DNA Nanoswitch

DOI: 10.1002/anie.200600353

A Surface-Bound DNA Switch Driven by a Chemical Oscillator**

*Tim Liedl, Michael Olapinski, and Friedrich C. Simmel**

The fabrication of autonomously moving molecular structures is one of the central challenges in the field of DNA nanodevices.[1] Some of the concepts introduced recently to achieve this goal rely on the sequence-dependent catalytic action of DNA-modifying enzymes such as restriction endonucleases or nicking enzymes[2] while others use the catalytic power of DNA itself by incorporating DNA enzyme sequences into DNA devices.[3] Both approaches have also been used to realize autonomous molecular computers.[4] Another strategy is based on controlled inhibition of DNA hybridization by formation of secondary structure and its acceleration by catalytic DNA strands.[5] These concepts were developed for the autonomous operation of DNA devices fueled by DNA hybridization. A different approach was recently taken by our research group[6] and we could show that the pH-sensitive conformational transition of a cytosine-rich DNA strand between a random conformation and the so-called "i motif" could be driven by the oscillating proton concentration generated by a chemical oscillator. In such a system, the temporal succession of the states of the DNA devices is determined by a nonlinear dynamical system rather than by an external operator. We report here how this system can be significantly improved by attaching the DNA conformational switches to a solid substrate. This attachment allows us to operate the chemical oscillator in a continuous flow stirred tank reactor (CSTR) into which a glass chip supporting the DNA devices is placed. In principle, the surface-bound DNA structures can undergo an infinite number of autonomous conformational switching events in this configuration.

We showed recently how proton-fueled DNA devices can be driven by an oscillating chemical reaction[6] by using a variant of the Landolt reaction to periodically change the pH value in a continuously fed reactor. To retain the DNA switches within the reaction solution, a reactor without an outlet had to be used. In such a configuration, one cannot reach a steady state since the continuous influx of reaction solution means the average concentrations of the reactants vary. As a result, this dynamic chemical system is driven out of its oscillatory region, thus causing the oscillations to die away after a few periods.

To overcome this limitation in the present work we operated the oscillator in a CSTR with two inlets and one outlet. In principle, an infinite number of homogeneous pH oscillations can be generated by using a continuous filling combined with the simultaneous removal of waste materials. However, the DNA devices had to be attached to a solid substrate to prevent loss of the DNA through the reactor's outlet. For these experiments, we used thiol-modified, fluorescently labeled DNA switches bound to an ultrathin transparent gold layer on a glass substrate. This allowed a firm covalent attachment of the DNA to the surface while at the same time energy transfer between the fluorophores and the gold layer[7] could be used to characterize the conformational transitions of the switches.

Schematic representations of the surface-bound DNA switches in their two states at low and high pH values are shown in Figure 1 a. The switches consist of 21 nucleotide (nt)

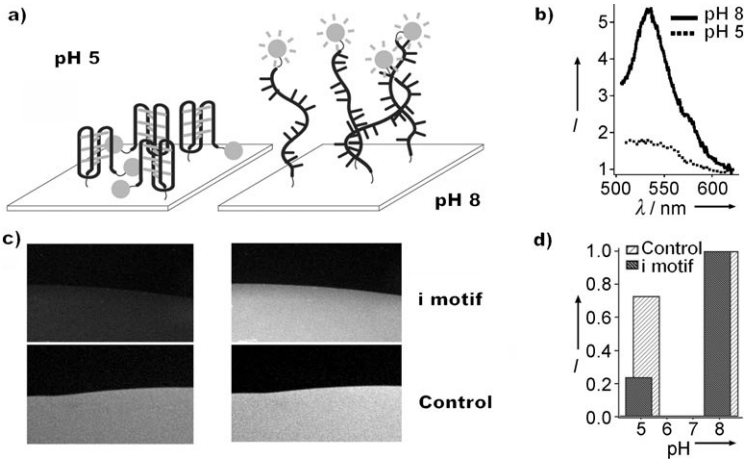

Figure 1. a) Schematic representation of single-stranded DNA bound to the gold/glass substrate through a 5′-thiol-C_6 spacer. In the closed i motif conformation at low pH values the dye attached to the 3′ end is in proximity to the surface and is thus quenched. In contrast, the fluorescence strongly increases at higher pH values where the DNA strand adopts a random single-stranded conformation. b) Emission spectra recorded from the i motif attached to the substrate at pH 8 (random) and pH 5 (i motif). c) Fluorescence microscopy images of the i motif (top) and a control strand (bottom) at pH 5 (left) and pH 8 (right). d) Corresponding normalized fluorescence intensities.

