Molecular Devices

DOI: 10.1002/anie.200800476

A Contractile DNA Machine**

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DNA is a material well suited to the building of structures and machines at the nanoscale.[1] Molecular DNA machines powered by changes in ionic conditions, DNA hydrolysis, or DNA itself have been constructed.[2–11] The idea of DNA-powered DNA machines was introduced by building DNA tweezers.[12] Molecular machines hold much promise for applications in molecular-scale production, synthesis, and medicine. The contractile DNA machine (CDM) reported here transduces DNA hybridization energy into controlled contraction movements in the nano- and micrometer ranges. An important element of its design is a long single-stranded (ss) DNA molecule. Such molecules have previously been used to build one-,[13–15] two-,[16–19] and three-dimensional[20] DNA structures.

The design of the CDM is shown in Figure 1. This molecular machine is a linear assembly of many copies of a molecular tweezers unit. Each tweezers unit is templated by one repeat unit of a long ss DNA molecule. Figure 1 a shows how this template is synthesized by using rolling-circle replication.[21] Figure 1 b shows part of the CDM in its fully extended state. Hybridized to the template with 20 nucleotides (nt) each are left and right tweezers arms; 32 nt of each arm remain ss. The arms perform an open–close movement. The spacer strand hybridizes to the template with its entire 95 nt. Its role is to separate adjacent units sufficiently to prevent interactions between tweezers. A 4-nt-long ss hinge segment, which is part of the template, connects the two arms of each tweezers unit and provides sufficient flexibility. The 135 double-stranded (ds) and four ss nt translate into a length of approximately 48 nm per fully extended repeat. The contracted state in which all tweezers units are closed is shown in Figure 1 d. The length of a repeat unit in its closed state is expected to be around 36 nm, 32 nm being contributed by the spacer segment and 4 nm (the width of two double helices lying side by side) by the two tweezers arms.

By using two "fuel" strands, an opening strand and a closing strand, the tweezers can be cycled between an open state (Figure 1 c1) and a closed state (Figure 1 c4). After being added, the ss closing strand hybridizes to the ss segment of the right arm of the tweezers. It then starts interacting with the ss segment of the left arm. This causes the tweezers to commence the closing process. The cooperative closing

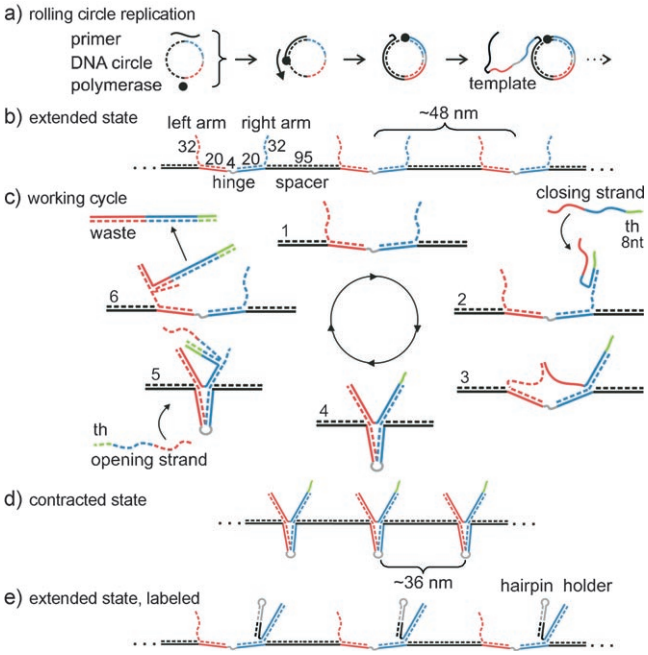

Figure 1. Design, synthesis, and working cycle of the CDM. Pairs of dashed and full lines of the same color indicate complementary sequences. a) Rolling-circle replication. The polymerase moves anticlockwise, adding nt to the 3′ end of the primer. b) Extended state of the CDM (three repeat units are shown). Numbers without a unit indicate the number of nt. c) Working cycle of the CDM. The operator adds a closing strand (th = toehold) before step 2 and an opening strand before step 5. The waste complex of the opening and closing strands is exhausted after step 6. d) Contracted state. e) Extended state labeled with a DNA marker consisting of hairpin and holder strands.

actions of all tweezers units translate into an overall contraction of the CDM. The ss arm and closing strand segments are longer than the arm segments that are hybridized to the template. This enables the closing strand to initiate hybridization to the ss parts of both arms without requiring energetically unfavorable contraction (Figure 1 c3), even when a tension acts on the device. The CDM is hence able to work against a load with a power stroke. The contracted state of the CDM is reached after the closing strand has completely hybridized to both arms.

