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Supporting Information

A simple mix-and-read bacteria detection system based on DNAzyme and molecular beacon

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Reagents and Apparatus

NaCl, Tris base and boric acid were obtained from Sigma-Aldrich (USA). 0.5 M EDTA (pH 8.0) and UltraPure distilled water were obtained from Life Technologies. Calcium chloride dihydrate was purchased from Amresco (USA). 1 M $MgCl_2$ and 1 M Tris–HCl (pH 8.0) were obtained from Thermo Fisher Scientific. SeaKem LE Agarose was obtained from Lonza (ME, USA). 5 X Ecoli buffer was prepared with final concentration of 250 mM Tris-HCl, 750 mM NaCl and 75 mM $CaCl_2$. DNA oligos were all ordered from Integrated Device Technology, Inc. (IDT) and listed in table S1.

Difco Agar and Difco LB (Luria-Bertani) broth, Miller were purchased from Becton, Dickinson and Company. They were sterilized by high-pressure steam sterilizer (TOMY Ditigal Biology, Japan). Thermo-shaker was used for bacteria heat incubation. High speed microcentrifuge was purchased from VWR. Orbital incubator shaker for bacteria culturing was purchased from Amerex Instruments Inc.

Crude intracellular mixture (CIM) extraction

E. coli K12 MG1655 (K12) were routinely cultured. A single *E. coli* K12 cell colony was taken from a LB agar (1.5%) plate and inoculated into 2 mL LB growth medium overnight with 250 rpm shaking speed. One mL culture solution was harvested and centrifuged at 11,000 g for 5 min at room temperature. The supernatant was discarded. The white cell pellet was suspended in 200 µL 200 mM Tris-HCl (pH 8.0) buffer and heated at 50 ^{o}C for 15 min. After centrifuging at 11,000 g for 5 min at room temperature, the clear supernatant was used as CIM of *E. coli* cells for the experiment. The CIM could be stored at -20 ^{o}C for a long time. *E. coli* BL12 strand (BL21), *Klebsiella Pneumoniae* (KP), *Bacillus Subtilis* (BS), *Staphylococcus Lugdunensis* (SL), *Staphylococcus Saprophyticus* (SS), and *Staphylococcus Hominis* (SH) were cultured and lysed as the same as *E. coli* MG1655.

E. coli K12 MG1655 cell counting was as followings: after shaking overnight, the *E. coli* culture was 10-fold serially diluted for seven times. 100 µL eighth dilution was inoculated onto an agar culture plate for 15 h at 37 °C. Count the number of colonies. Original *E. coli* cell concentration was calculated based on the average colonies number of three of these agar plates and the dilution times. The calculated concentration of the original bacteria culture solution was 1.57E9 CFUs/mL (CFUS: colony-forming unites, 1 CFU = a single live cell). For the single live cell detection, 100 µL bacteria solution was added into 900 µL LB broth. Repeat this 10-fold serial dilution for nine times. Split the ninth solution into ten 100 µL aliquots and put these aliquots into 2 mL freshen LB broth medium respectively. Label them from No.1 to No.10. Shake overnight and extract the CIM as described above.

Bacterial detection

5 µL 10 µM s-DNAzyme-Ecoli solution, 10 µL 5 X Ecoli buffer, 25 µL 1 mM $MgCl_2$ and 3 µL H_2O were premixed. Then add 5 µL CIM and incubate at 30 ^{o}C for 1 h. Cold the solution to room temperature and add 2 µL of 5 µM MB-rA solution. Six of 8 µL reaction solution were parallelly

added into Corning 384 low volume round bottom plate (USA) and the fluorescence signal was read by Synergy HT (BioTek, USA). Read parameters were as followings: fluorescence endpoint read, excitation 485/20, emission 528/20, top optics, 35 gain, normal read speed and 1 mm read height.

High-Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry (HPLC-ESI-MS)

HPLC-ESI-MS spectrum was performed with negative ion model on Dionex Ultimate 3000 UHPLC and Thermo Q-Exactive mass spectromer (Thermo Fisher, MA, USA). Elution was initiated with mobile phase A (20 mM ammonium acetate solution) and mobile phase B (acetonitrile). The MS detection range was set from 1000 to 6000 m/z. 25 µL CIM was added into 25 µL premix E coli DNAzyme solution and incubated for one hour. The final solution concentration was 1 µM E. coli DNAzyme, 0.5 mM $MgCl_2$ and 1 X E. coli buffer. After desalination by AidQuick Oligo Purification Kit (Aidlab, Beijing, China), the reaction mix was detected by HPLC-ESI-MS.

