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A Reversible pH-Driven DNA Nanoswitch Array

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Abstract: An array of surface-immobilized proton-fueled DNA nanomachines is reversibly actuated by cycling of the solution pH between 4.5 and 9, producing a conformational change between a four-stranded and a double-stranded structure, which elongates or shortens the separation distance between the 5′ and 3′ end of the DNA. By labeling the DNA 3′ end with a fluorophore and immobilizing it onto a thin-gold surface through its 5′ thiol modification, the nanoscale motion of the DNA produces mechanical work to lift up and bring down the fluorophore from the gold surface by at least 2.5 nm and transduces this motion into an optical "on-and-off" nanoswitch.

Introduction

DNA has been demonstrated to be an extremely versatile building material for the assembly of novel nanostructures and nanodevices with unique functions.[1] For example, DNA-based artificial nanomotors and nanomachines have been shown to be capable of producing reversible, well-defined nanometer-scale motions.[2] Furthermore, DNA-based nanowalkers can move along specifically designed DNA tracks unidirectionally.[3] Most of these DNA motors and walkers are fueled by competitive hybridization and can suffer system poisoning due to the accumulation of waste duplex DNA.[2,3] Recently, we demonstrated a new type of relatively clean DNA nanomachine that is fueled by protons. It could reversibly switch its conformation between a quadruplex, called *i*-motif [4] (closed state), and a double-stranded (open state) structure for over 20 cycles without observable degradation.[5] Simmel and co-workers have recently shown that the conformation of the *i*-motif DNA can also be switched by the pH changes produced from an oscillatory chemical reaction.[6] The nanomachine operation (conformational switching) was, however, as for most other artificial DNA motors or machines, demonstrated in buffer solutions without a solid support[2] and thus produced only nondirected random nanoscale motions. By immobilizing the *i*-motifs onto microfabricated silicon cantilevers, McKendry and co-workers have shown it is possible to convert the conformational change of the *i*-motifs into a microscopic cantilever bending.[7] To exploit the nanoscale motions of the DNA-based motors for nanotechnology, it is necessary to attach them onto defined surface locations of a solid support and to demonstrate their functions. To our knowledge, there has been only one previous example showing that a randomly immobilized DNA motor can maintain its function.[2h] Here, we have immobilized oriented proton-fueled motor DNA molecules onto defined locations of a microstructured surface to form a microarray. These immobilized motor DNAs maintain their motor function, and this motion can be harnessed to produce an on−off optical nanoswitch. This is an important step toward the construction of nanomachines that can perform useful work.

Experimental Section

Materials. 11-Mercaptoundecyl hexa(ethylene glycol) (EG_6OH) was synthesized following a literature procedure[8a] and purified as described

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Table 1. DNA Sequences and Their Modifications Used in This Study[a]

DNA name	sequence
motor DNA	HSC_6H_{12}-5′-CCCTAACCCTAACCCTAACCC-3′-rhodamine green
motor DNA-C	5′-G***T***GTTAGG***T***TTAGGGTTAG***T***G-3′
control-DNA-1	HSC_6H_{12}-5′-TCTATGCTGTTACTCTGACTC-3′-rhodamine green
control-DNA-1C	5′-GAGTCAGAGTAACAGCATAGA-3′
control-DNA-2	HSC_6H_{12}-5′-CACTCACCTCACACACACTCC-3′
control-DNA-2C	5′-GGAGTGTGTGTGAGGTGAGTG-3′

[a] The three base **T**s underlined shown in the motor DNA-C sequence indicate the DNA mismatch (to prevent it forming a G-quadruplex).

