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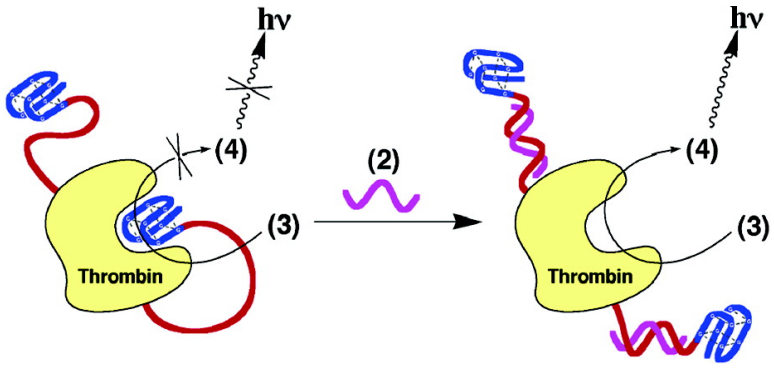

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Fluorescence Detection of DNA by the Catalytic Activation of an Aptamer/Thrombin Complex

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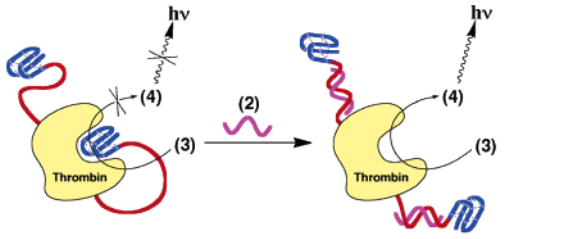
Aptamers are DNA or RNA strands that selectively bind to low molecular weight compounds or to specific domains of protein structures.[1] These binding features of aptamers were employed for the development of different sensor systems.[2] An interesting thrombin-binding aptamer was elicited,[3] and its self-organization in the form of a G-quadruplex that specifically binds to a sequence-specific peptide domain was elucidated.[4] Previously, the thrombin-specific aptamer was used for the optical detection of thrombin.[5] In the present study, we report on the use of a conjugate consisting of thrombin and covalently linked nucleic acid that includes thrombin aptamer units for the catalytic fluorescence detection of DNA. In addition to the analytical significance of the system, it adds a new concept to the rapidly developing area of DNA machinery.[6,7]

Thrombin (Th) was functionalized with a thiolated nucleic acid **1** by the primary reaction of the Th lysine residues with *N*-(γ-maleimidobutyryloxy)sulfosuccinimide ester (Pierce, USA) followed by the covalent linkage of **1** to the maleimide units. The unit **1** includes the G-quadruplex aptamer sequence that is tethered to Th by a nucleic acid that is complementary to the analyzed DNA **2**. The analysis of the Th/DNA conjugate indicates that the average loading of Th with **1** is 8. Quantification of the aptamer per thrombin ratio in the Th/aptamer conjugate was performed by the measurement of the total protein content[8] and total ssDNA content using ssDNA OliGreen quantitation reagent (Molecular Probes, USA).[9] Thrombin reveals biocatalytic activity toward the hydrolysis of bis(*p*-tosyl-Gly-Pro-Arg) derivative of rhodamine 110 (**3**) to the fluorophore, rhodamine 110 (**4**).[10] The association of the Th-specific aptamer to thrombin blocks the catalytic functions of Th, and this provides the principle for analyzing DNA by the Th/aptamer conjugate (Scheme 1).

Scheme 1. Optical Analysis of DNA by the Catalytic Activation of Thrombin through the Dissociation of a Thrombin/Aptamer Complex



The catalytic functions of Th toward the hydrolysis of **3** are blocked in the Th/aptamer conjugate structure due to the binding of the aptamer to the protein. Hybridization of the analyzed DNA with the sensing sequence of the nucleic acid attached to Th yields the double-strand duplex that distorts the aptamer/Th complex and activates Th toward the hydrolysis of **3**. A related scheme for the amplified detection of DNA has been reported by tethering a protease inhibitor to the protein by a nucleic acid and the allosteric activation of the enzyme by hybridization of the nucleic acid to the complementary DNA.[11]

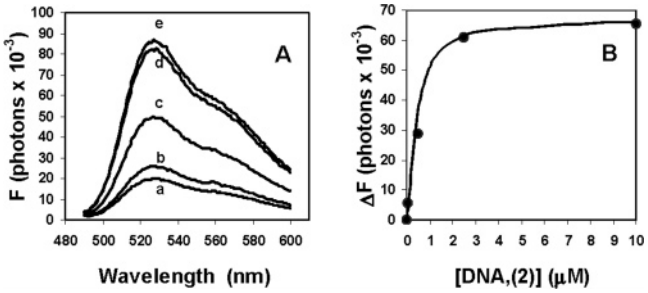


Figure 1. (A) Fluorescence spectra of the Th/aptamer conjugate after hybridization with different concentrations of the analyte DNA, **2**, and reaction with 5×10^{-6} M **3** for 5 min. (a) 0 M, (b) 5×10^{-8} M, (c) 5×10^{-7} M, (d) 2.5×10^{-6} M, (e) 1×10^{-5} M. The hybridization of the Th/aptamer complex with the different samples of **2** was performed in binding buffer[12] for 30 min. (B) Derived calibration curve.

