Electronic Supplementary Information

Amplified colorimetric detection of tetracycline based on enzyme-linked aptamer assay with multivalent HRP-mimicking DNAzyme

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Table S1. Sequences of oligonucleotides uesd in this work

Name	Sequence (5′ to 3′)	Modification
Bio-aptamer	CGTACGGAATTCGCTAGCCCCCCGGCAGGCCACGGCTTGGGTTGGTCCCACTGCGCGTGGATCCGAGCTCCACCGTG	3-biotin
H1	AGGGCGGGTGGGTGTTTAAGTTGGAGAATTGTACTTAAACACCTTCTTCTTGGGT	3-biotin
H2	TGGGTCAATTCTCCAACTTAAACTAGAAGAAGGTGTTTAAGTTGGGTAGGGCGGG	/
Trigger	AGAAGAAGGTGTTTAAGTA	/

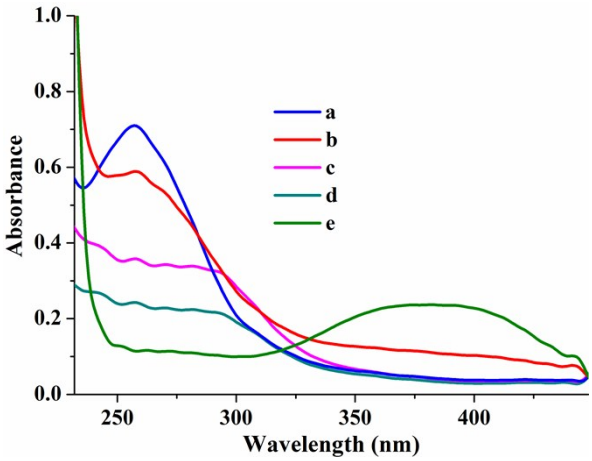

Fig. S1 UV-vis absorption spectra of multivalent HRP-mimicking DNAzyme synthesized with 300 nM G-quadruplex-rich nanowires solution, 300 ng/mL SA, 300 nM Bio-aptamer, and 20 μM hemin. a: 300 nM G-quadruplex-rich nanowires solution; b: multivalent HRP-mimicking DNAzyme; c: 300 ng/mL SA; d : 300 nM Bio-aptamer; e: 20 μM hemin

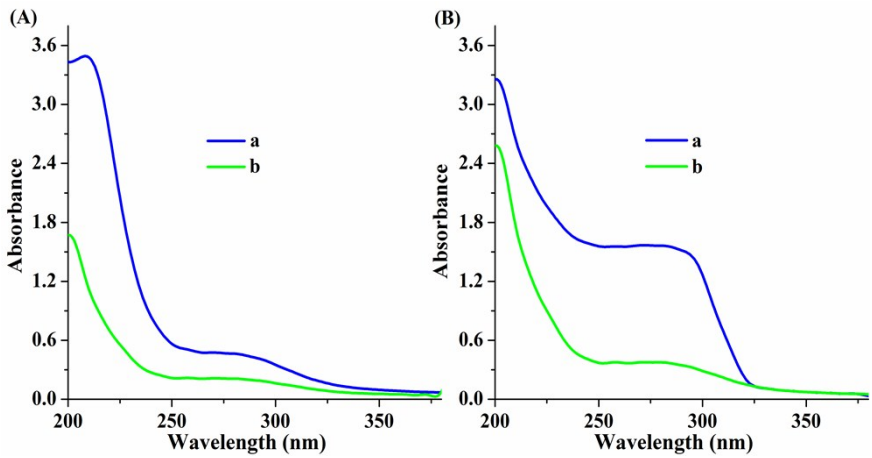

Fig. S2 (A) UV-vis absorption spectra of TC-BSA solution before (a) and after (b) immobilization, the concentration of TC-BSA was 10 μg/mL; (B) UV-vis absorption spectra of TC-BSA solution before (a) and after (b) immobilization, the concentration of TC-BSA was 50 μg/mL