previously.[8b] 2-Mercaptoethanol (99%), HPLC-grade ethanol, and other reagents were purchased from Sigma-Aldrich (Dorset, U.K.) and used as received unless otherwise stated. PBS buffer (100 mM sodium phosphate, 1 M NaCl, pH 4.5 or 9) was prepared with ultrapure MilliQ water (resistance > 18 MΩ.cm). The buffer was filtered through a Whatman syringe filter (0.20 μm pore size, Whatman Plc., Florham Park, NJ) before use. Flat transparent thin-gold coated glass coverslips (~5 nm thick of Au with a titanium adhesion layer and a transparency of ~50% at 519 nm, typical surface roughness < 1 nm over 25 μm^2) were coated by Ssens BV (Hengelo, The Netherlands).[9] Ultra-flat template-stripped gold (TSG) surfaces (typical surface roughness < 0.5 nm over 25 μm^2) were prepared following a literature procedure and stripped by using chemical stripping with THF.[10] Once prepared, the TSG surfaces were thoroughly rinsed with ethanol and MilliQ water and blown dry under a stream of N_2. They were used immediately to minimize contamination. The single-stranded motor DNA and control DNA strands and their complementary strands were all purchased from IBA GmbH (Göttingen, Germany) and used as received. Their sequences are shown in Table 1. They were dissolved and diluted to the desired concentrations with the PBS buffer before use.

Preparation of DNA SAM. A freshly prepared TSG surface was incubated with a solution of the motor DNA (1 μM in PBS, pH 4.5) for 12 h at room temperature (22 ± 2 °C), after which time the surface was thoroughly rinsed with PBS at the same pH before being mounted onto the sample stage of the AFM. The relatively long incubation period was used to ensure the formation of a more uniform SAM layer.[11a]

Microcontact Printing and Formation of Motor DNA Microarray. A PDMS stamp, with features of 2 μm stripes separated by 2 μm gaps, replicated from a photolithography patterned master was used to pattern the thin gold-coated coverglass surface.[12] The stamp was inked by wiping the stamp surface with a solution of EG_6OH in ethanol (2 mM) with a cotton swab. After being blown dry with N_2, the stamp was stamped twice (with the stamp being rotated 90° in the second print) on the thin-gold coated surface.[12b] This constituted the EG_6OH SAM background with squares of bare gold which were subsequently filled with a SAM of the motor DNA by incubation of the surface with a solution of the thiolated motor DNA (1 μM in PBS, pH 4.5) for 1 h. After being rinsed with PBS (pH 4.5), the surface was incubated with 2-mercaptoethanol (1 mM in PBS, pH 4.5) for 1 h to remove nonspecifically attached motor DNA[11b] and to reduce the density of the attached motor DNA. After removal of the 2-mercaptoethanol solution, followed by a thorough rinse with PBS (pH 4.5), the sample was mounted on an inverted fluorescence microscope to record the fluorescence images.

Atomic Force Microscopy. All of the AFM experiments were carried out in a custom-made fluid cell on a Digital Instrument (Veeco, Santa Babara, CA) Dimension 3100 AFM equipped with a Nanoscope IV controller.[13a] All images were collected in contact mode under the PBS buffer (pH 4.5 or 9) at 22 ± 1 °C. Standard oxide-sharpened V-shaped Si_3N_4 microlevers from Digital Instrument with a nominal spring constant of 0.12 N/m were used. Images were collected at either 256 × 256 or 512 × 512 pixels per image at 2 Hz and were analyzed using the Nanoscope image analysis software (version 5.12r3) with first-order flattening. Before conducting the nanografting experiment, the DNA SAM surface was first imaged under minimum loading force (0.2−0.5 nN) in PBS (pH 4.5) containing 1 mM of 2-mercaptoethanol to select a suitable small flat area avoiding domain boundaries. The chosen area was repeatedly scanned at 4−5 Hz under high loading forces (~30 nN) to scratch away the DNA SAM, creating a freshly exposed gold surface that was immediately grafted with a SAM of 2-mercaptoethanol that was already present in the solution by self-assembly.[13b] After scratching, the AFM tip was fully retracted from the surface, zoomed out to a bigger scan area, and then re-engaged with minimum force to take topographic images (~0.2 nN). After each manipulation, the remaining solution was removed from the fluid cell, and the surface was thoroughly washed with the PBS buffer before a new solution was introduced and incubated.

