

Scaling up genelet circuits

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DNA nanotechnology and synthetic biology both aim to expand the range of dynamic behaviours exhibited by rationally programmed biomolecules. Now, the programmability of synthetic transcriptional circuits has been improved to enable synthesis of dynamic biomolecular circuits with unmatched complexity.

Researchers in DNA nanotechnology and synthetic biology alike envision the realization and control of molecular structures and processes that perform useful functions. Whereas synthetic biologists typically make use of the full biochemical machinery of the cell, DNA nanotechnologists tend to create almost everything from nucleic acids^{1,2}. Both approaches have advantages, but also face major challenges. Now, writing in *Nature Chemistry*, a team led by Rebecca Schulman describes an approach that combines the programmability of DNA systems and the versatility of biochemical systems to greatly improve the ability to control non-equilibrium chemical reaction network kinetics³.

Synthetic biology aims to utilize the rich gene regulatory repertoire and the immense biosynthetic potential of biological cells to ‘reprogram’ cells to act as little soft robots and to generate synthetic cell-like systems *de novo*. However, the initial goal of rationally designing and programming living systems has turned out to be very challenging due to their sheer complexity. Synthetic gene regulatory circuits often do not behave as anticipated – compared with simplified computational models – due to unexpected interactions with other cellular components, or the influence of the cellular environment. Often synthetic biological systems therefore require fine-tuning using ‘irrational’ approaches such as screening or directed evolution to compensate for our ignorance of their molecular details.

On the other hand, DNA nanotechnology has been extremely successful in the generation of molecular systems composed entirely of DNA. The formation of DNA-based nanostructures can be very well predicted, and versatile computational tools are available for their design. Researchers have also developed dynamical chemical reaction networks composed of DNA – in most cases involving toehold-mediated strand displacement (TMSD) reactions – which, in principle, can perform the same functions as their complex biological counterparts⁴. However, pure DNA circuits are (by definition) extremely limited in terms of their biosynthetic capabilities. Moreover, many of the most interesting behaviours exhibited by biological systems – such as growth, replication and motion – are generated by intrinsically dissipative, out-of-equilibrium processes⁵. In the context of (pure) DNA nanotechnology, all processes are essentially driven by DNA hybridization reactions. DNA nanotechnology thus amounts to the rational design of a mix of DNA strands that generate interesting structures or dynamics, while they relax towards their equilibrium

state. There is no process to produce or regenerate molecules, which considerably limits the versatility of the systems.

The team use a ‘genelet’ strategy – one of several approaches that have been developed to overcome the limitations of both DNA nanotechnology and synthetic biology by generating simplified chemical reaction networks composed of DNA and a few enzymes. Genelet circuits use DNA molecules as templates for T7 RNA polymerase, which produces RNA molecules by transcription⁶. These can be used to switch on or off other transcription processes via TMSD, providing a means to generate synthetic gene regulatory networks⁷, or to actuate nucleic acid devices⁸. Nucleoside triphosphates are thus supplied as chemical fuels and converted into RNA species, which are later degraded by an additional ribonuclease (RNase) added to the system. This, in principle, allows the operation of such systems in a non-equilibrium steady state, and genelet circuits have already been demonstrated to generate bistability, oscillations and computational networks^{6–8}. Genelets thus benefit from the excellent sequence-programmability of DNA systems, but enable the implementation of more complex functions and richer dynamics more easily.

But the presence of only two enzymes (T7 RNA polymerase and RNase H) makes genelet systems much less predictable than naively expected. Schulman and colleagues have now systematically tackled the issues that have challenged genelet circuits so far, making major progress to make genelet circuits rationally designable and scalable³. The toehold sequences responsible for the interconnection of the different nodes of the circuits were hidden in so-called “hairpin clamps”, greatly reducing the crosstalk between the circuit components (Fig. 1a). In conventional genelet circuits, the polymerase tends to transcribe DNA molecules into RNA also in the absence of a promoter, poisoning the system with undesired RNA transcripts. Schulman and co-workers devised a novel scheme – based on the removal of “blocker strands” from a toehold – to activate transcription. To prevent promoter-independent transcription of the blockers, these were replaced by 2'-methylated RNA rather than DNA molecules. Finally, a set of 11 standardized, “regulatory domains” was extracted from a screening experiment, which exhibited uniform branch migration kinetics, and could be used interchangeably to build up large circuits (Fig. 1b). This contrasts with conventional genelet circuits based on branch migration processes with considerable kinetic variation, which has previously caused difficulties in computational prediction and design of circuit behaviour.

Bringing all of these improvements together, Schulman and colleagues were able to demonstrate excellent control and predictability of a variety of complex genelet circuits. In one example, they were able to show precise control over the timing and shape of pulses generated by an incoherent feedforward network, which could also be used to generate two pulses with a defined temporal separation. Other examples include a tri-stable network, and larger networks composed of up to ten different nodes. Further, these combined feedforward and feedback circuit dynamics could be captured by a simple kinetic model (Fig. 1c).

