

and controlled-release processes, or as semi-homogeneous micro-reactors for applications in organic, bioengineering and combinatorial synthesis. In these applications, Bragg diffraction from the arrays could be used to probe the existence and location of chemical species as a means to monitor physicochemical processes in real time. □

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A nanomechanical device based on the B–Z transition of DNA

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The assembly of synthetic, controllable molecular mechanical systems^{1–7} is one of the goals of nanotechnology. Protein-based molecular machines, often driven by an energy source such as ATP, are abundant in biology^{8,9}. It has been shown previously that branched motifs of DNA can provide components for the assembly of nanoscale objects¹⁰, links¹¹ and arrays¹². Here we show that

such structures can also provide the basis for dynamic assemblies: switchable molecular machines. We have constructed a supramolecular device consisting of two rigid DNA ‘double-crossover’ (DX) molecules connected by 4.5 double-helical turns. One domain of each DX molecule is attached to the connecting helix. To effect switchable motion in this assembly, we use the transition between the B and Z^{13,14} forms of DNA. In conditions that favour B-DNA, the two unconnected domains of the DX molecules lie on the same side of the central helix. In Z-DNA-promoting conditions, however, these domains switch to opposite sides of the helix. This relative repositioning is detected by means of fluorescence resonance energy transfer spectroscopy, which measures the relative proximity of two dye molecules attached to the free ends of the DX molecules. The switching event induces atomic displacements of 20–60 Å.

It has been recognized for a long time that the B–Z transition could be used to drive a DNA-based mechanical device¹⁵, but the development of such a device required the discovery of a rigid DNA motif to provide a fixed level arm: the rigidity of the DX molecule¹⁶ is the key element that enables the construction of this device.

A molecular model of the system is shown at the top of Fig. 1. A long central helix is flanked on either end by two ‘DAO’ molecules, where DAO (for double-crossover, antiparallel, odd-number) means that this is a DNA double-crossover molecule whose helices are antiparallel to each other, and whose crossover points are separated by an odd number (three) of double helical half-turns¹⁷. The ends of all helices have been closed with dT₄ hairpin loops (made up of four deoxythymidines); consequently, the entire construct consists of three cyclic strands of DNA, the two blue strands on the ends, and the red strand that contains a yellow central

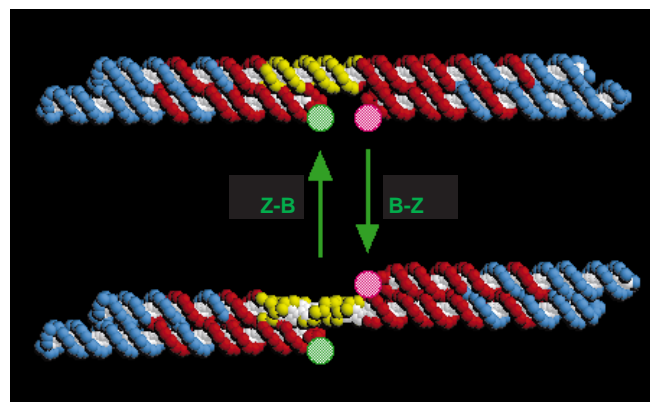


Figure 1 Design of the nanomechanical device. The device consists of two DNA double crossover (DX) molecules with the DAO motif¹⁷ connected by 4.5 turns of DNA between the nearest crossover points. Top, molecular model of the molecule constructed entirely from right-handed B-DNA. Each nucleotide is shown as two spheres, a coloured one for the backbone and a white one for the base. Three cyclic strands are shown, one in the centre drawn as a red strand with a central yellow segment, and two blue strands on the ends that are each triply catenated to the red strand. Fluorescent dyes are drawn schematically as stippled green (fluorescein) and magenta (Cy3) circles attached to the free hairpins near the middle of the molecule. At the centre of the connecting helix is a 20-nucleotide region of proto-Z DNA in the B-DNA conformation, shown in yellow. When the B–Z transition takes place, this same yellow portion becomes left-handed Z-DNA (bottom). When the transition occurs, the two DX molecules change their relative positions, increasing the separation of the dyes. It is possible to cycle this system in both directions.

