Differential Sensing of Antibiotics using Metal Ions and Gold Nanoclusters based on TMB-H_2O_2 System

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The feasibility of Using a Multifunctional Microplate Reader

In this experiment, the model of the multifunctional microplate reader we used is INFINITE 200 PRO, which can select the way of multi-point detection of the solution in a single well, and select whether to wait for a certain time when detecting different samples. We have studied the above detection methods, and the results are shown in Table S1. Mode 1 was a standard single-point detection mode, mode 2 was a single-point detection and stayed for 60 s, mode 3 was a four-point detection mode (point spacing is 1800 μm), and mode 4 was a four-point detection and stayed for 60 s (point spacing is 1800 μm). The samples used for the study contained a small amount of protein (0.1 mg ml^{-1}), and each sample was tested in three replicates. As shown in Table S1, the average values obtained by different detection modes were very close. Compared with the relative standard deviation (RSD), the RSD value of mode 3 was more minor. Therefore, mode 3 was used in this study. In addition, we compared the results of the microplate reader with those of the UV-Vis spectrophotometer. The samples tested contained a small amount of protein (0.1 mg ml^{-1}). As shown in Figure S1, the experimental data detected by the microplate reader was very stable, and the absorbance intensity detected by the microplate was about 0.4 times as much as the data detected by the spectrophotometer.

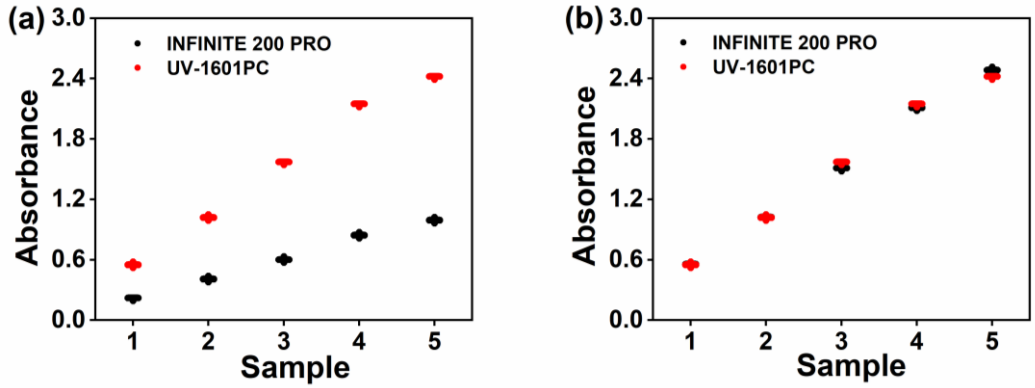

Figure S1. Comparison of multifunctional microplate reader and UV-Vis spectrophotometer. (a) Detection data obtained by a microplate reader (black dots) and a spectrophotometer (red dots). (b) The data obtained from the microplate reader were multiplied by a factor of 2.5.

Table S1. Comparison of detection modes of a multifunctional microplate reader.

Detection mode	1	2	3	4
average value	1.017	0.987	0.994	0.984
RSD	0.588	0.340	0.090	0.218

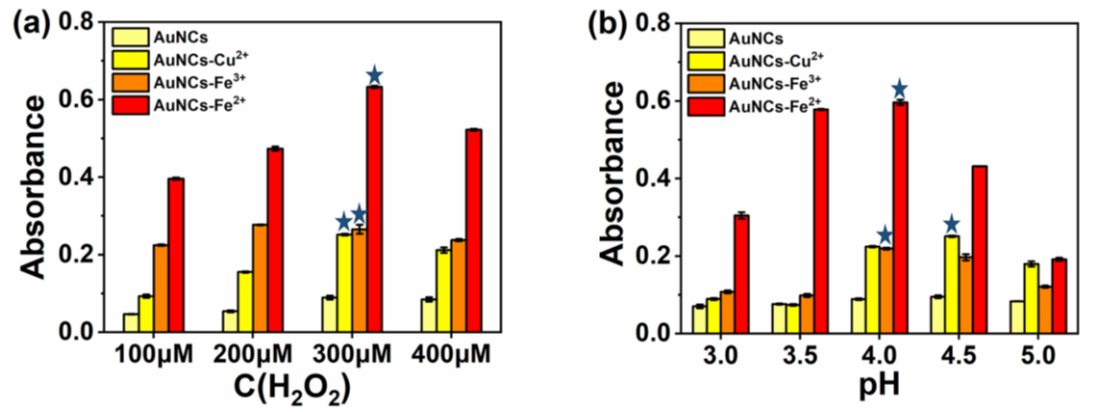

Figure S2. Optimization of the reaction conditions (a) the concentration of H_2O_2 and (b) the pH value of the reaction system (L-H_2O_2). The absorbance intensities of AuNCs (0.5 mg·mL^{-1}) catalytic system before and after the addition of 5 μM metal ions (Cu^{2+}, Fe^{3+}, Fe^{2+}).

