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3-D DNA nanodevices for on-site sensitive detection of antibiotic residues in food

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Experimental section

Chemicals and materials

Table S1. Sequence of oligonucleotides

Name	Sequence(5′-3′)
Aptamer hairpin (AP)	5'-TCA TGT CGT CGG CTT AGC CTT CCA GAC CGT GGG GGT TGA GGC TAA GCC GA-3'
Hairpin probe 1 (HP1)	5'-CTG GAA GGC TAA GCC GAC GAC ATG ATT AGC CTT CCA GCC TTG C-3'-Biotin
Hairpin probe 2 (HP2)	5'-CTG GAA GGC TAA TCA TGT CGT CGG CTT AGC CTA CAT GAT TAG CTT TTT TTT TT -3'-SH

In the Aptamer hairpin (AP), the sequences that bind to antibiotics are shown in green.In the Hairpin probe 1 (HP1), sequences that bind to AP are shown in red. In the Hairpin probe 2 (HP2), the underlined sequences

represent the sequences that are bound to HP1. SH refers to the modification of the invertase at 3' of HP2.

All oligonucleotide sequences (**Table S1**) were synthesized and purified by HPLC at Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Different types antibiotics (kanamycin sulfate, tetracycline, chloramphenicol, ampicillin, and streptomycin) were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Streptavidin magnetic beads (MBs, Mean diameter 1.5 μm) were obtained from Bangs Laboratories Inc (Fishers, IN). Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), and Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). All other reagents were of analytical reagent grade. Deionized water from a Millipore filtration system was used all throughout the experiment.

Synthesis of HP2-invertase

The prepared progress of HP2-invertase referred to our previously reported work. Firstly, the reaction mixture (50 μL), which consisted of 0.04 mM thiol-DNA hairpin probe 2 (HP2), 0.12 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 0.6 M sodium phosphate buffer (pH=5.5), was performed under gentle stirring at room temperature for 1 hour. After that, the mixture was purified by Amicon-10K using PBS buffer. Then, 1 mg of sulfo-SMCC was added to 200 μL 5 mg/mL invertase for invertase conjugation, and reacted under gentle stirring for 1 hour at room temperature. Subsequently, the resulting mixture was centrifuged and purified by Amicon-100K. Finally, the HP2-invertase conjugate were conducted with the purified sulfo-SMCC-activated invertase and the above processed thiol-DNA (HP2) at room temperature for 48 h. To remove unreacted thiol-DNA, the solution was purified by Amicon-100K for 5 times using PBS buffer.

Preparation of MBs-HP1

MBs functionalization with HP1 (MBs-HP1) was prepared with partial modification according to a method reported in the literature. Firstly, 2 μL of streptavidin-functionalized magnetic beads (MBs) (10 mg/mL) was rinsed with PBS for three times. Then, the rinsed MBs were resuspended in 100 μL PBS. Subsequently, 20 μL biotinylated HP1 (1 μM) was added into the MBs solution, and the mixture was incubated for 40 min at room temperature to prepare MBs-HP1. Finally, the product MBs-HP1 was rinsed with PBS for three times in a magnetic environment, and separated as each portion of 20 μL (1 mg/mL) in PBS solution for use.

Procedures of 3-D DNA nanomachine signal probe for digital quantification of Kanamycin

For the determination of kanamycin, the aptamer hairpin substrate was firstly denatured at 95 ^{o}C for 3 min,and cooled slowly to room temperature over a 10-min period. After that, 10 μL of various concentrations of kanamycin antibiotic was added to 15 μL of 20 nM aptamer hairpin to form different concentrations of kanamycin antibiotic-aptamer hairpin (A-A) binding complex. Referring to the reported literature,[1,2] the 25 μL A-A binding complex was mixed with 20 μL (1 mg/mL) of MBs-HP1, 25 μL of 2.5 mg/mL invertase-HP2. The reaction mixture was incubated at 37 ^{o}C for 2 h, assembling and forming 3-D DNA nanomachine signal probe of MB-HP1-HP2-invertase. Subsequently, the unreacted HP2-invertase was removed by magnetic separation, 50 μL 0.5 M sucrose were added to the MB-HP1-HP2-invertase signal probe and incubated at room temperature for 30 min. Finally, sucrose is hydrolyzed to glucose, a portion of 5 μL of the final solution was measured using a digital quantitative device PGM.

