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Robust and Photocontrollable DNA Capsules Using Azobenzenes

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ABSTRACT Various three-dimensional structures have been created on a nanometer scale using the self-assembly of DNA molecules. However, ordinary DNA structures breakdown readily because of their flexibility. In addition, it is difficult to control them by inputs from environments. Here, we construct robust and photocontrollable DNA capsules using azobenzenes. This provides a method to construct DNA structures that can survive higher temperatures and can be controlled with ultraviolet irradiation.

KEYWORDS 3D DNA structures, photo control, azobenzene, fast-scanning atomic force microscopy, melting temperature

DNA strands hybridize with each other in a sequence-specific manner; consequently, the binding between rationally designed DNA strands can be controlled to allow the assembly of various structures.[1] This makes DNA molecules some of the most powerful building blocks available for assembling new structures on a nanometer scale such as two-dimensional periodic patterns,[2−6] defined complex patterns,[7] and tube-shaped structures.[8] Many three-dimensional structures have also been created by folding single-stranded DNA,[9] assembling some DNA sequences,[10,11] or using DNA origami[12−15] or multibranched DNA motifs.[16−18] These structures are small and complex enough for applications such as drug delivery and nanoscale fabrication. However, ordinary DNA structures breakdown readily because of their flexibility. In addition, it is difficult to control them by inputs from environments.

Here, we construct robust and photocontrollable DNA capsules using azobenzenes. The robustness and photocontrollability of the capsules are clearly demonstrated by measuring the melting temperature (T_M) and using agarose gel electrophoresis and atomic force microscopy (AFM). Our DNA capsules with azobenzenes can survive higher temperature. A moving image obtained using fast-scanning AFM shows how the DNA capsules break into small structures on ultraviolet (UV) irradiation. Our strategy of using azobenzenes is universal in the sense that other structures can achieve this robustness and photocontrollability by inserting azobenzenes into their sequences. This enhances the potential for DNA structures to be used in technical applications such as drug-delivery systems as carriers.

To develop DNA capsules, we focused on polyhedra derived from three-branched structures called three-point-star motifs, which hybridize to one another and form tetrahedra, dodecahedra, and buckyballs, depending on their loop length and concentration.[16] Azobenzenes change their conformation from the trans to the cis form on UV irradiation at 300−400 nm, and from the cis to the trans form on visible light irradiation, at wavelengths >400 nm. When azobenzenes are inserted into a DNA strand, the cis-form prevents the formation of a double-stranded structure.[19] Consequently, the hybridization of DNA strands can be controlled by UV irradiation, allowing the formation of photocontrollable DNA structures.[20] In our system, azobenzenes were inserted into the sticky ends of three-point-star motifs. We expected DNA capsules to form and then breakdown into three-point-star motifs on UV irradiation (Figure 1). Additionally, azobenzenes can stabilize double-stranded DNA via stacking interactions.[21] This can be used to produce stable DNA structures.

To confirm the formation of DNA capsules, we observed the assembled structures of L&M&S-1azo (azobenzene-poor) and L&M-2azo&S-2azo (azobenzene-rich) using AFM (Figure 2). Both L&M&S-1azo and L&M-2azo&S-2azo formed sphere-shaped DNA capsules in air. As expected, the heights of both sphere-shaped structures were roughly twice those of three-point-star motifs (right in Figure 2). However, the yield of L&M&S-1azo was poorer than that of L&M-2azo&S-2azo. This was probably due to the position of the azobenzenes rather than the number of azobenzenes (see Figure 4).

