Electronic Supplementary Information (ESI) for Chemical Communications

Aptamer-biorecognition triggered DNAzyme liberation and Exo III assisted target recycling for ultrasensitive homogeneous colorimetric bioassay of kanamycin antibiotic

Zhichao Chen,[a] Feng Xiong,[a] Aimin Yu,[a,b] and Guosong Lai*,[a]

[a.] *Hubei Key Laboratory of Pollutant Analysis & Reuse Technology, Department of Chemistry, Hubei Normal University, Huangshi 435002, PR China*

[b.] *Department of Chemistry and Biotechnology, Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn VIC 3122, Australia*

* Corresponding author

E-mail: gslai@hbnu.edu.cn (G. Lai)

EXPERIMENTAL SECTION

Reagents and materials. Kana, florfenicol (FF), thiamphenicol (TAP), chloramphenicol (CAP), streptomycin (STR), gentamycin (GEN) and hemin were purchased from Aladdin Co. Ltd (Shanghai, China). Triton X-100 and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich Co., Ltd. Exo III with the concentration of 200 U/μL in 50 mM pH 8.0 Tris-HCl containing 5 mM $MgCl_2$ was obtained from Takara Biotechnology Co. Ltd (Dalian, China). The milk powder sample was purchased from one local supermarket. All other reagents were of analytical grade and directly used without further purification. Ultrapure water with a resistivity of 18 MΩ cm was used in whole experiments. All oligonucleotides were synthesized by Sangon Biotechnology Co. Ltd (Shanghai, China), and their base sequences are listed as follows:

(1) Bare DNAzyme (S0): 5'-GGG TAG GGC GGG TTG GG-3';

(2) DNAzyme sequence-contained strand (S1) 5'-GGG TAG GGC GGG TTG GGA ACC TCA AGA CCA CTT GGA CAT TTT-3';

(3) Kana-aptamer (S2): 5'-TGT CCA AGT GGT CTT GAG GTT TTT T-3';

(4) S2 partly complementary strand (S3): 5'-AAA AAA CCT GAC ACT AC-3';

(5) A-base enhanced DNAzyme (S4): 5'-GGG TAG GGC GGG TTG GGA-3';

(6) Two-base extended Kana-aptamer (S5): 5'-TGT CCA AGT GGT CTT GAG GTT CC-3';

(7) Four-base extended Kana-aptamer (S6): 5'-TGT CCA AGT GGT CTT GAG GTT CCC A-3';

(8) Nine-base extended Kana-aptamer (S7): 5'-TGT CCA AGT GGT CTT GAG GTT CCC AAC CCG-3'.

All oligonucleotides were resolved in 10 mM pH 8.0 Tris-HCl containing 1 mM EDTA. Prior to use, all solutions were heated for 5 min at 95 ^{o}C and then cooled to room

temperature gradually. A 10 mM pH 7.4 Tris-HCl containing 100 mM NaCl, 5 mM $MgCl_2$ and 5 mM KCl was prepared and used as the working solution. Hemin was dissolved in 1% DMSO containing 0.2 M KCl. It was used as a stock solution and diluted to the required concentration with 10 mM pH 7.4 Tris-HCl. A pH 5.0 buffer containing 52.1 mM citrate, 95.8 mM Na_2HPO_4, 40 mM KCl, 0.4 mM TMB and 0.4 mM H_2O_2 was prepared and used as the chromogenic solution.

Procedure of homogeneous assay. First, 990 μL of 10 mM pH 7.4 Tris-HCl containing 1% DMSO and 0.05% Triton X-100 were added into a 2-mL centrifuge tube. Next, 5 μL of S1 and 5 μL of S2 at the concentration of 10 μM were added. The mixture was vortexed at room temperature for 45 min to form a hybridized DNA duplex. Subsequently, 50 μL of Kana standard solution at different concentrations, 5 μL of 2 μM S3 solution, 5 μL of 5 U/μL Exo III, and 5 μL of 1 μM hemin were added into 100 μL of the hybridized DNA duplex solution prepared above to conduct a vortexed reaction at 37 ^oC for 70 min. Following that, the mixture was heated to 65 ^oC and kept at this temperature for 5 min to inactivate Exo III. The solution was finally cooled down to room temperature gradually.

