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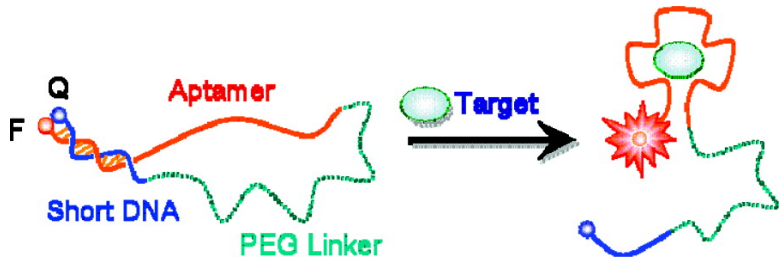

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Aptamer Switch Probe Based on Intramolecular Displacement

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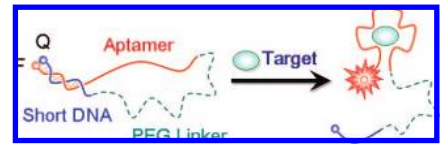
We have developed an effective molecular engineering mechanism to signal aptamer/target binding events. Aptamers are single-stranded oligonucleotides that can recognize and bind to target molecules with strong affinity and excellent specificity.[1,2] Aptamers are isolated from an *in vitro* selection process termed *s*ystem *e*volution of *l*igands by *e*xponential enrichment (SELEX) against a variety of targets, including small molecules, ions, proteins, and whole cells.[3,4] Since nucleic acid bases can be easily modified and molecularly engineered, aptamers can meet the stringent requirements for such uses as bioassay,[4–6] drug delivery,[7,8] signal transduction,[9,10] and gene expression mediation.[11,12]

Focusing on the development of bioassay probes, many different design principles have been advanced.[13,14] However, while each strategy has distinct advantages, each also presents its own unique set of limitations. One design type, for example, utilizes conformation alteration during the aptamer−target binding event, providing a simple approach for aptamer biosensor design.[5,6,15] Essentially, these probe designs use the spatial change of the aptamer sequence termini to produce a fluorescent or electrochemical signal. However, for any given aptamer sequence, this method commonly encounters unpredictable structural alteration such that the spatial change may not induce perceptible signal transduction.[16] Another strategy uses either DNA-intercalating dyes or photoactive polymers to report the recognition and binding between an aptamer and target molecule, thus giving a simple and label-free probe design.[17–19] This method, however, suffers from intrinsic limitations, such as high background, nonselectivity against specific aptamer, and lack of multiplex detection capability. Besides these strategies, a competition-based probe construction has been developed by introducing a DNA competitor to sense the binding between aptamer and target.[20–22] The DNA competitor hybridizes with partial or whole aptamer sequence but allows dehybridization when aptamer binds to target molecule. Similar to the methods described above, this strategy has advantages and disadvantages. For example, while this approach offers a general means of aptamer probe fabrication, it also requires a separate oligo competitor. This limits some applications, such as *in situ* detection, but favors others which require a long oligo competitor to achieve steady hybridization while maintaining a low background. At the same time, the introduction of a longer oligo competitor requires careful optimization to avoid conformational changes that would hinder the recognition and affinity of aptamer toward target.

To address many of these shortcomings, we report the development of a novel and versatile intramolecular signal transduction aptamer probe. As shown in Scheme 1, the probe is composed of three elements: an aptamer, a short DNA sequence complementary to part of the aptamer, and a PEG linker connecting these two. A fluorophore and a quencher are covalently attached at the two termini of the conjugated DNA sequences. Upon target binding, the conformation of the probe can then be "switched on". To explain, in the absence of a target molecule, the short DNA will hybridize with a small section of the aptamer, keeping the fluorophore and quencher in close proximity, thus switching off the fluorescence. Conversely, when the probe meets its target, the binding between aptamer and target molecule will disturb the intramolecular DNA hybridization and move the quencher away from the fluorophore, resulting in the restoration of fluorescence. Since the aptamer responds to its target molecule by switching on its fluorescent properties, we named this an "aptamer switch probe" (ASP). Compared with other aptamer probe designs, the ASP strategy provides a robust probe construction by integrating aptamer, competitor, and signaling moieties into one molecule. This design utilizes intramolecular hybridization/dehybridization and therefore requires a much shorter competitor, which reduces the impact on aptamer spatial conformation and simplifies probe optimization. Theoretically, the ASP design can be used for any aptamers.

Scheme 1. Aptamer Switch Probe (ASP) and Design of ATP-ASP and Human α-Thrombin ASP (Tmb-ASP)[a]



[a] F represents fluoropore and Q represents quencher. ATP-ASP: Ce6-CACCTGGGGGAGTATTGCGGAGGAAGGTT-$(CH_2CH_2O)_{36}$-CCAGGTG-BHQ2. Tmb-ASP: Ce6-CCAAC-$(CH_2CH_2O)_{30}$-GGTTGGTGTGGTTGG-BHQ2.