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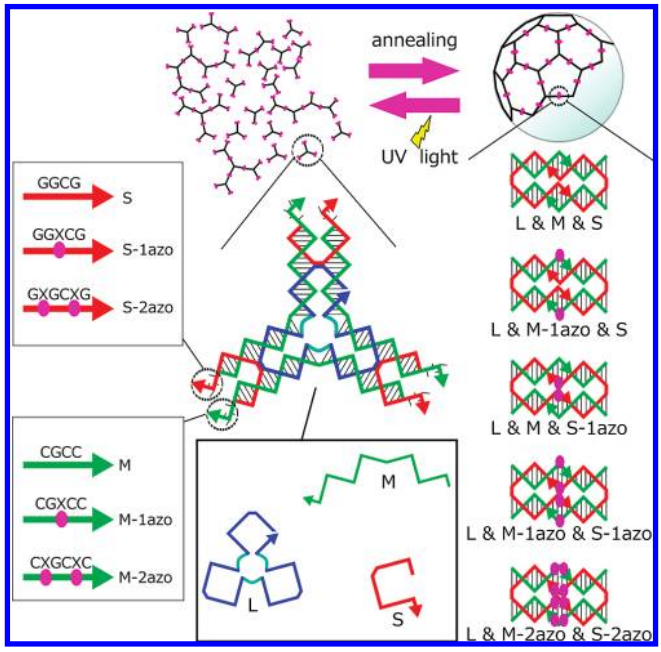

FIGURE 1. Design of DNA capsules using azobenzenes. The purple circles and X denote azobenzenes. A 1:3:3 mixture of L, M, and S (by concentration) formed three-point-star motifs, which further hybridized to one another to form polyhedra.[16] The sequences M, M-1azo, and M-2azo contained 0, 1, and 2 azobenzenes, respectively, as did the sequences S, S-1azo, and S-2azo. L&α&β (α is M, M-1azo, or M-2azo; β is S, S-1azo, or S-2azo) denotes a mixture of L, α, and β at a concentration ratio of 1:3:3.

The robustness of the DNA capsules made with azobenzenes was confirmed by measuring T_M. The T_M values between the sticky ends of three-point-star motifs were measured using other DNA sequences that form hairpin structures (see Experimental Section). In Table 1, hpM&hpS, hpM-1azo&hpS, hpM&hpS-1azo, hpM-1azo&hpS-1azo, and hpM-2azo&hpS-2azo correspond to the hybridization between the sticky ends of three-point-star motifs formed by L&M&S, L&M-1azo&S, L&M&S-1azo, L&M-1azo&S-1azo, and L&M-2azo&S-2azo, respectively. All of the T_M values of the azobenzene-modified strands were higher than that of the original strands without azobenzenes. This result supported our hypothesis that azobenzenes stabilize DNA structures and was consistent with previous reports.[21] Interestingly, the T_M of hpM-2azo&hpS-2azo, which contains four azobenzenes, was higher than that of hpM&hpS by as much as 6.7 °C, indicating that much more stable capsules were formed by using L&M-2azo&S-2azo. In fact, the T_M of DNA capsules formed by L&M-2azo&S-2azo was much higher (56.3 °C) than that formed by L&M&S (50.1 °C), although the melting profiles were not as clear (see Supporting Information).

The stabilizing effects did not increase in proportion to the number of azobenzenes. The structures formed by hpM-1azo&hpS were much more stable than those formed by hpM&hpS-1azo, although they had the same number of azobenzenes. This indicates that the stability of the DNA capsules was also affected by the position of the azobenzenes in the sequences. This is probably because the positioning of the azobenzenes had a greater effect on the conformation of the assembled structure in L&M&S-1azo than in L&M-1azo&S.

The photocontrollability of the DNA capsules was clearly demonstrated using fast-scanning AFM and agarose gel electrophoresis at 4 °C (Figure 3 and Figure 4). In Figure 3, the assembled L&M-2azo&S-2azo still formed sphere-shaped structures for 49 s without UV irradiation (see Supporting Information for the moving image). This shows that the fast-scanning AFM observation did not destroy the DNA capsules. However, soon after UV irradiation (t = 50 s), the capsules started to breakdown into three-point-star motifs, revealing the photocontrollability of the capsules. The height of a DNA capsule before UV irradiation was roughly twice that after UV irradiation (see the lower images in Figure 3). This also proves that before UV irradiation the assembled structures successfully formed the expected DNA capsules rather than single-layered sheetlike structures.

