Electronic Supplementary Material (ESI) for Nanoscale.

An Enzyme-Mediated Universal Fluorescence Biosensor Template for Pathogen Detection Based on Three-dimensional DNA Walker and Catalyzed Hairpin Assembly

Dan Li[†], Enlai Yang[†], Zewei Luo[‡], Qiyue Xie[†], Yixiang Duan[†,*]

[†] Research Center of Analytical Instrumentation, Key Laboratory of Bio-resource and Eco-environment, Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610065, Sichuan, P.R. China

[‡] Research Center of Analytical Instrumentation, Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry & Materials Science, Northwest University, Xi'an 710069, Shanxi, P.R. China

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Table S1. DNA sequences used in this experiment.

DNA name	Sequence (5'-3')
Apt-*S.ty*	TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG
c-Apt-*S.ty*	GACGCCGCCATAACCAGCTGAGGAG
Apt-*E.coli*	TGAGCCCAAGCCCTGGTATGTTACAGTATGCTACCTCTACTTGAAG GTTGGTCGACGCGGGGCAGGTCTACTTTGGGATC
c-Apt-*E.coli*	CATACCAGGGCTTGGGCTCAACCAGCTGAGGAG
PolyA$_5$-DNA	AAAAATATTAACCCTCCTCAGCTGGTTAATAGTCCGAAT
PolyA$_{10}$-DNA	AAAAAAAAAATATTAACCCTCCTCAGCTGGTTAATAGTCCGAAT
PolyA$_{20}$-DNA	AAAAAAAAAAAAAAAAAAAATATTAACCCTCCTCAGCTGGTTAAT AGTCCGAAT
PolyA$_{30}$-DNA	AAAAAAAAAAAAAAAAAAAAAAAAAAAAATATTAACCCTCCTC AGCTGGTTAATAGTCCGAAT
PolyA$_{40}$-DNA	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATAT TAACCCTCCTCAGCTGGTTAATAGTCCGAAT
SH-DNA	SH-TTTTTTATTAACCCTCCTCAGCTGGTTAATAGTCCGAAT
H1	GCACAGAGACATTCGGACTATTAACCAGCTGAACTCTGGTATCTG GTCTCAGCTGGTTAATAGTCTTGAGTTAGAGCAG
H2	CCAGCTGAGAACCAGAGTTCAGCTGGTTAATAGT
F	FAM-AACTCAAGACTATTAATGTGGCAA
Q	TAGTGTTAGTCTTGAGTT-DABCYL

Table S2. Comparison results of different methods for *S.ty* and *E.coli* detection.

Target	Method	LOD (CFU mL^{-1})	Reaction Time	Ref.
S.ty	ICP-MS	100	40 min	[1]
	FOLSPR biosensor	128	2 h	[2]
	Colorimetric detection	42	3 h	[3]
	microfluidic biosensor	58	2 h	[4]
	LIBS	61	65 min	[5]
	Fluorescence biosensor	22.8	85 min	This work
E.coli	SERS	1000	No mentioned	[6]
	Electrochemical biosensor	100	3 h	[7]
	Colorimetric detection	50	1 h	[8]
	Biomimetic receptors	100	140 min	[9]
	Fluorescence biosensor	28.1	85 min	This work

a. *S.ty* and *E.coli* detection methods with higher LOD than this work.

Target	Method	LOD (CFU mL^{-1})	Reaction Time	Ref.
S.ty	PCR	7-9	<5 h	[10]
	Immunology-based assay	10	No mentioned	[11]
	SERS	4	130 min	[12]
E.coli	Interferometric Reflectance Imaging	2.2	2 h	[13]
	LIBS	8.89	135 min	[14]
	SERS	1	45 min	[15]

b. *S.ty* and *E.coli* detection methods with lower LOD than this work.

Table S3. The sequences of *S.ty* and *E.coli* primers of qPCR.