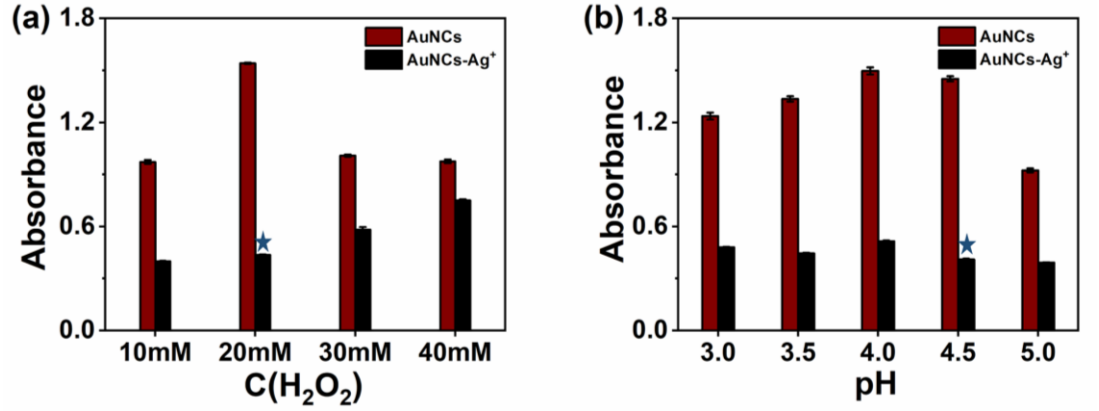

Figure S3. Optimization of reaction conditions (a) the concentration of H_2O_2 and (b) the pH value of the reaction system (H-H_2O_2). The absorbance intensities of AuNCs (0.5 mg·mL^{-1}) catalytic system before and after the addition of 10 μM Ag^+ ions.

Table S2. Summary of the interaction between the antibiotic and metal ions reported in the literature.

Metal Ions	Antibiotics					
	TC	OTC	CTC	Cip	Van	GM
Cu^{2+}	○	○	○	○		
Fe^{3+}	○	○	○	○	○	
Fe^{2+}	○	○	○	○		
Ag^{+}						○

The results of the detection of different antibiotics

Table S3. LDA identification results of 6 antibiotics with different concentrations. The value in the table is I/I_0.

Grouping variable	Independent variable						Verified
	AuNCs (L-H_2O_2)	AuNCs -Cu^{2+}	AuNCs -Fe^{3+}	AuNCs -Fe^{2+}	AuNCs (H-H_2O_2)	AuNCs -Ag^{+}	
TC (50 nM)	1.154	0.881	2.194	1.453	0.963	1.114	TC
TC (250 nM)	1.216	0.681	2.725	1.575	0.952	1.175	TC
TC (500 nM)	1.238	0.466	3.066	1.637	0.972	1.253	TC
TC (2500 nM)	1.293	0.341	3.240	1.667	0.966	1.253	TC
TC (5000 nM)	1.309	0.227	3.302	1.675	0.999	1.269	TC
OTC (50 nM)	1.184	0.824	2.331	1.424	1.013	1.122	OTC
OTC (250 nM)	1.352	0.660	2.624	1.456	1.051	1.234	OTC
OTC (500 nM)	1.524	0.505	3.077	1.523	1.056	1.399	OTC
OTC (2500 nM)	1.561	0.383	3.385	1.569	1.080	1.557	OTC
OTC (5000 nM)	1.621	0.340	3.698	1.634	1.239	1.617	OTC
CTC (50 nM)	1.185	0.896	1.864	1.136	1.086	1.233	CTC
CTC (250 nM)	1.380	0.780	1.274	1.137	1.089	1.247	CTC
CTC (500 nM)	1.413	0.528	1.441	1.203	1.109	1.263	CTC
CTC (2500 nM)	1.529	0.357	1.457	1.242	1.132	1.335	CTC

CTC (5000 nM)	1.646	0.232	1.536	1.301	1.389	1.376	CTC
Cip (50 nM)	1.605	1.141	1.945	1.462	1.074	1.254	Cip
Cip (250 nM)	2.020	1.151	2.491	1.705	1.098	1.321	Cip
Cip (500 nM)	2.060	1.164	3.275	1.851	1.114	1.555	Cip
Cip (2500 nM)	2.199	1.244	4.572	1.987	1.188	1.661	Cip
Cip (5000 nM)	2.340	1.267	4.733	2.030	1.251	1.743	Cip
Van (50 nM)	1.020	0.995	0.862	0.916	1.016	0.967	Van
Van (250 nM)	1.022	1.000	0.835	0.911	1.015	0.973	Van
Van (500 nM)	1.021	0.970	0.832	0.902	1.017	1.005	Van
Van (2500 nM)	0.958	1.019	0.829	0.889	1.024	1.015	Van
Van (5000 nM)	1.004	0.952	0.809	0.888	1.031	0.972	Van
GM (50 nM)	0.831	0.871	0.370	0.706	1.004	1.313	GM
GM (250 nM)	0.829	0.860	0.353	0.704	0.996	1.316	GM
GM (500 nM)	0.804	0.817	0.340	0.679	1.004	1.462	GM
GM (2500 nM)	0.781	0.794	0.302	0.633	1.004	1.593	GM
GM (5000 nM)	0.729	0.745	0.316	0.629	1.001	1.711	GM

