

Supplementary Information

A hairpin probe-mediated DNA circuit for the detection of *mecA* gene of *Staphylococcus aureus* based on exonuclease III and DNAzyme-mediated signal amplification

Jiafeng Pan,^{a,b} Dongqin Bao,^c Enhu Bao^c and Junhua Chen^{*b}

^a College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha 410128, China.

^b National-Regional Joint Engineering Research Center for Soil Pollution Control and Remediation in South China, Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-environmental and Soil Sciences, Guangdong Academy of Sciences, Guangzhou 510650, China.

^c Shuyang Hospital Affiliated to Xuzhou Medical University, Suqian 223800, China.

*Corresponding author:

E-mail: 222chenjunhua@163.com; jhchen@soil.gd.cn

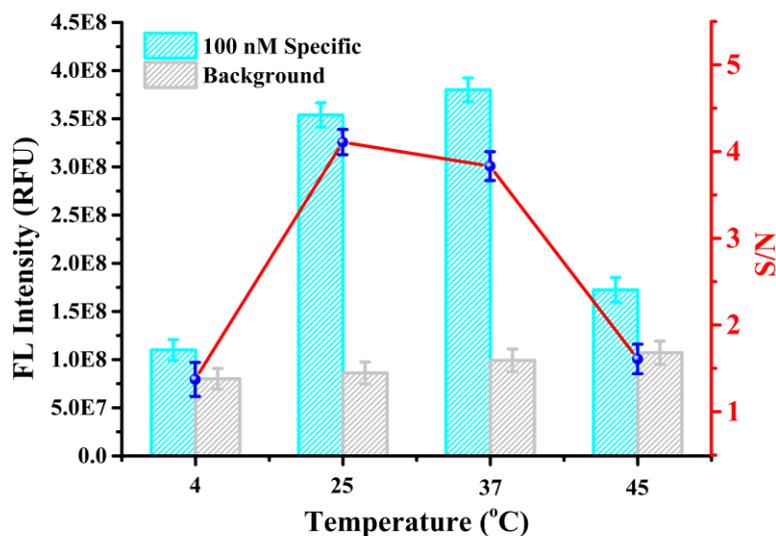


Fig. S1. Effect of the reaction temperature on the response of the sensing system. The histograms represent fluorescence intensity of the solution in the presence of 10 nM *mecA* gene (cyan) and in the absence of target (gray), respectively. The red line represents the S/N ratio. The corresponding error bars represent the standard deviation of three independent measurements obtained at each reaction temperature.

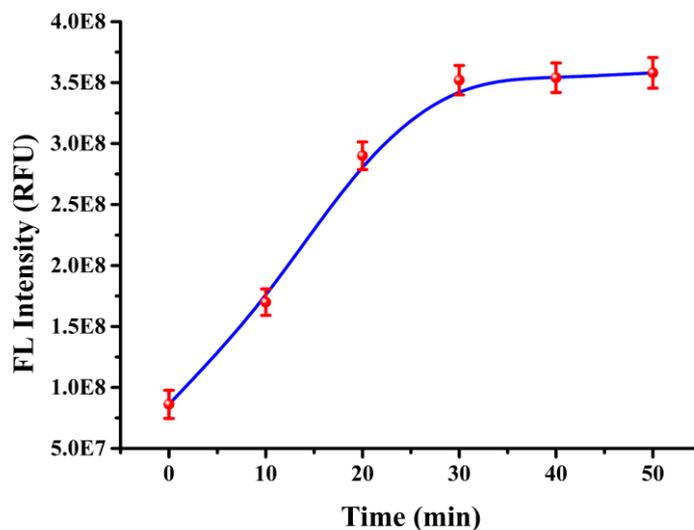


Fig. S2. Effect of the reaction time of Exo III-assisted signal amplification on the fluorescence intensity of the proposed method for the detection of *mecA* gene (10 nM). Reactions were performed at room temperature.

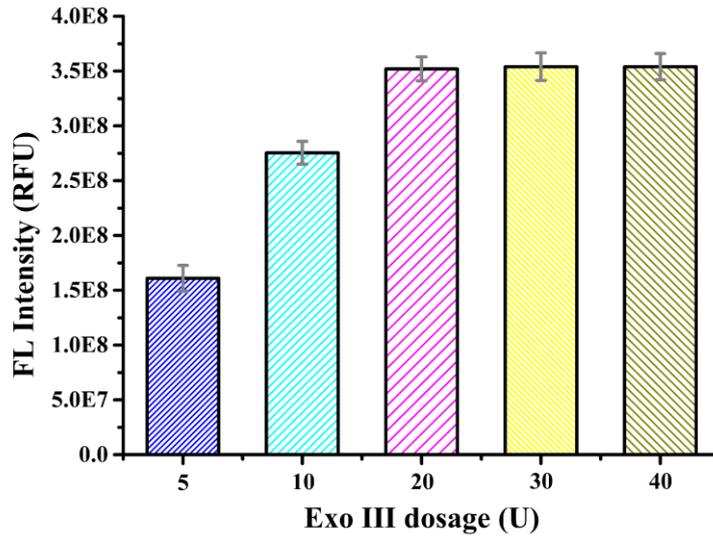


Fig. S3. The effect of the Exo III dosage on the aptasensor performance. The experiments were carried out at room temperature.

