J|A|C|S ARTICLES

Published on Web 03/05/2010

DNA Scissors Device Used to Measure MutS Binding to DNA Mis-pairs

Hongzhou Gu,[†] Wei Yang,[‡] and Nadrian C. Seeman*,[†]

Department of Chemistry, New York University, New York, New York 10003, and Laboratory of Molecular Biology, NIDDK, National Institutes of Health, 9000 Rockville Pike, Bldg 5, Rm B01-13, Bethesda, Maryland 20892

Received December 10, 2009; E-mail: ned.seeman@nyu.edu

Abstract: MutS is a DNA repair protein that recognizes unpaired and bulged bases. When it binds to DNA, it bends the double helix. We have developed a novel DNA-based nanomechanical device that measures the amount of work that a DNA-bending protein can do when it binds to the double helix. The device we report here is a scissors-like device consisting of two double-crossover (DX) molecules connected to each other by a flexible Holliday junction. The two DX components are connected by a double helix that contains the binding site for MutS; when the binding site duplex is bent, the scissors contracts. The two DX molecules are also joined by sticky ends on an edge adjacent to the binding site; the sticky ends can be disrupted if the protein binds with sufficient free energy. Those sticky ends are flanked by a pair of dyes; when the sticky ends are disrupted, the dyes separate, and the fluorescence resonance energy transfer signal can monitor the disruption. The strength of the sticky ends is readily varied, so that the ability of the protein to disrupt them can be quantitated. We use this device to measure work in conjunction with a second device that measures the bending angle resulting from protein binding, so as to calibrate the system. Our data are in good agreement with previous measurements of MutS binding, indicating that this device is able to measure the strength of binding correctly.

DNA-based nanomechanical devices are capable of performing tasks and measurements of biophysical interest; a variety of such devices have been reported in the past few years (e.g., ref 1). Several years ago, we built a device that can be used to estimate the amount of work that a protein can do if it bends DNA when it binds to it;[2] this measurement is closely related to the free energy, ΔG, of the interaction. The protein used in that initial study was integration host factor (IHF). The device contains a binding site for the protein that is flanked by a pair of triple-crossover (TX) DNA motifs, as seen in Figure 1a. The binding event causes the separation of the relative positions of the two TX components, as shown in Figure 1b. A dye is attached to each of the TX motifs (Figure 1a), and the change in separation of the two motifs (D_B and D_A, before and after, in Figure 1, a and b, respectively) can be monitored by fluorescence resonance energy transfer (FRET); the FRET signal is decreased when the TX motifs separate. The double-helical domains of the TX components furthest from the binding domain are connected weakly by cohesive ends. By varying the length (and thereby the strength) of this cohesive tract, it is possible to estimate the amount of additional work that can be done by a protein binding to the DNA binding site (Figure 1b). The strength of the cohesive ends can be calculated from the data collected by SantaLucia and his colleagues[3] that measure the sequence dependence of DNA hybridization free energy. By using a series of cohesive ends of increasing strength, the ability of the protein to separate them can be titrated, until it fails completely to decrease the FRET signal.[2]

Here we extend this type of analysis to a new protein, MutS, which recognizes a variety of mismatched and unpaired DNA base interactions.[4] Its crystal structure in complex with mismatches has been determined,[5] and extensive thermodynamic data on its interaction with various substrates exist.[6] Thus, it would seem to be an ideal system to characterize with this approach. However, before applying the approach to MutS, it was necessary to generalize it, by converting the simple device used previously to a scissors-like molecule. This alteration retains the nature of the reporting signal (decreasing FRET with increasing cohesive strength based on a separation event), while rendering the device insensitive to the side of DNA to which the protein binds and the direction in which it bends the DNA; in addition, the scissors-like molecule allows a much larger protein to be characterized.

MutS is a large protein ($\sim$125 $\times$ 90 $\times$ 70 Å) that bends DNA in the same direction as IHF (toward the major groove), but it is positioned on the other side of the helix[5] from IHF. It is too large to be accommodated into the gap of the TX device unless the gap is extended markedly. Such an extension is likely to make the extended DNA construct more flexible, thus compro-

[†] New York University.
[‡] New York University.

(1) Seeman, N. C. *Trends Biochem. Sci.* **2005**, *30*, 119–235.
(2) Shen, W. Q.; Bruist, M. F.; Goodman, S. D.; Seeman, N. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 4750–4752.
(3) SantaLucia, J., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1460–1465.
(4) Modrich, P.; Lahue, R. *Annu. Rev. Biochem.* **1996**, *65*, 101–133.
(5) Obmolova, G.; Ban, C.; Hsieh, P.; Yang, W. *Nature* **2000**, *407*, 703–710.
(6) Schofield, M. J.; Brownewell, F. E.; Nayak, S.; Du, C.; Kool, E. T.; Hsieh, P. *J. Biol. Chem.* **2001**, *276*, 45505–45508.