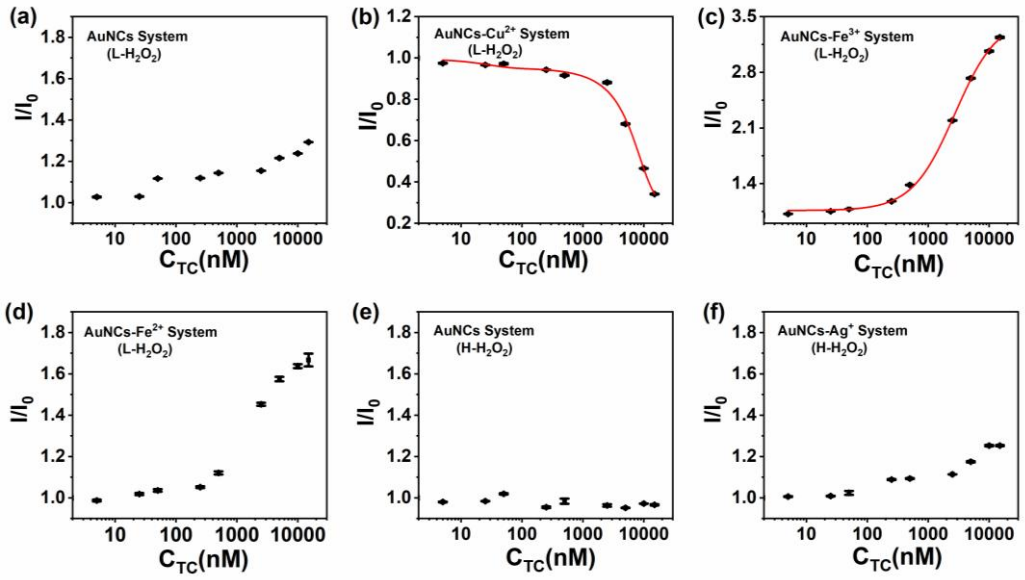

Figure S4. The dose-response curves for TC detection using (a) AuNCs, (b) AuNCs-Cu^{2+}, (c) AuNCs-Fe^{3+}, (d) AuNCs-Fe^{2+}, (e) AuNCs, (f) AuNCs-Ag^{+} reaction system, between the absorbance at 452 nm against the logarithmic concentration of TC. Reaction conditions: AuNCs, 0.5 mg · mL^{-1}; Cu^{2+}, 5 μM; Fe^{3+}, 5 μM; Fe^{2+}, 5 μM; Ag^{+}, 10 μM; H_2O_2, 300 μM (a-d) or 20 mM (e, f); pH value, 4 (c-e) or 4.5 (a, b, f); TMB, 0.1 mg · mL^{-1}. The error bars represent the standard deviation of the three measurements.

