Molecular Devices

A Proton-Fuelled DNA Nanomachine**

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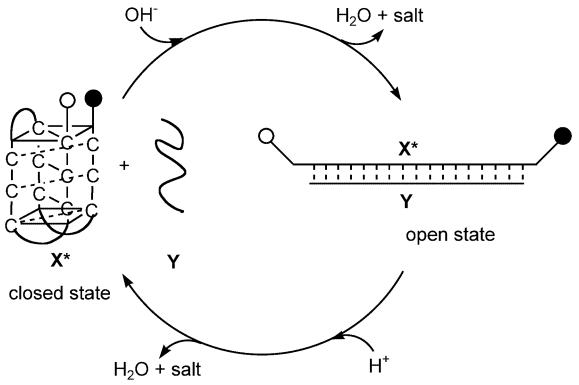
The molecular-recognition properties of DNA are sufficiently well understood to enable the self-assembly of defined structures and devices on the nanometer scale.[1,2] DNA machines based on a competing hybridization mechanism have been reported.[2] These nanomachines are fuelled by complementary oligonucleotides and accumulate double-stranded DNA waste products that poison the system. Herein we report a novel molecular machine driven by a distinct mechanism that is fast and relatively clean. It is based on a four-stranded DNA structure, called the *i*-motif, that can be formed from sequences containing stretches of cytosine (C) residues.[3–7] In this structure protonated C forms a noncanonical base pair with an unprotonated C (i.e., a $C{:}C^+$ base pair), and these base pairs interdigitate to form a quadruple helix that is stable under slightly acidic conditions.[3–7] A pH titration of the formation of the *i*-motif folded structure shows a sharp transition around pH 6.5;[4,5] thus, the design of a DNA nanomachine driven by pH changes is enabled.

As illustrated in Figure 1, our system comprises a 21mer single-stranded oligonucleotide X containing four stretches of CCC. The second component is a 17 mer DNA strand Y, whose sequence is complementary to X with the exception of two bases. The two mismatches are necessary to stop strand Y folding into a G-quadruplex, a four-stranded structure that results from stacked tetrads of hydrogen-bonded guanine residues,[8] and to moderate the melting point of the double helix formed (see Supporting Information). At acidic pH (pH 5.0) strand X folds into the closed *i*-motif structure.[3,4,7] In this closed state, complementary strand Y adopts a floppy random-coil conformation. When the pH value is raised to 8.0, strand X unfolds and is captured by hybridization to Y with formation of an extended duplex structure (the open state). Interconversion of the closed and open states of the machine is thus mediated by alternating addition of H^+ and OH^-.

To visualize the open and closed states of this machine, a doubly labeled version of X (i.e., X*) was synthesized with a rhodamine green fluorophore at the 5′ end and a dabcyl quencher at the 3′ end. When the 5′ and 3′ ends of strand X*



X: 5′ CCCTAACCCTAACCCTAACCC 3′
X* 5′ Rhodamine Green-CCCTAACCCTAACCCTAACCC-Dabcyl 3′
Y: 3′ GATTGTGATTGTGATTG 5′

Figure 1. Oligonucleotide sequences and working cycle of the pH-driven DNA machine. The underlined DNA bases indicate the mismatched nucleotide positions in the duplex. The open circle represents rhodamine green dye, and the filled circle the dabcyl group.

are nearby, the fluorescence of rhodamine green is quenched by dabcyl, whereas rhodamine green fluorescence is strong when it is held away from the dabcyl moiety.[7,9] Rhodamine green was chosen since its fluorescence yield is insensitive to pH value in the range pH 4.0–9.0;[10] therefore, the fluorescence of this system depends only on whether the machine is open or closed.[2,7] Fluorescence quenching was also exploited as an internal indicator for titrating the required amount of H^+ or OH^- to switch between the open and closed states. In the fully extended, open state (pH 8.0), when rhodamine green is excited at 504 nm strong emission is observed at 534 nm; when closed by lowering the pH to 5.0, the fluorescence emission is reduced to 16% (Figure 2a).

The structures associated with the open and closed states were further confirmed by circular dichroism (CD) spectroscopy. At pH 5.0 the closed state (X/Y 1/1) has a CD spectrum which shows the distinct characteristics of the *i*-motif structure, with a strong positive band near 285 nm, a smaller negative band near 260 nm, and a crossover around 270 nm[11] (Figure 2b and Supporting Information). By studying the temperature dependence at 287 nm, the melting point of the closed structure was determined to be 60 °C. Examination of the open state at pH 8.0 by CD spectroscopy shows distinct characteristics of a *B*-form duplex DNA structure with a positive band near 275 nm, a negative band near 240 nm, and a crossover at 258 nm[11] In particular, the crossover at 258 nm rules out a random-coil structure (Supporting Information). By studying the variation of the UV absorbance at 260 nm with temperature, the melting temperature for the transition from the duplex (open state) to the random-coil structure was found to be 32 °C.