Fluorescence Imaging. Fluorescence images were recorded on a home-built confocal microscope under 5 μW laser illumination at 488 nm (Model 532-AP-01, Melles Griot, CA).[14] The fluorescence of Rhodamine green centered at 533 nm was collected by an oil-immersion objective (Nikon Plan 100 DIC, 100×, NA = 1.25). A beam-splitter (FITC/Cy5, AHF Analysentechnik AG, Germany), filters (510ALP and 535AF45, Omega Optical, VT), and a 75 μm pinhole were used to separate the fluorescence signal and to reject background signal and laser scattering. A photon-counting avalanche photodiode (SPCM AQR-13, Perkin-Elmer) was used as the detector. The optical resolution of the instrument determined by imaging 100 nm fluorescent microspheres was ~400 nm. The images were collected at 512 × 512 pixels per image with an integration time of 1 ms per pixel and were processed using the WSxM 3.0 program (Nanotec Electronica S.L., Spain). The average fluorescence intensity for each pixel from both the motor DNA features and the background was obtained by converting the fluorescence image into gray scale, and analyzed by using the histogram function of the Adobe Photoshop software (Version 6.0).[14c]

Results and Discussion

Our design is based on the immobilization of the motor DNA, with four stretches of *CCC* (*C* = cytosine) in sequence modified with a $-C_6SH$ linker at the 5′ and a rhodamine green fluorophore at the 3′ (Table 1) onto defined surface locations of a microstructured thin-gold coated glass coverslip, as shown schematically in Scheme 1.

This design enables the convenient attachment of the motor DNA onto the surface (via gold-thiol self-assembly)[11] and

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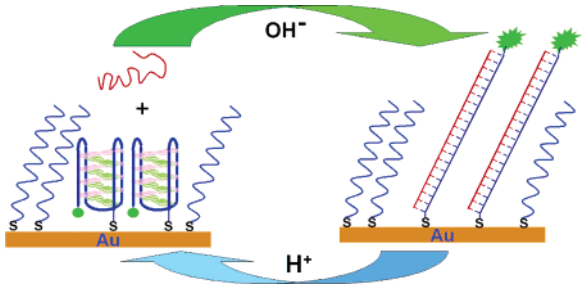
Scheme 1. Schematic Presentation of the Reversible Actuation of the Immobilized Motor DNA Array on a Microcontact Printing Patterned Surface[a]



[a] At low pH, the motor DNA adopts its closed *i*-motif structure, which brings the 3′-labeled fluorophore to close proximity of the gold surface and quenches its fluorescence. At high pH, the *i*-motif unfolds and hybridizes with its complementary strand in solution to form a rigid duplex, which lifts up the fluorophore away from the gold surface and becomes highly fluorescent.

sensitive optical detection (via rhodamine green fluorescence).[15] The fluorescence quantum yield of the fluorophore, rhodamine green, is insensitive to pH from 4 to 9.[15a] pH titration of the *i*-motif in solution showed a sharp transition at pH ~6.5; thus, a reversible operation of the DNA nanomachine can be triggered by the changes of the solution pH.[16] At low pH (acidic), the motor DNA adopts its quadruplex *i*-motif form, due to the formation of intramolecular noncanonical base pairs between cytosine (*C*) and protonated cytosine (CH^+).[4] This brings the 3′-end attached fluorophore to the close proximity to the gold surface (~1 nm assuming a well-packed *i*-motif structure with an extended C_6 chain) and quenches its fluorescence by the efficient energy transfer from the dye to the gold.[17] When the pH is increased to basic, the cytosine is no longer protonated and so the intramolecular $C-CH^+$ base-pairing, which was the driving force for the formation of *i*-motif structure, breaks down. The motor DNA unfolds to single-stranded and hybridizes to a complementary DNA in solution to form a rigid duplex. This lifts up the fluorophore away from the gold surface (~8 nm, if fully extended and vertically aligned) and greatly reduces the quenching efficiency, resulting in strong rhodamine green fluorescence when excited with 488 nm laser radiation. Consequently, a reversible operation of the fluorophore-labeled surface-immobilized motor DNA will lift up and bring down the 3′-labeled fluorophore reversibly from the gold surface, producing a reversible change in the fluorescence signal; i.e., the mechanical motion of the motor DNA is transduced into a fluorescence based on−off optical nanoswitch. The changes in fluorescence signal can be conveniently detected on our highly sensitive scanning confocal microscope, which is capable of detecting fluorophore-labeled single biomolcules.[14]

