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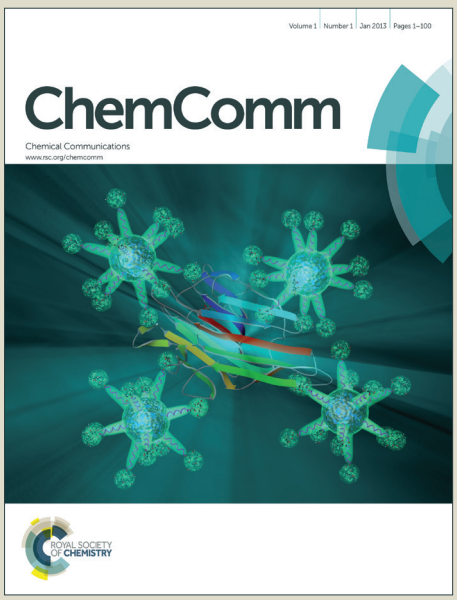

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Direct observation of the dual-switching behaviors corresponding to the state transition in a DNA nanoframe

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Yangyang Yang, [a] Masayuki Endo,* [bc] Yuki Suzuki,[ac] Kumi Hidaka [b] and Hiroshi Sugiyama*[abc]

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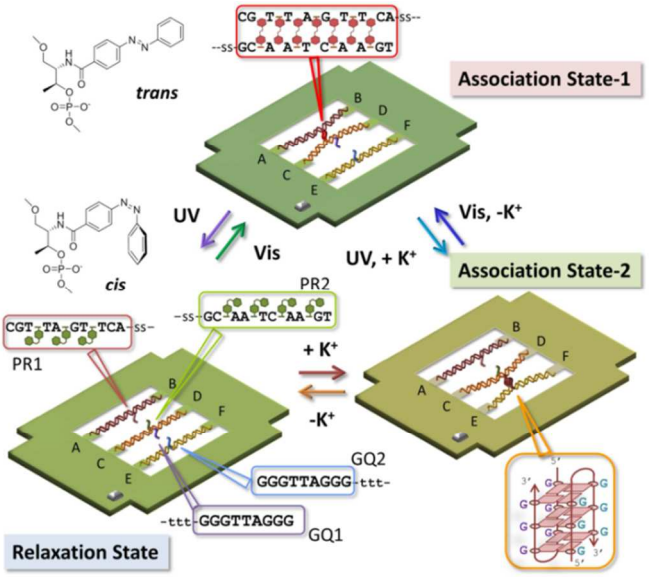
To create a nanoscale dual switch, two responsive DNA motifs, azobenzen-modified DNAs and G-telomeric repeat sequences, were introduced together into the nanoframe system. The dual-switching behaviors controlled by photoirradiation and K^+ were successfully visualized in real time by high-speed atomic force microscopy.

In recent years DNA-based biomaterials have been developed a lot and shown potential advantages over other synthetic materials.[1,2] In particular, various types of DNA based circuits have been reported for logical operations[3] or processing finite-state programs[4]. Currently, the DNA finite-state automaton often consists of free-floating DNA strands and is switched by specific triggers, such as aptamer-substrate complexes[5] and pH[6]. And the state change can then be read out as a quantitative and indirect output from fluorescence or gel electrophoresis.[4c] it is still often hard to achieve single-molecule level resolution and real-time detection. DNA origami in combination with high-speed atomic force microscopy (AFM) has been proven to allow for real-time monitoring DNA strand at single molecule level,[7] which provides a viable tool to decipher the DNA strand transformation. Previously, we have developed a series of frame-shaped DNA origami functionalized with DNA motifs, such as G-telomeric repeats,[8] photoresponsive oligonucleotides (ODNs),[9] and B-Z DNA transiting strands[10]. These nanosystems performed well to observe nanomechanical movements in response to one specific trigger at single molecule level in real-time.[11] In this study, we aim at examining the logical dual-switching behaviors in a unique nanoframe.

