Bottom-up construction of in vitro switchable memories

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AUTHOR SUMMARY

Keeping memory of past decisions is a fundamental operation of cellular information processing (1). Networks of reactions within cells may be bistable, and therefore exist in two mutually exclusive steady states. These bistable networks provide cells with long-term information storage. Such memory systems need not only to be robust but switchable; that is, able to update their state on detection of environmental stimuli. We show here that it is possible to reproduce these functions in vitro and to obtain addressable memories using simplified DNA biochemistry (2, 3). Our approach provides an unmatched opportunity to study the relationships between the structure of a biochemical reaction network and its dynamic function (4).

We describe a DNA-based toolbox for building in vitro reaction circuits. It is based on enzymatic polymerization and depolymerization of short DNA strands that mediate the communication between the elements of the circuit. The toolbox is composed of three modules: activation, autocatalysis, and inhibition (Fig. P1A). One can arbitrarily wire these modules in circuits encoding time-responsive behaviors. We use the DNA toolbox to rationally construct reaction circuits implementing memory functions of increasing complexity. We start with a foundational bistable system composed of two autocatalytic modules that mutually repress each other through two inhibition modules (Fig. P1B). This architecture is similar to that of in vivo bistable circuits. A simple mathematical model allows us to extract the important design parameters. These are then translated into the DNA sequences of the four modules. Experimentally, we incubate these DNA modules in presence of a DNA polymerase, a nicking enzyme, and an exonuclease. The resulting biochemical system indeed possesses two exclusive steady states. The state that the system ultimately displays depends only on the initial stimulus. The relative attractiveness of the two states can be adjusted by tuning the concentrations of the DNA modules. We also show that the two steady states are very resilient to perturbations in the sense that the system in one state will not switch to the other state unless a very strong perturbation is applied. On the flip side, this means that a dedicated strategy is necessary to use them as addressable memory elements.

We therefore connect the bistable core to two activation modules that read two separate exogenous inputs (γ and δ) (Fig. P1C) and amplify them into long-lasting stimulations. Experimentally, we obtain a system that can both flip reversibly between states (Fig. P1D) and robustly maintain the memoryized state. The behavior of this circuit is remarkably well described by a detailed model that takes into account the full set of reactions (Fig. P1D).

Our third target is a push-push memory circuit, that is, a bistable memory that can be switched back and forth by the same stimulus. This supposes that upon reading of its unique external input, the circuit integrates the current state of the memory and accordingly produces a long-lasting stimulus directed to the opposite state. Because our design process is modular, we are able to reuse the previous bistable element and to plug in four additional DNA modules to perform the switching function. It works as follows: Two activation modules receive and convert the same external input, but they are controlled by two inhibition modules that are linked to the state of the bistable system (Fig. P1E). The full experimental circuit contains eight modules, and it is indeed able to flip in both directions upon reading of a unique external input.

From three fully modular biochemical reactions, activation, autocatalysis, and inhibition, we show that it is possible to build dynamic circuits with predefined functions; here, memories. In this framework, simple circuit motifs can be reused for the assembly of more integrated functions. In the future, it should be possible to develop more complex circuits with different memory states.

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build even more complex dynamic in vitro circuits by iterating this modular approach. This design strategy is also very close to the one observed in cellular reaction networks, where elementary biochemical processes (e.g., regulation of gene expression) are repeatedly used in the assembly of large systems driving complicated functions (5). By building in vitro information-processing systems at the chemical scale, we are therefore also exploring the underlying design rules of the reaction circuits controlling cells.