Electronic Supplementary Information for

pH-triggered visual detection of *Escherichia Coli* based on the co-assembly of bacitracin and thymolphthalein

Tianran Lin[a], Yunping Lai, Gaoyan Jiang, Xinlian Chen, Li Hou[b]*, Shulin Zhao[c]**

School of Chemistry and Pharmaceutical Sciences, State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, Guangxi Normal University, Guilin 541004, P. R. China.

[a.] *Tianran Lin*, E-mail: tianranlin@163.com

[b.] *Li Hou*, E-mail: houli@gxnu.edu.cn

[c.] *Shulin Zhao*, E-mail: zhaoshulin001@163.com

Table of Contents

1. **Experimental Section**
2. **Supporting Figures and Tables:**

Fig. S1. Optimization of the amount of AMP in the synthesis of AMP/TP NPs.

Fig. S2. Condition optimization of (A) volume of AMP/TP NPs, (B) volume of Apt-MBs, (C) incubation time, and (D) temperature.

Fig. S3 The absorbance changes of AMP/TP nanoprobes after incubation in PBS buffer (pH 7.4) or human urine sample for different times.

Fig. S4 Particle size distribution of (A) AMP/TP NPs, (B) E. coli, and (C) AMP/TP NPs@E. coli.

Fig. S5. A brief description of how to use the App.

Fig. S6. Effect of (A) AMP/TP NPs and (B) TP NPs solution on bacterial growth.

Table S1. Performance comparison of different detection methods for *Escherichia coli* based on different recognition modules.

Table S2. Determination of Escherichia coli in human urine samples by standard addition method.

Supporting References

Experimental Section

1. Materials and reagents

Dimethyl sulfoxide (DMSO), potassium dihydrogen phosphate (KH_2PO_4), and dipotassium phosphate (K_2HPO_4) were purchased from Shanghai Aladdin Biochemical Technology Co., LTD. The synthetic DNA strands (DNA sequence: 5'-Bio-CCGGACGCTTA TGCCTTGCCA TCTACAGAGCAGGTGTGACGG-3') were purchased from Shanghai Sangong Bioengineering Co., LTD. Bovine serum albumin (BSA) was purchased from Beijing Solebo Technology Co., LTD. NaOH was purchased from Sinopharm Chemical Reagent Co., LTD. Bacitracin (AMP) was purchased from Shanghai Maclean Biochemical Technology Co., LTD. Streptavidin-labeled Fe_3O_4 was purchased from Tianjin Beisler. *E. coli* (ATCC 25922) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538) were purchased from Guangzhou Huankai Microbial Technology Co., LTD. All chemicals are analytically pure and can be used without additional purification. The water used in the experiment was ultrapure.

2. Experimental apparatus

Hc-2064 high-speed centrifuge (Zhong Jia, China) was applied to the centrifugal separation of AMP/TP NPs. Jinyi constant temperature magnetic stirrer 85-2B was used for AMP/TP NPs synthesis. JEOL 200 kV field emission transmission electron microscope (JEM-2100F, Japan) was applied to morphology characterization. Microplate reader (Tecan Spark, USA) was used for the optical density (OD) detection. The Zetasizer Nano ZS90 laser particle size/potentiometer was used to characterize the particle size distribution before and after the reaction between AMP/TPs and *E. coli*.

3. The synthesis of AMP/TP NPs and TP NPs

AMP/TP NPs synthesis: dissolve 10 mg BSA in 10 mL water and stir at a low temperature (4°C); 4 mg TP was dissolved in 1.6 mL DMSO and dropped into the BSA solution. Then 2 mg of AMP was dissolved in 0.5 mL water and dropped into the above solution, stirred for 3 h, and the product was centrifuged and washed with ultrapure water, and dispersed into 2 mL ultrapure water for later use. TP NPs were prepared by the same operation steps as above without the addition of AMP solution.