10.1021/ja910188p

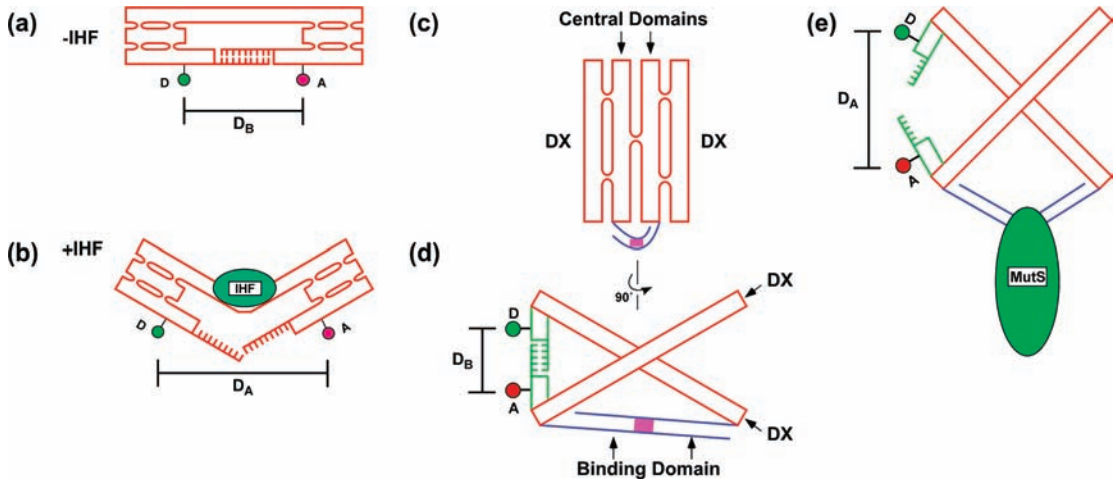

Figure 1. Scissors-based device to measure the work done by DNA-bending proteins. Double-helical DNA is shown as rectangular boxes. The double-crossover motifs (DX) are shown as two fused rectangular boxes. The triple-crossover motifs (TX) are shown as three fused rectangular boxes. Panels (a) and (b) show the TX device[2] used to measure the work done by IHF. The FRET pair is shown as green (donor) and magenta (acceptor) filled circles. The distances between the pair are indicated in (a) as D_B (before binding IHF) and (b) as D_A (after binding IHF). The sticky ends are indicated at the bottom of (a), and their disruption is indicated at the bottom of (b). Panels (c), (d), and (e) show the scissors-like device built in this work. Panel (c) is a view of the device showing that it consists of two DX molecules joined by a flexible Holliday junction. The binding DNA domain containing the MutS recognition site is shown in blue, and the actual binding site is indicated by a lavender segment. The DX blades of the scissors are labeled, and the central domains are labeled. Panel (d) shows a view rotated 90° about the vertical direction. The FRET pair is indicated again as green (D) and a magenta (A) filled circles separated by distance D_B. They are attached to a green domain containing the cohesive ends. The MutS binding domain is shown again as a blue curve, and the binding site is again a lavender portion within that linear duplex domain. The scissors-like nature of the device is evident. Panel (e) shows that when MutS binds, the scissors contracts, lengthening the distance between the dyes from D_B to D_A by disrupting the cohesive ends.

mising the structural integrity of the device. Consequently, we have taken a different approach and have designed a new device.

Design of the Device

We have replaced the simple TX-based device with a scissors-like machine. This device consists of two double-crossover (DX) molecules joined by a flexible Holliday junction. Figure 1c is a view showing the double connections of the helices that form the DX motifs, as well as the single Holliday junction in the middle that joins the two DX motifs. Each DX molecule is a stiff motif[7] that rigidly reflects the state of the scissors device, open or closed. A DNA duplex (blue), containing the *Taq* MutS binding site (magenta), acts as a bridge to connect the ends of the central double-helical domains of the DX components. Figure 1d shows a view perpendicular to the view in Figure 1c, corresponding to a 90° rotation about the vertical. It illustrates that the two DX molecules are likely to form an angle of about 60° with each other.[8,9] Figure 1d also shows another DNA duplex component (green) that was occluded in Figure 1c. This piece of DNA contains cohesive ends similar to those in Figure 1a; it bridges the ends of the central domains of the DX components on an adjacent side of the scissors. Pendent from the green domains are a pair of fluorescent dyes that are close enough (distance D_B) for energy to transfer between them, thus leading to a FRET signal.

