

1 **Supplementary information**

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3 **An all-in-one enzyme-free fluorescent aptasensor**
4 **integrating localized catalyzed hairpin assembly**
5 **for sensing of antibiotic in food with improved detection efficiency**

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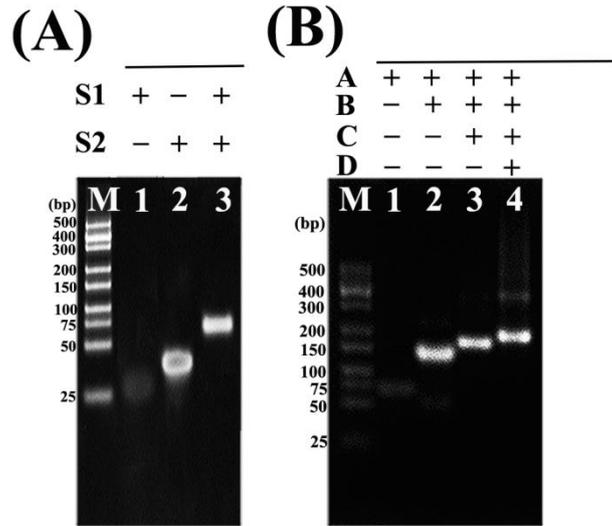
21 **Table S1** Sequences of oligonucleotides used in this study.

22 The red sequence in S2 is the primer sequence, and the green sequence in S2 is the

23 aptamer sequence.

Name	Sequences (5'-3')
S1	CCCACAGTTAGATTCTTTTTTTTTTTTTTTTTTTTTTTTTTTT
S2	AATCAACTGCGAGAATCTAACTGTGGGGGTTGAGGCTAAGCCGA
H1	AGATTGAGGGTTTGGGTGATTTTCAGTTAGATTCTCGCAGTTGATTCCATGT GTAGAAATCAACTGCGAGAA
H2	TAGTTAGTATGCTTGGCTGATTTAGTTGATT(BHQ2)-CTACACATGGAATCAA CTGCGAGAACCATGTGTAGA-(Cy5)
A	ATCACCCAAACCCTCAATCTTTTACATTCTAAGTCTGAAACATTACAGCTT GCTACACGAGAAGAGCCGCATAGTA
B	TCAGCCAAGCATACTAACTATTTTATCACCAGGCAGTTGACAGTGTAGCAA GCTGTAATAGATGCGAGGGTCCAATAC
C	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCT CTTCAAAAAAAAAAAAAAAAAAAAAA
D	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCTGTTTGTATTGGACCCTCG CAT

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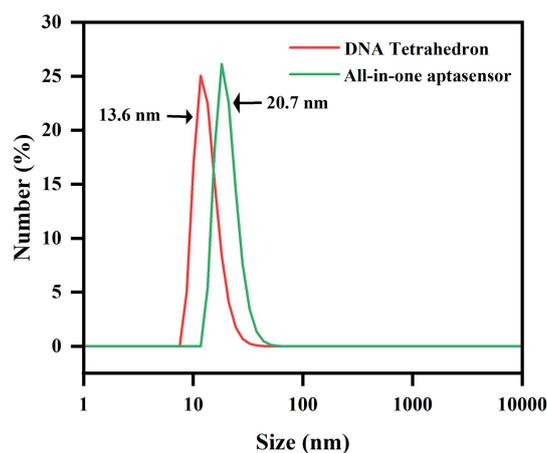


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26 **Fig. S1.** (A) Polyacrylamide gel electrophoresis (15%) characterization the fabrication
 27 of dsDNA S1-S2. (B) Agarose gel electrophoresis (3%) characterization the assembly
 28 of DNA tetrahedron.

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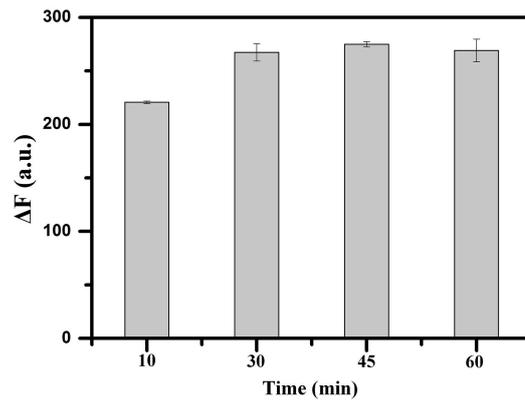
32 **Fig. S2.** DLS analysis of DNA tetrahedron and L-CHA-based all-in-one enzyme-free
 33 aptasensor.

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35 DLS characterization was performed to provide the sizes of DNA tetrahedron
 36 before and after modification. DNA tetrahedron (50 μ L, 100 nM) and L-CHA-based
 37 all-in-one enzyme-free aptasensor (50 μ L, 100 nM) were respectively added into
 38 50- μ L disposable cuvettes for DLS measurements. As shown in Fig. S2, the size of
 39 DNA tetrahedron was 13.6 nm whereas the size of the all-in-one aptasensor was 20.7
 40 nm. The increase in size was attributed to the connection of S1-S2, H1, and H2 to the
 41 DNA tetrahedron.

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45 **Fig. S3.** Optimization the reaction time of dsDNA S1-S2, hairpins H1, and H2

46 binding to DNA tetrahedron. Condition: $C_{S1-S2} = C_{H1} = C_{H2} = C_{DNA\ tetrahedron} = 100\ nM$,

47 $C_{kanamycin} = 100\ \mu g/mL$. Error bars were the standard derivation (n=3).