Stretching and Transporting DNA Molecules Using Motor Proteins

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ABSTRACT

Inside cells, motor proteins perform a variety of complex tasks including the transport of vesicles and the separation of chromosomes. We demonstrate a novel use of such biological machines for the mechanical manipulation of nanostructures in a cell-free environment. Specifically, we show that purified kinesin motors in combination with chemically modified microtubules can transport and stretch individual λ-phage DNA molecules across a surface. This technique, in contrast to existing ones, enables the parallel yet individual manipulation of many molecules and may offer an efficient mechanism for assembling multidimensional DNA structures.

The size, strength, and sequence specificity of DNA molecules make them promising building blocks for the assembly of 2D and 3D structures on the nanometer scale.1 One potential application for such DNA-based structures is in nanoelectronics. Although the intrinsic conductance of DNA is very low,2 linearly stretched DNA molecules have proven to be valuable as templates for metallization,3–5 for metal-ion insertion,6 and for the nucleation of metal and semiconductor nanoparticles.7,8 Such metallized DNA has sufficient conductance for use as a nanowire.4,9,10

To use DNA to link the components of a nanocircuit, the molecules must be aligned mechanically. This poses a major challenge. Recently, a number of physical techniques have been developed to manipulate single DNA molecules.11 These techniques include the employment of magnetic12,13 or optical tweezers,14–16 microneedles,17,18 or an atomic force microscope (AFM).19 However, a major limitation of these techniques is that only one DNA molecule can be manipulated at a time. Although this is not a problem for the investigation of the mechanical properties of individual DNA molecules, it severely limits the efficiency of these techniques for DNA-based molecular construction. In addition, these techniques require large, sophisticated, and expensive apparatus, thus hampering parallelization.

One way to circumvent these difficulties and to manipulate multiple DNA molecules simultaneously is to use hydrodynamic flow,20,21 an electric field,22,23 or a moving water—air interface.24,25 In this way, many DNA molecules can be simultaneously aligned and stretched. However, to create truly 2D structures, these procedures would have to be applied sequentially in different directions, or additional steps such as the cutting and moving of individual DNA fragments by an AFM26 would have to be added.

We present an alternative approach in which biological machines, specifically, kinesin motor proteins, are used to manipulate simultaneously many individual λ-phage DNA molecules near a surface. Although the interaction of motor proteins with filamentous structures in cells has been studied extensively,27 its applicability to nanotechnological tasks has been explored only recently.28–36 An intriguing feature of motor proteins, such as kinesin, is that they perform very precise nanometer steps in a highly controlled manner: for example, kinesin takes 8-nm steps along a microtubule,37 each step coupled to the hydrolysis of one ATP molecule.38 However, because the force produced by one kinesin molecule27 (about 6 pN) is barely sufficient to stretch a λ-phage DNA molecule to its contour length19 of 16.5 μm, many motors have to be used together. Our approach is therefore based on a gliding motility assay in which the substrate surface is coated with kinesin motor proteins and microtubules are propelled across the surface by a number of kinesin molecules in the presence of ATP. The attachment of DNA to microtubules was achieved using a biotin–streptavidin linkage (Figure 1).

To visualize the transport and stretching of the DNA molecules by motile microtubules, a fluorescence microscopy assay was performed. A 5-mm-wide flow cell was built from
a microscope slide (FISHERfinest Premium Plain, 75 × 25 × 1 mm²), a coverslip (Menzel-Gläser, 18 × 18 mm²), and two pieces of double sided tape (Scotch 3M, thickness 0.1 mm). First, a casein-containing solution (0.5 mg/mL in BRB80) was perfused into the flow cell, and the casein was allowed to adsorb to the surfaces for 5 min to reduce the denaturation of kinesin and to prevent the sticking of microtubules. Then the motor-containing solution (5 µg/mL kinesin, 1 mM Mg-ATP, 0.2 mg/mL casein in BRB80) was perfused. After 5 min, the solution was exchanged for a motility solution containing the microtubules with bound DNA³⁹ (50 nM biotinylated tubulin, 0.5 µg/mL DNA, 1 mM Mg-ATP, 0.2 mg/mL casein, 10 µM taxol in BRB10). Total internal reflection fluorescence (TIRF) microscopy (through the objective, 100×, NA = 1.45, Zeiss, Germany) was used to image the YOYO-labeled λ-phage DNA molecules.⁴⁰ Individual DNA molecules, which condense into 1-µm-diameter random coils because of their high flexibility, were clearly observed as they were transported across the kinesin-coated surface on the gliding microtubules (Figure 2, supplementary movie S1). Typically, some 10–50% of the microtubules had DNA bound to them.

The forces generated by the motor proteins can be used to stretch condensed DNA molecules. In one method, the pH of the motility solution was lowered to 6.0 resulting in a number of DNA molecules binding to the surface by one end.⁴¹ However, using this method, it cannot been ruled out that a number of the DNA molecules do bind directly to the surface with a midsegment or via streptavidin. Moving microtubules were then able to pick up the other end of the DNA, via the biotin-streptavidin linkage. The DNA was stretched (Figure 3a, supplementary movie S2), sometimes up to its 16.5-µm contour length (supplementary movie S1), until the DNA detached from the surface or broke. Breakage of the DNA in our experiments was presumably due to
producing forces up to 600 pN) interact with a 5- to about 100 kinesin molecules (potentially capable of moving microtubules were not dramatically influenced by the high motor densities of up to 1000 motor molecules per square micrometer of surface area. At these densities, as many as seven simultaneous stretching events. Because the frequency of events is expected to depend on the microtubule density (as well as on other factors), we believe that a substantial increase in the event number should be possible. More rarely, DNA was stretched between two moving microtubules (Figure 3b, supplementary movie S3). In these experiments, the speed of the microtubules was independent of whether DNA molecules were attached to a moving microtubule. Furthermore, the trajectories of the moving microtubules were not dramatically influenced by the binding to the DNA molecule. We attribute these findings to the high motor densities of up to 1000 motor molecules per square micrometer of surface area. At these densities, up to about 100 kinesin molecules (potentially capable of producing forces up to 600 pN) interact with a 5-µm-long microtubule.

