Synthetic Biology

Domitilla Del Vecchio
Department of Mechanical Engineering, MIT
Cambridge, 02139
ddv@mit.edu

***Abstract*—Synthetic biology, that is, the ability to engineer biology for useful functionalities will have remarkable impact in a number of applications ranging from health and medicine, to environment and energy. Examples include engineered bacteria that recognize and kill cancer cells, nautralize radioactive waste, transform feedstock into fuel, and programmable mammalian cells that control the differentiation of tissue *in vivo*. Engineering biological circuits that are sufficiently sophisticated to accomplish these functions is becoming possible, but at the same time is proving challenging due to a number of obstacles. Many of these obstacles require a system-level understanding of the dynamical and robustness properties of interacting systems and hence the field of control and dynamical systems theory may highly contribute. In this paper, we review the basic technology employed in engineering biology, simple example modules and complex systems created using this technology, and discuss key system-level problems along with challenging research questions for the field of control theory.**

***Index Terms*—biomolecular systems, gene expression, robustness, scalability, modularity.**

I. Introduction to Synthetic Biology

Synthetic biology is an engineering discipline in which the biochemical and biophysical principles present in living organisms are used to engineer new systems [12]. These systems will have the ability to accomplish a number of remarkable tasks, such as turning waste into energy sources [46, 57], detecting pathogens [45], or recognizing cancer cells with the aim of targeting them for deletion [18]. While synthetic biology can be employed to create new functionalities, it can also enable the understanding of fundamental design principles of living systems. In fact, implementing a circuit with a prescribed behavior provides a powerful means to test hypotheses regarding the underling biological mechanisms.

The functions of living organisms are controlled by biomolecular circuits, in which, at the most basic level, proteins and genes interact with each other through activation and repression interactions forming complex networks. A common signal carrier is the concentration of the active form of a protein, which can be controlled through a number of mechanisms, including gene expression regulation, post-transcriptional, and post-translational modification. Through the process of gene expression, proteins are produced by their corresponding genes, whose production rates can be activated or repressed by other proteins (transcription factors). Once the proteins are produced they can be activated or inhibited, by other proteins or small molecules, through post-transcriptional modification of mRNA and post-translation modification to proteins, such as phosphorylation, and allosteric modification [5, 21]. We next describe some salient aspects of gene expression focusing, for simplicity, on prokaryotic systems.

A gene is a piece of DNA whose expression rate can often be controlled by a DNA sequence upstream of the gene itself, called promoter. The promoter contains the binding regions for the RNA polymerase, an enzyme that transcribes the gene into a messenger RNA molecule, which is then translated into protein by the ribosomes (central dogma of molecular biology [4]). The promoter also contains operator sites, which are binding regions where other proteins, called transcription factors, can bind. If these proteins are activators, they will help the RNA polymerase in binding the promoter to start transcription. By contrast, if these proteins are repressors, they will prevent the RNA polymerase from binding the promoter. These activation and repression interactions are nonlinear and stochastic, therefore the most commonly used modeling frameworks include systems of nonlinear ordinary differential equations, stochastic differential equations, or the chemical master equation [21].

The basic technique for constructing synthetic circuits is that of assembling, through the process of cloning, DNA sequences with prescribed combinations of promoters and genes such that a desired network of activation and repression interaction is created. For example, if we would like to create an "inverter" where protein A represses protein B, we can simply place the gene of B under the control of a promoter repressed by protein A. Currently, there is an increasing library of parts that one can use to assemble a desired circuit this way [1, 2]. The set of parts includes promoters, gene coding sequences, terminators, and ribosome binding sites. Terminators are DNA sequences placed at the end of a gene to make the RNA polymerase terminate transcription, while ribosome binding sites are DNA sequences placed at the beginning of a gene, which establish the rate at which ribosomes will bind to the mRNA, determining the overall translation rate [21]. An area of intense research is expansion of the library by creating mutations of existing parts or by assembling new ones.

