

Self-adaptive biosystems through tunable genetic parts and circuits

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Abstract

Biological systems often need to operate in complex environments where conditions can rapidly change. This is possible because of their inherent ability to sense changes and adapt their behavior in response. Here, we detail recent advances in the creation of synthetic genetic parts and circuits whose behaviors can be dynamically tuned through a variety of intracellular and extracellular signals. We show how this capability lays the foundation for implementing control engineering schemes in living cells and allows for the creation of biological systems that are able to self-adapt, ensuring their functionality is maintained in the face of varying environmental and physiological conditions. We end by discussing some of the broader implications of this technology for the safe deployment of synthetic biology.

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Introduction

A key characteristic of all living organisms is their ability to adapt. From altering metabolism to best utilize shifts in nutrients, to regulating ion transport to maintain cellular

homeostasis, adaptive responses are crucial to many aspects of life. To support such adaptive processes, cells have evolved a wide array of sensors able to capture information about their local environment and their internal state. These sensors are connected to cellular circuits that both monitor and modify internal processes with the goal of maintaining specific functionalities (e.g. homeostasis) no matter the perturbations experienced by the cell.

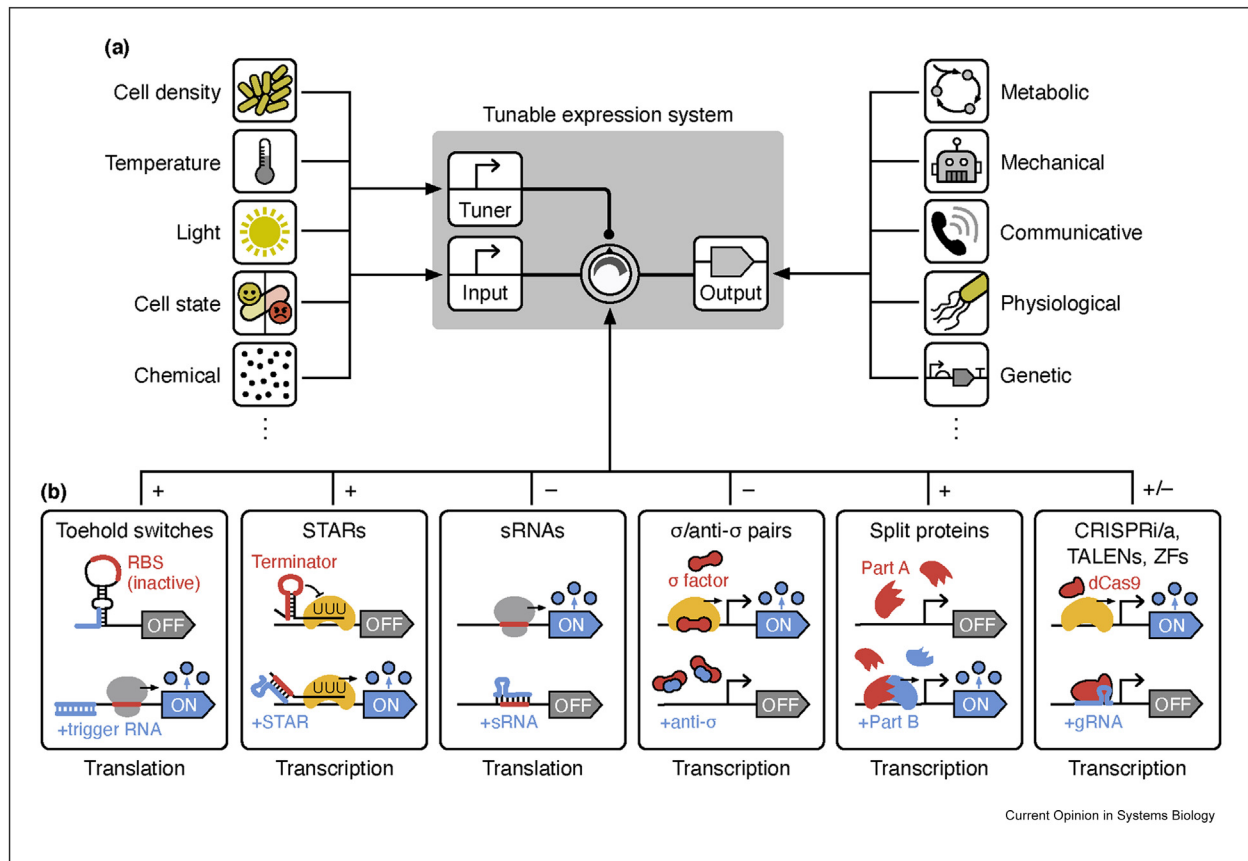
Unlike in nature, engineered biological systems often lack the ability to adapt to changing conditions, making them fragile and causing them to break easily [1–6]. This stems historically from an absence of genetic parts that can be used to dynamically tune the response of a system and the additional burden of implementing control processes on top of a basic functioning system. This view, however, is beginning to change [7–9]. Recent developments in synthetic biology have led to a wide variety of biological parts able to precisely regulate the transcription [10–14] and translation [15–19] of genes in response to diverse intracellular and extracellular signals [20]. Furthermore, the benefits of exploiting control engineering principles to create robust biosystems is also becoming recognized [7–9,21,22]. This stems from a growing need in many applications for reliable and guaranteed functionalities no matter the strain of cell used, or the environment deployed [23].

