DNA Switch

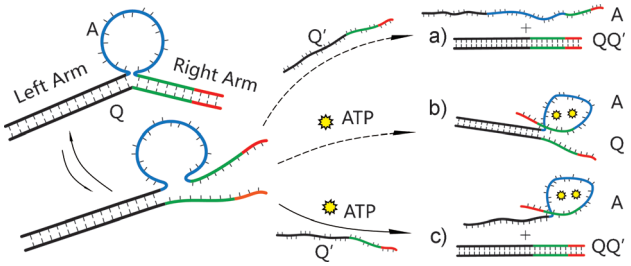
DOI: 10.1002/anie.201105923

A Responsive Hidden Toehold To Enable Controllable DNA Strand Displacement Reactions**

*Yongzheng Xing, Zhongqiang Yang, and Dongsheng Liu**

Over the past two decades, DNA nanotechnology[1] has developed rapidly as an emerging research field. Through rational sequence design and DNA self-assembly processes, a series of dynamic DNA nanostructures,[2] for example, molecular machines,[3] catalytic circuits,[4] amplification reactions,[4c,5] and logic gates,[6] have been successfully constructed, most of which are driven by toehold-mediated DNA strand displacement reactions.[7] However, it remains a challenge to confer environmental responsiveness to the DNA strand displacement reaction, which limits the versatility of regulative methods towards the control of strand displacement reactions and hinders their utilization in biomedical areas. Herein, in contrast to well-established overhanging-toehold systems, we develop a responsive "hidden toehold" on a metastable DNA bulge-loop structure, which allows regulation of a DNA strand displacement reaction with environmental stimuli. This design will expand future applications of DNA nanotechnology.

Our strategy is illustrated in Scheme 1: Two single-stranded DNA (ssDNA) strands, A and Q, both contain two domains complementary to each other, and strand A also contains an extra unpaired domain in between. After hybridization, the complementary parts form two arms, and the unpaired domain forms a loop in the middle, which results in a bulge-loop structure, denoted as AQ complex. The bulge-loop structure has two arms of different lengths: a longer left arm (15 bp) and a shorter right arm (10 bp). The hidden toehold (green and red domains on strand Q, Scheme 1) is located on the right arm. The structure is metastable, because the loop domain has a constraint effect over the whole structure, and the right arm is less stable and vulnerable to be dehybridized at room temperature. An equilibrium exists between closed and opened AQ complex, because a fraction of the AQ complex can spontaneously open, while the exposed single strands can hybridize back to the closed state. As a result, the hidden toehold of an opened AQ complex can be exposed and hybridize with its complementary strand Q′ to initiate the DNA strand displacement reaction (pathway a). A 27-mer ATP aptamer (ATP = adenosine triphosphate)[8] is incorporated into the loop domain and the adjacent seven nucleotides on the right arm of strand A (blue and green domains, Scheme 1), so it can bind ATP molecules when the complex is open (pathway b). The binding event can keep the opened structure from restoring its closed state, thereby increasing the availability of the toehold hidden inside the AQ complex. When both strand Q' and ATP are added, the strand displacement reaction can be enhanced by the binding of ATP (pathway c).



Scheme 1. The DNA bulge-loop structure (AQ complex) and its reaction pathways. The AQ complex is composed of two ssDNA strands, A and Q. Both strands have two domains complementary to each other that form a left arm (15 bp, black) and a right arm (10 bp, green and red). For strand A, the loop domain (blue) and its adjacent 7 nt domain (green) on the right arm comprise an ATP aptamer sequence, which can bind ATP molecules when the right arm gets dehybridized. The additional 3 nt domain (red) at the end of the right arm imparts the structure the appropriate stability. Three reaction pathways exist: after adding a) strand Q', b) ATP, or c) both to the AQ complex solution. The reactions for pathways (a) and (b) are slow (dashed arrows), while pathway (c) is fast (solid arrow). bp = base pair, nt = nucleotide.

To verify whether the DNA assemblies can form and the reaction can proceed as designed, a PAGE experiment was carried out (Figure 1). Firstly, stoichiometric amounts of strands A and Q were added to Tris-HCl (20 mM, pH 8.3, Tris = tris(hydroxymethyl)aminomethane) buffer containing $MgCl_2$ (5 mM) and NaCl (300 mM) to give a final concentration of 20 μM for each strand. The resulting solution was heated to 95 °C for five minutes and subsequently cooled to room temperature in two hours. The single band with the smallest shift in lane 4 (Figure 1) indicated that the AQ complex was effectively formed as designed. The addition of ATP (lane 5) did not cause an obvious change in band shift compared to the AQ complex alone. However, after the addition of strand Q′ (lane 6), the band of the AQ complex got weaker and the bands of QQ′ and A can be seen in the gel.

