Over the past 30 years, tremendous effort has contributed to the successful development of DNA nanostructures and nanodevices. Attention has recently shifted from designing DNA nanostructures/devices to exploring their potential functions in biological systems, including regulating cell activities, delivering therapeutic compounds, and amplifying detection signals. Successful applications of DNA assembly systems have been limited to nucleic acids and a few small molecules, due to the limited approaches to trigger the DNA assemblies. Herein, we describe a binding-induced DNA strand displacement strategy that can convert protein binding to the release of a predesigned output DNA at room temperature with high conversion efficiency and low background. This strategy allows us to construct dynamic DNA assembly systems that are able to respond to specific protein binding, opening an opportunity to initiate dynamic DNA assembly by proteins.

Our strategy is illustrated in Figure 1. The binding-induced strand displacement strategy is designed to have target recognition and signal output elements. Target recognition is achieved by two specific affinity ligands binding to the same target molecule. One affinity ligand is conjugated to the output DNA (OT) that is formed by prehybridizing the output DNA (O) and the support DNA (T), and the other is conjugated to the competing DNA (C). The complementary sequence of OT was designed to have the same length as C. Thus, in the absence of the target molecule, the rate of the strand exchange reaction between OT and C is extremely slow at 25 °C. However, in the presence of the target molecule, binding of the target molecule to the two affinity ligands that are linked to OT and C brings C in close proximity to OT. This process greatly increases the local concentration of C and accelerates the strand displacement reaction between OT and C. As a consequence, the output DNA O is released from its support T. The subsequent dynamic DNA assembly can be triggered by O, e.g., using the principle of toehold-mediated strand displacement. To be more specific, the toehold part of O is designed to be embedded in the complementary part of OT (Figure 1, black), so no dynamic DNA assembly can be triggered unless the target molecule is present and the toehold part of the output DNA is released.

We first designed a binding-induced strand displacement strategy for streptavidin using biotin as the affinity ligand (Figure S1). Streptavidin was selected for its extremely high binding affinity to biotin \( (K_d = 10^{-15} \text{ M}) \). This strong interaction ensures that the target binding process will not limit the performance of
the binding-induced strand displacement. T and C were each conjugated with a biotin molecule. The output O was designed to hybridize to T with a complementary length of 12 nt. O was extended with another 15 nt to help direct further DNA assemblies.

Figure 2 shows the characterization of the relevant oligonucleotides using polyacrylamide gel electrophoresis (PAGE). In the absence of the target streptavidin, the incubation of the two probes OT and C for 45 min does not lead to the release of O (Figure 2, lane 4), indicating that the rate of strand exchange between OT and C was extremely slow. However, in the presence of streptavidin, the observed strong bands of O and TC-target complex indicate the release of O from OT and the formation of TC-target complex according to Figure 1. These results suggest that the binding between streptavidin and biotin accelerated the kinetics of strand displacement reaction between OT and C.

As many dynamic DNA assembly systems, e.g., DNA catalytic circuits and nanomachines, use longer DNA molecules (e.g., 50 nt), we further tested the versatility of our strategy to output DNA assemblies, using two examples: a strand displacement beacon to the detection of PDGF protein.

Having achieved isothermal binding-induced strand displacement, we further show that this strategy is able to direct dynamic DNA assemblies, using two examples: a strand displacement beacon and a catalytic DNA circuit. We first designed a toehold-mediated strand displacement beacon that was able to respond to the output DNA O (Figure 3A). Briefly, two complementary DNA strands are labeled with a fluorophore (F) and a quencher (Q), respectively. Q is designed to have 7 nt longer than F, which serves as a “toehold” for the hybridization of Q to the output DNA O. In the absence of O, a stable DNA duplex is formed between F and Q, and the fluorescence signal is quenched. However, in the presence of O, the toehold-mediated strand displacement reaction is initiated and F is released from Q, turning on the fluorescence signal (Figure S3). Thus, the binding-induced displacement beacon can be used to determine protein binding through monitoring of the displaced O.

