Microchemomechanical devices using DNA hybridization

Guolong Zhu\textsuperscript{a,1}, Mark Hannel\textsuperscript{a}, Ruojie Sha\textsuperscript{a}, Feng Zhou\textsuperscript{a}, Matan Yah Ben Zion\textsuperscript{a}, Yin Zhang\textsuperscript{a}, Kyle Bishop\textsuperscript{a}, David Grier\textsuperscript{b}, Nadrian Seeman\textsuperscript{b,1}, and Paul Chaikin\textsuperscript{a,1}

\textsuperscript{a}Department of Physics, New York University, New York, NY 10003; \textsuperscript{b}Department of Chemistry, New York University, New York, NY 10003; and \textsuperscript{c}Department of Chemical Engineering, Columbia University, New York, NY 10027

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The programmability of DNA oligonucleotides has led to sophisticated DNA nanotechnology and considerable research on DNA nanomachines powered by DNA hybridization. Here, we investigate an extension of this technology to the micrometer-colloidal scale, in which observations and measurements can be made in real time/space using optical microscopy and holographic optical tweezers. We use semirigid DNA origami structures, hinges with mechanical advantage, self-assembled into a nine-hinge, accordion-like chemomechanical device, with one end anchored to a substrate and a colloidal bead attached to the other end. Pulling the bead converts the mechanical energy into chemical energy stored by unzipping the DNA that bridges the hinge. Releasing the bead returns this energy in rapid (~20 μm/s) motion of the bead. Force-extension curves yield energy storage/retrieval in devices that is very high. We also demonstrate remote activation and sensing—pulling the bead enables binding at a distant site. This work opens the door to easily designed and constructed micromechanical devices that bridge the molecular and colloidal/scale.

For large extensions, we combine 10 rods into an “accordion,” attached to the substrate at one end and to a 500-nm colloidal bead (10–15) at the other end (Fig. 2A and SI Appendix, Fig. S5). Force-extension measurements (16, 17) on the accordion were made by pulling on the tethered bead with optical tweezers until the bead escapes the optical trap (Fig. 2B and SI Appendix, Fig. S7 and Movie S1). Mechanical advantage amplifies 6 pN on the bead to ~180 pN on DNA zippers. The total throw of the accordion is 7.4 μm. The equilibrium particle position distribution and Boltzmann statistics yield the initial restoring force and equilibrium position (SI Appendix, Fig. S8). Force calibration is detailed in SI Appendix, Fig. S6.

In the force-extension curve (Fig. 2B and C), bridging DNA unzips in the range 8 to 20 pN or 0.27 to 0.67 pN applied to the accordion ends. A control with no bridging DNA zippers (blue) is well fit as a freely jointed chain, Langevin function (black) (SI Appendix, Fig. S10). The force-extension curve agrees well with the rms displacement (SI Appendix, Fig. S9) from an elastic spring model at low force and an entropic freely jointed chain at large force. The integrated area (shaded green) between the accordion (red) and the control (blue), 408 kBT (±8.3%), is the measured work done and energy stored in the unzipped DNA. By comparison, the calculated ΔG for the particular sequences

\textbf{Significance}

With simple DNA origami lever arms arranged in hinges and accordion structures, we amplify the nanometer displacements from DNA hairpin zippers to 4-μm motion, easily observable and quantified in real space and real time with conventional optical microscopy. Mechanically pulling a bead tethered on the accordion end, we measure high-energy recovery and retraction speeds up to 50 μm/s. On longer time scales, we have also opened and closed the hinges with light and heat. DNA nanotechnology, and particularly DNA origami, combined with colloids and emulsions can provide powerful architectures. The present study is a step toward activating such colloidal/cellular scale devices using DNA as a power source/fuel. We envision artificial active flagella, cilia, micropumps, and other cellular scale devices.

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1To whom correspondence may be addressed. Email: gz429@nyu.edu, ned.seeman@nyu.edu, or chaikin@nyu.edu.

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used is 418 k_BT (±5%) (18): The mechanical work done on the extension has been converted to recoverable DNA hybridization chemical energy that can be used to move the tethered bead through the viscous medium.