Figure 1(A) shows the fluorescence spectra observed upon the interaction of the Th/aptamer conjugate with different concentrations of the DNA **2**, allowing the system to catalyze the hydrolysis of **3**. The Th/aptamer conjugate reveals a residual fluorescence due to unbound, noninhibited thrombin that exists in equilibrium with the Th/aptamer complex. The hybridization of DNA **2** with the Th/aptamer conjugate releases the aptamer unit and activates the Th toward the hydrolysis of **3** and the generation of the fluorophore **4**. As the concentration of **2** increases, the resulting fluorescence is enhanced due to the increase of catalytically active free Th. Figure 1(B) shows the derived calibration curve. In a control experiment, the noncovalently bound aptamer **1** was complexed with Th, and its catalytic activity was blocked. The hybridization of the aptamer with **2** did not activate Th toward the hydrolysis of **3**. This result indicates that the aptamer G-quadruplex structure is not affected by the hybridization with **2**, and that the covalently linked Th/aptamer configuration is essential to activate the biocatalytic functions of Th. That is, hybridization of **2** with the "beacon-type" structure of the Th/aptamer conjugate introduces a strain into the aptamer tether that is released by the dissociation from the aptamer/Th complex. Furthermore, interaction of the Th/aptamer conjugate with a foreign nucleic acid or with a short, partially complementary nucleic acid (**2a**) did not activate Th toward the biocatalytic process.

The analysis of the DNA by means of the Th/aptamer conjugate was further examined on glass supports. Glass surfaces were functionalized with a 3-aminopropylsiloxane film, and the Th/

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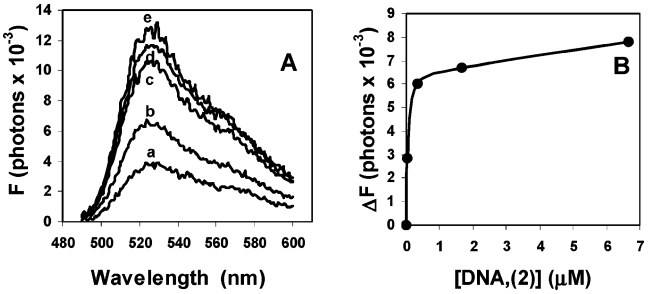


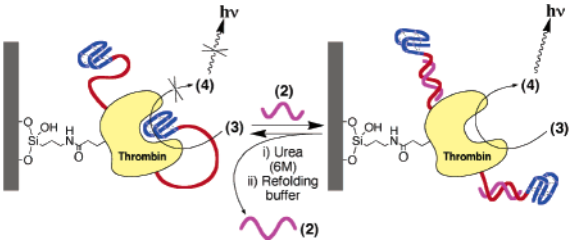
Figure 2. (A) Fluorescence spectra of the Th/aptamer conjugate linked to a glass support upon analyzing different concentrations of **2**: (a) 0 M, (b) 3.3×10^{-8} M, (c) 3.3×10^{-7} M, (d) 1.6×10^{-6} M, (e) 6.6×10^{-6} M. Spectra were recorded after reacting the interface with **3** for 30 min. Hybridization of the interface with **2** was conducted for 1 h. (B) Derived calibration curve for the optical detection of **2** by the Th/aptamer-modified surface.

aptamer conjugate was covalently coupled to the surfaces. The resulting interfaces were hybridized with different concentrations of **2**, and the hybridized interfaces were subjected to the biocatalytic hydrolysis of **3**. The fluorescence of the resulting fluorophore was monitored in a volume of 380 μL.

Figure 2 shows the fluorescence generated by the Th/aptamer conjugate upon analyzing different concentrations of **2** and the resulting calibration curve.

Besides the functions of the Th/aptamer conjugate as an active sensing matrix, the system contributes a new approach for developing a biomaterial-based machine.[6,7] The dissociation of the Th/aptamer conjugate, by the hybridization of **2**, and the activation of the biocatalytic functions of Th represent a mechanical process at the molecular level driven by **2** that acts as a fuel and the catalytic reaction as a readout process. The addition of the hybridized interface into 6 M urea solution followed by the rinsing of the interface with the binding buffer[12] dehybridizes **2**, which is converted into a waste. The subsequent insertion of the Th/aptamer into the G-quadruplex generation and hybridization buffer refolds the aptamer and generates the switched-off machine. The rehybridization of the system with the **2**-fuel reactivates the machine, and the fluorescence from the system is regenerated (Scheme 2 and Figure 3).

