

Self-adaptive biosystems through tunable genetic parts and circuits

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1 **Abstract**

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

11

12 **Highlights**

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

19 Introduction

20 A key characteristic of all living organisms is their ability to adapt. From altering metabolism
21 to best utilize a shift in nutrients, to regulating ion transport to maintain cellular homeostasis,
22 adaptive responses are crucial to many aspects of life. To enable such adaptive processes,
23 cells have evolved a wide array of sensors able to capture information about their local
24 environment as well as their internal state. These sensors are connected to cellular circuits
25 that both monitor and modify internal processes with the goal of maintaining a desired
26 functionality (e.g. homeostasis) no matter the perturbations experienced by the cell.

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

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

45

46 Tunable genetic parts

47 To develop an adaptive system, it is necessary to be able to dynamically alter/tune the input-
48 output relationship of parts within the system. These ‘tunable’ components come in many
49 different forms, however, conceptually have a common structure (**Figure 1a**). Each tunable
50 element consists of an input and output, and a further tuner input that is able to alter the input-
51 output relationship in a useful way [24]. Input, output and tuner signals can take many forms,
52 from gene expression rate to protein phosphorylation state. However, one of the most
53 commonly used is transcriptional activity [3,6,25]. This is captured by the RNA polymerase
54 (RNAP) flux along DNA and can be directed to particular points by positioning

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

57 While there are many ways that the behavior of biological parts can be tuned, the most
58 widespread and easiest to apply is through the control of gene expression. By incorporating
59 additional regulatory parts to modify the rate of transcription and/or translation of an output
60 gene it is possible to create a tunable expression system (TES) that can vary the amount of
61 output protein produced for a given input transcriptional activity [24]. As gene expression
62 underlies many core cellular behaviors this approach is a flexible means to control a variety
63 of biological functionalities in a dynamic and tunable way.

64 The core structure of a TES comprises of promoters acting as signals for the input and
65 tuner, a gene that is expressed as output, and internal regulators that allow the input and tuner
66 promoters to dynamically alter output protein expression rate (**Figure 1a**). For the input and
67 tuner promoters, a variety of sensors with transcriptional outputs now exist to sense
68 environmental conditions such as chemical concentrations [27] and light [28,29], as well as
69 internal cellular states (e.g. stress responses) [9] and population level features like cell density
70 through quorum sensing [30]. Similarly, many output genes exist that enable control of cellular
71 behaviors from modifying their metabolic state [31–33] to cell movement [34] and even cell-
72 to-cell communications [30]. The final component in the TES is the internal regulator used to
73 modulate how transcriptional activity of the input promoter is transformed into a protein
74 expression rate. To make this relationship a function of the transcriptional activity of the tuner
75 promoter, numerous types of transcriptional and translational regulators can be used (**Figure**
76 **1b**). These include: 1. toehold switches (THSs) where translation rate is controlled through
77 expression of a trigger RNA that is able to disrupt secondary structures around the RBS of the
78 output gene [15,16,24]; 2. small transcription activating RNAs (STARS) which interact with
79 transcriptional terminators that are placed in the 5' untranslated region (UTR) of a gene and
80 regulate premature RNAP termination [11,12,35]; 3. small RNAs (sRNAs) that can be
81 designed to bind the ribosome binding site for a gene of interest and suppress translation
82 initiation [18]; 4. σ /anti- σ pairs where the anti- σ protein is expressed by the tuner promoter to
83 reduce the expression rate of input promoters driven by the cognate σ -factor [36,37]; 5. split
84 T7 RNAPs where the input and tuner promoters express different halves and the gene of
85 interest is connected to the cognate promoter of the RNAP [38]; and 6. other programmable
86 transcription factors like CRISPRi/a [14], transcription activator-like effector nucleases
87 (TALENs) [39] and zinc fingers (ZFs) [40] that can be expressed by the tuner promoter and
88 interfere or enhance transcription initiation or elongation from the input promoter.

89 Although it is more common for the input and tuner promoters to be different, recently
90 it has been shown that by using identical promoters to control both regulatory inputs in unison,
91 more stringent control of a protein expression can be achieved as well as sharp digital-like

92 transitions between OFF and ON states [35,41] (Greco et al. bioRxiv doi:
93 10.1101/2020.07.04.187500).