Primers	Sequence (5’ - 3’)	Product size (bp)
Forward primer	ATTTCTGGATATGGTACTGAGGC	123
Reverse primer	AAATGAGTTGCTTTACGCTGC	

a. The primer sequences of *S.ty.*

Primers	Sequence (5’ - 3’)	Product size (bp)
Forward primer	TGTTCTGACTGGTGACGTGAAA	126
Reverse primer	TGCTTGGTCGGGATAGTGG	

b. The primer sequences of *E.coli.*

Table S4. Reaction system for qPCR.

Reagents	Volume (μL)
Distilled water	3.2
SYBR green real-time PCR master mix	5
Forward primer (10 μM)	0.4
Reverse primer (10 μM)	0.4
DNA template	1
Total volume	10

Table S5. The detection results of the natural samples by the proposed biosensor.

Natural samples	Detected by qPCR (CFU mL^{-1})	Detected by the biosensor (CFU mL^{-1})

Rain-water	Negative	BDL
Tap-water	Negative	BDL
Pool water	Negative	BDL

a. The detection results of *S.ty* in natural samples.

Natural samples	Detected by qPCR (CFU mL^{-1})	Detected by the biosensor (CFU mL^{-1})
Rain-water	Negative	BDL
Tap-water	Negative	BDL
Pool water	Negative	BDL

b. The detection results of *E.coli* in natural samples.

"BDL" means that natural sample signals are below detection limit.

Figures

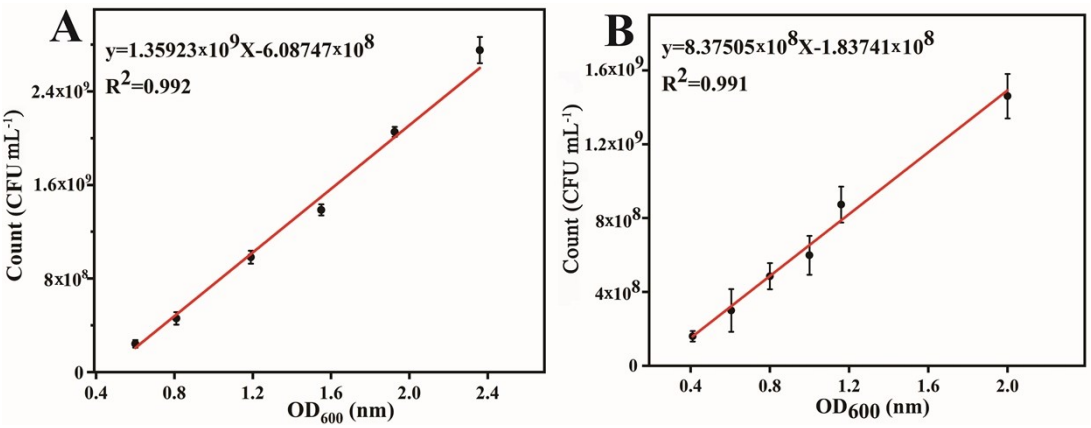

Figure S1. Standard linear calibration curve for *Salmonella Typhimurium* (A) and *Escherichia coli* (B). Each OD_{600} was repeated for three times.

