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Reversible Regulation of Protein Binding Affinity by a DNA Machine

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Supporting Information

ABSTRACT: We report a DNA machine that can reversibly regulate target binding affinity on the basis of distance-dependent bivalent binding. It is a tweezer-like DNA machine that can tune the spatial distance between two ligands to construct or destroy the bivalent binding. The DNA machine can strongly bind to the target protein when the ligands are placed at an appropriate distance but releases the target when the bivalent binding is disrupted by enlargement of the distance between the ligands. This "capture–release" cycle could be repeatedly driven by single-stranded DNA without changing the ligands and target protein.

Polyvalent interactions are characterized by the simultaneous binding of multiple ligands on one biological entity to multiple receptors on another.[1] In comparison with the corresponding monovalent interactions, polyvalent interactions often have a cooperative effect, resulting in a significant enhancement of binding affinity and specificity. The better performance of polyvalent interactions strongly relies on precise control of the relative spatial position of multiple ligands. In the past decades, DNA has been demonstrated as an ideal material for fabrication of nearly arbitrary one-, two-, or three-dimensional nanostructures.[2] Moreover, the well-established modification[3] and accurate addressability[4] of DNA make these nanostructures ideal scaffolds to hold ligands in position and show polyvalent effects.[5] More recently, successes with DNA nanomachines[6] and dynamic DNA nanotechnology[7] have provided a promising method for studying dynamic behavior at the nanometer scale.[8] Here we report a DNA machine that can tune the spatial distance between two functional domains and reversibly regulate their target binding affinity. This strategy provides a universal platform for a polyvalent system and will also benefit the understanding of the mechanism of the polyvalent interactions.

Our strategy is shown in Figure 1. Two DNA double-crossover (DX)[9] motifs were joined by a Holliday junction to form a tweezers-like DNA machine. A DNA motor, which can cycle between stem–loop and double helix structures driven by a strand-displacement reaction, was incorporated into the middle of it. This design could amplify the small distance generated by the DNA motor into a much bigger spatial change between the two rear ends of the DNA machine. We considered the following factors during the design of this DNA machine. First, a DX motif was selected as a scaffold because it is more rigid than a duplex and can provide more

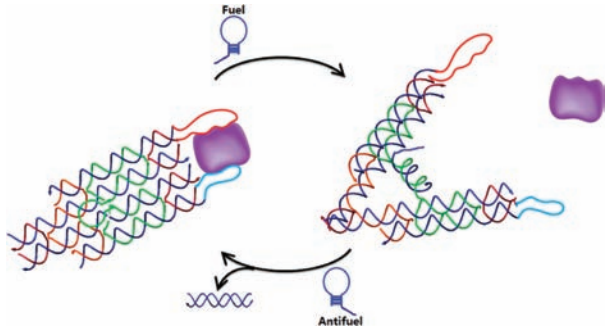

Figure 1. Schematic illustration of reversible regulation of target binding affinity by the DNA machine.

variability in terms of modulating the distance between the end groups, and most importantly, it provides an internal connecting position for a hairpin loop motif without a significant decrease in rigidity. Experimental results demonstrated that similar nicks in the duplex arms break the linearity of the arms, eliminating the distance change for such a DNA machine (data not shown). Second, the position connected with the hairpin loop was carefully chosen to keep the link position inside, and three bases were added at each end of the hairpin loop to minimize the possible steric hindrance. Third, an eight-base toehold[10] was added to the "fuel" strand of the DNA machine, enabling it to be displaced efficiently from the DNA machine by the fully complementary "antifuel" strands. The two ligands were incorporated at the terminals of the two DX motifs. In the closed state, their spatial distance is ideal for cooperative binding to the target "thrombin"; upon addition of "fuel" DNA strands, the DNA machine drives the two ligands apart, releasing the target protein. This regulation could run in the reverse direction upon the cycle of the DNA machine.