Colorimetric analysis. The TMB-H_2O_2 system was adopted for the chromogenic signal transduction of the peroxidase-mimicking DNAzyme liberated from above homogeneous assay. Typically, 70 μL of the chromogenic solution containing 0.4 mM TMB and 0.4 mM H_2O_2 was added into the homogeneous assay product. After reaction at room temperature for 5 min, 30 μL of 2 M H_2SO_4 was further added to terminate the chromogenic reaction. The resultant yellow solution was finally measured with a UV-1901 spectrophotometer (Purkinje, Beijing) to construct the linear relationship between absorbance and the Kana concentration for quantitative analysis.

Polyacrylamide gel electrophoresis characterization. For the polyacrylamide gel

electrophoresis (PAGE) assay, the polyacrylamide gel (20%) was prepared by a 1×TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.0). The tested sample was prepared by mixing 10 μL of each oligonucleotide with 2 μL 6×loading buffer, and subjected to gel electrophoresis at a Bio-Rad PowerPac universal electrophoretic device (USA). The final concentrations of all oligonucleotides and Kana were adjusted to 2 μM and 100 ng mL^{-1}, respectively. After running at 80 V for 150 min and staining with ethidium bromide (EB) for 10 min, the photographing was scanned with a Bio-Rad ChemiDoc™ XRS+ gel image analysis system (USA).

SUPPLEMENTARY DATA

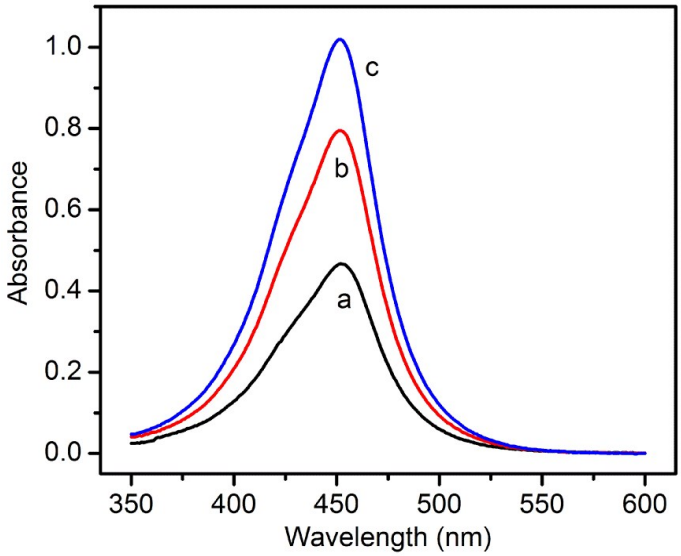

Fig. S1 Absorbance responses of the peroxidase-mimicking DNAzymes produced from 0.019 μM of S0 (a), S1 (b) and S4 (c), respectively.

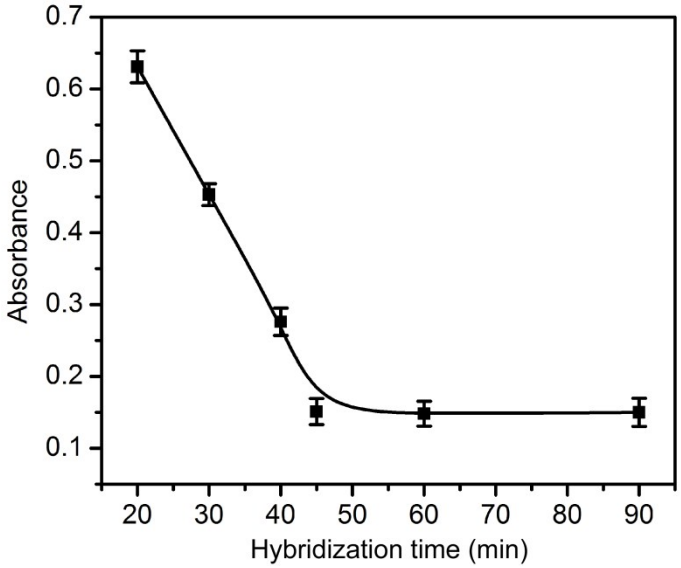

Fig. S2 Effect of the DNA hybridization time between 0.019 μM S1 and S2 on the absorbance response of the resultant product.