Once a DNA sequence is created that encodes the desired circuit, it is inserted in a cell either on the chromosome itself or on DNA plasmids. Once in the cell, the circuit can "run" using the cellular resources required for gene expression, including RNA polymerase and ribosomes, amino acids, and ATP. Alternatively, a cell extract can be created for running the circuits in cell-free setups [47, 44], a technology that has

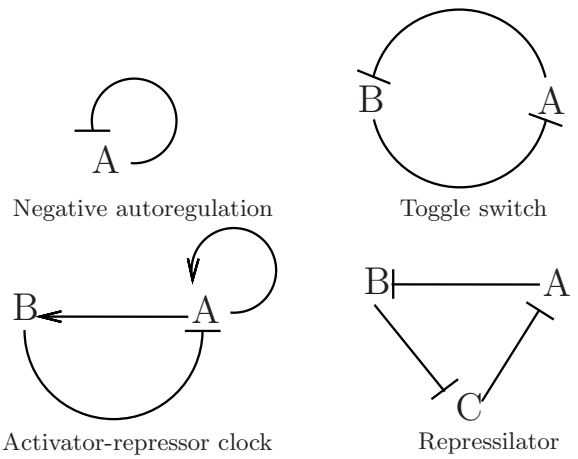

Fig. 1. Early gene circuits that have been fabricated in bacteria *E. coli*: the negatively autoregulated gene [9], the toggle switch [26], the activator-repressor clock [7], and the repressilator [25].

open the way to a number of applications, including diagnostic [45, 54]. In this sense, the cell, or the cell extract, can be viewed as a chassis for the synthetic genetic circuits. The operation of the circuit can then be observed by monitoring the concentration of reporters, that is, of proteins that are easy to detect and quantify. These include fluorescent proteins, that is, proteins that exhibit bright fluorescence when exposed to light of a specific wave length. Examples include the green, red, blue, and yellow fluorescent proteins. These fluorescent proteins are mainly employed in two different ways to measure the amount of a protein of interest. Specifically, one can fuse the gene of the fluorescent protein with the gene expressing the protein of interest. Alternatively, one can use the protein of interest as a transcription factor of the fluorescent protein. In both cases, the concentration of the fluorescent protein will provide an indirect measurement of the concentration of the protein of interest.

It is also possible to apply external inputs to a circuit to control the activity of transcription factors. This is accomplished through the use of inducers, which are small signaling molecules that can be injected in the cell culture and enter the cell wall. These inducers bind specific transcription factors and either activate them, allowing the transcription factor to bind the promoter operator sites, or inhibit them, reducing the transcription factor ability to bind the promoter operator sites [39].

II. Early Synthetic Biology Modules

A number of modules comprising two or three genes have been fabricated in the earlier days of synthetic biology [7, 9, 25, 26, 53]. We can group them into oscillators [7, 25, 53], mono-stable systems [9], and bistable systems called toggle switches [26].

Oscillators. The creation of circuits whose protein concentrations oscillate periodically in time has been a major focus. In fact, the ability of creating an oscillator has the potential of shedding light into the mechanisms at the basis of natural clocks, such as circadian rhythms and the cell cycle. Oscillator designs can be divided into two types: loop oscillators [25], in which repression/activation interactions occur in a loop topology, or oscillators based on the interplay between an autocatalytic loop and negative feedback [7, 53] (see Figure 1).

The design requirements of synthetic circuits are usually explored through models of varying detail, starting with the use of low-dimensional models, which are composed of a set of nonlinear ordinary differential equations describing the rate of change of the circuit's proteins. These models allow application of a number of tools from dynamical systems theory to infer parameter or structural requirements for a desired behavior. After simple models are analyzed, larger scale mechanistic models are constructed, which include all the intermediate species taking part in the biochemical reactions. These models can be either deterministic or stochastic. Simulation is usually required for the study of these more complicated models and the Gillespie algorithm is often employed for stochastic simulations [27].

As an example of a simple model and related analysis, consider the activator-repressor clock of Atkinson et al. [7] shown in Figure 1. This oscillator is composed of an activator A activating itself and a repressor B, which, in turn, represses the activator A. Both activation and repression occur through transcription regulation. Denoting in italics the concentration of species, a toy model of this clock can be written as

$$\begin{aligned} \dot{A} &= \frac{\beta_A (A/K_a)^n + \beta_{0,A}}{1 + (A/K_a)^n + (B/K_b)^m} - \gamma_A A, \\ \dot{B} &= \frac{\beta_B (A/K_a)^n + \beta_{0,B}}{1 + (A/K_a)^n} - \gamma_B B, \end{aligned} \tag{1}$$