The DNA machine was prepared in its closed state. In general, all the strands were mixed and then annealed from 95 to 20 °C in 12 h. As shown in lane 2 of Figure 2a, only one clear single band appeared in 8% native polyacrylamide gel electrophoresis (PAGE), suggesting that the designed structure was formed with high assembly efficiency. After addition of the "fuel" strands, an obvious mobility shift was observed, and the lower mobility was attributed to the introduction of "fuel" strands at the hairpin loop region. Moreover, the structure became more extended as the DNA machine opened, and the size of the scaffold increased, thus further lowering the mobility

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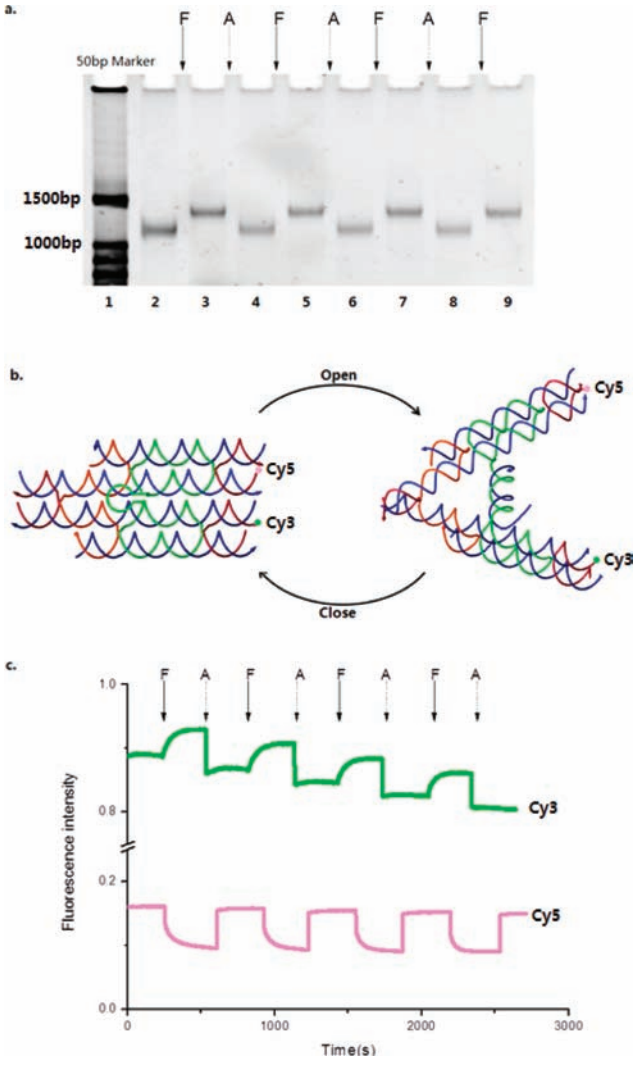

Figure 2. Cycling of the DNA machine. (a) Characterization of the cycling of the DNA machine by native PAGE analysis. (b) Design of DNA machine with fluorophores. (c) FRET measurement of the DNA machine upon successive additions of fuel and antifuel strands.

(lane 3). Removing the "fuel" strands by addition of the "antifuel" strands drove the DNA tweezers back to the initial closed state, which showed the same mobility as the previous band (lane 4). This cycle could be repeated several times without obvious efficiency decay by alternatively adding "fuel" and "antifuel" strands into the system (lanes 3–9).

As designed, the opening and closing of the DNA machine should induce a significant change in the distance between the ends of the DX motifs, and this was verified by Förster resonance energy transfer (FRET).[11] As shown in Figure 2b, the two DX motifs of the DNA tweezers were modified with fluorophores at their ends, one with Cy3 (donor) and the other with Cy5 (acceptor). The emission signals of the dyes were monitored at 565 nm (Cy3) and 664 nm (Cy5), while only Cy3 was photoexcited continuously at 550 nm; the results are shown in Figure 2c. In the closed state, the dyes on the DNA machine were close enough to lead to efficient FRET, resulting in a fluorescence decrease for Cy3 and an increase for Cy5. When "fuel" strands were added, the DNA machine was opened by the newly formed double helix at the hairpin loop region, making the distance between Cy3 and Cy5 too large for efficient FRET. This process could also be cycled many times, suggesting that the DNA machine indeed undergoes opening and closing processes and changes the distance between the dye molecules effectively.

On the basis of the above achievements, we chose human α thrombin as a model object to demonstrate the designed DNA machine's ability to regulate bivalent interactions. According to the literature, human α thrombin has a heparin-binding exosite that binds to aptamer A (apt-A: 29-mer, 5′-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3′)[12] and a fibrinogen-recognition exosite that binds to aptamer B (apt-B: 15-mer, 5′-GGT TGG TGT GGT TGG-3′).[13] These two binding sites are at almost opposite sides of the thrombin molecule,[12,14] and previous works have proved that the distance between the two aptamers significantly influences their binding affinity.[5b,15] Upon rational sequence design, we introduced the two closed-loop aptamers to the outboard helix ends of the two DX motifs (Figure 1). Two thymine bases were added at each end of each aptamer to provide a certain degree of three-dimensional flexibility, allowing the closed-loop aptamers to rotate and adjust to attain an optimal orientation for binding with the thrombin molecule. In the closed state, these two aptamers were kept 4–6 nm apart as designed, consistent with the distance between the two binding sites of thrombin that has been proved to be the ideal distance to get strong binding.[5b] As shown in Figure 3a, after incubation of 20 nM DNA structures

