

SYNTHETIC BIOLOGY

Universal control in biochemical circuits

A module for implementing robust feedback control in synthetic cellular networks has been reported. Its design is first proved mathematically to be universal for all networks, and then implemented in living cells.

NOAH OLSMAN & JOHAN PAULSSON

Anyone who has lived without central heating and cooling has had to learn the right combinations of opening windows, turning on radiators or adjusting blinds to get the temperature just right. Modern thermostats eliminate all that: you set them once and the built-in controllers do the rest, regardless of changes in the weather or the type of home. The temperature might still vary a little, but as long as the heaters and coolers are designed correctly, it should vary around the set point, rather than merely taking the edge off the cold or heat.

Writing in *Nature*, Aoki and colleagues report¹ an analogous system for chemical reactions in living cells. Specifically, they design a reaction module in which two components sequester each other, and show that adding this to almost any network can force the output of the system to maintain a precise value that is proportional to an input signal, in a way that is robust to both external disturbances and uncertainty in the internal parameters — a behaviour known as robust perfect adaptation.

The results are striking for two reasons. First, most self-corrective biochemical circuits merely dampen the effects of external changes, rather than compensate for them perfectly. For example, by auto-repressing their own production, proteins can make their abundances less responsive to parameter changes than they would otherwise be, but still respond to some extent (Fig. 1a). Such systems are therefore known as homeostatic regulators because they maintain similar (homeo), rather than the same (homo), protein levels. Second, the impact of adding extra reactions to a biomolecular network usually depends on context. For instance, adding a repression step could create a positive or a negative feedback loop, depending on the rest of the network. Most systems have therefore been modelled and engineered on a case-by-case basis, and it has been hard even to imagine that any universal synthetic control could be found.

The approach taken by Aoki and co-workers

is as striking as their results. Anyone working with synthetic biologists will eventually hear them quote the last words that physicist Richard Feynman wrote on his blackboard: “What I cannot create, I do not understand.” However, Feynman was referring to mathematical derivations rather than to the building of real-world systems such as biological networks, and Aoki and colleagues’ paper is one of the few in synthetic biology that truly lives up to the quote. The authors started by deriving exact mathematical rules that apply to broad classes of chemical-reaction system, and only then proceeded to physically build systems that illustrate the rule.

Although the authors’ results pertain to average abundances of molecules across a population of cells, they were derived using frameworks that account for the inherent

randomness of individual reaction events in individual cells. That might seem like a subtle distinction — mathematically accounting for probabilistic mechanisms but then predicting only averages of the resulting statistical distributions. But for most chemical networks, in which reaction rates often depend nonlinearly on concentrations, accounting for probabilistic reactions is necessary even to predict the right averages. Aoki and colleagues’ unusual level of rigour in this respect thus makes their results much stronger.

More specifically, the authors focused on a system architecture known as antithetic integral feedback control², in which feedback is implemented by actuator and sensor molecules that bind irreversibly to each other (Fig. 1b). If each sensor molecule consistently finds a partner actuator molecule, then the system detects that the output is correctly matching the input. If, instead, there are too many or too few sensor molecules, the actuator molecules automatically adjust the production of sensors to try to get the balance right, like a molecular ‘buddy system’. Aoki *et al.* prove mathematically not only that this circuit has the capacity to implement robust perfect adaptation in any chemical-reaction network, but also that all networks that exhibit robust perfect adaptation must at some level embed this kind of antithetic feedback motif.

The authors went on to demonstrate that their theoretical control architecture can be