in which γ_A and γ_B represent protein decay (due to dilution and/or degradation). The functions $(\beta_A(A/K_a)^n + \beta_{0,A})/(1 + (A/K_a)^n + (B/K_b)^m)$ and $(\beta_B(A/K_a)^n + \beta_{0,B})/(1 + (A/K_a)^n)$ are called Hill functions and are the most commonly used models for transcription regulation [21]. The first Hill function in system (1) increases with A and decreases with B while the second one increases with A, as expected since A is an activator and B is a repressor. The key mechanism by which this system displays sustained oscillations is a supercritical Hopf bifurcation with bifurcation parameter the relative time scale of the activator dynamics with respect to the repressor dynamics [21]. Specifically, as the activator dynamics become faster than the repressor dynamics, the system goes through a supercritical Hopf bifurcation and a stable periodic orbit appears (Figure 2(b)).

Mono-stable systems. The first mono-stable system engineered through negative autoregulation was fabricated with the aim of understanding the role of negative feedback in attenuating biological noise. The results of Becskei and Serrano [9] clearly showed that negative autoregulation can reduce intrinsic noise. Furthermore, the results of Austin et al. [8]

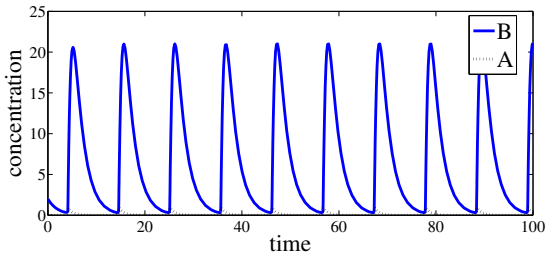

Fig. 2. Activator-repressor clock time trajectory.

demonstrated that while low frequency noise is attenuated, noise at high frequency can be amplified by negative autoregulation in accordance with Bode's integral formula [3].

Bistable systems. The toggle switch of Gardner et al. [26] was the first bistable system constructed. It constitutes the simplest circuit with memory, in which the state of the system can be switched from one equilibrium (low, high) to the other (high, low) by external inputs. Once the system state is switched to one of these two equilibria, it will stay there unless another external perturbation is applied. Based on this circuit, a number of applications have been developed, including systems to control endogenous cell factors [35], kill switches for programmed cell death [16], and programmable bacteria that detect and record events in the guts [36].

While the early circuits described so far were fabricated mainly to investigate design principles for limit cycles, memory, and robustness to noise, many more circuits after these have been fabricated with the aim of solving concrete engineering problems, including incoherent feedforward loops for robustness to DNA copy number [11, 51], logic gates [41], communication modules [17], load drivers [40, 43], and various types of feedback controllers [29, 34, 6, 52, 31, 37]. For a more extensive review, the reader is referred to [23, 50, 30, 38].

III. From Modules to Systems: Opportunities and Challenges

One approach to creating systems that can accomplish sophisticated tasks is to assemble together simpler modules, such as those described in the previous section [48]. Increasingly large systems are being built by composing modules together in a layered architecture [41] and software tools such as Cello are being developed in order to automate the process that goes from design concept to choice of genetic parts [42]. Layered logic gates are often necessary in order to integrate multiple signals in several applications. One such application is a cell type classifier based on RNA signatures [55]. Here, a synthetic gene circuit is created that integrates sensory information from a number of molecular markers to determine whether a cell is in a specific state, that is, cancer, and, in such a case, produces a protein output triggering cell death. The design of this circuit is based on the composition of three key modules. Specifically, a double inversion module senses high levels of a molecular marker, a single inversion module senses low levels of a molecular marker, and a logical "and" module finally integrates the outputs of the other two modules to produce the output protein.

While increasingly sophisticated circuits composed of multiple modules are being built, the behavior of these circuits becomes more difficult to predict and engineer as their size increases. A major problem is that circuits characterized in isolation often fail to behave as intended once they are part of a larger system. This type of failure is commonly referred to as *context-dependence* of genetic circuits [13, 20], that is, the fact that modules behave in a poorly predictable way once interacting together in the cell environment. This is a bottleneck to creating larger circuits that behave predictably.

Problems of context-dependence can be divided into three qualitatively different types: genetic context; direct inter-module interactions; and indirect interactions among genetic modules. While the first one can be dealt with via appropriate genetic engineering of DNA parts [20, 56, 39], the other two forms of context-dependence require a system-level understanding of dynamic interactions among circuit components. We therefore focus mostly on the latter two aspects here.