A nanoframe DNA origami observation system was designed to integrate photoresponsive ODNs and G-telomeric repeats together. As shown in Fig. 1, a vacant nanoframe holding three pairs of connection sites (A-B, C-D, and E-F) inside was integrated with three different (parallel) double-stranded DNAs (dsDNAs). Two dsDNAs containing different azobenzene-modified (pseudocomplementary) photoresponsive ODNs (PR-1 and PR-2) were hybridized to the A-B and C-D sites (Table S1; Fig. S12), respectively. The photoresponsive ODNs can hybridize in the *trans*-form or dissociate in the *cis*-form of azobenzene by photoirradiation with different wavelengths.[12] Meanwhile, two G-telomeric overhangs rendering G-quadruplex formation: GQ-1 and GQ-2 were introduced to the CD and EF strands, respectively (Table S1; Fig. S12).[13] The "kissing" and "unkissing" between AB and CD strands, representing Association State-1 (AS-1) and Relaxation State (RS), can be regulated by UV/visible light. The interaction between CD and EF can be switched by G-quadruplex via addition/removal of potassium ion, resulting in RS and Association State-2 (AS-2),



respectively. It was attempted to visualize the logical association/dissociation behaviors among three parallel dsDNAs analogous to finite state transition in real time.

Fig. 1 Schematic presentation of the dual-switching behaviors of three parallel dsDNAs in a nanoframe DNA origami system. There are three dsDNA configurations in a single nanoframe system, representing AS-1, RS and AS-2, respectively. Each state is triggered by combination of photo irradiation and K+.

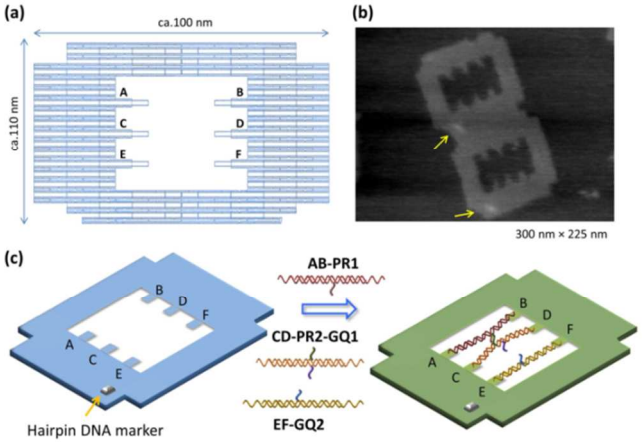


Fig. 2 (a) Design of nanoframe with six connection sites and (b) its AFM image. (c) three dsDNAs: AB, CD and EF, each carrying different DNA motifs assembled with nanoframe. AB: photoresponsive ODNs with three azobenzene molecules (PR-1); CD: photoresponsive ODNs with four azobenzene molecules (PR-2) and two G-telomeric repeats sequence (GQ1); EF: two G-telomeric repeats sequence (GQ2). A hairpin DNA marker was introduced adjacent to "E" site pointed by yellow arrow in the AFM image (b).

First, the nanoframe containing three pairs of connection sites along inner edges was designed (Fig. 2a), which was prepared by reported method[14]. The assembled structure was imaged by AFM (Fig. 2b and Fig. S1). A hairpin marker pointed by yellow arrows was introduced close to the "E" site in order to distinguish three dsDNAs. The final nanostructure was then constructed by hybridizing three different dsDNAs carrying pre-assigned DNA motifs with purified nanoframe and again confirmed by AFM.

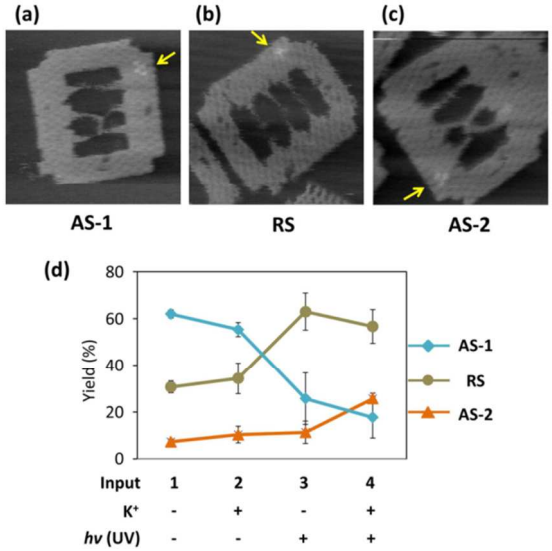


Fig. 3 AFM images of three states and the yield of them under four independent inputs. **Input-1:** without any stimulation; **Input-2:** under buffer containing 50 mM K^+; **Input-3**: UV irradiation for 10 min at 35 °C; **Input-4:** integrating 50 mM K^+ with UV irradiation for 10 min at 35 °C. The hairpin marker was pointed out by yellow arrow. The experiments were all repeated three times, and the error bars represent S.D. Image size: 150 nm × 150 nm.