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

100

101 **Adaptive genetic circuitry**

102 To implement more complex functionalities, it is often necessary to connect together many
103 genetic parts into a circuit [6]. In other engineering fields such as electronics, specifying the
104 connections between components would generally be sufficient to create a working system.
105 This is due to electronic components having standardized operating ranges to ensure
106 compatibility and reliable functionalities no matter the context they are used in. For example,
107 complementary metal-oxide-semiconductor (CMOS) electronic logic gates expect inputs of 0–
108 1.5 V for an OFF state and 3.5–5 V for an ON state. In biology, such standardization is difficult
109 due to the diversity of biochemical components used and challenges in engineering them to
110 ensure a common level of response [26,43]. Therefore, rather than imposing constraints on
111 biology that are near impossible to implement, it is instead necessary to work with the diversity
112 present and ensure that components connected have inputs and outputs that are ‘matched’
113 to guarantee signals propagate correctly [6,20,44]. Many of the advances in automated
114 genetic circuit design have revolved around ensuring parts perform consistently when used in
115 different ways (e.g. insulating their function from varying genetic context [45,46]) and
116 automating the selection of combinations of parts within a circuit such that their inputs and
117 outputs are optimally matched [6,26,43].

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

127 Beyond the tuning of steady-state response functions, circuits capable of exhibiting
128 dynamic behaviors such as oscillations have also been developed, where characteristics such
129 as period and amplitude can be varied through diverse inputs to the system. In one such
130 oscillator for *Escherichia coli* cells, positive and negative feedback loops are implemented
131 using the P_{BAD} (positive) and P_{tac} (negative) systems which can be further regulated using
132 arabinose and isopropyl β -D-1-thiogalactopyranoside (IPTG), respectively [48]. It was found
133 that increasing the concentration of Arabinose caused a lengthening of the oscillatory period,
134 while increasing an IPTG concentration or temperature led to a shortening of the oscillatory
135 period. Other tunable oscillator circuits have also been developed to allow for control via light
136 [49], to synchronize behaviors across a population of cells [50] and to function in mammalian
137 cells [51]. Furthermore, they've been modelled to demonstrate regulatory motifs capable of
138 having their oscillatory amplitude and frequency tuned independently [52].

139

140 **Towards self-adaptive systems**

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

146 Some simple feedback control schemes have already been implemented in living cells.
147 Many of these focus on the development of dynamic regulatory schemes for metabolism to
148 maximize the yield of desired products [33,54,55]. Feedback is created by either using
149 endogenous transcription factors that respond to intermediate metabolites of interest [31,56],
150 or by the design of RNA aptamers able to sense and then actuate gene expression or shifts
151 in metabolic fluxes in response to changes in metabolite concentrations (Glasscock et al.
152 bioRxiv doi: 10.1101/529180). Related to this, general cellular stress responses have also
153 been used as triggers for feedback control. Specifically, the σ^{32} heat-shock response of *E. coli*
154 was found to be rapidly activated when cells were burdened by excessive protein expression
155 [57]. By connecting the endogenous P_{htpG1} σ^{32} -promoter to a CRISPRi based feedback control
156 system (**Figure 3a**), it was shown that protein expression of burdensome synthetic genetic
157 constructs could be dynamically regulated to reduce cellular burden [9]. This both increased
158 overall protein yield, as there was less impact of cellular growth, and the evolutionary stability
159 of the synthetic genetic constructs as there was less selective pressure for mutations. Similar
160 approaches have been implemented using repressor proteins for negative feedback regulation
161 and the P_{ibpAB} σ^{32} -promoter as a sensor of burden [58]. Dynamic regulation of protein
162 expression has also been performed in mammalian cells using translation-based negative

163 feedback control [59] and general-purpose gene expression controllers based on quorum-
164 sensing [32].

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

170 Molecular titration has also been shown to be an effective mechanism to implement
171 ‘comparator’ devices able to produce an output function of the mismatch between the levels
172 of two different inputs, an essential component of any biomolecular controller [36,60].
173 Implementations of more sophisticated control strategies have also been recently presented,
174 such as the biomolecular PID controller presented in [61]. As the complexity of biomolecular
175 control designs increases, to successfully implement the control function, the parts needed to
176 construct the control strategy must be finely tuned to guarantee the right balance between the
177 sensing and actuating parts of a circuit [62]. The use of tunable parts could open the way to
178 the development of adaptive biomolecular controllers able to self-tune themselves in order to
179 guarantee the robust execution of the control task they are assigned to perform even in the
180 presence of perturbations, cell-to-cell variability, etc. This might be even more crucial when
181 the control functions are spread among different populations in a microbial consortium as
182 recently suggested in [63].

