DNA machines for molecular self-assembly

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ABSTRACT

The molecular recognition properties of DNA are used to construct and power two prototype nanomachines, a pair of molecular tweezers and a nanoactuator. The motion of these machines is driven by hybridization with DNA 'fuel' strands. Cyclic operation between two distinct mechanical states is possible for both machines and is demonstrated by fluorescence resonance energy transfer experiments. DNA serves as structural material, as fuel and as information carrier.

Keywords: Nanotechnology, DNA, self-assembly, molecular machines.

1 INTRODUCTION

Conventional molecular self-assembly is restricted in flexibility by the interactions which dictate the assembly process. Programmable molecular machines with well-defined interactions and motions that can be switched on and off would greatly increase the versatility of such molecular assembly processes. Here we present two prototypes of such machines which are made from and powered by DNA. DNA has proven to be a versatile self-assembly molecule as it has simple and predictable molecular recognition properties. Two single strands of DNA form a double helix only if their base sequences are complementary, i.e. adenine (A) binds to thymine (T) and guanine (G) to cytosine (C) [1]. This simple construction rule has allowed the assembly of a variety of structures including polyhedra [2] and two-dimensional sheets [3]. In addition, the B-Z transition of DNA has been employed to induce motion on the molecular scale [4]. Here, we show how DNA 'fuel' strands can be used to drive artificial molecular machines also made from DNA. In particular, we have devised two DNA-based molecular devices [5], [6] which both consist of two double-stranded arms held together at one end by single-stranded DNA that serves as a flexible hinge. One device can be used to bring two molecular components close together, whereas the other can push components several nanometers apart from each other.

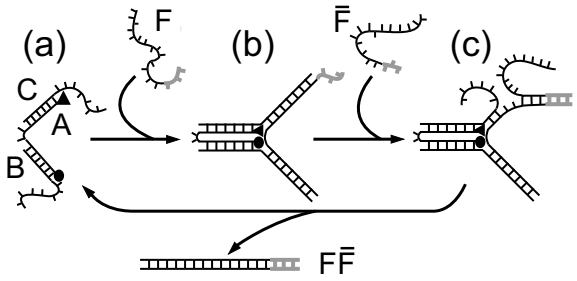

Figure 1: One operation cycle of the molecular tweezers in a schematic representation: Starting from the open tweezer configuration (a), self-assembled from three DNA strands A, B, and C, the tweezers can be closed (b) upon addition of a DNA fuel strand F. The removal strand $\bar{F}$ can remove the fuel strand F by branch migration (c) and restore the original state (a). An inert waste product $F\bar{F}$ is ejected in each cycle. $\bar{F}$ can attack F at a special 'toehold' section (colored in gray). This machine can reversibly bring two molecules attached to its arms (indicated by a circle and a triangle) into close proximity.

2 DNA MACHINES

2.1 Molecular Tweezers

The principle of operation of the first of our DNA machines, termed 'molecular tweezers', is schematically depicted in Fig. 1. In the starting configuration the device consists of three strands of DNA A, B, C. The base sequences of these strands are chosen in such a way that A, B, and C self-assemble into the 'open state' shown in Fig. 1 (a). Two double-stranded arms are held together by a short, flexible single-stranded region. The two single-stranded extensions of the arms define the 'motor domain' of the machine which enables its operation. Motion is induced by the addition of a fuel strand F which hybridizes with these single-stranded extensions. The hybridization process pulls the tweezers shut (Fig. 1 (b)), thereby bringing molecular components (indicated by circles and triangles in Fig. 1) attached to the arms close together. The fuel strand F contains a short single-stranded section which remains unhybridized in

the closed configuration (gray in Fig. 1). This section serves as a toehold for the removal strand $\bar{F}$ which is completely complementary to F. $\bar{F}$ can attach to F at this toehold and then wrest F from the closed tweezers in a process called branch migration (Fig. 1 (c)) [9]. This mechanism allows re-opening of the tweezers and thereby restoring the original open state. Repeated addition of fuel and removal strands enables cyclic operation of our molecular device. This is one of the main characteristics of a 'machine' [7].

In the experimental realization of the tweezers, the arms are chosen to be 18 base pairs or 6.12 nm long, joined by a four base long hinge section. The single-stranded extensions of the arms are 24 bases and the toehold section is 8 bases long. In the open state the mean separation of the ends of the arms is 6 nm corresponding to an opening angle of $\approx 50^o$ [8].

