Communication

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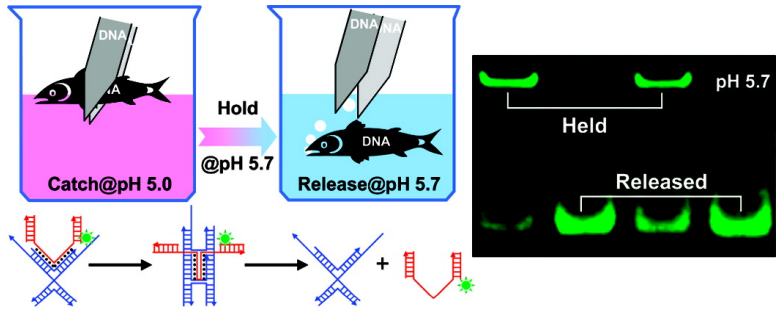
J. Am. Chem. Soc., **2008**, 130 (44), 14414-14415 • DOI: 10.1021/ja805945r • Publication Date (Web): 14 October 2008



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Published on Web 10/14/2008

Catch and Release: DNA Tweezers that Can Capture, Hold, and Release an Object under Control

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In this communication, we report a pair of DNA tweezers that can capture, hold, and release a target accompanying its "close" and "open" actions. DNA nanomechanical devices have become the focus of intensive researches over the past years.[1] These devices have potentials in many applications such as microsurgery, nonviral gene delivery, targeted medicine delivery, in-cell cargo transportation, and even nanoscaled assembly lines. Starting from a B-Z transformation-based DNA mechanical device[2] and DNA-fueled molecular tweezers,[3] various forms of DNA machines have been built.[1−6] Although DNA tweezers, as the most basic and simplest form of DNA mechanical devices, are expected to be the first to demonstrate some close-to-reality functions, one open challenge is how to make them really behave like real tweezers that can be handled to grasp and transfer an object. Previous work has shown that a DNA tetrahedron can encapsulate a protein with a size matching the inside cavity of the tetrahedron.[7] DNA polyhedra and even a DNA buckyball should also be able to serve as such nanocontainers.[8] Capture and release of protein has also been tried using a DNA-cross-linked polyacrylamide gel.[9] Back to DNA tweezers, the challenge still remains: how to engineer the tweezers so that they can mimic real tweezers to capture, hold, and release an object?

Here we try to address this challenge by taking advantage of the formation of a DNA triplex[10] via Hoogsteen hydrogen bonding between a target DNA and the two arms of DAE[6] structured (evolved from Yan's design)[6b] DNA tweezers. Three steps are involved to operate such a device (refer to Figure 1): (1) Four DNA strands are annealed to form opened tweezers. A DNA target preassembled from three DNA strands is introduced. The single stranded part of this target has a polypyrimidine sequence that will form triplexes with the tweezers' arms[10] at pH 5.0. (2) A DNA set (locker) strand is then employed to close the tweezers by hybridizing with two single strand overhangs on the tweezers' arms. The target has five consecutive adenines in the middle to ensure sufficient flexibility when the tweezers are closed. The pH is then switched to 5.7, and at this increased pH the tweezers still firmly hold the target via a closing action and some remaining Hoogsteen bonding. (3) Finally, an unset (unlocker) strand that fully complements the set strand is added to remove the set strand from the closed tweezers. This process utilizes some yet unpaired bases on the set strand to initialize a strand displacement reaction.[3] The tweezers then are opened, and the DNA target is released.

The pH at which the DNA target was held should be optimized to allow some remaining yet significant Hoogsteen hydrogen bonding to help stabilize the target in the closed tweezers. However, this Hoogsteen bonding should be sufficiently weak so that the target could easily detach from opened tweezers. After some trial-and-error experiments (easy to work with thanks to the monotonous change of Hoogsteen bonding stability with pH), a pH of 5.7 was found to meet the above criteria. In addition, the target had two duplex parts flanking the polypyrimidine domain, which were found to create some extra steric hindrance to further secure the target in the closed tweezers.

