Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Rapid and sensitive detection of *E. coli* O157:H7 based on antimicrobial peptide functionalized magnetic nanoparticles and urease-catalyzed signal amplification

Zhaohui Qiao[a], Chunyang Lei[a,b]*, Yingchun Fu[a], Yanbin Li[a,c]*

[a] *College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China*

[b] *State Key Laboratory of Chem/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China*

[c] *Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, AR 72701, USA*

* Email: chunyangly@126.com; yanbinli@zju.edu.cn.

1. Fluorescent Microscopy

The prepared solution of *E.coli* O157:H7 at a concentration of 10^7 cfu mL^{-1} was pelleted by centrifugation and resuspended in 70% ethanol to fix overnight at 4 °C, protected from light. Then the fixed cells were then incubated with 0.01mg mL^{-1} solution of propidium iodide (PI) for 15 min. After incubation, the cells were pelleted by centrifugation and washed three times by PBS buffer. The samples of stained bacterial cells were then incubated with MNP-AMP for 10 min in the dark and observed by an upright motorized microscope (Ni-E, Nikon). The stained *E. coli* O157:H7 of the same concentration was used as a control sample.

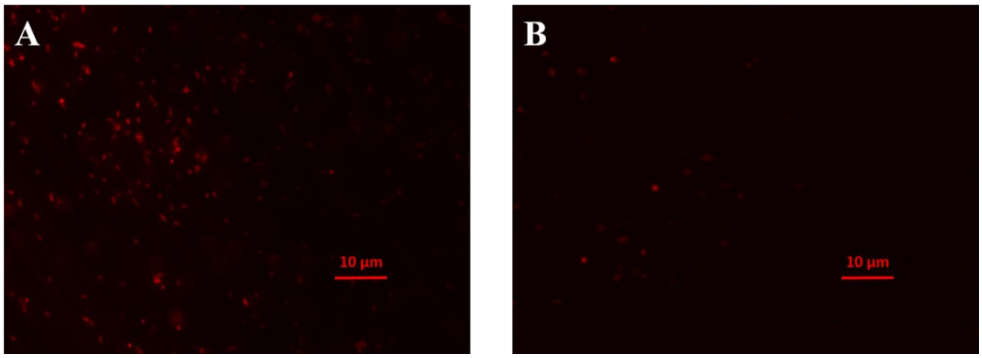

Fig. S1 (A) Representative fluorescence image of *E. coli* O157:H7 (control). (B) The fluorescence image of the supernatant of *E. coli* O157:H7 incubated with MNP-AMP after magnetic separation. The concentration of *E. coli* O157:H7 was 10^7 cfu mL^{-1}. All the fluorescence images were obtained under magnification of 10×100.

2. Verification of the feasibility of the proposed principle

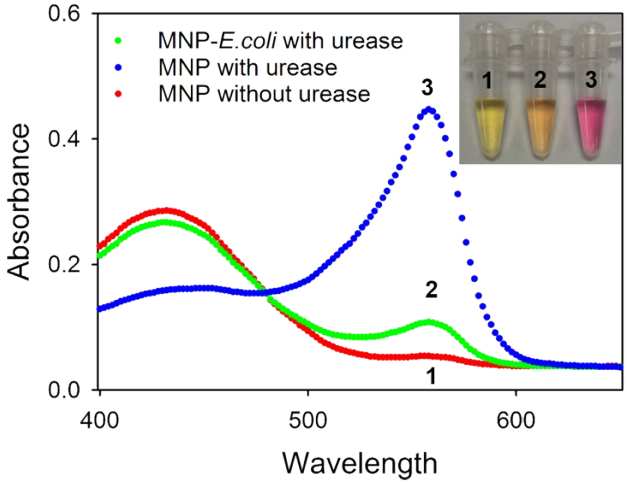

Fig. S2 Verification of the feasibility of the proposed principle by measuring the concentration

of urease attached on MNP-AMP. The concentrations of *E. coli* O157:H7, urease and MNP-AMP were 10^7 cfu mL^{-1}, 1μg mL^{-1} and 0.035 mg mL^{-1}, respectively.

3. Capture efficiency of MNP-AMP with and without antibody against *E. coli* O157:H7 and non-target bacteria.

Different species of bacteria, including *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, nonpathogenic *E. coli* DH5a and BL21 were prepared in PBS at the concentration of 10^3 cfu mL^{-1}. 10 μL of the prepared MNP-AMP or MNP-AMP with antibody was mixed with 200 μL of culture and rotated at 15 rpm for 10 min at RT. The MNP-AMP-bacteria complexes were washed two times and re-suspended in 200 μL of PBS and the MNP-AMP/Ab-bacteria complexes were washed by PBST (PBS + 0.05% tween). The uncaptured cells in supernatant were also collected. A 100 μL of the captured samples and uncaptured samples was plated on corresponding selective agars and incubated at 37°C for enough time for bacterial enumeration. The same level for all the original cultures were used as positive controls. All enumeration experiments were performed in triplicate. The capture efficiency (CE, %) was calculated with the following equation [1]: CE (%) = $(1 - N_U/N_O)$ × 100%, where N_O is the number of original cells, N_U is the number of uncaptured cells (in supernatant and washed solution). and N_C is the number of captured cells. The calculation was considered valid only when N_C was in the same magnitude as N_O.

