Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

A Fluorescein Labeled Aptamer Switch for Thrombin with Fluorescence Decrease Response

Lihua Hao[1], Qiang Zhao[1, 2*]

[1]Institute of Environmental Science, College of Chemistry and Chemical Engineering, Shanxi University, Taiyuan, P. R. China, 030006

[2]State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, P. R. China

Table S1 List of tested DNA oligonucleotides and labeling Sites

Name	Sequences
Th27	5'-GTC CGT GGT AGG GCA GGT TGG GGT GAC-3'
Th29	5'-A GTC CGT GGT AGG GCA GGT TGG GGT GAC T-3'
Th27-3-FAM	5'-GTC CGT GGT AGG GCA GGT TGG GGT GAC-3'(FAM)
Th27-5-FAM	5'(FAM)-GTC CGT GGT AGG GCA GGT TGG GGT GAC-3'
Th27-3-TMR	5'-GTC CGT GGT AGG GCA GGT TGG GGT GAC-3'(TMR)
Th27-3-Cy3	5'-GTC CGT GGT AGG GCA GGT TGG GGT GAC-3'(Cy3)
Th29-3-FAM	5'-A GTC CGT GGT AGG GCA GGT TGG GGT GAC T-3'(FAM)

Table S2 Comparison of various aptamer-based fluorescence assays for thrombin with respect to methods, limit of detection (LOD) and linear dynamic range (LDR)

Methods	LOD	LDR	Ref.
Molecular beacon with signal-decrease response	0.373 nM	NA[a]	10
Molecular beacon with signal-increase response	10 nM	10-40 nM	11
Assay using guanine as quencher in aptamer	0.34 nM	NA	13
Assay using dual pyrene labeled aptamer	0.042 nM	0.042-17 nM	14
Fluorescence polarization assay	0.20 nM	0.6-100 nM	15
Assay using aptamer and ethidium bromide	2.8 nM	2.8-20 nM	16
Time resolved fluorescence assay	0.14 nM	1-10 nM	17
Assay using aptamer with fluorescent nucleotide analogues	9.7 nM	NA	18
Assay using FAM labeled aptamer	0.062 nM	0.062-2 nM	This work

a: NA means not available

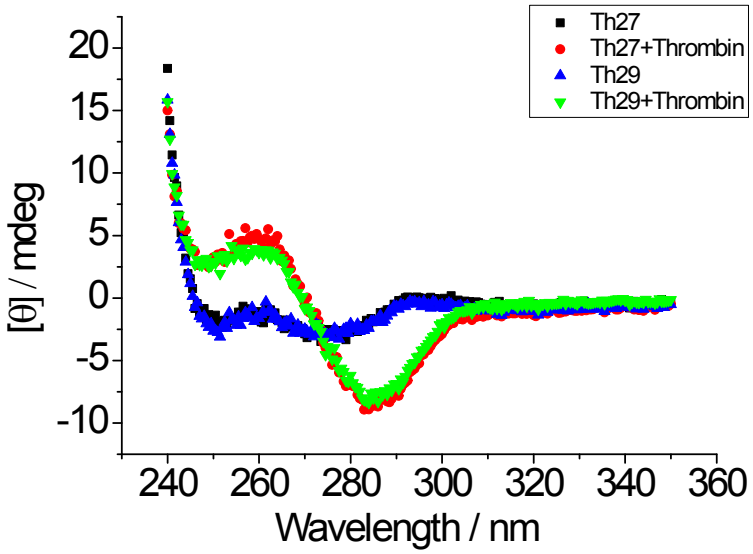

Fig. S1 The circular dichroism spectra of 27-mer aptamer (Th27) and 29-mer aptamer (Th29) against thrombin in absence of thrombin and in presence of thrombin in the binding buffer solution containing 20 mM Tris–HCl, 10 mM NaCl, 0.5 mM MgCl2, 0.5 g/L BSA (pH 7.5). CD spectra measurements were conducted on a Jasco J-815 CD spectrometer (Tokyo, Japan). The cuvette with a path length of 1 cm was used. The concentration of used aptamers was 3 μM, and the concentration of used thrombin was 3 μM.