4. Preparation of aptamer-modified magnetic beads

The aptamer tube was centrifuged at 4000 rpm for 60 s before opening the cover. Add 250 μL H_2O into the aptamer tube and configure the concentration of aptamer into a 10 μmol L^{-1} solution. Streptavidin-labeled Fe_3O_4 was dispersed evenly by ultrasound for 2 min before use. 100 μL magnetic bead stock (5 mg mL^{-1}) was separated and washed twice with PBS (pH 7.4, 10 mmol L^{-1}). Add 500 μL H_2O to prepare a concentration of 1 mg mL^{-1} magnetic bead stock. Subsequently, 75 μL biotin-modified aptamers (Bio-Apt, 10 μmol L^{-1}) were added to the magnetic bead stock and incubated at 37 °C for 60 min. Excessive Bio-Apt was removed by magnetic separation for 5 min. The aptamer-coupled magnetic beads (Apt-MB) were washed twice with PBS (pH 7.4) and dispersed in 300 μL PBS for later use.

5. Determination of E. coli

The bacterial stock solution was centrifuged with PBS buffer (pH 7.4) 3 times (5000 rpm, 5 min), and the bacterial precipitation was dispersed to PBS buffer. Then 200 μL of the bacterial solution ($OD_{600\ nm}$ = 0.32) was diluted 10^1, 10^2, 10^3, 10^4, and 10^5 times. Then, 100 μL of the above diluted bacterial solutions were used for the plate count

method. After amplification at 37 °C for 16 h, colony counting was performed. The bacterial concentration of the bacterial solution ($OD_{600\ nm}$ = 0.32) is calculated as 2.08 × 10^8 CFU mL^{-1}.

500 μL bacterial solutions with different bacterial concentrations of 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 CFU mL^{-1}) were mixed with 10 μL APT-MB (1.67 mg mL^{-1}) and 25 μL AMP/TP NPs (8 mg mL^{-1}) at 37 °C for 90 min. Excessive AMP/TP NPs were removed by magnetic separation and the AMP/TP NPs@*E. coli*@Apt-MB sandwich composite was washed twice by magnetic separation. 180 μL NaOH (1 mol L^{-1}) was added to the sandwich composite, and magnetic separation with 5 min was performed. Meanwhile, the color change of the AMP/TP NPs@*E. coli*@Apt-MB solution was observed, and the 150 μL supernatant was taken to a 96-well plate to measure the $OD_{590\ nm}$ value. Control group: the above bacterial solution was replaced with PBS, and other steps remained unchanged.

6. Determination of E. coli in the human urine samples

Human urine samples were prepared by the standard addition method. 80 mL of human urine from healthy people was dispersed into 45 mL PBS (pH 7.4). Bacterial standard solutions with different concentrations (2.08 × 10^1, 2.08 × 10^2, 2.08 × 10^3, 2.08 × 10^4, 2.08 × 10^5, 2.08 × 10^6, 2.08 × 10^7 CFU mL^{-1}) were prepared by dilution method. 500 μL of each of the above bacterial standard solutions was added into 4500 μL human urine to prepare a series of urine samples with different bacterial concentrations. 500 μL of the above urine samples ($C_{E.\ coli}$: 0, 10^1, 10^2, 10^3, 10^4, 10^5 CFU mL^{-1}) was incubated with 10 μL Apt-MB (1.67 mg mL^{-1}) and 25 μL AMP/TP NPs (8 mg mL^{-1}) at 37 °C for 90 min. Excessive AMP/TP NPs were removed by magnetic separation. 180

μL NaOH (1 mol L^{-1}) solution was added to the AMP/TP NPs@*E. coli*@Apt-MB sandwich composite and magnetic separation was performed. The color change of the AMP/TP NPs@*E. coli*@Apt-MB solution was observed, and 150 μL supernatant was taken to a 96-well plate to measure the $OD_{590\ nm}$ value.

All experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional committee has approved the experiments.

7. Bacterial growth inhibition ability of AMP/TP NPs and TP NPs

Preparation of bacterial suspension: The purchased *E. coli* strains were coated on an agar plate by plate marking method and cultured overnight at 37 °C. 1~3 single colonies were scraped and cultured in 10 mL fluid medium overnight on a shaker (160 rpm, 37 °C). Then the bacteria were collected by centrifugation at 5000 rpm for 5 min and washed with PBS (pH 7.4 and 10 mmol L^{-1}) three times to remove the fluid medium from the suspension. Finally, the bacterial suspension ($OD_{600\ nm}$ = 0.32) was diluted into 10^1 and 10^4 CFU mL^{-1} in PBS.