183 Beyond simple feedback motifs, it can be difficult to implement complex control
184 algorithms using biochemical components because the feedback strengths and dynamics
185 required may be difficult to match to available parts. Therefore, an intermediate step is
186 sometimes taken whereby a computer is used to implement controller logic within the
187 feedback loop and create what is termed a cybergenetic system [22] (**Figure 3b**).
188 Cybergenetic systems often rely on single-cell microscopy platforms and microfluidics to image
189 engineered cells whose current state is displayed via fluorescent reporter proteins and use
190 chemical inducers [64,65] or light [28,29,66,67] as inputs to perturb the cells states in a pre-
191 defined way (i.e. the cells are engineered to sense and update their state in response to a
192 stimulus). The computer-based controller runs in real-time analyzing microscopy images to
193 extract the current states of cells and then immediately computes a control action, which is
194 then administered by varying chemicals concentrations or light that the cells are exposed to.
195 Such systems have been shown capable of controlling both population [8,64,66,68] and
196 single-cell behaviors [67]. Moreover, toolkits have emerged to simplify their creation by
197 handling image analysis, tracking and calculation of control actions (Pedone et al. bioRxiv doi:
198 10.1101/2020.06.25.171751). The major advantage of this hybrid approach is that the

199 computer controllers are cell-agnostic, allowing them to be used with any biosystem that has
200 the same types of control inputs and observable outputs.