In this work, we discuss some of the recent advances toward engineering self-adaptive biological systems. We begin by providing an overview of the wide variety of parts now available for sensing and tuning cellular behaviors and show some of the ways these can be used to create adaptive genetic circuits. We then discuss recent steps toward using these circuits to implement closed-loop feedback control within living cells to create self-adaptive systems and end by outlining some of the future applications that such capabilities could support.

Tunable genetic parts

To develop an adaptive system, it is necessary to be able to dynamically alter/tune the input–output relationship of parts within a system. These ‘tunable’ components come in many different forms, however, conceptually have a common structure (Figure 1a). Each tunable element consists of an input and output, and a further

Figure 1



Tunable genetic parts. (a) The schematic of a tunable expression system (TES) where a variety of different inputs and output can be selected. Typically, inputs are transcriptional signals related to environmental or cellular states, and the output is the expression of a gene that influences cellular behavior or acts as an input to another part of a larger circuit. (b) Major regulatory mechanisms that can be used to tune gene expression in a TES. Both active and inactive states shown in addition to whether the tuner will cause activation (+) or repression (–) of the output. The stage in gene expression (i.e. transcription or translation) where regulation takes place is shown below the box for each mechanism. Ribosomes and RNA polymerase (RNAP) shown by light gray and orange shapes without an outline, respectively. For the CRISPRi/a, TALENs and ZFs box a repressive CRISPRi system is shown. This can be modified to be an activator by fusing dCas9 to an activator domain to recruit RNAP to the promoter. In general, the additional blue element would be expressed by the tuner input to modulate expression of the output. RBS, ribosome-binding site; sRNA, small RNA; STAR, small transcription-activating RNA; CRISPRi/a, clustered regularly interspaced short palindromic repeats interference/activation; TALEN, transcription activator–like effector nuclease; ZF, zinc finger; gRNA, guide RNA.

tuner input that is able to alter the input–output relationship in a useful way [24]. Input, output, and tuner signals can take many forms, from gene expression rate to protein phosphorylation state. However, one of the most commonly used is transcriptional activity [3,6,25]. This is captured by the RNA polymerase (RNAP) flux along a strand of DNA and can be directed to particular points by the placement of promoter sequences that control the transcriptional initiation of RNAP. This makes it easy to connect individual parts. You simply make the output promoter of one part the input promoter of another [10,26].

Although there are many ways that the behavior of biological parts can be tuned, the most widespread and easiest to apply is through the control of gene

expression. By incorporating additional regulatory elements to modify the rate of transcription and/or translation of an output gene, it is possible to create what we term a tunable expression system (TES) that can vary the amount of output protein produced for a given input transcriptional activity [24]. As gene expression underlies many core cellular behaviors, this approach is a flexible means to control a variety of biological functionalities in a dynamic and tunable way.