After addition of the ss opening strand, it interacts with and binds to the closing strand through an 8-nt-long toehold (th). The th mediates a strand displacement reaction[22] by which the closing strand is displaced from the tweezers unit, thus allowing it to open and thereby prompting the CDM to extend. The displacement of the closing strand proceeds from the right arm to the left arm. Only after complete removal of the closing strand from the tweezers unit can the tweezers close again and contribute to the next contraction. The closing

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[**] This work was supported by NUS grants R144000143112 and R144000146133 and by the NUS Nanoscience and Nanotechnology Initiative. We thank Andrew Turberfield and Johan Van Der Maarel for comments and discussions.

and opening strands form an inert waste complex of 72 base pairs (bp). It is expected that tweezers units close and open independently of each other, but that the rapid speed of hybridization and strand displacement[22–24] leads to fast overall contraction and extension reactions.

To measure the difference in extension between contracted and extended states, the extended state was labeled with a marker (Figure 1e) consisting of two DNA strands. The first, the holder, is complementary to the ss segment of the right arm of the tweezers. In addition, it has a 15-nt ss overhang. The second is a 12-nt DNA hairpin with a 15-nt ss overhang on its neck. By way of this overhang it can bind to the holder. The total number of bp in the marker is 59. In contrast, closed tweezers have 104 bp.

The CDM was investigated with atomic force microscopy (AFM) and polyacrylamide gel electrophoresis (PAGE). Figure 2a1 shows the CDM in its original extended state. Its appearance is very similar to that of common ds DNA, shown in Figure 2d for comparison. The ss segments of the arms and the 4-nt-long ss hinge connecting them are not resolved by the AFM tip. Figure 2a2 shows the extended state after adding the DNA markers. Fairly regularly spaced "blobs" are visible, which can be identified as the DNA markers. The gap between labeled tweezers units was determined by section height measurements (Figure 2a2, inset). The results of a large number of such measurements on CDM segments that were not crossing over or had other factors affecting the reliability of data are plotted in the histogram. The average intertweezers distance is (47 ± 4) nm, which is in line with the geometry of the CDM.

