Synthetic biology by controller design

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Abstract

Natural biological systems display complex regulation and synthetic biomolecular systems have been used to understand their natural counterparts and to parse sophisticated regulations into core design principles. At the same time, the engineering of biomolecular systems has unarguable potential to transform current and to enable new, yet, to be imagined, biotechnology applications. In this review, we discuss the progression of control systems design in synthetic biology, from the purpose of understanding the function of naturally occurring regulatory motifs to that of creating genetic circuits whose function is sufficiently robust for biotechnology applications.

Introduction

Feedback and feedforward control motifs are highly represented in natural regulatory networks, and their synthetic counterparts have been extensively used to investigate the properties of these motifs within tightly maintained experimental conditions [1, 2, 3, 4]. In the past decade, it has become apparent that engineered biological systems can be used not just to understand biology, but also, and especially, to create new functionalities for a wide range of applications, from biosensing, to programmable probiotics, to regenerative medicine and cell therapies [5]. However, for practical adoption, engineered biological systems must be sufficiently predictable when operating across different cellular and environmental contexts. Indeed, achieving sufficient robustness to changes in intra-cellular and extra-cellular conditions is one of the greatest challenges in engineering biology [6, 7]. On the other hand, control system design is an integral component of most modern human-made systems, from mechanical, to electrical, to aerospace, and is often employed to ensure that performance is robust to environmental and system uncertainty. It is therefore natural that in recent years, the field of synthetic biology has crossed roads with control system design [8, 9].

In this paper, we provide a review of synthetic biomolecular feedback and feedforward control systems, starting with studies of their dynamical input/output properties (Section 1). We then progress through more sophisticated feedback and feedforward designs, often relying on control systems theory, aimed at engineering robustness of a genetic circuit's output to specific perturbations (Section 2). We conclude the paper with a discussion and outlook.

1 Input/output properties of biomolecular feedback and feedforward systems

Feedback and feedforward control are widely used in traditional engineering systems for a variety of tasks, ranging from stabilizing unstable processes, to shaping the temporal and steady state response to specific inputs, to filtering noise in a frequency-dependent manner, to achieving robustness to uncertainty and perturbations [10]. In this section, we review how several of these properties were recapitulated by simple experimental implementations of biomolecular feedback and feedforward systems.

Negative autoregulation. Negative feedback regulation (negative autoregulation) is highly represented in bacteria, yeast, plant, and animal cells, wherein a gene expresses a protein, also called a transcription factor (TF), that represses its own expression by binding to its own gene [4]. For example, 40% of known TFs in *E. coli* negatively autoregulate [11]. A simple genetic circuit representing a TF that negatively autoregulates is shown in Fig. 1-a. This circuit was implemented by using a variety of specific TFs and its

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dynamical input/output properties, as predicted from mathematical models, were experimentally characterized. Specifically, it was demonstrated that this motif decreases cell-cell variability of the TF level by decreasing the magnitude of outputs noise (Fig. 1-b) [12]. Interestingly, it was demonstrated by performing single cell measurements that the motif also shifts the noise spectra of the output to high frequency (Fig. 1-c) [13]. This spectral shift is in agreement with the well-known "water-bed" effect in control systems theory, wherein, depending on the system's order, a negative feedback system can decrease the noise on the output for low-frequency noise, but it increases it at high frequency [10]. From a biological viewpoint, this can still be overall beneficial for accurate performance since high-frequency noise can be filtered away by downstream processes that take the TF in question as an input, such as through other regulated genes or post-translational modification processes [14]. Accordingly, it was shown that negative autoregulation decreases transcriptional response time, although with the potential downside of having overshoot (Fig. 1-d) [1]. This is also consistent with well-known downsides of negative feedback in systems with substantial phase lag, which include damped oscillations and the potential for instability, possibly leading to sustained oscillations. Contributors to a phase lag between transcriptional input and functional TF include the temporal dynamics of transcription, translation, and that of protein folding, the latter of which is known to be potentially slow depending on the protein type.

One problem of the negative autoregulation circuit is that the feedback strength is coupled to the output level, wherein stronger feedback leads to lower output. This can create some challenges when comparing the behaviors of open loop and closed loop systems as they operate about different steady state levels. Ways to handle this difficulty include making comparisons within a relative scale, in which both open loop and closed loop system outputs are internally normalized, or varying other parts in the closed (open) loop system, such as increasing (decreasing) the promoter or ribosome binding site (RBS) strengths.