Figure 1e shows that when MutS binds, the scissors contracts somewhat because the protein bends the DNA; this bending correspondingly lengthens the distance between the dyes and disrupts the sticky ends. The drawing schematizes the effect of binding MutS to the molecule: The bridge is distorted, causing the contraction of the scissors device, which in turn leads to an increase in the distance between the dyes (D_A) and a corresponding decrease in the FRET signal. The efficiency of the transfer (E_{FRET}) depends inversely on the sixth power of the distance separating the fluorophores[10] and is given by $E_{FRET} = R_0^6/(R_0^6 + R^6)$, where R is the scalar distance between the fluorophores and R_0 is the characteristic Förster distance for the donor−acceptor combination at which E_{FRET} equals 0.5. $R_0 =$ 56 Å for the pair of FRET dyes (fluorescein and cyanine-3) used in our study.[11]

Figure 1, d and e, also illustrates the way the scissors device is used to measure the amount of work done when MutS binds. The left sides of the two DX molecules are connected by a pair of cohesive strands. The cohesion can be broken when MutS binds to its recognition site on the bridging binding domain. If an excess of free energy is available when MutS binds, it can disrupt the base pairs. However, if the binding free energy of MutS is not larger than that of the DNA cohesion, the FRET signal will not change. Intermediate changes in the FRET signal have been interpreted to reflect part of the ensemble of devices being separated, but not all of it.[2] By using a range of cohesive lengths, each with a different free energy of association, one can use this nanodevice to estimate the amount of work of which MutS is capable when it binds to DNA.

Figure 2 shows the two scissors devices used in this work. Figure 2a shows the sequence of the scissors device lacking a cohesive domain, which we use to estimate the bending angle of the binding domain, termed the "angle device". By contrast, Figure 2b contains the sequence of a scissors device that contains a cohesive domain and is used to measure the work done by the protein, termed the "work device". All devices with varied cohesion energies used in this study migrate identically on

(7) Sa-Ardyen, P.; Vologodskii, A. V.; Seeman, N. C. *Biophys. J.* **2003**, *84*, 3829–3837.
(8) Duckett, D. R.; Murchie, A. I. H.; Diekmann, S.; Von Kitzing, E.; Kemper, B; Lilley, D. M. J. *Cell* **1988**, *55*, 79–89.
(9) Mao, C.; Sun, W.; Seeman, N. C. *J. Am. Chem. Soc.* **1999**, *121*, 5437–5443.
(10) Förster, T. *Ann. Phys.* **1948**, *2*, 55–75.
(11) Norman, D. G.; Grainger, R. J.; Uhrin, D.; Lilley, D. M. J. *Biochemistry* **2000**, *39*, 6317–6324.