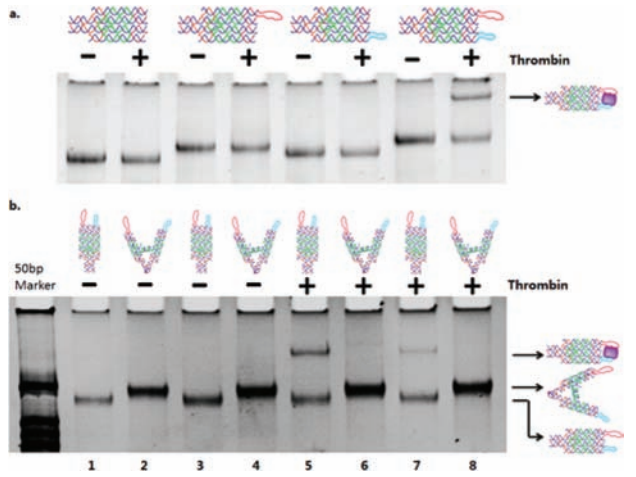

Figure 3. Native PAGE analyses of DNA machine binding with thrombin. (a) Closed DNA machines containing different aptamers were incubated with (+) and without (−) thrombin and then analyzed by native PAGE. (b) The addition of fuel and antifuel strands drove the DNA machine to catch and release thrombin molecules.

with 40 nM thrombin, a band moving much slower than the DNA machine in the closed state, which represents the DNA machine/thrombin complex, was clearly observed. Under the same conditions, the DNA machine in the closed state but with neither aptamer or only one aptamer did not show such a slower-moving band. These results suggested that the bivalent binding had a much higher binding affinity than monovalent binding and that the spacing between the two aptamers was appropriate for simultaneous binding with thrombin when the DNA machine was closed. We also demonstrated that by addition of "fuel" strands the caught thrombin could easily be released along with the opening of DNA machine, and this "capture–release" cycle could be repeated several times (Figure

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3b, lanes 5−8). The apparent dissociation constants (K_d) for the open and closed forms of the tweezers toward thrombin were also estimated by titration of the thrombin concentration in the gel mobility shift assay (see the Supporting Information). The closed DNA machine showed an apparent K_d of ∼15 nM, which is much smaller than that for the open tweezers (∼500 nM). These results illustrated that the bivalent effects are still based on the noncovalent interactions: the strong binding affinity of the closed state originates from the spatial tie-up of two weak interactions, and this tie-up can be interrupted by a tiny but very specific stimuli, which brings the binding reversibility. With this strategy, we can further investigate the origin of polyvalent interactions in natural biological processes with high binding affinity, specificity, and reversibility.

In summary, we have built a DNA machine that can manipulate target binding affinity by constructing and destroying distance-dependent bivalent interactions without changing ligands and target proteins. Recent progress in structural DNA nanotechnology has enabled the study of more complex polyvalent interactions in three dimensions. With well-established DNA modification methods, the binding ligands of such a system could be expanded to small molecules and polymers,[16] peptides,[17] and even nanoparticles.[18] We believe that these advantages and developments of DNA nanotechnology will make our strategy a universal platform for studying dynamic polyvalent interactions and developing highly specific target binding entities, which could be a potential tool in biology and pharmacology. Such a target-responsive DNA machine may also possibly be used to develop biosensing devices.[19]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and DNA sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755−2794.

(2) (a) Seeman, N. C. *Nature* **2003**, *421*, 427−431. (b) Shih, W. M.; Quispe, J. D.; Joyce, G. F. *Nature* **2004**, *427*, 618−621. (c) Goodman, R. P.; Schaap, I. A. T.; Tardin, C. F.; Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. *Science* **2005**, *310*, 1661−1665. (d) Rothemund, P. W. K. *Nature* **2006**, *440*, 297−302. (e) He, Y.; Ye, T.; Su, M.; Zhang, C.; Ribbe, A. E.; Jiang, W.; Mao, C. D. *Nature* **2008**, *452*, 198−201. (f) Andersen, E. S.; Dong, M.; Nielsen, M. M.; Jahn, K.; Subramani, R.; Mamdouh, W.; Golas, M. M.; Sander, B.; Stark, H.; Oliveira, C. L. P.; Pedersen, J. S.; Birkedal, V.; Besenbacher, F.; Gothelf, K. V.; Kjems, J. *Nature* **2009**, *459*, 73−76. (g) Douglas, S. M.; Dietz, H.; Liedl, T.; Hogberg, B.; Graf, F.; Shih, W. M. *Nature* **2009**, *459*, 414−418.

