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Switching the Conformation of a DNA Molecule with a Chemical Oscillator

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ABSTRACT

pH oscillations generated by a nonequilibrium chemical reaction are used to switch a pH-sensitive DNA structure between two distinct conformations. The utilization of a chemical oscillator represents a novel method for achieving autonomous motion in molecular devices. The oscillatory reaction is a variant of the Landolt reaction and produces pH variations in the range between pH 5 and 7. In this range, a cytosine-rich DNA strand can be switched between a random coil conformation and the folded i-motif structure. The conformational changes are monitored simultaneously with the pH value in fluorescence-resonance energy-transfer experiments.

DNA-based nanomachines are DNA supramolecular structures, which can cyclically undergo large conformational changes in the presence of certain trigger molecules such as other DNA strands or small molecules. These conformational changes are accompanied by rotatory,[1,2] stretching,[3−12] or, as demonstrated quite recently, even translatory[13−18] movements. In most cases, these molecular machines have to be controlled externally, that is, an external operator has to add certain chemical compounds to the reaction mixtures to cycle the devices through their various mechanical states. Autonomous behavior of nanodevices has been achieved in only a few cases with the help of deoxyribozymes[9,17,19] or enzymes.[14,18] Here we present a novel approach to achieving autonomous motion by demonstrating how a proton-fueled DNA conformational change can be driven by pH variations generated by a nonequilibrium oscillatory chemical reaction.

Conformational changes in artificially constructed DNA nanomachines are usually enforced by the addition of a DNA "fuel" strand, leading to the stiffening of a previously flexible single-stranded part of the device or connecting distant sections of it. Other DNA devices are driven by a change of buffer conditions, which favor one of several possible conformational states. To achieve cyclical operation, fuel strands had to be removed by "anti-fuel" strands using DNA branch migration or the original buffer conditions had to be restored. Continuous operation of these devices requires keeping track of the state of the devices and the external addition of fuel at the right moment. Under closed conditions, waste products accumulate and soon become the dominant chemical species in the system. In a conceptually different approach, here we demonstrate how the conformational transition of a single-stranded DNA molecule can be driven autonomously by an oscillating chemical reaction occurring in a continuously fed reactor.

Inspired by spatiotemporal ordering processes in biological systems, oscillatory chemical reactions have been under investigation for a long time.[20] The canonical example is the Belousov−Zhabotinsky (BZ) reaction,[21,22] but there are numerous other examples of reactions in which chemical concentrations oscillate in time[23,24] or travel in space as chemical waves.[25] Several criteria have to be met in order to drive DNA-based devices with these chemical oscillators: First, the oscillating chemical species should be able to drive or influence a DNA conformational transition. Among the possibilities are to use the influence of certain ion species on DNA whose concentration may be varied by coupling pH oscillations to complexation and precipitation equilibria[24] or to use the oscillations in pH itself, as is done in the present work. Second, the pH range in which the oscillator operates should be compatible with biochemistry; this rules out, for example, the original BZ reaction. Furthermore, the reaction solution should be sufficiently "biocompatible" to avoid rapid degradation of DNA by oxidation. This requirement rules out, for example, oscillators using hydrogen peroxide in the presence of copper ions.[26] On the basis of these considerations, here we adopt an oscillatory variant of the Landolt reaction to change the pH value periodically in a continuously fed chemical reactor.[26−28] The oscillating proton concentration is then utilized to drive proton-fuelled DNA nanodevices through their conformational states periodically.

Our artificial reaction network is depicted schematically in Figure 1a. The alternating oxidation of sulfite and thiosulfate by iodate is accompanied by a periodic production