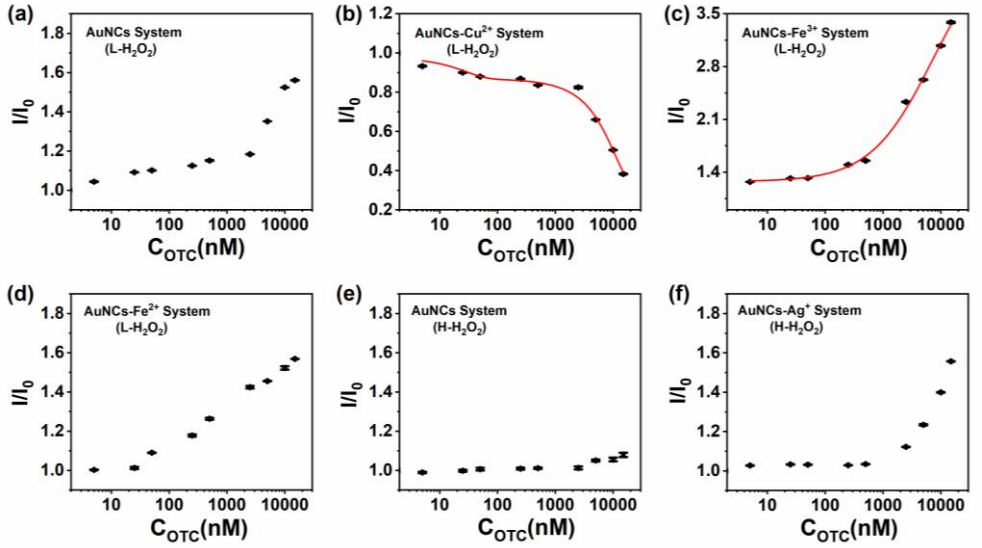

Figure S5. The dose-response curves for OTC detection using (a) AuNCs, (b) AuNCs-Cu^{2+}, (c) AuNCs-Fe^{3+}, (d) AuNCs-Fe^{2+}, (e) AuNCs, (f) AuNCs-Ag^{+} reaction system, between the absorbance at 452 nm against the logarithmic concentration of OTC. The reaction conditions were the same as those of TC.

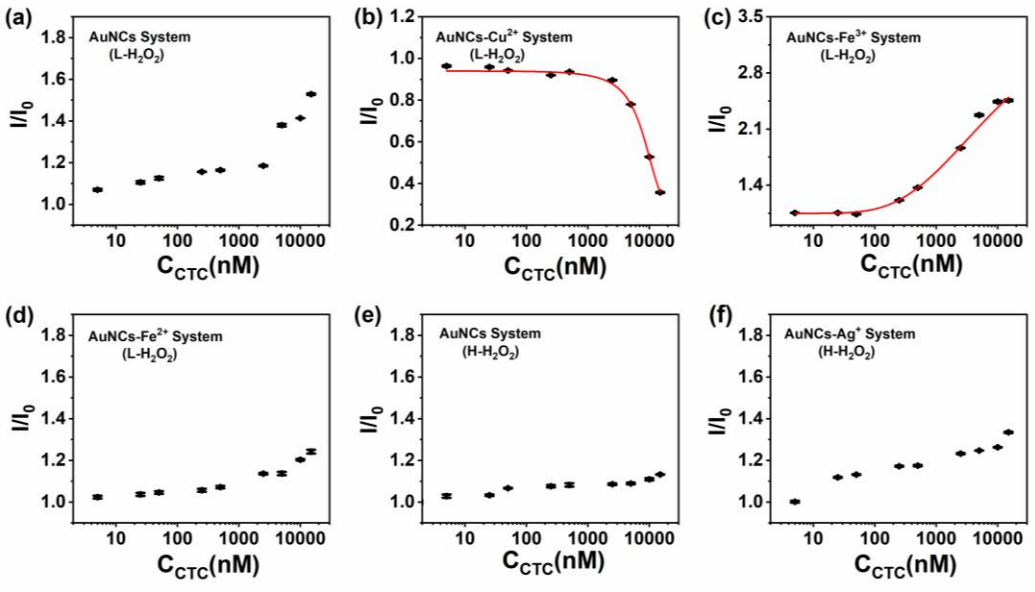

Figure S6. The dose-response curves for CTC detection using (a) AuNCs, (b) AuNCs-Cu^{2+}, (c) AuNCs-Fe^{3+}, (d) AuNCs-Fe^{2+}, (e) AuNCs, (f) AuNCs-Ag^{+} reaction system, between the absorbance at 452 nm against the logarithmic concentration of CTC. The reaction conditions were the same as those of TC.

