Supplementary Information (SI) for Analyst.

Electronic supplementary information

Construction of a smartphone-assisted colorimetric nanosensor with multiple antibiotic response based on silver-ceria nanocomposites mimicking peroxidase activity

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1. Supplementary experimental section

1.1 Materials and reagents

Sodium hydroxide (NaOH) was purchased from Xilong Scientific Co., Ltd. (Shantou, China). Cerium(III) nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$), silver nitrate ($AgNO_3$) and Kana were bought from Adamas-beta Co., Ltd. (Shanghai, China). TC was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Penicillin (PG), chloramphenicol (CL), fosfomycin sodium (FF), ampicillin (AMP), penicillin G potassium salt (PEN), cloxacillin sodium (CLO), oxytetracycline (OTC), chlortetracycline (CTC) and AMX were acquired from Meizheng Biological Co., Ltd. (Beijing, China). 2, 2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). TMB and o-phenylenediamine (OPD) were bought from Aladdin Co., Ltd. (Shanghai, China). Citrate, sodium chloride (NaCl), potassium carbonate (K_2CO_3), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), zinc sulfate ($ZnSO_4$), potassium nitrate (KNO_3), glucose (Glu), glycine (Gly) and threonine (Thr) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Except for methanol and acetonitrile which are of chromatography grade, all other chemical reagents are analytical reagent (AR) grade. No additional treatment was performed on the reagents prior to the experiment. High-purity water (18.2 MΩ.cm) from a Milli-Q purification system was used throughout the experiment. The tap water sample was gained from the campus of Hunan Agricultural University, the drinking mineral water and the milk sample were procured from a local supermarket (Changsha, China).

1.2 Instruments and apparatus

An ultraviolet-visible (UV-Vis) microplate reader (SPARK 20 M, TECAN, Switzerland) was employed to record absorption spectra in the wavelength range of 500-800 nm. Transmission electron microscopy (TEM) image of AgNPs@CeO_2 and energy-dispersive X-ray spectrum (EDS) profiles were acquired using a JEM-2100F field emission transmission electron microscopy (JEOL, Japan). The surface elemental composition and chemical states of the samples were characterized by a smartlab X-ray photoelectron spectrometer (XPS) (Thermofisher, USA). Meanwhile, powder X-ray diffraction (XRD) date were collected using a D8 Advance X-ray diffractometer (Bruker, Germany). Electron paramagnetic resonance (EPR) spectroscopy was applied to test the reactive oxygen species (ROS) by a Bruker-A300 spectrometer (Bruker, GER). Photographs were captured using a Redmi K50 Ultra (Xiaomi, China).

1.3 POD-like activity assay of AgNPs@CeO_2

The POD-like activity of AgNPs@CeO_2 was assessed as follows: 50 μL of H_2O_2 (1 M) and 50 μL of TMB (1 mM) were added to 50 μL of AgNPs@CeO_2 solution (4 μg/mL, dissolved in 10 mM pH 3.8 acetate buffer), running under a water bath (40°C) for 25 min.

The free radical trapping experiment was conducted in solutions of isopropanol (IPA), L-histidine (HD) and p-benzoquinone (PBQ) separately mixed with AgNPs@CeO_2/TMB/H_2O_2 under optimal conditions to capture hydroxyl radicals (•OH), singlet oxygen($^1O_2$), and superoxide anion ($O_2^{-\bullet}$), respectively.

1.4 Steady-state kinetics determination of AgNPs@CeO_2

The steady-state kinetics of AgNPs@CeO_2 was carried out by varying the TMB concentration (0-2 mM) and fixing H_2O_2 concentration (1 M) in the presence of AgNPs@CeO_2 (4 μg/mL), and through changing the concentration of H_2O_2 (0-3 M) in the presence of 1 mM TMB and 4 μg/mL AgNPs@CeO_2. The kinetic parameters (K_m and V_{max}) were derived from the Michaelis-Menten equation, which is expressed as follows:

$$v = V_{max} [S]/(K_m + [S])$$

where v denotes the reaction velocity; V_{max} represents the maximum reaction velocity; K_m is the Michaelis constant, and [S] stands for the substrate concentration.

