Electronic Supplementary Information

A TiS_2 nanosheet-enhanced fluorescence polarization biosensor for ultra-sensitive detection of biomolecules

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Table S1. List of the oligonucleotides used

oligonucleotide	Sequences (from 5’ to 3’)
Probe 1	5’-TAT CTA GTT GAG CTG TCT AGT C-folate-3’
Probe 2	5’-G ACT AGA CGT TGA ↓ AGG ATA-FAM-3’
Probe 3	5’-TAT CTA GTT GAG CTG TCT AGT CGG TTG GTG TGG TTG G-3’
Probe 4	5’-G ACT AGA CGT TGA ↓ AGG ATA -3’
3-F	5’-ATA-FAM-3’
6-F	5’-AGG ATA-FAM-3’
10-F	5’-T TGA AGG ATA-FAM-3’
15-F	5’-AGA CGT TGA AGG ATA-FAM-3’
19-F	5’-G ACT AGA CGT TGA AGG ATA-FAM-3’
25-F	5’- C GTA CTG ACT AGA CGT TGA AGG ATA-FAM-3’

Table S2. Comparison of fluorescence methods for the determination of FR

technique and method	LOD; linear range	ref
Electrochemical	0.3 ng/mL; 1.0-20.0 ng/mL	S1
Electrochemical, Nicking endonuclease	0.19 ng/mL ; 0.3-15 ng/mL	S2
Electrochemical, single-walled carbon nanotubes	0.114 ng/mL (3 pM); 0.01-1 nM	S3
Electrochemical	0.114 ng/mL (3 pM); 0.01-500 nM,	S4
Electrochemical; Hybridization chain reaction	0.003 ng/mL , 0.01-100 ng/mL	S5
Colorimetry; AuNPs	0.33 ng/mL, 0.5- 50 ng/mL	S6
Colorimetry , Hemin/G-quadruplex DNAzyme	0.19 ng/mL (5 pM), 0-100 pM	S7
Colorimetry; Gold nanoparticle , Exonuclease III	0.0019 ng/mL (50 fM) , 0.1-10 pM	S8
Fluorescence; Graphene oxide	0.77 ng/mL, 1-800 ng/mL	S9
Fluorescence; Rolling circle amplification; Exonuclease III	0.030 ng/mL (0.8 pM),1 pM to 1 nM	S10
Fluorescence polarization; TiS_2 nanosheet, Zn^{2+} depended-DNAzyme	0.003 ng/mL; 0.01-20 ng/mL	this study

It should be noted that 1 pM ≈ 38 pg/mL

Table S3. Comparison of the amplification method developed in this work with the traditional ELISA kit approach for the detection of FR in the serum samples.

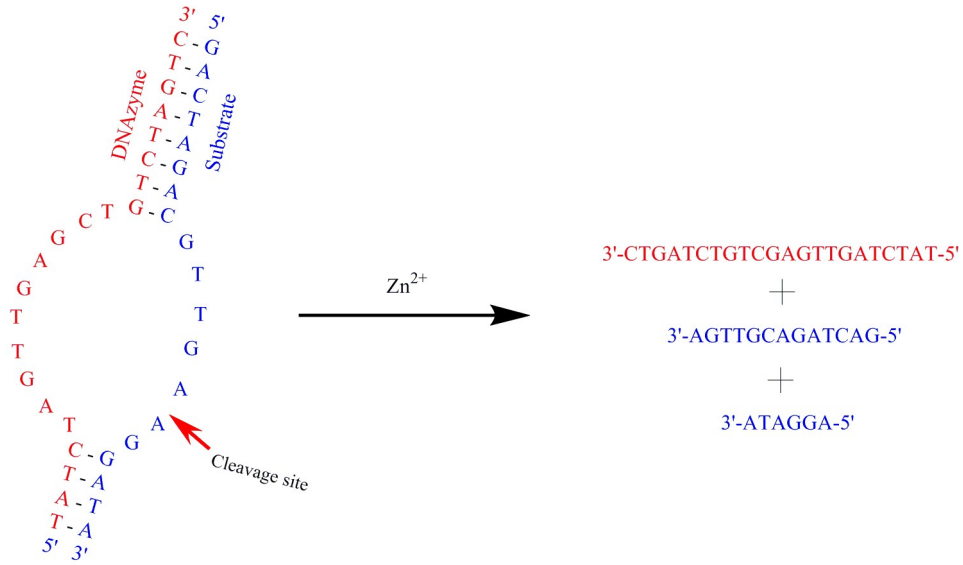
Sample[a]	This work (ng/mL)[b]	RSD (%, n=3)	ELISA (ng/mL)[b]	RSD (%, n=3)
1	1.23	2.5	1.27	2.6
2	1.35	2.3	1.32	2.4
3	1.28	2.2	1.29	2.1
4	0.075	3.2	0.080	3.0
5	0.086	2.8	0.089	3.1
6	0.077	3.1	0.070	2.9

[a] Samples 1, 2 and 3 were from advanced ovarian cancer patients; samples 4, 5 and 6 were from healthy women.