Interestingly, DNA can also be used to control the movement of the microtubuses. This was achieved by lowering the density of the motors and increasing the stability of the DNA by reducing the YOYO labeling. DNA-leashed microtubules were then stopped completely (supplementary movie S1) or had their trajectories altered (Figure 4, supplementary movies S4). A further example where a microtubule is forced onto a circular path is given in Supporting Information (supplementary movie S5).

Here, we have demonstrated a novel method for the manipulation of individual DNA molecules based on force generation by motor proteins. Thus, we have used the transport machinery of living cells to fulfill specific tasks in an engineering environment. In contrast to conventional DNA manipulation methods, our approach offers the potential for simultaneous yet individual operation on multiple biomolecules. However, to be useful for building nanostructures, the manipulation needs to be more precise in both position and direction. We believe that the further combination of concepts from biology and materials science might overcome these challenges. For example, to create nanocircuits, microtubules gliding along predefined tracks could be used to link DNA between the appropriate contact points in a nanofabricated array. Prior to metallization, sequence-specific information within the DNA strands could be used by restriction enzymes to cut unwanted connections. Because each step in such a procedure would allow simultaneous operation on many DNA molecules, complex network structures might be produced with high efficiency. Beyond the application of our approach to DNA manipulation, we foresee the potential of this techniques in the transport and positioning of other nanostructures such as functionalized carbon nanotubes.

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Supporting Information Available: Quicktime movies of DNA stretching and microtubule movement. This material is available free of charge via the Internet at http://pubs.acs.org.

References


Figure 4. Motile microtubule guided on a “DNA leash” that is attached to its leading edge. In contrast to the other microtubules observed in this experiment (straight dotted lines), the guided microtubule is forced onto a bent trajectory by the pulling force of the anchored DNA molecule. (See also supplementary movie S4.) Another example, where a microtubule performs about five full circular turns on an anchored DNA leash is given in Supporting Information (supplementary movie S5).

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Rhodamine-labeled, biotinylated microtubules were polymerized from 10 μL of bovine brain tubulin (4 mg/mL, mixture of 3 rhodamine-labeled/4 biotinylated/9 unlabeled tubulin units) in BRB80 buffer (80 mM potassium PIPES, pH 6.9, 1 mM EGTA, 1 mM MgCl₂) with 4 mM MgCl₂, 1 mM Mg-GTP, and 5% DMSO at 37 °C. After 30 min, the microtubule polymers were stabilized and 100-fold diluted with room-temperature BRB80 containing 10 μM taxol. To remove unpolymerized tubulin, 400 μL of the resulting microtubule solution (0.32 mM) was centrifuged at 178000g in a Beckman airfuge for 5 min. The pellet was resuspended in 200 μL of BRB10 (10 mM potassium PIPES, pH 6.9, 1 mM EGTA, 1 mM MgCl₂) containing 10 μM taxol, yielding a microtubule solution with about 0.64 μM polymerized tubulin (also termed B-MT 50). The ends of B-phase DNA were biotinylated using the Kleenow fragment of polymerase I and biotinylated dCTP (13), thus yielding fully double-stranded DNA with four biotin molecules on one end and six biotin molecules on the other. For fluorescent labeling, 5 μL of biotinylated λ-DNA (100 μg/mL) was mixed with 4 μL of Nanopure water and 1 μL of YOYO-1 iodide stock solution (25× diluted in TBE buffer (45 mM Tris-borate, 1 mM EDTA)). The solution was incubated for at least 30 min at 4 °C in the dark. Streptavidin was bound to the biotinylated tubulin sites on the microtubule lattice by mixing B-MT 50 with streptavidin (40 nM in BRB10). To achieve rapid binding and thus avoid any cross linking of microtubules, the mixing was performed while vortexing the solution. The labeled λ-DNA (diluted to 2 μg/mL in BRB 10 containing 10 μM taxol) was then added to the microtubule–streptavidin solution.

For objective-type TIRF, the 488-nm line from an argon/krypton ion laser (Innova 90, Coherent, U.K.) was coupled into an optical single mode, polarization-maintaining fiber (ÖZ optics, Canada) and injected off-center into the epi-illumination port of a Zeiss Axiovert 200M optical microscope. Rhodamine-labeled microtubules were imaged by conventional epifluorescence microscopy. Sets of rhodamine (red) and YOYO (green) fluorescence images were acquired every 5 s with an exposure time of 100 ms using an intensified cooled CCD camera (I-PentaMax, Princeton Instruments, Trenton, NJ) and the Metamorph imaging system (Universal Imaging Corp., Downingtown, PA). To obtain dual color images, fast switching between TIRF and epi-illumination was realized using an optical shutter system (Ludl Electronic Products, Hawthorne, NY) and the motorized filter turret of the Zeiss Axiovert 200M microscope. Red and green images were obtained sequentially with a lag time of about 0.9 s.


