Programming the Dynamics of Biochemical Reaction Networks

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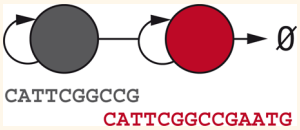
ABSTRACT The development of complex self-organizing molecular systems for future nanotechnology requires not only robust formation of molecular structures by self-assembly but also precise control over their temporal dynamics. As an exquisite example of such control, in this issue of *ACS Nano*, Fujii and Rondelez demonstrate a particularly compact realization of a molecular "predator–prey" ecosystem consisting of only three DNA species and three enzymes. The system displays pronounced oscillatory dynamics, in good agreement with the predictions of a simple theoretical model. Moreover, its considerable modularity also allows for ecological studies of competition and cooperation within molecular networks.



Biological systems are a major source of inspiration for researchers in nanotechnology. For instance, "bottom-up" nanotechnology is based in large part on the concept of molecular self-assembly, where a multitude of relatively weak, noncovalent interactions between molecular building blocks is utilized to generate larger molecular structures by self-association, without external guidance. Self-assembly is, of course, prevalent in biology: it is the basis for the recognition between two complementary strands of DNA to form a DNA double helix, for the folding of proteins, and for the assembly of lipid membranes and vesicles.

As one of the prototype building materials for molecular self-assembly, DNA molecules have been utilized extensively in recent decades for the fabrication of static nanostructures. Most prominently, this has led to the realization of two- and three-dimensional molecular "lattices" made from DNA as well as to the fabrication of discrete molecular objects.[1] In particular, the development of the so-called "DNA origami" technique[2,3] has led to a true surge in activity, and first applications of DNA nanostructures in molecular biophysics and nanoscience are emerging. Apart from the assembly of static nanostructures, DNA (and RNA) molecules have been used extensively for the realization of nanomechanical devices that can be switched between a variety of functionally distinct conformations. Such devices have already been utilized as molecular sensors, artificial molecular motors, and prototypical molecular "robots".[4]

Self-assembly is only one aspect of biology that informs nanotechnology, however. Whereas self-assembled structures simply attain their structure of lowest free energy, many peculiar features of biological systems are in fact caused by dynamical processes, such as motility, sensing and signaling, information processing, or self-replication. These processes involve biochemical reaction cascades and networks that often exhibit emergent dynamical behavior such as multistability, oscillations, or more complex dynamics.

Dynamical functions such as motility, sensing, information processing, and replication would also be interesting capabilities for future nanosystems. Again, synthetic DNA-based systems can be used to explore the design principles of and challenges for such dynamic functions. In recent years, a new and exciting research area at the boundary of DNA nanotechnology and biomolecular computing has emerged—termed "molecular programming"—whose goal is the realization of artificial molecular reaction systems with designed dynamical behavior. There are several approaches toward molecular programming. Some rely exclusively on DNA molecules and so-called strand displacement reactions.[5] Others use DNA and a handful of enzymes. For instance, Kim and Winfree recently demonstrated bistable switches[6] and oscillators[7] based on only a few strands of DNA and two enzymes, RNA polymerase and the nuclease RNase H. In another approach, Rondelez and co-workers realized molecular oscillators based on DNA, a DNA polymerase, and

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Published online January 07, 2013
10.1021/nn305811d

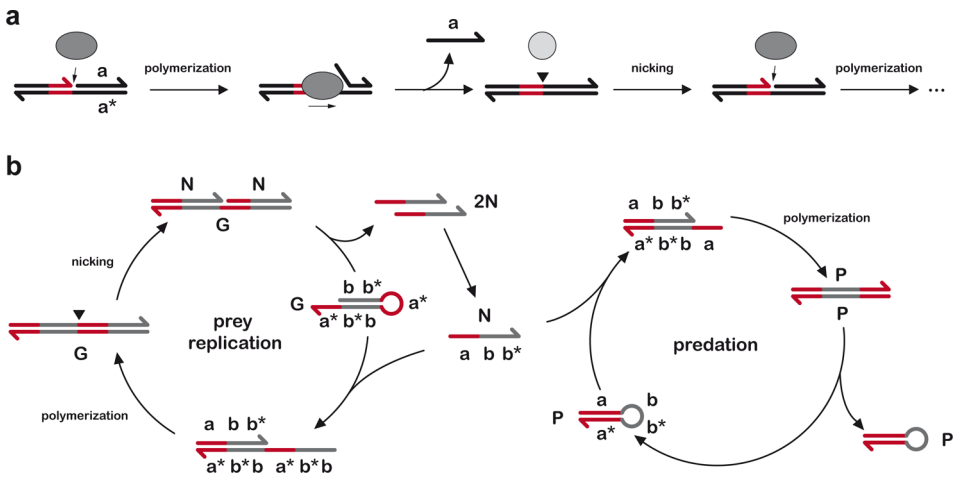

Figure 1. (a) Schematic representation of nicking enzyme amplification. A DNA polymerase with strong strand displacement capabilities synthesizes the complement of sequence a*, starting from a primer strand with an accessible 3′ end (symbolized by an arrowhead). During polymerization, already present a-strands are displaced. The polymerization process results in a new binding/nicking site for a nicking endonuclease, which, upon nicking, generates a new primer. Repetition of these steps leads to an effective amplification of a-strands. (b) Predator–prey system by Fujii and Rondelez. Prey strands N either replicate through the left cycle with the help of auxiliary hairpin G, or they are consumed by interaction with prey molecules P, which produces additional P hairpins. All species involved simply contain different combinations of the sequences a, b, or their complements, as indicated (potential side reactions are omitted in the diagram).