[b] Each sample was analyzed in triplicate, and the results were the average values

Table S4. Sensing platforms for the detection of Tb

Technique and method	LOD; linear range	real sample	ref
Fluorescence; Gold nanoparticle	2.5 nM; 5.0- 2500 nM	No	S11
Fluorescence; Graphene oxide nanosheet	2 nM; 0-100 nM	No	S12
Fluorescence; Carbon nanotube	1.8 nM; 4.0-150 nM	No	S13
Fluorescence; MoS_2 nanosheet	300 pM; 0-100 nM	No	S14
Fluorescence; Exo III- amplification molecular aptamer beacon	89 pM; 0-2 nM	No	S15
Fluorescence; Nicking Enzyme -amplification molecular aptamer beacon	100 pM; 0-1 nM	human serum	S16
Fluorescence polarization	100 pM; 0.1−2 nM	human serum	S17
Fluorescence polarization; Nicking Enzyme - amplification; Graphene oxide nanosheet	1 fM; 2 fM–200 nM	human plasma	s18
Fluorescence polarization; Zn^{2+}-depented - DNAzyme amplification; TiS_2 nanosheet	0.01 pM; 0.05 pM-50nM	human plasma	this study



Scheme S1. The structure of Zn^{2+}-depended self-hydrolyzing deoxyribozymes.

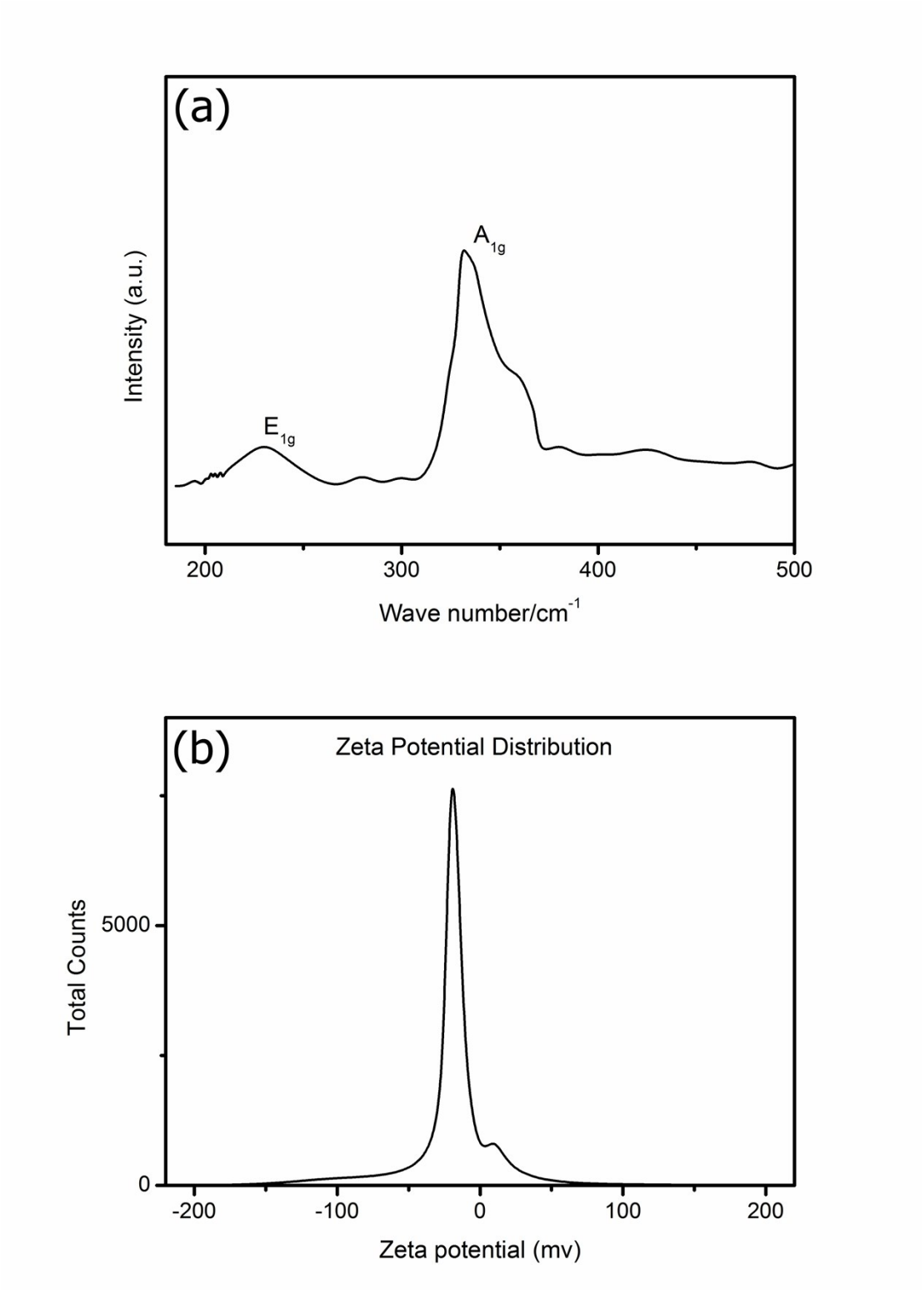


Fig. S1. (a), Raman spectra of the as-prepared TiS_2 nanosheets; (b), values of the zeta potential for the as-prepared TiS_2 nanosheets.