Figure 3B shows the fluorescence signal increase of the binding-induced displacement beacon for streptavidin as a function of time. Within a period of 45 min, fluorescence intensities from 10 nM streptavidin (red curve) are readily distinguishable from the blank (green curve) that contained all reagents but not the target streptavidin. To confirm that the binding-induced displacement beacon is target specific, we tested our system using the same 10 nM streptavidin that was fully saturated with 500 μM of free biotin (Control-1). The results are similar to those of the blank. Likewise, in the absence of O (Control-2), C (Control-3), or OT (Control-4), only background fluorescence was detectable. These results suggest that specific binding is responsible for the fluorescence signals from the binding-induced displacement beacon. Having established the binding-induced displacement beacon, we further estimated its efficiency of converting target streptavidin to the output DNA O (details in Supporting Information (SI) and Figure S4) at different target concentrations. By comparing the experimentally determined concentrations of O with their theoretical concentrations, we found that the average converting efficiency was 99.3 ± 7.6% throughout a wide range of target concentrations (160 pM to 10 nM) (Figure 4).

To demonstrate the general applicability of our strategy, we applied the binding-induced displacement beacon to the analysis of a clinically relevant protein, platelet derived growth factor (PDGF). A DNA aptamer for PDGF-BB was incorporated into the DNA probes OT and C, forming Apt-OT and Apt-C (Table S2). Binding of PDGF-BB to its aptamer sequences in OT and C brought the two DNA probes together, resulting in the displacement of output DNA O (Figure S6A). The released output DNA O triggered a subsequent toehold-mediated strand displacement reaction, releasing F from FQ. Fluorescence intensity from F provided a measure for the detection of PDGF-BB. The fluorescence intensity increases with the increase of PDGF concentration (Figure S6B). These quantitative results demonstrate an application of the binding-induced strand displacement beacon to the detection of PDGF protein.

The success of binding-induced displacement beacon opens up opportunities for directing further dynamic DNA assemblies,
have optimized the designs of oligonucleotides, OT and C, to minimize target-independent strand displacement. This optimization is based on the previous discovery that increasing the length of DNA duplex could slow down the rate of strand exchange reactions drastically. As shown in Figure 5A,B, in the presence of 10 nM streptavidin, the fluorescence intensities decrease with increasing length of OT and C from 12 to 16 nt. An extended incubation period (e.g., 150 min) results in noticeable increases in background (Figure 5B), suggesting the target-independent displacement of output DNA O. To eliminate the target-independent displacement, we fixed the competing DNA C to be 12 nt in length, and increased the length of OT from 12 to 20 nt. In principle, shorter competing DNA is thermodynamically unfavorable to displace a longer DNA strand, and thus should be able to suppress nonspecific release of O. Indeed, Figure 5C,D shows that the nonspecific displacement can be eliminated even after incubation for 150 min. To maximize signal-to-background, we chose a 2-nt difference between OT (14 nt) and C (12 nt). We also examined this optimized condition with PAGE (Figure S7), and found that no output DNA O band was observed on the gel without target molecule (lane 4), while a strong O band appeared with target (lane 5). These results confirm that we are able to eliminate the target-independent displacement of output DNA O.

Upon eliminating the target-independent displacement, we further designed a binding-induced catalytic DNA circuit to demonstrate the ability of our strategy to direct dynamic DNA assemblies with higher structural complexity. The principle of our binding-induced catalytic DNA circuit strategy is shown in Figure 6A: a pair of DNA hairpins (H1 and H2) is designed to...

Figure 4. Estimation of the conversion efficiency from target streptavidin to O at different streptavidin concentrations through the binding-induced displacement beacon. The streptavidin test solutions contained 20 nM OT, 20 nM C, 20 nM QF, and varying concentrations of streptavidin. Error bars represent one standard deviation from duplicate analyses.

Figure 5. Optimization of the binding-induced DNA strand displacement to minimize the target-independent strand displacement. Streptavidin test solutions contained 5 nM streptavidin, 10 nM OT, 10 nM C, and 20 nM QF. In the blank, all reagents were the same as streptavidin sample solution, but with no streptavidin added. Effects of simultaneous increases in the length of both OT and C on the performance of the binding-induced strand displacement were monitored at 45 (A) and 150 min (B). Effects of the length difference between OT and C were also monitored at 45 (C) and 150 min (D). The negative control (N.C.) contained only 20 nM QF in TE-Mg buffer. Error bars represent one standard deviation from duplicate analyses.