A flat thin-gold coated (~5 nm thick, with a titanium adhesion layer) glass cover-slip was used as the substrate to immobilize the motor DNA because it is optically transparent (~40%), enabling convenient and sensitive fluorescence-based optical detection.[9] Further, the quenching efficiency of gold is strongly dependent on the separation distance between the fluorophore and the gold surface.[17] To define the location of the motor DNA on the surface, the substrate was patterned on the microscale by microcontact printing (μCP).[12] This was achieved by stamping a PDMS stamp (2 μm stripes separated by 2 μm gaps), inked with 11-mercaptoundecylhexa(ethylene glycol) alcohol (EG_6OH), twice on the same gold surface, with the stamp being rotated 90° for the second stamping.[12b] This constituted a self-assembled monolayer (SAM) background of EG_6OH that can effectively resist the nonspecific adsorption of biomolecules,[8] with micron-sized squares of unstamped bare gold surface. The motor DNA was immobilized to the unstamped bare gold region through gold-thiol self-assembly by incubation of the post-stamped surface with the motor DNA (1 μM) in pH 4.5 PBS (100 mM sodium phosphate, 1 M NaCl, all PBS buffers used here have the same components and differ only in their pH) for 1 h. A low pH buffer was used to ensure the motor DNAs were immobilized in their folded *i*-motif states, producing a relatively loosely packed motor DNA SAM. This allows for sufficient surface space for the immobilized motor DNA to switch its conformation freely and reversibly.[17b] The surface was then treated with 2-mercaptoethanol (MCE, 1 mM in PBS) for 1 h to remove nonspecifically adsorbed DNA[11b] and then thoroughly rinsed with PBS. All of these treatments were carried out using the same pH 4.5 PBS to avoid inducing any conformational changes to the immobilized motor DNAs.

This surface exhibited only weak fluorescence when excited with an Ar^+ laser beam at 488 nm on our scanning confocal fluorescence microscope under pH 4.5 PBS buffer.[14] Incubation of this surface with a complementary DNA (motor DNA-C, three mismatches were engineered to prevent it forming G-quadruplex,[4c] 1 μM in pH 4.5 PBS, Table 1) for 0.5 h produced no detectable change in the fluorescence from the motor DNA patterns. A low fluorescence signal-to-background ratio of only ~3 was observed (Figure 1a). This is consistent with the motor DNA adopting its closed *i*-motif structure,[4b] where the fluorophore is brought to close proximity of the gold surface and therefore quenched. This is presumably because the *i*-motif form is more stable than the single-stranded or duplex (with three mismatches) form for the motor DNA under such experimental conditions and agrees with our earlier solution-based results.[5] Replacing the pH 4.5 solution with a pH 9 PBS containing the same complementary DNA strand (1 μM) for 0.5 h produced a 4-fold increase in the fluorescence intensity from the motor DNA patterns alone, with little change from the EG_6OH background region (Figure 1b). As a result, the fluorescence signal-to-background ratio is significantly improved to ~15. This agrees well with the motor DNA unfolding to single-stranded and hybridizing to its complementary strand in solution to form a rigid duplex structure at this pH. This extends the 3′ end-labeled fluorophore away from the gold surface and greatly reduces the energy transfer between the fluorophore and the gold surface so the fluorophore becomes highly fluorescent when excited with the 488 nm laser.[17] The significant increase in fluorescence intensity observed here indicates that the fluorophore must have been moved vertically, not horizontally, from the gold surface; i.e., we can have some degrees of control