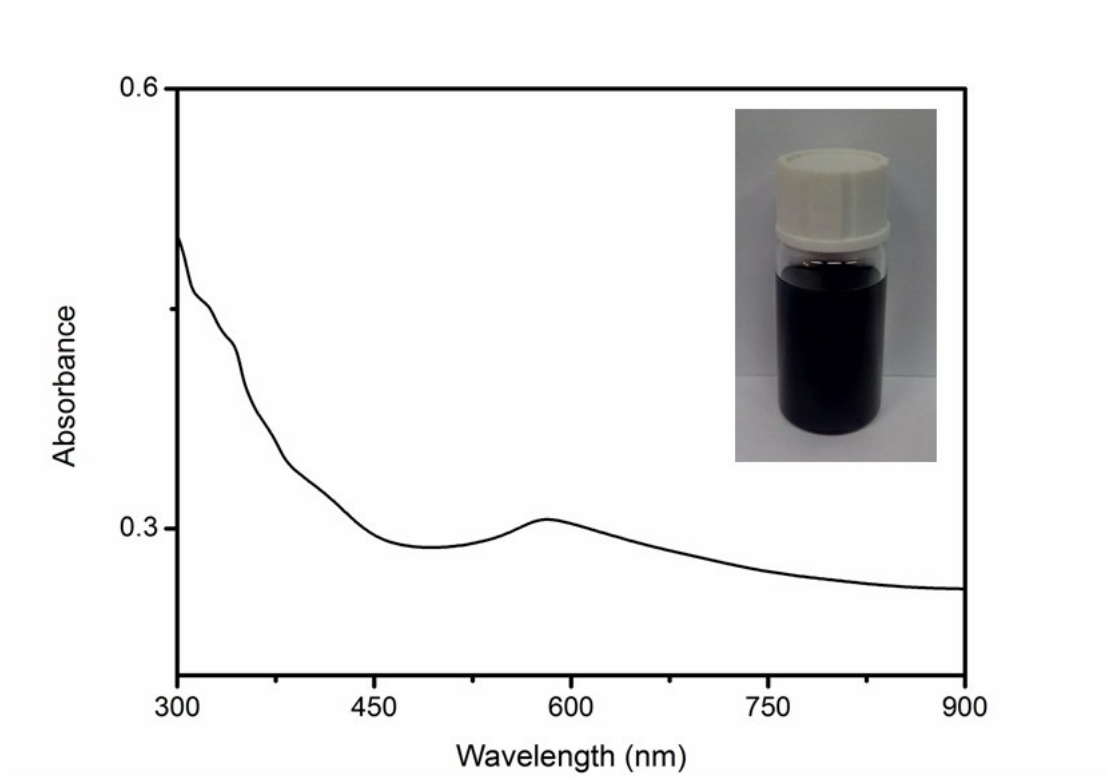


Fig.S2. UV-vis spectrum of the as-prepared layered TiS_2 nanosheet: Inset, the photograph of the TiS_2 nanosheet in aqueous solution.