The bridging DNA zippers used in Fig. 2 have 30 nt pairs plus 4 nt spacers at midpoint and remain hybridized with at least 8 nt pairs upon complete accordion extension. Shorter zippers (21 nt pairs), which are completely unzipped at full extension, show hysteresis (Fig. 3). At large extension, the only reactive force is entropic; therefore, the completely unzipped states lie on the same curve as the control, the freely jointed chain that lacks any bridging DNA zippers. At shorter extension, DNA zippers are partially hybridized, exerting an enthalpic force, in addition to the entropic force. The hinges are at least partially closed, and the force is transmitted from one hinge to the next by the elastic bending of the 6HB rods; see SI Appendix, Fig. S3. The losses only arise upon completely unzipping and then renucleating the hybridization, similar to losses in forming secondary structures in RNA or proteins on folding (19, 20).

To quantify the time scale for such DNA devices, we pull the bead to near full extension and release, using the accordion with zippers long enough that they are always partially bound, and exhibit no hysteresis (Fig. 2). We use beads of two sizes, 500 (blue) and 1,000 nm (red). Both show a fast pull back within a fraction of second (Fig. 4B and Movie S2), with a peak velocity of 50 and 25 μm/s, respectively (Fig. 4C). The time and speed depend on the load rather than the folding time (10 to 100 μs) of the DNA hairpin (21). A model including particle and accordion Stokesian drag near a wall agrees with our observations (SI Appendix, Fig. S11). The force is mostly entropic at large extension and mostly elastic at short distances. The extension range in which the DNA hybridization is the dominant driver is from 3,000 to 1,000 nm. The collapse in this region happens within 0.1 s and at speeds exceeding 25 μm/s. Using shorter DNA zippers (SI Appendix, Fig. S12), it takes ~1 s for the devices to return to 1,000 nm, as the complementary single strands must first diffuse to make contact, initiating hybridization.

To show directly that the bridging DNA is indeed unzipped upon accordion extension for short DNA zippers, we prepared a set of probe colloids coated with 21-base ssDNA, complementary to one side of a particular DNA zipper. Probe colloids held close to the unstretched accordion do not bind. When the accordion bead is pulled to full extension, the probe particle attaches to the designated open hinge strand. In three designs, with the probes programmed to bind to zippers at 400, 1,200 (Fig. 5A), and 2,000 nm (Fig. 5B), we observe binding. The binding is confirmed by pulling the bead around a circle at maximum extension and observing that each probe bead follows concentrically at its appropriate radius (SI Appendix, Fig. S3). Thus, our DNA accordion also exhibits remote sensing, a simple primitive form of mechanical allostery (22, 23), a displacement applied at one end of our construct allows binding at a distant site (Movie S3).

There have been many previous experiments pulling on DNA in clever configurations: long unhybridized sequences that explore DNA as a prototype entropic polymer (24), hairpin structures (25) that quantify hybridization free energy calculations and explore kinetics, or beautifully designed DNA springs (26) that demonstrate efficient purely elastic behavior with no dehybridization upon stretching. The present study builds on these studies and extends them to quantify the speed and reversibility of storing and recovering, specifically the dehybridization energy stored on the mesoscopic (many micrometers) scale.

We have demonstrated that the energy associated with DNA hybridization at the nanometer scale can be leveraged to produce controlled motion at the micrometer scale, with a 1.6-μm DNA construct activated by heat or by light. Furthermore, we have made an accordion, which demonstrates that DNA can be utilized at the ~4-μm scale to store and reversibly recover energy on a time scale of fractions of a second, producing speeds up to 50 μm/s. Unlike biological motors, in which the machine and the chemical energy storage are separate, DNA constructs incorporate...
both functions in a single molecule (3). It is worth emphasizing that in our devices we make use of reversible hydrogen bonds in contrast to the lossy use of ATP hydrolysis in molecular motors. The present system, combining mechanical advantage and short DNA strands that hybridize without initiation and without folding entanglements, is easily extended to even larger distances by adding more hinges (27). It has the advantage that it avoids the entanglements and distortion concomitant with folding large DNA hairpins. This system suggests a paradigm for the design of devices on the nanometer to cellular scales. Combined with the

![Diagram](image.png)

**Fig. 2.** Force-extension curves for the accordion construct. (A) An “accordion” of 10 6HBs, with nine DNA zippers and two 4T ssDNA at the vertices. The first 6HB is bound to the substrate with sticky “legs” and the last to a bead with sticky “hands.” (B) Each red point is an average over 40 measurements on each of five samples. Dashed red is calculated from the Boltzmann distribution, measured with no force. Solid blue is a control experiment with no bridging DNA zippers. Dashed blue is from the equilibrium distribution of the control. Solid black is the Langevin function for a 10-mer freely jointed chain. The green shaded area (408 k_BT) is the work done and energy stored in extending the accordion (ΔG for unzipped sequences is 418 k_BT). (C) Measured and calculated curves to full extension.