Quantitative detection of Kanamycin in authentic smaples

To verify the applicability in real sample, the milk sample purchased from a local supermarket was assayed through the developed digital quantitative

aptasensor. Before assay, the qualified milk samples 20 % milk was firstly obtained by mixing 2 mL of the milk and 8 mL of 10 mM PBS (pH 7.4). Then, different concentrations of kanamycin were spiked into the diluted milk samples. Finally, the spiked samples were detected directly without any pretreatments in terms of the progresses mentioned above. Meanwhile the spiked samples were further quantified by a classic ELISA method and compared with results obtained with our digital quantitative aptasensor.

Gel electrophoresis analysis

To verify the successful preparation of HP2-invertase, 5% polyacrylamide stacking gel and 8 % polyacrylamide resolving gel (SDS-PAGE) was carried out to evaluate the products of H2-invertase in tris-glycine electrophoresis buffer. Firstly, 5% polyacrylamide stacking gel was performed for 30 min at a current of 80 V. Then, 8 % polyacrylamide resolving gel was performed for 1.5 h at a current of 120 V. Finally, the SDS-PAGE gels was stained, decolorized, and photographed by Gel DocXR+ imaging system.

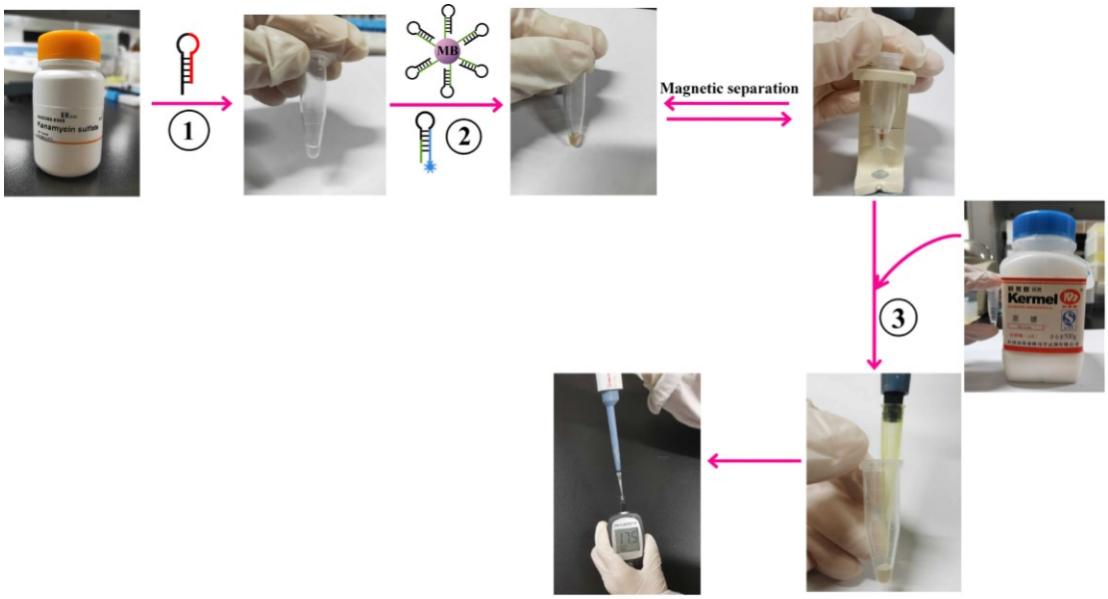


Fig. S1 The procedures of visualized digital quantitative aptasensor：(1) target binding and conversion, (2) the assembly of 3-D DNA nanomachine signal probe, and (3) PGM digital quantitative detection.