Table S1. DNA oligos used in the assay

Oligo ID	Oligo Sequence (5'-3')
s-DNAzyme-Ecoli	GAT GTG TGC GTT GTC GAG ACC TGC GAC CGG AAC ACT ACA CTG TGT GGG ATG GAT TTC TTT ACA GTT GTG TGC AGC TCC GTC CGA CTC TTC CTA GCT rATG GTT CGA TCA AGA[a]
MB-rA	/5'6-FAM/[b]CC GCG TCG AAC CAT AGC TAG CGC GG/3'Dab/[c]
clp-s	TG GTT CGA TCA AGA

(a). rA in red means adenine ribonucleotide. This rA base is also the cleavage site for this DNAzyme system.

(b)&(c). 5'6-FAM and 3'Dab mean FAM fluorophore modification in 5' end and Dabcyl quencher modification in 3' end.

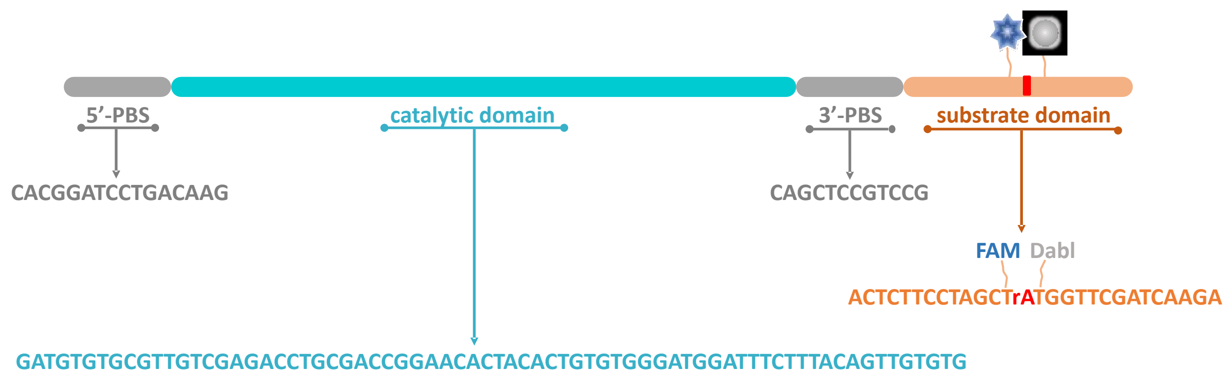
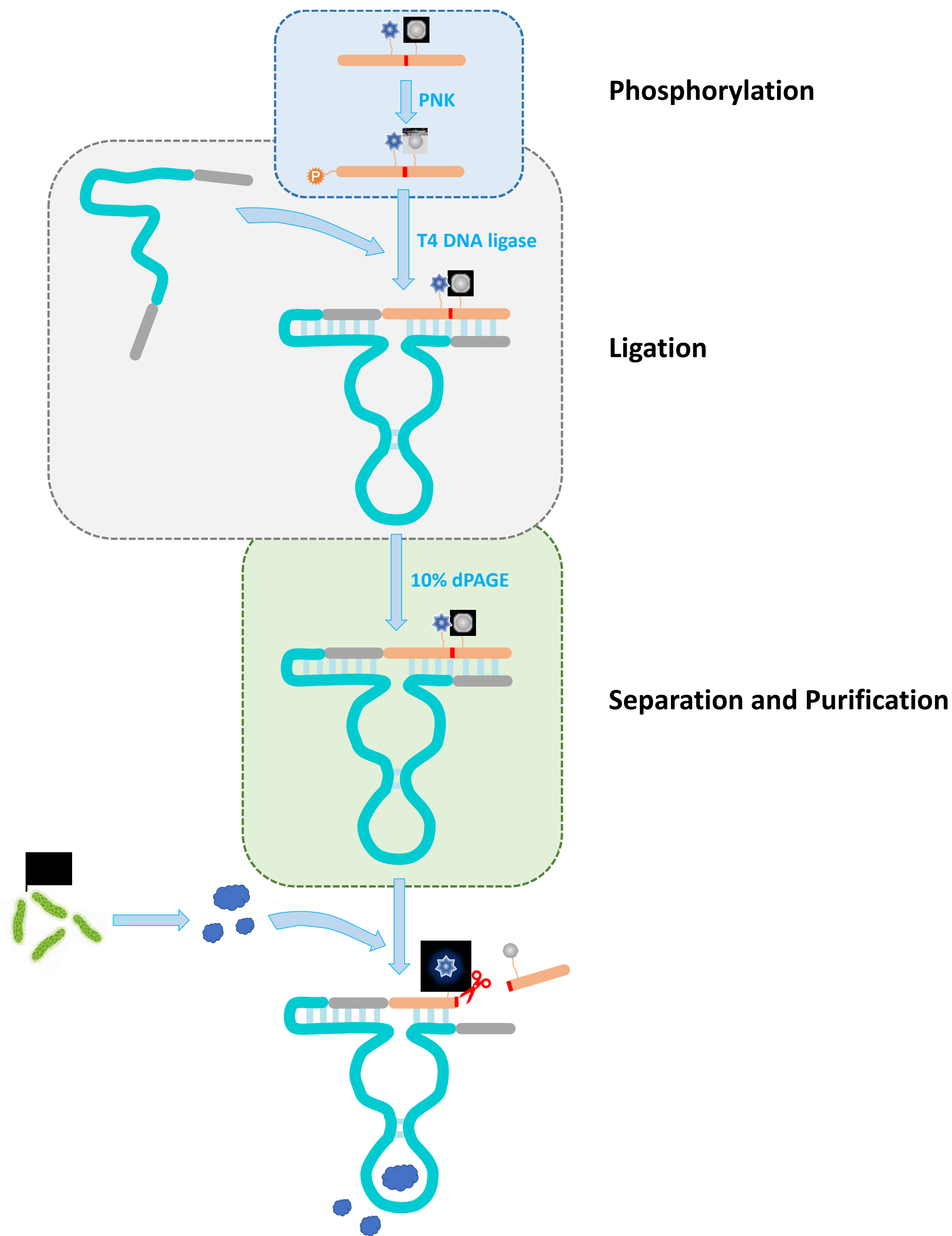


