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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 due to 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 intra- and extra-cellular 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.

Highlights

- Tunable genetic parts allow for their input-output relationship to be dynamically varied in response to intra- and extra-cellular signals.
- Self-adaptive biological systems can be built using a combination of control engineering principles and tunable genetic parts and circuits.
- An ability to engineer self-adaptive systems will be crucial in deploying synthetic biology into complex and changeable real-world environments.

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Introduction

A key characteristic of all living organisms is their ability to adapt. From altering metabolism to best utilize a shift in nutrients, to regulating ion transport to maintain cellular homeostasis, adaptive responses are crucial to many aspects of life. To enable such adaptive processes, cells have evolved a wide array of sensors able to capture information about their local environment as well as their internal state. These sensors are connected to cellular circuits that both monitor and modify internal processes with the goal of maintaining a desired functionality (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 is, however, 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 intra- and extra-cellular 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 to [23].

In this work, we discuss some of the recent advances towards 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 than discuss recent steps towards 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 the 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 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 DNA and can be directed to particular points by positioning

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promoters that control the transcriptional initiation of RNAP. This makes it simpler to connect individual parts by making the output promoter of one the input promoter of another [10,26].

While 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 parts to modify the rate of transcription and/or translation of an output gene it is possible to create 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 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 population level features like cell density through quorum sensing [30]. Similarly, many output genes exist that enable control of cellular behaviors from modifying their metabolic state [31–33] to cell movement [34] and even 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 a 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 (THSs) 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 (STARS) which interact with transcriptional terminators that are placed in the 5' untranslated region (UTR) of a gene and regulate premature RNAP termination [11,12,35]; 3. small RNAs (sRNAs) 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 and the gene of interest is connected to the cognate promoter of the RNAP [38]; and 6. other programmable transcription factors like CRISPRi/a [14], transcription activator-like effector nucleases (TALENs) [39] and zinc fingers (ZFs) [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, more stringent control of a protein expression can be achieved as well as sharp digital-like

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transitions between OFF and ON states [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 base mismatches in the guide RNA (gRNA) [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 (CMOS) 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 due to 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 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].

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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 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_{tac} (negative) systems which can be further regulated using arabinose and isopropyl β-D-1-thiogalactopyranoside (IPTG), respectively [48]. It was found that increasing the concentration of Arabinose caused a lengthening of the oscillatory period, while increasing an IPTG 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 cells [50] and to function in mammalian cells [51]. Furthermore, they've been modelled to demonstrate regulatory motifs capable of having their oscillatory amplitude and frequency tuned independently [52].

Towards 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 or shifts in 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_{htpG1} 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 overall protein yield, as there was less impact of 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

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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 while 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 of 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 PID controller presented in [61]. As the complexity of biomolecular control designs increases, to successfully implement the control function, the parts needed to construct the control strategy must be finely tuned to guarantee the right balance between the sensing and actuating parts 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 in order to guarantee the robust execution of the control task they are assigned to perform even in the presence of perturbations, cell-to-cell variability, etc. This might be even more crucial when the control functions are spread among different populations in a microbial consortium as recently suggested in [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 pre-defined 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 chemicals 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

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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 towards 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 towards the engineering of collective behaviors of vast populations of cells [71] or even entire synthetic ecologies [72].

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Author Contributions

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

Declaration of Interest

None.

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- of special interest
•• of outstanding interest

