Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Material for

Bridge-DNA synthesis triggered by allosteric aptamer for colorimetric detection of pathogenic bacteria

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This Supporting Information inlcudes Supplementary table S1, table S2, table S3 Figure S1, Figure S2, Figure S3, Protocol S1, and Protocol S2.

Table S1. Single-stranded DNA sequences used in this study

Name	Nucleic acid sequence, listed from 5' to 3'
AAP1	Cy3-AACCATACCTGACGACGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTT-Cy5
AAP2	Cy3-AACCATACCTGACGACCCGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTT-Cy5
AAP3	Cy3-ATACCTGACGACCAGTCGCGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTT-Cy5
AAP4	Cy3-CCTGACGACCAGTCGTGAATCGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTT-Cy5
AAP5	Cy3-CGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTTATACCTCGTTATCCG-Cy5
AAP6	Cy3-CGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTTCGTGAATACCTCGTTAT-Cy5
AAP5	biotin-CGGATAACGAGGTATTCACGACTGGTCGTCAGGTATGGTTATACCTCGTTATCCG

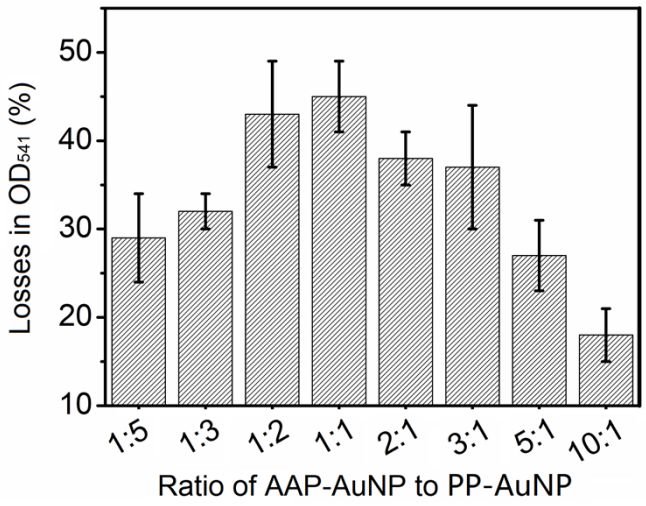
Note: Green and yellow letters represent complementary part and recognition part of the AAP, respectively. Shaded letters represent complementary site of the recognition part against to complementary part.

Supplementary table S2. Paired Primer sequences used in this study

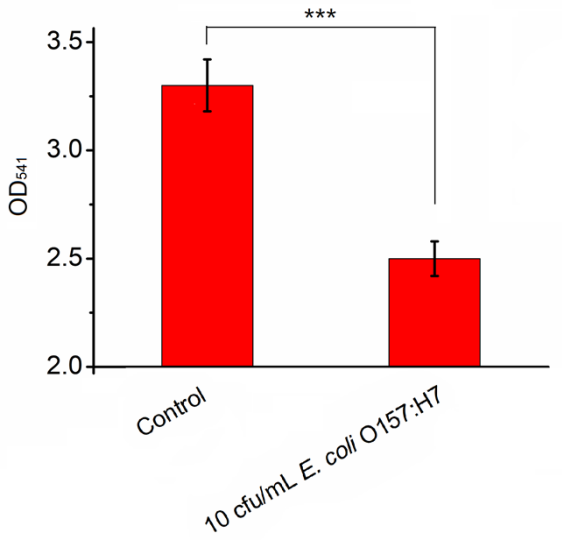
Name	Nucleic acid sequence, listed from 5' to 3'
PP6	Biotin-CGGATA
PP8	Biotin-CGGATAAC
PP10	Biotin-CGGATAACGA
PP12	Biotin-CGGATAACGAGG
PP14	Biotin-CGGATAACGAGGTA

Supplementary table S3. Comparison of different culture-independent methods for detection of pathogenic bacteria.