Figure S1. Conventional complete DNAzyme strand sequence for *E. coli* detection originally obtained via *in vitro* selection by Li Group.[1] 5'/3'-PBS means 5'/3' Primer Binding Sequence for PCR amplification in selection process. Catalytic domain and substrate domain mean catalytic and substrate active sequence in DNAzyme system.



Scheme S1. The conventional bacterium detection flowchart based on DNAzyme system originally proposed by Li Group.[1] PNK means T4 polynucleotide kinase. 10% dPAGE means 10% Urea-denaturing polyacrylamide gel electrophoresis. For the whole pre-treatment process, RNase inhibitor need to be added in case of the degradation of the DNAzyme substrate strand.

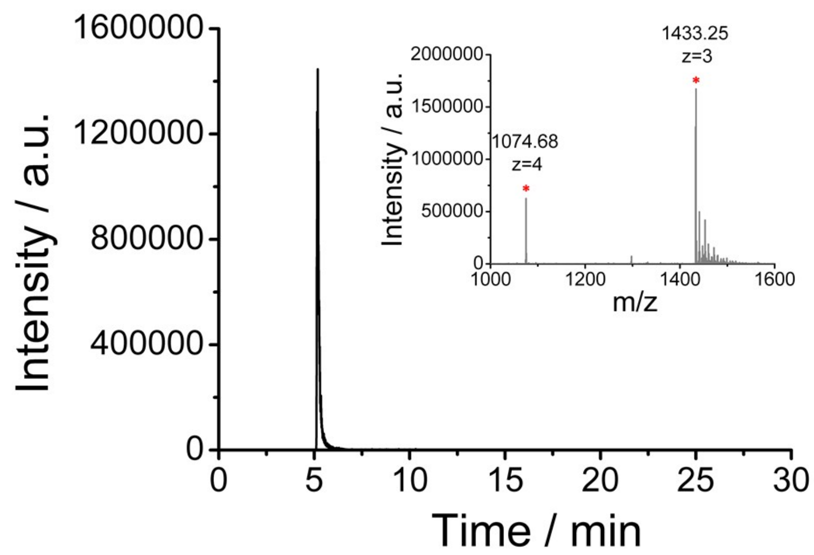


Figure S2. Chromatogram of 1 μM clp-s in H_2O (insert picture is corresponding mass spectrum data).