The effects of AMP/TP NPs and TP NPs on bacterial growth: 25 μL of AMP/TP NPs (8 mg mL^{-1}) and TP NPs (1.67 mg mL^{-1}) solutions were added to 100 μL bacterial suspensions (10^1 and 10^4 CFU mL^{-1}), respectively, and cultured in a shaking bed (1200 rpm) for 90 min at 37 °C. Add 100 μL of the mixture to 150 μL of bacterial culture. Then they were cultured in a shaker at 37 °C for 16 h, and their OD at 600 nm was measured by a microplate reader every 2 h. Note: A blank control group was set for AMP/TP NPs alone and TP NPs to subtract the effect of material absorption at 600 nm.

Supplementary Figures and Table

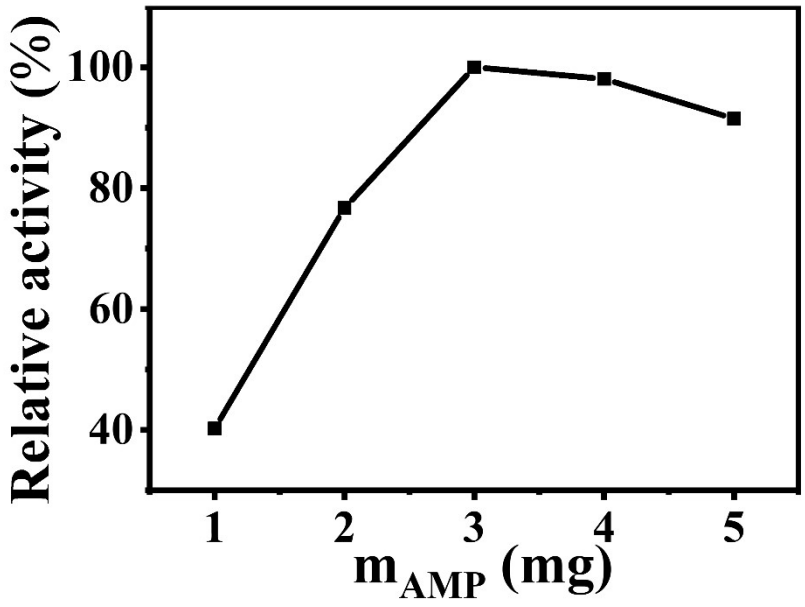

Fig. S1. Optimization of the amount of AMP in the synthesis of AMP/TP NPs.