Direct inter-module interactions. These are unwanted interactions that occur directly between modules. A well known example is that of off-target binding of transcription factors to promoters. These undesirable bindings couple genetic modules that should be decoupled and can be tackled by co-optimizing the genetic sequences of promoters and transcription factors [39]. A different type of undesirable interaction occurs when modules are connected to each other and a protein in an upstream module is used as an "input" to a downstream module. This creates a "load" on the upstream system due to the fact that the output protein cannot take part in the upstream module's reactions whenever it is taking part in the downstream module's reactions. As a consequence, the behavior of the upstream module changes compared to when the system functions in isolation [22, 33]. These loading effects have been called retroactivity to extend the notion of loading and impedance to biomolecular systems. Solutions to mitigate this problem have appeared [43, 40]. These are based on the design of "insulation devices", systems that are placed between a sending module and receiving load modules such that arbitrarily large loads can be driven without a deterioration of the transmitted signal. The design of these load drivers uses principles of disturbance attenuation from control theory in order to reject retroactivity [32].

Indirect interactions among genetic modules. Ideally, the cell should function as a "chassis" for engineered biological circuits. In practice, this is not the case because the cellular endogenous circuitry interacts with externally introduced circuits at multiple levels, chiefly by sequestering resources such as ATP, RNA polymerase, and ribosomes, which are required for the operation of synthetic circuits. This sequestration often reduces cell fitness, with deleterious consequences also for synthetic circuits, a phenomenon that has been broadly called "metabolic burden" [10]. This phenomenon has been studied

and characterized more precisely in recent years, by showing that it is possible to create a "burden monitor" that senses the level of stress by cells and correlates with growth rate [14]. A more subtle phenomenon than purely reducing cell fitness is that synthetic circuits compete with each other for the same resources. This fact creates implicit and unwanted coupling among circuits with unpredictable consequences. Specifically, it was demonstrated that competition for shared gene expression resources, chiefly ribosomes, by multiple synthetic genes couples the expression levels of these genes in such a way that inducing one gene causes a drop in the expression of the other [28]. These "hidden" interactions are a major cause of lack of modularity in the design of genetic circuits and have been shown to lead to emergent genetic circuit behaviors that are arbitrarily far from the theoretically prescribed one [49].

Approaches to mitigate these problems have recently appeared in the literature. Specifically, in order to mitigate defects on cell growth rate imparted by induction of synthetic genes, a control system has been built, which uses the burden monitor to sense burden and, based on this, reduces the expression of synthetic genes [15]. Concurrently, solutions have appeared to mitigate the coupling among synthetic genetic circuits due to competition for ribosomes. Two types of solutions have appeared. The first type is a decentralized control architecture in which each genetic module is equipped with a feedback controller that aims at making output protein level robust to fluctuations in available ribosomes [52, 31]. Specifically, in [31], the authors built a quasi-integral controller that is embedded within a genetic module via post-transcriptional modifications, which allows to keep the inputs and outputs of the genetic module unchanged while reaching adaptation to variable ribosome demand. In the second type of solution, a centralized controller was proposed to dynamically allocate orthogonal ribosomes to synthetic genetic circuits [19].

Finally, the external environment where a cell operates has a number of physical attributes, which may also be subject to perturbations. These physical attributes include temperature, acidity, nutrients' level, etc. Perturbations in these attributes often lead to poor cell fitness or to non-standard growth conditions, ultimately leading to synthetic circuits malfunctions. Recently, a universal integral control architecture has been proposed to mitigate some of these disturbances, such as growth rate changes [6].

IV. Summary and Future Directions

The future of synthetic biology highly depends on the ability of scaling up the complexity of design to create more sophisticated functions, which yet are robust and reliable. While a number of issues can be successfully addressed by (non-trivial) fabrication of new parts, issues such as context-dependence require a system-level dynamic understanding of circuits and their interactions. Here is where control and dynamical systems theory could greatly contribute. Control theory has proven critical to reason about and engineer robustness in a number of concrete applications including aerospace and automotive systems, robotics and intelligent machines, manufacturing chains, electrical, power, and information networks. Similarly, control theory could enable the understanding of principles that ensure robust behavior of synthetic genetic circuits. An extensive review on this topic can be found in [24].

