Single-Molecule Observation of the Photoregulated Conformational Dynamics of DNA Origami Nanoscissors

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Abstract: We demonstrate direct observation of the dynamic opening and closing behavior of photocontrollable DNA origami nanoscissors using high-speed atomic force microscopy (HS-AFM). First, the conformational change between the open and closed state controlled by adjustment of surrounding salt concentration could be directly observed during AFM scanning. Then, light-responsive moieties were incorporated into the nanoscissors to control these structural changes by photoirradiation. Using photoswitchable DNA strands, we created a photosensitive nanoscissors variant and were able to distinguish between the open and closed conformations after respective irradiation with ultraviolet (UV) and visible (Vis) light by gel electrophoresis and AFM imaging. Additionally, these reversible changes in shape during photoirradiation were directly visualized using HS-AFM. Moreover, four photoswitchable nanoscissors were assembled into a scissors–actuator-like higher-order object, the configuration of which could be controlled by the open and closed switching induced by irradiation with UV and Vis light.

A key goal of nanotechnology is to construct molecular robots, mechanical devices, and nanomachines. To create such objects, stimuli-responsive molecular actuators are required. DNA-based nanomachines with a stimuli-responsive switching function have been widely investigated, and systems have been reported that can perform rotational and reciprocating motions with nanometer-scale precision.[1] However, regulating the mechanical movements in response to specific stimuli remains a challenging aspect in the construction of dynamic DNA nanodevices.[2–4] Typically, DNA nanomachines are regulated by the addition of specific metal ions, proteins, and DNA strands for strand displacement reactions.[1,5–13] However, these methods unavoidably generate byproducts and their accumulation. By contrast, light is an attractive regulating entity because it can be spatially and temporally controlled and it is non-invasive. Photochemical reactions including photocleavage and photoisomerization can be initiated by light irradiation and exploited to control the conformation of a target object. In this study, we employed photochromic molecules to control conformational switching dynamics of DNA actuators adsorbed to a surface.[14–16]

Here, we adapted a previously described multi-layer DNA origami switch “nanoscissors”[17] to study light-induced conformational transitions in real-time on the level of single particles. To achieve the photoirregulation, we employed photosensitive DNA oligonucleotides containing azobenzene molecules which can hybridize in the trans-form and dissociate in the cis-form of the azobenzenes by photoirradiation of different wavelengths.[18,19] In the previous work, we used azobenzene-tethered DNA strands functioning as a photosensitive unit for duplex formation/dissociation to control the assembly and disassembly of DNA origami, to open and close a DNA nanocapsule,[20] and to control a rotary movement.[21,22] The key goal of the present work is to resolve the photoinduced conformational transitions in real-time on the level of single particles using high-speed atomic force microscopy (HS-AFM) with rapid scanning and high-resolution imaging, to gain insight into switching dynamics and to study the effect of surface interactions on the conformational switching.[23,24] To perform this technically challenging experiment, we adapted the multi-layer DNA origami switch because we anticipated that its well-defined pivot axis and greater rigidity as compared to single-layer designs may render the object less susceptible to surface-induced deformations which may inhibit conformational dynamics.[17]

We first used NS with the stacking and shape-complementary system, which was designed using caDNAo (Figure 1a).[17,25] We prepared DNA nanoscissors (NS) according to the previously reported method. After assembly of the p8064 plasmid and staple strands, the structures were imaged by AFM in a buffer solution. Open and closed NSs were clearly observed on the mica surface (Figure 1b). By increasing the Mg²⁺ concentration, the ratio of the closed structures increased (Figure S1). The midpoint in salt concentration of open and closed structures was approximately 13.5 mm from our AFM observation and characterization. We subsequently
observed the time-lapsed opening/closing behavior by changing the Mg\(^{2+}\) concentration during HS-AFM scanning (Figure 1c, see Movie S1 in the Supporting Information). During AFM imaging, the NSs fluctuated in 5 mM MgCl\(_2\). We also added 1 mM NaCl to facilitate conformational change on the mica surface.\(^{[26]}\) To induce closing of the NSs, high concentration of MgCl\(_2\) was added to the observation buffer (ca. 20 mM final). We clearly observed the open-to-closed change after the addition of a high concentration of MgCl\(_2\) (Figure 1c left to middle). On the other hand, a low concentration of MgCl\(_2\) was added to lower the concentration (ca. 7 mM final), and opening of the structures was observed (Figure 1c middle to right). Three individual NSs marked by dashed circles change their structure in a reversible fashion (open-close-open). We successfully observed the conformational change of NSs. However, tracking the structural changes on the single particle level requires the addition of MgCl\(_2\) solution to the observation buffer from outside, and this type of operation is technically challenging in the context of an HS-AFM.