Scheme 2. Optical Detection of DNA by the Catalytic Activation of a Th on a Surface by the Dissociation of a Th/Aptamer Complex and the Cyclic Operation of the Biomolecular Activated/Deactivated Machine



To conclude, the present study has introduced a functional thrombin/aptamer conjugate for the amplified analysis of DNA. The system also reveals biomolecular machinery operations that are transduced by the Th-generated fluorophore **4**.

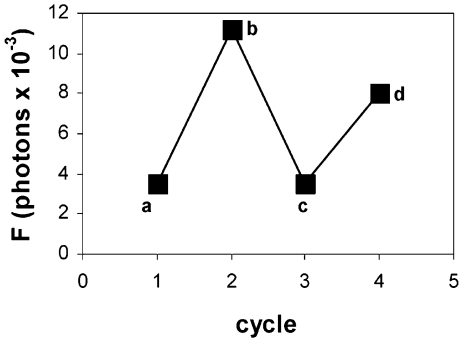


Figure 3. Fluorescence imaging of the cyclic activation/deactivation of the Th/aptamer complex by the hybridization/dehybridization of **2**: (a and c) catalytic inactive Th/aptamer complex; (b and d) catalytic activation of thrombin by the hybridization of **2** with the aptamer.

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References

(1) Luzi, E.; Minunni, M.; Tombelli, S.; Mascini, M. *Trends Anal. Chem.* **2003**, *11*, 810−818.
(2) (a) Fukusho, S.; Furusawa, H.; Okahata, Y. *Chem. Commun.* **2002**, 88−89. (b) Kawakami, J.; Hirofumi, I.; Yukie, Y.; Sugimoto, N. J. *Inorg. Biochem.* **2000**, *82*, 197−206. (c) Kleinjung, F.; Klussman, S.; Erdmann, V. A.; Scheller, F. W.; Furste, J. P.; Bier, F. F. *Anal. Chem.* **1998**, *70*, 328−331. (d) Potyrailo, R. A.; Conrad, R. C.; Ellington, A. D.; Hieftje, G. M. *Anal. Chem.* **1998**, *70*, 3419−3425. (e) Myoyong, L.; Walt, D. R. *Anal. Biochem.* **2000**, *282*, 142−146. (f) Heyduk, E.; Heyduk, T. *Anal. Chem.* **2005**, *77*, 1147−1156.
(3) (a) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564−566. (b) Macaya, R. F.; Waldron, J. A.; Beutel, B. A.; Gao, H. T.; Joesten, M. E.; Yang, M. H.; Patel, R.; Bertelsen, A. H.; Cook, A. F. *Biochemistry* **1995**, *34*, 4478−4492. (c) Padmanabhan, K.; Tulinsky, A. *Acta Crystallogr.* **1996**, *D52*, 272−282.
(4) (a) Kelly, J. A.; Feigon, J.; Yeates, T. O. *J. Mol. Biol.* **1996**, *256*, 417−422. (b) Tasset, D. M.; Kubik, M. F.; Steiner, W. *J. Mol. Biol.* **1997**, *272*, 688−698. (c) Wu, Q.; Tsiang, M.; Sadler, J. E. *J. Biol. Chem.* **1992**, *267*, 24408−24412.
(5) (a) Ho, H. A.; Leclerc, M. *J. Am. Chem. Soc.* **2004**, *126*, 1384−1387. (b) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 11768−11769.
(6) (a) Shin, J.-S.; Pierce, N. A. *J. Am. Chem. Soc.* **2004**, *126*, 10834−10835. (b) Alberti, P.; Mergny, J.-L. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1569−1573. (c) Yin, P.; Yan, H.; Daniell, X. G.; Turberfield, A. J.; Rief, J. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4906−4911.
(7) (a) Xiao, Y.; Pavlov, V.; Niazov, T.; Dishon, A.; Kotler, M.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 7430−7431. (b) Dittmer, W. U.; Reuter, A.; Simmel, F. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 3550−3553. (c) Yurke, B.; Turberfield, A. J.; Mills, A. P., Jr.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605−608.
(8) Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. *Anal. Biochem.* **1985**, *150*, 76−85.
(9) A calibration curve for the OliGreen fluorescence responses at variable concentrations of **1** was extracted. The fluorescence intensity of **1**-modified Th was then monitored to determine the total concentration of **1**. Knowing the protein concentration, we extracted the average loading of Th.
(10) Zweig, S. E.; Meyer, B. G.; Sharma, S.; Min, C.; Krakower, J. M.; Shohet, S. B. *Biomed. Instrum. Technol.* **1996**, *30*, 245−256.
(11) Saghatelian, A.; Guckian, K. M.; Thayer, D. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 344−345.
(12) Composition of binding buffer: 20 mM Tris-HCl (pH = 7.4), 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5% glycerol (w/v).

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