Figure 4 shows that other DNA capsules can be also controlled by using UV light. Before UV irradiation, each lane had two bands: the upper band corresponded to DNA capsules and the lower band corresponded to thrree-point-star motifs. The brightness of the bands containing L&M-2azo&S-2azo was weak because azobenzenes quench fluorescence. The 12 azobenzenes in one three-point-star motif formed by L&M-2azo&S-2azo greatly weakened the strength of the fluorescence from SYBR Gold. As expected, only DNA capsules formed by azobenzene-containing sequences were destroyed by UV light irradiation. Despite the stability of DNA capsules formed by L&M-2azo&S-2azo (see Table 1), they were more sensitive to UV irradiation than other DNA capsules. In fact, they were destroyed by UV irradiation for only 3 s. This is probably because L&M-2azo&S-2azo contained the most azobenzenes (12 in each three-point-star motif) of the five structures. Thus, azobenzene-rich sequences can both stabilize the assembled structures and increase their sensitivity to UV light.

In contrast, azobenzene-poor sequences were insensitive to UV irradiation. Nonetheless, the DNA capsules formed by L&M&S-1azo could be broken easily. This is probably due to their instability and the inefficiency of capsule formation. The structures formed by L&M-1azo&S were difficult to break because of their stability (Table 1) and insensitivity to UV light, because they had the fewest azobenzenes (three in each three-point-star motif).

The yields of DNA capsules were 49.4 ± 3.3% (L&M&S), 45.4 ± 2.0% (L&M-1azo&S), 22.0 ± 5.5% (L&M&S-1azo), 39.7 ± 3.5% (L&M-1azo&S-1azo), and 45.9 ± 3.6% (L&M-2azo&S-2azo). These were lower than those obtained by He et al.[16] In particular, without azobenzenes (i.e., original DNA buckyballs), our yield was also worse (49.4%) than theirs (68.9%). We believe that our experimental conditions, especially the DNA concentrations, were not as optimal as theirs. Additionally, the yields differed depending on the

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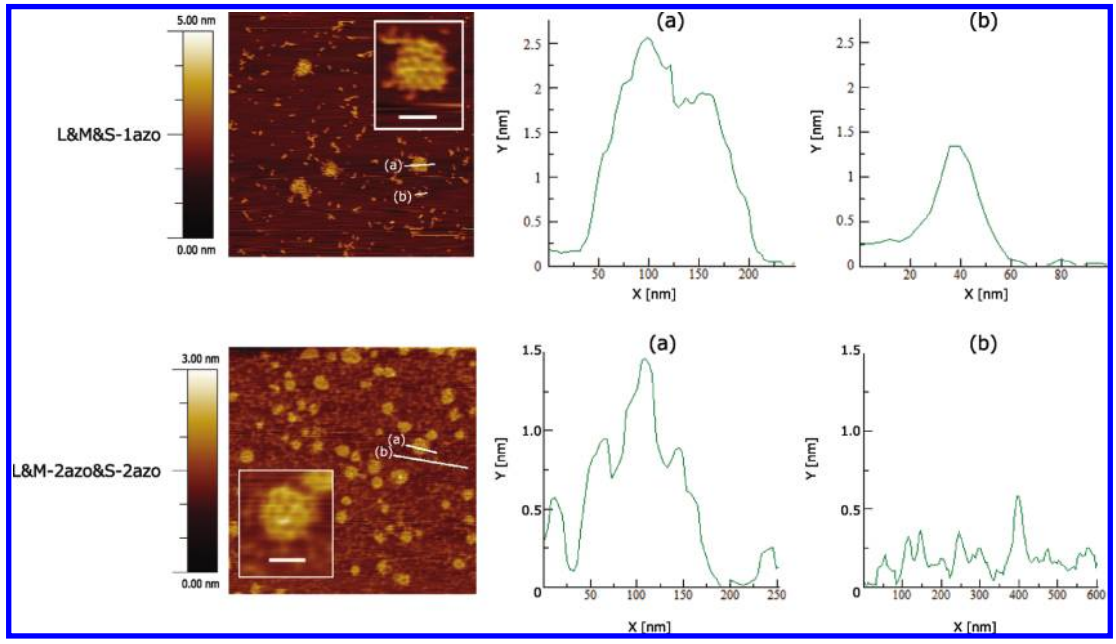

FIGURE 2. The leftmost figures are AFM images of L&M&S-1azo (upper) and L&M-2azo&S-2azo (lower) in air. The scale bars in the insets are 100 nm. The heights at the lines denoted by (a) and (b) in the AFM images are shown in the two rightmost figures.