segment. The outer strands are each linked three times to the central strand. The double helix connecting the two DAO molecules contains the base-paired sequence $d(CG)_{10}$, a 'proto-Z' sequence that can be converted to left-handed Z-DNA at high ionic strength; this is the portion of the red strand drawn in yellow. The top part of Fig. 1 shows this yellow region in the right-handed form, B-DNA, and the bottom part shows it in the left-handed form, Z-DNA. The change in twist from B-DNA to Z-DNA is estimated to be about -128° for each $d(CG)$ dinucleotide¹³. Thus, ignoring the B-Z junctions, whose twist is unknown¹⁸, the change in twist between the B and the Z forms of this molecule is expected to be about -3.5 turns, resulting in a transition roughly like that depicted in Fig. 1. Overall, the transition consists largely of a rotary motion; however, the separation of the base pairs along the helix axis (3.7 \AA) is slightly longer in Z-DNA than in B-DNA (3.4 \AA) (ref. 13), which is expected to result in a 6-\AA expansion (visible in Fig. 1) in the length of the central helix axis.

Two fluorescent dyes—a donor, fluorescein, and an acceptor, the sulphoindocarbocyanine, Cy3 (ref. 19)—are represented in Fig. 1 as green and magenta stippled circles, respectively. The drawing shows that the distance between them is expected to change when the transition occurs. The signal in fluorescence resonance energy transfer (FRET) is proportional to the inverse sixth power of the separation of the dyes, providing a means to monitor the transition. The experiment is predicated on the assumption that well-defined distances will characterize the separation of the dyes in the two states. Well-defined distances can exist only if the parts of the molecule maintain their structural integrity; initial attempts to demonstrate motion in devices containing three-arm DNA

branched junctions instead of DX molecules were unsuccessful, owing to the flexibility²⁰ of those components. This problem has been solved, because the DX motif behaves as a rigid unit¹⁶.

The device was constructed by ligating a series of oligonucleotides, using methods described previously¹⁰. The molecule was isolated from denaturing gels, and its successful construction was demonstrated by restricting the individual strands to yield target linear, circular and catenated products (data not shown). To account for the uncertainty involving the twist of the B-Z junction, we have made similar molecules containing $d(CG)_9$ and $d(CG)_{11}$; moreover, we have varied the separation of the inner crossover points in steps of two nucleotides, over the range 45–49. Of the nine molecules tested, the lowest relative energy transfer under Z-promoting conditions (compared with a non-proto-Z control) was obtained from the molecule whose separation of 47 nucleotides contained $d(CG)_{10}$. To confirm that the B-Z transition can occur in this molecule, we have prepared the central 47-nucleotide double helix, and have shown that a mixed B-Z circular dichroism spectrum is obtained in 5M NaCl (data not shown), unlike a control lacking the proto-Z sequence.

Figure 2 illustrates the results of taking this system through successive cycles of B-promoting and Z-promoting conditions. The molecule used for these experiments contains $d(m^5CG)_{10}$ (in which the 5-position of cytosine is methylated, a modification that increases the propensity of DNA to undergo the B-Z transition²¹); as a result, we are able to promote the transition with a 10 mM cacodylate buffer (pH 7.5) containing 0.25 mM $Co(NH_3)_6Cl_3$, 100 mM $MgCl_2$ and 100 mM NaCl, whereas B-DNA is present when the same buffer contains 10 mM $MgCl_2$, 100 mM NaCl and no $Co(NH_3)_6Cl_3$. Figure 1 shows that the larger separation is designed to occur in the Z-form, and that the response to Z-promoting conditions should be to lengthen the distance and decrease the FRET signal between the two dyes. This is seen to happen in Fig. 2, both when energy transfer is measured by decrease of donor fluorescence or by increase of acceptor fluorescence. Figure 2 also illustrates the results obtained with a control molecule, one designed to be incapable of undergoing the B-Z transition. In this case, energy transfer appears to be at roughly the same level throughout the experiments, a level appropriate only to the B-state of the molecule.