2.2 Nanoactuator

In our second nanomechanical device, a DNA 'nanoactuator', the single-stranded extensions of the tweezers are joined together, leading to a loop-like structure (see Fig. 2 (a)). Here, the fuel strand F hybridizing with the single-stranded motor domain of the actuator pushes the two double-stranded arms apart (Fig. 2 (b)). Using the same operation principle as for the tweezers, a removal strand $\bar{F}$ can attach at the toehold of F and restore the relaxed configuration of Fig. 2 (a)) by branch migration (Fig. 2 (c)). This machine can be cycled between a relaxed and a straightened configuration, thereby pushing two components apart from each other.

The single-stranded section of the actuator is 48 bases long, 40 of which constitute the motor domain which hybridizes with the fuel strand F. The other 8 bases are needed as two 4 base long flexible spacers. The arms and the hinge section are identical to those in the tweezers. In the relaxed state the components attached to the ends of the double-stranded arms are ≈ 5.1 nm apart. In the straightened state this distance is 13 nm.

3 EXPERIMENTS

The proper operation of both molecular machines was verified in several experiments involving fluorescence resonance energy transfer (FRET) and gel electrophoresis [8]. For the fluorescence measurements, the fluorescent dyes TET (tetrachlorofluorescein) and TAMRA (tetramethylrhodamine) were attached to the double-stranded arms of the tweezers and the actuator (see Figs. 1 and 2). These dyes constitute a 'FRET pair', i.e. the emission spectrum of TET and the absorption spectrum of TAMRA overlap. If TET and TAMRA come in close proximity (typically a few nm), TET can transfer its fluorescence energy to TAMRA, thereby decreasing the TET fluorescence signal. Due to its pronounced distance dependence, this energy transfer effect can be used as a 'spectroscopic ruler' [10] and for characterization of motion on the nanometer scale [4], [5],[6]. In Fig. 3 the TET fluorescence signal during cyclic operation of the two DNA machines is displayed. In the case of the tweezers, the two dyes are brought together upon closing the tweezers, leading to an increased energy transfer and therefore to a drop in fluorescence (Fig. 3 (a)). Conversely, the actuator pushes the two dyes apart, decreasing the energy transfer and therefore increasing the TET fluorescence (Fig. 3 (b)). The typical times needed to switch between the different mechanical states of the DNA devices are 13 s for opening and closing the tweezers, 21 s for straightening the actuator and 59 s for relaxing the actuator. These are the times needed for half-completion of the corresponding reactions in Figs. 1 and 2. In addition to the FRET measurements, extensive gel electrophoresis experiments were performed [5], [6]. Except for the occasional formation of unwanted tweezer or actuator dimers consisting of several DNA devices linked together, all findings were consistent with the operation schemes depicted in Figs. 1 and 2. The amount of dimer formation is approximately 20% for the tweezers and 10% for the actuator.

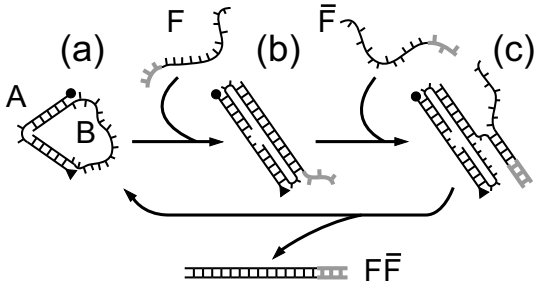

Figure 2: One operation cycle of the DNA nanoactuator. The actuator is assembled from two DNA strands A and B to from a loop-like relaxed configuration (a). The fuel strand F straightenes the DNA construct (b). By branch migration (c) the removal strand $\bar{F}$ restores the relaxed configuration (a). Toehold sections are colored in gray.

4 DISCUSSION

The molecular tweezers and the DNA nanoactuator are very simple examples of molecular machines, basically consisting of a motorized hinge. They can be repeatedly switched between two distinct mechanical states, thereby consuming DNA 'fuel'. The motion of these machines is driven by base pair hybridization. Hybridization of one base pair is accompanied by a mean free energy change of -78 meV [11] and motion on the length scale of the distance between two base pairs, i.e.