![Diagram](image.png)

**Fig. 3.** Hysteresis with complete unzipping of short DNA zippers. (A) The bead is pulled to 3,800 nm with 6 pN. Held with these forces, the extension is decreased. The trapping force is then lowered until the accordion pulls the bead out from the trap. The force and extension are recorded (red points). Without changing the trapping force, the bead is then pulled from its equilibrium position to where it is pulled out from the trap. Force and extension are recorded (blue points). Each trapping force corresponds to two extensions. An example is the two points just inside the dashed gray ellipse. The two states correspond to zipped and unzipped bridging DNA zippers, as illustrated above and below the ellipse. (B) Snapshots of the experiment for one set of points with green as full extension, red the position where the bead escapes the trap, yellow the equilibrium position to which the bead retracts, and blue the position that the bead can be pulled to with the same (escape) force, ~0.33 pN. (Scale bar, 1 μm.)
The extension is measured from micrograph movies at 30 frames/s, averaged over 40 runs. Red/blue are for 1-μm/500-nm beads, respectively. A total of 10 different 6HBs was mixed, filter at 2.4 k RCF for 3 min. Following the final step, the remaining material was retrieved by centrifuging the inverted centrifuged at 2.2 k relative centrifugal force (RCF) for 12 min. Following the last step, the remaining material was retrieved by centrifuging the inverted filter at 10 °C/h for retrieval.

**Methods**

**Preparation of the 6HB DNA Origami and Accordion.**

6HB DNA origami buffer. 1x Tris-acetate-EDTA buffer with 10.5 mM Mg (12.5 mM Mg, 2 mM EDTA), the annealing 6HB monomer, is created when the solution was ramped up to 68 °C, remained at that temperature for 30 min, ramped down at 10 to 28 °C/h, left for 5 h, and then ramped at 60 °C/h down to 4 °C for retrieval.

**Purification.** Different 6HBs are purified separately with a 100 Kd Amicon filter. A total of 200 μL 6HB was prepared with M13 (10 nM) and each staple strand (100 nM). The material was purified three times by filling the 400-μL spin filter with its maximum volume (total ~400 μL). The filter was centrifuged at 2.2 k relative centrifugal force (RCF) for 12 min. Following the last step, the remaining material was retrieved by centrifuging the inverted filter at 2.4 k RCF for 3 min.

**Annealing the accordion assembly.** A total of 10 different 6HBs was mixed, ramped up to 43 °C, annealed for 5 h, ramped down to 28 °C at 0.1 °C/h, and left for 10 h; the material was then ramped down to 4 °C at 1 °C/h for retrieval.

**Annealing the trimer assembly.** Three different 6HBs were mixed, ramped up to 43 °C, and left for 5 h. The solution was then ramped down to 28 °C at 0.5 °C/h and annealed for 10 h. The solution was then ramped down to 4 °C at 10 °C/h for retrieval.

Preparation of the DNA-coated colloidal beads follows the protocol of ref. 11.

**Preparation of the Accordion-Colloid Assembly.** The assembly buffer consists of the following: 1x phosphate-buffered saline (155 mM Na), MgCl2 (10.5 mM Mg), and 0.1% F127 (wild type/wild type).

**Assembly.** A total of 2.5 μL DNA-coated beads (100 pM) were mixed with 2.5 μL 2x assembly buffer and 4 μL 1x assembly buffer, to which 1 μL DNA origami (diluted 1x assembly buffer to keep the ratio of origami:beads = 1:5) was added. Then, 1 μL tracking reference particle solution was added and annealed 10 min before washing (60 μL, eight times). Finally, the sample cell was sealed with ultraviolet (UV) glue.

**AFM Imaging.** Bruker atomic-force microscopy, ScanAsyst, Peak Force Tapping in Air mode was used.

The DNA origami monomer was diluted to a concentration of 0.2 nM. Then, 3 to 5 μL was deposited on the mica surface, annealed for 5 to 10 min, washed with 100 μL DI water quickly, and dried with an air gun.

**Agarose Gel Electrophoresis.** Around 0.8% (wild type/wild type) agarose gels were used. Gels were stained with ethidium and imaged with UV (365 nm) light.

**Light Microscopy and Particle Tracking.** Conventional inverted bright field or fluorescent microscopy was used with oil or air lenses; the Interactive Data Language two-dimensional, particle-tracking method follows ref. 17.
Holographic Optical Trapping and Video Microscopy. The holographic, optical-trapping instrument is described in ref. 36.

Data Availability. All study data are included in the article and/or supporting information.

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