Supporting information for

Logic-Based Dual-Functional DNA Tweezers with Protein and Small Molecule as Mechanical Activators

Xue-Mei Li\textsuperscript{a,b,*}, Wei Li\textsuperscript{b}, An-Qing Ge\textsuperscript{b}, Hong-Yuan Chen\textsuperscript{a,**}

\textsuperscript{1} Department of Chemistry, Nanjing University, Nanjing 210093, P. R. China
\textsuperscript{2} State Key Laboratory Base of Eco-chemical Engineering, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China
Formation of dimer or larger structures

After one cycle of opening and closing, higher molecular weight systems than were expected from the simple tweezer assembly were observed for all systems (lane 4 in Figure S1). These structures are attributed to dimer or larger structures as shown in Scheme S1. The formation of the dimer or larger structures might be explained as follows. For the construction of the closed tweezers, a solution consisting of A-F in a buffer was incubated at 90 °C for 3 min and slowly cooled to room temperature to allow the components hybridize. The closed state (in scheme 1 in the main text) was formed through self-assembly in a homogeneous phase. The introduction of C and D lead to the open state. To the resulting solution were added C and D. The dimer or larger structures were formed, possibly due to the presence of an open-stated structure. Similar dimer configurations were observed with other DNA-tweezers, and according to the references the dimer formation could in principle be avoided completely by tethering the tweezers to a solid substrate.

Scheme S1. The dimer or larger structures reconstructed from the open state with additions of strands C and D.

Figure S1. Polyacrylamide gel electrophoresis results of the tweezers. Lane 1 is the markers. Lane 2 and 3 are the open and closed tweezers, respectively. Lane 4 is the closed tweezers after one cycle of opening by C and D and closing with addition of strands C and D.
Activator selectivity of the system

The selectivity for the activators of the present DNA tweezers was based on the high affinity between aptamers and their target molecules. We carried out important control experiments to validate the hypothesis that the tweezers operate through thrombin and ATP-dependent initiation of action. In order to test that the DNA motors were activated by mechanical activators instead of small molecules or proteins, control experiments were carried out. By adding thrombin and ATP to the motor system, no fluorescence increases were observed (Figure S2). When adding C_{in} and D_{in} to the motor system, the fluorescence intensities increased. These results indicated that the mechanical activation for DNA motors was due to the mechanical activator, instead of thrombin and ATP.

![Fluorescence spectra](image)

**Figure S2.** Fluorescence spectra of Cy5 (A) and TAMRA (B) corresponding to the closed tweezers (a) and following addition of thrombin and ATP (b).
Experiments were also conducted on BSA and lysozyme to serve as control to assess the specificity of thrombin aptamer. CTP and GTP were also chosen as control for ATP. Different concentrations of BSA, lysozyme, CTP and GTP were added to MBs containing thrombin aptamer and ATP aptamer. As shown in Figure S3, all the analogues showed no fluorescence signal change, irrespective of the concentrations of analogues.

Figure S3. Comparison of CL signals for the DNA tweezers with adding different targets to cleave the input DNA strands: (a) thrombin and ATP, (b) BSA and ATP, (c) lysozyme and ATP, (d) thrombin and CTP, (e) thrombin and GTP, (f) BSA and CTP, (g) lysozyme and GTP. The concentrations of each components were of 10 µM for (a) to (e), and were of 50 µM for (f) and (g). All results are the mean values of three parallel experiments.
References
