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Supporting information

Transcription aptasensor: amplified, label-free and culture-independent detection of foodborne pathogens via light-up RNA aptamers

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Experimental Procedures

Materials and reagents

The sequences used were synthesized by Invitrogen (Shanghai, China) as followings:

1) S. aureus aptamer (SA31):

5'-TCCCACGATCTCATTAGTCTGTGGATAAGCGTG GGACGTCTATGA-3'

2) Blocker:

5'-TATCCACAGACT AATGAGATCGTGGGA-3'

3) L-broccoli:

5'-GAGCCCACACTCTACTCGACAGATACGAATATCTGGACCCGACCGTCTCTAACCC

TATAGTGAGTCGTATTATCCCACGATCTCATT-3'

All of them were purified by polyacrylamide gel electrophoresis (PAGE). The concentration of nucleic acid probe solutions was quantified by UV-Vis absorption spectroscopy. 5-difluoro-4-hydroxybenzylidene imidazolidinone (DFHBI-1T) was purchased from Lucerna (Brooklyn, USA). Phi29 DNA polymerase (10 U/μL), 10000×Sybr Gold dye, 10000×Sybr Green II, and T7 RNA polymerase (20 U/μL) were bought from Thermo Fisher Scientific (Waltham, USA). DNTPs (dATP, dTTP, dCTP, dGTP) and 50×Tris-acetate-EDTA (TAE) buffer were bought from by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). RNTPs (ATP, UTP, CTP, GTP) was purchased from New England Biolabs (Beijing, China). 10000× Gelred^{TM} Nucleic Acid Gel Stain, agarose and 6× loading buffer (DNA) were bought from Beijing DingGuo Biotechnology Co., Ltd. (Beijing, China). All reaction solutions were prepared by Molecular biology grade water (Corning, New York, USA). The culture medium was bought from Qingdao Hope Bio-Technology Co.,

Ltd. The bacterial strains *S. aureus* (ATCC 29213), a methicillin-resistant *S. aureus* (MRSA, ATCC 43300) *Escherichia coli* (*E. coli*, ATCC 43889), *Listeria monocytogenes* (*L. monocytogenes*, ATCC 54002), *Bacillus cereus* (*B. cereus*, ATCC 14579), *Salmonella enterica* (*S. enterica*, ATCC 50094), and *Enterobacter sakazakii* (*E. sakazakii*, ATCC 29544) were provided from the American Type Culture Collection (ATCC).

Bacteria preparation

Before lineation to activate the growth on nutrient agar (NA) plates at 37 °C for 20 h, all of the bacterial strains were stored at frozen state under -80 °C. At the experiment eve, the cells were enriched by inoculated to nutrient broth (NB) in orbital shaker (37 °C, 120 rpm) overnight. The required concentrations of cells were prepared by suspending with 1×PBS buffer after washed once by the same buffer. To get the specific concentration with colony counting experiments, the samples were surface-coated on plate counting agar (PCA) plates and incubated in the air oven for 24 h at 37 °C, and the single colony forming units (CFU) per milliliter were counted.

Analytical procedures using transcription aptasensor

The hybridization reaction was carried in a volume of 10 μL with 2 μL 10×phi29 DNA polymerase reaction buffer (33 mM Tris-acetate (pH 7.9 at 37 °C), 10 mM Mg-acetate, 66 mM K-acetate, 0.1% (v/v) Tween 20, 1 mM DTT), 2 μL H_2O, 3 μL Blocker (6 μM) and SA31 aptamer (6 μM). It was preprocessed at 90 °C for 3 min followed by annealing at room temperature (r. t.) for 30 min. The displacement step was then carried with the addition of 3 μL L-broccoli (6 μM) and 4 μL prepared *S. aureus* (ATCC 29213), allowing to react at r. t. for 30 min. Sequentially, 2 μL dNTPs (2.5 mM) and 1 μL phi29 DNA polymerase were

added with an incubation of 30 min at 30 °C. The extending product was used for *in vitro* transcription. The system was added with 6 μL 5× T7 RNA polymerase buffer, 1 μL rNTPs (10mM) and 3 μL T7 RNA polymerase (20 U/μL) before cultivated at 37 °C for 2.5 h. The transcribed RNA aptamers were stained with DFHBI-1T, Sybr Gold or Sybr Green II, and ready for the fluorescence and electrophoresis analysis.

