these cases, we can interpret the entire cell containing the synthetic system as the controller and the environment as the plant. We need to consider strategies for the cell to sense and influence its environment appropriately to achieve a desired function.

Additionally to designing cells to respond to particular environmental cues, as described in Section III-B, the intended function of the cell may also be to actuate control the environment. One important application of using synthetic cells as environmental controllers is in therapeutics [19]. Cells have been programmed to detect nearby environmental features and display a phenotypic response [68]. In particular, synthetic cells can localise to the environment of tumours and selectively respond to cancerous cells [69], [70].

As functional requirements increase further, the next stage of Synthetic Biology will be the coordination of diverse systems through the construction of larger, multi-cellular synthetic biological structures [71]. Cell–cell signalling has already been used for population control [72] and pattern formation [73] in populations of synthetic cells. For example, AHL signalling was used to construct oscillating synthetic predator–prey cell populations [74]. Coordinating multiple cell types [75] may also be a very efficient way to implement complicated designs that single cells cannot realise [76].

V. Conclusions

This review discusses some of the complementary relationships between Synthetic Biology and Feedback Control Theory. The latter is a tool which has enabled the optimisation of a large number of simple biomolecular motifs and, by ensuring that modules remain robust when implemented in the highly uncertain cellular environment, is invaluable for the implementation of next-generation synthetic networks. Feedback control is also an extremely important application of Synthetic Biology, in particular for the sensing and actuation of the cellular environment. The interface between the two research areas will remain an important source of exciting innovation in both fields.

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