V. Acknowledgements

The author would like to thank Richard M. Murray for having provided feedback on the initial form of the current manuscript.

References

[1] *BioBricks Foundation*. `https://biobricks.org`.
[2] *Registry of Standard Biological Parts*. `http://parts.igem.org`.
[3] K. J. Åström and R. M. Murray. *Feedback Systems*. Princeton, 2008.
[4] B. Alberts, A. Johnson, J. Lewis, M. Raff, and K. Roberts. *Molecular Biology of the Cell*. Garland Science, fifth edition, 2007.
[5] U. Alon. *An introduction to systems biology. Design principles of biological circuits*. Chapman-Hall, 2007.
[6] S.K. Aoki, G. Lillacci, A. Gupta, A. Baumschlager, D. Schweingruber, and M. Khammash. A universal biomolecular integral feedback controller for robust perfect adaptation. *Nature*, 570: 533–537, 2019.
[7] M. R. Atkinson, M. A. Savageau, J. T. Meyers, and A. J. Ninfa. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. *Cell*, 113:597–607, 2003.
[8] D. W. Austin, M. S. Allen, J. M. McCollum, R. D. Dar, J. R. Wilgus, G. S. Sayler, N. F. Samatova, C. D. Cox, and M. L. Simpson. Gene network shaping of inherent noise spectra. *Nature*, 439:608–611, 2005.
[9] A. Becskei and L. Serrano. Engineering stability in gene networks by autoregulation. *Nature*, 405:590–593, 2000.
[10] W. E. Bentley, N. Mirjalili, D. C. Andersen, R. H. Davis, and D. S. Kompala. Plasmid-encoded protein: the principal factor in the "metabolic burden" associated with recombinant bacteria. *Biotechnol Bioeng*, 35(7):668–81, 1990.
[11] L. Bleris, Z. Xie, D. Glass, A. Adadey, E. Sontag, and Y. Benenson. Synthetic incoherent feedforward circuits show adaptation to the amount of their genetic template. *Molecular Systems Biology*, 7:519, 2011.
[12] D. E. Cameron, C. J. Bashor, and J. J. Collins. A brief history of synthetic biology. *Nature Reviews Microbiology*, 12:381–390, 2014.
[13] S. Cardinale and A. P. Arkin. Contextualizing context for synthetic biology – identifying causes of failure of synthetic biological systems. *Biotechnology Journal*, 7:856–866, 2012.
[14] F. Ceroni, R. Algar, GB. Stan, and T. Ellis. Quantifying cellular capacity identifies gene expression designs with reduced burden. *Nat. Methods*, 12(5):415–422, 2015. doi: 10.1038/nmeth.3339.
[15] F. Ceroni, A. Boo, S. Furini, T.E. Gorochowski, O. Borkowski, Y.N. Ladak, A.R. Awan, C. Gilbert, G.B. Stan, and T. Ellis. Burden-driven feedback control of gene expression. *Nat. Methods*, 15:387–393, 2018.
[16] C. T. Chan, J. W. Lee, D. E. Cameron, C. J. Bashor, and J. J. Collins. 'deadman' and 'passcode' microbial kill switches for bacterial containment. *Nature Chemical Biology*, 12:82–86, 2016.

[17] M-T. Chen and R. Weiss. Artificial cell-cell communication in yeast *Saccharomyces cerevisiae* using signaling elements from *Arabidopsis thaliana*. *Nature Biotechnology*, 23(12):1551–1555, 2005.

[18] T. Danino, A. Prindle, G.A. Kwong1, M. Skalak, H. Li, K. Allen, J. Hasty, and S. N. Bhatia. Programmable probiotics for detection of cancer in urine. *Science Translational Medicine*, 7, 2017.

[19] A. P. S. Darlington, J. Kim, J. I. Jiménez, and D. G. Bates. Dynamic allocation of orthogonal ribosomes facilitates uncoupling of co-expressed genes. *Nat. Comm.*, (9), 2018.

[20] D. Del Vecchio. Modularity, context-dependence, and insulation in engineered biological circuits. *Trends in Biotech.*, 2015.

[21] D. Del Vecchio and R. M. Murray. *Biomolecular Feedback Systems*. Princeton University Press, 1st edition, 2014.

[22] D. Del Vecchio, A. J. Ninfa, and E. D. Sontag. Modular cell biology: Retroactivity and insulation. *Molecular Systems Biology*, 4:161, 2008.