Gel electrophoresis analysis

The different experimental samples of each steps were gathered and analyzed by 3% (w/v) agarose gel electrophoresis added in 1×TAE. The gel was stained by 10×Gelred. 1 μL 6×loading buffer was mixed with 5 μL nucleic acid mixtures, and prepared for gel electrophoresis. Gel electrophoresis was performed with the prepared solutions running through the prepared gel in 1×TAE at 150 V for 30 min. After electrophoresis, the gel was visualized via Gel Doc XR+ system (BioRad, USA).

Fluorescence spectra and real-time fluorescence analysis

The fluorescence spectra were measured by the fluorescence microplate reader Synergy H1 (BioTek, USA). The fluorescent spectra of DFHBI-1T were recorded at the emission ranged from 498 nm to 650 nm under the excitation of 468 nm. The excitation wavelength of Sybr Gold was 490 nm, with emission spectra recorded ranging from 520 to 650 nm. The excitation wavelength of Sybr Green II was 480 nm, with emission spectra recorded ranging from 510 to 650 nm.

A 50 μL volume of L-broccoli and Blocker transcription product with DFHBI-1T was used for real-time fluorescence analysis, and the concentrations of these sequences were the same as used for detection procedures (6 μM). The sample without T7 RNA polymerase

was set as a control. The real-time fluorescence signal was recorded using fluorescence microplate reader Synergy H1 (BioTek, USA). The excitation wavelength was 468 nm, while the emission wavelength was 506 nm, and the time interval of the measurement was 10 min.

Specificity test of transcription aptasensor

E. coli, *L. monocytogenes*, *B. cereus*, *S. enterica*, and *E. sakazakii* were used as interfering pathogens for the specificity test. And a methicillin-resistant *S. aureus* (MRSA) (ATCC 43300) was also tested to investigate the ability of transcription aptasensor to profiling drug-resistant pathogens. The pathogens were all cultured by NB and diluted with 1×PBS to be required concentration (10^7 CFU/mL). Then the reagents were measured under the same condition as *S. aureus* ATCC 29213.

Application of *S. aureus* (ATCC 29213) detection in water and food samples

The lake and tap water samples were collected from the campus of Sichuan University. Milk and pork were obtained from local supermarket. For the water samples, dilute concentrations of *S. aureus* were spiked directly for further analysis without any other treatment. For the pork sample, 1 g minced pork was added into 10 mL 1×PBS and sterilized with UV light. The suspension was centrifuged at 10,000 rpm for 15 min. Then the liquid supernatant and milk were both diluted tenfold by water before *S. aureus* were spiked. All of samples were detected as the procedures mentioned above.