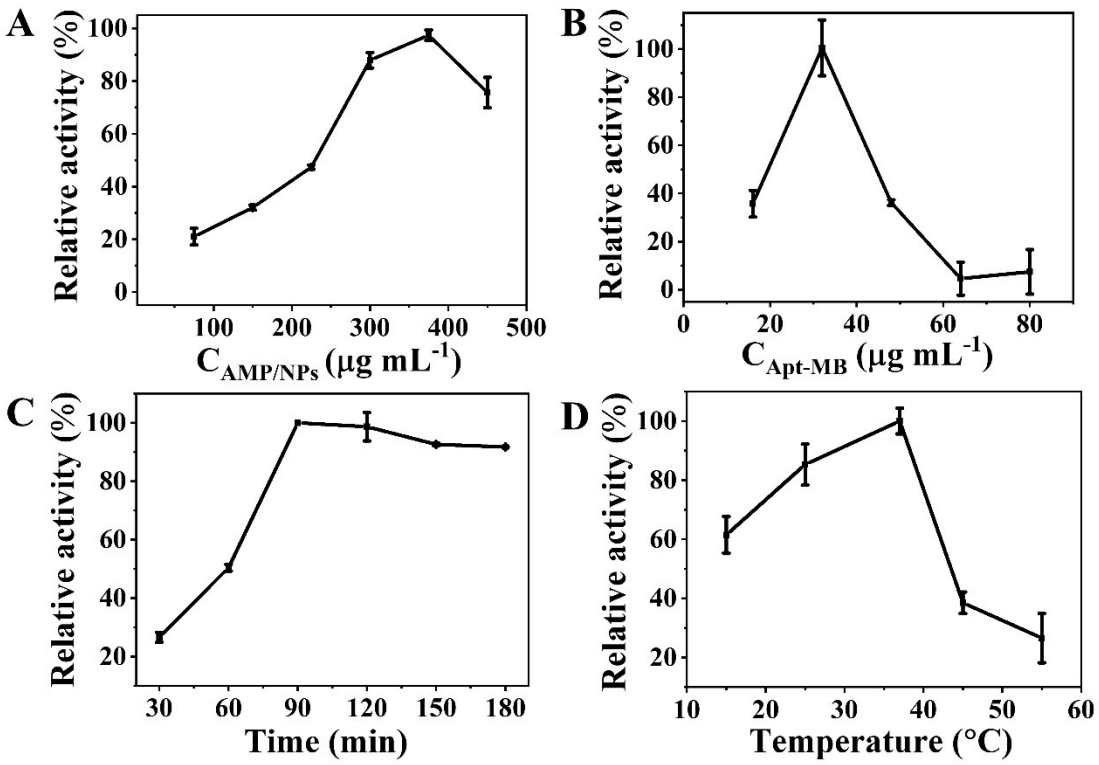

Fig. S2. Condition optimization of (A) volume of AMP/TP NPs, (B) volume of Apt-MBs, (C) incubation time, and (D) temperature

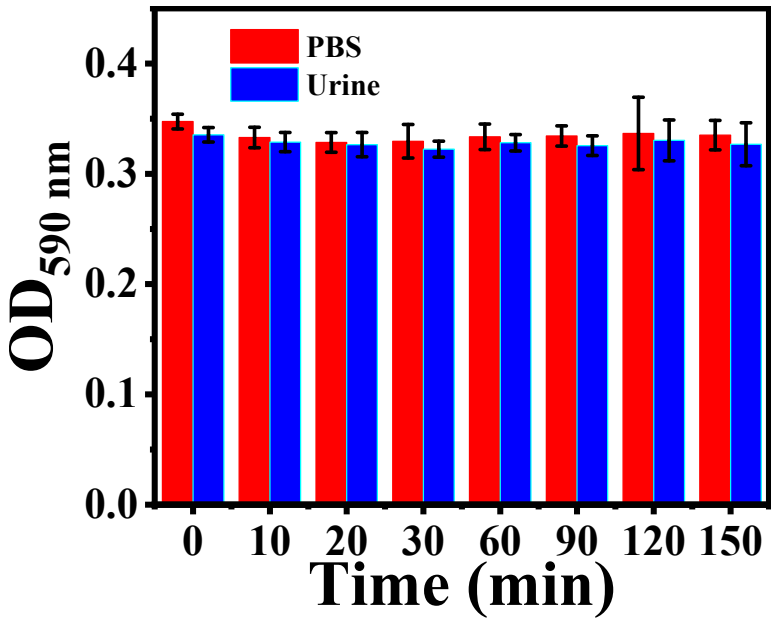

Fig. S3 The absorbance changes of AMP/TP nanoprobes after incubation in PBS buffer (pH 7.4) or human urine sample for different times. Detailed procedures: 50 μL AMP/TPs (1.32 mg mL^{-1}) was incubated with 500 μL PBS (pH 7.4) or healthy human urine at 37 ℃ for 0, 10, 20, 30, 60, 90, 120, 150 min, respectively, and then centrifuged at 5000 rpm min^{-1} for 5 min.Take 500 μL supernatant and add 50 μL NaOH (1 mol L^{-1}). Take 150 μL solution to 96-well plate to measure the OD_{590nm} value.