Thermodynamic data were obtained for the open and closed states by analysis of the respective melting curves with an established method[12] (see Supporting Information). The ΔG value for the formation of the *i*-motif structure is $-19.15\ \mathrm{kcal\,mol^{-1}}$ (pH 5.0, 20 °C), while ΔG for duplex

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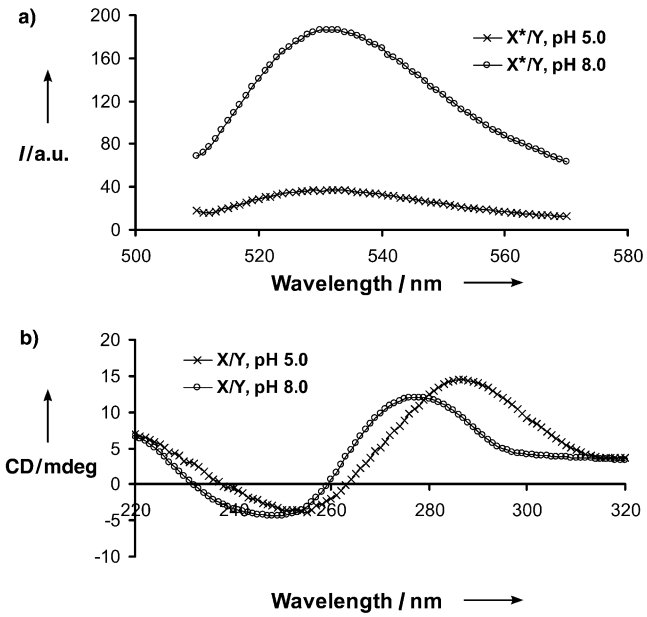

Figure 2. Characterization of the open and closed structures by CD spectroscopy (for X/Y) and fluorescence spectroscopy (for X*/Y). a) Fluorescence emission spectra of the open and closed states at 20 °C with excitation at 504 nm. 1 μM X*/Y in 50 mM MES/50 mM NaCl buffer at the pH value indicated. b) CD spectra of the open and closed states at 20 °C. 1 μM X/Y in 50 mM MES/50 mM NaCl buffer at the pH value indicated.

formation by X/Y is $-8.03\,\mathrm{kcal\,mol^{-1}}$ (pH 8.0, 20 °C). Given that the stability of the X/Y duplex will decrease when the pH value is reduced from 8.0 to 5.0, the magnitude of the change in ΔG (i.e., $\Delta\Delta G$) associated with the transition from open to closed state must have a lower limit of $-11.12\,\mathrm{kcal\,mol^{-1}}$. In the closed state of the machine the 5′ and 3′ ends of X are held 0.8 nm apart according to an X-ray crystal structure of the *i*-motif.[6] In the open state X/Y forms a 17-nucleotide duplex in which the ends are held 5.8 nm apart.[13] Thus, in a complete cycle the machine undergoes a linear movement of about 5 nm. Therefore, we estimate the closing force of this system to be 15.5 pN, while the opening (or extending) force is 11.2 pN.

Multiple cycling of the machine (X*/Y 1/1) can be demonstrated by alternating addition of HCl and NaOH. Figure 3a shows the cyclical changes in fluorescence emission that result from controlled opening and closing of the system. Over 30 cycles the decrease in the amplitude of the system is negligible (206 ± 7 a.u. throughout). The same output was observed by monitoring cyclical changes in the CD signal at 287 nm that result from driving the machine with pH changes (data not shown). Evidently, the accumulating "waste products" (H_2O and NaCl) do not interfere with the molecular mechanism of this machine. The response time of the system can be gauged by analysis of a single opening or closing event. For a strand stoichiometry of X*/Y = 1/1 at 20 °C, the opening (Figure 3b) and closing (Figure 3c) processes are both completed in about 5 s.