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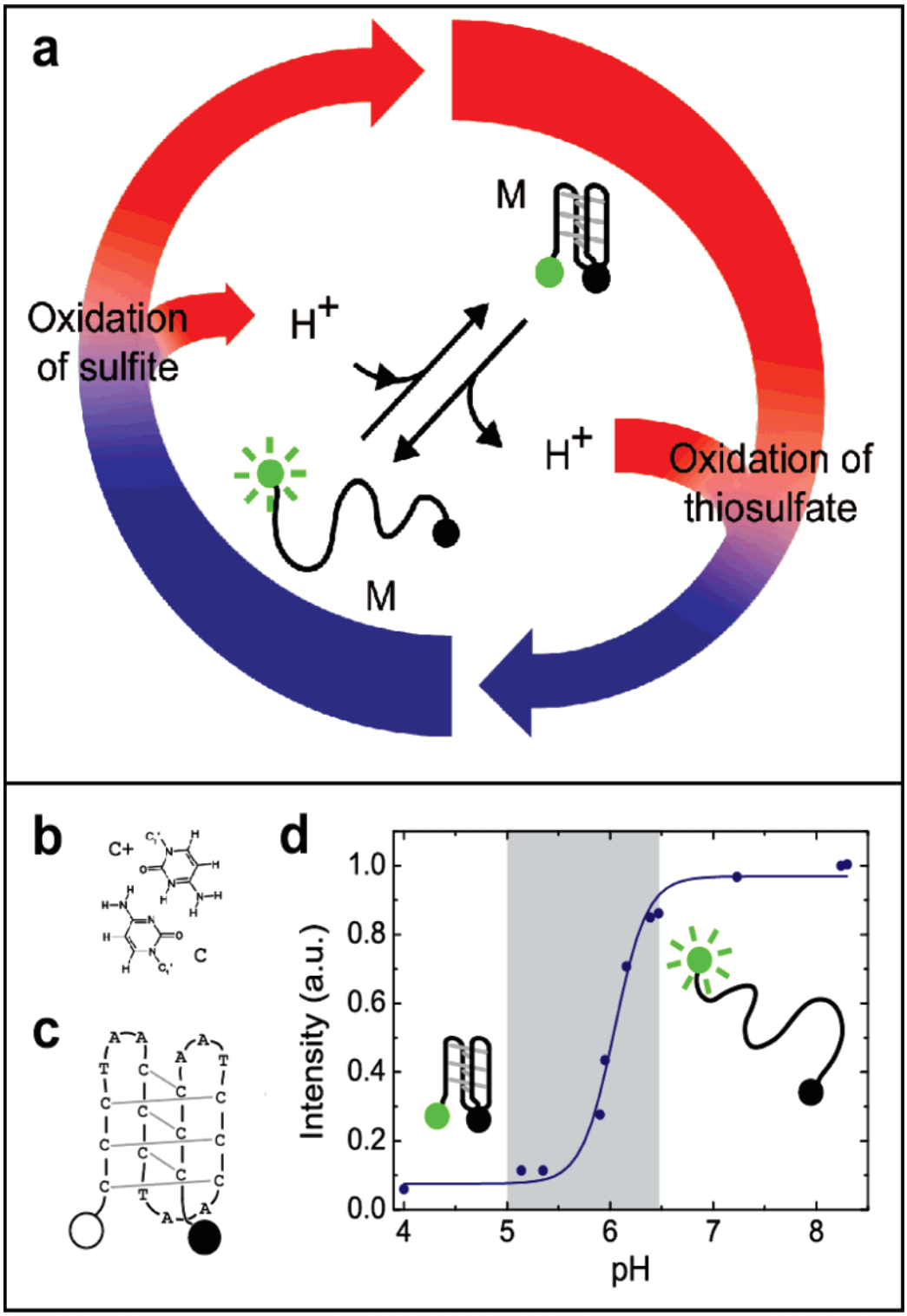

Figure 1. Principle of operation. (a) Operation cycle for DNA switch M driven by a chemical oscillator. An oscillatory variant of the Landolt reaction changes the pH value of the reaction solution periodically. In one-half of the reaction cycle, protons produced during the oxidation of sulfite induce a conformational transition to a folded DNA structure, the so-called i-motif (see part c of this Figure). In the other half of the reaction cycle, the oxidation of thiosulfate consumes protons and leads to an unfolding of the i-motif. (b) Base-pairing scheme between protonated and nonprotonated cytosin. (c) Sequence and folding scheme of DNA strand M. Strand M is labeled with Alexa 488 on the 5′ end and BHQ on the 3′ end. At low pH values, M folds into the i-motif upon formation of six C+−C pairs (gray lines). In this conformation, the dye and the quencher are in close proximity and the fluorescence intensity is considerably lower than that in the open state. (d) Fluorescence titration of strand M against the pH value. At low pH, the conformational switch M assumes the i-motif conformation. Between pH 5.5 and 6.5, strand M unfolds into a random coil conformation. Because strand M is labeled with a fluorescent dye and a quencher, the transition can be monitored in the fluorescence signal (dots). In the folded state, fluorescence is quenched, whereas in the random coil conformation it is not. A sigmoidal fit to the titration values is drawn as a continuous line.

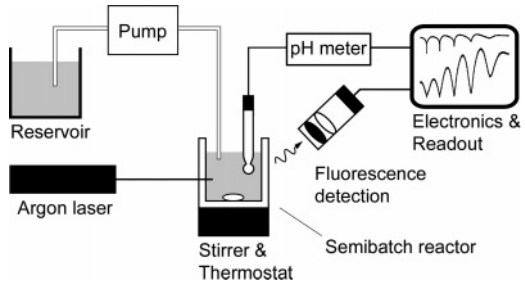

Figure 2. Experimental setup. A solution of sodium sulfite, sodium thiosulfate, and sulfuric acid is pumped from a reservoir into a continuously stirred reactor containing a solution of sodium iodate and the fluorescent-labeled DNA switches M. Fluorescence is excited with an argon ion laser, detected, and recorded simultaneously with the pH value.