Optimization of transcription aptasensor

Transcription aptasensor was dependent on strand-displacement triggered transcription reaction, mainly involved the transcription, light-up of transcribed RNA aptamers and extending processes. And the conditions for these processes were optimized to improve the detection performance of transcription aptasensor. The effect of transcription time was firstly investigated by real-time fluorescence analysis with the presence of DFHBI-1T (Fig. S1). With the addition of T7 RNA polymerase, the fluorescence intensity gradually increased, and reaching to the maximum at 2.5 h. The control group without the addition of T7 RNA polymerase would remain with a low-level fluorescence. Thus, the transcription process was proceeded for 2.5 h for transcription aptasensor. The concentration of DFHBI-1T dye would be pivotal for the light-up process of transcribed RNA aptamers. Both of the fluorescence intensity of background ("- *S. aureus*") and positive samples ("+ *S. aureus*") raised up with the increase of the concentration of DFHBI-1T dye (Fig. S2). The S/B ratio firstly raised, and reached a peak at 10 mM of DFHBI-1T (the S/B ratio reached the maximum-5.08). And it was selected as the optimal concentration for further experiments. The extending process was optimized with the concentration of phi29 DNA polymerase. The addition of a very low concentration of phi29 DNA polymerase (0.001 U/μL) would lead to a sharp increase of fluorescence compared to that with no addition of phi29 DNA polymerase (from 233 to 2259) (Fig. S3), and the S/B ratio jumped from 0.96 to 6.15. This further confirmed the necessity of the extending process for the transcription amplification process. When enzyme activity increased from 0.001 to 0.05 U/μL, both of the fluorescence intensity of background ("- *S. aureus*") and positive samples ("+ *S. aureus*") would increase, while the S/B ratio tend to be reduced. Therefore, phi29 DNA polymerase with a concentration of 0.001 U/μL would be sufficient for the extending

process, and contribute a high S/B ratio for *S. aureus* detection.

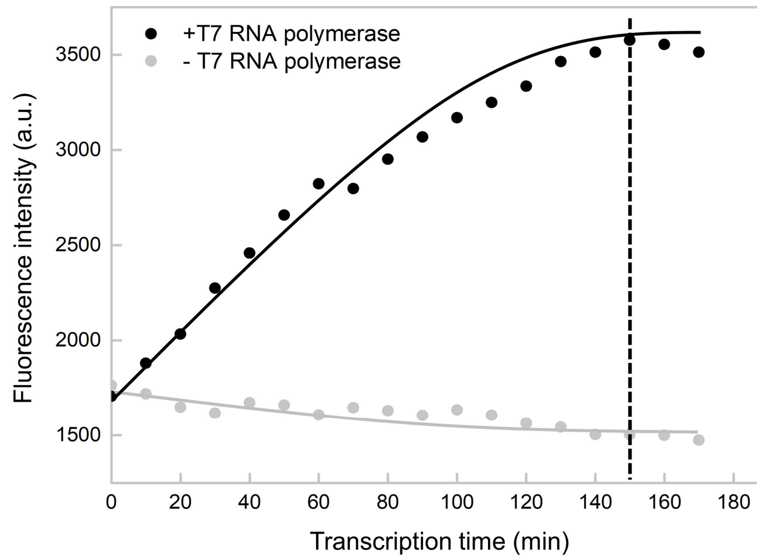

Fig. S1 Real-time fluorescence intensity of transcription aptasensor in the presence and absence of T7 RNA polymerase. The excitation wavelength was 468 nm, and the emission wavelength was 506 nm.