Our system is based on just two strands of DNA, one of which reversibly undergoes a precise structural change driven by a pH change. The linear motion generated by this machine is associated with extending and shrinking forces in the range of 10–16 pN. The magnitudes of these forces are at the upper end of the range of forces generated by natural single-

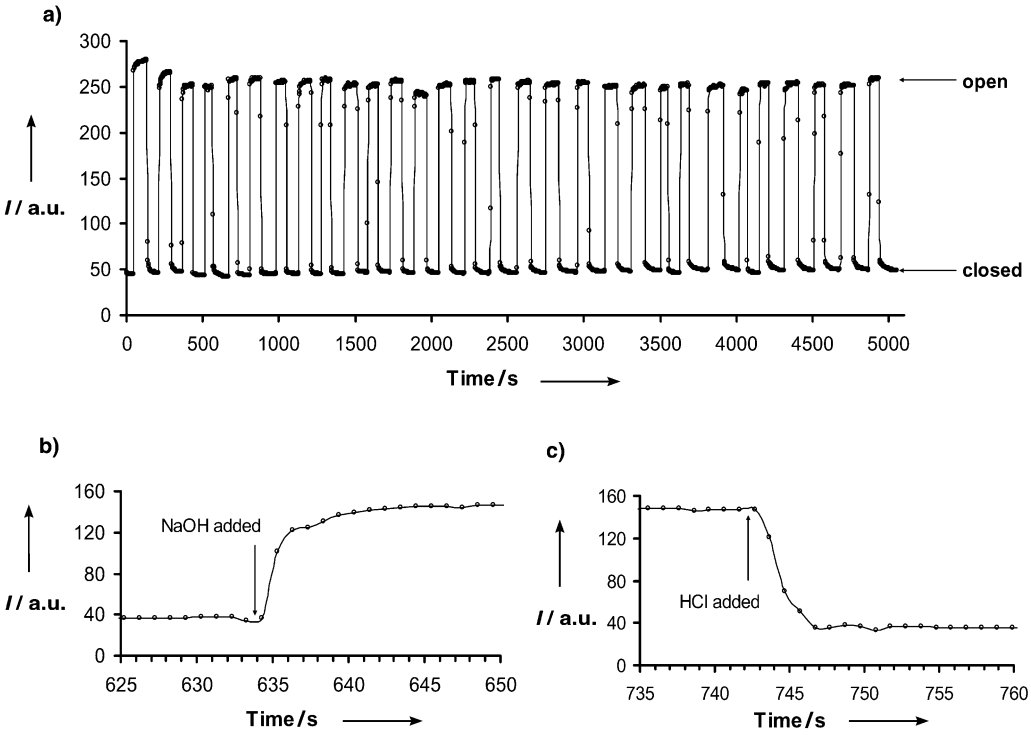

Figure 3. Cycling of the machine (X*/Y), as observed by fluorescence spectroscopy. Excitation at 503 nm and emission monitored at 534 nm at a sampling interval of 0.3 s. a) Cycling of the machine (X*/Y, 1.5 μM). b) Opening of the machine (X*/Y, 1 μM). c) Closing of the machine (X*/Y, 1 μM).

molecule protein motors, such as kinesin[14] and myosin,[15] and also compare favorably with those of other artificial DNA machines.[2] Furthermore, since the machine exerts comparable opening and closing forces, it could be used to do work in both strokes of a working cycle. The switching mechanism has the advantage of being fast compared to DNA machines that employ competitive hybridization mechanisms. As the waste products are nontoxic, one can envisage many working cycles without apparent loss in efficiency. The modular design of this system could be employed to generate a wider range of motion that spans multiples of 5 nm by connecting *i*-motif-forming DNA sequences in tandem. By chemically tethering the ends to components such as proteins, nucleic acids, or other, synthetic molecules, adapted X/Y systems could reversibly and controllably bring two selected entities together in space, and the resulting interaction could be explored. One could also readily immobilize the X or the Y strand using DNA recognition or established covalent chemistry for integration into more complex devices. This system provides new possibilities for exploiting pH-driven motion in nanoscale structures.

Experimental Section

Oligonucleotides X and Y were purchased from Qiagen (salt-free, resuspended in H_2O prior to use). Oligonucleotide X* was purchased from Sigma Genosys.

All CD spectra were recorder on a JASCO-810 spectropolarimeter equipped with a programmable temperature-control unit. All UV spectroscopic studies were conducted on a Varian CARY 1E UV/Vis spectrophotometer. Fluorescence spectroscopy was carried out on a Varian fluorescence spectrometer.

Cycling of the system (X*/Y) visualized by fluorescence spectroscopy: X* and Y were dissolved in a buffer solution (400 μL) containing 2-morpholinoethanesulfonic acid (MES, 50 mM) and NaCl (50 mM) to give a final concentration of 1.5 μM X*/Y in a quartz fluorescence cuvette. The pH value of the buffer was cycled between 5.0 and 8.0 by alternatively adding 16 μL of 1M HCl and 1.5 μM X*/Y (to maintain a constant concentration of X* and Y), or 16 μL of 1M NaOH and 1.5 μM X*/Y to 400 μL of buffer solution at 20 °C. Under these conditions the ionic strength would gradually increase to a maximum of 1M NaCl. A lower buffer concentration may allow switching with smaller additions of NaOH/HCl and hence lead to reduced accumulation of NaCl.

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