long DNA strands with the sequence 5′-CCCTAACCC-TAACCCTAACCC-3′ (strand M). Below pH 6.5, DNA molecules of this sequence are known to undergo a conformational transition to the so-called "i motif", in which four DNA strands are held together by a number of semiprotonated $C{\cdot}C^+$ base pairs (in this case six intramolecular $C{\cdot}C^+$ pairs). This particular DNA sequence has been utilized previously for the fabrication of other DNA-based nanodevices[8] and is also the same sequence as used in our previous bulk experiments.[6] The DNA strands were modified with a thiol-C_6

[*] T. Liedl, M. Olapinski, Dr. F. C. Simmel
Department of Physics and Center for Nanoscience
LMU Munich
Geschwister Scholl Platz 1, 80539 Munich (Germany)
Fax: (+49) 89-2180-3182
E-mail: simmel@lmu.de

[**] This work was supported by the Deutsche Forschungsgemeinschaft (Emmy Noether grant DFG SI 761/2-2). T.L. and M.O. are supported by the LMU International Graduate School "Nanobiotechnology".

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

spacer at the 5′ end and with the fluorescent dye Rhodamine Green (RG) at the 3′ end. The fluorescence of RG is pH-insensitive between pH 4 and 9 (see the Supporting Information). At low pH values, the transition to the i motif brings the 3′ end of the molecule into proximity to the 5′ end (ca. 1.5 nm[9]). The fluorophore is brought closer to the substrate when the DNA switches are attached to a surface through the 5′ end.

A glass coverslip with an ultrathin layer of gold was used as the substrate. To prepare the substrate, first a thin gold layer was evaporated onto a clean coverslip, and then nearly completely removed by sputtering with argon ions (experimental details are given in the Supporting Information). The substrate is then nearly transparent, but it is still possible to attach DNA strands to the remaining gold on the surface (see the Supporting Information). The modified coverslips were mounted on an epifluorescence microscope and the fluorescence was monitored while subsequently adding phosphate buffer of pH 8 and pH 5. Switching the DNA strands between a random conformation at high pH values and the i motif at low pH values resulted in strong changes in the fluorescence intensity (Figure 1 c,d). Such clear changes in the intensity could be monitored only at sites on the chip spotted with the i motif strand. Sites spotted with a control strand with a random sequence only showed a small change in intensity.

A fluorescence spectrometer was extended with a custom built module which allowed characterization of the sample when placed in a CSTR to allow for fluorescence spectroscopic and energy-transfer measurements during the operation of the pH oscillator. The setup is shown schematically in Figure 2. Fluorescence spectra recorded with this setup for the substrate-attached i-motif switches at low and high pH values are displayed in Figure 1 b.

The pH oscillator was operated by pumping two separate solutions at 150 μL min^{-1} into the cuvette, which was initially filled with 20 mL H_2O. One solution contained 19 mM $NaIO_3$ while the other contained 30 mM Na_2SO_3, 21 mM $Na_2S_2O_3$, and 5 mM H_2SO_4. A second pump removed excess volume at 300 μL min^{-1}. Typically, after a period of 4 h prior to oscillation, the pH value started to oscillate between pH 6.3 and pH 5.5 with a period of 20 minutes. The oscillations persisted until the reactant reservoirs were exhausted (typically after 24 h).