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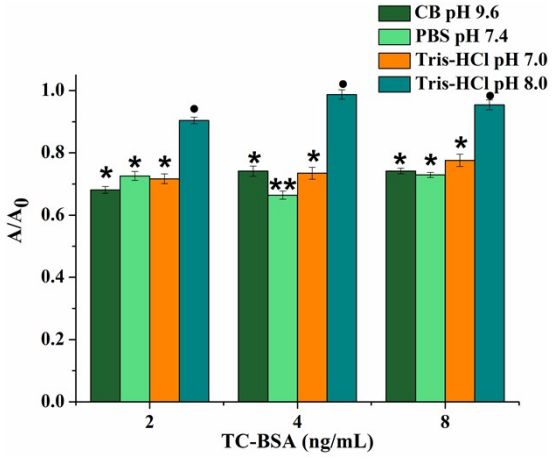

Fig. S3 Effect of coating buffer on the detection system, 10 mM Bicarbonate buffer (CB, 3 mM Na_2CO_3, 7 mM $NaHCO_3$, pH 9.6), 10 mM phosphate buffer saline (PBS, 100 μM KH_2PO_4, 500 μM $Na_2HPO_4(12H_2O)$, 10 mM NaCl, 200 mM KCl, pH 7.4), and 10 mM Tris–HCl buffer with different pH.

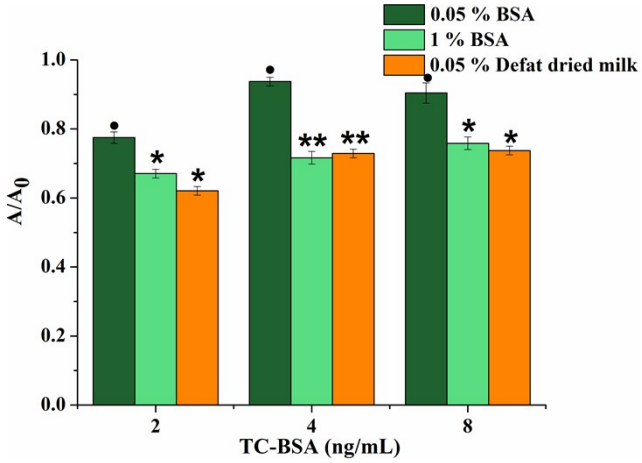

Fig. S4 Effect of blocking agents on the detection system, including 0.05% BSA, 1% BSA, and 0.05% defat dried milk

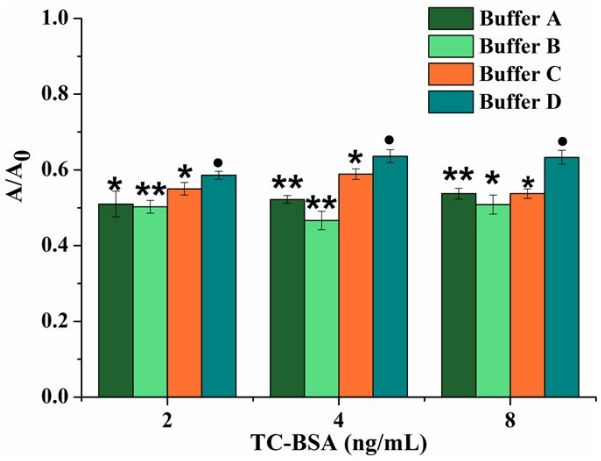

Fig. S5 Effect of binding buffers on the detection system, buffer A: 20 mM Tris-HCl (pH 8.0), buffer B: 20 mM Tris-HCl (pH 8.0), 100 mM NaCl; buffer C: 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 2 mM $MgCl_2$; buffer D: 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$

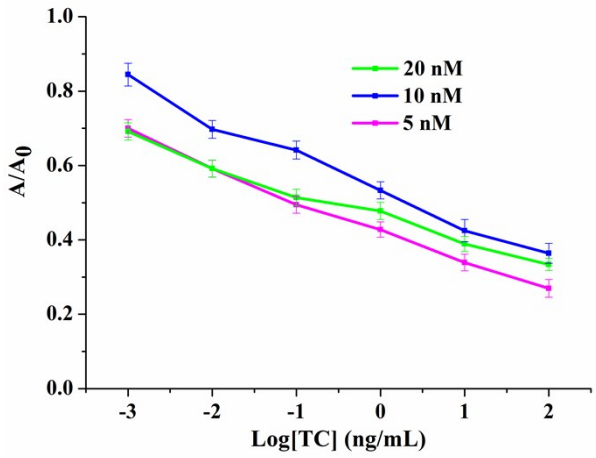

Fig. S6 Indirect competitive assay for TC detection using different concentrations of aptamer modified HRP-mimicking DNAzyme system (5, 10, 20 nM), the concentration of TC-BSA was 4 μg/mL