The noise attenuation property combined with a decreased output range for the same input range (input in Figure 1e) was later exploited to create the linearizer genetic circuit (Fig. 1-e). In this circuit, the negatively autorgulated TF is used to repress a gene of interest that expresses an output protein. In this case, the inducer input not only tunes the feedback strength as in Fig. 1-a, but also tunes the expression of the output protein's level (Fig. 1-e). The presence of the negative feedback allows an input/output response that is nearly linear for input levels below a threshold (Fig. 1-f) and also carries less noise than the corresponding system where autoregulation is not present [15]. These results are consistent with early uses of negative feedback in electronics for extending the linear regime of amplifiers [16]. The linearizer circuit has been implemented in mammalian cells [17], in which graded and accurate input/output responses are often desired in order to set TF levels in applications such as cell fate reprogramming [18].

While negative autoregulation can tune the

inptut/output dynamical properties of gene expression as described above, a genetic circuit that temporally follows (tracks) dynamic biomolecular input signals is also highly desired. Indeed, reference trajectory tracking is one of the other widespread uses of feedback control in common engineering systems, such as in robot manipulators and in satellite systems [19]. For synthetic biology, the ability to make endogenous TFs follow specified temporal trajectories is central to many applications, such as in guided cell differentiation [20] and cell fate reprogramming [21].

Therefore, in [22], a genetic circuit known as the "concentration tracker" was used to achieve robust input reference tracking. The circuit is based on a negative feedback mechanism implemented through the sequestration of an input scaffold protein through an antiscaffold output protein as shown in Fig 1-g. The circuit parameters can be tuned such that the output protein concentration tracks the input protein concentration with a time delay as shown in Fig 1-h.

Feedfoward Control. In addition to negative autoregulation, incoherent feedforward loops (iFFL) are highly abundant in bacteria and yeast, for example, in the gene networks of *E. coli*, their frequency exceeds the mean value of their frequency in an ensemble of randomized gene networks by more than 30 standard deviations [23]. Because of their abundance, their function has been extensively investigated in systems biology [2] and validated using synthetic biology on re-engineered natural motifs [24].

The general architecture of the most common iFFL is one where the input signal upregulates the output through one branch and downregulates it through another branch as shown in Fig. 1-i. iFFLs can provide adaptation of the output to step changes in the input as shown in Fig. 1-j, provided the repressive branch is designed to exactly offset the effect of the input on the activating branch [2, 24]. When the activating and repressive branches do not cancel each other's effect, it was shown that iFFLs decrease gene expression response time to a transcriptional input and can provide fold change detection, where the output depends only on fold changes in the input, and not on absolute levels [3, 25].