Figure 3 shows the fluorescence intensity recorded from the surface-immobilized DNA switches together with the oscillations in the pH value. The fluorescence strongly oscillates in concordance with the pH value, thus indicating that the chemical oscillator enforces the conformational transition of the switches as designed. We performed a variety of test experiments to verify that these fluorescence oscillations are indeed caused by the conformational transitions of the switches to the i motif and back. A conventional fluorescence titration experiment shows that the immobilized DNA switches undergo a conformational change when the pH value is lowered below about 6.5, as expected (see the Supporting Information). A plot of the

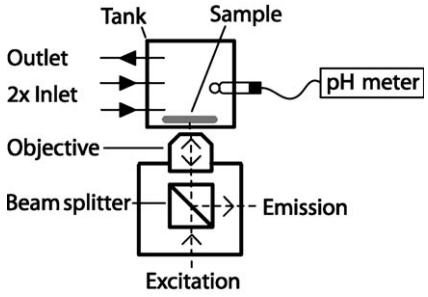

Figure 2. Experimental setup: Excitation light coming from a fluorescence spectrometer is focused onto the sample chip with a long working distance objective. Light emitted from the chip is collected by the same objective and reflected into the spectrometer with a beam splitter. The sample chip resides in a CSTR which consists of a large volume (V = 25 mL) fluorescence cuvette with two inlets and one outlet for the reactants. The pH value is monitored with a conventional pH meter.

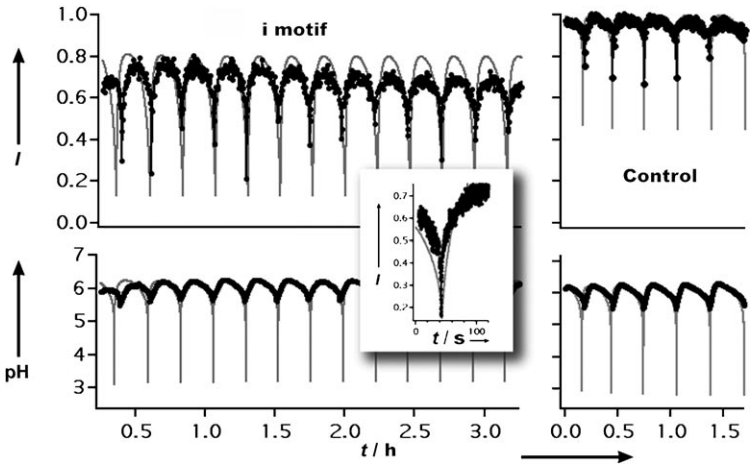

Figure 3. Left: In the CSTR setup, a large number of pH oscillations can be generated (bottom, black trace). The simultaneously recorded fluorescence intensity originating from the i motif bound to a gold/glass chip follows the pH oscillations (top, black trace). The simulated pH values (bottom, gray trace) coincide with the measured values except for regions where the low pH spikes occur. This deviation is caused by the slow response of the pH meter. The simulated fluorescence trace in the top graph (gray) is generated from data from a titration experiment (see the Supporting Information). Inset: In an experiment at higher time resolution it became apparent that the fluorescence intensity at the position of the low pH spikes indeed drops to 20% of its maximum value, as expected from the simulation. Right: Measured (bottom, black trace) and predicted (bottom, gray trace) pH oscillations and corresponding fluorescence intensity (top, black trace: experimental data, gray curve: calculated values) of the control strands. The fluorescence is normalized to the maximum value at pH 7.4 for both the device and control strand. In the case of the device strand, the i motif is already partly formed under the conditions of the oscillator, which results in a decreased fluorescence signal. The fluorescence values of both strands are in complete agreement with the titration experiments (see the Supporting Information), which shows that the strands essentially behave in the same way in the CSTR as under ordinary buffer conditions. The fluorescence of the device strand is consistent with its transition to the i motif, while the control strand does not show such a behavior.

fluorescence intensity obtained in the CSTR during the operation of the pH oscillator essentially shows the same transition (see the Supporting Information). By contrast, the immobilized control strands show a less pronounced response to pH changes, in particular no transition around pH 6 is observed. Accordingly, a different fluorescence trace is recorded when they are put under the influence of the oscillator (Figure 3). The fluorescence for the control strand only drops to about 50% of its maximum value, whereas the signal decreases to below 20% for the device strand. Both fluorescence traces are fully consistent with those obtained by conventional titration experiments, thus indicating that the immobilized DNA molecules undergo the same conformational changes when driven by the oscillator. It has to be noted, however, that when the pH is below 4 the fluorescence may not reflect conformational changes of the DNA alone, but can be influenced by a variety of other factors (see the Supporting information).