[*] Y. Xing, Prof. Dr. Z. Yang, Prof. Dr. D. Liu
Key Laboratory of Organic Optoelectronics & Molecular Engineering of the Ministry of Education
Department of Chemistry, Tsinghua University
Beijing 100084 (China)
E-mail: liudongsheng@tsinghua.edu.cn
Y. Xing
National Center for Nanoscience and Technology
Beijing 100190 (China)

[**] We thank National Basic Research Program of China (973 program, No. 2007CB935900 & 2011CB935701), the National Natural Science Foundation of China (No. 20725309 & 91027046), and NSFC-DFG joint project TRR61 for financial support.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201105923.

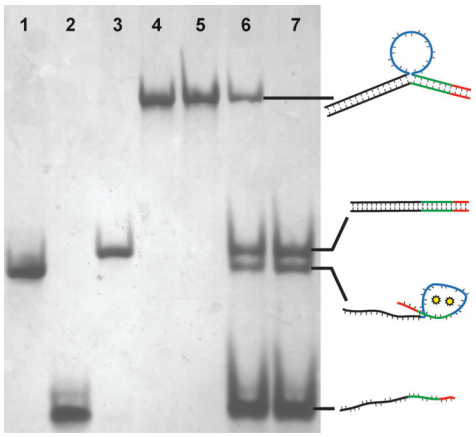


Figure 1. Native PAGE (15%) analysis of the formation of the AQ complex and its strand displacement reaction after adding either ATP, strand Q′, or both. In a typical experiment, AQ complex (6 μM) was mixed with ATP (1 mM), strand Q′ (60 μM), or both, and incubated at room temperature for 30 min. Lane 1: strand A, lane 2: strand Q′, lane 3: QQ′, lane 4: AQ complex, lane 5: AQ and ATP, lane 6: AQ and Q′, lane 7: AQ, ATP, and Q′.

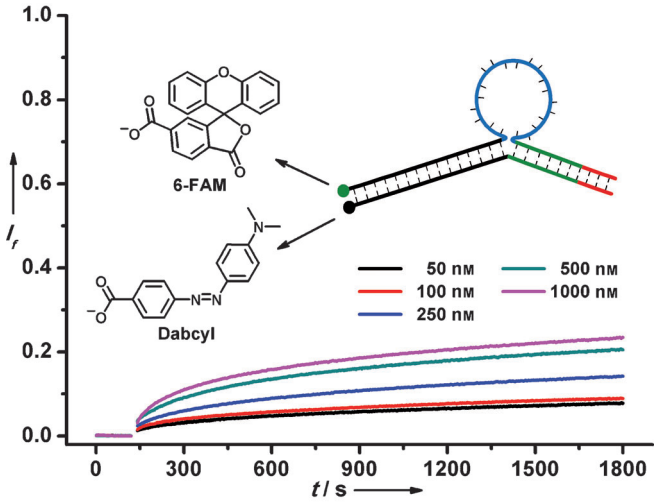


Figure 2. Fluorescence spectroscopy test of the reaction between left-arm-labeled AQ complex (AQ_L) and strand Q′. In a typical test, AQ_L in buffer solution (400 μL, 50 nM) was placed in a cuvette, and strand Q′ (1 μL) was added and mixed quickly within 20 s to initiate the reaction. The final concentration of strand Q′ was increased from 50 nM to 1000 nM. The fluorescence signals were normalized using the equation $I_f = (F - F_0)/(F_{max} - F_0)$, where F is the fluorescence intensity of each sample, F_0 is the lowest fluorescence intensity for each sample in the initial state, and F_{max} is the fluorescence intensity detected after annealing the sample of AQ_L complex with strand Q′ (500 nM).

This finding suggests the existence of the equilibrium that produces a fraction of opened AQ complex and further suggests that the exposed "hidden toehold" indeed initiates the DNA strand displacement reaction. In the presence of both ATP and strand Q′ (lane 7), the band of the AQ complex almost completely disappeared, thus indicating that the opening of the AQ complex can be enhanced by binding of ATP molecules and the exposed toehold can initiate the strand displacement reaction.

To quantitatively evaluate the reaction and the properties of the hidden toehold, fluorescence spectroscopy experiments were performed by using fluorophore-labeled strands A and Q. Left-arm-labeled AQ complex (AQ_L) was obtained by annealing 3′-6-FAM-labeled A (6-FAM = 6-carboxyfluorescein) and 5′-Dabcyl-labeled Q strands (Figure 2, inset) in reaction buffer (20 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, 300 mM NaCl). Initially, when the AQ complex stayed intact, the fluorophore 6-FAM ($\lambda_{em} = 518$ nm and $\lambda_{ex} = 494$ nm) and the quencher Dabcyl were in proximity to each other, so the fluorescence was quenched. However, when strand A was released from strand Q, the quenching interaction was eliminated and the fluorescence intensity increased significantly. Time-dependent fluorescence spectroscopy tests were carried out, in which the excitation wavelength was fixed at 494 nm and the emission passing through a 515 nm filter was recorded by a fluorescence detector.