The core structure of a TES comprises of promoters acting as signals for the input and tuner, a gene that is expressed as output, and internal regulators that allow the input and tuner promoters to dynamically alter the output protein expression rate (Figure 1a). For the input and tuner promoters, a variety of sensors with

transcriptional outputs now exist to sense environmental conditions such as chemical concentrations [27] and light [28,29], as well as internal cellular states (e.g. stress responses) [9] and even population level features such as cell density through quorum sensing [30]. Similarly, many output genes exist that enable the control of cellular behaviors from modifying the metabolic state of a cell [31–33] to controlling cell movement [34] and even coordinating cell-to-cell communications [30]. The final component in the TES is the internal regulator used to modulate how transcriptional activity of the input promoter is transformed into an output protein expression rate. To make this relationship a function of the transcriptional activity of the tuner promoter, numerous types of transcriptional and translational regulators can be used (Figure 1b). These include (1) toehold switches where translation rate is controlled through expression of a trigger RNA that is able to disrupt secondary structures around the RBS of the output gene [15,16,24]; (2) small transcription-activating RNAs which interact with transcriptional terminators that are placed in the 5' untranslated region of a gene and regulate premature RNAP termination [11,12,35]; (3) small RNAs that can be designed to bind the ribosome-binding site for a gene of interest and suppress translation initiation [18]; (4) σ /anti- σ pairs where the anti- σ protein is expressed by the tuner promoter to reduce the expression rate of input promoters driven by the cognate σ -factor [36,37]; (5) split T7 RNAPs where the input and tuner promoters express different halves of the T7 RNAP and the gene of interest is connected to a cognate T7 promoter [38]; and (6) other programmable transcription factors such as clustered regularly interspaced short palindromic repeats interference/activation (CRISPRi/a) [14], transcription activator-like effector nucleases [39] and zinc fingers [40] that can be expressed by the tuner promoter and interfere or enhance transcription initiation or elongation from the input promoter.

Although it is more common for the input and tuner promoters to be different, recently it has been shown that by using identical promoters to control both regulatory inputs in unison, a more stringent control of protein expression and sharp digital-like transitions between OFF and ON states can be achieved [35,41] (Greco et al. bioRxiv doi: 10.1101/2020.07.04.187500).

It should also be noted that other approaches to tuning gene expression have been developed. For example, two-component systems where phosphorylation rates can be modified by the concentration of specific kinases [42] and CRISPRi systems where the strength of repression is controlled by basepair mismatches in the guide RNA [9]. However, in most cases tuning of such systems requires the physical modification of the encoding DNA making it impossible to dynamically regulate behavior.