Simulated time traces of the pH oscillations based on a model developed by Rabai and Beck[10] are also shown in Figure 3. Experimentally obtained data agree well with the model's prediction at pH values above 5.5. However, the model also predicts sharp "spikes" down to pH values of about 3. In fact, these spikes also occur experimentally, but their short duration ($t_{1/2} \approx 2$ s) means they cannot be resolved by our pH meter, which has a response time of 30 s. We independently checked the occurence of the spikes at low pH values with the pH-sensitive dye methyl orange, which changes its color from yellow to pink at pH 4.4.[10] Indeed, during the operation of the oscillator we observed this color change for fractions of a second at the position of the low pH spikes. This also explains why we observe fluorescence values at the minima of the pH oscillations which are lower than those expected from the recorded pH values. For comparison, the upper panel of Figure 3 contains time traces calculated from the simulated pH values and the titration curves for DNA strand M and the control strand. The experimental intensities agree well with the simulated values, except that the experimental traces again do not follow the low intensity spikes. This is simply caused by "undersampling". Only a limited number of data points could be taken for observations over long times, because of excessive photobleaching, and therefore the spacing of the data points is not close enough to resolve the pH spikes. In the inset of Figure 3, a fluorescence trace recorded at a higher rate is shown which reproduces very well the predicted values. This also indicates that the response time of the immobilized switches is on the order of a second.

Further evidence for the formation of the i motif in the surface-bound DNA switches is obtained from temperature-dependent measurements: a sharp melting transition is observed for DNA switches immobilized on the chip surface and in solution, whereas no such transition is seen for the control strand (Figure 4). These observations are in agreement with previous circular dichroism studies on the i motif.[11]

In summary, a chemical oscillation generated in a continuous flow stirred tank reactor was utilized to periodically switch a DNA molecular structure immobilized on a glass chip between two distinct conformations. In a CSTR, these oscillations occur with a regular period and can in principle occur infinitely often. To realize and characterize this molecular-switching system experimentally, the DNA oligonucleotides were immobilized on a glass surface covered with an ultrathin gold layer, which allowed characterization of the surface-bound switches within a CSTR in an epifluorescence setup. This system represents the first example of an autonomously driven DNA switch immobilized on a solid substrate. It was shown recently in a related study by Shu et al.[8b] that an immobilized DNA device based on the i motif can cyclically generate forces during the consumption of H^+ ions and can thus even be construed as a periodically working motor. Such and similar systems may find application as actuators or sensors in biomolecular hybrid nanostructures. It is expected that surface-immobilized DNA switches could

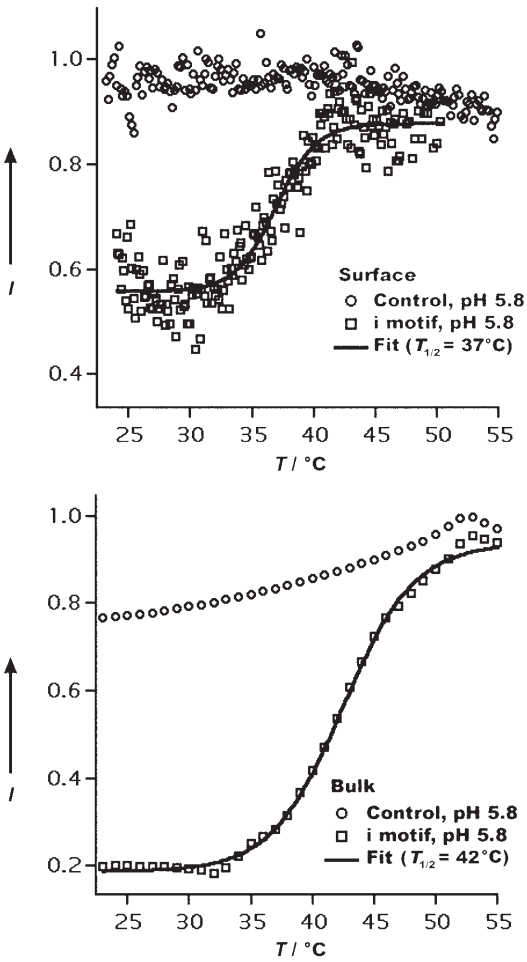

Figure 4. Fluorescence intensities of the i motif and the control strand in a melting experiment at pH 5.8 and comparison between surface-bound and solution-phase switches. Top: The surface-bound i motif unfolds at 37°C, while a surface-bound control strand does not show any change in fluorescence. Bottom: For the experiment in solution, the i motif strand labeled with a dye on one end and a quencher on the other end[10] displays essentially the same behavior and unfolds at a slightly higher temperature than on the surface. The control strand exhibits only a small change in the fluorescence in the solution experiment.

also display spatiotemporal patterns and oscillations under the influence of chemical reaction waves.