[23] D. Del Vecchio, A. J. Dy, and Y. Qian. Control theory meets synthetic biology. *J. R. Soc. Interface*, 13(120), 2016. doi: 10.1098/rsif.2016.0380.

[24] D. Del Vecchio, Y. Qian, R. M. Murray, and E. D. Sontag. Future systems and control research in synthetic biology. *IFAC Reviews in Control*, 45:5–17, 2018.

[25] M. B. Elowitz and S. Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403:339–342, 2000.

[26] T.S. Gardner, C.R. Cantor, and J.J. Collins. Construction of the genetic toggle switch in *Escherichia Coli*. *Nature*, 403:339–342, 2000.

[27] D. T. Gillespie. Exact stochastic simulation of coupled chemical reactions. *The Journal of Physical Chemistry*, 81:2340–2361, 1977.

[28] A. Gyorgy, J. I. Jiménez, J. Yazbek, H.H. Huang, H. Chung, R. Weiss, and D. Del Vecchio. Isocost lines describe the cellular economy of gengene circuits. *Biophys. J.*, 109(3):639–646, 2015. doi: 10.1016/j.bpj.2015.06.034.

[29] V. Hsiao, E. De Los Santos, W. R. Whitaker, J. E. Dueber, and R. M. Murray. Design and implementation of a biomolecular concentration tracker. *ACS Syn. Biol.*, 4(2):150–161, 2015. doi: 10.1021/sb500024b.

[30] V. Hsiao, A. Swaminathan, and R. M. Murray. Control theory for synthetic biology: Recent advances in system characterization, control design, and controller implementation for synthetic biology. *IEEE Control Systems Magazine*, 38:32–62, 2018.

[31] H.H. Huang, Y. Qian, and D. Del Vecchio. A quasi-integral controller for adaptation of genetic modules to variable ribosome demand. *Nature Communications*, 9(1), dec 2018. doi: 10.1038/s41467-018-07899-z.

[32] S. Jayanthi and D. Del Vecchio. Retroactivity attenuation in biomolecular systems based on timescale separation. *IEEE Trans. Aut. Control*, 56:748–761, 2011.

[33] S. Jayanthi, K. Nilgiriwala, and D. Del Vecchio. Retroactivity controls the temporal dynamics of gene transcription. *ACS Synthetic Biology*, DOI: 10.1021/sb300098w, 2013.

[34] C.L. Kelly, A. Harris, H. Steel, E. J. Hancock, J. T. Heap, and A. Papachristodoulou. Synthetic negative feedback circuits using engineered small RNAs. *Nucleic Acids Research*, (46): 9875–9889, 2018. doi: 10.1021/sb500024b.

[35] H. Kobayashi, M. Kaern, M. Araki, K. Chung, T. S. Gardner, C. R. Cantor, and J. J. Collins. Programmable cells: Interfacing natural and engineered gene networks. *Proc. National Academy of Sciences*, 101:8414–8419, 2004.

[36] J. W. Kotulaa, S. J. Kernsa, L. A. Shaketb, L. Sirajb, J. J. Collins, J. C. Wayb, and P. A. Silver. Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proc. Natl. Acad. Sci, USA*, 111:4838–4843, 2014.

[37] Gabriele Lillacci, Yaakov Benenson, and Mustafa Khammash. Synthetic control systems for high performance gene expression in mammalian cells. *Nucleic Acids Research*, 46(18):9855–9863, sep 2018. doi: 10.1093/nar/gky795.

[38] C. McBride, R. Shah, and D. Del Vecchio. The effect of loads in molecular communications. *Proceedings of the IEEE*, 107: 1369–1386, 2019.

[39] A. J. Meyer, T. H. Segall-Shapiro, E. Glassey, J. Zhang, and C. A. Voigt. *Escherichia* coli "marionette" strains with 12 highly optimized small-molecule sensors. *Nature Chemical Biology*, 15:196–204, 2019.

[40] D. Mishra, P. M. Rivera-Ortiz, A. Lin, D. Del Vecchio, and R. Weiss. A load driver device to engineer modularity in biological circuits. *Nat. Biotechnology*, 32:1268–1275, 2014.

[41] T. S. Moon, C. Lou, A. Tamsir, B. C. Stanton, and C.A. Voigt. Genetic programs constructed from layered logic gates in single cells. *Nature*, 491:249–253, 2012.