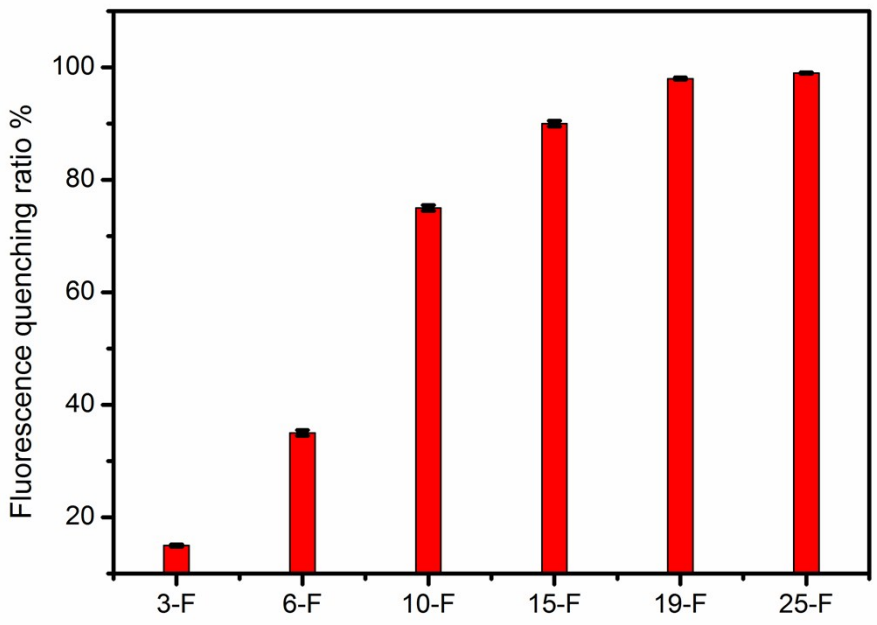


Fig. S3. Comparison of the fluorescence quenching ratio of TiS_2 nanosheet to ssDNA with different lengths: the concentration of TiS_2 nanosheet was fixed at 25 μg/mL, and the 3-F, 6-F, 10-F, 15-F, 19-F and 25-F indicate FAM-labeled ssDNA containing 3, 6, 10, 15, 19 and 25 bases, respectively.

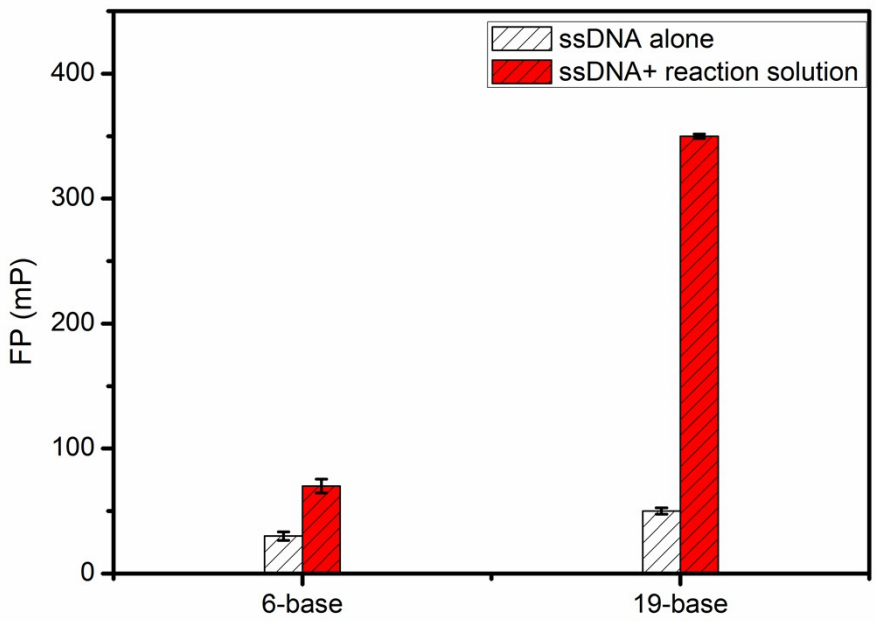


Fig. S4. The changes in the fluorescence polarization value of 6-base FAM-ssDNA (6-F) and 19-base FAM-ssDNA (19-F) with or without addition of the reaction solution.

In order to verify whether the low fluorescence polarization value may be caused by incomplete adsorption of the fragments, additional control experiments have been performed. For this purpose, a new substrate strand is designed as probe 4. Compared with probe 2, probe 4 has the same base length but has no fluorophore label. The experiment procedure is the same as that shown in the assay procedure using probe 2. After the reaction is completely accomplished, we measure the fluorescence polarization value of the reaction solution upon addition of two different lengths of ssDNA (one is 6-base length FAM-ssDNA, the other is 19-base length probe 2), respectively. The results are displayed below in Fig. S4. It can seen that the FP value of the solution with addition of 6-base length FAM-ssDNA (20 nM) is 70. However, the FP value of the solution with addition of 19-base length FAM-probe 2 (20 nM) is 350. Obviously, the short length ssDNA has weaker affinity to TiS_2 nanosheet compared with the long length ssDNA. This result confirms that the TiS_2 nanosheet

has enough adsorption sites for the ssDNA. Thus, the low fluorescence polarization value is not caused by incomplete adsorption of the fragments.