To demonstrate the feasibility of this principle, two aptamers were selected with which to construct ASP models: one targets the small biomolecule, adenosine triphosphate (ATP), and the other binds to human α-thrombin (Tmb). Chlorin e6 (Ce6) and FAM were chosen as fluorophores, while Black Hole Quencher 2 (BHQ2) was used to quench the fluorescence of Ce6 and FAM. Ce6 is chosen in addition to FAM because we want to use the same design for singlet oxygen generation in the future in target-controlled phototherapy. Five to six PEG spacer 18s were added as the linker to connect the aptamer and the partially cDNA sequence. The synthesis of ASPs was conducted on a DNA synthesizer, followed by an in-tube coupling of Ce6. The purification of probes was carried out on a HPLC, and only the product with all three absorptions of DNA, Ce6/FAM, and BHQ2 was collected. UV spectra of products further confirmed the quality of the probe synthesis. To test the fluorescence signaling of the ATP-ASP with the addition of ATP, three samples were prepared in 10 mM Tris-HCl buffer with 6 mM $MgCl_2$ and irradiated at a wavelength of 404 nm, which is the maximum absorption of Ce6.

The ATP-ASP showed a significant fluorescence increase upon target binding. As shown in Figure 1, the ATP-ASP presents up to a 30-fold enhancement of fluorescence immediately after the addition

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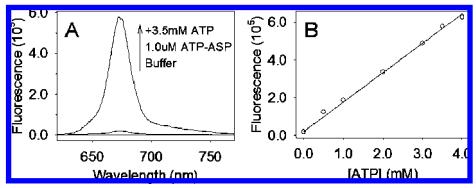

Figure 1. (A) Fluorescence spectra of the buffer, ATP-ASP, and ATP-ASP+ATP. (B) Plot of the fluorescence of ATP-ASP as a function of ATP concentration.

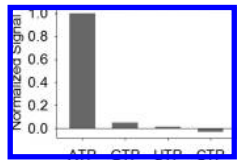

Figure 2. Selectivity of ATP-ASP toward NTPs. The fluorescence signal enhancements were normalized to the ATP sample.

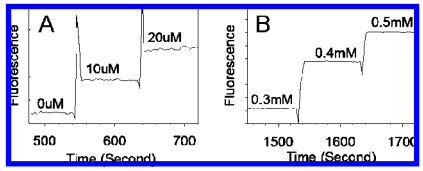

Figure 3. Kinetics of ATP-ASP response. The final concentration of ATP after each addition is labeled above the curve. The peaks were caused by the stir during spiking. (A) Response at low concentration of ATP. (B) Response at high concentration of ATP.

of 3.5 mM ATP, proving the feasibility of the ASP design. The response of ATP-ASP toward a series concentration of ATP was also investigated, and the results present a linear relationship between the fluorescence enhancement and the concentration, demonstrating that the fluorescence of ATP-ASP can be quantitatively mediated by ATP concentration.

The ATP-ASP also shows excellent specific response against ATP analogues. As shown in Figure 2, the fluorescence signal does not show much change after the addition of GTP, UTP, or CTP at the concentration of 1.0 mM, but it does show significant enhancement after the introduction of 1.0 mM ATP. The excellent selectivity of ATP-ASP is similar to that of the original ATP aptamer.[23] This result clearly confirms the fact that the ASP design does not affect the selectivity of the ATP aptamer.

The response kinetics of ATP-ASP was then tested by real-time monitoring of the sample with several successive additions of ATP. As shown in Figure 3, the response of ATP-ASP to the introduction of ATP is prompt. The ATP-ASP delivered >90% response and reached equilibrium within 5 s, regardless of the concentration of ATP. The fast kinetics, which derives from the short oligo, is an advantage of the intramolecular hybridization design. Apparently, the shorter complementary oligo has less effect on the aptamer's spatial folding, keeping, as a result, higher recognition affinity, better selectivity, and a faster hybridization/dehybridization rate, which are all required for optimal aptamer probe design and application.[24,25] Compared with previous designs, the ASP is simpler and efficient in signaling aptamer−target binding process for both analytical applications and binding mechanism studies.