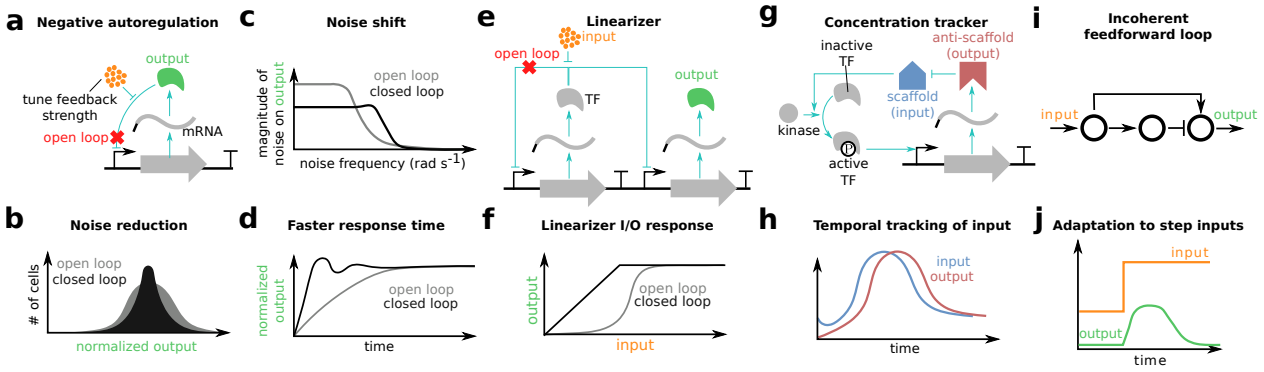

Figure 1: **Properties of biomolecular feedback and feedforward systems. (a) Negative feedback regulation genetic circuit**. The output protein (green) represses its own production by preventing transcription. The input inducer (orange) is a signaling molecule that once bound to the output protein prevents it from binding to its own DNA to block transcription, hence decreasing the strength of the feedback. The open loop system (red) is one where the output protein does not block its own transcription. **(b) Noise reduction.** The plot shows on the x-axis the normalized output protein concentration and on the y axis the number of cells with the given output. The gray shade represents the open loop system while the black shade represents the closed loop system [12]. **(c) Frequency-dependent noise filtering.** The x-axis represents the noise frequency while the y axis depicts the magnitude of the noise on the output protein concentration. The gray line represents the open loop system while the black line represents the closed loop system [13]. **(d) Shaping temporal response.** The plot shows the normalized protein concentration as a function of time. The gray line represents the open loop system while the black line represents the closed loop system [1]. **(e) The linearizer genetic circuit.** The circuit takes as an input an inducer (orange) that binds to a negatively autoregulated TF, thereby preventing repression of its own DNA and of the output protein's (green) DNA [15]. The open loop system is one where the TF is not autoregulated. **(f) Input/output steady state response linearization.** The gray line represents the open loop system while the black line represents the closed loop system. **(g) Concentration tracker genetic circuit.** Negative feedback occurs via the sequestration of the input scaffold (blue) protein by the anti-scaffold (red) protein [22]. The scaffold protein enables the transfer of a phosphate group (denoted by a circled "P") from a kinase to an inactive TF that, when phosphorylated (active TF), activates transcription of the anti-scaffold protein. **(h) Input/output temporal tracking.** The output (red) and input (blue) concentration over time for the concentration tracker circuit. **(i) Feedforward control.** The architecture depicts a common incoherent feedforward loop (iFFL) where the input signal (orange) upregulates the output (green) through one branch and downregulates through another branch. The circles can be implemented by a variety of genetic modules whose output protein is either upregulated or downregulated by the indicated input (see Section 2 for example implementations). **(j) Step input rejection.** The y-axis depicts the input concentration in orange and in green the output concentration versus time (x-axis). The iFFL motif can reject a step disturbance input [2, 24]. The black arrows in the genetic circuit diagrams of panels (a), (e), and (g) represent the promoter. The promoter is a DNA sequence upstream the gene's coding region where TFs bind and either activate or repress the transcription of the gene [14]. In all diagrams, " $\rightarrow$ " represents upregulation while " $\dashv$ " represents repression.

Altogether, we have discussed how feedback and feedforward motifs shape the steady state, temporal properties, and noise profiles of gene expression systems. In the next section, we discuss how feedback and feedforward control are employed to make gene expression robust to disturbances and uncertainty.

2 Feedback and feedforward control systems for robustness

Altogether, the early studies on feedback and feedforward biomolecular circuit motifs described in the previous section recapitulated in the context of biological systems many of the known properties of control systems employed today in engineering. On the one hand, this suggests that nature may employ principles that humans have been "re-discovering" and using for engineering purposes. It is therefore plausible that nature may be "hiding" many more, yet to be discovered, principles that could be game changers for current and future engineering challenges. On the other hand, since simple biomolecular implementations of control systems display potentially useful properties, one may think of investigating how more sophisticated versions of them could be used to tackle open challenges in engineering biology. One such open challenge is the lack of robustness of engineered biomolecular systems to uncertainty and to variability in intra-cellular, inter-cellular, and extra-cellular context [18]. One of the celebrated uses of control system design in traditional engineering

systems has been for conferring robustness to uncertainty, perturbations, and unmodeled dynamics [10]. In light of these facts, there have been substantial efforts in the past several years in designing biomolecular feedback and feedforward control systems to make genetic circuit operation robust to perturbations. We discuss some of these efforts in this section.

Feedback controllers. The general structure of a feedback controller for robustness to disturbances is depicted in Fig. 2-a, where a module produces an output of interest denoted as y that is sensed by the controller, which then compares such an output to a reference input u and actuates the module to compensate for the discrepancy.

The desired outcome is that, with the controller, the output will eventually reach back its unperturbed value after presentation of the disturbance input as shown in Fig. 2-b. Although the subdivision of the feedback system into controller and module and of the signals into sensor and actuator signals are convenient abstractions to help us match the design to that of traditional control systems, such subdivisions are often non-intuitive in the biomolecular implementation and may not even hold. Nevertheless, we use this abstraction here for the sake of presentation clarity.

