Supporting Information

Amplified Fluorescence Polarization Aptasensors Based on Structure-Switching-Triggered Nanoparticles Enhancement for Bioassays

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Materials and Methods

Materials: All oligonucleotides were purchased from the Sangon Biotech Co. (Shanghai, China) and purified by HPLC. The sequences of the involved oligonucleotides were given in Table S1. H1 and DNA-1 were used for adenosine triphosphate (ATP) detection; H2 and DNA-2 were used for assaying thrombin (Tb). Cytosine triphosphate (CTP), guanosine triphosphate (GTP), ATP, uridine triphosphate (UTP), Tb, and other proteins were obtained from Sigma-Aldrich. The amino-modified silica nanoparticles (SiO_2 NPs) of different diameter sizes, i.e. 25, 50, 75, and 100 nm, were obtained from Polyscience. All other chemicals were of analytical grade. The water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work.

Preparation of DNA-SiO_2 NP conjugates: The SiO_2 NPs functionalized with each DNA were based on a previous publication with minor modifications.[1] The amino-modified SiO_2 NPs (6 mg) were activated by reaction with the heterobifunctional crosslinker 3-maleimidopropionic acid N-hydroxysuccinimide ester (4 mg) in DMSO (1 mL) at room temperature for 4 h. After centrifugation and washing with DMSO and water, the maleimido-activated particles were then mixed with 25 nmol of thiolated oligonucleotide in 5 mL phosphate buffer (0.1 M, pH 7.4) for 8 h. After that, a cysteine solution (250 μL, 0.1M) was added into the mixture and stirred for 1 h to block free maleimido groups. Finally, the resulting particles were washed with water and phosphate buffer (0.1 M, pH 7.4). The DNA-modified SiO_2 NPs were dispersed in an appropriate amount of phosphate buffer, and stored at 4 °C before use.

Procedures for fluorescence polarization measurements: Fluorescence polarization is a technique that provides a quantitative measure for the rotational motion of a fluorescently labeled molecule.[2] The fluorescence polarization value ***P*** can be calculated by Perrin equation.[3] In a particular surrounding, the ***P*** value of a fluorophore is proportional to its rotational relaxation time, which depends on its molecular volume. If the fluorescently labeled molecule is free in solution, it will rotate faster and, hence, will have relatively small ***P*** value. When the fluorescently labeled molecule is bound with another large molecule to from a complex, it rotate slowly and the ***P*** value will increase. From the principle described above, it is clearly that the ***P*** value will significantly increase when a fluorophore-labeled oligonucleotide binds a larger molecule (SiO_2 NP-functionalized DNA).

For amplfied assay, 1.0 μL DNA-1-modified SiO_2 NPs solution (corresponding to 4 μM of DNA) and 1.0 μL FAM-labeled aptamer hairpin (2.0 μM) were added to a 1.5 mL vial containing 997 μL of 20 mM Tris-HCl solutions (100 mM NaCl, 5 mM $MgCl_2$, pH 7.4). Subsequently, 1μL of target solution with different concentration or other analogue molecules solution was added, and the solution was incubated in the vial for 1 h before fluorescence polarization measurement. 300 μL of final solution was used for fluorescence polarization measurements. The control experiments using non-modified DNA and FAM-labeled aptamer hairpin were carried out under identical conditions. All experiments were repeated three times. Each sample was measured five times. FP measurements were performed on an FL3-P-TCSPC system (Jobin Yvon, Inc., Edison, NJ, USA). Fluorescence polarization of the sample solution was monitored by exciting the sample at 494 nm and measuring the emission at 520 nm. And slits for both the excitation and the emission were set at 3 nm.

Application study: To investigate the application potential of the proposed FP aptasensor in real samples, we applied the proposed assay for the detection of Tb (as a model) in healthy human serum. As shown in Figure S3, the FP values of the system remained almost unchanged. This indicated that no Tb was detected in the serum sample. This was due to the fact that healthy human serum sample does not contain thrombin.[4,5] To examine the applicability of this aptasensor in serum sample, we performed spike experiments. The 10-fold diluted serum sample was spiked with 5 pM, 100 Pm and 100 nM Tb, and then analyzed. The FP values in Tb spiked serum samples were slightly lower (< 4%) than that obtained in Tris buffer solutions (Figure S3). These results indicated the potentiality of the proposed FP aptasensor for Tb detection in real biological samples.

Methods comparison: Table S2 compares the sensitivities and range of response of the proposed FP aptasensors with other homogeneous optical aptasensors. As can be seen, the sensitivities of the proposed FP aptasensors were much higher than that of traditional homogeneous aptasensors,[6,7,9,10,12-15] and also better than other amplification methods.[8,11,16] Moreover, the proposed FP aptasensors also exhibited wider dynamic range than other methods. In addition, the relative standard deviation (n=10) for ten replicate detections of both ATP (80 pM, 10 nM, and 1 μM) and Tb (4 pM, 100 pM, 500 nM) was less than 4.3%. This indicated the good reproducibility of our method.