201

202 **Conclusions**

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

216

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223

224 **Author Contributions**

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

226

227 **Declaration of Interest**

228 None.

229 **References**

230 • of special interest

231 •• of outstanding interest

232

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264 contribution is the detailed analysis of endogenous cellular responses to find a suitable
265 burden-responsive promoter and the use of a tunable CRISPRi feedback regulatory
266 mechanism that enables any synthetic construct to be targeted. It is shown that this
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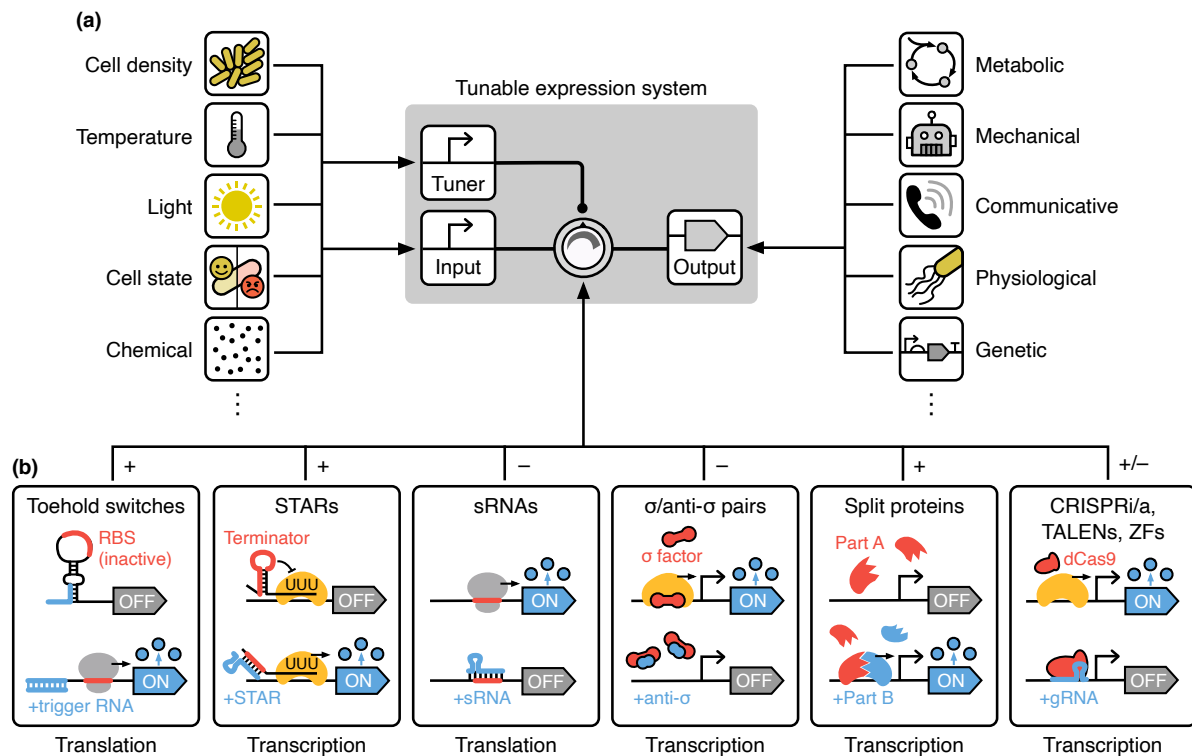
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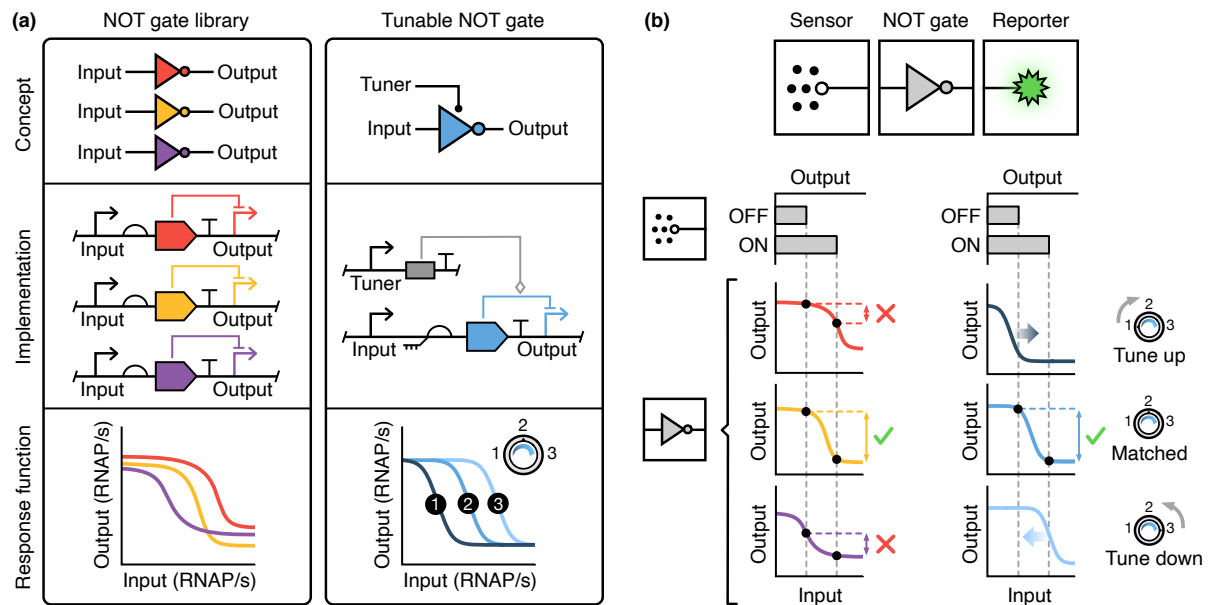
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470 **Figures and captions**