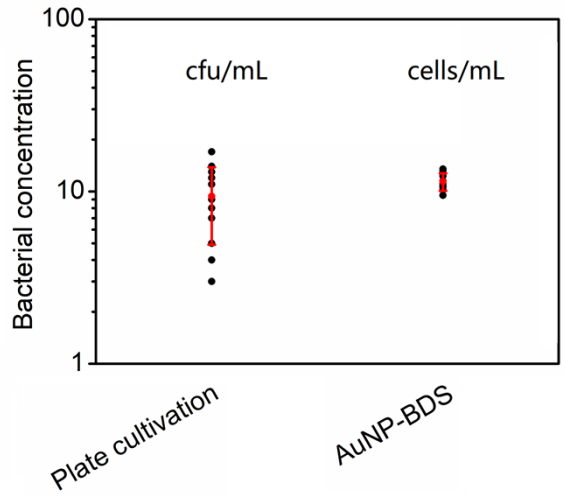
Methods **Parameters**	Testing time	Detection limits	Necessary instrumentation and cost	References
AuNP-BDS	30-60 min	10^1-10^2 cells/mL	UV Spectrophotometer (very cheap)	-
ELISA	90-150 min	10^4-10^5 cells/mL	UV Spectrophotometer (very cheap)	1, 2
Electrochemical sensor	120-180 min	10^1-10^2 cells/mL	Electrochemical workstation (cheap)	3, 4
Flow Cytometry	10-120 min	10^3-10^5 cells/mL	Flow Cytometer (very expensive)	5-7
qPCR	60-120 min	1-10 cells/mL	qPCR instrument (expensive)	8
SERS	20-60 min	10^1-10^2 cells/mL	Raman spectroscopy (expensive)	9, 10



Supplementary Fig. S1. 1. The effect of AAP-AuNP/PP-AuNP ratio on the signal-to-background ratio (S/N) of the AuNP-BDS for detecting 100 cfu/mL E. coli O157:H7. The S/N was defined as the loss of OD_{541} value of AuNP-BDS in respond to E. coli O157:H7.



Supplementary Fig. S2. Responses of OD_{541} value to 10 cfu/mL *E. coli* O157:H7 after bridge DNA synthesis for 35 min. Ten independent experiments were carried out for each group. The three asterisks represented P value was less than 0.01.



Supplementary Fig. S3. Repeatability of the AuNP-BDS assay against to plate cultivation for detecting E. coli O157:H7 at a concentration of about 10 cfu/mL. Eleven repeats were carried out for each methods.

Protocol S1. Sample pretreatment for milk

Milk was diluted 100 times with pure water, and vortexed for 1 min. Then 10 mL dilution was pipetted into a tube, and centrifuged at 12000 rpm for 5 min to separate cream from bacteria and protein particles. The cream on the top and in the supernatant were removed, and the pellet along with 1 mL supernatant were left in the tube. After cleaning the cream adsorbed on the tube wall by a Q-tip cotton swab, 8.95 mL water and 50 μL proteinase K (Solarbio Life Sciences, Beijing, China, 20 mg/mL) were added and the tube was vortexed to mix well. The tube was incubated at 37 °C for 40 min to digest protein particles, and then re-centrifuged to remove cream again as mentioned above. The pellet was mixed with residual 1 mL supernatant in the tube to obtain 1 mL purified bacterial suspension.

Protocol S2. Sample pretreatment for orange juice

The juice sample was diluted 10 times with ultrapure water, and filtered through a PP membrane (5 μm pore size). Then 10 mL filtered solution was pipetted into a tube, and centrifuged at 5000 g for 5 min. Nine hundred microliter supernatant was removed carefully, and the pellet along with 1 mL residual supernatant were left undisturbed in the tube. After that, 9 mL cellulase solution (50 mg/mL) was added to the tube to lyse plant cells and debris for 60 min at 37 °C. The digested sample was centrifuged at 5000 g for 5 min. Nine milliliter supernatant was removed carefully, and the pellet along with 1 mL residual supernatant were left undisturbed in the tube. After that, 9 mL ultrapure water was added to the tube and mixed well. The sample was again centrifuged at 5000 g for 5 min. Nine milliliter supernatant was removed carefully, and the pellet was resuspended with the residual supernatant (1 mL).

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