1.5 Real sample analysis

Real samples including drinking mineral water, tap water, and skim milk were measured. Water sample was simply obtained by filtering the sample through a 0.22 μM syringe filter to remove impurities. The pretreatment of milk was performed by a previously reported method. First, skim milk was diluted with deionized water (1:10), and then 20% (v/v) acetic acid was added to adjust the pH to 4.6 to denature the milk protein precipitate. The precipitated protein was removed by centrifugation at 11,000 rpm for 20 min. It was then filtered through a 0.22 μM syringe filter and diluted 50-fold with deionized water before measurement.

1.6 Detection of antibiotic by HPLC

For TC detection using HPLC, TC was separated via an HPLC column, and then detected by UV detection. In accordance with the Chinese national standard method (GB 31658.6-2021), the HPLC was operated under the following parameters: a C18 chromatographic column was maintained at a temperature of 30°C throughout the detection process; the mobile phase consists of solution A (0.1% acetic acid) and solution B (acetonitrile) (8:2) with the flow rate of 1 mL/min and the absorption wavelength of the UV detector was tuned to 350 nm.

2. Additional figures

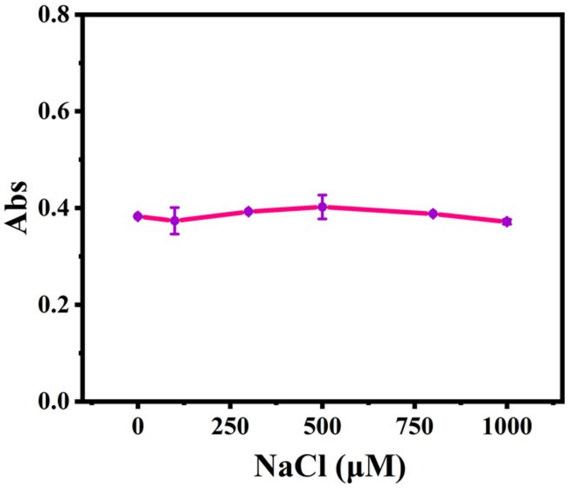

Fig. S1. Salt tolerance of the AgNPs@CeO_2 nanozyme.

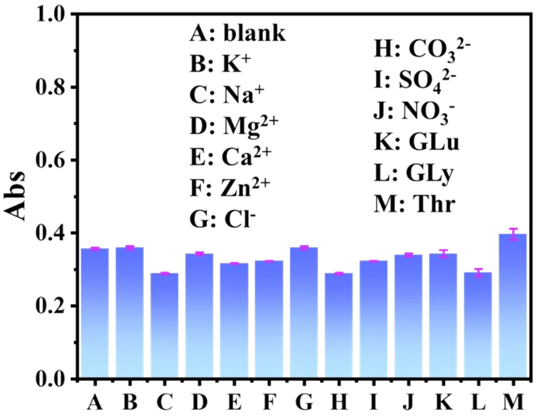

Fig. S2. The catalytic reaction of AgNPs@CeO_2 under different interfering substances.