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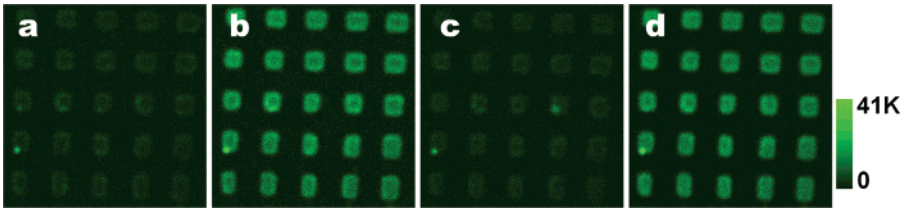

Figure 1. Fluorescence images (size: 21 μm × 21 μm) showing the reversible actuation of the motor DNA microarray. The images were recorded from the same sample surface region in PBS (100 mM phosphate, 1 M NaCl, with 1 μM motor DNA-C) with the pH sequence as follows: (a) pH 4.5, (b) pH 9, (c) pH 4.5, and (d) pH 9. All images were recorded at 512 × 512 pixels per image with an integration time of 1 ms for each pixel using 5 μW laser excitation at 488 nm.

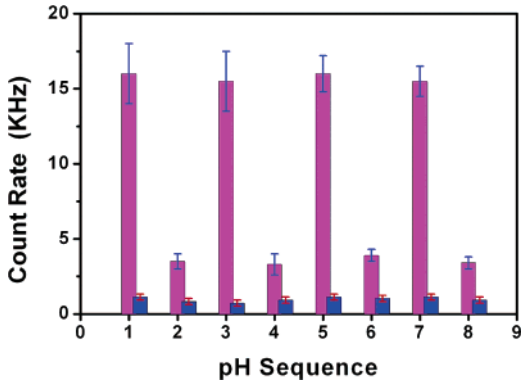

Figure 2. Plot of the average fluorescence intensity from the motor DNA patterns (pink column) and background (blue column) as a function of pH. The pH is 9 for sequence 1, 3, 5, 7 and 4.5 for sequence 2, 4, 6, 8. The error bar is determined from the average of 20 different spots on the sample.

in the direction of the nanoscale movement of the immobilized DNA motors.

The quenching efficiency, defined as $(I_O - I_C)/I_O$, where I_O and I_C are fluorescence intensities at the open (pH 9) and closed (pH 4.5) states, respectively, is estimated to be ~80%. The quenching efficiency is similar to those observed in solutions with organic quenchers.[2h,5] The process is found to be highly reversible; the fluorescence decreases to the original folded value when the solution pH is changed to 4.5 (Figure 1c) but can be fully recovered as the pH is changed back to 9 (Figure 1d). All of these solutions contained the same concentration of the complementary DNA (motor DNA-C, 1 μM). This suggests that immobilization of the motor DNA onto the gold surface does not affect its ability of reversible conformational change. The switching process completes in less than 5 min in both the opening and closing states (data not shown). Figure 2 shows the average fluorescence intensities from the motor DNA patterns and the background as a function of the pH cycles. It is clear that the immobilized motor DNA arrays are quite robust and can withstand multiple buffer washings, and their operation (conformational change) is highly reversible. No reduction in operational amplitude (fluorescence intensity changes) is observed after four full cycles. We note that the orientation (lying down or standing up) of short DNAs immobilized on metal surfaces can also be modulated electrically to produce a fluorescence-based optical output.[17b] Here, we used the intrinsic conformational change of the immobilized motor DNA, induced by the change of the solution pH without the need of injecting external energy, to lift up and bring down the end-tethered fluorophore from the gold surface and to produce the fluorescence output. Further, we can also control the location of these motor DNAs immobilized on the solid support by using a microstructured surface and direct their nanoscale motions. This opens up opportunities for harvesting such well-defined nanoscale motions of the DNA motors.