Received: January 26, 2006
Revised: April 4, 2006
Published online: June 29, 2006

Keywords: chemical oscillations · DNA · molecular switches · nanotechnology

[1] a) N. C. Seeman, *Trends Biochem. Sci.* **2005**, *30*, 119 – 125; b) F. C. Simmel, W. U. Dittmer, *Small* **2005**, *1*, 284 – 299.
[2] a) P. Yin, H. Yan, X. G. Daniell, A. J. Turberfield, J. H. Reif, *Angew. Chem.* **2004**, *116*, 5014 – 5019; *Angew. Chem. Int. Ed.* **2004**, *43*, 4906 – 4911; ; b) J. Bath, S. J. Green, A. J. Turberfield, *Angew. Chem.* **2005**, *117*, 4432 – 4435; *Angew. Chem. Int. Ed.* **2005**, *44*, 4358 – 4361.
[3] a) Y. Chen, M. S. Wang, C. D. Mao, *Angew. Chem.* **2004**, *116*, 3638 – 3641; *Angew. Chem. Int. Ed.* **2004**, *43*, 3554 – 3557; b) Y. Tian, Y. He, Y. Chen, P. Yin, C. D. Mao, *Angew. Chem.* **2005**, *117*, 4429 – 4432; *Angew. Chem. Int. Ed.* **2005**, *44*, 4355 – 4358.
[4] a) Y. Benenson, T. Paz-Elizur, R. Adar, E. Keinan, Z. Livneh, E. Shapiro, *Nature* **2001**, *414*, 430 – 434; b) M. N. Stojanovic, D. Stefanovic, *Nat. Biotechnol.* **2003**, *21*, 1069 – 1074.
[5] A. J. Turberfield, J. C. Mitchell, B. Yurke, A. P. Mills, M. I. Blakey, F. C. Simmel, *Phys. Rev. Lett.* **2003**, *90*, 118102.
[6] T. Liedl, F. C. Simmel, *Nano Lett.* **2005**, *5*, 1894 – 1898.
[7] a) B. Dubertret, M. Calame, A. J. Libchaber, *Nat. Biotechnol.* **2001**, *19*, 365 – 370; b) H. Du, M. D. Disney, B. L. Miller, T. D. Krauss, *J. Am. Chem. Soc.* **2003**, *125*, 4012 – 4013; c) C. S. Yun, A. Javier, T. Jennings, M. Fisher, S. Hira, S. Peterson, B. Hopkins, N. O. Reich, G. F. Strouse, *J. Am. Chem. Soc.* **2005**, *127*, 3115 – 3119.
[8] a) D. S. Liu, S. Balasubramanian, *Angew. Chem.* **2003**, *115*, 5912 – 5914; *Angew. Chem. Int. Ed.* **2003**, *42*, 5734 – 5736; b) W. M. Shu, D. S. Liu, M. Watari, C. K. Riener, T. Strunz, M. E. Welland, S. Balasubramanian, R. A. McKendry, *J. Am. Chem. Soc.* **2005**, *127*, 17054 – 17060; c) D. Liu, A. Bruckbauer, C. Abell, S. Balasubramanian, D. Kang, D. Klenerman, D. Zhou, *J. Am. Chem. Soc.* **2006**, *128*, 2067 – 2071.
[9] K. Gehring, J. L. Leroy, M. Gueron, *Nature* **1993**, *363*, 561 – 565.
[10] a) G. Rabai, M. T. Beck, *J. Phys. Chem.* **1988**, *92*, 2804 – 2807; b) G. Rabai, M. T. Beck, *J. Phys. Chem.* **1988**, *92*, 4831 – 4835.
[11] G. Manzini, N. Yathindra, L. E. Xodo, *Nucleic Acids Res.* **1994**, *22*, 4634 – 4640.