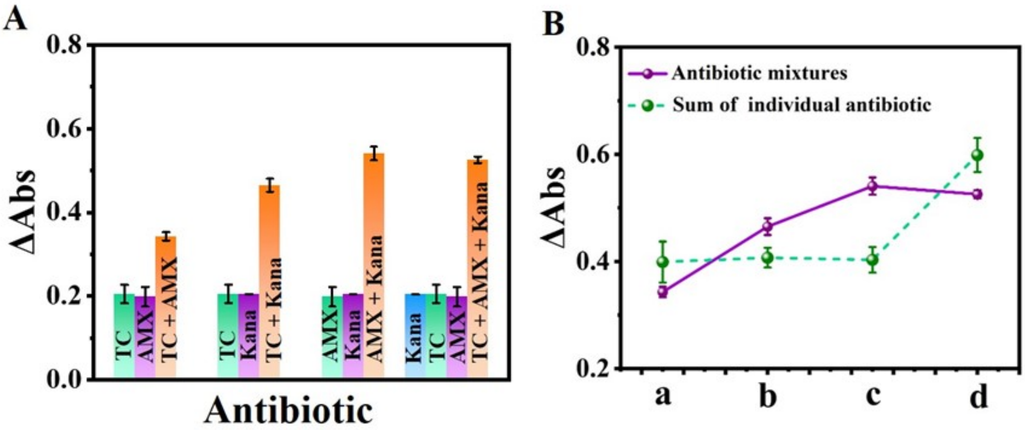

Fig. S3. (A) Response of the AgNP@CeO$_2$ nanozyme sensor to different antibiotic systems. (B) Analysis of the response of antibiotic mixtures, and the groups are (a) TC + AMX, (b) TC + Kana, (c) AMX + Kana, and (d) TC + AMX + Kana. Concentration: TC, 300 μM ; AMX, 80 μM; Kana,1000 nM).

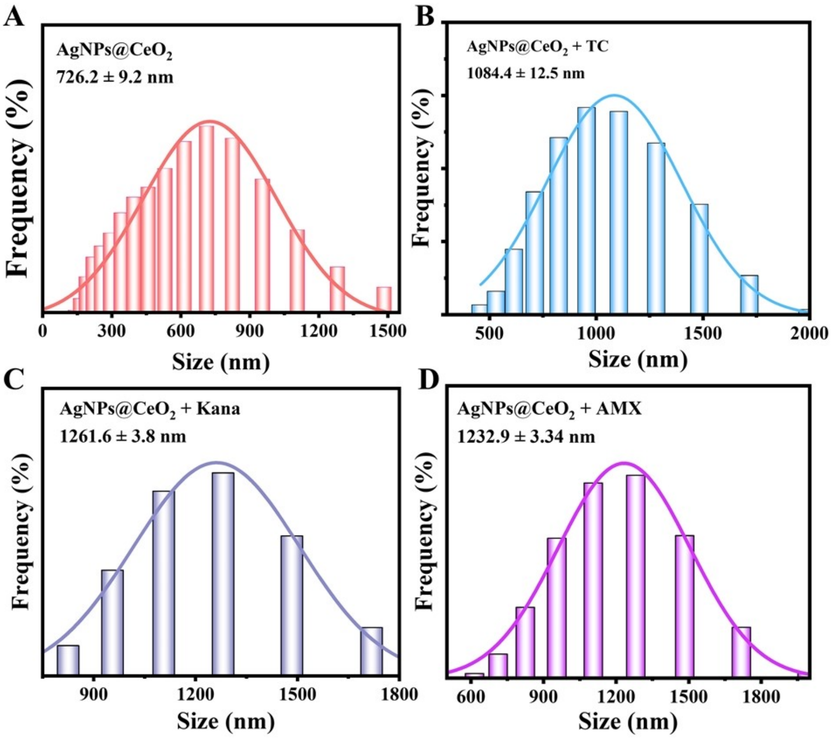

Fig. S4. (A-D) Particle size distribution diagram of AgNPs@CeO$_2$ without and with antibiotic (Gaussian fitting).

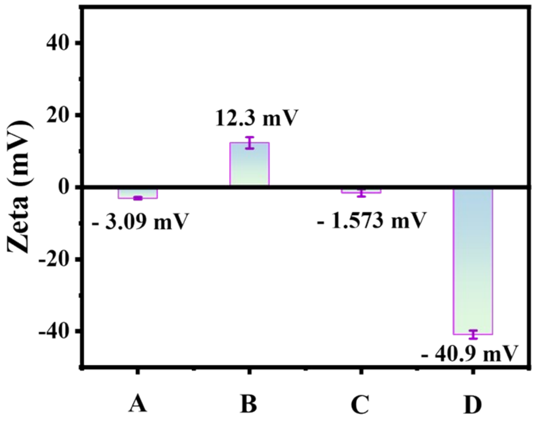

Fig. S5. Zeta potential of AgNPs@CeO_2 without and with antibiotics. A, B, C, and D represent the zeta potentials of AgNPs@CeO_2, AgNPs@CeO_2 after TC adsorption, AgNPs@CeO_2 after Kana adsorption, and AgNPs@CeO_2 after AMX adsorption, respectively.