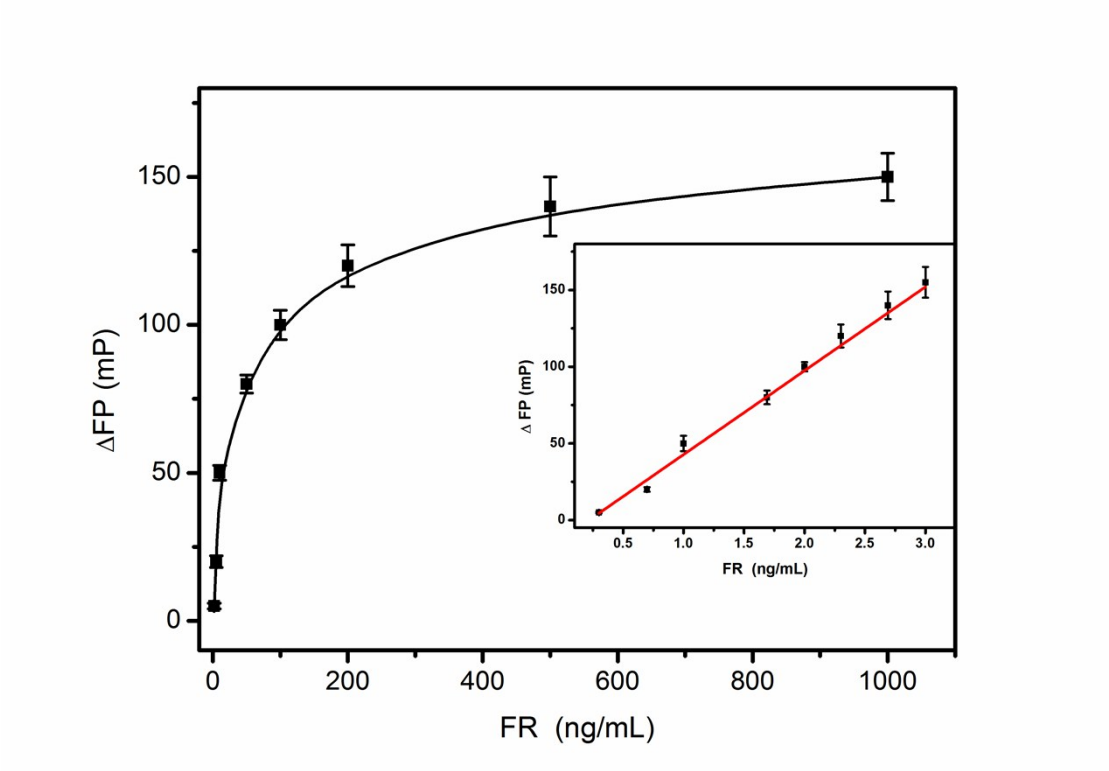


Fig. S5. Fluorescence polarization changes of probe 1 + probe 2 + TiS_2 system upon addition of different concentrations of FR. Inset is the relationship of *ΔFP* with the FR concentration. [Probe 1] = 48 nM, [probe 2] = 40 nM and [TiS_2] = 35 μg/mL. The error bar was calculated from three independent experiments.

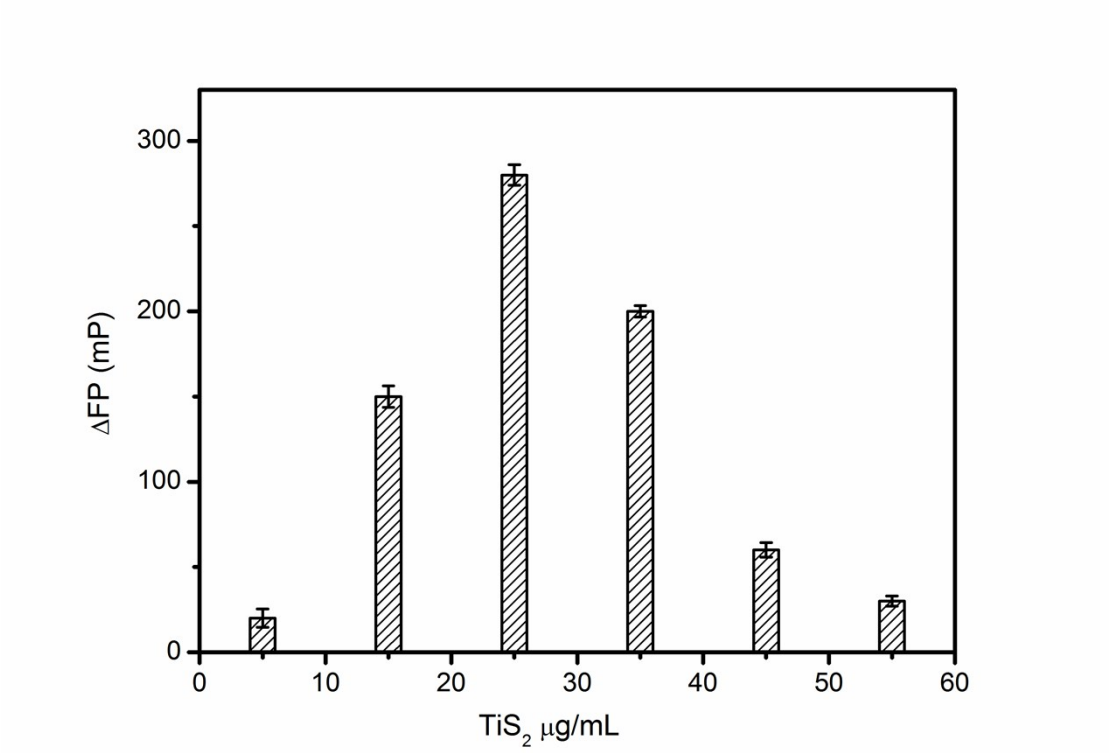


Fig. S6. The effect of TiS_2 concentration on the *ΔFP* response of the sensing system. $\Delta FP = FP_0 - FP$, where FP_0 and FP are the fluorescence polarization in the absence and presence of Tb, respectively. [probe 3] = 30 nM, [probe 2] = 25 nM, [Tb] = 5 ng/mL, $[Zn^{2+}]$ = 2 mM and [Exo I] = 10 U.