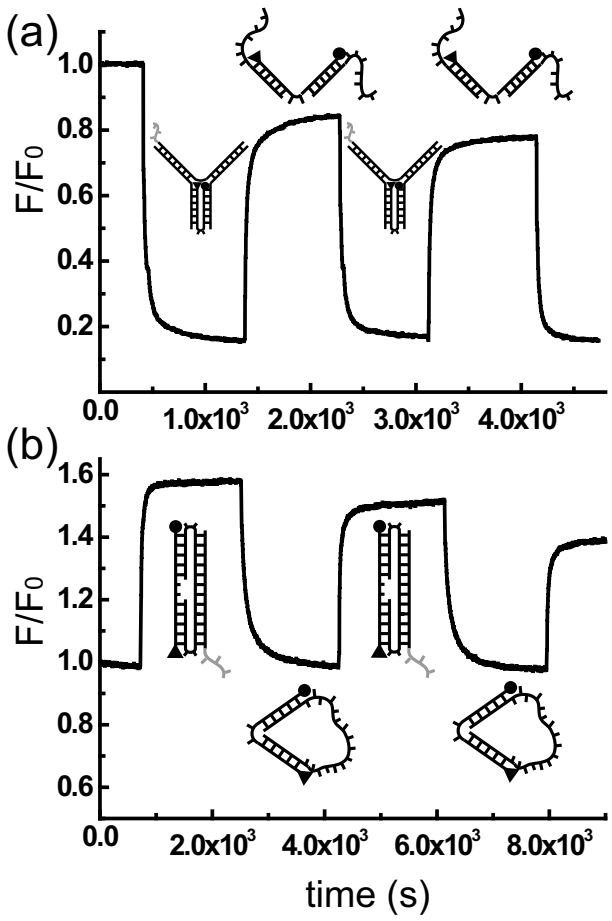

Figure 3: Fluorescence signal F/F_0 during cyclic operation of the molecular tweezers (a) and the nanoactuator (b): The double-stranded arms of the DNA machines are labeled with the fluorescence resonance energy transfer pair TET and TAMRA. TET fluorescence is excited with Ar ion laser light at 514.5 nm and detected at 536 nm. The fluorescence signal is normalized to the initial value F_0 for open tweezers or relaxed actuator. Fuel and removal strands F and $\bar{\text{F}}$ are alternately added to both machines in stoichiometric amounts leading to closing and opening of the tweezers or straightening and relaxing of the actuator as indicated by the schematic drawings. The TET fluorescence changes due to energy transfer between the dyes, demonstrating proper operation of both devices. Closing the tweezers in (a) quenches the fluorescence, as the dyes are brought close together, straightening the actuator in (b) increases the fluorescence, as the dyes are pushed apart from each other. The overall decrease in fluorescence is due to the dilution of the sample resulting from the addition of F and $\bar{\text{F}}$. Initially, the molecular machines have a concentration of 1μM in SPSC buffer (50mM Na_2HPO_4, 1M NaCl, pH 6.5) [8].

0.34 nm [1]. From these values, it is estimated that forces on the order of 10 pN or more can be generated by DNA machines, which is comparable to forces exerted by biological molecular motors such as actin [12] or RNA polymerase [13]. As biological motors are involved in molecular assembly processes in living cells, this indicates that our DNA machines are good candidates for molecular self-assembly in nanotechnology.

The task of the tweezers and the nanoactuator is bringing molecular components close together or apart from each other, respectively. In our first experiments, these components were fluorescent dyes which enabled the characterization of the motion of these machines. However, in future work they can be replaced by other molecules having a special chemical, biological or electronic function. Tweezers functionalized with chelating agents could serve as 'ion grabbers', attachment of electronic donors, acceptors and molecular wires could become useful in the assembly of molecular electronic circuits.

The usage of DNA as material for molecular self-assembly has several advantages over other approaches to self-assembly, which lie in the nature of this biopolymer as information-carrying molecule: The existence of a four letter alphabet {A,T,G,C} together with the high specifity of the base-pairing interaction allow for programmable self-assembly, where interactions between molecules can deliberately be chosen or avoided. The huge combinatorial space of different DNA sequences (e.g., there are $4^{10} \approx 1.0 \times 10^6$ different 10 base long oligomers) offers great flexibility for the design of molecular constructs. In the case of DNA machines, the information-carrying nature of DNA can be exploited even further: in an ensemble of several different machines, the fuel strands could be used to specifically address particular machines to perform their task. Furthermore, it is conceivable to arrange molecular machines in such a way, that after completion of its performance one machine triggers the action of another.

In DNA nanotechnology, the similarity of self-assembly and computation becomes obvious [14]. DNA is code and structural material at the same time. The execution of the 'program' encoded on the DNA strands results in the assembly of complicated structures and the performance of specific tasks. DNA hybridization interactions serve as a low-level programming language for molecular self-assembly.

5 CONCLUSION

We have demonstrated the assembly and proper operation of two simple molecular machines made of and powered by DNA. One of these devices can bring two molecules close together, whereas the other can push two molecular components apart. The DNA machines presented here are prototypes which may help under-

standing the basic mechanisms underlying motion on the molecular scale both in biological systems as well as in artificial nanosystems. Moreover, more elaborate versions of these molecular machines can possibly be utilised to direct self-assembly of complicated structures on the nanometer-scale such as molecular electronic circuits. In contrast to other approaches to molecular self-assembly DNA offers the advantage of programmability and hence a greatly increased versatility in building nanometer-sized structures.

6 ACKNOWLEDGMENTS

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