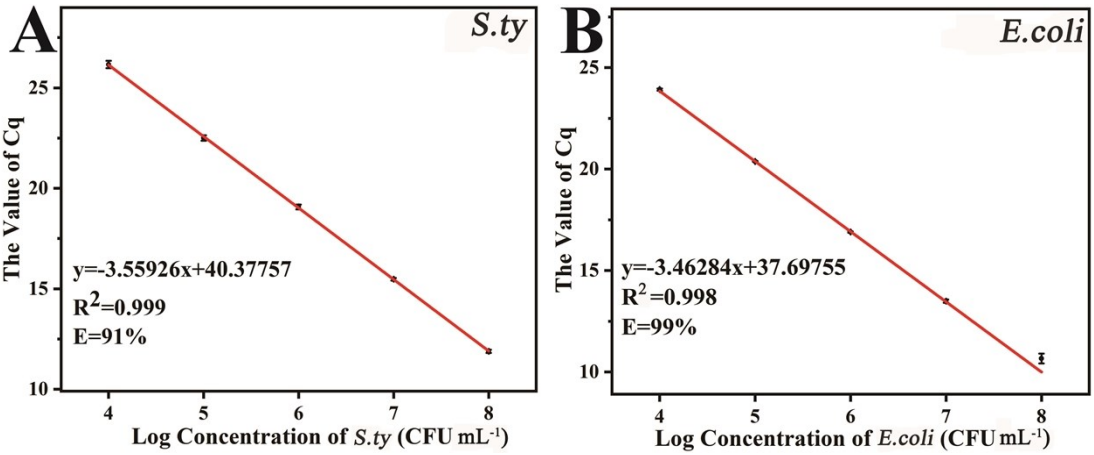

Figure S2. The relationship between the bacteria concentration and the valve of Cq. (A) The relationship between the different *S.ty* concentration and the valve of Cq. (B) The relationship between the different *E.coli* concentration and the valve of Cq. All experiments were repeated three times.

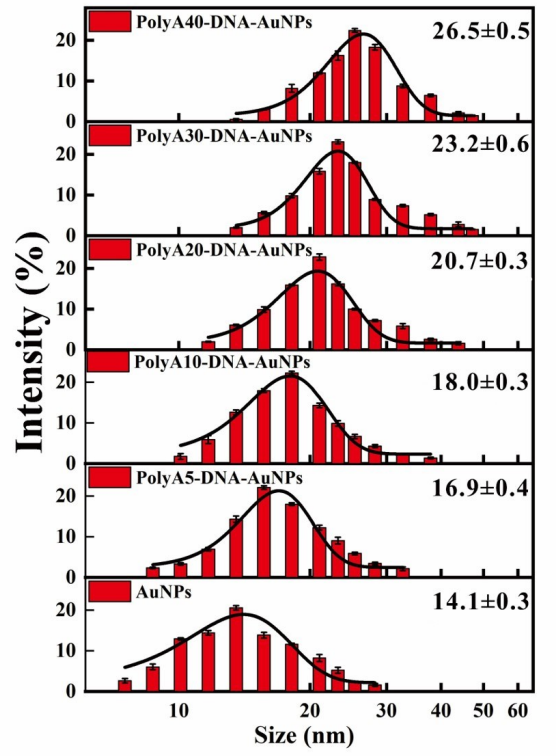

Figure S3. The DLS results of the bare AuNPs, PolyA$_5$-DNA-AuNPs, PolyA$_{10}$-DNA-AuNPs, PolyA$_{20}$-DNA-AuNPs, PolyA$_{30}$-DNA-AuNPs and PolyA$_{40}$-DNA-AuNPs.

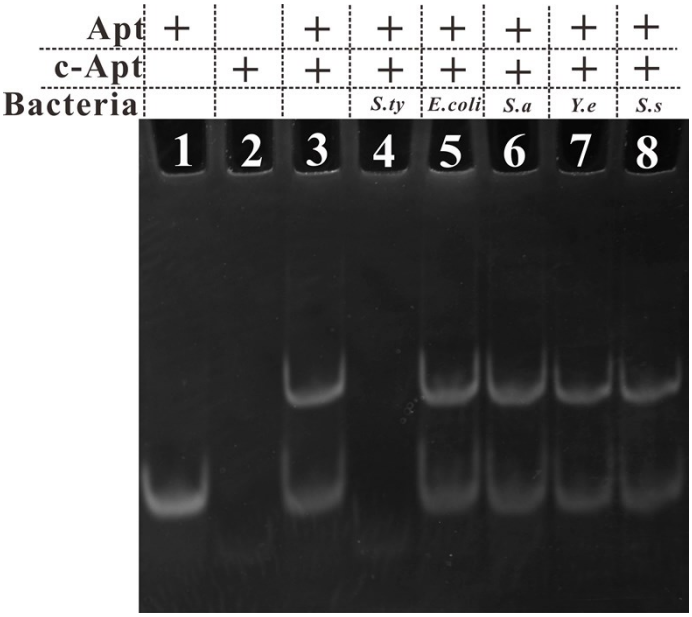

Figure S4. PAGE band intensity changes of the aptamer specificity for detecting different bacteria. Reaction conditions: 16 μL (3 μM) Apt, 16 μL (1 μM) c-Apt, 20 μL (10^3 CFU mL^{-1}) *S.ty, E.coli, S.a, Y.e, S.s.*