Results showed that the AQ complex can react with strand Q′ (Figure 2), which indicates that a part of the AQ complex can indeed spontaneously switch from the closed to the opened state, and the hidden toehold of the opened AQ complex hence becomes available to be anchored by strand Q′. When the concentration of strand Q′ was increased even up to 20 times (1000 nM) that of the AQ complex, the fluorescence signal was only enhanced less than 30% in 30 min, thus suggesting that the DNA strand displacement reaction between the AQ complex and strand Q′ is quite slow when the reaction only depends on the spontaneous switching of the AQ complex to expose the hidden toehold. Adjusting only the concentration of strand Q′ is not sufficient to effectively regulate the reaction, and therefore we tried to verify if the addition of ATP can function as an effective regulator of the reaction.

Moreover, the right-arm-labeled AQ complex (AQ_R) was also tested in a similar fluorescence spectroscopy experiment as described for AQ_L (Figure S1 in the Supporting Information). Only 12% signal enhancement was observed when it reacted with strand Q' alone, showing a lower background than AQ_L (29%). However, after the addition of ATP (1 mM), the fluorescence signal can be enhanced by 93% for AQ_L, compared to only 43% of AQ_R. Therefore, fluorescence of AQ_L can be regulated over a wider window (64%) than that of AQ_R (31%). The difference can be explained by the change in stability of the AQ complex owing to the different positions of the fluorophore pairs, which is supported by the fact that the melting temperature of AQ_L was 4°C lower than that of AQ_R (Figure S2). These results show that the AQ_L complex was more sensitive under the same conditions than the AQ_R complex, and we chose the AQ_L complex for further experiments. Considering that the stability of the right arm plays an important role in the exposure of the toehold, we also tried different sequences and lengths to demonstrate the effect of different right arms on the performance of the AQ complex (see Figure S3).

Finally, to investigate whether ATP can regulate the reaction between the AQ complex and strand Q′, fluorescence spectroscopy experiments were performed after the

addition of ATP with different concentrations. At a fixed concentration of strand Q' (500 nM), the strand displacement reaction can be enhanced in an ATP-concentration-dependent way (Figure 3). Also, the reaction showed a high specificity for ATP, because the addition of 1 mM GTP

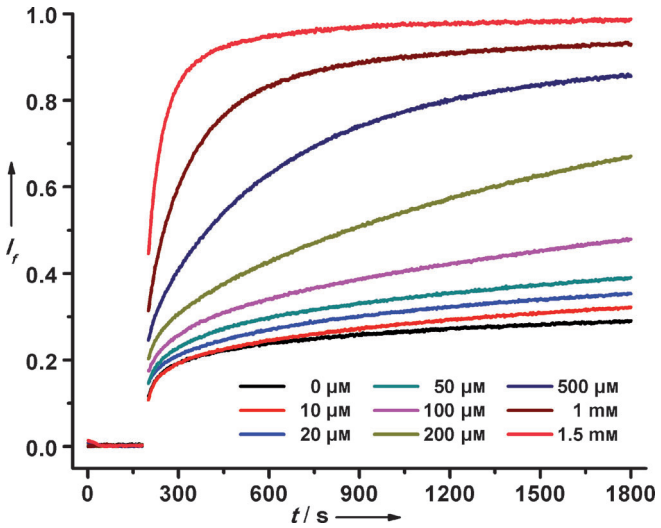


Figure 3. Fluorescence experiments with mixtures of AQ_L complex with strand Q′ and ATP of different concentrations. In a typical test, AQ_L complex in buffer solution (400 μL, 50 nM) was placed in a cuvette, and both strand Q′ (1 μL, 200 μM) and ATP (1 μL at the proper concentration) were added and mixed quickly within 20 s to initiate the reaction. The concentration of strand Q′ was fixed at 500 nM. The fluorescence signals were normalized using the equation $I_f = (F - F_0)/(F_{max} - F_0)$, where F is the fluorescence intensity of each sample, F_0 is the lowest fluorescence intensity for each sample in the initial state, and F_{max} is the fluorescence intensity detected after annealing the sample of AQ_L complex in the presence of both ATP (1 mM) and strand Q′.

(guanosine triphosphate) only induced a negligible change of the fluorescence signal (Figure S4). Therefore, it is demonstrated that the presence of ATP can enhance the strand displacement reaction between the AQ complex and the complementary strand Q′, and the kinetics of the reaction can be regulated by adjusting the addition of ATP molecules.