Figure 6. (A) Principle of the binding-induced catalytic DNA circuit. The fluorescence intensity was normalized such that 1 n.u. corresponds to 1 nM positive DNA P (details in the SI). An output DNA test solution contained 10 nM output DNA O, 125 nM H1, 200 nM H2, and 125 nM FQ. Streptavidin test solutions contained 20 nM OT, 20 nM C, 125 nM H1, 200 nM H2, 125 nM FQ, and varying concentrations of streptavidin. In the blank, all reagents were the same as in the streptavidin test solutions, but without streptavidin. (B) Evaluation of the binding-induced catalytic DNA circuit. The magnitude of amplification was determined by the linear fitting between fluorescence intensity and concentration of streptavidin. Error bars represent one standard deviation from duplicate analyses.
Communication

To test the signal amplification ability of our binding-induced DNA circuit, we monitored the fluorescence intensity increase as a function of time over a period of 4 h. As shown in Figure 6B, the fluorescence intensity generated from 10 nM streptavidin is close to 100 normalized units, which corresponds to 100 nM positive DNA (P) (Figure S8). Essentially no background fluorescence signal was observed for the blank. Compared to the toehold-mediated catalytic DNA circuit that is triggered directly by the output DNA O (Figure 6B, red curve), the binding-induced catalytic DNA circuit (Figure 6B, green curve) demonstrates comparable signal amplification capability. Furthermore, the measured fluorescence intensities are responsive to the concentrations of streptavidin in the range of 10 pM to 10 nM (Figure 6C), demonstrating the capability for quantification. We estimated from the standard curve (Figure 6C) that the fluorescence signal has been amplified by over 10-fold throughout this concentration range.

In conclusion, we have successfully developed a binding-induced DNA strand displacement strategy that functions at room temperature with high conversion efficiency and low background. Our success in constructing the binding-induced displacement beacon and binding-induced catalytic DNA circuit has demonstrated the feasibility of our strategy to direct dynamic DNA assemblies that are able to respond to protein binding. The concept and strategies have potential to further expand the existing dynamic DNA nanotechnology to proteins for diverse applications. One such application could be in the area of point-of-care analysis of proteins that could be performed under ambient temperature and without requiring the use of enzymes for signal generation and/or amplification. It is necessary to have the DNA strand displacement process faster than the dissociation of the target from affinity ligands. This requirement can be achieved by using affinity ligands with slow dissociation rate, e.g., slow off-rate modified aptamer (SOMAmer), stabilizing the binding complex by photo or chemical cross-linking, and/or increasing the rate of intramolecular DNA strand displacement by tuning the length of DNA probes or increasing the incubation temperature.

As has been shown in proximity ligation assays and binding-induced DNA assembly assays, diversity of affinity ligands, including antibodies, peptides, and aptamers, can be conjugated to DNA probes and form affinity complexes with target molecules, thereby triggering DNA assemblies. We anticipate that our strategy can also be applied to studies of molecular interactions, e.g., protein–protein and DNA–protein interactions.

**ASSOCIATED CONTENT**

Supporting Information

Supporting figures, DNA sequences, and detailed experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

**REFERENCES**

(1) (a) Seeman, N. C. J. Theor. Biol. 1982, 99, 237. (b) Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. Nature 1998, 394, 539. (c) Aldaye, F. A.; Palmer, A. L.; Sleiman, H. F. Science 2008, 321, 1795. (d) Haider, T. H.; Liedl, T.; Hobiger, B.; Graf, F.; Shih, W. M. Nature 2009, 459, 414. (e) Wei, B.; Dai, M.; Yin, P. Nature 2012, 485, 623. (f) Zhang, D. Y.; Seelig, G. Nat. Chem. 2011, 3, 103. (g) Pinheiro, A. V.; Han, D.; Shih, W. M.; Yan, H. Nat. Nanotechnol. 2011, 6, 763. (h) Wang, Z.; Tang, L.; Tan, L.; Li, J.; Lu, Y. Angew. Chem., Int. Ed. 2012, 51, 9078.

(2) Venkataraman, S.; Dirks, R. M.; Ueda, C. T.; Pierce, N. A. Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 16777.