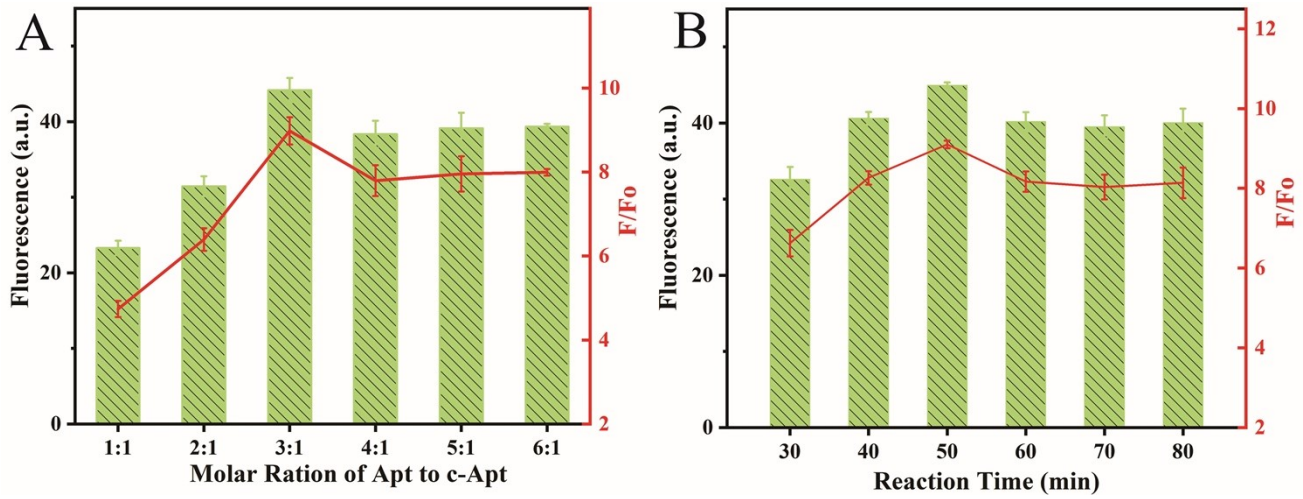

Figure S5. Optimization of key parameters. (A) Fluorescence analysis of Apt/c-Apt at different molar ratios, including 1:1, 2:1, 3:1, 4:1, 5:1 and 6:1. Reaction conditions: 16 μL Apt including 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, 6 μM; 16 μL (1 μM) c-Apt, 20 μL (10^3 CFU mL^{-1}) *S.ty* and 16 μL H1, 16 μL H2, 32 μL F@Q and 0.06 U μL^{-1} Nt.BbvCI. (B) Effect of reaction time. Reaction conditions: 16 μL (3 μM) Apt, 16 μL (1 μM) c-Apt, 20 μL (10^3 CFU mL^{-1}) *S.ty*, 16 μL PolyA10-DNA-AuNPs; 16 μL H1, 16 μL H2, 32 μL F@Q and 0.06 U μL^{-1} Nt.BbvCI using different reaction time.