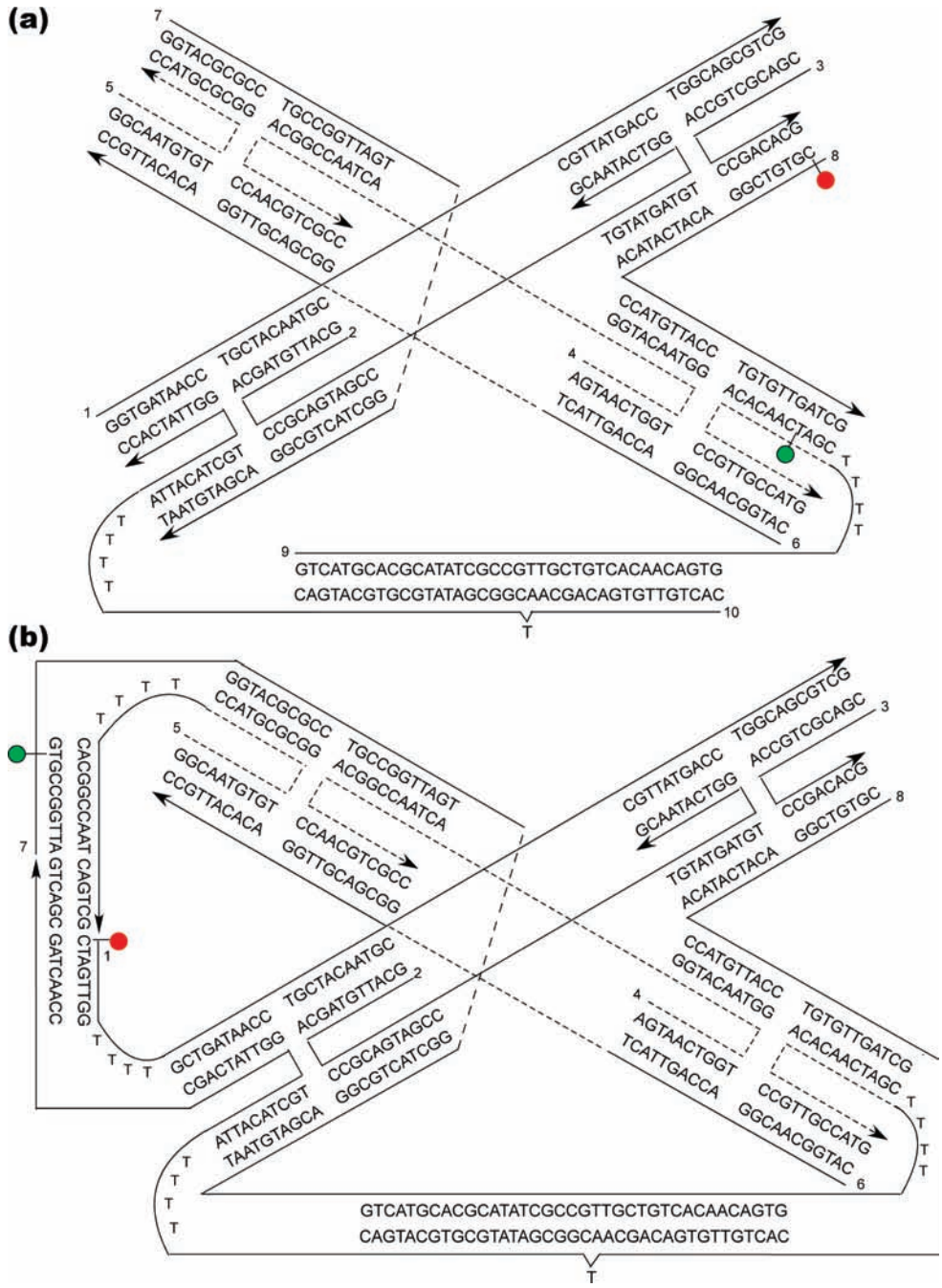

Figure 2. Sequence of the scissors-like measuring devices. (a) The scissors device without the cohesive tract that measures the bending angle. Ten individually numbered strands are displayed. Arrowheads indicate the 3′ ends of the strands. The site of fluorescein is indicated by a green filled circle and that of Cy3 is shown as a red circle. Crossovers between strands are drawn as connections. Here a T bulge is shown as the MutS binding site. A_3, A_5, and A_7 bulges have also been introduced into the same position into the linear duplex DNA, to calibrate the expected relationship between bulge size and bending angle. (b) The scissors-like device containing the cohesive tract that measures the work performed by MutS. The same conventions apply as in (a). Each of the eight mismatches (including the C:C mismatch as a control) has been substituted into the site of the T bulge for MutS binding experiments. Cohesive strands are shown in the left domain. Shifting the positions of the nicks in the 3′ direction provides the series of molecules used for the measurements (Table S1). Variation of the sequence enables interpolation of values.

nondenaturing gels, with mobilities similar to that of a topologically closed standard. As a baseline structure, we have chosen a T bulge in the duplex bridge as the MutS binding site. It has been reported[12] that base bulges can kink linear duplex DNA. The bending angles for three, five, and seven adenines (A) are 50−70°, 85−105°, and ∼90°, respectively.[12] We have introduced A_3, A_5, and A_7 bulges into the duplex bridge of the angle device shown in Figure 2a, so as to calibrate the distortions of the scissors device.

Results and Discussion

Figure 3 contains nondenaturing gels that show the formation of the DNA complexes in Figure 2 and of some of the MutS−DNA complexes. The angle device in Figure 2a contains a T bulge that causes it to move faster than those molecules with adenine bulges on the gel mobility shift assay (lanes A−E).

(12) Lilley, D. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7140–7142.

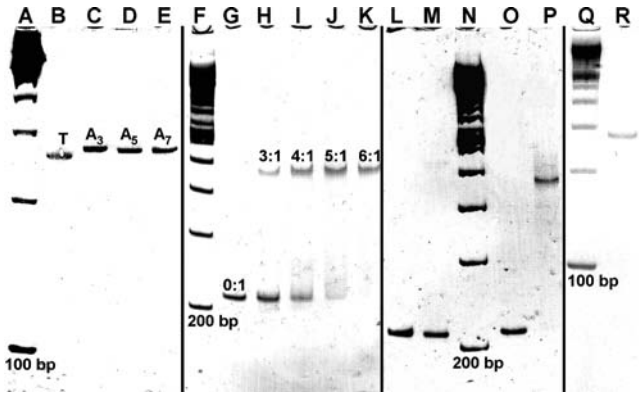

Figure 3. Formation of the DNA complexes and MutS−DNA complex. Four 5% nondenaturing gels run at 25 °C are shown. Lane A: 100 bp marker. Lanes B, C, D, and E: annealing products of the angle device in Figure 2a with a T, A_3, A_5, or A_7 bulge, respectively. Lane F: 100bp marker. Lanes G, H, I, J, and K: the mixture of MutS protein and the angle device of Figure 2a at the indicated ratios. Lane L: the mixture of MutS and annealing product of the angle device in Figure 2a, but lacking the bulge. The molar ratio of MutS protein to DNA device is 6:1. Lane M: annealing product of the angle device in Figure 2a without any bulge. Lane N: 100 bp marker. Lane O: annealing product of the angle device in Figure 2a with the MutS binding site, a T bulge. Lane P: the mixture of MutS and annealing product of the angle device in Figure 2a with a T bulge. The molar ratio of MutS protein to the DNA device is 6:1. Lane Q: 100 bp marker. Lane R: annealing product of the work device in Figure 2b with a 6 base-pair sticky end in the domain of cohesive tract.