References

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Table S1. Sequences of DNA used in this work

Strand	Sequence*
H1	5'-CCCAGGTAACAAGAAAGCCAAACCTCTTGTT*ACCTGGGGGA GTATTGCGGAGGAAGGT*-FAM-3'
H2	5'-CACCAACCAACAAGAAAGCCAAACCTGATGTT*GGTTGGTGT GGTTGG*-FAM-3'
DNA-1	5'-TTGTTACCTGGGTTTTTTTTTT-SH-3'
DNA-2	5'-TTGGTTGGTGTTTTTTTTTT-SH-3'

*In the hairpin sequences, stems are underlined, and aptamer nucleotides are italicized.

Table S2. Performance comparison between homogeneous optical aptasensors.

Type	Sensitivity	Dynamic range	Reference
ATP			
Gold nanoparticle-based aptasensor	48 nM	100 nM-2000 nM	[6]
G-quadruplex-based aptasensor	1-10 μM	10 μM-1000 μM	[7]
Exonuclease III amplification	0.25 μM	0.25 μM-500 μM	[8]
Carbon nanotube-based aptasensor	0.5 μM	0.8 μM-80 μM	[9]
	4.5 nM	10 nM-800 nM	[10]
Nuclease amplification	40 nM	0.1 μM-1000 μM	[11]
SiO_2 NP-enhanced FP aptasensor	20 pM	40 pM-100 μM	This work
Tb			
Carbon nanotube-based aptasensor	1.8 nM	4.0 nM-150 nM	[12]
Graphene oxide-based aptasensor	2.0 nM	5 nM-100 nM	[13]
Quantum dot-based aptasensor	30 pM	0.1 nM-10 nM	[14]
FP aptasensor	250 pM	250 pM-8 nM	[15]
Exonuclease III amplification	89 pM	360 pM-14 nM	[8]
Nicking enzyme amplification	2 pM	5 pM-50 nM	[16]
SiO_2 NP-enhanced FP aptasensor	0.3 pM	1 pM-1 μM	This work