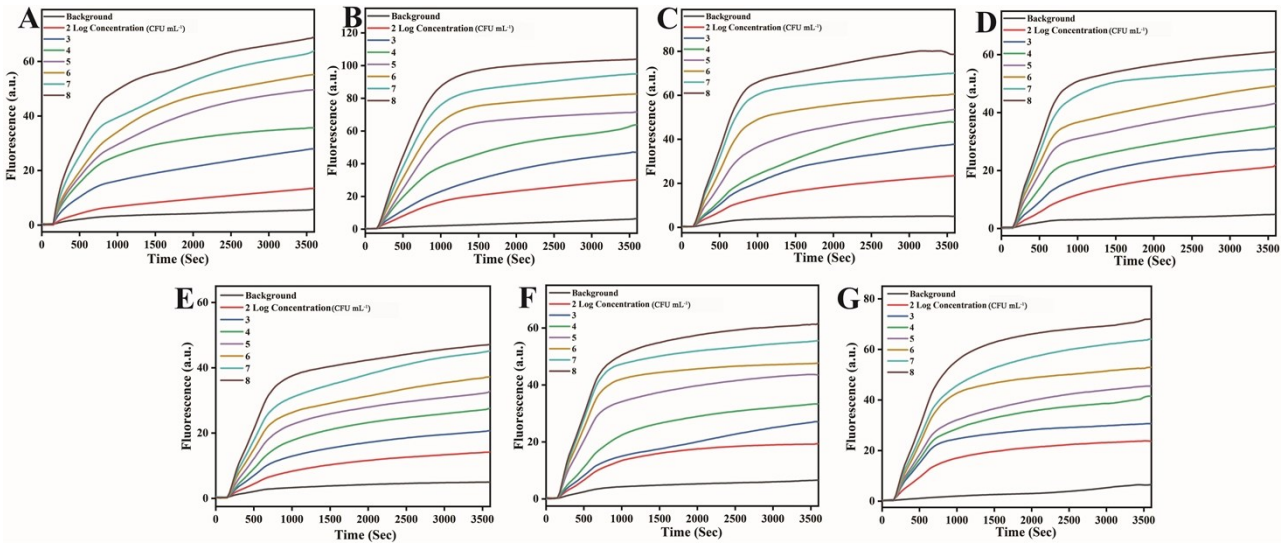

Figure S6. The fluorescence kinetic curve in real-time monitor. (A) PolyA_5-DNA-AuNPs; (B) PolyA_{10}-DNA-AuNPs; (C) PolyA_{20}-DNA-AuNPs; (D) PolyA_{30}-DNA-AuNPs; (E) PolyA_{40}-DNA-AuNPs; (F) SDAs; (G) MSDAs. Reaction conditions: 16 μL (3 μM) Apt, 16 μL (1 μM) c-Apt, 20 μL *S.ty* with different concentration, 16 μL PolyA-DNA-AuAPs with different length, 16 μL H1, 16 μL H2, 32 μL F@Q and 0.06 U μL^{-1} Nt.BbvCI.