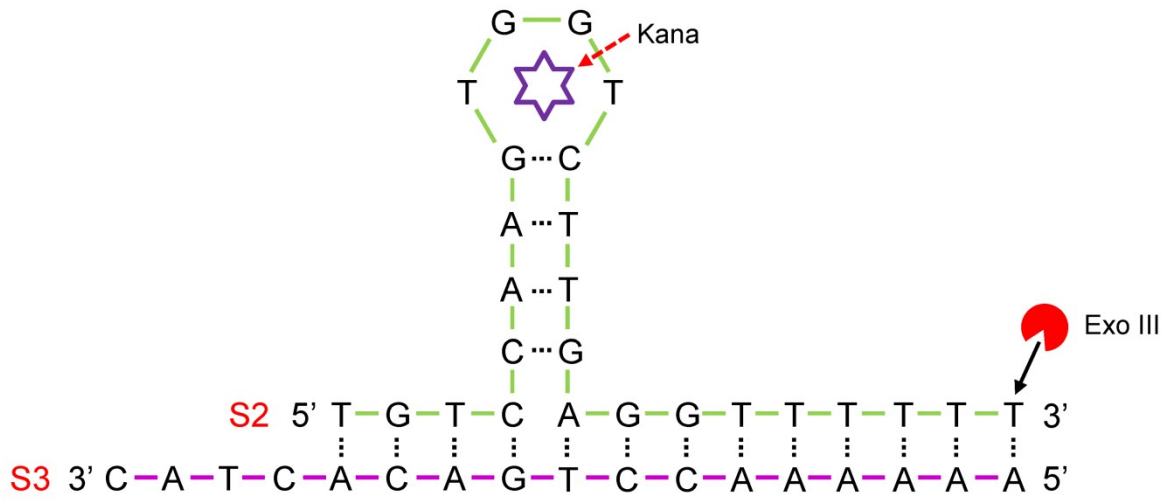

Fig. S3 Schematic illustration of the DNA hybridization between S3 and the aptamer-Kana biocomposite for inducing the catalyzed digestion reaction of Exo III.

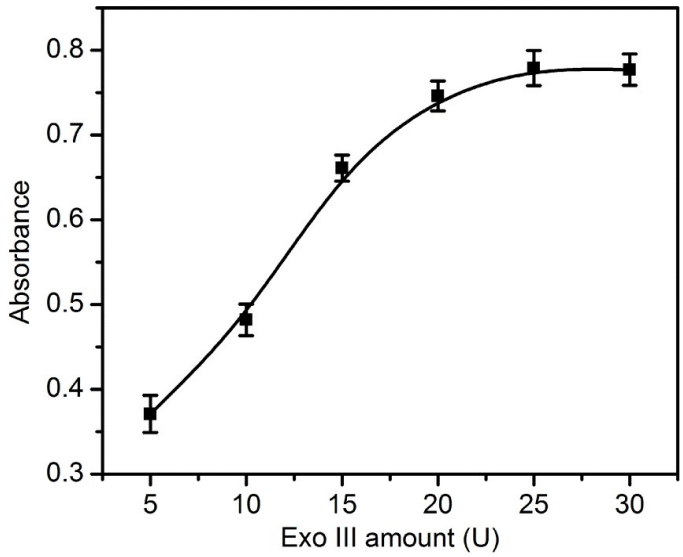

Fig. S4 Effect of the Exo III amount on the absorbance response of 10 ng mL^{-1} Kana.

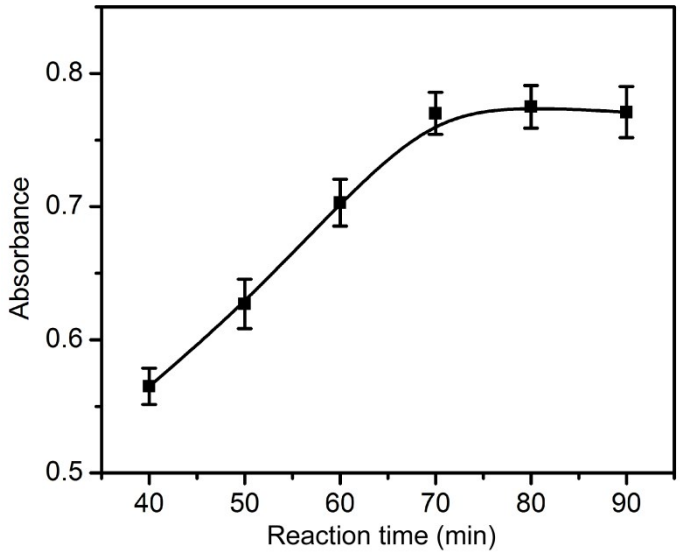

Fig. S5 Effect of the homogeneous reaction time on the absorbance response of 10 ng mL^{-1} Kana.