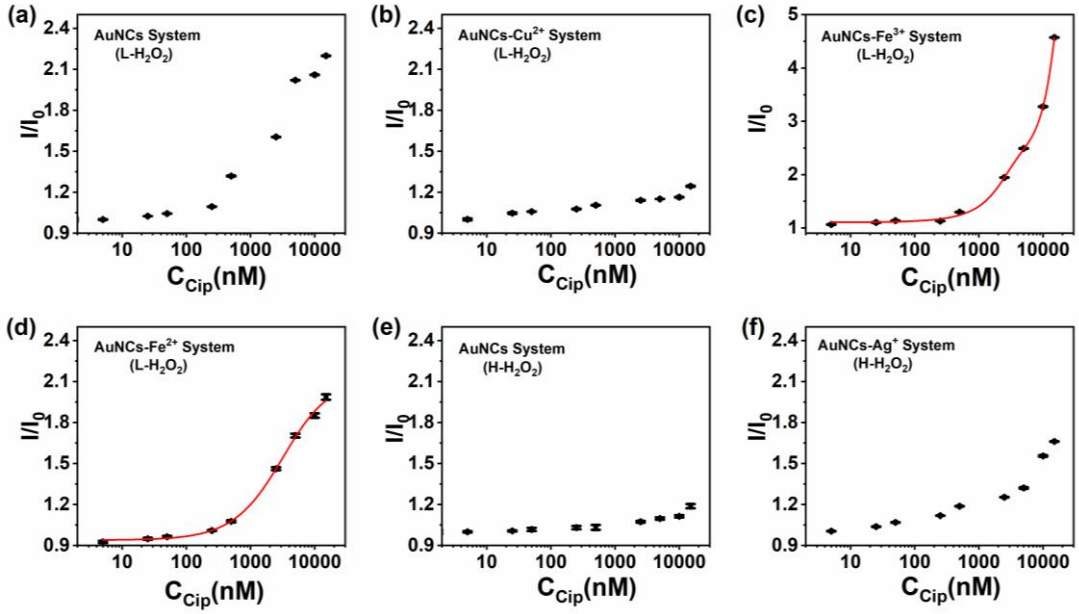

Figure S7. The dose-response curves for Cip detection using (a) AuNCs, (b) AuNCs-Cu^{2+}, (c) AuNCs-Fe^{3+}, (d) AuNCs-Fe^{2+}, (e) AuNCs, (f) AuNCs-Ag^{+} reaction system, between the absorbance at 452 nm against the logarithmic concentration of Cip. The reaction conditions were the same as those of TC.

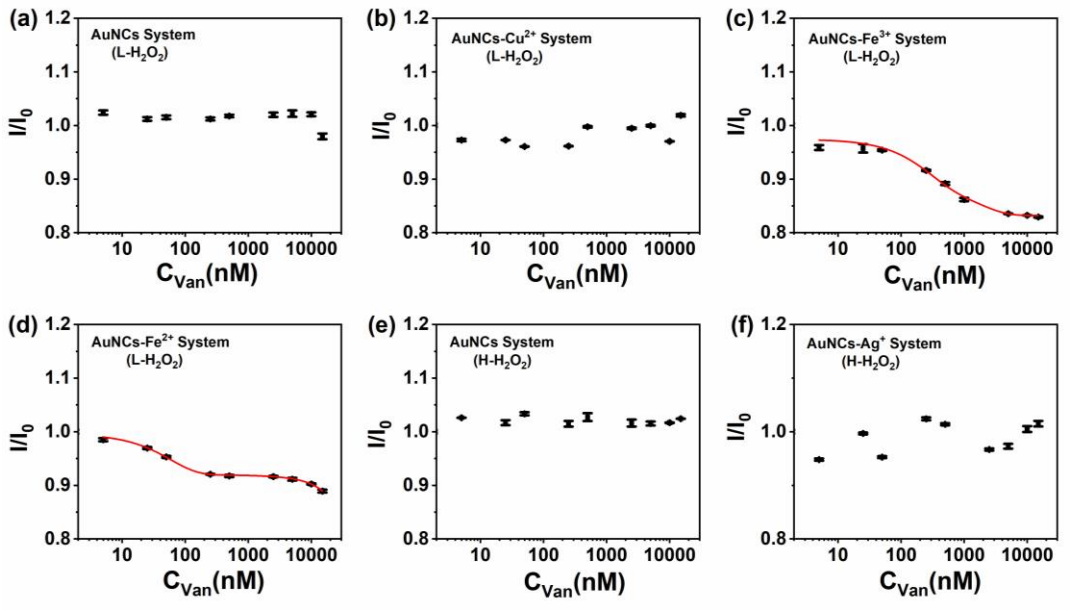

Figure S8. The dose-response curves for Van detection using (a) AuNCs, (b) AuNCs-Cu^{2+}, (c) AuNCs-Fe^{3+}, (d) AuNCs-Fe^{2+}, (e) AuNCs, (f) AuNCs-Ag^{+} reaction system, between the absorbance at 452 nm against the logarithmic concentration of Van. The reaction conditions were the same as those of TC.