In biomolecular systems, the reference input often represents a desired concentration for an output protein of interest. Notable examples of disturbances include reaction fluxes that directly affect the protein of interest y due to on or off-target binding (retroactivity) [26], variations in the concentration of transcriptional (TX) and/or translational (TL) resources due to resource competition [27, 28, 29], variations in the decay rates of the module's internal species due to growth rate changes or to competition for degradation resources [30, 31], uncertainty in biomolecular parameters such as due to DNA copy number variability from cell to cell, and variations of conditions such as temperature, pH, or nutrients, although these perturbations will affect also the controller. In what follows, we review some specific implementations aimed at making the system's output robust to specific disturbances in these classes.

Feedback control for robustness of gene expresson to transcripitonal and translational disturbances. Disturbances on free TX and TL resources pose one of the challenges in designing genetic circuits with predictable behavior [27, 32, 33, 29]. Indeed, any time one gene is activated, TX and TL resources are temporarily sequestered by this gene and, as such, they become unavailable to other genes, thereby also changing other genes' expression levels [27]. Therefore, a problem that has attracted notable attention is that of making a genetic module's output robust to the fluctuating availability of TX and TL resources. Notably, in bacterial cells, the resources that are mostly perturbed by gene expression are translational [27], while in mammalian cells are transcriptional [33]. Specifically, to make gene expression robust to TL resource fluctuations in *E. coli*, a feedback controller based on small RNA (sRNA) sequestration was implemented as shown in Fig. 2-c [34]. This design is known as a quasi-integral controller since in the limit that the controller dynamics are much faster than molecular decay it approximates an integral controller where zero steady state error is expected for a range of constant disturbance values [35]. Integral controllers are ubiquitous in traditional engineering systems and represent a leading approach to ensure adaptation to constant or slowly changing perturbations [10]. While the physical implementation of such controllers in engineering can be easily achieved in a variety of ways, their implementation in the context of biomolecular systems has proven challenging. Major difficulties include identifying physically achievable network architectures of biochemical reactions that result in an integral action and the presence of molecule dilution/degradation, which causes integrator leakiness [35, 36]. A similar design to that of [42] was implemented using protein based sequestration instead of sRNA enabled sequestration [37]. This system, however, does not display a (quasi-)integral control function.

In mammalian cells, a phosphorylation cycle based feedback controller was implemented to make gene expression robust to perturbations in both TX and TL resources and off-target effects, which are a major cause of unintended coupling among genetic modules (Fig. 2-d) [33]. This design, under suitable parameter regimes, can also function as a quasi-integral controller.

Although promising, these solutions present scalability challenges as each gene requires its own controller in order to make its expression robust to resource variations. An alternative approach to make gene expression robust to variable loads on ribosomes (a central TL resource) that bypasses the scalability issue, is to use a single controller to regulate ribosome concentration directly, such that the free ribosome concentration is unaffected as heterologous proteins are expressed. This solution only requires a single controller independent of the number of heterologous proteins expressed.

To this end, in [38] a feedback controller of orthogonal ribosomes was implemented, where the orthogonal 16S rRNA (o-rRNA) concentration was regulated to ensure that the orthogonal ribosome concentration is less sensitive to transnational demand (Fig. 2-e).

Part of the controller, however, still interfaces with the cell's natural regulatory systems such as through

the use of r-proteins. Thus, an open question remains how these controller-host interactions impact performance.

Feedback control for robustness of CRISPRi-based circuits to dCas9 loading. The clustered regularly interspaced short palindromic repeats interference (CRISPRi) enables the creation of large libraries of transcriptional repressors, and for this reason it has been considered a promising route to scale up the complexity of genetic circuit design [39]. Specifically, each represssor contains a catalytically inactive Cas9 (dCas9) bound to a single guide RNA (sgRNA) whose DNA sequence matches that of the target promoter. Similar to the transciptional/translational resource sharing problem, dCas9 is shared by multiple sgRNAs, each within a different genetic regulatory module. Thus, dCas9 has the role of a shared resource in CRISPRi-based genetic circuits and is subject to variable loads as multiple sgRNAs are expressed in a circuit. The loads cause cross-talk among otherwise orthogonal sgRNA regulatory paths [40, 41]. To mitigate this problem, a negative feedback controller of dCas9 concentration was implemented in [42] by having dCas9 repress its own promoter through CRISPRi and thus making dCas9 concentration robust to the demand for dCas9 by sgRNAs (Fig. 2-f.) Controllers such as this one will be likely required for true modular and scalable genetic circuit designs that use CRISPRi.