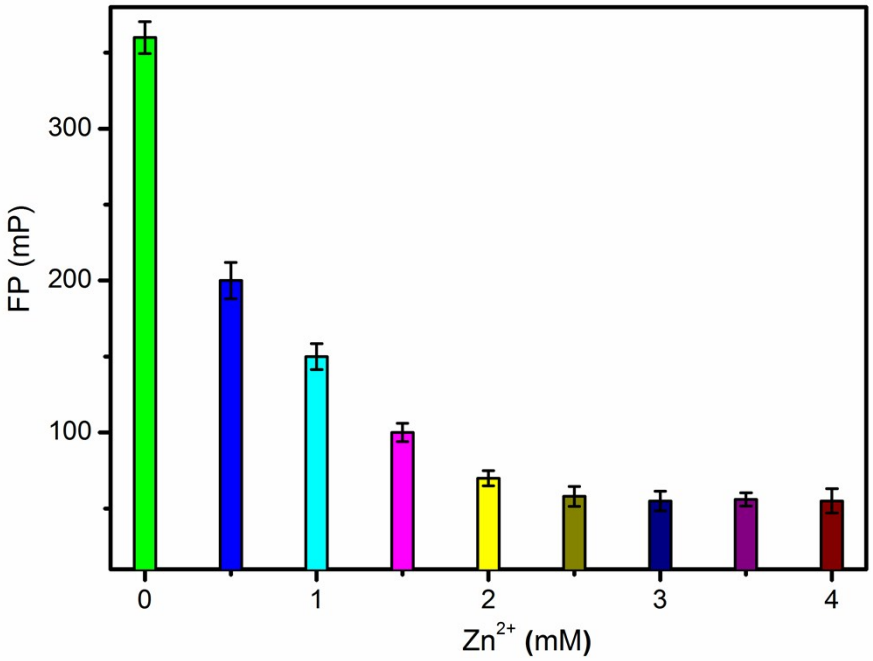


Fig. S7. The effect of Zn^{2+} concentration on the fluorescence polarization of the detection system. [probe 3] =30 nM, [probe 2] = 25 nM, [TiS_2] = 25 μg/mL.