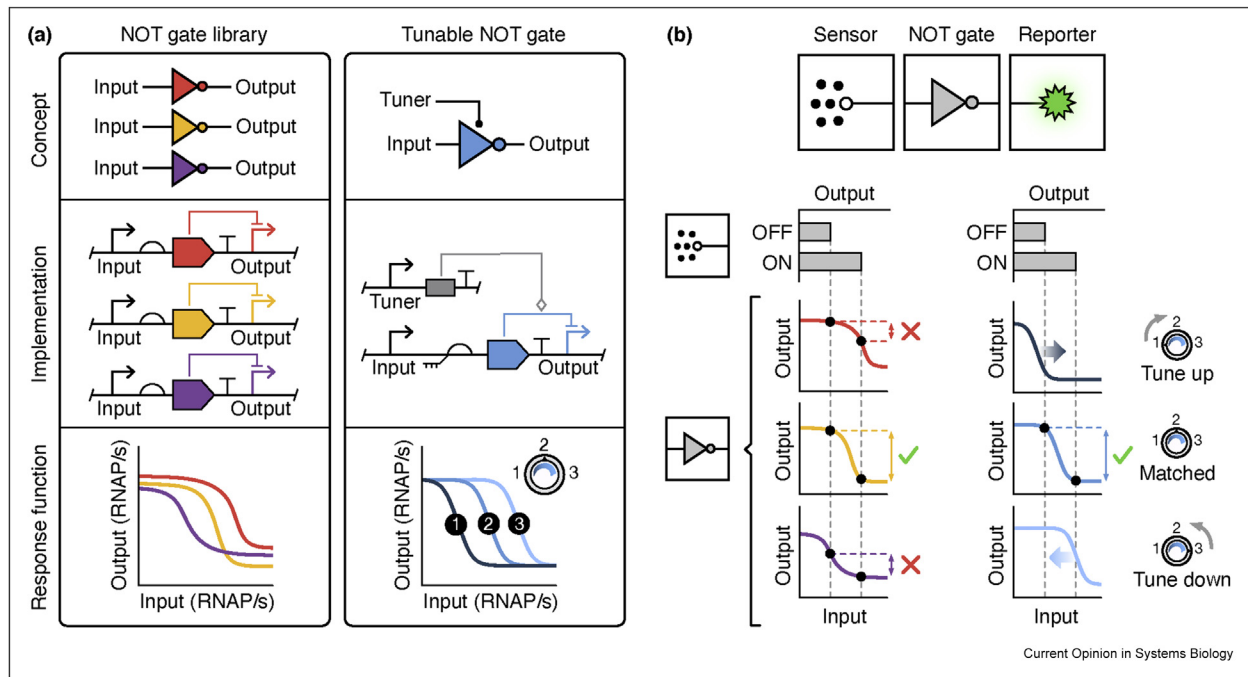
Adaptive genetic circuitry

To implement more complex functionalities, it is often necessary to connect together many genetic parts into a circuit [6]. In other engineering fields such as electronics, specifying the connections between components would generally be sufficient to create a working system. This is due to electronic components having standardized operating ranges to ensure compatibility and reliable functionalities no matter the context they are used in. For example, complementary metal oxide–semiconductor electronic logic gates expect inputs of 0–1.5 V for an OFF state and 3.5–5 V for an ON state. In biology, such standardization is difficult because of the diversity of biochemical components used and challenges in engineering them to ensure a common level of response [26,43]. Therefore, rather than imposing constraints on biology that are near impossible to implement, it is instead necessary to work with the diversity present and ensure that components connected have inputs and outputs that are ‘matched’ to guarantee signals propagate correctly [6,20,44]. Many of the advances in automated genetic circuit design have revolved around ensuring parts perform consistently when used in different ways (e.g. insulating their function from varying genetic context [45,46]) and automating the selection of combinations of parts within a circuit such that their inputs and outputs are optimally matched [6,26,43].

Tunable genetic parts can greatly simplify this process by removing the need to reassemble a circuit if two parts are found to be mismatched when connected. At the cost of additional tuning inputs to a circuit, tunable genetic parts can have their response function dynamically varied after circuit assembly (Figure 2a). This allows parts to be dynamically matched and opens up the possibility of rapidly optimizing overall circuit function without the need to reassemble the underlying DNA (Figure 2b). In addition to simplifying the creation of optimized circuits, the ability to dynamically vary the response dynamics of individual parts is also valuable for systems that must function in highly changeable environments, where shifts might cause physiological changes that impact some or all parts in a circuit [4,5,47].

Beyond the tuning of steady-state response functions, circuits capable of exhibiting dynamic behaviors such as oscillations have also been developed, where characteristics such as the period and amplitude can be varied through diverse inputs to the system. In one such oscillator for *Escherichia coli* cells, positive and negative feedback loops are implemented using the P_{BAD} (positive) and P_{lac} (negative) systems which can be further regulated using arabinose and isopropyl β -D-1-thiogalactopyranoside, respectively [48]. It was found that increasing the concentration of arabinose caused a

Figure 2



Tunable genetic parts enable the construction of adaptive circuits. (a) Libraries of genetic parts (e.g. NOT gates) are commonly created that cover a range of different behaviors (left box). These differences are shown by the specific response function of each part, which captures the steady-state input–output relationship. For most genetic parts, the response function is fixed and, therefore, physical replacement is necessary if a part is not compatible when used in a system. In contrast, tunable genetic parts (right box) have additional tuner inputs that allow the shape and position of the response function to be dynamically varied as required. (b) The schematic of a simple genetic circuit where a sensor input is inverted to give a desired output reporter (e.g. green fluorescence). For the sensor and NOT gate parts to work effectively, the output of the sensor must ‘match’ the response function of the NOT gate (dotted gray lines). If the parts are matching, then a large change in the NOT gate output will occur when the sensor switches between OFF and ON states. For standard NOT gates (left column) entire libraries need to be assembled and screened to find a working combination. Furthermore, if the environment changes then too might the behavior of the parts making reassembly necessary. For a tunable NOT gate (right column), the tuner input can be varied until the gate perfectly matches the sensor’s outputs. No reassembly is required, allowing the circuit to be dynamically tuned to changing conditions. Genetic circuits shown using Synthetic Biology Open Language (SBOL) Visual notation [73]. RNAP, RNA polymerase.