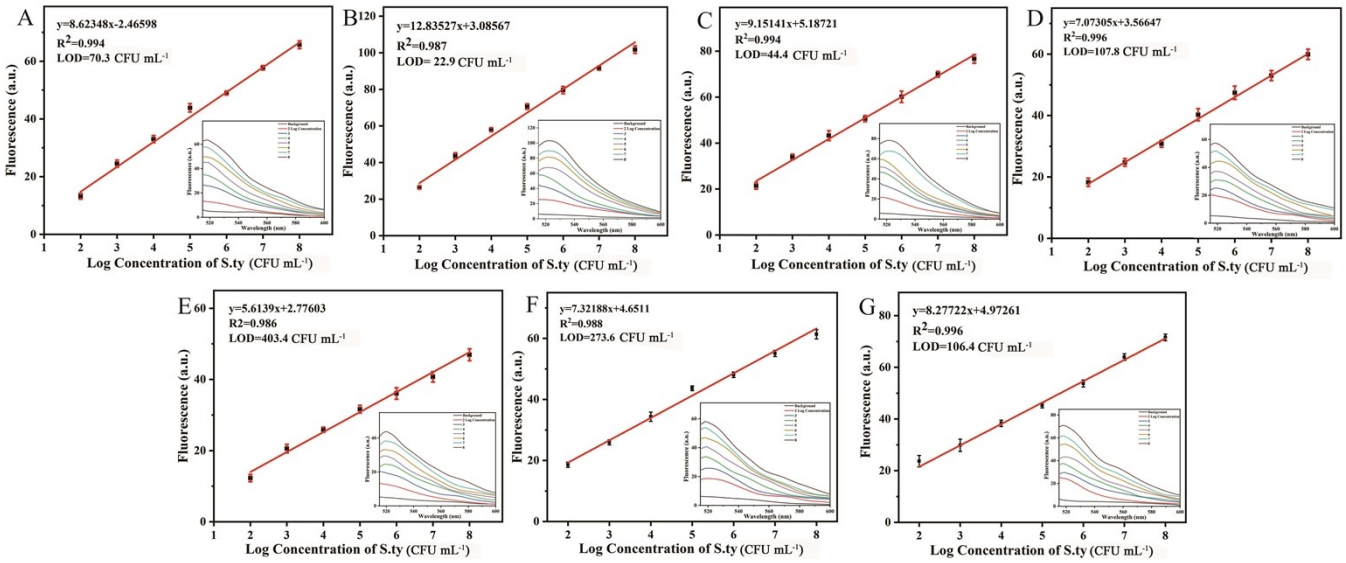

Figure S7. The relationship between the *S.ty* concentration and the fluorescence intensity change at 520 nm. (A) PolyA$_5$-DNA-AuNPs; (B) PolyA$_{10}$-DNA-AuNPs; (C) PolyA$_{20}$-DNA-AuNPs; (D) PolyA$_{30}$-DNA-AuNPs; (E) PolyA$_{40}$-DNA-AuNPs; (F) SDAs; (G) MSDAs. Reaction conditions: 16 μL (3 μM) Apt, 16 μL (1 μM) c-Apt, 20 μL *S.ty* with different concentration, 16 μL PolyA-DNA-AuAPs with different length, 16 μL H1, 16 μL H2, 32 μL F@Q and 0.06 U μL^{-1} Nt.BbvCI.