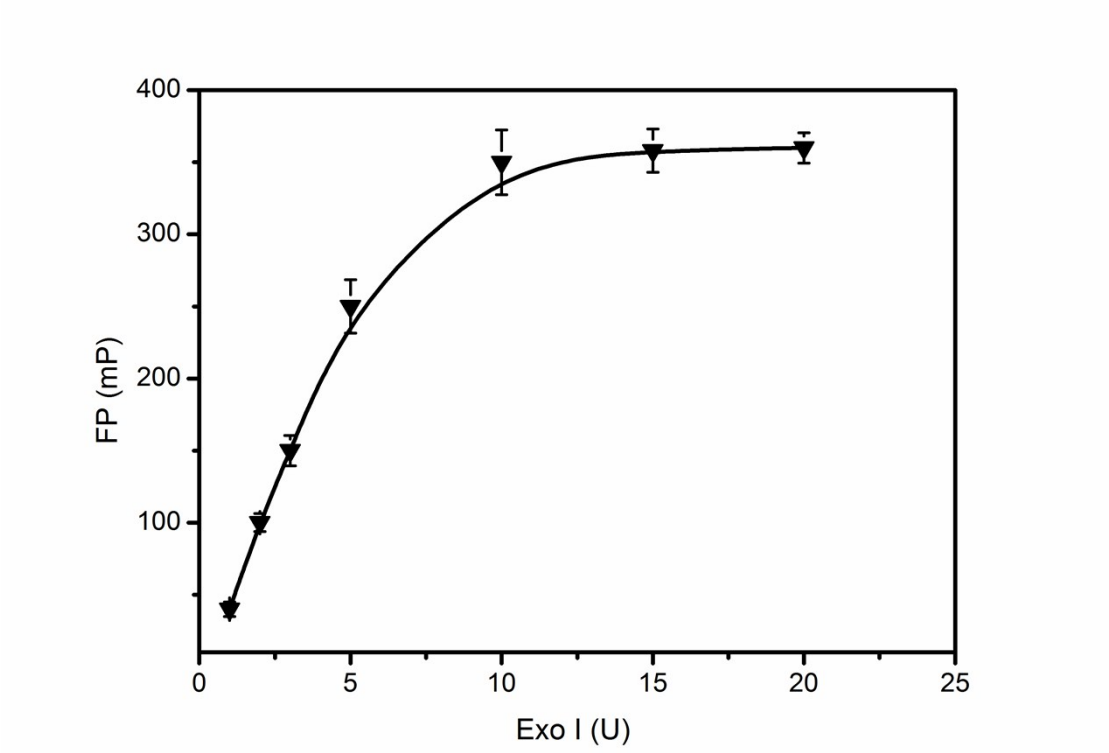


Fig. S8. The effect of Exo I concentration on the fluorescence polarization response of the sensing system. [probe 3] = 30 nM, [probe 2] = 25 nM, [TiS_2] = 25 μg/mL, [Zn^{2+}] = 2.0 mM.

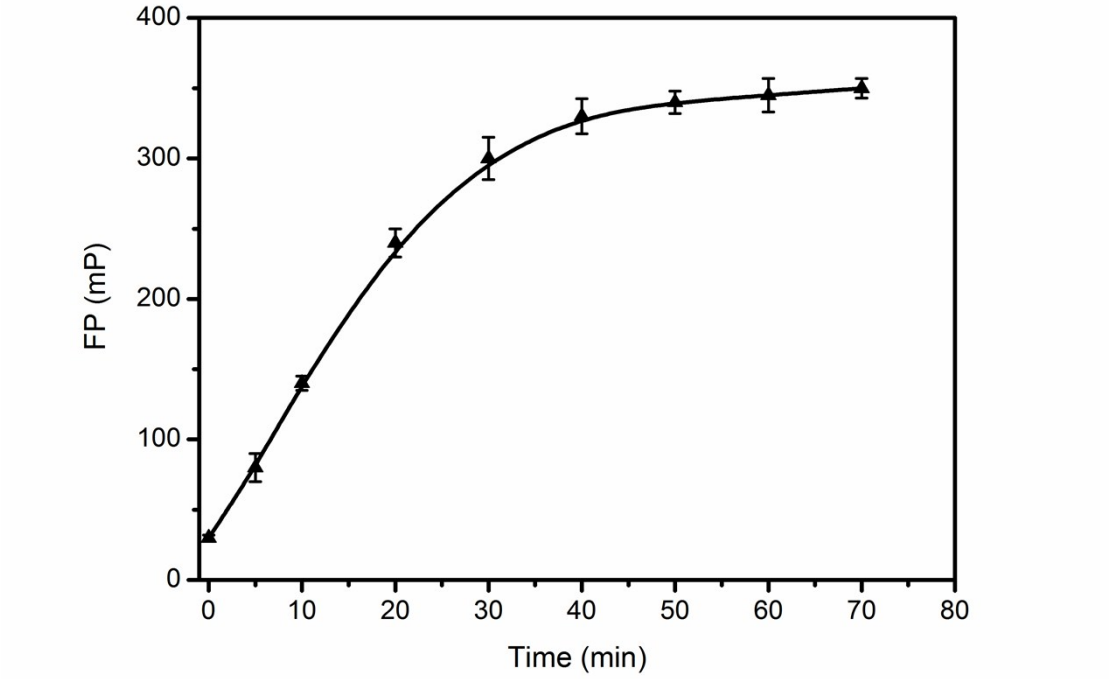


Fig. S9. The fluorescence polarization intensity of the sensing system as a function of the reaction time between probe 1 and Exo I. [probe 3] = 30 nM, [probe 2] = 25 nM, [TiS_2] = 25 μg/mL, [Exo I] = 10 U, [Zn^{2+}] = 2.0 mM.