Feedback control for growth rate robustness to gene expression burden. Another critical challenge when adding genetic circuits within cells is that the burden on cellular resources also decreases growth rate. This is especially problematic in applications to materials production where the yield can substantially decrease as a consequence of decreased growth rate. To overcome this problem, in [43] the authors created a burden-driven feedback controller of gene expression that downregulates the expression of a heterologous protein when its expression results in significant growth rate decrease as shown in Fig. 2-g. A critical component of this feedback system is the sensor, which is composed of a burden sensitive promoter. The burden sensitive promoter was extracted from an endogenous gene in *E. coli* that is responsible for the heat-shock response. The transcription rate of the burden sensitive promoter increases rapidly in response to the expression of a heterologous protein that burdens the cell. As shown in Fig. 2-g, CRISPRi downregulates the modules transcription rate when the corresponding sgRNA is activated by cell burden.

Feedback control for robustness of gene expression to uncertain parameters. To address the issue of uncertain and variable parameters, such as varying degradation rates and temperature, a sequestration based integral feedback controller was implemented via σ/anti-σ sequestration in *E. coli* as shown in Fig. 2-h [44]. The controller ensures that protein concentration is robust to variations in the protein's degradation rate. The authors also demonstrated an application of the feedback controller to make growth rate robust to fluctuations in temperature. As for the controllers designed for robustness to TX/TL resource fluctuations, these controllers are also subject to scalability limitations and further work will be required to understand how these solutions can scale to the level of multi-gene circuits.

Feedforward control for robustness. In addition to negative feedback control, feedforward controllers have been implemented for robustness to various perturbations, including those described for feedback controllers. The general structure of a feedforward controller consists of a disturbance sensor and an actuator that applies a forcing to the module of opposite sign to that applied by the disturbance, as shown in Fig. 3-a. If the sensor and actuator are well tuned, the controller action can offset the effect of the disturbance on the output (Fig. 3-a). In [45], a trancriptional repression-based feedforward controller, as shown in Fig. 3-b , was designed in *E. coli* to make gene expression robust to DNA copy number.

Indeed, DNA plasmid copy number variability from cell to cell is a major source of variability of gene expression within a population of cells and ways to mitigate it have been researched for long time. In particular, one of the first implementations of feedforward control to attenuate the effects of DNA copy number variability was implemented in mammalian cells and used microRNA-enabled degradation of mRNA as the actuation mechanism (Fig. 3-c) [46]. More recentely, feedforward controllers have been engineered in mammalian cells by either using microRNA-enabled or endoribonuclease-enabled (Fig. 3-d) degradation of mRNA as the actuation mechanism to make the concentration of a protein of interest robust to depletion of TX resources [33, 29].

A feedforward controller is typically designed to make the output of the system robust to one specific disturbance input and additional disturbances that affect the module only and are not input to the controller cannot be compensated for. By contrast, a feedback controller can, in principle, ensure robustness of the output to multiple disturbances and parameter uncertainties that are affecting the module because it indirectly senses their effect on y by measuring the discrepancy between y and the desired reference input u. Moreover, feedforward controllers built to date, are focused on making a specific output level, as opposed to the module entire input/output response, robust to disturbances, which is instead achieved by feedback controllers. On

the other hand, feedforward controllers are often easier to implement from a practical standpoint. So, an interesting future extension of feedforward controllers will be to make them address the robustness of an input/output response as opposed to only an output level. Future designs may also combine the use of feedback and feedforward controllers. For example, in [47] a hybrid negative feedback and feedforward controller was engineered that makes gene expression robust to plasmid copy number variation and transactivator dosage variability in mammalian cells. More generally, it has been shown that concatenating well-characterized feedback controllers to make gene expression simultaneously robust to several disturbances is non-trivial and is an open area of research [48]. When designing controllers, it is typically assumed that noisy signals do not appear in the controller dynamics, sensors, or actuators, however in practice every part of the module and of the controller are affected by intrinsic noise [49]. Thus, an open question is how to engineer controllers such that they achieve the robustness specification despite noise within the controller dynamics. Finally, we have only considered temporally constant disturbances, but, in practice, these are expected to be time varying in many instances. Thus, in the future we should consider how to design controllers to achieve robustness to time varying disturbances, while responding to desired, possibly time varying, input signals.