SDS-PAGE experiment was carried out for the verification of the invertase-HP2 conjugate. As displayed in Fig. S2 (ESI†), lane 1 shows the band of invertase, which has a molecular weight of around 135 kDa. Lane 2 represents the HP2, for which there is no band. Lane 3 is the band of the HP2-invertase conjugate, which exhibits a molecular weight of around 200 kDa. Obviously, the migration of the HP2-invertase conjugate band is slower than that of invertase, since the conjugation increases the molecular weight of invertase, indicating the successful preparation of the invertase-HP2 conjugate.

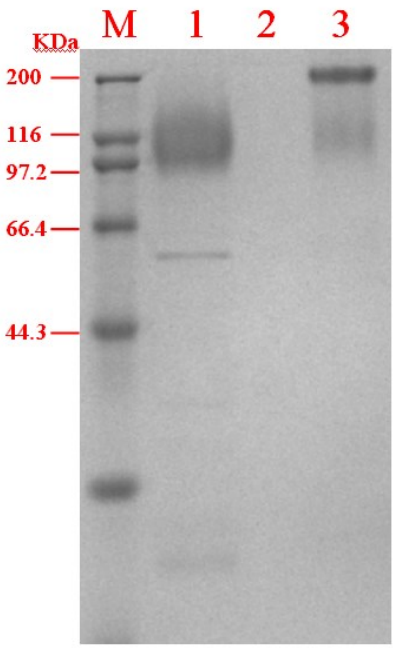


Fig. S2 Protein-staining image of the conjugation of invertase to HP2 : Lane M: protein marker, Lane 1: invertase, Lane 2: HP2, Lane 3: HP2-invertase conjugate.

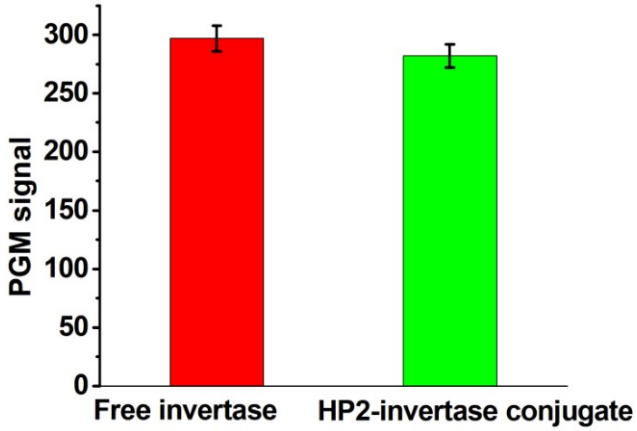
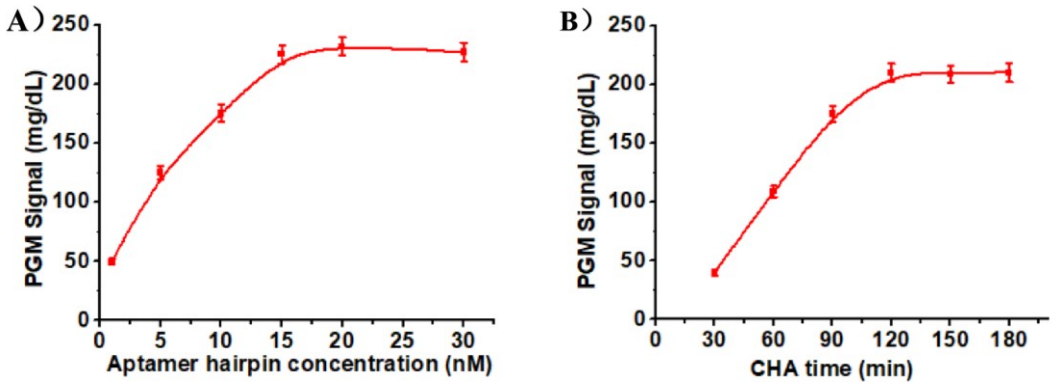


Fig. S3 The activity of invertase in free solution and the conjugated invertase. The concentration of invertase in free solution and the conjugated invertase are equal at 100 nM.