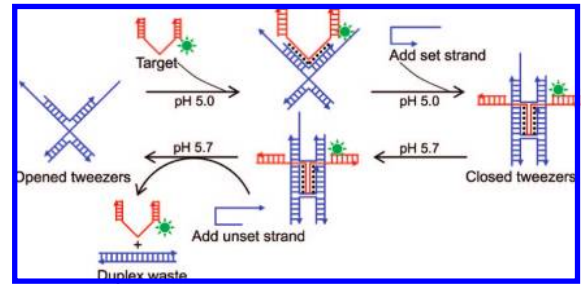

Figure 1. Schematic illustration of a pair of DNA tweezers (in blue) being operated to capture, hold, and release a DNA target (in red). Bars and dots (not to show exact numbers of base pairs) represent Watson−Crick and Hoogsteen bondings, respectively. See Figure S1 for all DNA sequences.

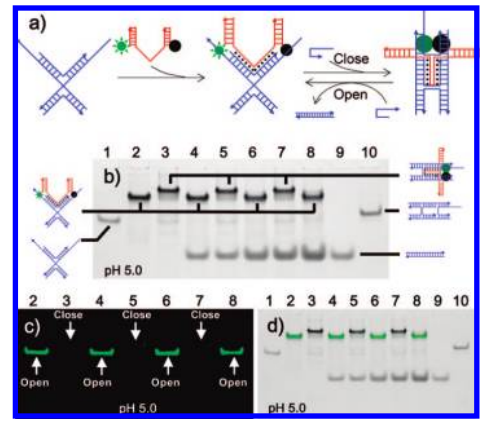

Figure 2. A 12% native PAGE run at pH 5.0 and viewed by dye staining and fluorescent imaging (based on emission from a labeled DNA target) to show that DAE tweezers can be effectively operated between "open" and "close" states and exert folding and unfolding actions on a captured DNA object: (a) "open" and "close" cycling of the tweezers; (b) gel stained by stains-all; (c) a fluorescent gel; and (d) superimposed gel images of (c) on (b). Note that the fluorescence image was taken prior to gel staining.

We first used a fluorescent resonance energy transfer (FRET) technique[11] in combination with a native polyacrylamide gel electrophoresis (PAGE) to check if the tweezers could be properly closed and opened and if these closing and opening actions could effectively fold and unfold the captured DNA target. We used blackhole quencher (BHQ-1) and fluorescein (FAM) dual-labeled DNA target (see Figure 2a) to monitor this process: fluorescence emission from FAM would be efficiently quenched by BHQ-1 when they were brought adjacent to each other by closing the tweezers. As shown in Figure 2b, the DNA target was successfully captured at pH 5.0 and the tweezers could be opened and closed by alternate additions of the set and unset strands. Strong evidence came from

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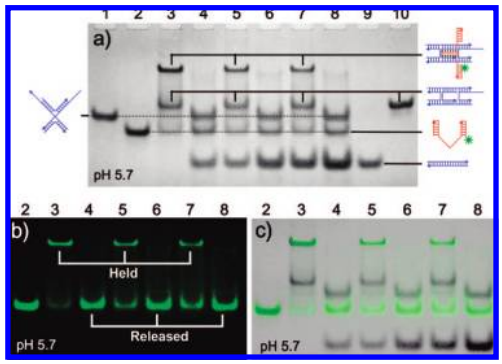

Figure 3. A 12% Native PAGE run at pH 5.7 and viewed by nonspecific dye staining and fluorescence imaging to demonstrate that DAE tweezers can be cycled to hold and release a DNA object: (a) a dye-stained PAGE gel; (b) a fluorescent image of the same gel in (a); (c) superimposed images of (b) on (a). Samples were first set to pH 5.0 to finish the capturing processes and then switched to pH 5.7 for gel loadings (see Figure S3 for more details about the cycling process).