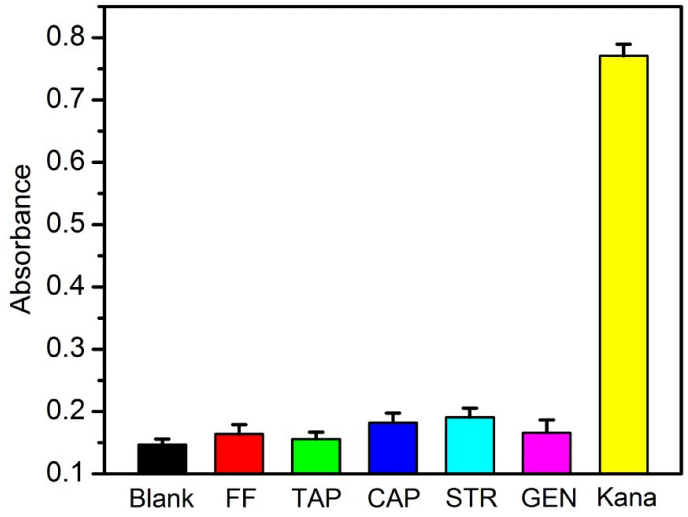

Fig. S6 Absorbance response of blank control and 1.0 μg mL^{-1} FF, TAP, CAP, STR, GEN and 10 ng mL^{-1} Kana added for homogeneous assay.

Table S1. An overview on the specific features of the bioassay methods recently reported for Kana analysis.

Assay format	Signal transduction strategy	Linear range	LOD	Ref.
Heterogeneous assay	Aptamer-biorecognition induced electrochemical signal decrease of enzyme-nanoprobe at an electrode	2 pg mL^{-1}-100 ng mL^{-1}	0.88 pg mL^{-1}	3*c*
Homogeneous assay	Inhibition of fluorescence resonance energy transfer induced by aptamer-biorecognition	0.01-3 nM	9 pM	4*b*
Homogeneous assay	Aggregation of silver nanoparticles induced by aptamer-biorecognition for colorimetric analysis	0.05-0.6 μg mL^{-1}	2.6 ng mL^{-1}	12*a*
Homogeneous assay	Target-activated split peroxidase DNAzyme for colorimetric analysis	0.05-500 nM	14.7 pM	12*b*
Heterogeneous assay	Aptamer-biorecognition induced gold nanoparticle release at a lateral flow strip for color change	1-30 nM	0.0778 nM	12*c*
Homogeneous assay	Aptamer-biorecognition induced nanozyme activity recovery of gold nanoparticles for colorimetric analysis	1-100 nM	1.49 nM	12*d*
Heterogeneous assay	Aptamer-biorecognition induced electrochemical signal decrease of a graphene-thionine nanocomposite at an electrode	0.5 pg mL^{-1}-50 ng mL^{-1}	0.42 pg mL^{-1}	13*a*
Heterogeneous assay	Target-triggered cascade enzymatic recycling to form DNAzymes at an electrode for electrocatalytic reaction	1 pM-10 nM	0.5 pM	13*b*
Homogeneous assay	Target binding-induced DNAzyme liberation for colorimetric analysis	0.1 pg mL^{-1}-10 ng mL^{-1}	0.045 pg mL^{-1}	This work

Table S2 The results of the recovery tests of Kana added in milk sample solutions (n=3).

no.	Added concentration (ng mL^{-1})	Found concentration[a] (ng mL^{-1})	Recovery (%)	RSD (%)
1	5.0	5.46	109	9.2
2	1.0	0.981	98.1	5.6
3	0.1	0.0911	91.1	9.3
4	0.01	0.0107	107	8.7
5	0.005	0.0052	104	5.6
6	0.001	0.00105	105	8.8

[a] The linear regression equation between the concentrations of Kana added and found in the milk samples is as follows: $y = 1.005x + 0.000062$ ($R = 0.998$) (x-axis: the added Kana concentrations; y-axis: the found Kana concentrations).