a DNA nicking enzyme.[8] The basic process of their concept is an isothermal nicking enzyme amplification reaction.[9] In this reaction, a DNA polymerase, starting from a short primer region, initially produces a DNA double strand, which contains the recognition sequence of a nicking endonuclease. After nicking, the two resulting DNA fragments may dissociate from the original template, releasing it for another round of polymerization. Alternatively, a DNA polymerase may start DNA polymerization with one of the fragments as primer and actively displace the other fragment during this process (Figure 1a). In any case, this leads to an amplification of the DNA sequence synthesized by the polymerase.

On the basis of this general concept, in this issue of *ACS Nano*, Teruo Fujii and Yannick Rondelez[10] report on an exciting accomplishment in molecular programming. They created an artificial biochemical reaction network modeled after the well-known predator–prey system that was initially discussed by Lotka in 1920 in the context of—then still hypothetical—oscillatory chemical reactions[11] and six years later by Volterra in the context of population dynamics.[12] In the Lotka–Volterra system, the number of predators P increases only in the presence of prey N, while prey replicates "just by itself", in equations

$$\dot{N} = \alpha N - \beta NP$$

$$\dot{P} = \gamma NP - \delta P$$

In chemical terms, prey molecules replicate autocatalytically in the presence of nutrients, while predator "molecules" catalyze their own production from prey molecules, thereby consuming them.

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Fujii and Rondelez implemented these basic functions using three species of DNA molecules and three enzymes (Figure 1b). Prey molecules N replicate themselves by interaction with an auxiliary DNA hairpin molecule G, whose sequence is given by two consecutive copies of the complementary sequence of N. Upon hybridization between N and G, the hairpin opens up and allows DNA polymerase to synthesize the full complement of G. As described above, this process generates the recognition sequence for a nicking enzyme, which cleaves off the newly synthesized strand, resulting in two copies of N.

Predator molecules P were implemented as self-complementary DNA hairpins, with a section fully complementary to the prey strands. Hybridization of predator to prey again generates a primer/template complex for DNA polymerase, whose action results in two copies of predator molecules hybridized to each other. In this case, no cleavage site for the nickase is generated, so the resulting homodimeric complex can only disassemble into two copies of P. In order to observe interesting dynamics in the system, the lengths of the sequences as well as the reaction temperature had to be chosen carefully to ensure sufficiently fast disassembly of reaction products from the template strands and to reduce the effect of competing reactions. An additional crucial ingredient of the

system is a degradation pathway for both predator and prey, which is provided here by the action of the thermostable exonuclease ExoN.

Fujii and Rondelez first checked the dynamics of the prey and predator modules independently, which also allowed them to determine the relevant rate parameters for the mathematical model of the system. Importantly, upon mixing predators with prey strands, oscillations occurred exactly as anticipated. In contrast to other chemical oscillators demonstrated previously, the dynamics are faithfully described by only two differential equations—a slightly modified version of the original Lotka–Volterra equations above, which accounts for the finiteness of resources in the system.

In the spirit of the original ecological meaning of "predator–prey" dynamics, Fujii and Rondelez then extended their system to study two important standard scenarios of population dynamics—competition and mutualism (*i.e.*, cooperation between different species). To study competition, they generated a second predator–prey pair—as both pairs rely on the action of the same enzymes, they effectively compete for common resources. This competition results in quite distinct dynamical behavior—from time to time, the predator–prey dynamics may transiently synchronize or otherwise show chaotic concentration fluctuations. Both of these behaviors are observed experimentally as well as in the model.

Mutualism—or symbiotic interaction—was realized by adding two auxiliary generator hairpin molecules in addition to the generator G for the prey species. The first hairpin served as a template for the production of an additional symbiont species S from N, while the purpose of the second hairpin was to template production of prey species N from S. For large amounts of symbionts, the resulting predator–prey–mutualist system showed a transition from limit cycle behavior to stable coexistence of the three species. Finally, a system composed of two predators and two symbiotic prey species displayed phase-locking and synchronization with increasing coupling as expected for two such coupled oscillator systems.