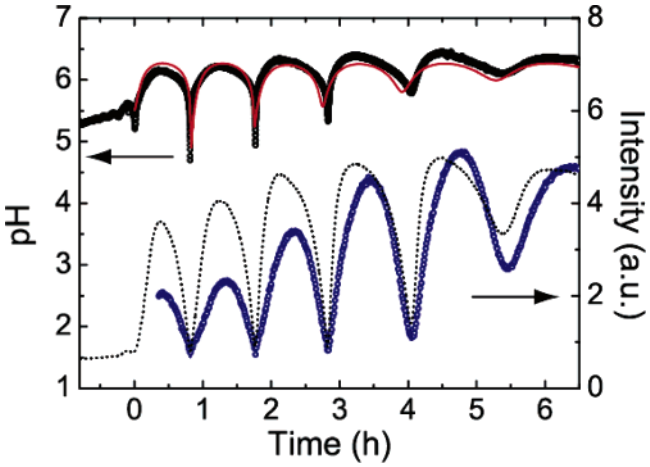

Figure 3. Measurements. Upper curves: measured (circles) and simulated (red solid line) time course of the pH value of the chemical oscillator in the semibatch configuration. See the Supporting Information for a detailed description of the kinetic model used for the simulation. Lower curves: fluorescence trace (blue circles) recorded simultaneously with the pH oscillations monitoring the motion of the DNA switches. Also shown is the fluorescence intensity expected from the pH values using the titration data from Figure 1 (dotted line). The measured fluorescence values roughly agree with the calculated values, in particular for the later oscillations that have a larger temporal spacing and reach slightly higher pH values. This indicates that the unfolding kinetics may not be fast enough at lower pH values in order for the DNA switches to follow the oscillator.

or consumption of protons. This pH oscillation is coupled to a DNA conformational change. As the proton-sensitive DNA structure, a molecular device based on the i-motif is chosen.[7,29] The i-motif is a four-stranded DNA structure that forms because of intramolecular noncanonical base-pairing between protonated and unprotonated cytosine residues (Figure 1b). In the particular case of the device used here, a DNA strand containing 12 cytosines can form 6 intramolecular C+−C base-pairs, resulting in the folded DNA structure depicted in Figure 1c. The p*K* value for cytosine is approximately 4.2, and the transition to the i-motif conformation, in which only half of the cytosines are protonated, takes place in the pH range between 6 and 7. At higher pH values, the cytosines are deprotonated and the DNA strand adopts a random coil conformation. To monitor its conformational transition, we labeled the DNA molecule with a pH-insensitive fluorophore at the 5′ end and a quencher at the 3′ end. If the fluorophore and quencher are in close proximity, then the fluorescence of the fluorophore is suppressed by the quencher because of energy transfer. If the two chromophores are far apart, then the fluorescence intensity is high. The transition between the folded single-stranded i-motif structure and the relaxed coil structure is therefore accompanied by a strong increase in fluorescence (Figure 1d).

The experimental setup is displayed in Figure 2. For the reaction, a solution of Na_2SO_3, $Na_2S_2O_3$, and H_2SO_4 is fed slowly with a pump into a continuously stirred reaction vessel initially containing only a solution of $NaIO_3$. Qualitatively, the chemical processes taking place are the following: At low H^+ concentrations, iodate predominantly oxidizes hydrogen sulfite to sulfate, a reaction that produces iodide plus protons and therefore lowers the pH. At high H^+ concentrations, however, the oxidation of iodide to iodine and of thiosulfate to tetrathionate becomes dominant, consuming protons and thus raising the pH value again.[30,31] Because we wish to keep the same population of DNA molecules in the reactor permanently, we currently use a "semibatch" reactor that has an inlet but no outlet.[26] As shown in Figure 3, in this setup the oscillations slowly die out because of the continuous decrease in reactant concentrations. This behavior can be well reproduced in model calculations based on a set of rate equations[30,31] describing the dominant kinetic processes of the reaction that we adapted for the semibatch case. If the reaction is performed in an open reactor with an inlet and an outlet, then pH oscillations with a very regular amplitude can be produced (see the Supporting Information).