To realize the control of the open/closed conformational change of the NS without the addition of solutions from outside, we prepared the photoresponsive NS by incorporating photoswitching DNA strands which respond to irradiation with UV and Vis light. For the construction of photoswitchable NS (ps-NS), two pairs of photoswitching DNA strands connected to the specific staple strands were incorporated into specific positions of the concavity/convexity domains of the non-stacking system inside both arms (Figures 2a and S2).\(^{[17]}\)

The reversible opening and closing behavior of the ps-NS was controlled by irradiation with UV (350 nm) and Vis (450 nm) light, respectively. DNA origami and photoswitching DNA strands are stable enough for irradiation with UV light employed in this experiment, and the photoswitching strands are substantially stable for repeated switching.\(^{[14,16,21]}\)

After the DNA origami assembly, the formation of the ps-NS was confirmed by AFM. The conformational change stemming from time-dependent photoirradiation was examined using gel electrophoresis (Figure 2). Comparing the migration speed of the two states, the mobility of the open and closed structures with UV and Vis irradiation was clearly changed. We first investigated the irradiation with UV light in order to open the closed ps-NS. During the irradiation with UV light, the population of the closed ps-NS shifted to the open state in a time-dependent manner (Figures 2c and S4). The UV-light-irradiated (10 minutes) ps-NS (open-form) was treated with Vis light irradiation, and the closed-form formation saturated almost in 5 minutes to yield approximately 70% (10 minutes Vis light irradiation, Figures 2d and S4). The reversible
Photoinduced open/closed behavior of the ps-NS was investigated by switching between irradiation with UV and Vis light for 10 minutes. As shown in Figure 2e, by alternating the irradiation wavelength, the open and closed conformations can be switched reversibly. These results show that the state of the ps-NS can be controlled reversibly and repeatedly by alternating irradiation with UV and Vis light.

After the irradiation with UV light, the closed form opened because of the dissociation of photoswitching DNA strands caused by photoisomerization of the azobenzene moieties. The following Vis light irradiation to the open ps-NS produced the closed ps-NS in a few minutes due to the hybridization of the photoswitching DNA strands. In addition, during the alternating UV/Vis light irradiation of the same sample, the closed structure of the ps-NS still functioned properly. Figure 2f shows the proportion of the open/closed ps-NS after alternating UV/Vis light irradiation, and the fraction of the open structure and closed structure in each cycle remains at 85% and 65%, respectively. The ps-NS showed reversibility for 30 times alternating UV and Vis light irradiation without causing any damage (Figure S5). The cycling shows that the photoswitching strands can be employed to regulate the conformation of the DNA nano-scissors reversibly and repeatedly.

We analyzed the structural change by AFM and acquired AFM images of the closed ps-NS in the initial state (Figure S6). Then UV light was irradiated to the sample, and AFM images were obtained. In the initial state, 45% of ps-NS were observed in the closed form. After the UV light irradiation, 11% of the ps-NS remained closed. Then after the subsequent Vis light irradiation, 33% closed ps-NS could be observed. These results indicate that the photoirradiation-induced open/closed movement of the ps-NS should be observable during HS-AFM scanning, in spite of possible surface interactions that may affect the opening and closing transitions.