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Figures and captions

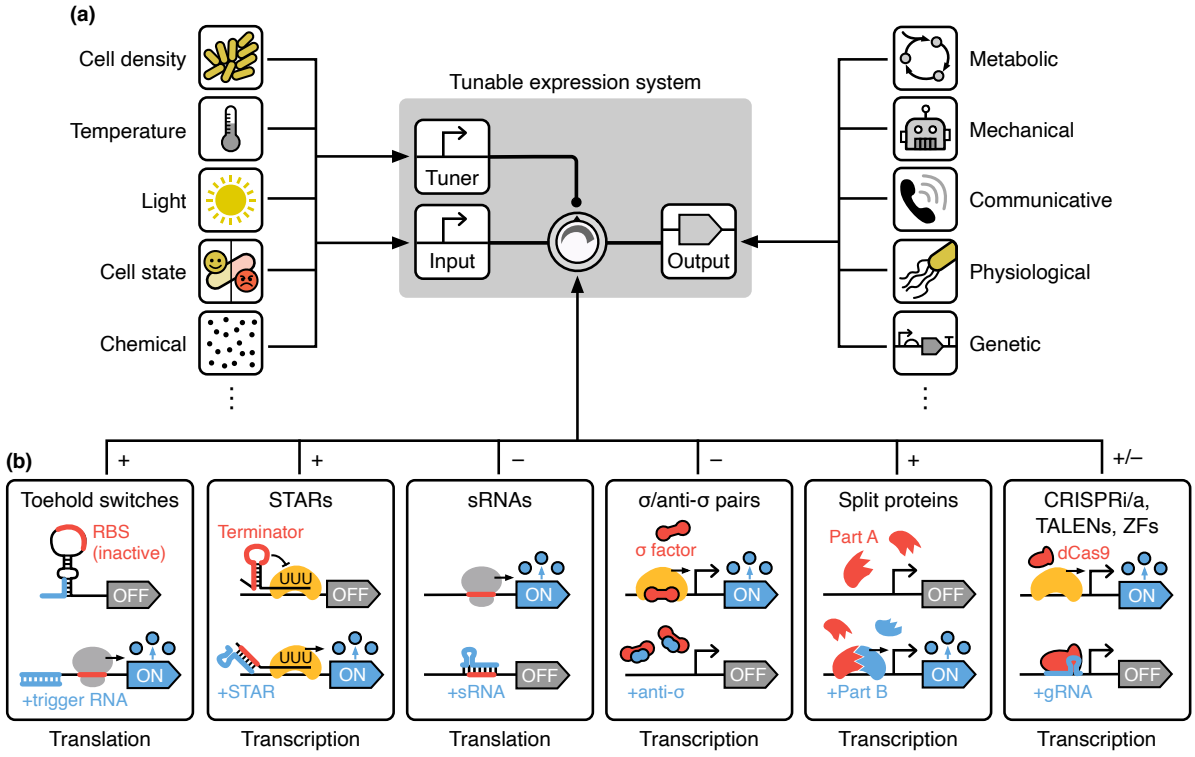

Figure 1: Tunable genetic parts. (a) Schematic of a tunable expression system (TES) where a variety of different inputs and output can be selected. Typically, inputs are transcriptional signals related to environmental or cellular states and the output is the expression of a gene that influences cellular 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 grey 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 RNAs; siRNA, small interfering RNA; CRISPRi/a, clustered regularly interspaced short palindromic repeats interference/activation; TALEN, transcription activator-like effector neclease; ZF, zinc finger; gRNA, guide RNA.

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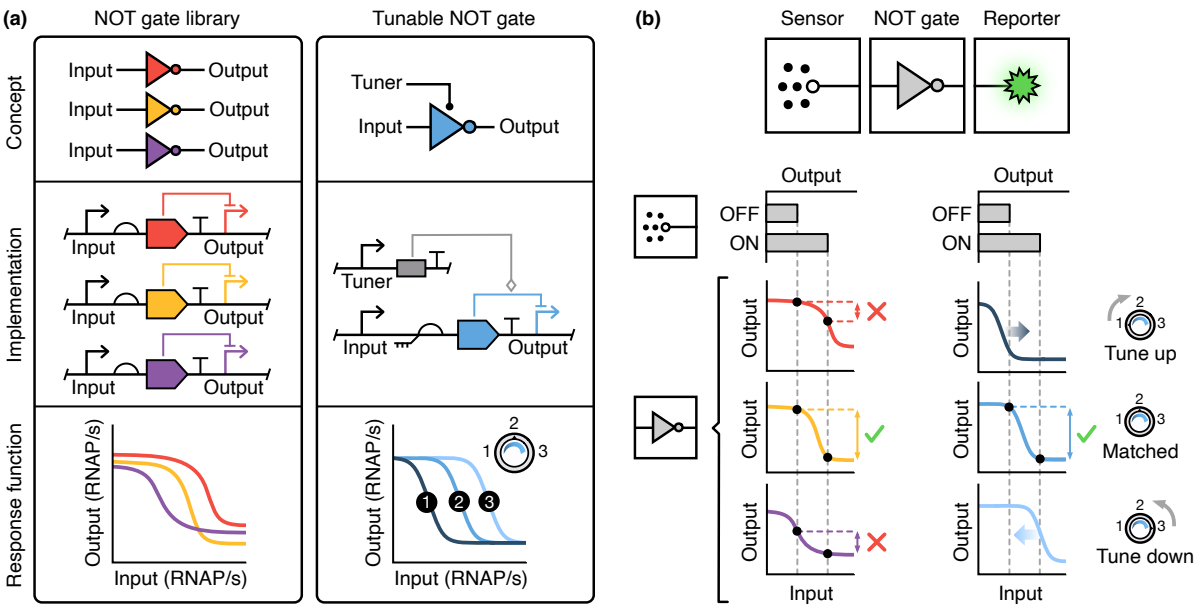