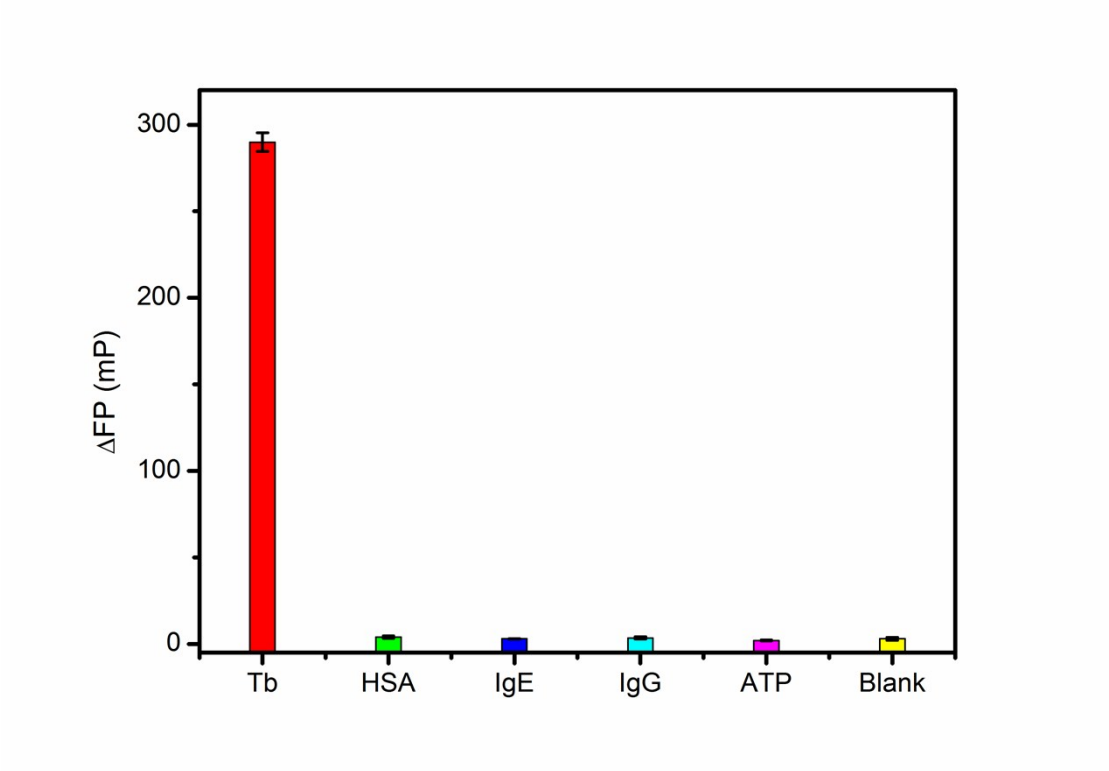


Fig. S10. Selectivity of the developed sensing system for Tb (1 nM) against the interfering molecules HSA, IgE, IgG and ATP at 1 μM. The error bar was calculated from three independent experiments.

References:

S1 G. F. Wang, X. P. He, L. Wang, X. J. Zhang, *Biosens. Bioelectron.,* **2013,** 42 337-341.

S2 Y. Cao, S. Zhu, J. C. Yu, X. J. Zhu, Y. M. Yin, G. X. Li, *Anal. Chem.,* **2012,** 84, 4314−4320.

S3 Z. Wu, Z. Zhen, J. H. Jiang, G. L. Shen, R. Q. Yu, *J. Am. Chem Soc.,* **2009,** 131, 12325–12332.

S4 H. B. Wang, H. D. Zhang, S. P. Xu, T. Gan, K. J. Huang, Y. M. Liu, *Sensor. Actuat B-Chem.,* **2014,** 194, 478-483.

S5 J. Zhao, S. S. Hu, Y. Cao, B. Zhang, G. X. Li, *Biosens. Bioelectron.,* **2015,** 66 327-331.

S6 Y. H. Zhu, G. F. Wang, L. Sha,Y. W. Qiu, H. Jiang, X. J. Zhang, *Analyst,* **2015,** 140, 1260-1264.

S7 X. Gong, W. J. Zhou, Y. Q. Chai, Y. Xiang, R. Yuan, *RSC Adv.,* **2015,** 5, 6100-6105.

S8 X. J. Yang, Z. Q. Gao, *Nanoscale,* **2014,** 6, 3055-3058.

S9 Y. He, X, J. Xing, H. W. Tang, D. W. Pang, *Small,* **2013,** 9, 2097-2101.

S10 L. J. Ou, H. B. Wang, X. Chu, *Analyst,* **2013,** 138, 7218-7223.

S11 F. Luo, L. Zheng, S. Chen, Q. Cai, Z. Lin, B. Qiu, G. Chen, *Chem. Commun.,* **2012,** 48, 6387-6389.

S12 C. H. Lu, H. H. Yang, C. L. Zhu, X. Chen, G. N. Chen, *Angew. Chem. Int. Ed.,* **2009,** 48, 4785 -4787.

S13 R. Yang, Z. Tang, J. Yan, H. Kang, Y. Kim, Z. Zhu, W. H. Tan, *Anal. Chem.,* **2008,** 80, 7408-7413.

S14 X. Liu, R. Freeman, I. Willner, *Chem. Eur. J.,* **2012,** 18, 2207-2211.

S15 J. Ge, E. C. Ou, R. Q. Yu, X. Chu, *J. Mater. Chem. B,* **2014,** 2, 625-628.

S16 L. Y. Xue, X. M. Zhou, D. Xing, *Anal. Chem.,* **2012,** 84, 3507-3513.

S17 D. P. Zhang, Q. Zhao, B. L. Zhao, H. L. Wang, *Anal. Chem.,* **2012,** 84, 3070-3074.

S18 Y. Huang, X. Liu, L. Zhang, K. Hu, S. Zhao, B. Fang, Z. F. Chen, H. Liang, *Biosensor. Bioelectron.,* **2015,** 63, 178-184.