Figure 3. Repeated open/closed motion of the nanoscissors with photoswitches (ps-NS) imaged by HS-AFM during photoirradiation. a) HS-AFM images of the ps-NSs. Successive HS-AFM images were extracted by 50 s intervals. AFM images during UV light irradiation (100–300 s) (top); Vis light irradiation (400–650 s) (middle); second time UV light irradiation (750–1000 s). Two ps-NSs are marked by 1 and 2, and opened and closed ps-NSs are indicated with blue and red arrow, respectively. b) Time-lapsed images (0.2 frames s⁻¹) of the ps-NSs. Closed-to-open change during UV light irradiation (top); open-to-close change during Vis light irradiation (middle); close-to-open change during the second UV light irradiation (bottom). c) Summary of the change of the appearance of two ps-NSs during successive UV/Vis-Ul light irradiation. The scale bar is 50 nm.
We next aimed to visualize opening and closing events of the ps-NS using HS-AFM. First, the opening event of the ps-NS with UV light irradiation was observed by HS-AFM (Figure 3, Movie S2). We used 10 mM MgCl₂ and added 1 mM NaCl to weaken the interaction to the mica surface to promote the dynamic movement. UV light irradiation of the sample was carried out during continuous AFM scanning. Time-lapsed AFM images (0.2 frame s⁻¹) were obtained and are shown in Figure 3 (Movie S2). The ps-NS moving on the mica surface remained closed during the AFM scanning, and then after UV light irradiation, the closed ps-NS opened during fluctuation (Figure 3a top). This indicated that the ps-NS needs to be attached weakly to the mica surface for the open/closed behavior. High Mg²⁺ concentration (20 mM) also affected the opening of the ps-NS (Figure S7), and the response after the UV light irradiation was slower compared to the usual Mg²⁺ concentration (12 mM).

After the opening of the ps-NSs, Vis light was subsequently irradiated and the ps-NS moving on the mica changed its structure to the closed form (Figure 3a middle). Now, the closed ps-NS was treated with UV light again, and we observed the opening of the structures, including the fully opened and half-opened structures (Figures 3a bottom). This could suggest that the surface interaction prevents the opening of the arms of the ps-NS. The appearance of the two ps-NSs (closed, half-open, and open form) during the photoirradiation is summarized in Figure 3c. The slow response of the ps-NSs to open and close after photoirradiation was caused by the interaction between the ps-NS and the mica surface. Closing by irradiation with Vis light occurred slower (ca. 70 s) compared to opening by UV light irradiation (ca. 20 s), indicating the different surface interaction with the open and closed ps-NS. Using the dynamic DNA origami structure modified with photoswitches, the reversible movement of the single ps-NS was directly visualized by HS-AFM imaging.

Finally, we prepared a square-shaped tetramer using four ps-NS units and controlled the configurational change of the tetramer by photoirradiation (Figure 4). Assembly of the four ps-NS units to form the desired patterns was also performed in a programmed fashion by connecting the specific ends of the ps-NS units (Figure S8). Using the unmodified NS units, we obtained the square-shaped assembly by joining two dimers (Figure S9). Using the same method, we assembled the UV-irradiated opened ps-NS units in the square-shape by assembling two opened ps-NS dimers in 15% yield on the gel image (Figure S10). We also characterized the change in shape of the tetramers by photoirradiation using AFM. In the initial state using the opened ps-NS units, we observed the opened square-shaped structures. After irradiation with Vis light, the assemblies changed their forms to the closed structures (Figure 4c). By observing the individual units, some parts were closed and some parts remained open as a result of the structural stress of the assembly. Closing of the tetramer did not work perfectly or the assembled structures partially opened when attaching onto the mica for AFM observation. After the tetramer formation, we alternately irradiated UV and Vis light to the square-shaped assembly and observed the repeating band shifting on the agarose gel (Figure S10c). These indicate that the reversible actuation of the ps-NS assembly can be controlled by irradiation with UV/Vis light.

In conclusion, we have demonstrated the direct observation of the opening and closing transitions of a DNA nanosissor on the single particle level using HS-AFM imaging. The photocontrollable open/closed system was introduced into the NS, and the reversible open/closed states of the ps-NS were switched by irradiation with UV and Vis light. The opening and closing events of the single ps-NS were directly observed on the mica surface using HS-AFM during alternating irradiation with UV/Vis light. In addition, the change in shape of the square-shaped assembly from four photo-functionalized units was achieved using photoirradiation. This reversibly open and closed system is used to initialize the configuration of the nanostructures and change into the specific shapes repeatedly by just irradiating the specific wavelength of light. These nanosized photofunctionalized molecular devices could be applied for shape-changeable nanomaterials and may potentially become useful tools to regulate biochemical reactions. As we have seen, the capability for switching is preserved even while particles are attached on surfaces, which may offer interesting possibilities for constructing light-responsive electronic or photonic devices[27] that use DNA origami switches.

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Conflict of interest

The authors declare no conflict of interest.

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