We can see that this device is completely bound by MutS protein when the molar ratio for MutS to DNA is 6 to 1 (lanes F−K). For the device with a Watson−Crick complementary duplex bridge, the DNA band stays at the same position before and after MutS is added to a molecule containing a T bulge, confirming that MutS cannot bind to a normal DNA duplex without a mismatch or a bulge (lanes L−O); when MutS is added, the mobility of the DNA is clearly decreased (lane P). MutS readily binds to the work device containing the sticky-ended species (lanes Q, R). Note that under the conditions where the experiments are performed there is no indication of other nucleic acid species.

Figure 4 shows the calibration of the system, comparing A_3, A_5, and A_7 bends observed using the angle scissors device with those observed previously for free DNA;[12] we also compare the crystallographic observations of MutS binding to the T bulge.[5] These measurements are shown in Figure 4a, and they are based on the model of bending shown in Figure 4b. The results are summarized at the bottom of the figure. There is qualitative agreement between the measurements of A bulges, within 5° of the previous measurement range. The value we measure for MutS bending a T bulge with the scissors device indicates somewhat stronger bending than seen in the crystal structure: The estimate for the bending of the T bulge in the presence of MutS is 75°, rather than 60°. Thus, as a structural tool, the scissors is in good agreement with other solution methods, but it does differ somewhat from the crystallographic observations. A sample calculation of the bending angle is shown in the Supporting Information (Figure S1).

Figure 5a contains bar graphs that show the results of experiments monitoring energy transfer from donor quenching for the work device in Figure 2b with the T bulge binding site. In the experiment, we first took a sequence of DNA [5′-CCAATCAGTCG/CT-3′ : 5′-AGCGACTGAT/TGG-3′, where the slashes indicate nicks] and changed the length of the overlap region systematically over a range of four to nine nucleotide pairs. The donor energy transfers are plotted as light blue bars (before MutS binding) and dark red bars (after MutS binding). The abscissa corresponds to the standard free energy of the overlap region, using the parameters of ref 3 plus others for the nicks, generously supplied by Dr. SantaLucia. Each measurement was performed three times. We used a modification of the basic sequence to interpolate the values available from the initial sequence: data for [5′-CCAATCATACA/CT-3′ : 5′-AGTGTATGA/TTGG-3′], containing two AT pairs substituted for GC pairs, are shown in dark green (unbound) and yellow (bound). We ascribe the relatively small amount of transfer before addition in the low-energy measurements to fraying of the small cohesive ends present. Nevertheless, it is clear that the energy transfer observed following addition of MutS increases as we increase the number of nucleotide pairs holding the device together. The transition appears to be complete at about 11 kcal/mol, as indicated by the similar amounts of energy transfer with and without MutS. We interpret the data for higher values of ΔG to indicate that the cohesion is too strong for the transition to occur.

The structure with a strand-disruption energy of less than 5 kcal/mol shows little energy transfer when MutS is bound, while the structures with a disruption energy of 6−10 kcal/mol have an intermediate value for energy transfer in the presence of MutS. It is unlikely that this intermediate level comes from partial conversion of a device; these signals are more likely the average of a mixed population of completely disrupted molecules and intact molecules to which MutS was unable to bind.[2] Once more than 11 kcal/mol is needed to disrupt the cohesive strands, essentially none of the devices are bent by MutS. Thus, as done previously,[2] we select the end point of the transition as the value we report. Schofield et al.[6] estimated the dissociation constant for Taq MutS to a T bulge binding site to be 0.1−4.3 nM. From the relationship $\Delta G = -RT \ln K$, at 298 K, this value corresponds to a 11.4−13.7 kcal/mol binding energy, a value for a system with no sticky ends to disrupt. Our measurement (∼11 kcal/mol) is in good agreement with this value.