TABLE 1. Melting Temperatures (T_M) between Two Sticky Ends with or without Azobenzenes

name of hairpin	hpM&hpS	hpM-1azo&hpS	hpM&hpS-1azo	hpM-1azo&hpS-1azo	hpM-2azo&hpS-2azo
no. of Azos	0	1	1	2	4
T_M (°C)	37.3	40.4	38.5	39.2	44.0

number and position of azobenzenes. In particular, the yield of DNA capsules formed by L&M&S-1azo was lower than the others. The upper band of L&M&S-1azo was not clear, indicating that the main product was not a DNA capsule. This result was consistent with the AFM results, which showed only a few DNA capsules (Figure 2). The difference in yields of DNA capsules is probably due to the effect of the position of azobenzenes on the conformation of DNA capsules. The stabilization effect of the position of the azobenzenes needs further analysis.

In conclusion, we successfully used azobenzenes to construct robust and photocontrollable DNA capsules. We showed that the number and position of azobenzenes in the capsules dramatically affected their stability and sensitivity to UV light and demonstrated that azobenzenes can stabilize DNA capsules. These results suggest that using azobenzene-rich DNA strands enables us to form DNA capsules that can be opened at a designated time and place by short exposure to UV irradiation and that are robust against changes in the environment such as temperature and ionic strength. This strategy is applicable to other DNA motifs such as DX tiles,[2] cross tiles,[4] and five-point-star motifs[17] on inserting azobenzenes into the sticky ends. This enhances the potential for DNA structures to be used in technical applications, such as drug-delivery systems as carriers.

Experimental Section. ***Oligonucleotides.*** The DNA sequences used in this study were the same as those described by He et al.:[16] central long-strand L, AGG CAC CAT CGT AGG TTT CTT GCC AGG CAC CAT CGT AGG TTT CTT GCC AGG CAC CAT CGT AGG TTT CTT GCC; medium strand M, TAG CAA CCT GCC TGG CAA GCC TAC GAT GGA CAC GGT AAC GCC; and short peripheral strand S, TTA CCG TGT GGT TGC TAG GCG. To confirm the formation of a single three-point-star motif, we prepared a short strand without a sticky end (S-noEnd): TTA CCG TGT GGT TGC TA. DNA sequences modified with azobenzenes were also prepared: medium strand with one azobenzene (M-1azo), TAG CAA CCT GCC TGG CAA GCC TAC GAT GGA CAC GGT AAC GXC C; medium strand with two azobenzenes (M-2azo), TAG CAA CCT GCC TGG CAA GCC TAC GAT GGA CAC GGT AAC XGC XC; short strand with one azobenzene (S-1azo), TTA CCG TGT GGT TGC TAG GXC G; short strand with two azobenzenes (S-2azo), TTA CCG TGT GGT TGC TAG XGC XG, where X denotes the azobenzene. All azobenzenes were inserted into the sticky ends of three-point-star motifs.

Sample Preparation. Oligonucleotides without azobenzenes were supplied by Sigma-Aldrich, Japan, and synthesized using polyacrylamide gel electrophoresis (PAGE) purification. Oligonucleotides with azobenzenes were supplied by Tsukuba Oligo Service and purified by high-performance liquid chromatography (HPLC). All oligonucleotides were dissolved in Tris-acetate-EDTA-Mg^{2+} (TAE/Mg^{2+}) buffer containing 40 mM Tris-acetate (pH 8.0), 1 mM EDTA, and 12.5 mM magnesium acetate. The oligonucleotide concentrations were determined by the difference in absorbance at 260 and 320 nm using extinction coefficients calculated from the nucleotides and