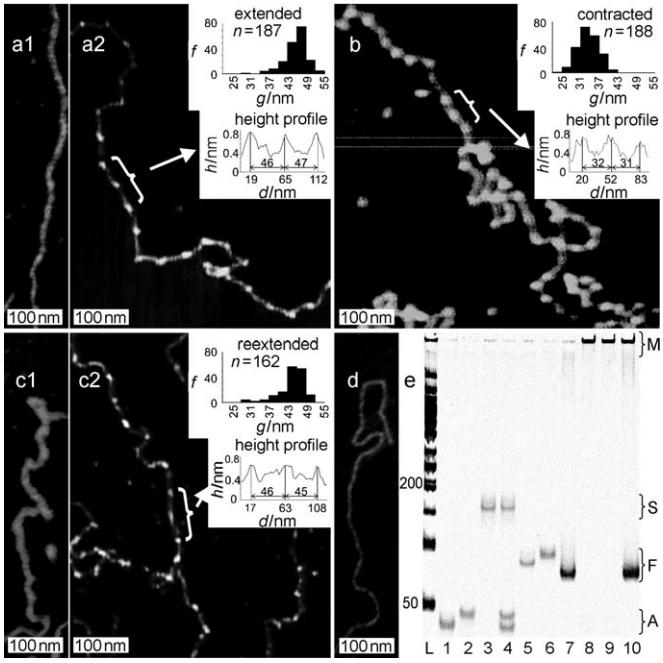

Figure 2. AFM and PAGE of the CDM. The height scale is 2.5 nm. The insets in (a)–(c) show the intertweezers distance (top) and height profiles for the areas marked with curly brackets (bottom); f = frequency, g = gap size, h = height, d = distance. a1) Original extended state. a2) Original extended state labeled with DNA marker. b) Contracted state. c1) Reextended state. c2) Reextended state labeled with DNA marker. d) ds DNA (linearized PhiX-174). e) 15% PAGE analysis. M = CDMs; S = spacer; F = closing strand, opening strand, and waste; A = arms. Lanes: L, 50-bp ladder; 1, left arm; 2, right arm; 3, spacer; 4, arms and spacer; 5, closing strand; 6, opening strand; 7, waste complex; 8, original CDM comprised of template, arms, and spacer; 9, contracted CDM after addition of closing strand; 10, reextended CDM after further addition of opening strand.

The image in Figure 2b shows the CDM in its contracted state. Visible again are regularly spaced blobs, which can be identified as closed tweezers units. Gaps between closed tweezers were again obtained by section height measurements and plotted in a histogram (Figure 2b, inset). The average intertweezers gap is (34 ± 3) nm, in line with expectations. Figure 2c1 shows the reextended CDM after adding the opening strand. The appearance is very similar to that of the original extended state. Figure 2c2 shows a reextended CDM labeled with DNA marker. The average intertweezers gap was measured to be (45 ± 4) nm. This is slightly less than that in the original extended state, but is within statistical error. Not shown in the histograms are a small number of measurements of approximately twice the expected gap sizes. These can occur when an open tweezers unit fails to be labeled or when a tweezers unit fails to close. Figure 2e shows a gel of the CDM components and assembly as well as the working cycle. Lanes 8, 9, and 10 correspond to the AFM images in Figure 2a1, b, and c1, respectively. The appearance of the waste complex band upon adding an opening strand to the contracted CDM in lane 10 confirms that it can be cycled.

Although the ds spacer segments are rigid and prevent interactions of adjacent units, interactions between nonadjacent tweezers are still possible. This may be the reason for some of the entanglements visible in Figure 2b. However, the CDM is designed to work under load and to actively contract against it. The load would stretch the CDM, thereby preventing entanglements. The maximum force against which the CDM can contract is expected to be given by the force required to unzip ds DNA, approximately 15 pN.[25]

In conclusion, we have built a contractile molecular DNA machine that is powered by DNA and can be cycled between contracted and extended states. It is conceivable to build contractile devices based on alternative designs. These could, for example, utilize the opening and closing of DNA hairpin loops integrated into a long DNA template, or the refolding of a long DNA template from an extended state into a compact state through a number of short DNA bracing strands. The tweezers-based design was chosen because of its active power stroke and its simple fuel system. A number of labeling options for the extended state, such as biotin–streptavidin markers,[26] were available. DNA markers were chosen for their simplicity and their single binding site. Although the molecular mechanism of the CDM and its energy source are different, its mechanical contraction action is similar to that of microtubule depolymerization and myosin–actin motors which are found in nature.[27] The forces that microtubule depolymerization[28] and myosin[29] motors can exert are somewhat smaller than the maximum load against which the CDM is expected to contract. Putting several CDMs in parallel would increase the maximum force that can be produced. The CDM may find applications in nanotechnology and its interface to the micrometer scale.

Experimental Section

Rolling-circle replication of the template was performed by first synthesizing a DNA circle by hybridization of two phosphorylated oligonucleotides with two linker strands at a concentration of 1 μM. The DNA circle was then ligated with T4 DNA ligase in T4 DNA ligase buffer (NEB, Ipswich, MA, USA) for 30 min at 16 °C. Noncircular DNA was digested for 30 min with the exonucleases Exo I and Exo III (NEB) in T4 DNA ligase buffer at 37 °C. DNA circle and primer (both 0.1 μM) were then hybridized in Φ29 buffer (NEB) at room temperature (20 °C). Φ29 polymerase (0.1 μM) and dNTPs (2 mM; NEB) were added to the circle–primer mix, thus starting the reaction. Rolling-circle replication was allowed to proceed for 2 h at 30 °C. All three enzymatic reactions were stopped by heat inactivation. A final phenol–chloroform extraction was performed to purify the template. The concentration of repeat units on the template was measured by titration against the spacer strand, the concentration of which was known from UV absorption spectroscopy (260 nm). To assemble the CDM, template, arms, and spacer were mixed at a repeat unit concentration of 10 nM in $MgCl_2$/2 × TE buffer (10 mM; TE = Tris/edta; tris(hydroxymethyl)aminomethane (Tris), ethylenediamine tetraacetate (edta)) at pH 8.0 and then annealed from 95 °C to room temperature over 12 h.