Next, to demonstrate the versatility of the ASP design, we used the same principle and molecular scheme to design and synthesize a Tmb-ASP targeting human α-thrombin, which is an important protein in human blood. In this design, the fluorophore was conjugated at the competitor side to verify the effect of fluorophore position on fluorescence restoration. It is worth noting that the length of the competitor is only 5 bases, which is much shorter than the intermolecular hybridization design. The fluorescence of Tmb-ASP was enhanced up to 17.6 times after the addition of 300 nM thrombin (Figure S5). The fluorescence signal of Tmb-ASP could also be quantitatively mediated by the addition of various thrombin concentrations. Furthermore, the Tmb-ASP presented selective response toward thrombin. In contrast, when tested with IgG, IgM, and BSA at the concentration of 200 nM, the Tmb-ASP did not produce fluorescence enhancement compared with the sample containing 200 nM thrombin (Figure S6).

The success of ATP-ASP and Tmb-ASP unquestionably supports the feasibility of the ASP design targeting protein and small biomolecules. The ASP design has several significant advantages over conventional designs. First, compared to intermolecular DNA hybridization, the intramolecular DNA hybridization requires a shorter oligo to achieve the same melting temperature. The shorter competitor hybridizes to a smaller partial aptamer and leaves more aptamer sequence free, thus increasing the binding affinity between aptamer and target, as well as improving the sensitivity. Second, the equilibrium of intramolecular hybridization and dehybridization between oligos is more stable and faster than intermolecular events, enabling faster response and lower background, which are important for signaling transduction in probe construction. Third, and most importantly, this design conjugates the aptamer, competitor oligo, and signaling groups into one molecule, preventing the dissociation of probe during applications and making ASP a robust molecular probe. In summary, the ASP strategy holds promising potential for future applications, such as biochip and *in situ* imaging, which require reusability, excellent stability, prompt response, and high sensitivity.

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Supporting Information Available: Synthesis, experimental details, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818–822.
(2) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505–510.
(3) Shangguan, D.; Li, Y.; Tang, Z.; Cao, Z.; Chen, H.; Mallikaratchy, P.; Sefah, K.; Yang, C.; Tan, W. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 11838–11843.
(4) Navani, N. K.; Li, Y. F. *Curr. Opin. Chem. Biol.* **2006**, *10*, 272–281.
(5) Yang, C. J.; Jockusch, S.; Vicens, M.; Turro, N. J.; Tan, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17278–17283.
(6) Hamaguchi, N.; Ellington, A.; Stanton, M. *Anal. Biochem.* **2001**, *294*, 126–131.
(7) McNamara, J. O.; Andrechek, E. R.; Wang, Y.; Viles, D.; Rempel, R. E.; Gilboa, E.; Sullenger, B. A.; Giangrande, P. H. *Nat. Biotechnol.* **2006**, *24*, 1005–1015.
(8) Lee, J. F.; Stovall, G. M.; Ellington, A. D. *Curr. Opin. Chem. Biol.* **2006**, *10*, 282–289.
(9) Cho, E. J.; Yang, L.; Levy, M.; Ellington, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 2022–2023.
(10) Shlyahovsky, B.; Li, D.; Weizmann, Y.; Nowarski, R.; Kotler, M.; Willner, I. *J. Am. Chem. Soc.* **2007**, *129*, 3814–3815.
(11) Bayer, T. S.; Smolke, C. D. *Nat. Biotechnol.* **2005**, *23*, 337–343.
(12) Friedrichs, E.; Simmel, F. C. *Chembiochem* **2007**, *8*, 1662–1666.
(13) Mairal, T.; Ozalp, V. C.; Sanchez, P. L.; Mir, M.; Katakis, I.; O'Sullivan, C. K. *Anal. Bioanal. Chem.* **2008**, *390*, 989–1007.
(14) Willner, I.; Zayats, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 6408–6418.
(15) Stojanovic, M. N.; de Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* **2001**, *123*, 4928–4931.
(16) Lin, C. H.; Patel, D. J. *Chem. Biol.* **1997**, *4*, 817–832.
(17) Li, B. L.; Wei, H.; Dong, S. J. *Chem. Commun.* **2007**, 73–75.
(18) Jiang, Y. X.; Fang, X. H.; Bai, C. L. *Anal. Chem.* **2004**, *76*, 5230–5235.
(19) Ho, H. A.; Leclerc, M. *J. Am. Chem. Soc.* **2004**, *126*, 1384–1387.
(20) Li, N.; Ho, C. M. *J. Am. Chem. Soc.* **2008**, *130*, 2380–2381.
(21) Rupcich, N.; Nutiu, R.; Li, Y. F.; Brennan, J. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 3295–3299.
(22) Nutiu, R.; Li, Y. F. *J. Am. Chem. Soc.* **2003**, *125*, 4771–4778.
(23) Huizenga, D. E.; Szostak, J. W. *Biochemistry* **1995**, *34*, 656–665.
(24) Bonnet, G.; Krichevsky, O.; Libchaber, A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8602–8606.
(25) Yang, C.; Martinez, K.; Lin, H.; Tan, W. *J. Am. Chem. Soc.* **2006**, *128*, 9986–9987.

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