In conclusion, we have constructed a DNA bulge-loop structure as a substrate for a DNA strand displacement reaction, which is different from previously reported toehold systems. We did not employ an overhanging toehold but instead developed a hidden toehold, the activity of which is firstly limited by the formation of a duplex in the metastable structure and can then be adjusted by the addition of ATP. The kinetics of the strand displacement reaction can be regulated by tuning the concentration of ATP, thus allowing ligand-responsive regulation. Furthermore, with over two decades' development of SELEX (systematic evolution of ligands by exponential enrichment) technology,[9] hundreds of aptamer sequences[10] that can bind specifically to different ligands have been screened and applied in biosensing systems.[11] Therefore, by alternatively inserting other aptamer sequences and carefully tailoring the stability of the bulge-loop structure, our system can be extended to couple with a large number of other ligands and shows a great potential to construct ligand-responsive dynamic molecular systems.

Received: August 22, 2011
Published online: October 20, 2011

Keywords: aptamers · DNA structures · hidden toehold · nanotechnology · strand displacement reaction

[1] a) N. C. Seeman, *Angew. Chem.* **1998**, *110*, 3408–3428; *Angew. Chem. Int. Ed.* **1998**, *37*, 3220–3238; b) N. C. Seeman, *Nature* **2003**, *421*, 427–431; c) N. C. Seeman, *Nano Lett.* **2010**, *10*, 1971–1978.

[2] a) J. Bath, A. J. Turberfield, *Nat. Nanotechnol.* **2007**, *2*, 275–284; b) H. Liu, D. S. Liu, *Chem. Commun.* **2009**, 2625–2636; c) Y. K. Krishnan, F. C. Simmel, *Angew. Chem.* **2011**, *123*, 3180–3215; *Angew. Chem. Int. Ed.* **2011**, *50*, 3124–3156.

[3] a) B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel, J. L. Neumann, *Nature* **2000**, *406*, 605–608; b) A. J. Turberfield, J. C. Mitchell, B. Yurke, A. P. Mills, M. I. Blakey, F. C. Simmel, *Phys. Rev. Lett.* **2003**, *90*, 1181021; c) P. Yin, H. Yan, X. G. Daniell, A. J. Turberfield, J. H. Reif, *Angew. Chem.* **2004**, *116*, 5014–5019; *Angew. Chem. Int. Ed.* **2004**, *43*, 4906–4911; d) J. Bath, S. J. Green, A. J. Turberfield, *Angew. Chem.* **2005**, *117*, 4432–4435; *Angew. Chem. Int. Ed.* **2005**, *44*, 4358–4361; e) S. Venkataraman, R. M. Dirks, P. W. K. Rothemund, E. Winfree, N. A. Pierce, *Nat. Nanotechnol.* **2007**, *2*, 490–494.

[4] a) G. Seelig, B. Yurke, E. Winfree, *J. Am. Chem. Soc.* **2006**, *128*, 12211–12220; b) D. Y. Zhang, A. J. Turberfield, B. Yurke, E. Winfree, *Science* **2007**, *318*, 1121–1125; c) P. Yin, H. M. T. Choi, C. R. Calvert, N. A. Pierce, *Nature* **2008**, *451*, 318–323; d) D. Y. Zhang, E. Winfree, *J. Am. Chem. Soc.* **2008**, *130*, 13921–13926.

[5] a) R. M. Dirks, N. A. Pierce, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15275–15278; b) D. Lubrich, S. J. Green, A. J. Turberfield, *J. Am. Chem. Soc.* **2009**, *131*, 2422–2423.

[6] G. Seelig, D. Soloveichik, D. Y. Zhang, E. Winfree, *Science* **2006**, *314*, 1585–1588.

[7] a) D. Y. Zhang, E. Winfree, *J. Am. Chem. Soc.* **2009**, *131*, 17303–17314; b) A. J. Genot, D. Y. Zhang, J. Bath, A. J. Turberfield, *J. Am. Chem. Soc.* **2011**, *133*, 2177–2182.

[8] a) D. E. Huizenga, J. W. Szostak, *Biochemistry* **1995**, *34*, 656–665; b) R. Nutiu, Y. F. Li, *J. Am. Chem. Soc.* **2003**, *125*, 4771–4778; c) J. W. Liu, Y. Lu, *Angew. Chem.* **2006**, *118*, 96–100; *Angew. Chem. Int. Ed.* **2006**, *45*, 90–94.

[9] a) A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818–822; b) C. Tuerk, L. Gold, *Science* **1990**, *249*, 505–510.

[10] J. F. Lee, J. R. Hesselberth, L. A. Meyers, A. D. Ellington, *Nucleic Acids Res.* **2004**, *32*, 95D–100D.

[11] a) J. W. Liu, Z. H. Cao, Y. Lu, *Chem. Rev.* **2009**, *109*, 1948–1998; b) E. J. Cho, J. W. Lee, A. D. Ellington, *Annu. Rev. Anal. Chem.* **2009**, *2*, 241–264.