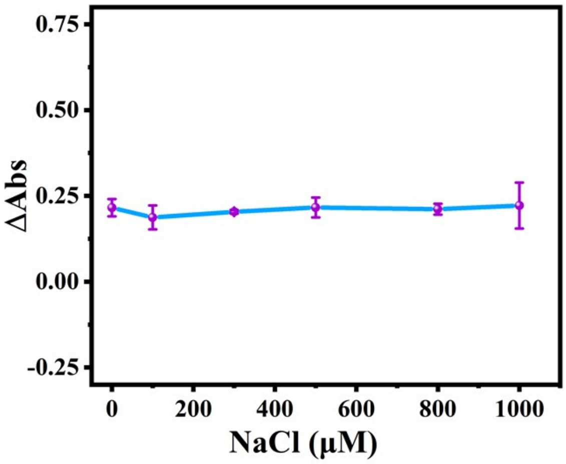

Fig. S6. Salt tolerance of TC.

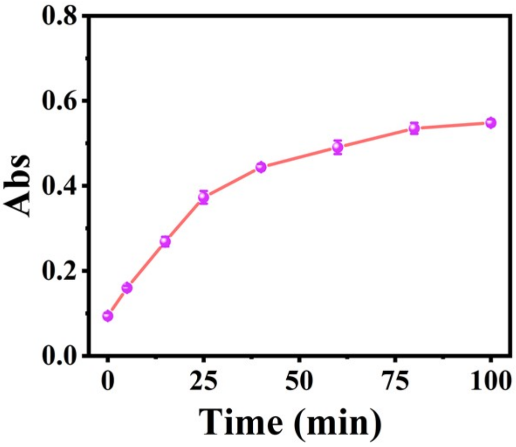

Fig. S7. Optimization of reaction time. Conditions: pH 3.8, 10 mM NaAc-HAc solution; TC, 300 μM; AgNPs@CeO$_2$, 4 μg/mL; H$_2$O$_2$, 1 M; TMB, 1 mM; 40°C.

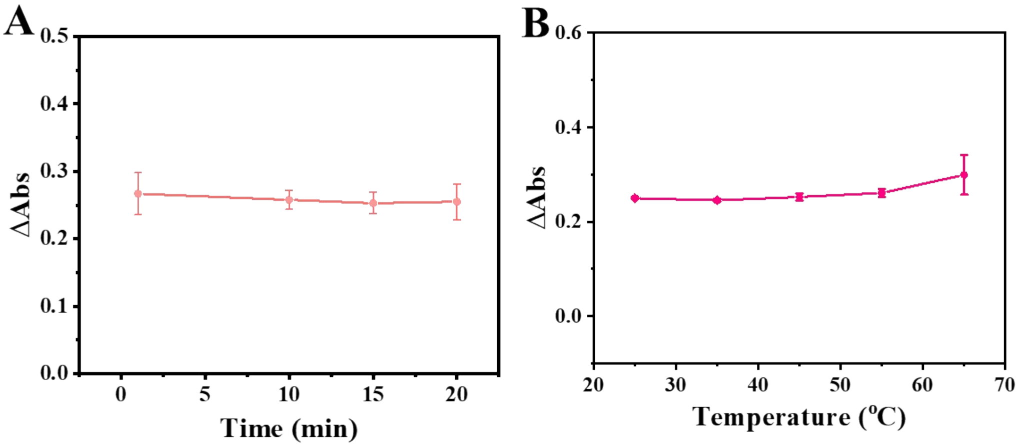

Fig. S8. The effect of TC on the incubation time (A) and temperature(B) of AgNPs@CeO$_2$ on the catalysis of the TMB chromogenic reaction. Conditions: pH 3.8, 10 mM NaAc-HAc solution; TC, 300 μM; AgNPs@CeO$_2$, 4 μg/mL; H$_2$O$_2$, 1 M; TMB, 1 mM; 25 min, 40°C.