(3) Endo, M.; Sugiyama, H. *ChemBioChem* **2009**, *10*, 2420−2443.

(4) (a) Lund, K.; Liu, Y.; Lindsay, S.; Yan, H. *J. Am. Chem. Soc.* **2005**, *127*, 17606−17607. (b) Chhabra, R.; Sharma, J.; Ke, Y. G.; Liu, Y.; Rinker, S.; Lindsay, S.; Yan, H. *J. Am. Chem. Soc.* **2007**, *129*, 10304−10305. (c) Wilner, O. I.; Weizmann, Y.; Gill, R.; Lioubashevski, O.; Freeman, R.; Willner, I. *Nat. Nanotechnol.* **2009**, *4*, 249−254. (d) Xu, L.; Liu, D. *Chem. Soc. Rev.* **2010**, *39*, 150−155.

(5) (a) Melkko, S.; Scheuermann, J.; Dumelin, C. E.; Neri, D. *Nat. Biotechnol.* **2004**, *22*, 568−574. (b) Rinker, S.; Ke, Y. G.; Liu, Y.; Chhabra, R.; Yan, H. *Nat. Nanotechnol.* **2008**, *3*, 418−422. (c) Rosenzweig, B. A.; Ross, N. T.; Adler, M. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2010**, *132*, 6749−6754.

(6) (a) Bath, J.; Turberfield, A. J. *Nat. Nanotechnol.* **2007**, *2*, 275−284. (b) Liu, H.; Liu, D. S. *Chem. Commun.* **2009**, 2625−2636. (c) Lund, K.; Manzo, A. J.; Dabby, N.; Michelotti, N.; Johnson-Buck, A.; Nangreave, J.; Taylor, S.; Pei, R.; Stojanovic, M. N.; Walter, N. G.; Winfree, E.; Yan, H. *Nature* **2010**, *465*, 206−210.

(7) (a) Zhang, D. Y.; Seelig, G. *Nat. Chem.* **2011**, *3*, 103−113. (b) Krishnan, Y.; Simmel, F. C. *Angew. Chem., Int. Ed.* **2011**, *50*, 3124−3156.

(8) (a) Dittmer, W. U.; Reuter, A.; Simmel, F. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 3550−3553. (b) Harris, D. C.; Chu, X. Z.; Jayawickramarajah, J. *J. Am. Chem. Soc.* **2008**, *130*, 14950−14951.

(9) Fu, T. J.; Seeman, N. C. *Biochemistry* **1993**, *32*, 3211−3220.

(10) (a) Yurke, B.; Turberfield, A. J.; Mills, A. P.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605−608. (b) Zhang, D. Y.; Winfree, E. *J. Am. Chem. Soc.* **2009**, *131*, 17303−17314.

(11) Stryer, L.; Haugland, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 719−726.

(12) Tasset, D. M.; Kubik, M. F.; Steiner, W. *J. Mol. Biol.* **1997**, *272*, 688−698.

(13) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564−566.

(14) Padmanabhan, K.; Padmanabhan, K. P.; Ferrara, J. D.; Sadler, J. E.; Tulinsky, A. *J. Biol. Chem.* **1993**, *268*, 17651−17654.

(15) Kim, Y.; Cao, Z.; Tan, W. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5664−5669.

(16) Sun, Y.; Liu, H.; Xu, L.; Wang, L.; Fan, Q.-H.; Liu, D. *Langmuir* **2010**, *26*, 12496−12499.

(17) Williams, B. A. R.; Diehnelt, C. W.; Belcher, P.; Greving, M.; Woodbury, N. W.; Johnston, S. A.; Chaput, J. C. *J. Am. Chem. Soc.* **2009**, *131*, 17233−17241.

(18) Lo, P. K.; Karam, P.; Aldaye, F. A.; McLaughlin, C. K.; Hamblin, G. D.; Cosa, G.; Sleiman, H. F. *Nat. Chem.* **2010**, *2*, 319−328.

(19) Li, D.; Song, S.; Fan, C. *Acc. Chem. Res.* **2010**, *43*, 631−641.