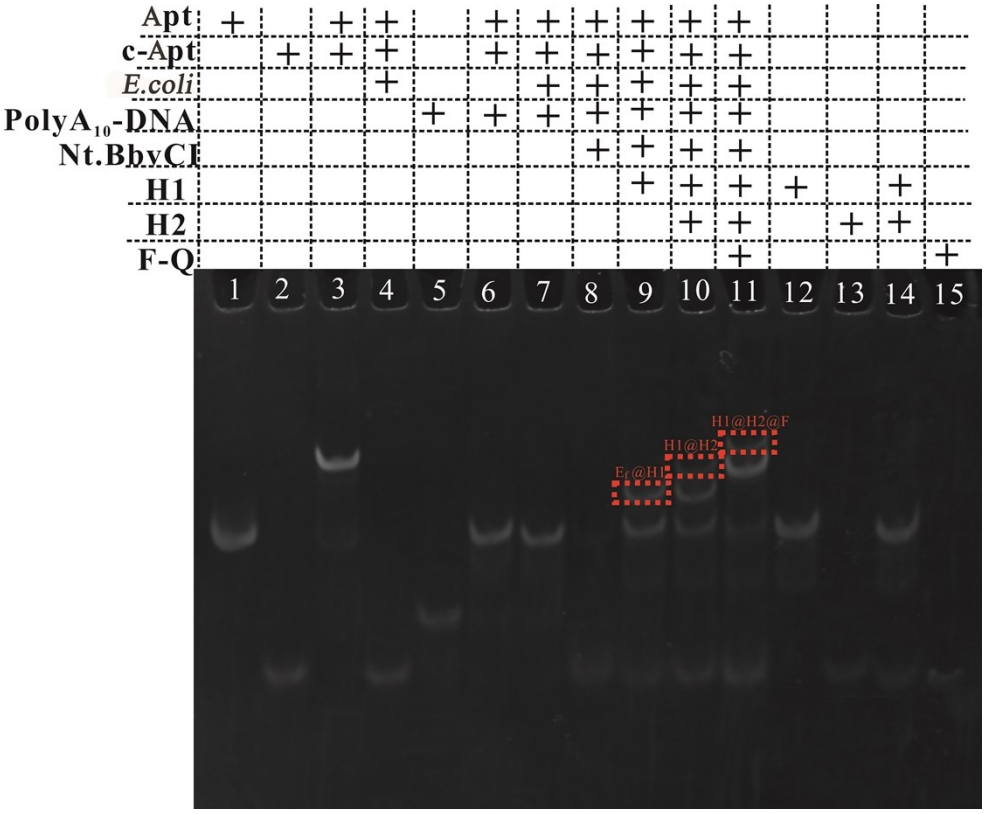

Figure S8. PAGE image of verifying the feasibility. Lane 1: 16 μL (3 μM) Apt; Lane 2: 16 μL (1 μM) c-Apt; Lane 3: lane 1 + lane 2; Lane 4: lane 3 + 20 μL (10^3 CFU mL^{-1}) *E.coli*; Lane 5: 16 μL $PolyA_{10}$-DNA, Lane 6: 16 μL c-Apt + lane 5; Lane 7: lane 4 + lane 5; Lane 8: lane 7 + 0.06 U μL^{-1} Nt.BbvCI; Lane 9: lane 8 + 16 μL H1; Lane 10: lane 9 + 16 μL H2; Lane 11: lane 10 + 32 μL F@Q; Lane 12: 16 μL H1; Lane 13: 16 μL H2; Lane 14: lane 12 + lane 13; Lane 15: 32 μL F@Q.

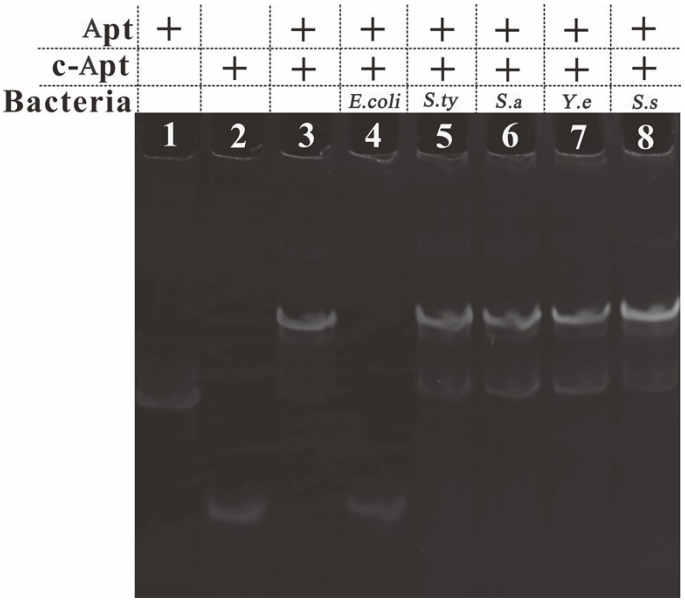

Figure S9. PAGE band intensity changes of the aptamer specificity for detecting different bacteria. Reaction conditions: 16 μL (3 μM) Apt, 16 μL (1 μM) c-Apt, 20 μL (10^3 CFU mL^{-1}) *E.coli, S.ty, S.a, Y.e, S.s.*

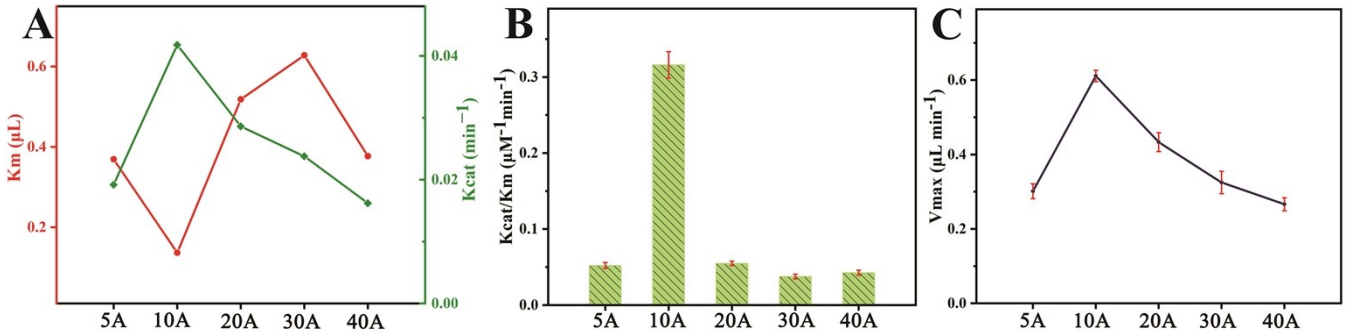

Figure S10. Effect of surface coverage on the performance of biosensor system. (A) Comparison of kinetic parameters of Nt.BbvCI in different length of PolyA; (B) The comparison of the specificity constant of Nt.BbvCI in solution; (C) The programmable change of Vmax with the change of the surface coverage. All experiments were repeated five times.