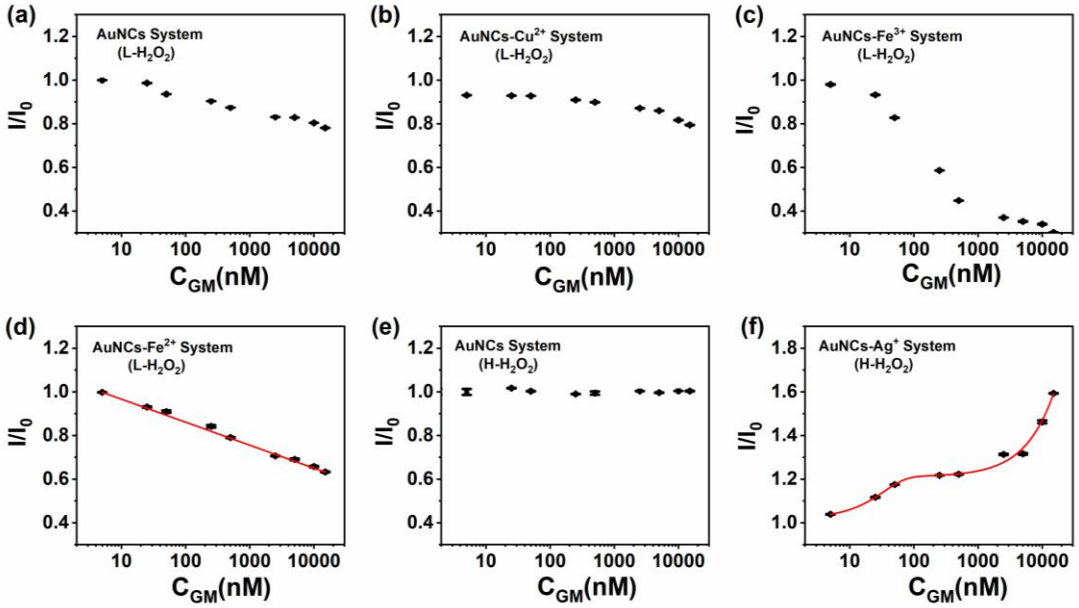

Figure S9. The dose-response curves for GM detection using (a) AuNCs, (b) AuNCs-Cu^{2+}, (c) AuNCs-Fe^{3+}, (d) AuNCs-Fe^{2+}, (e) AuNCs, (f) AuNCs-Ag^{+} reaction system, between the absorbance at 452 nm against the logarithmic concentration of GM. The reaction conditions were the same as those of TC.

Table S4. Summary of the fitting curve formula in Fig. S4-9.

Figure		Fitting formula	Parameter	R^2
Figure S4	(b)	$y = A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = 0.288 A2 = 1.260 LOGx01 = -12.130 p = 0.127 LOGx02 = 4882.946 h1 = -0.021 h2 = -1.137E-4	0.996
	(c)	$y=START+(END-START)\frac{x^n}{k^n+x^n}$	START = 1.061 k = 2730.197 END = 3.489 n = 1.235	0.996
Figure S5	(b)	$y=A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = 0.276 A2 = 1.576 LOGx01 = -14.245 p = 0.247 LOGx02 = 2627.965 h1 = -0.021 h2 = -7.034E-5	0.993
	(c)	$y=START+(END-START)\frac{x^n}{k^n+x^n}$	START = 1.275 END = 4.507 K = 7234.212 n = 0.805	0.992
Figure S6	(b)	$y=A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = 0.269 A2 = 1.024 LOGx01 = -1.921E8 LOGx02 = 7660.305 h1 = -2.704E-12 h2 = -1.262E-4 p = 0.036	0.990
	(c)	$y=A_{min}+\frac{A_{max}-A_{min}}{\left(1+\left(\frac{x_0}{x}\right)^h\right)^s}$	A_{min} = 1.047 A_{max} = 3.502 x_0 = 0.302 h = 0.403 s = 41.481	0.994
Figure S7	(c)	$y=A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = 0.610 A2 = 8.455 LOGx01 = 1484.531 LOGx02 = 17000.648 h1 = 4.193E-4 h2 = 8.335E-5 p = 0.168	0.996
	(d)	$y=A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = -5.397 A2 = 2.900 LOGx01 = -5363.434 LOGx02 = 15767.646 h1 = 1.543E-4 h2 = 0.001 p = 0.877	0.997
Figure S8	(c)	$y=A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = 0.832 A2 = 61.947 LOGx01 = -1678.077 LOGx02 = -9261.123 h1 = -0.002 h2 = -2.489E-4 p = 0.806	0.993

	(d)	$y=A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = 0.607 A2 = 3.977 LOGx01 = -212.260 LOGx02 = 40113.907 h1 = -0.007 h2 = -2.991E-5 p = 0.901	0.993
Figure S9	(d)	$y=a+b*x$	a = 1.072 b = -0.105	0.999
	(f)	$y=A1+(A2-A1)\left[\frac{p}{1+10^{(LOGx01-x)h1}}+\frac{1-p}{1+10^{(LOGx02-x)h2}}\right]$	A1 = -2.589 A2 = 3.269 LOGx01 = -2.655 p = 0.071 LOGx02 = -23961.641 h1 = 0.019 h2 = 9.033E-6	0.994

Table S5. Identification of unknown 18 antibiotics samples in river water.