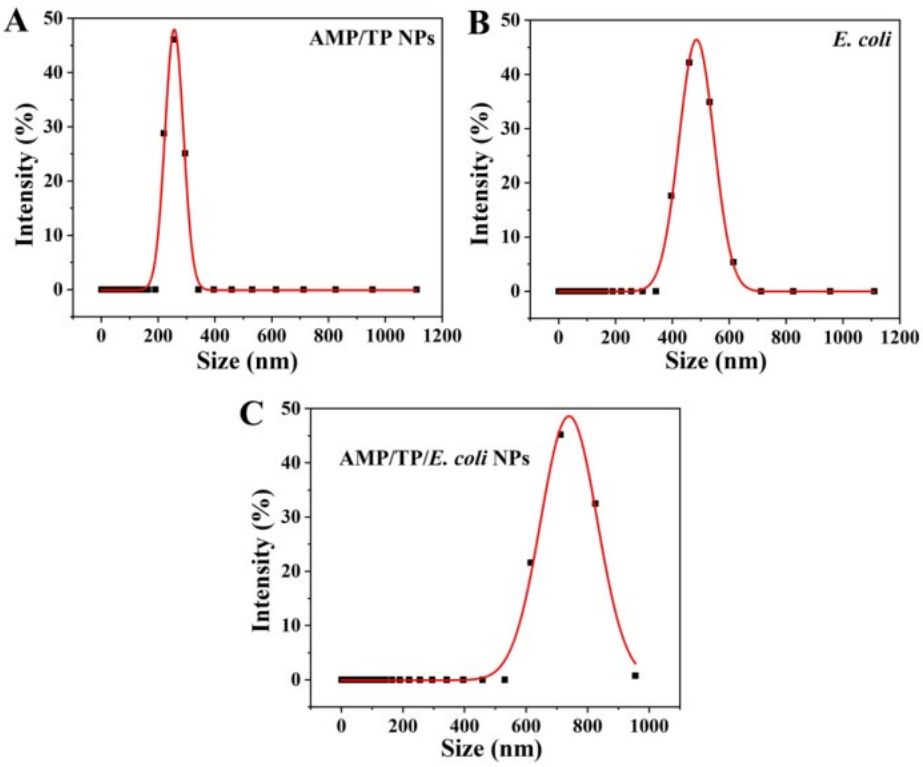

Fig. S4 Particle size distribution of (A) AMP/TP NPs, (B) *E. coli*, and (C) AMP/TP NPs@*E. coli*.

The materials used in this experiment are prepared by the following procedures.

AMP/TP NPs@*E.coli*: 50 μL 8 mg mL^{-1} AMP/TP NPs and 500 μL 2.08×10^6 bacterial solution were incubated at 37 ℃ for 90 min. Centrifuge at 5000 rpm min^{-1} for 5 min, wash with water 3 times, disperse into 1 mL water, and dilute 50 μL AMP/TP NPs@E.coli solution to 1 mL for particle size test.

E. coli: The bacterial solution was centrifuged and washed with PBS (pH 7.4, 10 mmol L^{-1}) for 3 times (5000 rpm min^{-1}, 5 min), and the final bacteria were dispersed to PBS (pH 7.4, 10 mmol L^{-1}) with an OD_{600nm} value as 0.32. The dispersed bacterial solution was diluted 1000 times for this experiment.

AMP/TP NPs: 0.08 mg mL^{-1} AMP/TP NPs was used for this experiment.

Fig. S5. A brief description of how to use the App.

(A) The user selects and opens the App.

(B) Input the information of the sample source.

(C) Import the photo or take a photo of the samples.

(D) Set the point number.

(E) Select the area for analysis by adjusting the green point position.

(F) Select the area for analysis by adjusting the red point position.

(G) Fine adjustment of the position of some point.

(H) Output R, G, B (red, green, blue) color channel values of the multiple points.

The requirements for the operation of the mobile app are as follows. 1) The number of mobile phone models is not limited. 2) Operating system: Android 4.4 or higher, or Harmony OS 2.0.0 or higher. 3) Java as a software development environment. 4) Application permissions required: store and read data.