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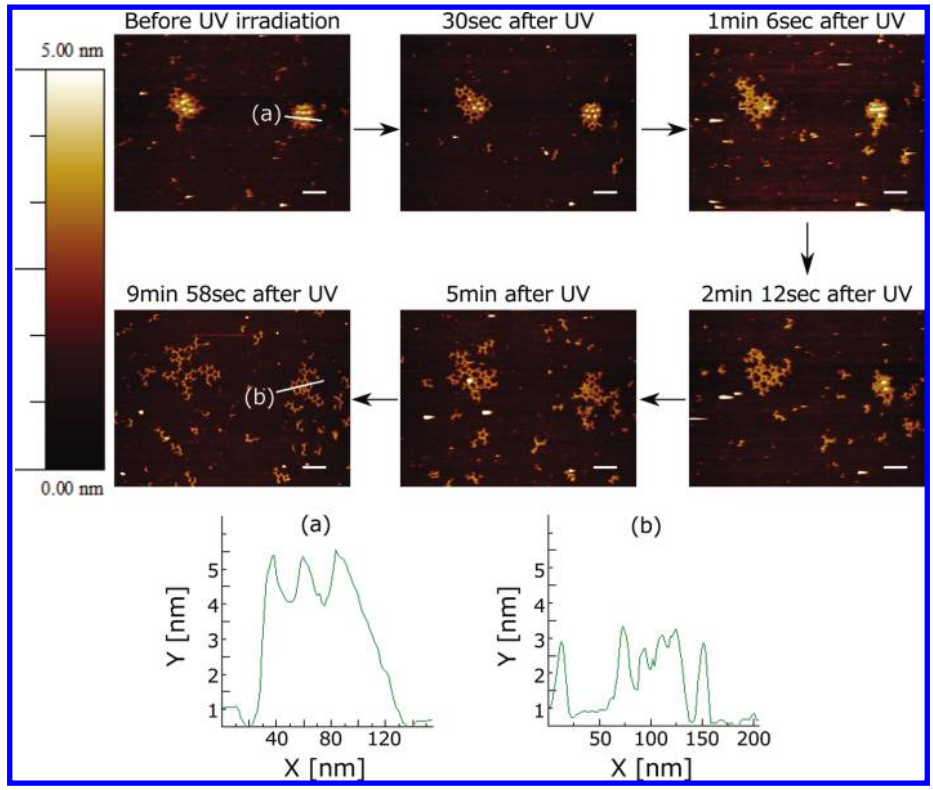

FIGURE 3. The upper figures are AFM images of azobenzene-rich DNA capsules (L&M-2azo&S-2azo) using fast-scanning AFM. Scale bars are 100 nm. The lower figures are the heights at the lines denoted by the symbols (a) and (b) in the AFM images.