Figure 5b also shows FRET measurements for the A:G mis-pair, where the convergence of the bound and unbound values occurs at 8.6 kcal/mol. Values for other mis-pairs are summarized in Table 1 and compared, where appropriate with previous measurements. Changes of enthalpy and entropy are also shown in Table 1 and in the Supporting Information. The other FRET measurement plots are shown in the Supporting Information (Figure S2). The C:C mismatch is a control since MutS does not bind to it. For the FRET measurement, we estimate the systematic error to be ∼1.5% because the precision of each measurement suggests that number. As noted above, our data match the ΔG values generated from K_d.[6]

One exception is the A:C mismatch, which is smaller. All the measurements above have been done at pH 7.8, but it is known that the A:C mismatch involves protonation of the adenine.[13] Lowering the pH from 7.8 to 6.0 yields a different result for the A:C mismatch: the work goes up to 10.01−11.16 kcal/mol, but there is no change seen for the T bulge. This result suggests that MutS binds more tightly to a somewhat more protonated base pair. It is not possible to lower the pH further and retain binding activity.

We have demonstrated that the scissors-like work device introduced here can be used to measure the amount of work

(13) Hunter, W. N.; Brown, T.; Anand, T. T.; Kennard, O. *Nature* **1986**, *320*, 552–555.

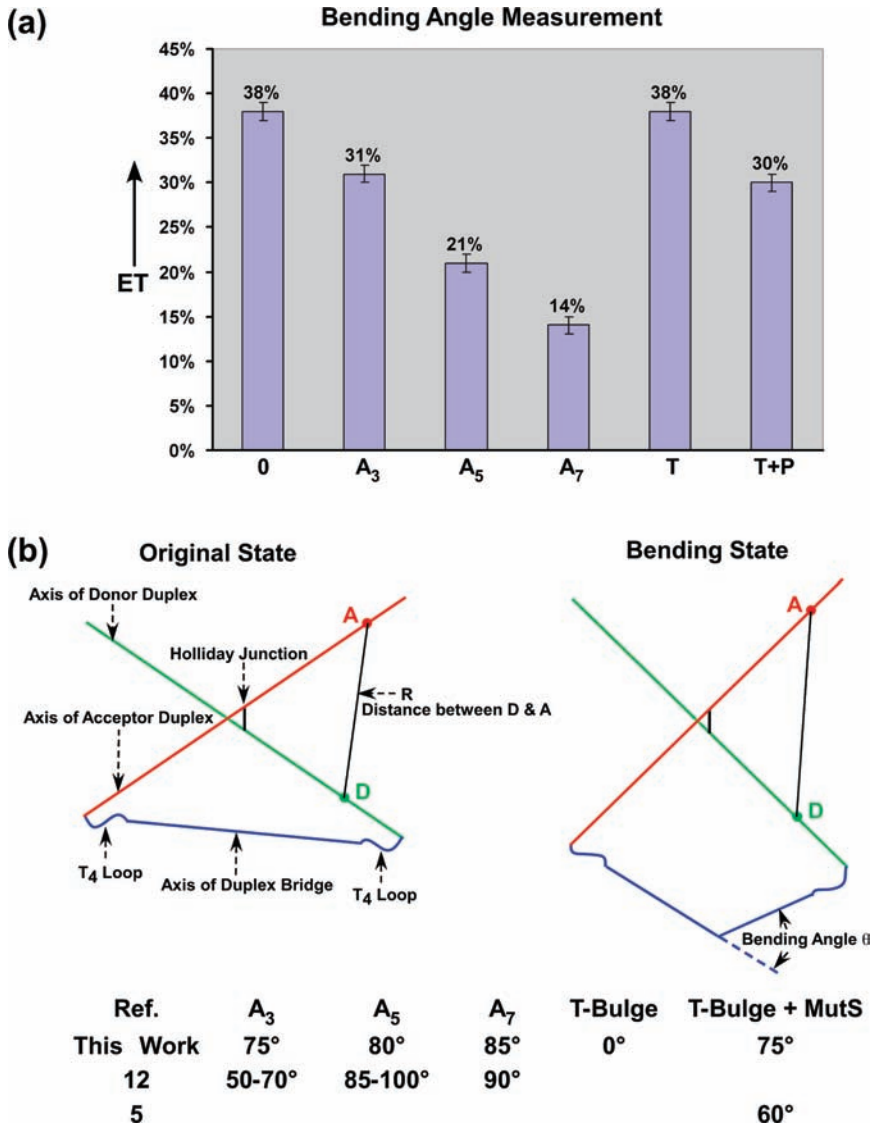

Figure 4. FRET signal calibration. (a) With a Watson−Crick complementary bridge domain, the scissors device shows a 38% donor energy transfer signal. For calibration, A_3, A_5, and A_7 bulges were introduced into the binding domain and the energy transfer signals decreased to 31%, 21%, and 14%, which corresponded to a 75°, 80°, and 85° bending angle, respectively, based on the calculation from the model shown in (b). Introducing a T bulge to the MutS binding domain did not change the energy transfer signal. When MutS bound to the scissors with a T bulge, the energy transfer signal decreased to 30%, suggesting a 75° bending angle. (b) Schematic drawing of the model used to calculate the bending angle according to the FRET signal. Before binding, the original state of the scissors is shown in the left panel. The axes of the duplex segments with donor and acceptor dyes are shown as green and red lines, respectively. They are joined together by a Holliday junction (black). The axis of the MutS binding duplex is illustrated as a blue line. It is connected to the scissors device by two T_4 loops, which are also drawn in blue. The right panel shows the bending state when MutS binds to the device. Upon MutS binding, it bends the DNA duplex bridge, so the scissors contracts, lengthening the distance (R) between FRET pairs, which leads to a decrease in FRET signal.