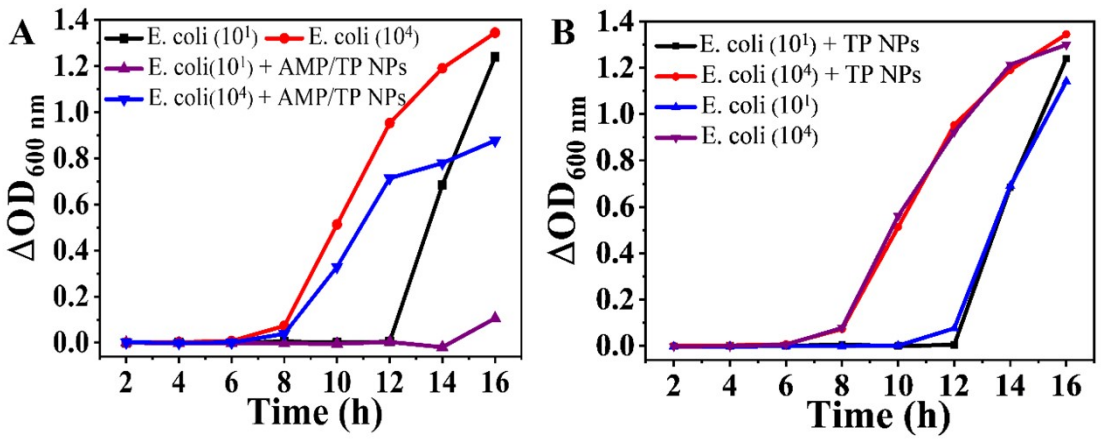

Fig. S6. Effect of (A) AMP/TP NPs and (B) TP NPs solution on bacterial growth.

Table S1. Performance comparison of different detection methods for *Escherichia coli* based on different recognition modules.

Detection method	Recognition modules	Linear range (CFU mL^{-1})	Detection limit (CFU mL^{-1})	Reference
Surface plasmon resonance	Magainin I	$1 \times 10^3 \sim 5 \times 10^7$	5×10^2	[1]
Colorimetric/Electrochemical	p-benzoquinone	$1 \times 10^3 \sim 1 \times 10^9$	1×10^4/1×10^3	[2]
Electrochemical	Ig G	/	3×10^1	[3]
Electrochemical	16S rDNA	$1 \times 10^3 \sim 1 \times 10^8$	100	[4]
Lateral flow immunoassay	Antibody	CF	78	[5]
Fluorescence	/	$1 \times 10^2 \sim 1 \times 10^6$	89	[6]
Colorimetric	Bacteriophage	□ 10^3	50	[7]
Colorimetric	Aptamer	$1.2 \times 10^2 \sim 3.6 \times 10^3$	40.46	[8]
Colorimetric	Bacitracin	$2.08 \times 10^1 \sim 2.08 \times 10^5$	1.7（Microplate Reader）/1.3（Smartphone）	This work

Table S2. Determination of *Escherichia coli* in human urine samples by standard addition method.

Original value (CFU mL^{-1})	Added (CFU mL^{-1})	Detected ± SD (CFU mL^{-1})	Recovery (%)
6.2	20.8	27.4±1.3	101.9
6.2	208.0	212.2±2.8	99.1
6.2	2080.0	2120.5±161.5	101.6
6.2	20800.0	20237.5±914.9	97.3

References

1. H. Bai, S. Bu, C. Wang, C. Ma, Z. Li, Z. Hao, J. Wan and Y. Han, *Mikrochim. Acta*, 2020, **187**, 220.
2. C. Zhou, H. Zou, M. Li, C. Sun, D. Ren and Y. Li, *Biosens. Bioelectron.*, 2018, **117**, 347-353.
3. J. Sun, A. R. Warden, J. Huang, W. Wang and X. Ding, *Anal. Chem.*, 2019, **91**, 7524-7530.
4. A. Gumustas, M. G. Caglayan, M. Eryilmaz, Z. Suludere, E. Acar Soykut, B. Uslu, I. H. Boyaci and U. Tamer, *Anal. Methods*, 2018, **10**, 1213-1218.
5. M. Cimafonte, A. Fulgione, R. Gaglione, M. Papaianni, R. Capparelli, A. Arciello, S. Bolletti Censi, G. Borriello, R. Velotta and B. Della Ventura, *Sensors*, 2020, **20**, 274.
6. R. Derda, M. R. Lockett, S. K. Tang, R. C. Fuller, E. J. Maxwell, B. Breiten, C. A. Cuddemi, A. Ozdogan and G. M. Whitesides, *Anal. Chem.*, 2013, **85**, 7213-7220.
7. J. Zhang, J. Wang, X. Zhang and F. He, *Biosens. Bioelectron.*, 2018, **118**, 9-15.
8. Y. Xie, Y. Huang, J. Li and J. Wu, *Sens. Actuators B-Chem.*, 2021, **339**, 129865.