With our choice of experimental conditions, the oscillator varies the pH value between 5 and 7 periodically with a temporal period of about 1 h. In consequence, the conformational transitions of the DNA strands are enforced periodically by the oscillator. In Figure 3, the pH oscillations in the reactor are displayed together with the simultaneously measured fluorescence-intensity signal. The fluorescence signal indeed follows the pH value, indicating that the DNA strands present in solution undergo their conformational change as designed. Also indicated in Figure 3 is the fluorescence signal calculated from the titration curve in Figure 1d on the basis of the pH oscillations. Obviously, for the first few oscillations (corresponding to lower pH values) the amplitude of the fluorescence oscillations is lower than expected. We assume that in this regime the kinetics of the conformational change between the i-motif and the relaxed coil is actually slower than the time-scale of the oscillations. Control experiments with the fluorophore alone and with DNA strands not prone to fold into the i-motif confirmed that the observed oscillations in the fluorescence signal are in fact caused by the structural transition of the DNA switch (see the Supporting Information for control experiments).

The i-motif has been used previously for the construction of a DNA-based molecular machine that could stretch and contract cyclically, driven in response to changes in the pH value.[7] In this case, the stretching motion was caused by hybridization of the i-motif strand to its complement strand.

We therefore also added the complement of M to our oscillator solution. The fluorescence signals recorded from the two DNA strands were indistinguishable from the signals recorded from strand M alone, indicating that hybridization does not take place under the present reaction conditions (Supporting Information Figure 2). To make full use of chemical oscillators for the autonomous operation of DNA nanomachines, the main challenge will be the optimization of the reaction conditions to allow for efficient DNA hybridization. Optimization of the reaction conditions will also be necessary to achieve faster oscillations, which may be more relevant technologically. Temporal periods of 2−5 min have been demonstrated for pH oscillations very similar to the ones used in this work.[31] Among the parameters influencing the period are temperature and flow speed. Another challenge will be the immobilization of DNA nanodevices to solid supports. Immobilized devices could, in principle, be driven indefinitely by the regular pH oscillations that occur in a flow-through reactor (Supporting Information Figure 1). A general limitation of this approach, as generally is the case for DNA devices that are driven by changes in buffer composition, is that no use is made of sequence specificity and DNA devices cannot be addressed individually within the reaction solution.

Here we have shown how a proton-sensitive DNA structure can be driven autonomously through its conformational states by a chemical oscillator. This constitutes the first example of a DNA device maintained in nonequilibrium within a permanently fed chemical reactor and opens up a novel field of applications for oscillating and pattern-generating chemical reactions. It should be possible to generalize the approach presented here to operate DNA nanodevices within spatiotemporal chemical patterns or oscillations that could be helpful for applications in which molecular machines assist in nanoassembly tasks or in which transport of nanocomponents along supramolecular tracks is desired. Our approach should be applicable to other pH-dependent DNA machines such as devices based on the duplex−triplex transition;[10,12] however, it need not be restricted to DNA-based systems. It should also be possible to drive other pH-dependent molecular machines by chemical oscillators, for example, the recently demonstrated rotaxane-based supramolecular "elevator".[32]

Experimental Section. Unless stated otherwise, all chemicals were purchased from Sigma-Aldrich, Germany. DNA strand M with the sequence 5′-CCCTAACCCTAAC-CCTAACCC-3′ was synthesized by biomers.net, Ulm, Germany and labeled at the 5′ end with Alexa Fluor 488 (Molecular Probes, Eugene, OR) and at the 3′-end with BHQ-1 (Biosearch Technologies, Novato, CA).

The semibatch reactor consisted of a reservoir, a reactor, and a peristaltic pump (Minipuls 2, Gilson, Bad Camberg, Germany). The reservoir contained a solution of 30 mL of 26 mM Na_2SO_3, 15 mM $Na_2S_2SO_3$, and 5 mM sulfuric H_2SO_4. This solution was pumped at 30 μl/min into the reactor containing 30 mL of a 50 mM solution of $NaIO_3$. The reaction solution was stirred constantly at 300 rpm. After approximately 4 h, the pH value started to oscillate and DNA strand M was added to the reactor at an initial concentration of 10 nM. The titration of strand M was performed in a solution of 100 mM NaCl, and the pH was adjusted by the addition of NaOH and HCl.

For oscillator measurements, fluorescence was excited with an argon ion laser (λ = 488 nm) and detected with a Si photodiode at an angle of 90°. The signal was amplified, detected by a lock-in amplifier, and recorded with a data acquisition card. The pH value was measured and recorded simultaneously with a pH-Electrode (Schott, Mainz, Germany). Fluorescence titrations were performed with a spectrofluorometer (Fluorolog 3, Jobin Yvon GmbH, Munich, Germany).

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Supporting Information Available: Simulation of the kinetics of the oscillator, reaction with the complementary strand of M, and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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