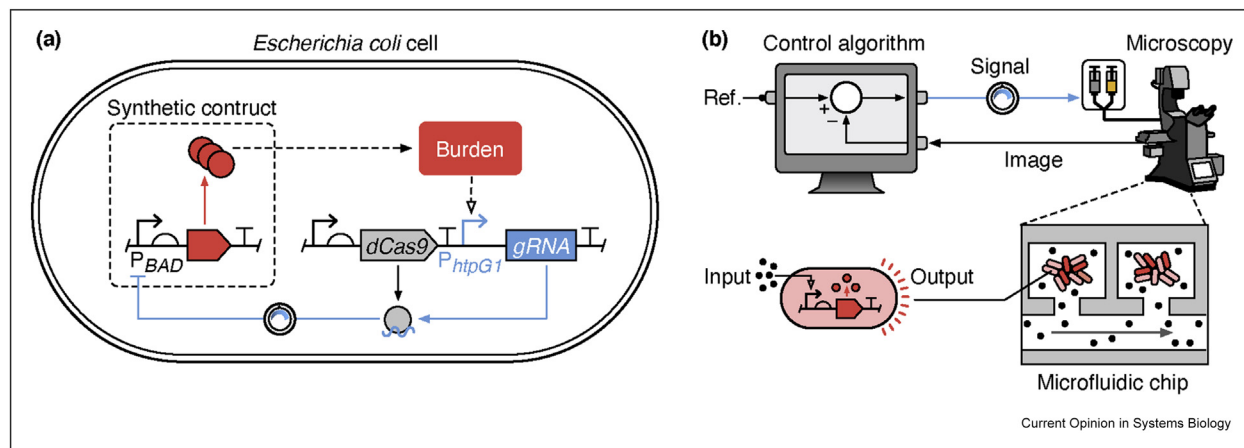
lengthening of the oscillatory period, whereas increasing an isopropyl β -D-1-thiogalactopyranoside concentration or temperature led to a shortening of the oscillatory period. Other tunable oscillator circuits have also been developed to allow for control via light [49], to synchronize behaviors across a population of bacteria [50] and to function in mammalian cells [51]. Furthermore, they have been modeled to demonstrate regulatory motifs capable of having their oscillatory amplitude and frequency tuned independently [52].

Toward self-adaptive systems

A limitation of using tunable genetic parts and circuits is the need for external inputs to be continually provided. A solution to this is to connect the output of a cellular process to the tuner input of the circuit, creating a closed-loop, self-adaptive system. There has been growing interest in the application of closed-loop feedback control in biology and the role that control engineering principles might play in creating robust biosystems [8,21,22,53].

Some simple feedback control schemes have already been implemented in living cells. Many of these focus on the development of dynamic regulatory schemes for metabolism to maximize the yield of desired products [33,54,55]. Feedback is created by either using endogenous transcription factors that respond to intermediate metabolites of interest [31,56], or by the design of RNA aptamers able to sense and then actuate gene expression and therefore shift metabolic fluxes in response to changes in metabolite concentrations (Glasscock et al. bioRxiv doi: 10.1101/529180). Related to this, general cellular stress responses have also been used as triggers for feedback control. Specifically, the σ^{32} heat-shock response of *E. coli* was found to be rapidly activated when cells were burdened by excessive protein expression [57]. By connecting the endogenous P_{hspG1} σ^{32} -promoter to a CRISPRi-based feedback control system (Figure 3a), it was shown that protein expression of burdensome synthetic genetic constructs could be dynamically regulated to reduce cellular burden [9]. This both increased the overall protein yield, as there

Figure 3



Self-adaptive systems. (a) Embedded burden-based controller. A synthetic construct expresses a burdensome protein. Endogenous cellular processes (dashed arrows) lead to the activation of the P_{htpG1} promoter under high levels of protein expression burden causing expression of a guide RNA (gRNA). This gRNA forms a complex with a constitutively expressed dCas9 protein that then targets the promoter of the synthetic construct, downregulating its expression. The strength of this negative feedback loop is dynamically 'tuned' by the endogenous burden signal and mismatches in the gRNA to the target promoter that reduce the binding affinity of the dCas9:gRNA complex. Panel adapted from the study by Ceroni et al. [9]. (b) Schematic of an external *in silico* control system. Living cells grow in a microfluidic chip that is continually imaged by a microscope. These images are sent to a computer and analyzed, and an output signal from the cells (e.g. fluorescence) was compared with a desired reference value. A control algorithm assesses this difference and emits a control signal, which actuates syringes and changes the concentration of a signaling molecule provided to the cells. The cells sense this change and alter their gene expression in response. The strength of feedback in this system can be tuned by modulating the control signals produced. The gray arrow in the microfluidic chip represents the flow of nutrients and signaling molecules. gRNA, guide RNA.