Discussion

In this paper, we first reviewed how simple feedback and feedforward synthetic genetic circuits have been employed to study the properties of their natural counterparts, focusing on noise suppression and filtering, temporal dynamics, and input/output characteristics. We then discussed the application of more sophisticated genetic circuit implementations of both feedback and feedforward control systems for obtaining robustness of specific molecule levels to perturbations and uncertainty. Indeed, the need to achieve robust performance of engineered biomolecular circuits is a critical premise for the application of synthetic biology in concrete setups, wherein lack of robustness and performance guarantees may ultimately impede concrete use. Although the biomolecular control systems implementation that we reviewed showcase the potential of control design for empowering circuits with robustness and increased accuracy, there are significant limitations that warrant substantial new research where novel concepts and theory will likely be required.

First of all, to reach a future where genetic circuits will incorporate several feedback and feedforward controllers that enable more accurate circuit operation across changing intra and extra-cellular contexts, the problem of scalability will need to be resolved. Not only there is a question of how multiple part-wise orthogonal controllers can be designed but, even then, the problem of whether they can function concurrently is non-trivial [51]. Indeed, controllers for robust module operation in the face of resource perturbations do not generate more resources; instead, they essentially make the module work in "sub-optimal" conditions such that there is a sufficient buffer of resources that can be re-allocated when needed. Multiple such controllers working concurrently may need to coordinate to ensure they do not interfere with one another. Controllers also need cellular resources to proper function, and this can cause performance trade offs [52] and increased cellular burden, which will need to be compensated for through other means.

In classical control design problems stemming from electrical, mechanical, or aerospace engineering, there is a clear separation between the feedback system's components (sensor, compensator, and actuator) and the process to be controlled. However, this subdivision is non-trivial in biomolecular control systems implementations. Indeed, even though a simplified abstraction of this type may help conceptualize the design problem, strict structural mapping between the traditional separation and the biomolecular implementation may even be counterproductive and may not allow to exploit the physical features of biology. For example, a critical component of traditional feedback control systems is a means to compute the difference (the error) between the input reference signal u and the measured output y. In practice, however, to measure the discrepancy between the concentrations of u and y, it is not even needed to design a genetic circuit that computes the difference between input molecule concentrations since discrepancy can be measured in many other ways. One reason why the difference is used in traditional control system engineering is because it was easily implementable, initially by electrical circuits, then by processors and computers.

For traditional feedback control systems to perform as desired, the sensor path needs to be as accurate and precise as possible. Indeed, noise or disturbances in the sensory path can completely destroy regulation and tracking unless more sophisticated solutions are considered such as robust observers and Kalman filters. This is in net contrast with the biological picture, where all the parts of the feedback system are implemented by biomolecular reactions that are intrinsically stochastic, are subject to large uncertainty in the parameter values, and may even have unmodeled dynamics. Then, how can we design a feedback control system with accurate output despite all the composing elements are uncertain? This is still largely an open question and