At first sight, one might be tempted to view Fujii and Rondelez' predator–prey system as "just another chemical oscillator".[13] However, there are a number of highly remarkable features that differentiate this system from previous work. Most previously investigated chemical oscillators have relatively complex kinetics. In order to describe their kinetics mathematically, often many species and, correspondingly, many rate equations and rate parameters are required. Alternatively (and this is usually done), a simplified model description is chosen in which only the essential features of the dynamics are captured. What is, in a sense, different in Fujii and Rondelez' oscillator is that the chemical system was "modeled" after a basic predator–prey population dynamical system, and not *vice versa*. The system is described simply by two rate equations and a small number of parameters, and the agreement between model and experiment is quite astonishing. In fact, the system developed by Fujii and Rondelez is the first working biochemical implementation of predator–prey dynamics (there has previously been a proposal for a different biochemical implementation[14]). What is also remarkable is that pronounced and almost undamped oscillations can persist in the system for more than 24 h—which makes it one of the longest running oscillators demonstrated in a closed reactor so far. Last but not least, another exciting feature of this predator–prey system is its considerable modularity, which makes it possible to perform "ecological studies" with populations of oscillators and build "ecosystems" of molecular competitors and cooperators.

It is also interesting to discuss the significance of Fujii and Reondelez' work in the context of information processing and molecular programming. One of the goals of molecular programming is the efficient generation of complex molecular structures and behaviors, analogous to the efficient solution of computational problems using an algorithm or "code" in conventional computer science. In fact, given the "hardware"—DNA polymerase/nickase/exonuclease—the behavior of a predator–prey pair is entirely determined by the 20 bases of the generator hairpin G (whose sequence also determines the sequences for the prey and predator strands).

Another interesting "computational" aspect of this system is the fact that it was deliberately designed to behave like a predator–prey system. In this sense, one could also regard its dynamics as a molecular "simulation" of a different system. Simulation is one of the most basic notions of computer science—it also lies at the heart of concepts such as computational (Turing) universality. A universal computing machine can be fed the instructions for the behavior of another machine and therefore, in principle, perform any task the other can perform. It has previously been argued that computation by chemical kinetics—as a special type of computation by dynamical systems—is Turing universal.[15] One interesting recent result in this context was the demonstration that networks of DNA strand displacement reactions can be used, in principle, to simulate the kinetics of arbitrary other chemical reaction networks.[16]

OUTLOOK AND FUTURE CHALLENGES

One of the major practical obstacles for harnessing the power of chemical reaction networks for computation is the often complex interaction between all species that may occur within such networks—a problem molecular programming shares with the related discipline synthetic biology. Adding components to an existing network inevitably introduces many new interactions and may

change the behavior of the system in a complicated and effectively unpredictable manner. For scaling up molecular computational systems (*i.e.*, to be able to combine existing subsystems with larger systems in a robust and functional manner), modularity will be an important requirement. Here, sequence-based reaction networks such as that demonstrated by Fujii and Rondelez may be at an advantage over other chemical systems.

The system of Fujii and Rondelez is particularly compact in terms of "size" (*i.e.*, sequence length), but short sequences are necessary in order to ensure sufficient dissociation of predator dimers into monomers at experimentally acceptable temperatures (this is also one of the major problems in other schemes for molecular replication). Accordingly, the system runs only at 46.5 °C, which, in turn, requires the usage of thermostable enzymes. Sequence length restrictions and temperature requirements may ultimately limit the scalability and applicability of this particular system, but this is not a fundamental limitation of DNA-based reaction networks in general.

Keeping with the programming analogy, the "program" for the predator–prey system is written in machine code—you really have to know what your hardware is doing with it. One of the major challenges for molecular programming will be the development of sufficiently modular structures that enable more abstract symbolic representation of molecular processes, including molecular compilers that automatically translate high-level code into physical implementations of the networks. In fact, first attempts to generate such higher level descriptions have already been made in the context of strand displacement reaction networks and DNA circuits,[17–19] and it will be interesting to see whether these can be extended to enzyme-based networks in the future.

In the long run, the development of programmable reaction networks will generate the ability to design dynamical systems behavior in a systematic and robust way and to control the organization of molecular systems not only in space but also in time. This will have important consequences in a wide variety of contexts.

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On a fundamental level, artificial chemical reaction networks may be used to create reduced versions of naturally occurring systems or functional modules and to study their dynamical behavior experimentally in a systematic manner. This approach may be used to address important questions related to the general design principles of such systems including their modularity, parameter sensitivity, or simply their kinetics. Questions similar to those addressed by Fujii and Rondelez regarding the dynamics of molecular ecosystems have been discussed theoretically in the past in the context of molecular evolution and replicator dynamics.[20] It should therefore also be extremely interesting to introduce mutating replicator species into the predator–prey system and to study their resulting evolutionary dynamics.

A more practical aspect of artificial reaction networks is their application for the temporal control of molecular devices or assembly reactions. Oscillatory reactions can be simply used to "clock" the motion of nanomechanical devices[21] or to organize the growth of nanoassemblies into patterns or multilayers autonomously. Using more intricate "assembly logic", building blocks for nanoassemblies could be activated according to specific assembly rules, and growth of nanostructures could be made context-dependent with the help of "developmental programs" for nanoassembly.

Finally, the computational aspect of reaction networks may be used, well, for computation. Molecular computers based on dynamical chemical systems could evaluate or classify molecular concentration patterns, or, equipped with molecular memory, record their "chemical history". Among many other potential applications, this could be important for the development of powerful autonomous biosensors or theranostic nanodevices.

Conflict of Interest: The author declares no competing financial interest.

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