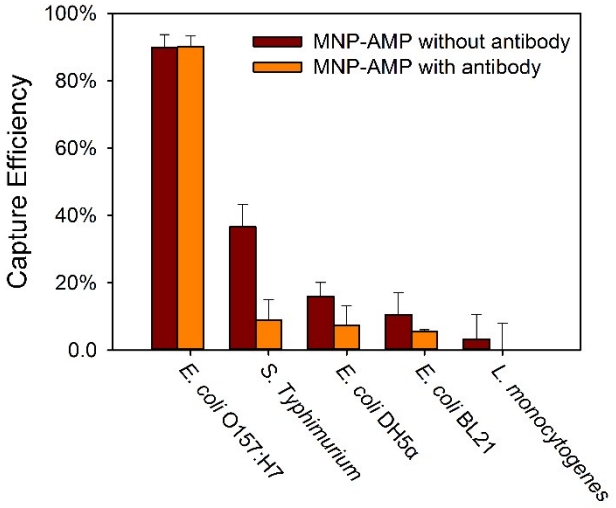

Fig. S3 Capture efficiency of MNP-AMP with or without antibody toward different species.

Table S1 Capture efficiency of MNP functionalized by different ligands.

	Capture efficiency (%)		
	Concentration of *E. coli* O157:H7 (cfu mL^{-1})		
	10^3	10^4	10^5
MNP-Ab	99.1 ± 0.4	99.8 ± 0.1	99.4 ± 0.5
MNP-AMP without antibody	91.9 ± 1.5	83.4 ± 1.5	80.1 ± 0.7
MNP-AMP with antibody	93.2 ± 0.6	93.4 ± 0.5	90.1 ± 1.2

Table. S2 Comparison of AMP-based methods for detection of bacteria with different techniques.

Technique	Target	AMPs	Analysis time	Detection limit	Specificity	Reference
Electrochemical method	*E.coli* O157:H7	Magainin I	1.5 H	10^3 cfu/mL	*E. coli* O157 > *E. coli* K12 > S.epidermidis and B.subtilis	2
	E.coli O157:H7	Magainin I	15 min	10^3 cfu/mL	*E.coli* O157:H7 > *S.typhimurium* > nonpathogenic *E.coli* > *Listeria*	3
	E.coli strains	Colicin V	15 min	10^3 cfu/mL	Gram-negative bacteria	4
Fluo-rescent method	*E.coli* O157:H7	poly-myxins	90 min	5×10^4 cfu/mL	*E.coli* and *Salmonella*	5
	E.coli O157:H7	Cecropin P1	30 min	10^3 cfu/mL	[a]NA	6
QCM	*E.coli* O157:H7	Magainin I	10 min	400 cfu/mL	to be sensitive in presence of small concentrations of non-target bacteria	7
Colori-metric method	*E.coli* O157:H7	Magainin I	45 min	118 cfu/mL	*E.coli* O157:H7	8
	E.coli O157:H7	Magainin I	30 min	12 cfu/mL	*E.coli* O157:H7	This study

[a]NA = not available

References

1 Q. Xiong, X. Cui, J. K. Saini, D. Liu, S. Shan, Y. Jin and W. Lai, *Food Control*, 2014, **37**, 41-45.

2 Y. Li, R. Afrasiabi, F. Fathi, N. Wang, C. Xiang, R. Love, Z. She and H.-B. Kraatz, *Biosens. Bioelectron.*, 2014, **58**, 193-199.

3 M. S. Mannoor, S. Zhang, A. J. Link and M. C. McAlpine, *Proc. Natl Acad. Sci.,* 2010, **107**, 19207-19212.

4 K. Jiang, H. Etayash, S. Azmi, S. Naicker, M. Hassanpourfard, P. M. Shaibani, G. Thakur, K. Kaur and T. Thundat, *Analytical Methods*, 2015, **7**, 9744-9748.

5 N. V. Kulagina, K. M. Shaffer, G. P. Anderson, F. S. Ligler and C. R. Taitt, *Anal. Chim. Acta*, 2006, **575**, 9-15.

6 S. Arcidiacono, P. Pivarnik, C. M. Mello and A. Senecal, *Biosens. Bioelectron.*, 2008, **23**, 1721-1727.

7 Z.-M. Dong and G.-C. Zhao, *Talanta*, 2015, **137**, 55-61.

8 Z. Qiao, C. Lei, Y. Fu and Y. Li, *RSC Adv*., 2017, **7**, 15769-15775.