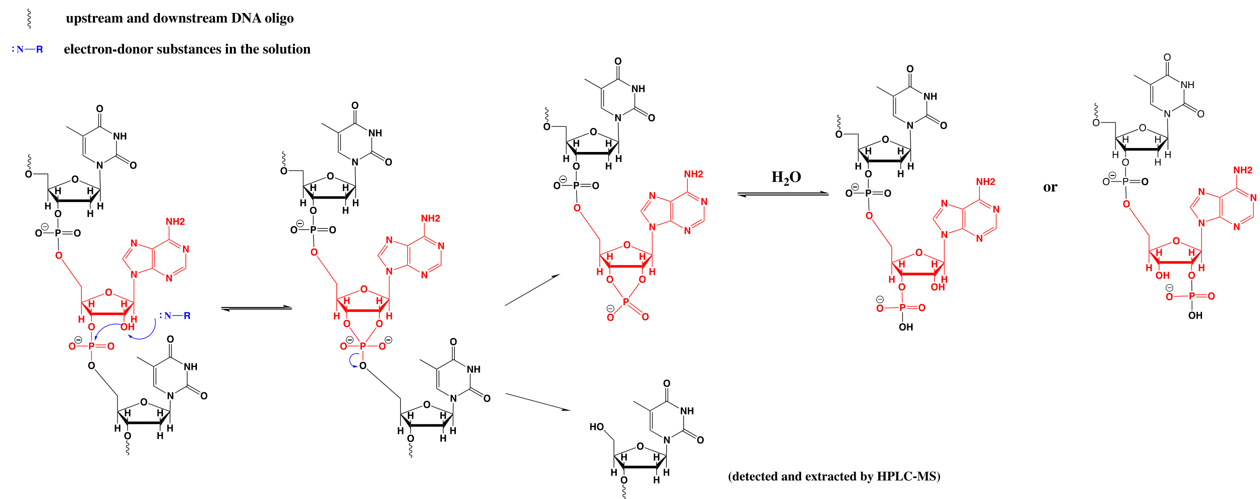


Figure S3. A possible cleavage mechanism for adenine ribonucleotide in s-DNAzyme-Ecoli.[2-3]

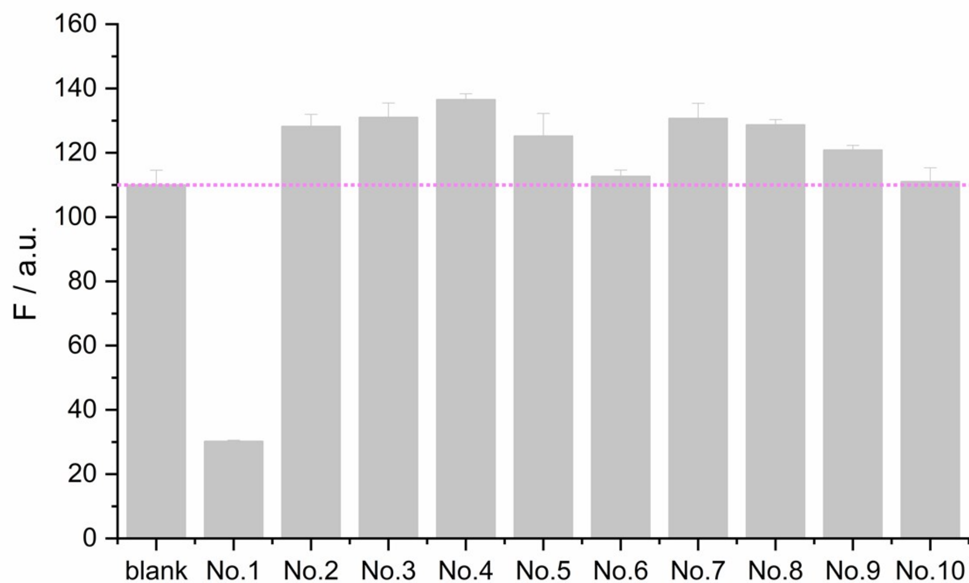


Figure S4. Single live bacterium detection integrated with cell-culturing step

References

[1]. M. M. Ali, S. D. Aguirre, H. Lazim, Y. Li, *Angew Chem Int Ed Engl*, **2011**, *50*, 3751-3754.

[2]. D. Voet, J. Voet, Biochemistry (4 ed.), *New York: J. Wiley & Sons*, **2011**, 85.

[3]. D. Elliot, M. Ladomery, Molecular Biology of RNA (1 ed.). *New York: Oxford University Press*, **2011**, 34–64.