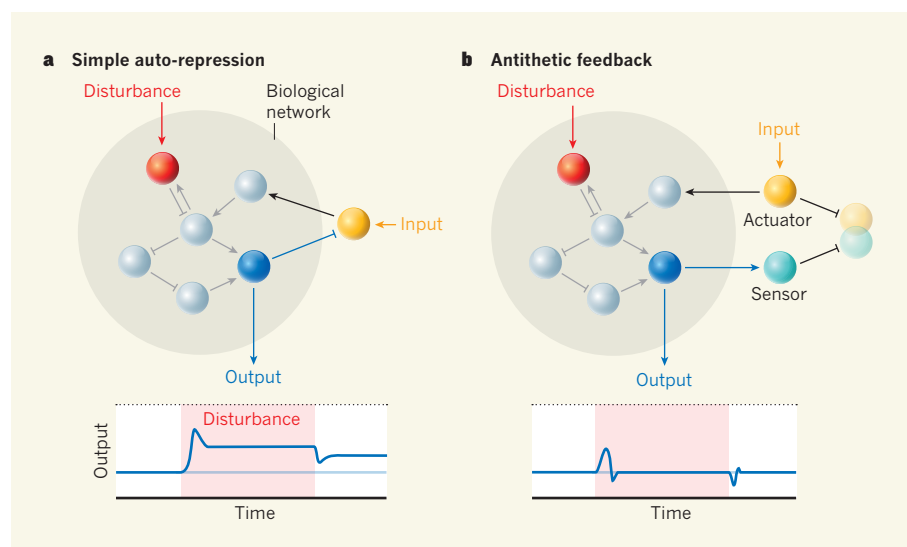


Figure 1 | Two modes of feedback regulation in biological networks. **a**, Auto-repression is a simple form of regulation for biological networks. In this general scheme, biomolecules (small spheres) in a network interact with each other, stimulated by an external input signal that acts on another molecule (yellow sphere). The molecule represented by the blue sphere inhibits the molecule acted on by the input, and produces a measurable output. When an external disturbance acts on part of the network (red sphere), altering the amplitude of the output, the network architecture partly compensates for the change, but does not precisely return the output to its original value. **b**, Aoki *et al.*¹ report an antithetic feedback module in which the input acts on an actuator molecule, the output acts on a sensor molecule, and the actuator and sensor molecules combine to cancel each other out. This architecture compensates for disturbances, and guarantees that the output returns to precisely its original value.

implemented in living cells. They focused on a system that incorporates proteins called σ factors, which regulate the initiation of gene expression in bacteria. Some σ factors are sequestered by binding partners (anti- σ factors), such as the σ factor SigW from the bacterium *Bacillus subtilis* and its anti- σ factor RsiW. The researchers integrated SigW into the model bacterium *Escherichia coli*, and used it to regulate the expression of a green fluorescent protein (GFP) as a reporter of gene expression. They then coupled the activation of the GFP-producing genes to the production of RsiW, which subsequently sequesters SigW. The levels of SigW were also regulated by a small molecule that induces the expression of the *sigW* gene, and which acts as an input to the circuit. If the circuit worked as expected, then the amount of green fluorescence produced by the *E. coli* cells should be proportional to the levels of SigW, and at steady state should be independent of any other parameters.

Sure enough, Aoki *et al.* showed that varying the concentration of the inducer could be used to control GFP output as expected. Yet when

the system was disturbed by adding a protease enzyme that degrades both GFP and a protein that affects RsiW production, the fluorescence signal transiently changed but then returned to a level that was indistinguishable from the starting value, demonstrating that the circuit does indeed exhibit robust perfect adaptation. By contrast, in an analogous system that lacked feedback control, the same disturbance systematically lowered the concentration of GFP to about half of its initial value. The authors even replaced GFP with a protein that regulates cell growth, and thereby produced an *E. coli* strain that grew at a constant rate, despite changes in factors that would otherwise alter growth rate.

One possible future direction for such work is to study the circuit in single cells, rather than its average effects across populations. On the one hand, recent work³ suggests that circuits of this type could increase spontaneous fluctuations, as has also been reported⁴ for related classes of reaction scheme. On the other hand, previously published theoretical work⁵ from the same research group as that of Aoki *et al.* suggests that more-complex circuit architectures could exhibit robust perfect adaptation

without amplifying spontaneous fluctuations. Such behaviour will be necessary to ensure that circuits can perform precise, quantitative functions in any given cell, despite inherent noise and uncertainty. In the same way that reducing error rates in digital circuits was essential for the development of modern computers, the ability to engineer sub-networks of cellular circuits that work precisely and robustly will probably be necessary as we seek to assemble complex synthetic cellular systems comparable to those found in nature. ■

Noah Olsman and Johan Paulsson are in the Department of Systems Biology, Blavatnik Institute at Harvard Medical School, Harvard University, Boston, Massachusetts 02115, USA. e-mail: johan_paulsson@harvard.edu

1. Aoki, S. K. *et al.* *Nature* <https://doi.org/10.1038/s41586-019-1321-1> (2019).
2. Briat, C., Gupta, A. & Khammash, M. *Cell Syst.* **2**, 15–26 (2016).
3. Olsman, N., Xiao, F. & Doyle, J. C. *iScience* **14**, 277–291 (2019).
4. Hilfinger, A., Norman, T. M., Vinnicombe, G. & Paulsson, J. *Phys. Rev. Lett.* **116**, 058101 (2016).
5. Briat, C., Gupta, A. & Khammash, M. *J. R. Soc. Interface* **15**, 20180079 (2018).