All further experimental steps were conducted at room temperature. The CDM was contracted by adding a stoichiometric amount of closing strand to the original sample. Reextension was achieved by adding a stoichiometric amount of opening strand to the contracted devices. Contraction and reextension reactions were allowed to proceed for 15 min. DNA markers consisting of hairpin and holder strands were annealed and, where applicable, added to samples at room temperature after annealing and contraction–extension reactions of the CDM had finished. The original, contracted, and reextended samples (20 μL each) were deposited on mica for 20 min. Mica pieces were then washed gently with deionized water and carefully dried with nitrogen.

AFM images were obtained in tapping mode in air with a Dimension 3000 microscope (Veeco, Woodbury, NY, USA). In addition, samples at 100 nM concentration of repeat units were analyzed with nondenaturing PAGE (15 % 29:1 polyacrylamide/Tris–acetate–EDTA buffer). DNA sequences (Table 1) were designed by using computational algorithms that minimize undesired interactions between noncomplementary sequences. DNA oligonucleotides were purchased from IDT (Coralville, IA, USA) and from Sigma Proligo (Singapore).

Table 1: DNA sequences (5′ to 3′).[a]

Name	Sequence
DNA circle part 1	*Pho*GTATGTGGTGATTAGTTGTTTAGGATGATAGATGA AGGTTTGAGTTTCGCGGCTTGCGGAAGCTAGTTTCGG GCATTCGCTGTGCTTAAGTCTAG
DNA circle part 2	*Pho*AAGTATAAGATGGCGAAAGCAGTGTCTATTGAGCC TACGTCCAG
linker 1	**CGCCATCTTATACTTCTAGACTTAAGCACAG**
linker 2	**CAACTAATCACCACATACCTGGACGTAGGCTC**
primer	**CTAGACTTAAGCACAGCGAA**
template (one repeat unit)	**(CTAGACTTAAGCACAGCGAATGCCCGAAACTAGCTT CCGCAAGCCGCGAAACTCAAACCTTCATCTATCATCC TAAACAACTAATCACCACATACCTGGACGTAGGCTCA ATAGA**CACT**GCTTTCGCCATCTTATACTT)**$_n$
left arm	TCTATTGAGCCTACGTCCAGAGTATCATGCCTTCAGTC TCAGTTGTAGTCAG
right arm	AGGAGTCAGGGTAAGTCGTATTGATGTGGTATAAGTA TAAGATGGCGAAAGC
spacer	GTATGTGGTGATTAGTTGTTTAGGATGATAGATGAAGG TTTGAGTTTCGCGGCTTGCGGAAGCTAGTTTCGGGCA TTCGCTGTGCTTAAGTCTAG
closing strand	**CTGACTACAACTGAGACTGAAGGCATGATACTATACCA CATCAATACGACTTACCCTGACTCCTCCTAGCAT**
opening strand	ATGCTAGGAGGAGTCAGGGTAAGTCGTATTGATGTGG TATAGTATCATGCCTTCAGTCTCAGTTGTAGTCAG
holder	**CCTGGACCGACTCTATTATACCACATCAATACGACTTAC CCTGACTCCT**
hairpin	TAGAGTCGGTCCAGGCGCGCCGCGTCTTTTT**AGACGC GGCGCG**

[a] Font colors correspond to the line colors in Figure 1. Bold font corresponds to full-line style and normal font to dashed-line style in Figure 1.

Received: January 30, 2008
Revised: June 24, 2008
Published online: August 4, 2008

Keywords: DNA structures · DNA · molecular devices · nanostructures · self-assembly

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