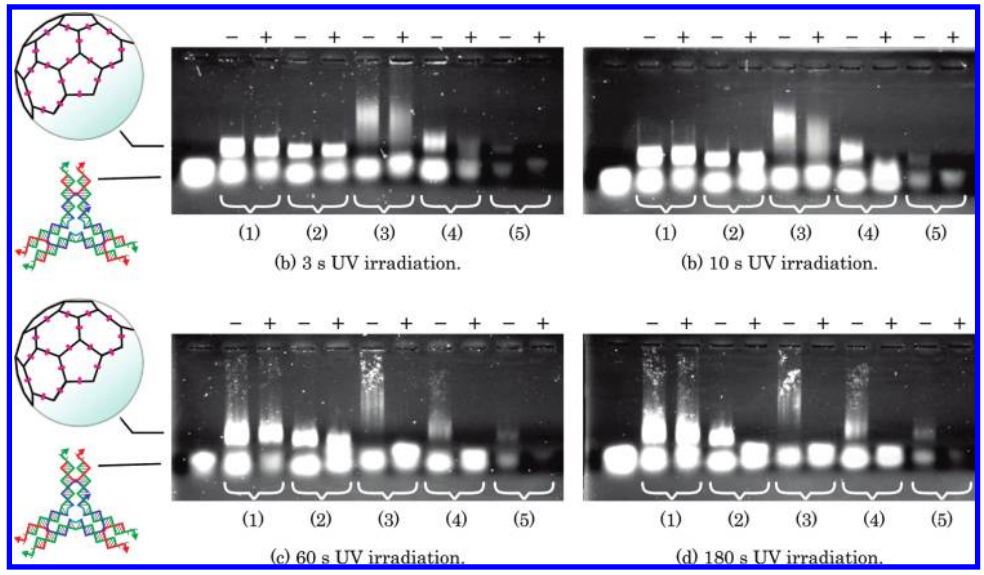

FIGURE 4. Agarose gel electrophoresis (0.5%) analysis of DNA capsules at 4 °C with different lengths of UV irradiation (shown at the bottom of the gel image). The symbols − and + at the top of the gel image denote without UV irradiation and with UV irradiation, respectively. Single bands in the leftmost lane in all gels correspond to three-point-star motifs formed by L&M&S-noEnd. Because the sequence S-noEnd did not have a sticky end, the assembled structure of L&M&S-noEnd remained as a single three-point-star motif. Pairs of lanes from left to right correspond to (1) L&M&S, (2) L&M-1azo&S, (3) L&M&S-1azo, (4) L&M-1azo&S-1azo, and (5) L&M-2azo&S-2azo.

dinucleoside phosphates based on a nearest-neighbor approximation.[22] The absorbance was measured at 90 °C on a Shimadzu UV-1650PC spectrophotometer to prevent the oligonucleotides from forming secondary structures.

Self-Assembly of DNA Complexes. DNA sequences, L, α (α is M, M-1azo or M-2azo), and β (β is S, S-1azo or S-2azo), were mixed at concentrations of 500 nM, 1.5 μM, and 1.5 μM, respectively. Under these conditions, three-point-star

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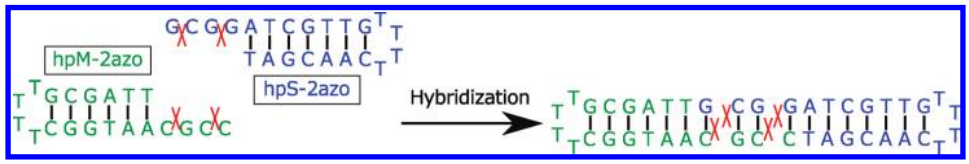

FIGURE 5. Hybridization between two hairpins, hpM-2azo and hpS-2azo. The X denotes azobenzenes.

motifs form buckyballs.[16] The mixed solutions were annealed from 95 °C to room temperature over 48 h using a hot water bath made of Styrofoam. The annealing schedule was confirmed by measuring the temperature with a data logger SuperThermochron (KN Laboratories).

AFM. Because AFM images are very sensitive to the experimental conditions such as probes, we performed the AFM observations carefully using various settings. The images in Figure 2 were obtained under dry conditions in AC mode on an MFP-3D AFM (Asylum Research) using NCH probes (NANOSENSORS). A 2 μL drop of DNA solution was spotted onto a freshly cleaved mica surface and left for 10 min to achieve adsorption. Then the drop was washed twice with 100 μL of 2 mM magnesium acetate solution and dried with compressed air. The images in Figure 3 and the movie in the Supporting Information were obtained under wet conditions using fast-scanning AFM (Nano Live Vision, RIBM) using BL-AC10EGS probes (Olympus). The scanning rate was 0.5 frames/s. A 1 μL drop of 2 mM $Mg(CH_3COO)_2$ was spotted onto a freshly cleaved mica surface. Then 1 μL of DNA solution diluted five times was added and left for 10 min to achieve adsorption. AFM imaging was performed with an additional 80 μL of 2 mM $Mg(CH_3COO)_2$. The image shown in Supplementary Figure 1 (Supporting Information) was taken using a 2 μL drop DNA solution spotted onto a freshly cleaved mica surface with 30 μL of TAE/Mg^{2+} buffer. DNA samples were imaged under wet conditions in tapping mode on a Multimode AFM with a Nanoscope IIIa controller (Veeco) using BL-AC40TS probes (Olympus). The images shown in Supplementary Figure 2 (Supporting Information) were obtained under wet conditions using dynamic mode AFM operated in the frequency modulation detection technique using NCH probes (Nanoworld). The AFM was modified so that it was suited for in-liquid measurements, based on a commercial AFM system (JEOL: JSPM-5200). A 2 μL drop of DNA solution was spotted onto a freshly cleaved mica surface and left for 10 min to achieve adsorption. Then 30 μL of TAE/Mg^{2+} buffer with 1 μL of 50 mM Ni^{2+} was added. The AFM images in Figure 2 and Figure 3 were analyzed using WSxM software.[23]