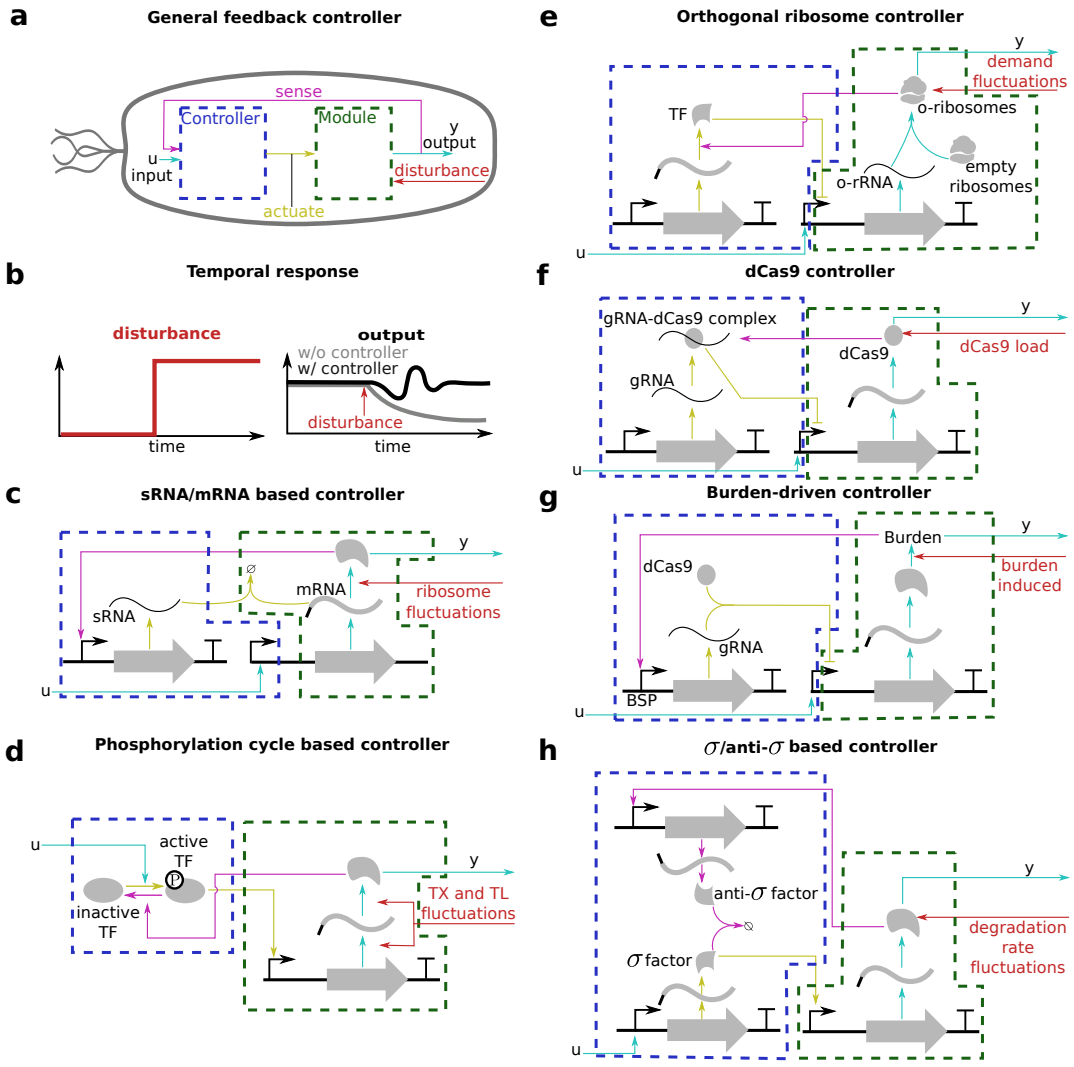

Figure 2: **Feedback control for robustness to perturbations. (a) Block diagram of a feedback controller.** To compensate for the effects of disturbances (red) on a module, a feedback controller actuates (yellow) the module based on the sensed (pink) output value y and the reference input u. **(b) Disturbance rejection.** The left-side plot shows a step increase of the disturbance in time. The right-side plot shows the expected temporal response of the output in the presence of a disturbance with and without the controller. **(c) The small RNA (sRNA) sequestration based controller.** This feedback controller makes protein expression robust to fluctuations in free ribosome concentration [34]. The output activates the transcription of a sRNA molecule that binds to the complementary sequence of the mRNA of the output protein y and annihilates it (null symbol). The input tunes the transcription rate of the module. **(d) The phosphorylation cycle based controller.** This feedback controller is used to make gene expression robust to transcriptional (TX) and translational (TL) disturbances [33]. The output protein dephosphorylates (phosphate group denoted by a circled "P") the active TF that upregulates the production of the output protein itself. The input promotes the phosphorylation of the TF into the active state. **(e) The orthogonal ribosome feedback controller.** This controller is used to make orthogonal ribosome (o-ribosome) concentration robust to loads on o-ribosomes themselves [38]. The o-ribosome translates the TF that represses the transcription of the orthogonal 16S rRNA (o-rRNA) used to build the o-ribosome. The input tunes the o-rRNA transcription rate. **(f) The dCas9 feedback controller.** This controller makes free dCas9 concentration robust to fluctuations in loads to dCas9 applied by sgRNAs [42]. dCas9 negatively autoregulates itself by binding to a sgRNA that targets the dCas9 promoter for CRISPRi. The input tunes the dCas9 transcription rate. **(g) The burden driven feedback controller.** This controller regulates the expression of the module's gene when it burdens the cell due to resource sequestration [43]. A burden sensitive promoter (BSP) expresses a sgRNA that binds to dCas9 to downregulate the output expression using CRISPRi when its resource usage is high. The input tunes the module's transcription rate. **(h) The σ/anti-σ factor based feedback controller.** This controller makes protein expression robust to variations in the proteins degradation rate [44]. The output protein activates the transcription of the anti-σ factor that sequesters (null symbol) the σ factor responsible for activating the transcription of the output protein. The input tunes the σ factor's transcription rate. In all diagrams, " $\rightarrow$ " represents upregulation while " $\dashv$ " represents repression.