[42] A. A. K. Nielsen, B. S. Der, J. Shin, P. Vaidyanathan, V. Paralanov, E. A. Strychalsk, D. Ross, D. Densmore, and C. A. Voigt. Genetic circuit design automation. *Science*, 352, 2016.

[43] K. Nilgiriwala, P. M. Rivera-Ortiz, J. Jimenez, and D. Del Vecchio. A tunable amplifying buffer circuit in *e. coli*. *ACS Synthetic BIology*, 4:577–584, 2015.

[44] K. Pardee, . A. Green, T. Ferrante, D. E. Cameron, A. Daleykeyser, P. Yin, and J. J. Collins. Paper-based synthetic gene networks. *Cell*, 159(4):940–954, mar 2014. ISSN 10974172. doi: 10.1016/j.cell.2014.10.004. URL `http://dx.doi.org/10.1016/j.cell.2014.10.004`.

[45] K. Pardee, A. Green, M. K. Takahashi, D. Braff, G. Lambert, J. W. Lee, T. Ferrante, D. Ma, N. Donghia, M. Fan, N. M. Daringer, I. Bosch, D. M. Dudley, D. H. O'Connor, L. Gehrke, and J. J. Collins. Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. *Cell*, 165(5):1255–1266, 2016. ISSN 10974172. doi: 10.1016/j.cell.2016.04.059. URL `http://dx.doi.org/10.1016/j.cell.2016.04.059`.

[46] P. P. Peralta-Yahya, F. Zhang, S. B. del Cardayre, and J. D. Keasling. Microbial engineering for the production of advanced biofuels. *Nature*, 488:320–328, 2012.

[47] J. G. Perez, J. C. Stark, and M. C. Jewett. Cell-free synthetic biology: Engineering beyond the cell. *Cold Spring Harb Perspect Biol.*, 12, 2016.

[48] P. Purnick and R. Weiss. The second wave of synthetic biology: from modules to systems. *Nature reviews. Molecular cell biology*, 10:410–22, 2009.

[49] Y. Qian, H.H. Huang, J. I. Jiménez, and D. Del Vecchio. Resource competition shapes the response of genetic circuits. *ACS Synthetic biology*, 6:1263–1272, 2017.

[50] Y. Qian, C. McBride, and D. Del Vecchio. Programming cells to work for us. *Annual Review Control Robotics, and Autonomous Systems*, 1(1), 2018. doi: 10.1146/annurev-control-060117-105052.

[51] T. H. Segall-Shapiro, E. D. Sontag, and C. A. Voigt. Engineered promoters enable constant gene expression at any copy number in bacteria. *Nature Biotechnology*, 36(4):352–358, mar 2018. doi: 10.1038/nbt.4111.

[52] T Shopera, L He, T Oyetunde, YJ Tang, and TS Moon. Decoupling resource-coupled gene expression in living cells. *ACS Synth. Biol*, 2017.

[53] J. Stricker, S. Cookson, M. R. Bennett, W. H. Mather, L. S. Tsimring, and J. Hasty. A fast, robust and tunable synthetic gene oscillator. *Nature*, 456:516–519, 2008.

[54] M. K. Takahashi, X. Tan, A. J. Dy, D. Braff, R. T. Akana, Y. Furuta, N. Donghia, A. Ananthakrishnan, and J. J. Collins. A low-cost paper-based synthetic biology platform for analyzing gut microbiota and host biomarkers. *Nature Communications*, 9(1):3347, 2018. ISSN 2041-1723. doi: 10.1038/s41467-018-05864-4. URL `https://doi.org/10.1038/s41467-018-05864-4`.

[55] Z. Xie, L. Wroblewska, L. Prochazka, R. Weiss, and K. Benenson. Multi-input rnai-based logic circuit for identification of specific cancer cells. *Science*, 333:1307–11, 2011.

[56] E. Yeung, A.J. Dy, K.B. Martin, A.H. Ng, D. Del Vecchio, J.L. Beck, J.J. Collins, and R.M. Murray. Biophysical constraints arising from compositional context in synthetic gene networks. *Cell Systems*, 5:11–24, 2017.

[57] F. Zhang, J. M. Carothers, and J. D. Keasling. Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids. *Nat. Biotechnol.*, 30:354–359, 2012.