The results of Schulman and her team are good news for the field: first, they demonstrate that a better understanding of the underlying

a Genelet design

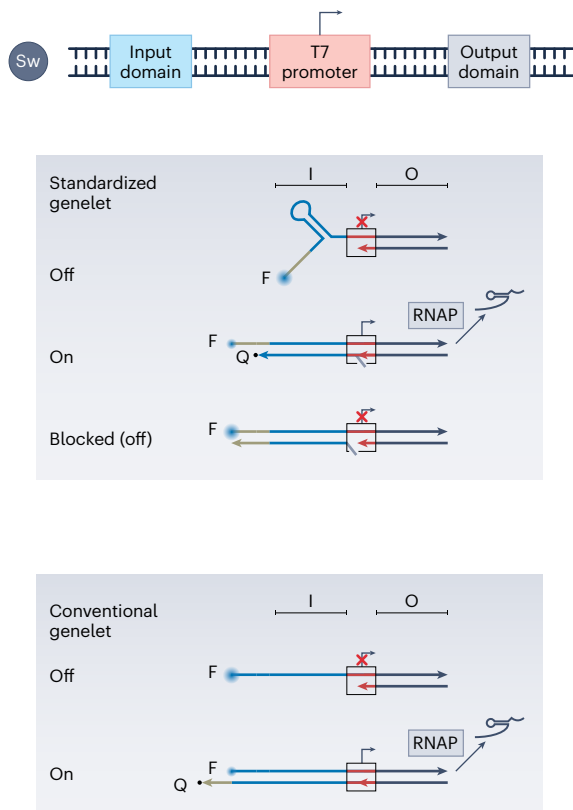
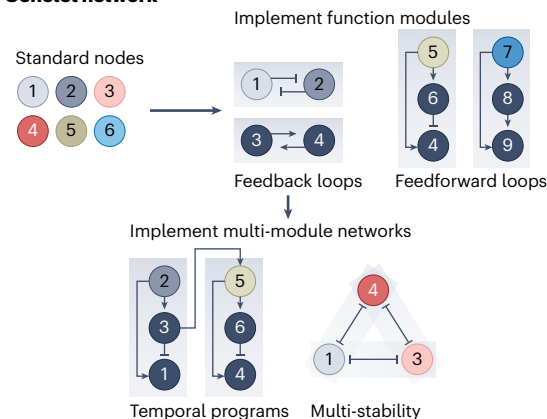


Fig. 1 | Programming the genelet circuits. **a**, Synthetic transcriptional switch (Sw) designs in standardized and conventional genelet circuits. Each consists of an input (I) domain and an output (O) domain. Complexation of the genelet with an activator (ON) completes the T7 RNA polymerase (RNAP) promoter sequence (red) and results in transcription. In the standardized genelet, addition of a blocker prevents binding of the activator and transcription. The

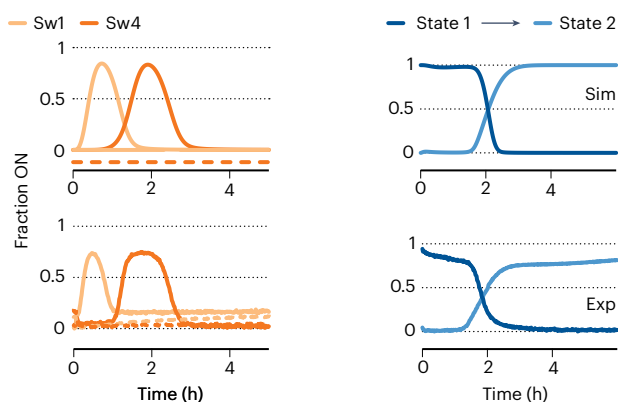
processes and careful design make the genelet approach more engineerable and thus allow scaling up of these systems to much larger networks than previously possible. This, in turn, allows the use of such systems in future applications, where they may be used as advanced sensors integrating and evaluating multiple inputs; as timers and pulse generators; or as circuits with memory and the capability for decision-making. Such circuits will be of great interest to endow bottom-up biological systems with some sort of ‘intelligence’ – for example, in the context of synthetic cells or microscale robots, together with recent extensions of genelet circuits with compartmentalization⁹, structural control¹⁰, and CRISPR/Cas activity¹¹. Importantly, the intrinsic out-of-equilibrium operation of such circuits allows them, in principle, to permanently monitor their surroundings and respond to a changing environment.

Finally, the improved understanding and scalability of these simplified biochemical circuits give hope that in the long run, more complex systems – such as gene circuits operating in a cell-free gene expression system, or even fully fledged synthetic biological circuits implemented *in vivo* – will become tractable through improved understanding and design rules.

b Genelet network



c Genelet modelling



state of the switches can be read out via the fluorescence of a fluorophore (F) and its reduction by a quencher (Q). **b**, Genelet network engineering workflow. Standardized circuit elements can be interchangeably assembled into larger networks. **c**, Circuit outputs predicted by the general genelet model simulation (Sim) and measured in experiments (Exp). Figure adapted from ref. ³, Springer Nature Ltd.

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Competing interests

The authors declare no competing interests.