T_M Measurement. Because three-point-star motifs hybridize to one another cooperatively and form into a large structure (polyhedron), it is difficult to analyze the hybridization between two sticky ends of three-point-star motifs. To simplify the measurement of T_M between the sticky-ends, we prepared special sequences that formed hairpin structures: hpM, TTA CCG TTT TCG GTA ACG CC; hpS, TAG CAA CTT TTG TTG CTA GGC G; hpM-1azo, TTA CCG TTT TCG GTA ACG XCC; hpS-1azo, TAG CAA CTT TTG TTG CTA GGX CG; hpM-2azo, TTA CCG TTT TCG GTA ACX GCX C; and hpS-2azo, TAG CAA CTT TTG TTG CTA GXG CXG, where X denotes the azobenzene. The sticky ends of these hairpin structures were designed to be the same as the sticky ends of the three-point-star motifs. Consequently, the hairpin formed by hpM, hpM-1azo, or hpM-2azo further hybridizes to that formed by hpS, hpS-1azo, or hpS-2azo (see Figure 5). This hybridization process reveals a more simple melting profile than the hybridization of three-point-star motifs. Thus, this hairpin system was relatively easy to analyze. The T_M values at 2 μM except for that of hpM-2azo&hpS-2azo were measured at 260 nm in TAE/Mg^{2+} buffer. For hpM-2azo&hpS-2azo, it was difficult to measure the T_M at 260 nm, because it was very close to that of the hairpin structure itself. Instead, we measured the T_M at 320 nm. When two azobenzenes interact with each other, the absorbance at 320 nm is changed by the interaction. In contrast, the absorbance of DNA sequences at 320 nm is very small and can be negligible. Thus, the hybridization between two sticky ends formed by hpM-2azo&hpS-2azo can be monitored by the absorbance change at 320 nm caused by the interaction between azobenzenes.

UV Light Irradiation. For gel electrophoresis, UV light at a wavelength of 365 nm was irradiated using an OMRON ZUV-C20H UV-LED irradiator with a ZUV-H10 M head unit and ZUV-L6T lens, after annealing. To increase the efficiency of UV irradiation, 10 μL of DNA solution was used. For fast-scanning AFM, we used another UV irradiator system that is optionally supplied with the AFM: a fluorescence mirror unit (U-MWU2, Olympus), a mercury lamp (USH-1030 L, Olympus), a mercury lamp housing (U-LH100HGAPO, Olympus), and a power supply unit (U-RFL-T, Olympus).

Agarose Gel Electrophoresis. Gels containing 0.5% agarose were run on a *M*upid-exu electrophoresis unit (Advance) at 4 °C and a constant voltage of 50 V for 75 min. The running buffer was TAE/Mg^{2+}. After electrophoresis, the gels were stained with SYBR Gold for 25 min and scanned. The bands were quantified using the ImageJ 1.43u software (National Institutes of Health, USA).

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us how to use AFM, and Motoharu Shichiri (RIBM) for taking some AFM images.

Supporting Information Available. Detailed structures of a three-point-star motif and an azobenzene, other AFM images of the structures formed by L&M&S-1azo or L&M-2azo&S-2azo, a melting curve of DNA capsules formed by L&M&S or L&M-2azo&S-2azo, and the movie file produced by fast-scanning AFM, which clearly demonstrated how the DNA capsules formed by L&M-2azo&S-2azo broke down. This material is available free of charge via the Internet at http://pubs.acs.org.

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