#	Independent variable						Identity	Verified
	AuNCs ($L\text{-}H_2O_2$)	AuNCs $-Cu^{2+}$	AuNCs $-Fe^{3+}$	AuNCs $-Fe^{2+}$	AuNCs ($H\text{-}H_2O_2$)	AuNCs $-Ag^{+}$		
1	1.228	0.561	2.982	1.612	0.981	1.218	TC	TC
2	1.483	0.644	2.950	1.491	1.051	1.353	TC	TC
3	1.396	0.631	1.818	1.189	1.087	1.263	TC	TC
4	2.075	1.154	3.302	1.832	1.086	1.451	OTC	OTC
5	1.022	1.007	0.840	0.893	1.004	1.097	OTC	OTC
6	0.811	0.845	0.315	0.677	1.000	1.455	OTC	OTC
7	1.225	0.605	2.889	1.606	0.962	1.213	CTC	CTC
8	1.423	0.591	3.214	1.484	0.990	1.337	CTC	CTC
9	1.394	0.599	1.320	1.168	1.089	1.270	CTC	CTC
10	2.073	1.171	3.281	1.859	1.079	1.567	Cip	Cip
11	1.008	1.001	0.816	0.893	0.995	0.987	Cip	Cip
12	0.832	0.875	0.349	0.697	1.020	1.340	Cip	Cip
13	1.194	0.840	2.367	1.509	0.959	1.147	Van	Van
14	1.192	0.849	2.361	1.422	0.996	1.139	Van	Van
15	1.218	0.899	1.442	1.107	0.990	1.233	Van	Van
16	2.176	1.216	4.610	1.986	1.199	1.679	GM	GM
17	0.983	1.022	0.811	0.880	1.062	1.005	GM	GM

18	0.827	0.849	0.344	0.678	1.001	1.457	GM	GM

Table S6. Recovery results for antibiotics detection in river water samples using AuNCs-metal ions system.

Antibiotics	Systems	Original (nM)	Spiked (nM)	Found (nM)	Recovery (%)	RSD (%)
TC	AuNCs-Cu^{2+}	not detected	80	88.63	110.78	0.88
		not detected	800	936.40	117.05	1.85
		not detected	8000	7859.12	98.24	0.20
	AuNCs-Fe^{3+}	not detected	80	77.07	96.33	1.63
		not detected	800	799.68	99.96	0.27
		not detected	8000	7531.90	94.15	0.58
OTC	AuNCs-Cu^{2+}	not detected	80	72.13	90.16	0.58
		not detected	800	732.51	91.56	0.64
		not detected	8000	7250.88	90.64	0.41
	AuNCs-Fe^{3+}	not detected	80	81.47	101.84	0.35
		not detected	800	763.78	95.47	0.54
		not detected	8000	8563.79	107.05	1.47
CTC	AuNCs-Cu^{2+}	not detected	80	84.19	105.24	1.00
		not detected	800	909.49	113.68	1.86
		not detected	8000	8560.02	107.00	0.74
	AuNCs-Fe^{3+}	not detected	80	84.96	106.20	1.11
		not detected	800	809.05	101.13	0.49
		not detected	80000	7616.94	95.21	0.21
Cip	AuNCs-Fe^{3+}	not detected	80	82.19	102.74	1.42
		not detected	800	808.52	101.07	0.74
		not detected	8000	9142.66	114.28	0.89
	AuNCs-Fe^{2+}	not detected	80	82.16	102.70	0.31
		not detected	800	860.50	107.56	0.75
		not detected	8000	8243.42	103.04	1.13

Van	AuNCs-Fe^{3+}	not detected	80	78.62	98.28	0.07
		not detected	800	843.00	105.37	0.55
		not detected	8000	7231.20	90.39	0.79
	AuNCs-Fe^{2+}	not detected	80	92.03	115.04	0.71
		not detected	800	852.00	106.50	1.41
		not detected	8000	8553.51	106.92	0.67
GM	AuNCs-Fe^{2+}	not detected	80	83.20	104.00	0.19
		not detected	800	738.59	92.32	0.31
		not detected	8000	8294.80	103.69	0.25
	AuNCs-Ag^{+}	not detected	80	87.89	109.86	0.99
		not detected	800	755.38	94.42	1.13
		not detected	8000	7279.47	91.00	0.91

Table S7. LDA identification results of 11 mixture antibiotics and 6 antibiotics with different concentrations. The value in the table is I/I_0.

Grouping variable	Independent variable						Verified
	AuNCs (L-H_2O_2)	AuNCs -Cu^{2+}	AuNCs -Fe^{3+}	AuNCs -Fe^{2+}	AuNCs (H-H_2O_2)	AuNCs -Ag^{+}	
TC + CTC (4000 nM)	1.699	0.521	2.286	1.679	1.541	1.305	TC + CTC
TC + CTC (1000 nM)	1.288	0.643	2.028	1.533	1.474	1.216	TC + CTC
TC + CTC (200 nM)	1.170	0.827	1.978	1.272	1.309	1.034	TC + CTC
TC + CM (4000 nM)	1.204	0.702	1.995	1.396	1.911	1.246	TC + CM
TC + CM (1000 nM)	1.161	0.747	1.837	1.347	1.846	1.130	TC + CM
TC + CM (200 nM)	1.032	0.922	1.530	1.282	1.222	1.063	TC + CM
OTC + CTC (4000 nM)	2.284	0.425	2.339	1.628	1.074	1.182	OTC + CTC
OTC + CTC (1000 nM)	2.039	0.698	2.250	1.530	1.092	1.128	OTC + CTC
OTC + CTC (200 nM)	1.951	0.785	1.902	1.404	1.087	0.998	OTC + CTC
OTC + Cip (4000 nM)	2.518	1.237	3.513	2.280	1.375	2.300	OTC + Cip
OTC + Cip (1000 nM)	2.287	1.200	3.412	2.179	1.348	1.514	OTC + Cip