As shown in Fig. 3 (Fig. S2), three patterns of nanostructures corresponding to AS-1, AS-2 and RS can be obtained. In primary samples (**input-1,** no triggering) the majority nanostructure (approximately 62%, Fig. 3d and S3, and Table S2) exists in AS-1 state, in which AB and CD were associated due to the hybridization of photoresponsive motif while the EF was left alone . By adding K^+ (50 mM, **input-2**) into the primary purified solution, the percentage of nanostructures at AS-1 state was slightly decreased while RS and AS-2 were slightly increased, indicating that the addition of K^+ alone had slight effect on the yields of three states. By exposing to photoirradiation (λ = 350 nm by band pass filter) for 10 minutes at 35 °C in the K^+-free buffer (**input-3**), the yield of AS-1 was decreased to 26% while RS was increased to 63% and AS-2 was slightly increased. Finally, by introducing K^+ (50 mM) and UV irradiation together (**input-4)**, the yield of AS-2 increased to 26%, which is 3-fold higher than the yield in **input-1**. The yields were all summarized in Fig. 3d, S4 and Table S2. From **input-4**, it can be seen that the cascading transformation from photoinduced dissociation to G-quadruplex formation was achieved via UV irradiation together with K^+ addition.

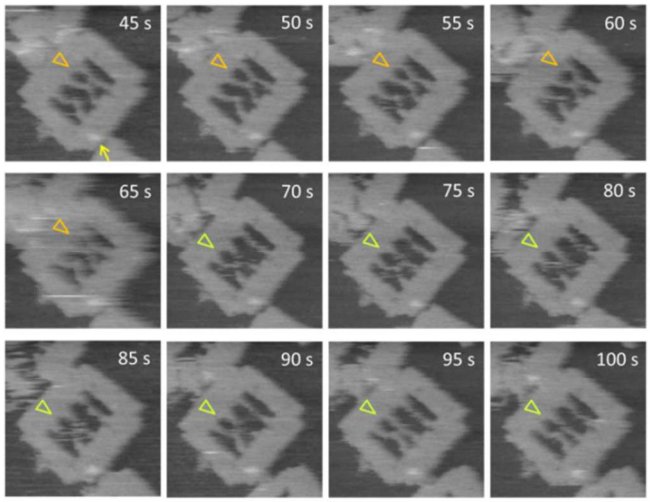
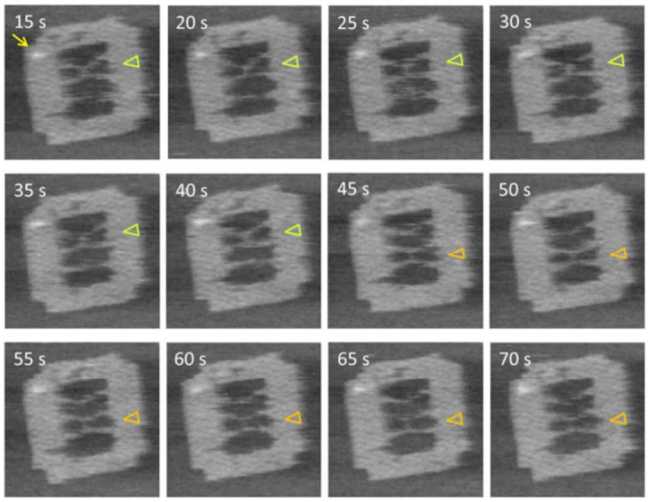


Fig. 4 Successive AFM images of the state change from AS-1 to AS-2 under buffer containing 10 mM K^+ with UV irradiation. Lapsed irradiation time was indicated on AFM image. The marker was pointed by yellow arrow. Green angle: association of AB and CD; orange angle: association of CD and EF; scanning rate: 0.2 frames/sec; image size: 180 nm ×180 nm.

Next, it was tried to visualize the logic-gated behaviors of three dsDNAs in the single nanoframe with the combination of K^+ and photoirradiation in real time similar as mechanical state transition. The conversions from any three states to other ones were initiated and directly monitored by the combinations of switching stimuli using high-speed AFM. The reversible conversion of AS-1 and RS was successfully observed by switching photoirradiation wavelength (see Fig. S7 and S8, Movies S1 and S2). And the transition between AS-2 and RS (Fig. S9 and S10, Movies S3 and S4) was also successfully monitored by regulating K^+.