RESULTS AND DISCUSSION

Optimization of Assay Conditions for the developed aptasensor



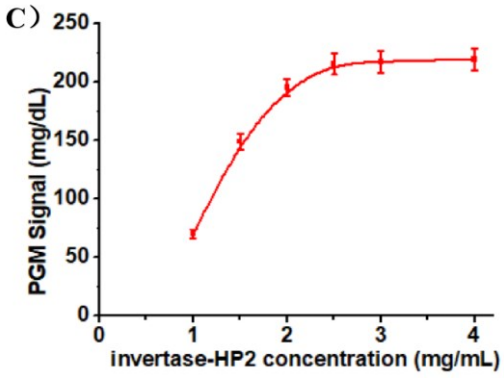


Fig. S4 Optimization of experimental conditions for the developed aptasensor. **(A)** Effect of the concentration of aptamer hairpin probe. **(B)** Effect of CHA reaction time. (C) Effect of HP2-invertase concentration.

To achieve the best sensing performance, Some crucial experimental parameters, such as the concentration of aptamer hairpin for target recognition and transduction, the reaction time and invertase-HP2 concentration in CHA reaction for efficient assembly of 3-D DNA nanomachine signal probe, were optimized. The PGM signal intensity was employed to evaluate the influence of different parameters in the aptasensor fabrication. **Fig. S4A** clearly shows that the sensitivity for detecting target antibiotic increased with increasing aptamer hairpin concentration, and after 15 nM the PGM signal reached the maximum and trends to a constant value. Taking into account the consumption of aptamer hairpin, 20 nM was selected as the optimum concentration for target recognition and transduction in the experiment. Furthermore, the effect of reaction time in CHA reaction was investigated in **Fig. S4B**. It is clear that the PGM signal intensity response exhibits a rapid increase with a further increase in the CHA reaction time and trends to a constant value at 120 min, indicating saturation of the CHA product due to the exhaustion of HP1 on MB. Thus, the incubation time was fixed at 2 h. Meanwhile, we also investigated the effect of the amount of invertase-HP2 on the sensitivity. The experimental results showed that the optima concentrations of invertase-HP2 was 2.5 mg/mL for efficient 3-D DNA nanomachine signal probe assembly(**Fig. S4C**).

Analytical performance of 3-D DNA nanodevices

The reproducibility and accuracy of the designed digital quantitative aptasensor were examined. The intra-assay imprecision of five paralleled tests in one assay and inter-assay imprecision in five different assays were

examined for 500 pM of antibiotic. The digital aptasensor displayed steady signals with relative standard deviations (RSD) of 3.6% in intra-assay, and 4.8% in inter-assay, indicating satisfactory precision and reproducibility.

Table S2 Comparison of different Sensing Platforms for the Detection of Kanamycin

readout	method	limit of detection	detection time	reference
fluorescence	gold nanocluster-functionalized MnO_2 nanosheets	1.2 pM	280 min	3
photoelectrochemical	rGO-Bi2WO6-Au based on branched hybridization chain reaction	0.78 pM	180 min	4
fluorescence	Multibranched DNA Nanostructures	0.176 pM	80 min	5
colorimetric	CHA-Assisted Target Recycling Amplification	0.34 pM	30 min	6
electrochemical	GR–PANI and PAMAM–Au nanocomposites	7.8 pM	50min	7
fluorescence	Enzyme Powered Three-Dimensional DNA Machine	1.23 pM	40 min	8
Digital PGM	Binding induced 3-D DNA Nanomachine Signal Probe	0.85 pM	150 min	This work

Software use

Photoshop and Origin software were used in the study. Photoshop was used

in Scheme 1, Fig. S1 a. Origin was used in Fig.1B, C, Fig. 2 and Fig. S1-4. Fig. 1A was obtained by Image Lab of ChemiDoc XRS+ Gel imaging apparatus.

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