was less impact on cellular growth, and the evolutionary stability of the synthetic genetic constructs as there was less selective pressure for mutations. Similar approaches have been implemented using repressor proteins for negative feedback regulation and the P_{ibpAB} σ^{32} -promoter as a sensor of burden [58]. Dynamic regulation of protein expression has also been performed in mammalian cells using translation-based negative feedback control [59] and general-purpose gene expression controllers based on quorum sensing [32].

More general feedback control schemes in living cells include the antithetic integral controller motif that uses sequestration mechanisms such as molecular titration to implement an embedded feedback controller [8]. This motif guarantees perfect adaptation, rejecting constant disturbances so that the output of the genetic system of interest initially responds to an external input but then returns to basal levels if the input persists [21].

Molecular titration has also been shown to be an effective mechanism to implement 'comparator' devices able to produce an output function capturing the mismatch between the levels of two different inputs – an essential component of any biomolecular controller [36,60]. Implementations of more sophisticated control strategies have also been recently presented, such as the biomolecular Proportional-Integral-Derivative (PID) controller presented in the

study by Chevalier et al. [61]. As the complexity of biomolecular control designs increases, to successfully implement the control function, parts must be finely tuned to guarantee the right balance between the sensing and actuating functions of a circuit [62]. The use of tunable parts could open the way to the development of adaptive biomolecular controllers able to self-tune themselves to guarantee the robust execution of the control task they are assigned, being able to function in the presence of perturbations, cell-to-cell variability, and so on. This might be even more crucial when the control functions themselves are spread among different populations in a microbial consortium as recently suggested by Fiore et al. [63].

Beyond simple feedback motifs, it can be difficult to implement complex control algorithms using biochemical components because the feedback strengths and dynamics required may be difficult to match to available parts. Therefore, an intermediate step is sometimes taken whereby a computer is used to implement controller logic within the feedback loop and create what is termed a cybergenetic system [22] (Figure 3b). Cybergenetic systems often rely on single-cell microcopy platforms and microfluidics to image engineered cells whose current state is displayed via fluorescent reporter proteins and use chemical inducers [64,65] or light [28,29,66,67] as inputs to perturb the cells states in a predefined way

(i.e. the cells are engineered to sense and update their state in response to a stimulus). The computer-based controller runs in real-time analyzing microscopy images to extract the current states of cells and then immediately computes a control action, which is then administered by varying the chemical concentrations or light that the cells are exposed to. Such systems have been shown capable of controlling both population [8,64,66,68] and single-cell behaviors [67]. Moreover, toolkits have emerged to simplify their creation by handling image analysis, tracking, and calculation of control actions (Pedone *et al.* bioRxiv doi: 10.1101/2020.06.25.171751). The major advantage of this hybrid approach is that the computer controllers are cell-agnostic, allowing them to be used with any biosystem that has the same types of control inputs and observable outputs.

Conclusions

The creation of self-adaptive biosystems that can function in the face of varying and uncertain environments will be a crucial step for the safe deployment of synthetic biology into everyday life. Recent advances in biological control engineering provide the theoretical foundations necessary to design such systems, and, as we have shown, tunable genetic parts and circuits can support their physical implementation [24]. While the self-adaptive systems built to date have mostly been small-scale proof-of-concepts, it is clear that the ability to synthesize and assemble entire genomes is in reach [69,70]. Demonstrating the value of integrating tunable parts and circuits within these cellular systems will be crucial to moving beyond the mere recoding of existing genomic information and toward the creation of synthetic cells built from the ground up to reliably implement novel functionalities. Furthermore, they will support the scale-up of these systems by enabling us to move beyond single cells and toward the engineering of collective behaviors of vast populations of cells [71] or even entire synthetic ecologies [72].

Author contributions

All authors contributed to the writing and editing. T.E.G. and V.B. produced the figures.

Conflict of interest statement

Nothing declared.

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