The experiments reported above show that it is possible to construct a nanomechanical device from DNA DX molecules. The absolute distances derived from energy-transfer measurements are 7.0 nm for B conditions and 8.9 nm for Z conditions, somewhat longer than those derived from model building²², 5.1 nm and 7.0 nm; nevertheless, the ratio of measured distances, 1.27, is similar to the model-building ratio of 1.37. It should be possible to incorporate this mechanical control in any figure or array produced by DNA nanotechnology²³, so long as a free swivel containing proto-Z DNA can be included in the design. In addition, it should be possible to change the relative positions of proteins or other large molecules connected to DX units²⁴, in the same way that the relative positions of fluorescent dyes have been changed here. This capability should enable the study of proximity effects in chemical and biological systems. It is difficult to predict whether this system could also be used to power a nanoscale motor; we have shown that the B-Z transition can provide two equilibrium structures in different positions, but the ability to transmit force depends on the structural and dynamic features of the transition itself.

We have recently reported a system in which double-stranded DNA branch migration, characterized by a screw motion, is controlled by the addition of ethidium to the solution²⁵. However, that system involves a long piece of circular DNA whose geometry is less well-defined than the molecule reported here. The synthetic systems mentioned above produce molecular motions of the order of 10 Å. By contrast, the atoms in the rotated DNA helical domain experience displacements that range from 20 to 60 Å. The fusion of those

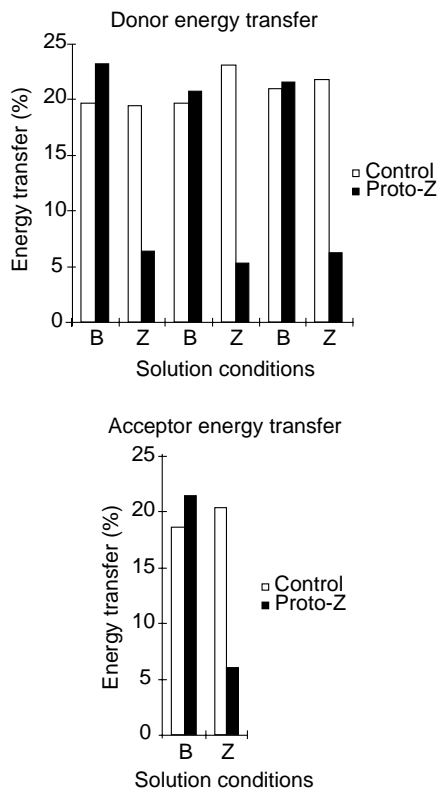


Figure 2 FRET demonstration of nanomechanical motion. Top, energy transfer measured from the decrease in donor fluorescence when cycling the system alternately through B and Z conditions. A similar result is obtained when the increase in acceptor fluorescence is used (bottom). In both graphs, the molecule containing the proto-Z segment is contrasted with a control that lacks it. Only the molecule containing the proto-Z segment shows a change in energy transfer in Z-promoting conditions.

differently triggered smaller systems with the synthetic DNA system reported here could provide a variety of complex nanomechanical motions. □

Methods

Molecular sequences. The sequences of the blue strands (Fig. 1) are: 5'-TCGAGCCTGTACCTGCCAAGTCAGACTGTGGACTTCGCATCACGGACGACTCATCTTTTGATGAGTCGTGGTACAGGCTCGAGTTGCTTTTGCAAC-3' and 5'-AATTCAGTGTAAACGGACCGCATTTGGCTGACACCATACGCCGGAATCCTGACCATCTGTTTTCAGATGGTACCGTTACACTGGAATTCGCACTTTTGTGCG-3'. The sequence of the red strand is 5'-TGCCGATCCTTT(Cy3)TTGGATCGGCAAGCTTCTGTGACCCAATGCGGTGGATTCCGGCGTATGGACGCAGTC*GC*GC*GC*GC*GC*GC*GC*GC*GAAGCCGCTCTGGCTCGACCACCGCGTTGGATCCTTFTTGATCCAACGGCTGGACAGTCTGACTTGGCACCCTGATGCGAAGTCTTGGTCGAGCCAGAGCGGCTTC*GC*GC*GC*GC*GC*GC*GC*GC*GACTGCGTGAAGCT-3', where Cy3 is attached to amino-modifier-C6-dT (Glen Research), F is the product of incorporating fluorescein-ON phosphoramidite (Clontech), and C* is 5-methyl-cytidine. The control molecule replaces the proto-Z sequence (yellow segment) with TGGACACTAAGCTATTCATC and its complement.