that a DNA-distorting protein can do when it binds to DNA. Assuming that we are examining the system at equilibrium, this number estimates the free energy of binding. The scissors-like device is much more general than the previous TX-based device,[2] because it is independent of the direction of bending and because it is largely independent of the size of the protein. The direction of bending can always be adjusted by rephasing the binding site by a half-turn, and the protein can always be put on the outside of the device space.

The angle device has produced results about the binding of MutS in good agreement with previous measurements. The bending estimate from FRET is about 15° from the crystallographic observations. Likewise, the disruption data for the sticky ends in the work device are in good agreement with previous values obtained by completely independent measurements. We expect that DNA-based nanodevices such as this one will find wide applications in molecular biology.

Materials and Methods

Design, Synthesis, and Purification of DNA. The sequences of the strands were designed by the program SEQUIN.[14] Custom DNA molecules used in this study were purchased from Integrated DNA Technology (www.idtdna.com) or were synthesized on an Applied Biosystems 394 automatic DNA synthesizer, removed from the support, and deprotected using routine phosphoramidite procedures.[15] DNA strands were purified by electrophoresis. Bands were cut out of 10−20% denaturing gels and eluted in a solution containing 500 mM ammonium acetate, 10 mM magnesium acetate, and 1 mM EDTA.

(14) Seeman, N. C. *J. Biomol. Struct. Dyn.* **1990**, *8*, 573–581.
(15) Caruthers, M. H. *Science* **1985**, *230*, 4273.

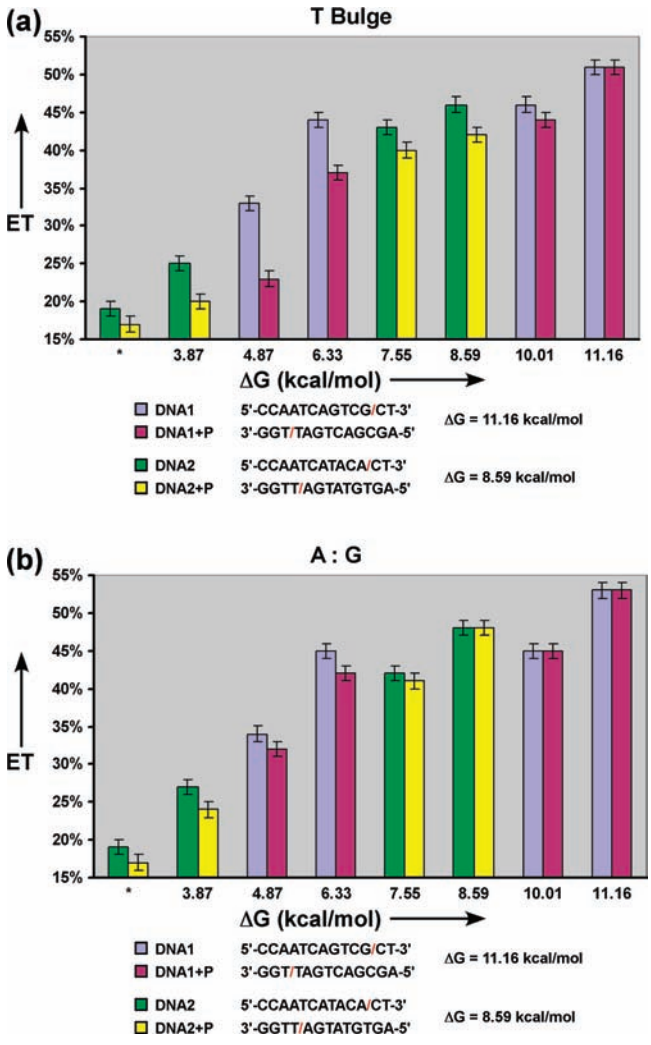

Figure 5. Donor energy transfer when MutS protein is bound to a bulge and a mismatch. Panels (a) and (b) show the energy transfer when MutS is bound to the scissors device with a T bulge and an A:G mismatch binding site, respectively. The pairs of colored bars indicate the difference in energy transfer (ET) between unbound DNA (left bar) and MutS-bound DNA (right bar) forms. Two different overlap regions were used. Their sequence and corresponding standard molar free energy available are shown below the figures. The abscissa shows the estimated standard molar free energy available, based on the nearest-neighbor approximation. Loop entropy was neglected. The energy transfer differences between the unbound and bound forms disappear at about 10.01−11.16 kcal/mol in (a) and 7.55−8.59 kcal/mol in (b).