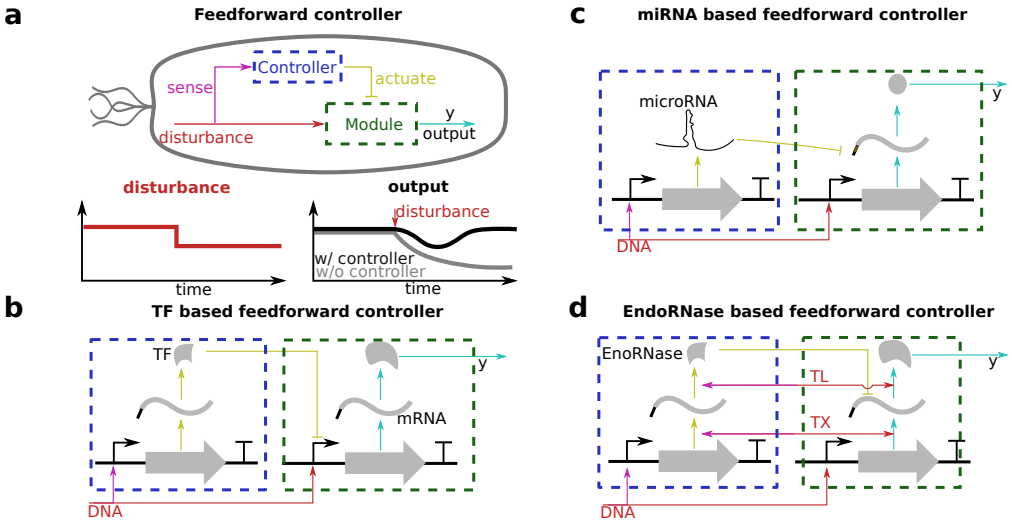

Figure 3: **Feedforward control for robustness to perturbations. (a) Block diagram of a feedforward controller.** To compensate for the effects of the disturbance (red) on a module's output y, a feedforward controller actuates (yellow) the module with a forcing of opposite sign to that applied by the disturbance based on the sensed (pink) signal. The plots show the expected output y with and without the controller for a step disturbance in the case that the controller action can perfectly off-set the effect of the disturbance on the module. **(b) A TF based feedforward controller.** This controller makes gene expression robust to DNA copy number variation in bacterial cells [45]. The DNA copy number increases the expression rate of the output protein and the TF that downregulates the output. **(c) A microRNA based feedforward controller.** This controller makes gene expression robust to DNA copy number variability in mammalian cells [50]. The DNA copy number increases the expression of both the output protein and the microRNA that binds and targets the output mRNA for degradation. **(d) An EnoRNase based feedforward controller.** This controller makes gene expression robust to DNA copy number variability and TX/TL resource perturbations in mammalian cells [28]. The TX/TL resources increase both the output protein's and the EndoRNase expression rate. The EndoRNase, in turn, degrades the output protein's mRNA. In all diagrams, " $\rightarrow$ " represents upregulation while " $\dashv$ " represents repression.

is even more puzzling when we consider that naturally occurring feedback and feedforward control systems perform accurate and precise functions despite uncertainty, variability, and noise in subsystem's components. Perhaps natural systems may have found a way to exploit all this uncertainty to their advantage. So, in our future designs, we may want to also learn from nature and perhaps consider the merging of rational design, which is the key premise of all the implementations described in this paper, with directed evolution approaches.

Conflict of interest

Declaration of interest: none

Acknowledgments

This work was supported in part by NSF Expeditions, Grant Number 1521925, NSF RoL Award # 1840257, the NSF Graduate Research Fellowships Program, and the Ford Foundation Predoctoral Fellowship. The authors thank Yili Qian for helpful discussions.

Annotated Bibliography

(*) Indicates references of interest.
(**) Indicates references of outstanding interest.

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