Calculation of energy transfer. Energy transfer is calculated following ref. 19. Energy transfer involving the donor, ET(D), is derived from $ET(D) = [F_{490}^{517}(D) - F_{490}^{517}(DA)]/F_{490}^{517}(D)$, where $F_{490}^{517}(D)$ 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 $F_{490}^{517}(DA)$ is the same quantity in the doubly labelled molecule. Energy transfer involving the acceptor is derived from $ET(A) = (\epsilon^{550}(DA)/\epsilon^{490}(DA)) \cdot \{(\text{ratio})_A - \{F_{490}^{565}(A)/F_{550}^{565}(A)\}\}$, where $(\text{ratio})_A = [F_{490}^{565}(DA) - cF_{490}^{565}(D)]/F_{550}^{565}(DA)$; $\epsilon^{550}(DA)$ and $\epsilon^{490}(DA)$ are the extinction coefficients at 550 and 490 nm, respectively, of the doubly labelled molecule (their ratio is 3.65 in B-DNA conditions and 3.0 in Z-DNA conditions), $F_{490}^{565}(A)$ is the fluorescence at 565 nm, the maximum emission wavelength, of the acceptor when excited at 490 nm, $F_{550}^{565}(A)$ is the fluorescence at 565 of the acceptor when excited at 550 nm, its maximum excitation wavelength; the quantities in $(\text{ratio})_A$ are similarly defined for the donor molecule (D) or the doubly labelled molecule (DA), and c is a normalization constant used to equalize donor emission in the (D) and (DA) molecules at 517 nm. The refractive indices of the B-DNA and Z-DNA promoting buffers are 1.338 and 1.339, respectively. The anisotropies of the dyes, measured in singly labelled molecules, were found to be 0.136 for fluorescein and 0.259 for Cy3 in both buffers, similar to values reported earlier¹⁹, and validating the use of 2/3 for the value of K^2 , the scalar orientation factor for dipole-dipole coupling²⁶. R_0 (the characteristic transfer distance) is calculated to be 55.7 Å (B-DNA conditions) or 56.1 Å (Z-DNA conditions), in agreement with previous work¹⁹. In experiments involving $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ the fluorescein fluorescence is quenched by 15%, but this appears to have no effect on the relative energy transfer or on the comparison between molecules with and without the proto-Z sequence.

Molecular modelling. The molecular model in Fig. 1 was drawn using the Insight II Molecular Modeling System, version 95.0.6 (Biosym Technologies). The coordinates for B-DNA and Z-DNA were taken from that program's data base. The dT₃ hairpin loops were sketched to resemble the dT₄ hairpin loops whose structure was determined in ref. 27.

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Sulphidic Mediterranean surface waters during Pliocene sapropel formation

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Sapropels—organic-matter rich layers—are common in Neogene sediments of the eastern Mediterranean Sea. The formation of these layers has been attributed to climate-related increases in organic-matter production^{1–3} and increased organic-matter preservation due to oxygen depletion in more stagnant bottom waters^{2,3}. Here we report that eastern Mediterranean Pliocene sapropels⁴ contain molecular fossils of a compound (isorenieratene) known to be synthesized by photosynthetic green sulphur bacteria, suggesting that sulphidic (euxinic)—and therefore anoxic—conditions prevailed in the photic zone of the water column. These sapropels also have a high trace-metal content, which is probably due to the efficient scavenging of these metals by precipitating sulphides in a euxinic water column. The abundance and sulphur-isotope composition of pyrite are consistent with iron sulphide formation in the water column. We conclude that basin-wide water-column euxinia occurred over substantial

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