Timing molecular motion and production with a synthetic transcriptional clock

Elisa Franco[a], Eike Friedrichs[b], Jongmin Kim[c], Ralf Jungmann[b], Richard Murray[a], Erik Winfree[c,d,e], and Friedrich C. Simmel[b,1]

[a]Control and Dynamical Systems, California Institute of Technology, Pasadena, CA 91125; [b]Lehrstuhl für Bioelektronik, Physik Department, Technische Universität München, Am Coulombwall 4a, 85748 Garching, Germany; [c]Bioengineering, California Institute of Technology, Pasadena, CA 91125; [d]Computation and Neural Systems, California Institute of Technology, Pasadena, CA 91125; and [e]Computer Science, California Institute of Technology, Pasadena, CA 91125

AUTHOR SUMMARY

Biological and computational systems need "timing" to operate properly. Digital clock generators synchronize the states of millions of transistors in silicon circuits, while circadian rhythms similarly orchestrate biological processes in complex organisms (1). Systematic methods for the design of clocked electronic circuits are available. However, the same is not true for biochemical circuits, which present challenges unique to the molecular context: for instance, it is not obvious whether a system can be subdivided into functional modules, and signals do not propagate through "wires." Here, we study such challenges through a model problem: we use a synthetic, cell-free biochemical oscillator to drive several different molecular "loads." In all cases the load causes a deterioration of the oscillator signal. We demonstrate that the perturbation can be efficiently reduced by placing a signal amplification device between the oscillator and the load process.

When operating in a cell-free environment ("in vitro") with a limited number of biological parts, we have the opportunity to gain insight into the design principles of complex natural circuits, following a bottom-up approach. We previously constructed and analyzed synthetic in vitro DNA switches ("genelets") that can be regulated through their corresponding RNA transcripts, allowing us to build a variety of different circuits (2, 3). Because of their simplicity, in vitro transcriptional circuits are amenable to systematic design and mathematical modeling. Therefore, genelet systems provide an attractive toolkit with which to investigate the design principles underlying interconnected biochemical circuits of increasing size and complexity.

Here, we use a synthetic biochemical oscillator by Kim and Winfree (3) as a clock to drive several types of molecular devices. The oscillator consists of two DNA transcription templates, SW21 and SW12. The output of SW21 is the RNA species rI2, which inhibits SW12. The output of SW12, rA1, is instead an activator for SW21. Overall, the two genelets form a delayed negative feedback loop, which is schematically represented in Fig. P1*A* (components inside the blue dashed circle). Two enzymes, RNA polymerase and RNase H, are present in solution that produce and degrade the RNA signals. The DNA switches are inhibited by displacing part of their promoter region through toehold-mediated branch migration (2). Additional activating and inhibiting DNA species set thresholds for the switching reactions. The amplitude and frequency of the oscillator can be tuned by choosing the concentrations of its components.

As a load to the oscillator, we use the well known DNA tweezers system (4). The term "DNA tweezers" refers to a nanomechanical structure consisting of two rigid double-stranded "arms" of 18 base pairs each, connected by a four nucleotide single-stranded molecular "hinge." Hybridization of single-stranded extensions of these arms—the "hands"—with a complementary strand brings the tweezers into a "closed"

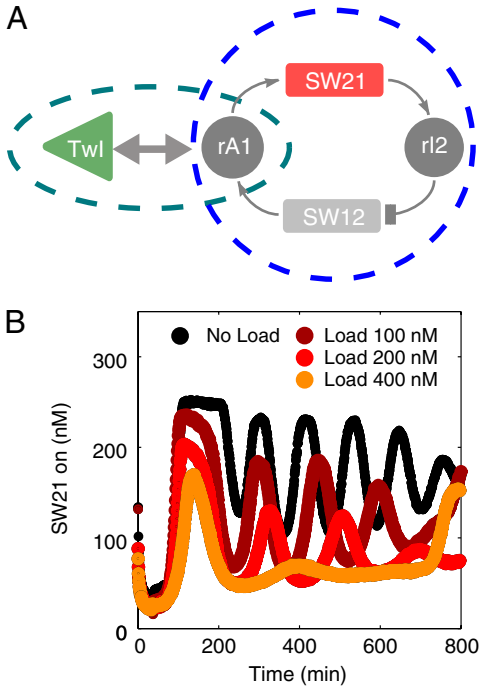

Fig. P1. We coupled our synthetic transcriptional oscillator (3) to several nanodevices, evaluating different direct and insulated modes of interconnection. Here we show the results for one of the direct modes, where the load is represented by the DNA tweezers system (4). (*A*): Schematic representation of the system. The oscillator consists of two "genelets" SW12 and SW21 that are connected via inhibiting and activating RNA species rI2 and rA1. The DNA tweezers load (TwI) is coupled to the activation reaction branch of rA1. The blue dashed circle highlights the oscillator components; the green dashed circle highlights the load and the oscillator components driving it. (*B*): In this coupling mode, the performance of the core oscillator deteriorates for increasing load: no load (black), 100 nM (dark red), 200 nM (red), and 400 nM (orange) tweezers load.

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[1]To whom correspondence should be addressed. E-mail: simmel@ph.tum.de.

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conformation. We evaluate different modes of coupling, by designing the tweezers hands to target different DNA or RNA components of the oscillator. We also interconnect the clock to additional genelets producing functional RNA signals. The molecular conformation of the oscillator templates and of the tweezers is tracked through suitably positioned fluorescent labels.

We find that some configurations more effectively transmit the oscillations, depending on the amplitude and mean value of the driving component. Fig. P1*A* shows a schematic representation of one of the coupling modes for our oscillator. Here the perturbations are proportional to the total amount of load, as is indicated by the reduction of the amplitude of the oscillations (Fig. P1*B*). We are able to reduce this undesired "retroactivity" (5) by the isolation of the source components from downstream loads by coupling the oscillator to an additional DNA switch, using its RNA transcript to drive the load. Transcription effectively represents an amplification stage, and the additional genelet therefore acts as an insulator (5).

Many of the general features of our experimental system can be understood on the basis of a simple theoretical model for the oscillator that only accounts for the basic feedback circuit and makes some generic assumptions about the nature of the load coupling. The simple model cannot offer a quantitative description of the experiments, however. A much more detailed understanding has therefore been attempted, and satisfyingly, this detailed model was able to semiquantitatively reproduce all of the experimental data with a single set of physically reasonable parameters.

The oscillator system under load represents the realization of an in vitro molecular clock that is used to drive other biochemical processes in a plug-and-play fashion. This work contributes to our understanding of two challenges: the synchronization of biomolecular processes and the design of modular and scalable biochemical circuits. In the future, in vitro oscillators could be used to orchestrate more diverse downstream processes, could be modified to effect more complex and conditional regulation (as in the cell cycle), and could be embedded in artificial vesicles as part of the quest to construct an artificial cell.

1. Goldbeter A (1997) *Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behavior* (Cambridge University Press, Cambridge, United Kingdom).
2. Kim J, White KS, Winfree E (2006) Construction of an in vitro bistable circuit from synthetic transcriptional switches. *Mol Syst Biol* 2:68.
3. Kim J, Winfree E (2011) Synthetic in vitro transcriptional oscillators. *Mol Syst Biol* 7:465.
4. Yurke B, Turberfield AJ, Mills AP, Jr, Simmel FC, Neumann JL (2000) A DNA-fuelled molecular machine made of DNA. *Nature* 406:605–608.
5. Del Vecchio D, Ninfa A, Sontag ED (2008) Modular cell biology: Retroactivity and insulation. *Mol Syst Biol* 4:161.