(3) (a) Douglas, S. M.; Bachelet, I.; Church, G. M. Science 2012, 335, 831. (b) Jiang, Q.; Song, C.; Nangreave, J.; Liu, X.; Lin, L.; Qiu, D.; Wang, Z.-G.; Zou, G.; Liang, X.; Yan, H.; Dung, B. J. Am. Chem. Soc. 2012, 134, 13396.

(4) (a) Yin, P.; Choi, H. M. T.; Calvert, C. R.; Pierce, N. A. Nature 2008, 451, 318. (b) Li, B.; Ellington, A. D.; Chen, X. Nucleic Acids Res. 2011, 39, e110. (c) Qian, L.; Winfree, E. Science 2011, 332, 1196. (d) Li, B.; Jiang, Y.; Chen, X.; Ellington, A. D. J. Am. Chem. Soc. 2012, 134, 13918. (e) Li, B.; Chen, X.; Ellington, A. D. Anal. Chem. 2012, 84, 8371.

(5) (a) Piciri, J. M.; Frezz, B. M.; Ghadiri, M. R. J. Am. Chem. Soc. 2009, 131, 9368. (b) Liao, S.; Seeman, N. C. Science 2004, 306, 2072. (h) Surana, S.; Bhat, J. M.; Koushika, S. P.; Krishnan, Y. Nat. Commun. 2011, 2, 340. (i) Modi, S.; Swetha, M. G.; Goswami, D.; Gupta, G. D.; Mayor, S.; Krishnan, Y. Nat. Nanotechnol. 2009, 4, 321. (j) Seferos, D. S.; Gijzen, H. A.; Hill, H. D.; Prigodich, A. E.; Mirkin, C. A. J. Am. Chem. Soc. 2007, 129, 15477. (k) Liu, X.; Yan, H. Nucleic Acids Res. 2007, 35, 701. (l) Pei, H.; Liang, L.; Yao, G.; Li, J.; Huang, Q.; Fan, C. Angew. Chem., Int. Ed. 2012, 51, 9020. (m) Liu, H.; Xiang, Y.; Lu, Y.; Crooks, R. M. Angew. Chem., Int. Ed. 2012, 51, 6925. (n) El-Hamed, F.; Dave, N.; Liu, J. Nanotechnology 2012, 22, 494011.

(6) (a) Xing, Y.; Yang, Z.; Liu, D. Angew. Chem., Int. Ed. 2011, 50, 11934. (b) Dirks, R. M.; Pierce, N. A. Proc. Natl. Acad. Sci. U.S.A. 2004, 101, 15275. (c) Zhang, D.; Seferos, D. S.; Giljohann, D. A.; Patel, P. C.; Mirkin, C. A. Nano Lett. 2009, 9, 3258.

(7) (a) Zhang, D. Y.; Winfree, E. J. Am. Chem. Soc. 2009, 131, 9368. (b) Genot, A. J.; Zhang, D. Y.; Bath, J.; Turner, A. J. J. Am. Chem. Soc. 2011, 133, 2177. (c) Chen, X. J. Am. Chem. Soc. 2012, 134, 263.

(8) (a) Li, Q.; Luan, G.; Guo, Q.; Liang, J. J. Nucleic Acids Res. 2002, 30, e5. (b) Zhang, D. Y.; Chen, S. X.; Yin, P. Nat. Chem. 2012, 4, 208.

(9) Reynaldo, L. P.; Vologodskii, A. V.; Neri, B. P. J. Mol. Biol. 2000, 297, 511.

(10) Gold, L.; Ayers, D.; Bertino, J.; et al. PLoS One 2010, 5, e15004.

(11) Vinkenborg, J. L.; Mayer, G.; Famulok, M. Angew. Chem., Int. Ed. 2012, 51, 9176.

(12) Li, F.; Zhang, H.; Lai, C.; Li, X.-F.; Le, X. C. Angew. Chem., Int. Ed. 2012, 51, 9317.

(13) (a) Fredriksson, S.; Gullberg, M.; Jarvius, J.; Olson, C.; Pietra, K.; Gustafsdottir, S. M.; Ostman, A.; Landegren, U. Nat. Biotechnol. 2002, 20, 473. (b) Heyduk, E.; Dummit, B.; Chang, Y.-H.; Heyduk, T. Anal. Chem. 2008, 80, 5152. (c) Zhang, H.; Li, X.-F.; Le, X. C. Anal. Chem. 2012, 84, 877.