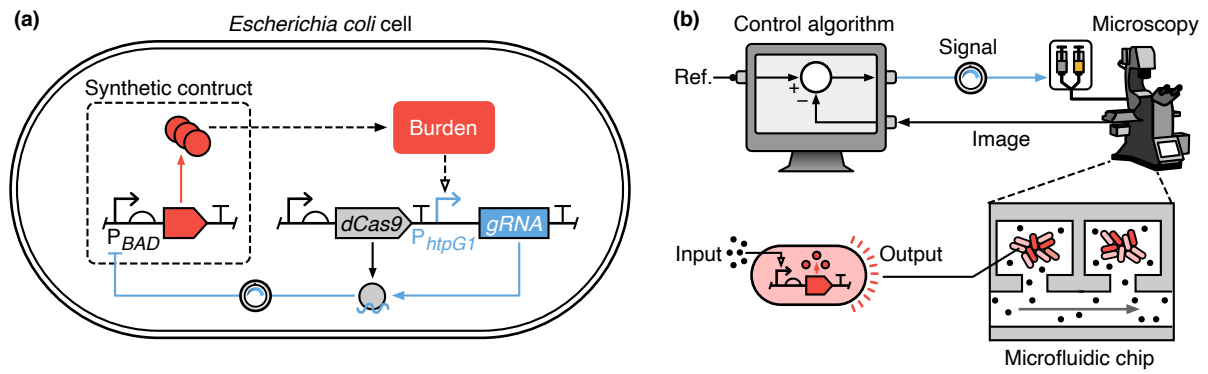
471

472 **Figure 1: Tunable genetic parts.** (a) Schematic of a tunable expression system (TES) where
 473 a variety of different inputs and output can be selected. Typically, inputs are transcriptional
 474 signals related to environmental or cellular states and the output is the expression of a gene
 475 that influences cellular behavior or acts as an input to another part of a larger circuit. (b) Major
 476 regulatory mechanisms that can be used to tune gene expression in a TES. Both active and
 477 inactive states shown in addition to whether the tuner will cause activation (+) or repression
 478 (-) of the output. The stage in gene expression (i.e. transcription or translation) where
 479 regulation takes place is shown below the box for each mechanism. Ribosomes and RNA
 480 polymerase (RNAP) shown by light grey and orange shapes without an outline, respectively.
 481 For the CRISPRi/a, TALENs and ZFs box a repressive CRISPRi system is shown. This can
 482 be modified to be an activator by fusing dCas9 to an activator domain to recruit RNAP to the
 483 promoter. In general, the additional blue element would be expressed by the tuner input to
 484 modulate expression of the output. RBS, ribosome binding site; sRNA, small RNA; STAR,
 485 small transcription activating RNAs; siRNA, small interfering RNA; CRISPRi/a, clustered
 486 regularly interspaced short palindromic repeats interference/activation; TALEN, transcription
 487 activator-like effector nuclease; ZF, zinc finger; gRNA, guide RNA.



488

489 **Figure 2: Tunable genetic parts enable the construction of adaptive circuits.** (a) Libraries
 490 of genetic parts (e.g. NOT gates) are commonly created that cover a range of different
 491 behaviors (left box). These differences are shown by the specific response function of each
 492 part, which captures the steady-state input-output relationship. For most genetic parts the
 493 response function is fixed, and so physical replacement is necessary if a part is not compatible
 494 when used in a system. In contrast, tunable genetic parts (right box) have additional tuner
 495 inputs that allow the shape and position of the response function to be dynamically varied as
 496 required. (b) Schematic of a simple genetic circuit where a sensor input is inverted to give a
 497 desired output reporter (e.g. green fluorescence). For the sensor and NOT gate to parts to work
 498 effectively, the output of the sensor must 'match' the response function of the NOT gate (dotted
 499 grey lines). If the parts are matching, then a large change in the NOT gate output will occur
 500 when the sensor switches between OFF and ON states. For standard NOT gates (left column)
 501 entire libraries need to be assembled and screened to find a working combination.
 502 Furthermore, if the environment changes then so too might the behavior of the parts making
 503 reassembly necessary. For a tunable NOT gate (right column), the tuner input can be varied
 504 until the gate perfectly matches the sensor's outputs. No reassembly is required, allowing the
 505 circuit to be dynamically tuned to changing conditions. Genetic circuits shown using Synthetic
 506 Biology Open Language (SBOL) Visual notation [73]. RNAP, RNA polymerase.



507

508 **Figure 3: Self-adaptive systems.** (a) Embedding burden-based controller. A synthetic
 509 construct expresses a burdensome protein. Endogenous cellular processes (dashed arrows)
 510 lead to the activation of the P_{htpG1} promoter under high levels of protein expression burden
 511 causing expression of a guide RNA (gRNA). This gRNA forms a complex with a constitutively
 512 expressed dCas9 protein that then targets the promoter of the synthetic construct, down
 513 regulating its expression. The strength of this negative feedback loop is dynamically 'tuned'
 514 by the endogenous burden signal as well as mismatches in the gRNA to the target promoter
 515 that reduce the binding affinity of the dCas9:gRNA complex. Panel adapted from [9]. (b)
 516 Schematic of an external *in silico* control system. Living cells grow in a microfluidic chip that
 517 is continually imaged by a microscope. These images are set to a computer, analyzed and an
 518 output signal from the cells (e.g. fluorescence) compared to a desired reference value. A
 519 control algorithm assesses this difference and emits a control signal, which actuates syringes
 520 and changes the concentration of a signaling molecule provided to the cells. The cells sense
 521 this change and alter their gene expression in response. The strength of feedback in this
 522 system can be tuned by modulating the control signals produced. Grey arrow in the
 523 microfluidic chip represents the flow of nutrients and signaling molecules. gRNA, guide RNA.