The following is to directly observe dissociation of AB and CD and sequential association of CD and EF using the dual-switch of UV irradiation and K^+. The nanoframe sample was firstly loaded

on a mica plate in K^+-free buffer. The high-speed scanning was carried out under the observation buffer containing 10 mM K^+ together with UV-irradiation (λ = 330 – 380 nm) for the *trans*- to *cis*-form isomerization of azobenzene molecules. The AFM images were obtained at a scan rate of 0.2 frames per second. After focusing on a nanoframe in the formation of AS-1, the AB and CD were unkissed during the UV irradiation (65 s - 70 s) owing to the dissociation of photoresponsive ODNs and the CD



immediately associated oppositely with EF (70 s) because of the formation of G-quadruplex in the presence of K^+ (Fig. 4 and Movie S5). The nanoframe was regulated to convert from "AS-1" to "AS-2" in a logical manner with the dual-switch of UV-photoirradiation and K^+. The state changing from AS-1 to AS-2 was directly visualized, during which no other transition state analogues were observed.

Fig. 5 Reverse AFM images of the state change from AS-2 to AS-1 under buffer without K^+ using visible light irradiation. Lapsed irradiation time was indicated on AFM image. The marker was pointed by yellow arrow. Green angle: association of AB and CD; orange angle: association of CD and EF; scanning rate: 0.2 frames/sec; image size: 180 nm ×180 nm.

The reversible conversion from "AS-2" to "AS-1" was also examined. By introducing K^+-free buffer for observation and alternating irradiation wavelength to visible light (λ= 440 – 470 nm) by bandpass filters, a nanoframe in the formation of RS-2 (pre-treated with UV irradiation and K^+) was focused and the AFM images were obtained under the same scanning rate. The scanning results were shown in Fig. 5 (Movie S6). At the beginning, the central position of CD and EF were first connected because of G-quadruplex formation. Under K^+-free buffer together with visible light irradiation, CD and EF were dissociated immediately and meanwhile AB and CD were associated (40 s – 45 s) because of the sequential hybridization of photoresponsive ODNs. Besides the above fast transition, another type of changes from AS-2 to AS-1 (Fig. S11, Movie S7) were also observed, in which the CD and AB did not associate immediately after the disruption of G-quadruplex, but the CD vacillated between AS-1 and an unidentified state for around 15 sec before the final formation AS-1. The difference of reversible changes between these two states might be attributed to different association/dissociation manners of two kinds of switching motifs. Finally, the sequential state transitions between AS-1 and AS-2 were directly visualized reversibly.

In conclusion, a two-step cascading transformation reaction was successfully performed in a single DNA nanostructure system by integrating two reversible switches: wavelength dependent photo-switch and metal ion dependent switch (K^+). Furthermore, a series of the dual-switching logical behaviors corresponding to conformational change were reversibly observed by cooperating high-speed AFM with DNA origami methodology. It was also displayed a new approach to trace signal conversion in DNA computing. Although the response of the self-assembled molecular devices is basically slow compared with the electronic devices, these logical molecular systems can be applied for creating versatile devices that sense various external environments.

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Notes and references

[a] Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan.
[b] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Yoshida-ushinomiyacho, Sakyo-ku, Kyoto 606-8501, Japan.
[c] CREST, Japan Science and Technology Corporation (JST), Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan.

† Electronic Supplementary Information (ESI) available: Additional AFM images, HS-AFM movies, and DNA sequences of DNA substrates and origami scaffold. See DOI: 10.1039/c000000x/

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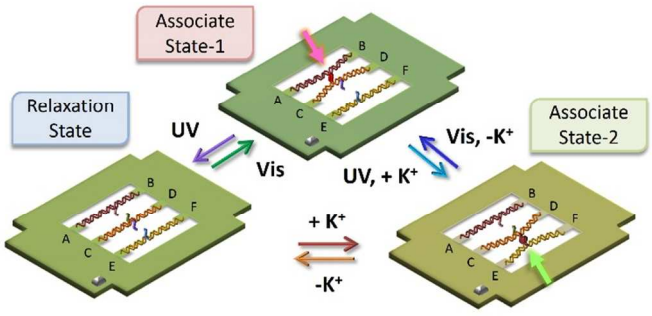
Graphical Abstract



The dual-switching behaviors of photoresposive DNAs and G-quadruplex in the DNA nanoframe were successfully visualized by high-speed atomic force microscopy.