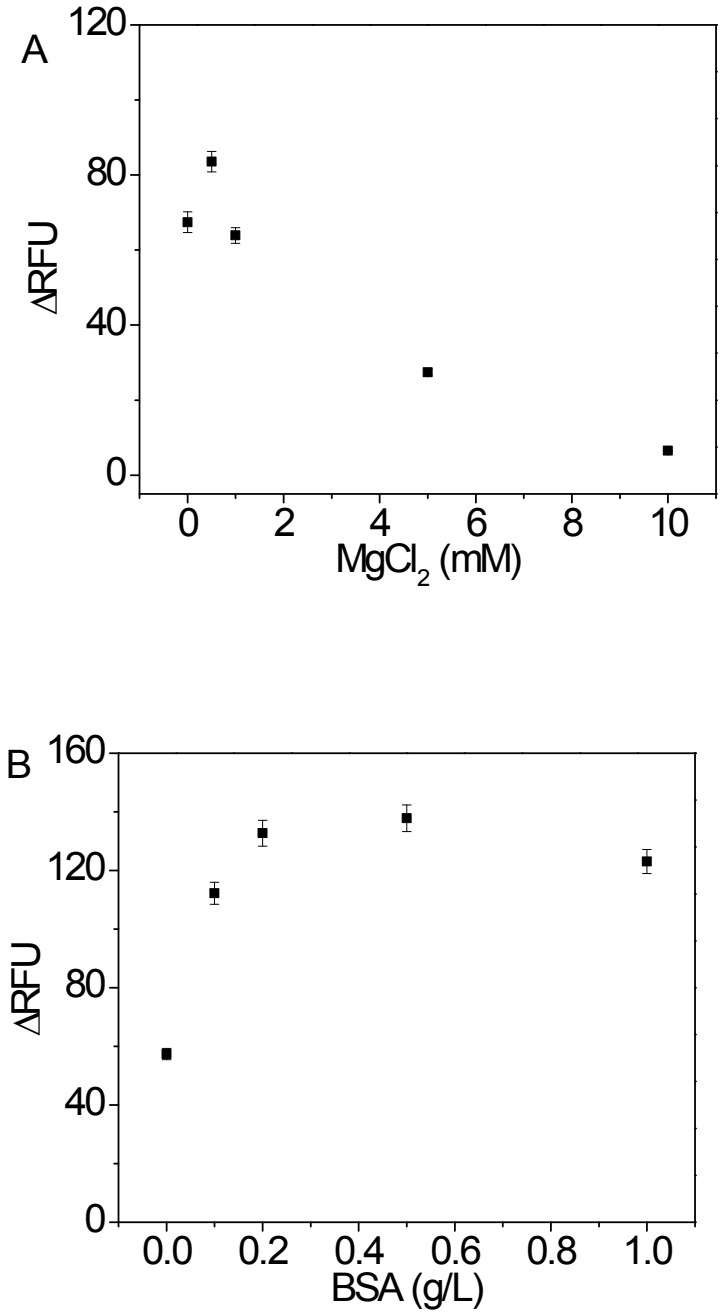

Fig. S2 (A) Effect of Mg^{2+} concentration on fluorescence change of the aptamer caused by thrombin in the binding solution containing 20 mM Tris–HCl (pH 7.5), 2 nM aptamer probe, 8.3 nM thrombin and varying concentrations of $MgCl_2$. (B) Effect of BSA concentration on fluorescence change of the aptamer caused by thrombin in the binding solution containing 20 mM Tris–HCl (pH 7.5), 2 nM aptamer probe, 8.3 nM thrombin and varying concentrations of BSA.

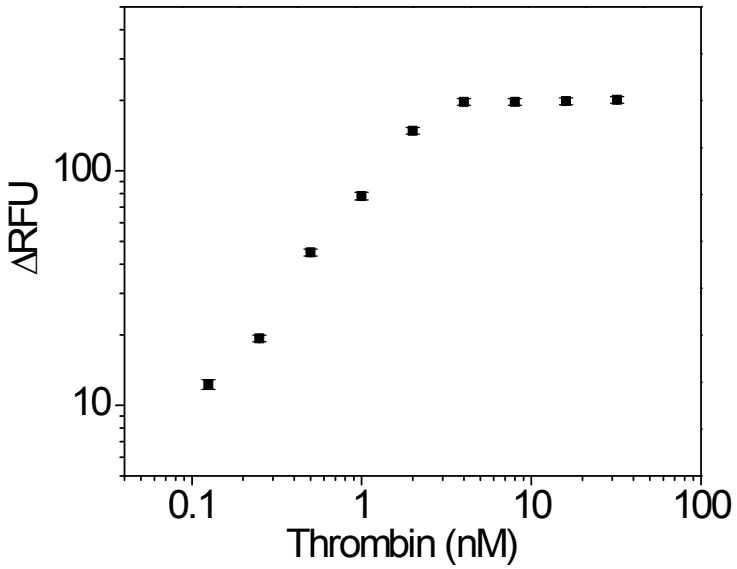

Fig. S3 Detection of thrombin spiked in 250-fold diluted human serum.