Figure 2: Tunable genetic parts enable the construction of adaptive circuits. (a) Libraries of genetic parts (e.g. NOT gates) are commonly created that cover a range of different behaviors (left box). These differences are shown by the specific response function of each part, which captures the steady-state input-output relationship. For most genetic parts the response function is fixed, and so 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)** Schematic of a simple genetic circuit where a sensor input is inverted to give a desire output reporter (e.g. green fluorescence). For the sensor and NOT gate to parts to work effectively, the output of the sensor must 'match' the response function of the NOT gate (dotted grey 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 so 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.

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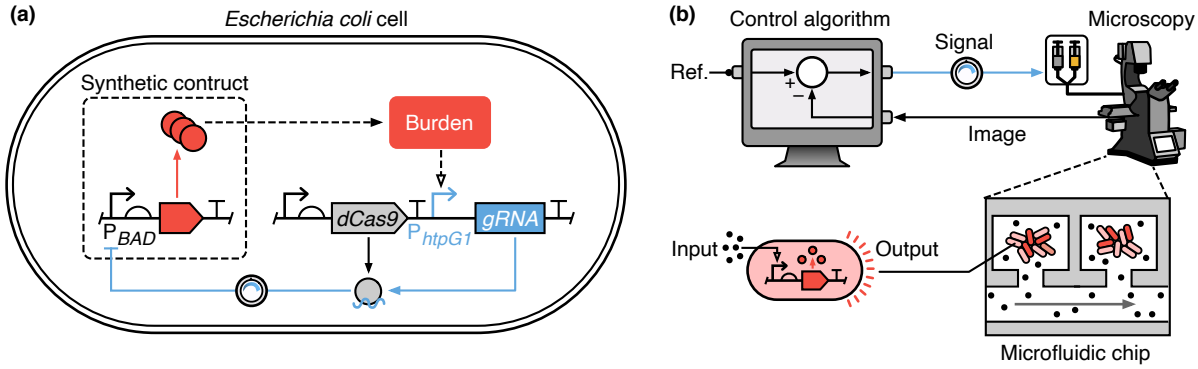

Figure 3: Self-adaptive systems. (a) Embedding burden-based controller. A synthetic construct expresses a burdensome protein. Endogenous cellular processes (dashed arrows) lead to the activation of the P_{htpG1} promoter under high levels of protein expression burden causing expression of a guide RNA (gRNA). This gRNA forms a complex with a constitutively expressed dCas9 protein that then targets the promoter of the synthetic construct, down regulating its expression. The strength of this negative feedback loop is dynamically 'tuned' by the endogenous burden signal as well as mismatches in the gRNA to the target promoter that reduce the binding affinity of the dCas9:gRNA complex. Panel adapted from [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 set to a computer, analyzed and an output signal from the cells (e.g. fluorescence) compared to 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. Grey arrow in the microfluidic chip represents the flow of nutrients and signaling molecules. gRNA, guide RNA.