

What Is the Role of Circuit Design in the Advancement of Synthetic Biology? Part 2

Embracing the constraints and principles of variation, environment, evolution, and ecology

Going beyond Analogy



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Circuit design lies at the heart of synthetic biology. Based on the view of gene networks as constructible circuitry, circuit design provides a formal and systematic conceptual foundation for the engineering of biological systems. It also establishes rational, forward engineering as the defining route of functionality programming. Circuit design further brings us valuable concepts (such as abstraction and modularity) and methodologies (like control theory) from other engineering disciplines.

After the successful creation of many proof-of-concept devices, synthetic biology is now moving into a new phase emphasizing real world applications. Implementing these applications entails reliable realization of desired functions in complex and uncontrollable natural settings. Thus, next-generation synthetic circuits must be functionally robust against perturbations, dynamically predictable for their behaviors, and ecologically more advantageous than their natural counterparts. Rational circuit design will play a more dominant role in developing such circuits, as increasing system complexity makes intuition and experience less reliable.

In order for circuit design to provide true guidance, it needs to modify the current design strategies, which are derived largely from simple genetic-electrical circuit analogies. We shall identify new fundamental design rules that are unique to gene circuits by considering the biological nature, cellular context, and systems dynamics of gene regulation. Establishing these rules will ultimately allow us to rapidly create robust and predictable functional circuits for future applications.

Desired Properties



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At the same 1961 Quantitative Biology Symposium where the concept of allostery was cemented, Monod and Jacob postulated that phenotypes representing physiological “differentiation” in microorganisms were controlled by gene regulatory circuits. They hypothesized a set of regulatory components could be arranged in small networks to compose biochemical circuits with “virtually any desired property”.

Since the toggle switch and repressilator were first described, increasingly complex synthetic circuits with exciting and powerful potential applications in medicine and bio-industry have been developed. Such circuits also advanced our fundamental understanding of biology, including an appreciation for biology’s inherent complexity and variability. For this latter reason, we remain far from rationally designing gene circuits with “any desired property,” despite surmounting technical difficulties with remarkable innovation spurred by synthetic and systems biology.

Circuit design will undoubtedly remain a critical driver of synthetic biology’s advancement, particularly logic-based designs harnessing naturally-evolved elements (e.g., riboswitches) used by cells to integrate signals and fine tune responses within narrow output ranges, on fast time scales with exquisite sensitivity. Persuasive arguments exist for complementing synthetic circuit design with systems biology approaches to quantify circuit performance in fluctuating environments, and holistically consider relationships with cellular testbeds. Ultimately these data can be integrated in new mathematical frameworks to robustly predict circuit behavior and stability, while highlighting opportunities for design refinement.

Design with Utility in Mind



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The last two decades have witnessed pioneering work in the synthetic biology field. Using simple interacting genetic components, several different small networks were constructed and shown to display activities ranging from bistability to oscillatory behaviors. In addition to such bottom-up construction efforts, experimental characterizations of modules embedded in natural gene networks also guided the field by providing elegant examples of functional topology-activity relationships.

In recent years, synthetic biology has started to move from being a field that merely studies interesting dynamic activity of synthetic elements to one whose products aim to contribute physiological utility in various organisms. This transition is guiding efforts towards designing and building synthetic circuits that not only function as desired but also deliver that functionality with high precision. Noise in gene expression, variability in cell size, and gene copy-number changes during the cell cycle are unavoidable realities for cells. Therefore, designing synthetic circuits that function with high precision by buffering or compensating for such variations is not a luxury but a necessity.

While the capacity for buffering natural variations may come at the expense of larger synthetic circuits, advances made in the CRISPR field are beginning to provide solutions. Efficient marker-free integrations into endogenous genome locations will translate into speed and precision in the synthetic biology field.

In Future (Cell) Generations



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Circuit design has become a touchstone of synthetic biology. Logic gates and oscillators have been constructed in an amazing variety of ways, from rewiring gene regulatory networks to harnessing enzymes that can rearrange and rewrite genome sequences. Modeling, optimization, and abstraction of these circuits has enabled this process to be scaled up so that one can program many sensing and logic capabilities into a cell.

The mesmerizing movies that show circuits functioning at the single-cell level foreshadow a remaining challenge. While the population overall will “light up” with the expected output, some cells will go dark, lose phase, or otherwise malfunction. Sometimes these defective “pixels” will eventually take over an entire population as the broken cells multiply.

Why? One reason is evolution: mutations stochastically yet inevitably arise in the DNA sequences encoding a circuit. Mutant cells will be rare at first, but if they malfunction in a way that alleviates a fitness burden associated with the circuit, then they will take over. Evolutionary pressures leading to circuit failure are expected to be even stronger in real-world environments and on longer timescales (e.g., for engineered cells deployed in the human microbiome or the soil). A foundational challenge for synthetic biology is to reliably maintain designed functions over many cell generations. Genetic circuits will remain a key proving ground for new approaches to solving this problem in the future.

Mirroring Biological Networks



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Synthetic biologists have used bottom-up assembly of biomolecular circuits and rewiring of networks to realize novel functions in cells. To advance the field, circuits need to operate in real-world environments, containing diverse resident organisms and heterogeneous abiotic factors. Circuits must be equipped to discriminate among diverse environmental inputs, balance engineered functions and cellular fitness and achieve ecological and evolutionary stability over multiple timescales.

Evolution by natural selection has sculpted biological networks to allow cell growth and survival in uncertain, ecologically competitive and fluctuating environments. Next-generation circuits should be informed by biological design rules to implement ecologically stable and robust functions in target environments. Such rules include stochastic and environmentally tunable phenotypic diversification across a population, advanced preparation for impending environmental shifts, optimal partitioning of energy, and division-of-labor among specialized populations.

Major challenges include equipping host cells with traits to secure a niche, while minimizing inadvertent impact on natural environments. Mirroring biological networks, synthetic circuit activity should be coupled to host-cell physiological states to mediate trade-offs between cellular fitness and target functions. Interactions between engineered organisms and constituent members of microbiomes represent major control parameters for broad applications in medicine, agriculture and bioenergy. Bridging the gap between synthetic circuits and ecosystem behaviors by programming microbial interactions is the next frontier of synthetic biology.

Encoding Collective Behavior



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A zygote generates the entire embryo via a self-organization process in which spatially controlled cell fate selection, programmed cell-cell interactions, timed proliferation, and death play significant roles. Substantial data that is vital for this collective cell behavior is wired in the mammalian genome in the form of natural genetic circuits. Circuit engineering in a homogeneous cell population has been a central element in synthetic biology and provided us the opportunity to reveal biological design principles through rational engineering. In the next chapter, we will move on to apply designer circuits to spatiotemporally control mammalian cell fate and function to set the stage for programmable multicellular systems.

In this journey, synthetic biology and circuit engineering will help us to go beyond a homogeneous cell population to understand collective cell behavior and design principles of tissue ecology and self-organization. This is challenging due to the diversity of intrinsic genetic circuits and limited data on temporal and spatial activity of such circuits with single cell resolution. However, integration with single cell genomics, machine learning, and advanced computational analyses can provide us with design substrates and candidate key targets for iterative processes of forward and reverse engineering. In the case of mammalian organogenesis, the products will be, first, understanding our own development and, second, emergence of programmable tissues and engineered multicellular machines.