the fluorescent gel in Figure 2c, which unambiguously shows that the gel bands strongly fluoresced for opened tweezers and became dark after the tweezers became closed. These bright and dark bands corresponded to the unfolded and folded states of the DNA object (refer to Figure 2d). It is also noteworthy that the DNA target could not be captured if the tweezers were closed first and then allowed to incubate with the target (Figure S5, lane 4), which demonstrated another interesting mechanical control opposite to the capturing process and indicated the tweezers were tightly closed benefiting from the rigidity of the short (one helical turn) duplex arms of the tweezers.

We then used a fluorescently labeled target (see Figure 1) combined with a native PAGE to see if the DNA tweezers could be manipulated to hold and release the DNA object. We prepared closed tweezers with the labeled DNA target captured inside at pH 5.0. The pH was then switched to 5.7 followed by adding the unset strand to open the tweezers and release the target. We demonstrated three such cycles in Figure 3 (see Figure S3 for a detailed cycling scheme). The dye stained gel (Figure 3a) shows that correct structures were formed and the DNA target could be held by the tweezers at pH 5.7 and released after the tweezers were opened. Lanes 1, 2, 9, and 10 in Figure 3a denote the opened tweezers (no target), the DNA target, the duplex formed between the set and unset strands, and the closed tweezers (no target). The upper band in lane 3 indicates a stably held target by the closed tweezers. Lane 4 shows the object was fully released after opening the tweezers. Lanes 5−8 demonstrate two further cycles of the "hold" and "release" processes. The fluorescent gel (only structures containing the labeled target are visible) in Figure 3b and the superimposed images in Figure 3c further verified the proper functioning of the tweezers. We also designed two control targets with the Hoogsteen sequence altered (one was changed to polyT; the other took a reversed sequence of the normal Hoogsteen domain) to block their interactions with the tweezers. Figures S6−S8 showed that neither of them could be properly captured, indicating the key role of Hoogsteen bonding.

It was also observed that there were some targets not being held by the tweezers at pH 5.7 (see Figure 3). This is reasonable considering the tweezer-binding domain in a target only contained two kinds of bases and thus unexpected binding modes might happen. Also, imperfections in the design and the operation conditions might exist that could lead to some improper clampings of the target. With further developments in noncanonical nucleic acid structures and a better understanding of the kinetics of the "catch and release" process, we believe these problems will be solved in the future. In addition, pH 5.7 at which the object was held can be a measure of the target stability in the closed tweezers, which is being used in our effort to further improve the tweezers' performance.

In conclusion, we have successfully built a pair of DNA tweezers that can capture, hold, and release an object with easy control. Most significantly, our work has disclosed the possibility of using synthesized DNA nanomechanical devices to undertake some close-to-reality work, which belongs to the long-term goals for DNA-structured nanomachines. Therefore, this work can be an important start for research on DNA mechanical devices toward more sophisticated functions. The structure of the tweezers is a DAE tile that can be integrated into various DNA lattices[4c,6a] toward cooperative and synergetic actions under multiplexed controls. After these tweezers are incorporated into periodical DNA structures, we will be expecting to use atomic force microscopy (AFM) to directly watch the capture and release processes. It would also be interesting to investigate if the mechanical force generated by a DNA nanomachine can be utilized to interfere with inter- or intramolecular interactions in a chemical[4d,10b,12a] or biological environment. Finally, we hope our results will be helpful to people who are seeking more advanced techniques for controlled chemical or biological processes, drug or gene delivery,[12b] and even DNA-based mechanosensors.

Acknowledgment. This work was supported by NSFC (Grant No. 20605019, 20873134), USTC (a start-up fund), CAS (a Bairen support), and PCSIRT (IRT0756).

Supporting Information Available: Experimental details and gels showing the correct assembly of the tweezers. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA805945R