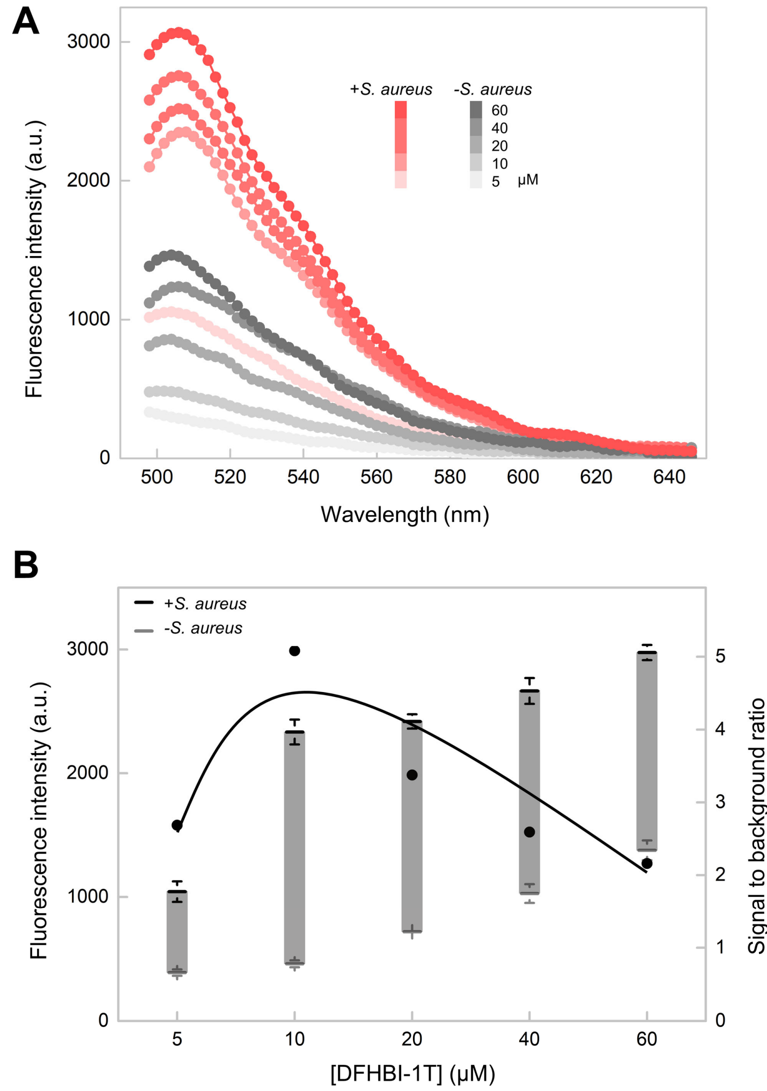

Fig. S2 Optimization of the concentration of DFHBI-1T dyes. (A) Fluorescence curves for transcription aptasensor with the addition of different concentrations of DFHBI-1T dyes in the presence or absence of *S. aureus*. (B) Fluorescence intensity of transcription aptasensor corresponding in (A). The excitation wavelength was 468 nm. Error bar=SD and all measured values are the average results of 3 paralleled determinations.