To verify that the operation (conformational change) is only specific to the motor DNA, a random DNA sequence of the same base length (so it cannot form *i*-motif structure, control-DNA-1, Table 1) was used as a control. The control-DNA-1 was modified with identical 5′ and 3′ modifications to the motor DNA and was immobilized on a microstructured thin-gold coated surface and treated under identical experimental conditions. No significant change (within 15%) in the fluorescence intensity from the DNA patterns was observed as the pH of the solution was switched between 4.5 and 9 for the control-DNA-1 (data not shown), whereas the change in fluorescence intensity for the motor DNA is 4-fold. This also confirms that the fluorescence of the rhodamine green fluorophore is insensitive to pH from 4.5 to 9 and the observed fluorescence change is indeed from the specific conformational changes of the motor DNA.

Because the density of the DNAs attached to the gold surface is different between the closed (containing only the motor DNA) and the open state (containing both the motor DNA and the complementary strand), this should be reflected in the thickness of the DNA layer and be detectable by AFM nanolithography.[13] This technique has previously been used to accurately measure the thickness of self-assembled monolayers (SAMs) of DNAs.[18] Here, we used this technique to directly measure the thickness of the motor DNA film at the two pHs.

A SAM of the motor DNA (identical sequence, without the fluorophore at the 3′) prepared on an ultra-flat template-stripped gold surface (typical roughness <0.2 nm over 25 μm^2)[10,18b] in PBS at pH 4.5 was found to be ~2.2 nm thick (see the Supporting Information, Figure S1a) and agrees with the dimension of the motor DNA in its closed *i*-motif form.[4] No change in thickness of the motor DNA SAM was observed when incubated with motor DNA-C (1 μM, pH 4.5) (Figure S1b), suggesting no hybridization has taken place. Replacing the pH 4.5 solution with a pH 9 PBS (with 1 μM motor DNA-C) increased the motor DNA thickness to ~3.6 nm, Figure S1c). This suggests that the fluorophore should have been lifted by at least 2.6 nm in the vertical direction, taking into account the difference of the fluorophore orientation between the two states.[19] The thickness is less than expected from a fully extended, densely packed, and vertically aligned motor DNA duplex SAM (~8.1 nm), suggesting the DNA is tilted at 64° from the surface normal. This is not unreasonable because the

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motor DNAs were originally immobilized in their *i*-motif form (4-stranded) and so would not be densely packed in the open state (duplex). Further, even densely packed SAMs of duplex DNAs have been found to be tilted at ∼45° from the surface normal.[18] The measured thickness should be a lower estimate because, despite imaging at low forces, there may still be tip compression to the DNA film. This tip compression should have a greater effect to the open (duplex) state than that to the closed (four-stranded) state. The change in DNA SAM thickness is reversible (all containing 1 μM motor DNA-C): the thickness decreased to ∼2.2 nm when the pH was changed to 4.5 (Figure S1d) but recovered to ∼3.5 nm when pH was cycled back to 9 (Figure S1e), suggesting the conformational switch of the surface immobilized motor DNA is reversible.

By contrast, control-DNA-2 SAM (identical length, modification, and the number of *C*s in sequence except for the *C*s being arranged randomly) prepared from pH 4.5 PBS was found to be ∼3.0 nm thick (Supporting Information, Figure S2a), ∼0.8 nm thicker than that of the motor DNA SAM. The thickness further increased to ∼4.6 nm after incubation with its complimentary DNA (control-DNA-2C, 1 μM, Figure S2b) at pH 4.5, suggesting specific hybridization did take place. The thickness increased to ∼5.5 nm when pH was increased to 9 (Figure S2c), but reverted back to ∼4.7 nm when the pH was decreased to 4.5 (Figure S2d), and the pH dependent thickness change (all with1 μM control-DNA-2C) is reversible.[20]