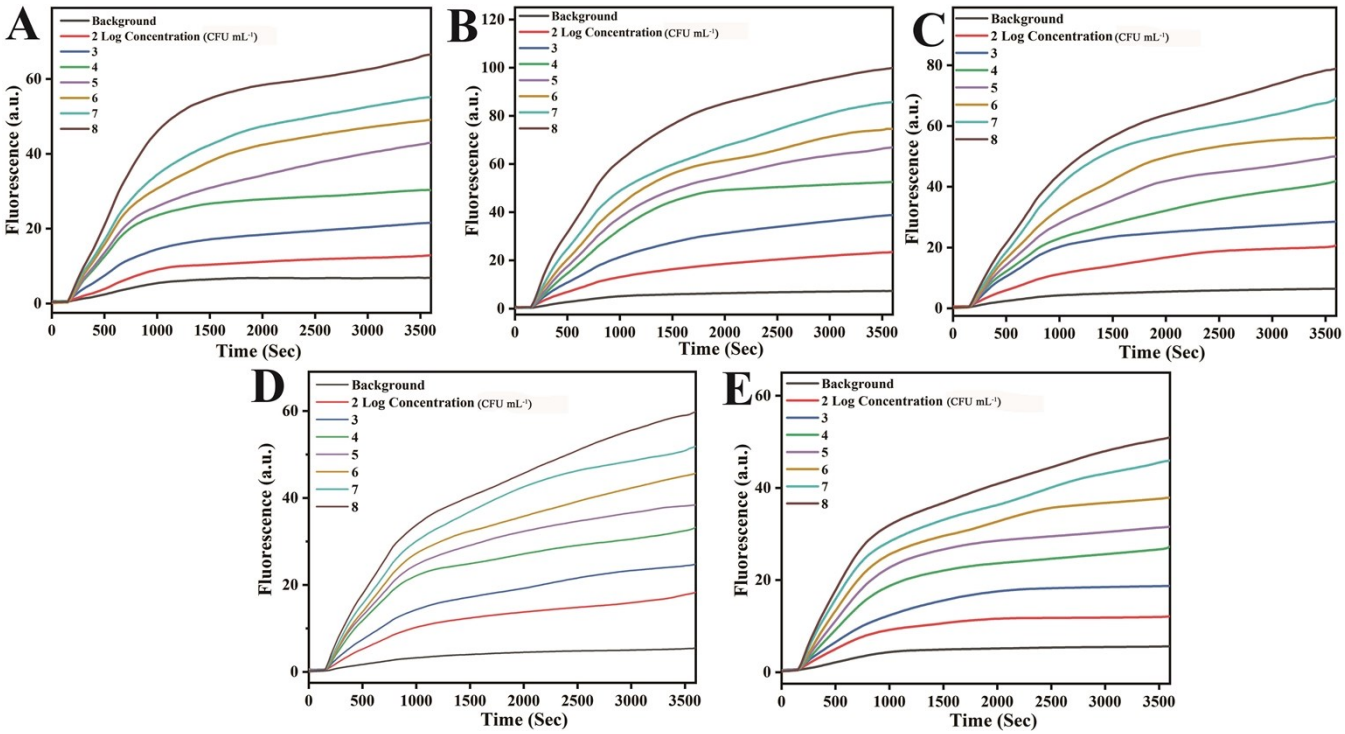

Figure S11. The fluorescence kinetic curve in real-time monitor. (A) PolyA_5-DNA-AuNPs; (B) PolyA_{10}-DNA-AuNPs; (C) PolyA_{20}-DNA-AuNPs; (D) PolyA_{30}-DNA-AuNPs; (E) PolyA_{40}-DNA-AuNPs. Reaction conditions: 16 μL (3 μM) Apt, 16 μL (1 μM) c-Apt, 20 μL *E.coli* with different concentration, 16 μL PolyA-DNA-AuAPs with different length, 16 μL H1, 16 μL H2, 32 μL F@Q and 0.06 U μL^{-1} Nt.BbvCI.

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