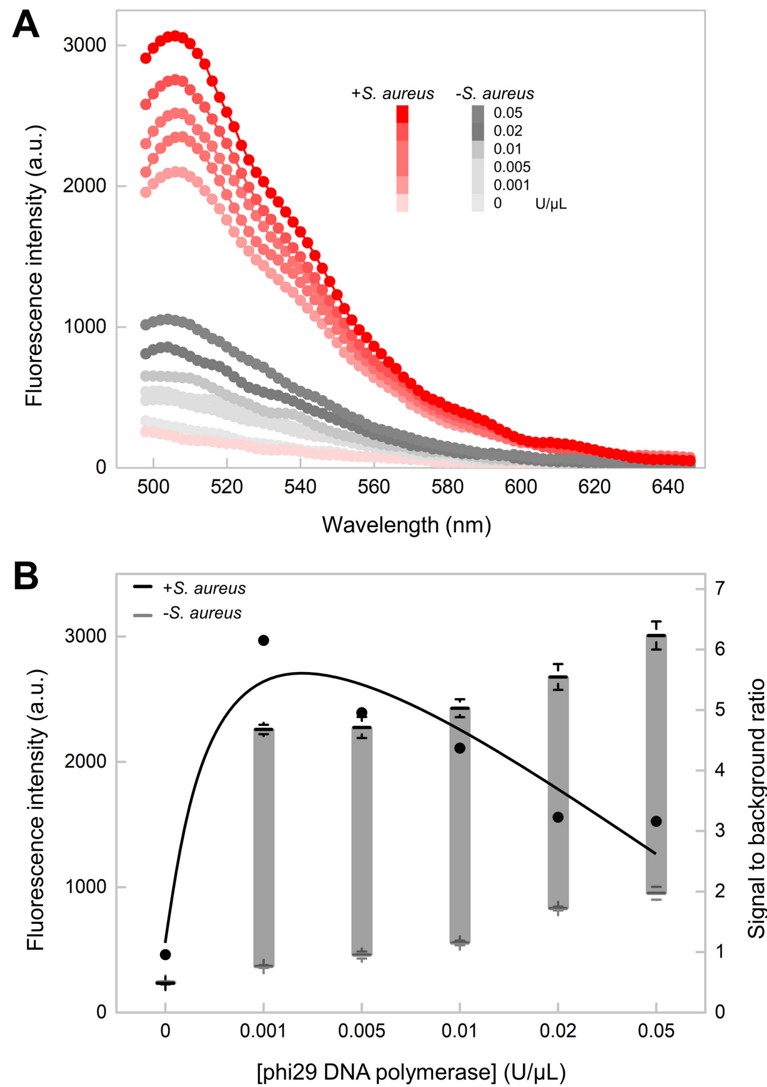

Fig. S3 Optimization of the concentration of phi29 DNA polymerase. (A) Fluorescence curves for transcription aptasensor with the addition of different concentrations of phi29 DNA polymerase in the presence or absence of *S. aureus*. (B) Fluorescence intensity of transcription aptasensor corresponding in (A). The excitation wavelength was 468 nm. Error bar=SD and all measured values are the average results of 3 paralleled determinations.

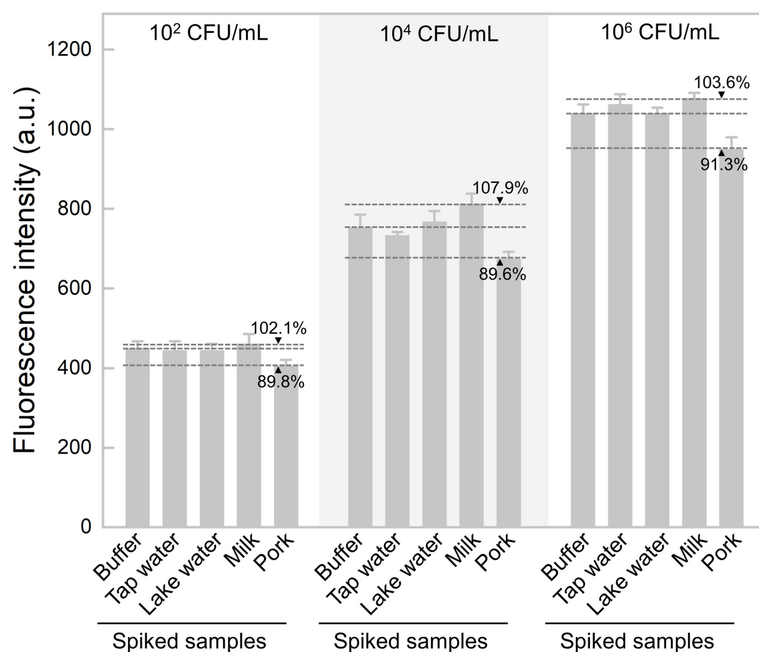

Fig. S4 Fluorescence intensity of transcription aptasensor for *S. aureus* detection in buffer, water and food samples. The concentrations of *S. aureus* were 10^2, 10^4, and 10^6 CFU/mL. The excitation wavelength was 468 nm. Error bar=SD and all measured values are the average results of 3 paralleled determinations.