It should be noted that the motor DNA layer thickness did not change when incubated with its complementary strand (motor DNA-C) at pH 4.5, while the control DNA showed a significant increase of 1.6 nm. Further, the thickness of the motor DNA SAM fully reverted to the original motor DNA only value when the pH switched from 9 to 4.5, suggesting a complete dehybridization. In contrast, the control-DNA-2 SAM thickness only dropped to the post-hybridized value at the same pH. All these observations confirm that the conformational switch of the motor DNA is reversible, and the complementary strands (with three mismatches) are completely dehybridized from the motor DNA strands when pH is switched from 9 to 4.5. However, this is not the case for control-DNA-2, where the hybridized strands remain bound when the pH is changed from 9 to 4.5. The reversible conformational switch is thus specific to the designed motor DNA sequence.

(19) The fluorophore at the 3′ end should point toward the gold surface in the closed *i*-motif structure, and thus, it is ∼1 nm above the gold surface estimated from the length of the HSC_6H_{12}-linker. In the open state, the fluorophore is pointing upward (see Scheme 1) so its distance from the gold surface should match the thickness of the DNA layer, i.e., at least 3.6 nm in this case.

(20) The AFM-measured DNA film thickness difference at pH 4.5 and 9 is possibly due to the difference in the electrostatic interactions between the tip and the DNA layer. At high pH (9), the tip terminal OH groups would be highly deprotonated and strongly negatively charged. It would be strongly repelled by the negatively charged DNA surface, leading to a thicker AFM measured DNA film. At the lower pH (4.5), the tip would be only partially deprotonated, so the electrostatic repulsion is weaker and thus leading to a thinner DNA layer measured by AFM under the same loading force.

The effect of the hybridization on the DNA conformation was investigated by measuring the fluorescence and thickness of the immobilized motor DNA in its *i*-motif, random coil and duplex states (Figures S3 and S4, Supporting Information). A significant increase (3.5-fold) in fluorescence was observed when the immobilized motor DNA was switched from the folded *i*-motif (pH 4.5) to the random coil (pH 9.0), while this produced only a small increase (∼0.3 nm) in film thickness. Further transformation from the random coil (pH 9.0) to duplex (pH 9.0 with motor DNA-C) produced a ∼0.9 nm increase in film thickness, but with only a marginal increase (∼10%) in fluorescence. These results show that hybridization has a major contribution to the observed film thickness increase, but only minor to the increase of the fluorescence signal.

In summary, we have shown that it is possible to controllably immobilize the motor DNA onto a microstructured thin-gold surface to produce a microarray of functional DNA nanomachines that can produce well-defined, highly reversible conformational changes when the pH is cycled between 4.5 and 9. This vertically lifts up and brings down the end-attached fluorophores from the gold surface and, hence, transduces these mechanical motions into an optical nanoswitch driven by the pH. This is one of the first examples showing the operation of substrate-bound DNA-based nanodevices. In principle, other DNA sequences that can produce well-defined conformational changes could also be used in a similar way, for example, G-quadruplex structures.[21] These nanoswitch arrays may have useful applications, such as sensors for detection of local pH and metal ion concentration. Furthermore, the ability to immobilize oriented and functional motor DNAs on a surface is an important first step toward the construction of functional DNA-based nanomachines. This opens up many opportunities to exploit this well-defined and controllable nanoscale motions, for example, to produce micromechanical work,[7] for pH-controlled drug release, movement of cargos, and changing the nature of the surface in response to a stimulus.

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Supporting Information Available: AFM topographic images of the nano holes showing the thickness of the motor DNA layers and fluorescence images showing the surface-immobilized motor DNAs at three different (*i*-motif, random coil, and duplex) states. This material is available free of charge via the Internet at http://pubs.acs.org.

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