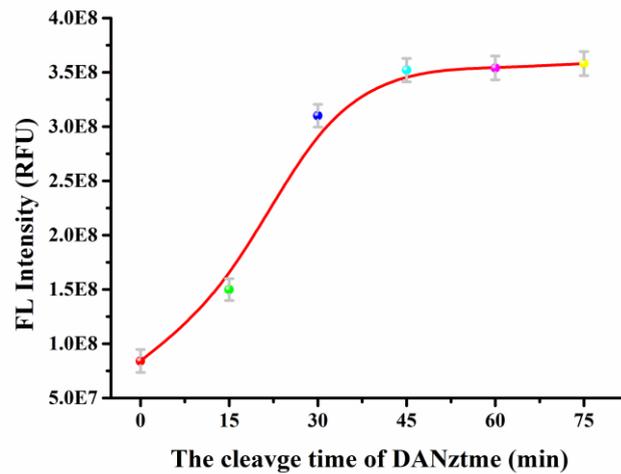


Fig. S4. Effect of cleavage time of DNAzyme on the performance of the sensing platform for the detection of *mecA* gene (10 nM). The experiments were performed at room temperature. Error bars represent the standard deviation of three independent measurements.

Table S1. Comparison of analytical methods capable of sensing *mecA* gene.

Method	Linear range	Detection limit	References
Fluorescent	10 nM-100 nM	1 nM	1
Electrochemical	75 fM-200 pM	63 fM	2
Electrochemical	50-250 pM	23 pM	3
Electrochemical	10 fM-100 nM	10 fM	4
Fluorescent	12.5 pM-3.125 nM	6.25 pM	5
Colorimetric	1 pM-100 pM	1 pM	6
Fluorescent	10 fM-100 nM	2.4 fM	7
Electrochemical	5 fM-500 pM	3.7 fM	8
Electrophoretic	50 pM-100 nM	12.3 pM	9
Visual	-	0.2 zM	10
Fluorescent	1 fM-1 nM	0.5 fM	This work

Table S2. Analysis of serum samples containing the *mecA* gene at different concentrations.

Sample	Spiked	Found ^a	qPCR ^b	Recovery	RSD
1	1 nM	0.94 nM	0.99 nM	94.0%	5.9%
2	100 pM	103.0 pM	100.5 pM	103.0%	4.3%
3	1 pM	0.97 pM	1.09 pM	97.0%	4.8%
4	100 fM	105.4 fM	99.5 fM	105.4%	5.4%
5	10 fM	9.83 fM	10.1 fM	98.3%	5.7%

^aEach sample was analyzed using our proposed biosensor, and the data reported in the table represents the average of five measurements. ^bThe concentration of *mecA* in clinical samples was certified using qPCR.

Reference

- [1] J. Shi, C. Chan, Y. Pang, W. Ye, F. Tian, J. Lyu, Y. Zhang and M. Yang, *Biosens. Bioelectron.*, 2015, **67**, 595-600.
- [2] T. Wang, Z. Zhang, Y. Li and G. Xie, *Sens. Actuators B*, 2015, **221**, 148-154.
- [3] M. Liu, H. Xiang, E. Hua, L. Wang, X. Jing, X. Cao, S. Sheng and G. Xie, *Anal. Lett.*, 2014, **47**, 579-591.
- [4] L. Xu, W. Liang, Y. Wen, L. Wang, X. Yang, S. Ren, N. Jia, X. Zuo and G. Liu, *Biosens. Bioelectron.*, 2018, **99**, 424-430.
- [5] S. Pang, Y. Gao, Y. Li, S. Liu and X. Su, *Analyst*, 2013, **138**, 2749-2754.
- [6] J. Klonoski, R. Mondesire, L. Rea, D.C. Ward and R.D. Jenison, *Anal. Biochem.*, 2010, **396**, 284-289.
- [7] Q. Li, D. Zhou, J. Pan, Z. Liu and J. Chen, *Analyst*, 2018, **143**, 5670-5675.
- [8] G. Dai, Z. Li, F. Luo, Y. Lu, Z. Chu, J. Zhang, F. Zhang, Q. Wang and P. He, *Microchimica Acta*, 2021, **188**, 39.
- [9] Y. Lu, F. Luo, Z. Li, G. Dai, Z. Chu, J. Zhang, F. Zhang, Q. Wang and P. He, *Talanta*, 2021, **222**, 121686.
- [10] Y. K. Kang, S. H. Im, J. S. Ryu, J. Lee and H. J. Chung, *Biosens. Bioelectron.*, 2021, **168**, 112566.