3. Additional tables

Table S1. Comparison of some reported sensing methods and the proposed sensor for TC detection in analytical performance.

Analytical method	Material	LOD	Linear range	Ref.
Colorimetric Luminometric Fluorescent	$TetX_2$@Au-NCs	1.3 mM 1.4 mM 1.6 mM	0 - 10 mM 0 - 10 mM 0 - 10 mM	S1
Fluorescent	TPE-CMP	1.23 μg/mL	0 - 100 μg/mL	S2
Fluorescent	CD	6 μM	10 - 400 μM	S3
Fluorescent	Dopamine and 4-fluororesorcinol	1.7 μM	10 - 350 μM	S4
Fluorescent	RBP-CDs	0.36 μM	0.5 - 30 μM 30 - 90 μM	S5
Colorimetric	**AgNPs@CeO_2**	**2.75 μM**	**5 - 500 μM**	**This study**

Table S2. Comparison of some reported sensing methods and the proposed sensor for Kana detection in analytical performance.

Analytical method	Materials	LOD (μM)	Linear range (μM)	Ref.
Colorimetric	WS_2 NSs	0.060	0.1 - 0.5	S6
Photothermal Colorimetric	CS/PtNPs	0.41 0.04	0.5–50 0.1 - 50	S7
Colorimetric Fluorescent	Au@PdNPs	0.0145 0.073	0.1 - 0.8 0.05 - 1	S8
Colorimetric	Co_3O_4 ND	0.057	0.2 - 0.8	S9
SPR	Au/graphene	0.29	1 - 100	S10
Fluorescent	CDs	0.018	0.04 - 0.24	S11
Colorimetric	**AgNPs@CeO_2**	**0.0083**	**0.02 - 1.5**	**This study**

Table S3. Comparison of some reported sensing methods and the proposed sensor for AMX detection in analytical performance.

Analytical method	Materials	LOD (μM)	Linear range (μM)	Ref.
Fluorescent	B-CQDs	0.825	1.43 - 429.12	S12
Voltammetric	MIPANI MIPMOA	2.6 0.61	10 - 5000 5 - 5000	S13
Electrochemical	PBE	8	29.8 - 126.7	S14
Electrochemical	e-LIG	5.4	9.6 - 103.3	S15
Electrochemical	SPE/MNP-TA/Ab	0.44	0.5 - 100	S16
Colorimetric	Qt AgNPs	4.46	10 - 95	S17
Colorimetric	**AgNPs@CeO$_2$**	**0.611**	**10 - 100**	**This study**

Table S4. Detection of TC in real samples using the developed colorimetric method (n = 3).

Sample	**Added (μM)**	**Found (μM)**	**Recovery (%)**	**RSD (%)**
Drinking water	0	＜LOD	/	/
	50	51 ± 3.18	102.66	6.19
	100	100 ± 0.45	99.97	0.45
	200	199 ± 17.33	99.26	8.73
	400	400 ± 0.09	100.00	0.02
Tap water	0	＜LOD	/	/
	50	55 ± 0.38	109.39	0.69
	100	104 ± 6.47	104.26	6.21
	200	208 ± 8.73	103.89	4.20
	400	386 ± 13.76	96.60	3.56
Milk	0	＜LOD	/	/
	50	49 ± 1.44	98.91	2.91
	100	128 ± 4.32	127.75	3.14
	200	241 + 19.51	120.46	8.10
	400	393 + 8.96	98.30	2.28

Table S5. Determination of TC in skimmed milk sample by HPLC method (n = 3).

Sample	**Added (μM)**	**Found (μM)**	**Recovery (%)**	**RSD (%)**
Milk	0	＜LOD	/	/
	50	46 ± 1.77	92.7	7.95
	100	87 ± 1.54	87.5	3.66
	200	214 + 4.53	107.2	4.40
	400	443 ± 2.73	110.8	1.28

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