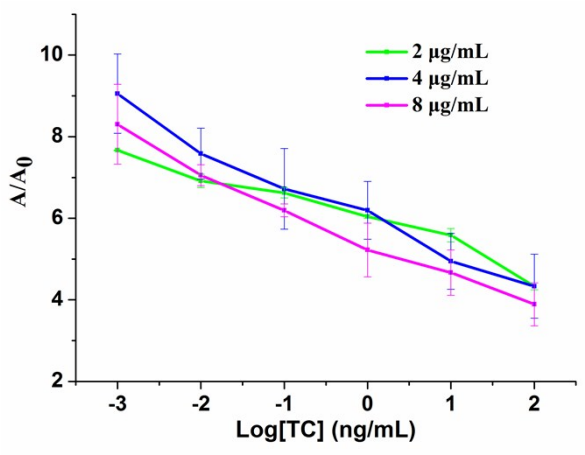

Fig. S7 Indirect competitive assay for TC detection in buffer using different concentrations of TC-BSA conjugate (2, 4, 8 μg/mL), the concentration of aptamer modified HRP-mimicking DNAzyme was 10 nM

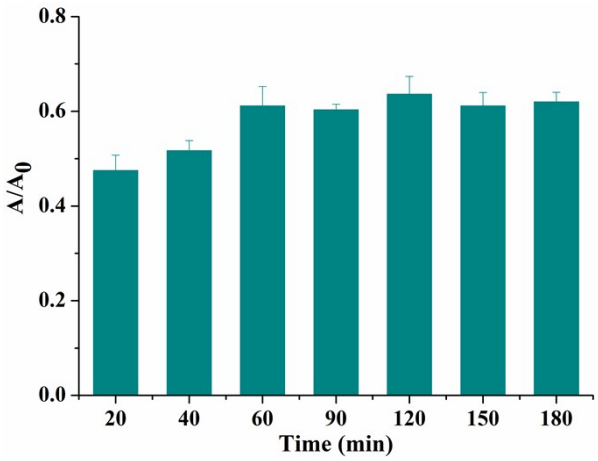

Fig. S8 Effect of competitive reaction time on the detection system, the concentration of TC-BSA and aptamer modified HRP-mimicking DNAzyme was 4 μg/mL and 10 nM, respectively

Table S2. Comparision of different methods for TC detection

Method	Keywords	Liner range	LOD	Ref.
ELISA	Biotin-avodin, HRP	3.16×10^{-10}-3.16×10^{-7} M	0.048 μg/ml	1
ELISA	Aptamer, HRP	0.01-100 ng/ml	9.6×10^{-3} ng/ml	2
HPLC	Liquid-liquid micro-extraction	5-15 μg/mL	0.95-3.6 μg/mL	3
HPLC	Solid phase extraction, Validation	50-500 ng/mL	21 ng/mL	4
Colorimeter	Colorimeter	0.5 -10 μg/mL	1.5 μg/mL	5
ELISA	Class-Specific monoclonal antibody	0.26 -2.0 μg/L	15 μg/L	6
Electrochemistry	Electrochemica aptasensor, M-shape structure	≤ 3000 nM	≈ 329 ng/mL	7
Electrochemistry	PtNPs/C/GCE.	9.99-44.0 μM	≈ 1.9 mg/ml	8
Electrochemistry	Aptamer biosensor	0.1-100 ng/mL	1 ng/mL	9
ELISA	Multivalent HRP-mimicking DNAzyme systems, Aptamer	10^{-2}-10^{4} ng/mL	8.1×10^{-2} ng/ml	This work

Table S3. Recovery study

Sample	Spiked concentration (ng/mL)	Detected concentration (ng/mL, mean±SD)	Recovery (%)
1	0.1	0.086±0.0032	86
2	1	1.014±0.0012	101.4
3	10	9.33±0.0021	93.3

References

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