Table S1 Comparation of transcription aptasensor with other assays for pathogens

Detection methods	Pathogens	Recognition	Strategy	Separation	Labeling	Detection range (CFU/mL)	LOD (CFU/mL)	Applications	Ref.
Fluorescence	*S. aureus*	Aptamer	Strands displacement assisted transcription	No	No	10^2-10^6	77	Water, milk, pork	*This work*
Fluorescence	*S. aureus*	Aptamer	Di-electrophoresis enrichment and nanoparticle labeling	No	Fluorescent silica nanoparticles	-	270	Water	*Analyst, 2015, 140, 4489*
Fluorescence	*S. aureus, V. parahemolyticus, S. typhimurium*	Aptamer	Upconversion nanoparticles labeling	Magnetic separation	Upconversion nanoparticles	50-10^6	25, 10, 15	Milk, shrimp	*Anal. Chem., 2014, 86, 3100*
Fluorescence	*S. aureus*	Aptamer	Measure nuclease from the bacteria via DNA capped mesoporous silica nanoparticles	Magnetic separation	No	800-10^4	682	Blood	*Biosens. Bioelectron., 2016,86, 27*
Fluorescence	*L. monocytogenes*	Aptamer	Sandwich-structured assay	Washing	FAM	10^2-10^7	75	Buffer	*Food Control, 2013, 33, 239*
Fluorescence	*S. typhimurium*	Aptamer	FAM-labeled aptamer/graphene	No	FAM	10^3-10^8	100	Milk	*Microchim. Acta, 2014, 181, 647*
Fluorescence	*L. monocytogenes, E. coli, S. enterica*	Antibody	Antibody-based fiber optic sensor	Washing	Alexa647	-	10^3	Beef, chicken, turkey meats	*Food Microbiol., 2013, 33, 166*
Fluorescence	*S. aureus*	Antibody	Sandwich assay	Washing	FITC	10^3-10^9	290	Apple juice	*Anal. Chem., 2015, 87, 9864*
Electrochemistry	*E. coli*	Aptamer	Electrokinetics-based capacitive sensing	Washing	No	10^2-10^6	2.8×10^2	-	*Biosens. Bioelectron., 2018, 112, 48*
Electrochemistry	*S. aureus*	Aptamer	Potentiometric biosensor using carbon nanotube	No	No	2.4×10^3-2.0×10^4	800	Pig skin	*Biosens. Bioelectron., 2012, 31, 226*
Immunochromatographic assay	*E. coli*	Antibody	Immunochromatograpic assay using personal glucose meter	No	Invertase	2.08×10^5-2.08×10^8	6.2×10^4	Milk	*Biosens. Bioelectron., 2018, 107, 266*
Immunochromatographic assay	*E. coli*	Antibody	Immunochromatograpic assay using graphene	No	Quantum dot nanocrystals	-	100	Milk	*Anal. Chem., 2015, 87, 8573*
Colorimetric	*S. aureus*	Peptide	Sandwich using magnetic nanobeads and gold surface	Magnetic separation	Magnetic nanobeads	75-7.5×10^6	40	Beef, turkey sausage, milk	*Biosens. Bioelectron., 2017, 90, 230*
Raman Spectroscopy	*S. aureus*	Aptamer	Magnetically assisted SERS bioassay	Magnetic separation	AuNR@Ag	10-10^5	10	Buffer	*ACS Appl. Mater. Inter., 2015, 7, 20919*
Raman Spectroscopy	*S. typhimurium, S. aureus*	Aptamer	SERS-based sandwich assay	Magnetic separation	Gold nanoparticles	10^2-10^7	35	Pork	*Biosens. Bioelectron., 2015, 74, 872*
Absorbance	*S. aureus*	Imprinting	Sandwich ELISA	Washing	SiO_2@CeO_2	-	500	Buffer	*Chem. Sci., 2015, 6, 2822*

Table S2. Recovery of *S. aureus* in the water from tap and lake, milk and pork samples.

Samples	PCA log (CFU/mL)	Fluorescence intensity (a.u.)	Transcription aptasensor log (CFU/mL)	Recovery (%)	RSD (%) n=3
Tap water	4.13	734	3.99	96.54	1.08
	5.95	1063	6.10	102.48	2.37
Lake water	4.13	768	4.21	101.85	3.52
	5.95	1040	5.95	100.04	1.85
Milk	4.13	813	4.50	108.89	3.34
	5.95	1078	6.20	104.15	1.79
Pork	4.13	675	3.61	87.51	2.33
	5.95	950	5.37	90.27	3.95