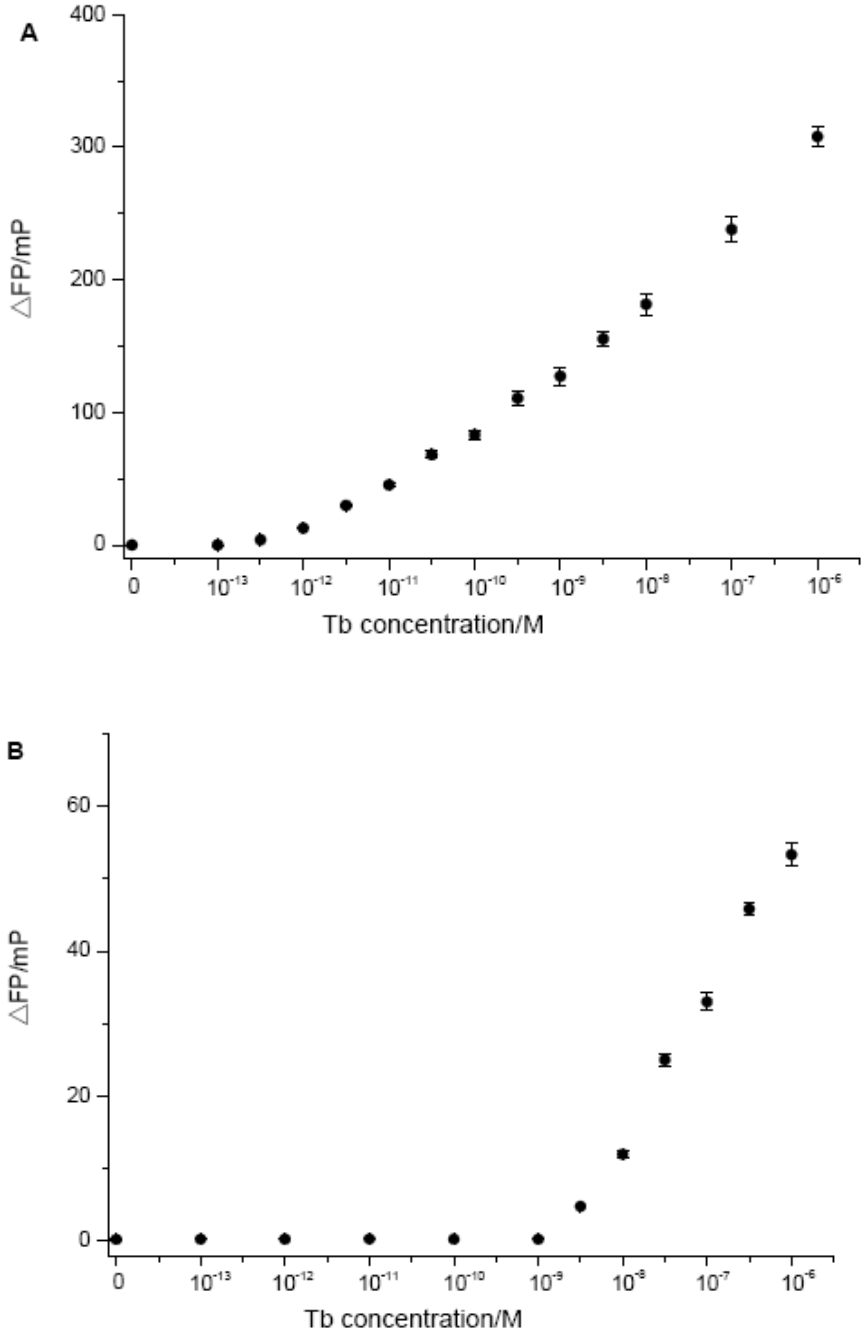

Figure S1. Plots of fluorescence polarization changes as a function of Tb concentrations for FAM-labeled H2/SiO_2 NP functionalized-DNA-2 system (A) and FAM-labeled H2/DNA-2 system (B).

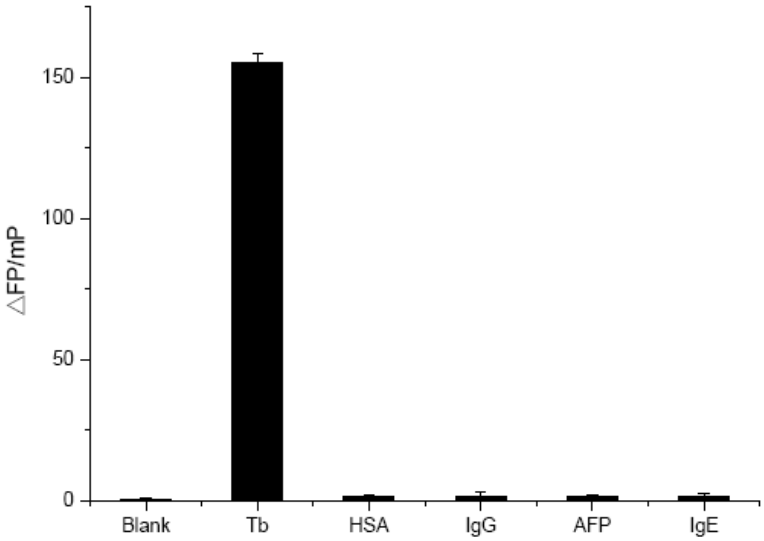

Figure S2. Specificity of the amplified FP aptasensor to Tb (5 nM) by comparing it to the interfering proteins at the 0.5 μM level: HSA, IgG, AFP and IgE.

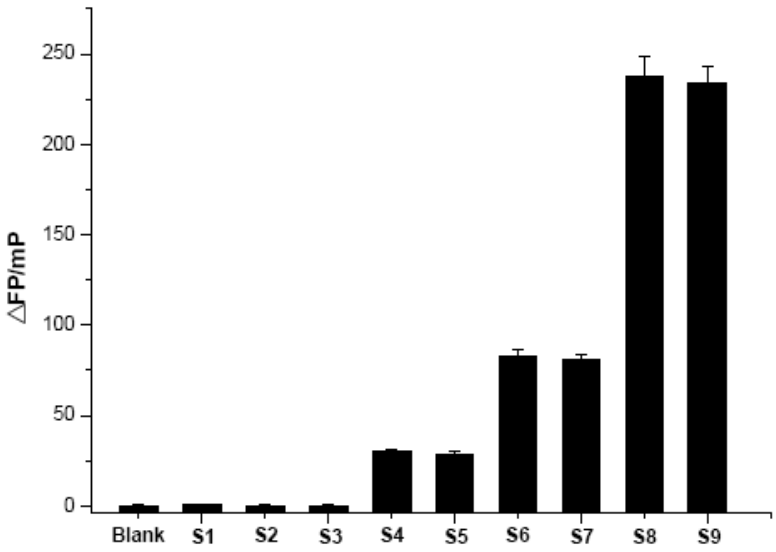

Figure S3. Fluorescence polarization changes upon analyzing different samples. Blank: without ATP; S1: human serum sample-1; S2: human serum sample-2; S3: human serum sample-3; S4: 5 pM Tb in Tris buffer; S5: 5 pM Tb in human serum; S6: 100 pM Tb in Tris buffer; S7: 100 pM Tb in human serum; S8: 100 nM Tb in Tris buffer; S4: 100 nM Tb in human serum.