Formation of Hydrogen-Bonded DNA Device Complexes. Complexes were formed by mixing a stoichiometric quantity of each strand (50 nM), as estimated by OD_{260}, in Hepes buffer (20 mM Hepes, pH = 7.8, 50 mM NaCl, 6 mM $MgCl_2$, 1 mM EDTA). This mixture was then heated to 90 °C and cooled slowly to room temperature during 40 h in a 2 L water bath insulated in a styrofoam box.

Nondenaturing Polyacrylamide Gel Electrophoresis. Gels contained 5% acrylamide (19:1, acrylamide/bisacrylamide). DNA complexes were formed in 100 μL of Hepes buffer according to the method described above. Samples were then brought to a final volume of 30 μL and a concentration of 50 nM, with a solution containing Hepes buffer, 50% glycerol, and 0.02% each of bromophenol blue and xylene cyanol FF tracking dyes. Gels were run on a Hoefer SE-600 gel electrophoresis unit at 11 V/cm at 4, 25, or 37 °C and stained with Stains-all dye.

Table 1. Thermodynamics of MutS Binding to DNA Mismatches

	ΔH^o(scissors device), kcal/mol	ΔS^o(scissors device), eu
ΔT	71.5−81.6	206−236.3
T:G	73.3−71.5	217.1−206
T:C	71.5−81.6	206−236.3
T:T	68.5−73.3	204.2−217.1
A:A	68.5−73.3	204.2−217.1
A:G	55.4−68.5	164.6−204.2
A:C	45.8−55.4 (pH 7.8); 71.5−81.6 (pH 6.0)	137.2−164.6 (pH 7.8); 206−236.3 (pH 6.0)
G:G	71.5−81.6	206−236.3

	ΔG^o_{25}(scissors device), kcal/mol	ΔG^o_{25}(Schofield et al.), kcal/mol
ΔT	10.01−11.16	11.42−13.65
T:G	8.59−10.01	8.76−9.05
T:C	10.01−11.16	8.57−8.78
T:T	7.55−8.59	7.47−8.08
A:A	7.55−8.59	7.38−7.58
A:G	6.33−7.55	7.20−7.25
A:C	4.87−6.33 (pH 7.8); 10.01−11.16 (pH 6.0)	7.61−8.13
G:G	10.01−11.16	N/A

FRET Dye Labeling. Fluorescein-labeled strand was the product of incorporating fluorescein-dT (Glen Research) into DNA strands. Cy3 labeling was done by incorporating Cy3 phosphoramidite (Glen Research) to the 5′ end of DNA strands.

Preparation of MutS-DNA Complex Sample. A 30−75 pmol amount of *Taq* MutS dimer was incubated with 50 nM DNA substrate in Hepes buffer in a total volume of 100 μL at room temperature for 30−60 min.

Spectroscopic Methods. Absorption measurements were taken on a Spectronic Genesys spectrophotometer. Steady-state fluorescence measurements were performed with an Aminco Bowman Series 2 spectrometer at room temperature. The emission spectra were corrected for instrument response, lamp fluctuations, and buffer contributions. Energy transfer is calculated following refs 16 and 17. Energy transfer involving the donor, ET(D), is derived from $ET(D) = [F^{517}{}_{490}(D) - F^{517}{}_{490}(DA)]/F^{517}{}_{490}(D)$, where $F^{517}{}_{490}(D)$ is the fluorescence of the donor at its wavelengths of maximum emission (517 nm) and excitation (490 nm) in the molecule containing only the donor, and $F^{517}{}_{490}(DA)$ is the same quantity in the doubly labeled molecule.

Acknowledgment. This research has been supported by grants GM-29544 from the National Institute of General Medical Sciences, CTS-0608889 and CCF-0726378 from the National Science Foundation, 48681-EL and W911NF-07-1-0439 from the Army Research Office, and N000140910181 and N000140911118 from the Office of Naval Research and a grant from the W.M. Keck Foundation.

Supporting Information Available: A sample calculation of thermodynamic changes (enthalpy and entropy) during the disruption of cohesive ends, the donor energy transfer when MutS protein binds to seven mismatches (Figure S1), the sequences of cohesive ends and the free energy involved (Table S1). References are at the end. This material is available free of charge via the Internet at http://pubs.acs.org.

JA910188P

(16) Jares-Erijman, E.; Jovin, T. M. *J. Mol. Biol.* **1996**, *257*, 597–617.
(17) Mao, C.; Sun, W.; Shen, Z.; Seeman, N. C. *Nature* **1999**, *397*, 144–146.