OTC + Cip (200 nM)	2.179	1.158	2.533	1.496	1.194	1.212	OTC + Cip
OTC + GM (4000 nM)	1.439	0.516	2.115	1.421	1.164	1.759	OTC + GM
OTC + GM (1000 nM)	1.228	0.679	1.868	1.387	1.187	1.688	OTC + GM
OTC + GM (200 nM)	1.138	0.821	1.572	1.332	1.181	1.114	OTC + GM
CTC + Van (4000 nM)	1.705	0.667	2.090	1.350	1.049	1.361	CTC + Van
CTC + Van (1000 nM)	1.689	0.671	2.039	1.347	1.050	1.314	CTC + Van
CTC + Van (200 nM)	1.590	0.844	1.936	1.317	1.094	1.255	CTC + Van
Cip + Van (4000 nM)	2.582	1.267	2.496	2.176	1.574	2.089	Cip + Van
Cip + Van (1000 nM)	2.278	1.296	2.387	2.141	1.298	1.644	Cip + Van
Cip + Van (200 nM)	1.999	1.289	1.956	1.589	1.178	1.503	Cip + Van
Van + GM (4000 nM)	0.498	0.702	0.401	0.630	1.383	1.591	Van + GM
Van + GM (1000 nM)	0.593	0.758	0.506	0.677	1.242	1.161	Van + GM
Van + GM (200 nM)	0.614	0.776	0.593	0.687	1.076	1.149	Van + GM
TC + OTC + CTC (4000 nM)	1.753	0.319	2.420	1.756	1.040	1.334	TC + OTC + CTC
TC + OTC + CTC (1000 nM)	1.689	0.343	2.389	1.736	1.035	1.067	TC + OTC + CTC
TC + OTC + CTC (200 nM)	1.623	0.633	1.488	1.203	1.086	1.059	TC + OTC + CTC
TC + Van + GM (4000 nM)	1.441	0.703	1.600	1.361	1.242	1.241	TC + Van + GM
TC + Van + GM (1000 nM)	1.398	0.843	1.591	1.239	1.214	1.213	TC + Van + GM
TC + Van + GM (200 nM)	1.237	0.854	1.525	1.137	1.098	1.080	TC + Van + GM
Six antibiotics (4000 nM)	1.637	0.387	3.579	1.550	2.209	2.989	Six antibiotics
Six antibiotics (1000 nM)	1.503	0.486	3.473	1.526	2.006	1.704	Six antibiotics
Six antibiotics (200 nM)	1.315	0.657	2.521	1.304	1.430	1.299	Six antibiotics
TC (500 nM)	1.238	0.466	3.066	1.637	0.972	1.253	TC
TC (2500 nM)	1.293	0.341	3.240	1.667	0.966	1.253	TC
TC (5000 nM)	1.309	0.227	3.302	1.675	0.999	1.269	TC
OTC (500 nM)	1.524	0.505	3.077	1.523	1.056	1.399	OTC
OTC (2500 nM)	1.561	0.383	3.385	1.569	1.080	1.557	OTC
OTC (5000 nM)	1.621	0.340	3.698	1.634	1.239	1.617	OTC
CTC (500 nM)	1.413	0.528	2.441	1.203	1.186	1.263	CTC

CTC (2500 nM)	1.529	0.357	2.457	1.242	1.211	1.335	CTC
CTC (5000 nM)	1.646	0.232	2.536	1.301	1.486	1.376	CTC
Cip (500 nM)	2.060	1.164	3.275	1.851	1.114	1.555	Cip
Cip (2500 nM)	2.199	1.244	4.572	1.987	1.188	1.661	Cip
Cip (5000 nM)	2.340	1.267	4.733	2.030	1.251	1.743	Cip
Van (500 nM)	1.021	0.970	0.832	0.902	1.017	1.005	Van
Van (2500 nM)	0.958	1.019	0.829	0.889	1.024	1.015	Van
Van (5000 nM)	1.004	0.952	0.809	0.888	1.031	0.972	Van
GM (500 nM)	0.804	0.817	0.340	0.679	1.004	1.462	GM
GM (2500 nM)	0.781	0.794	0.302	0.633	1.003	1.593	GM
GM (5000 nM)	0.729	0.745	0.316	0.629	1.001	1.711	GM

Table S8. Identification of unknown 11 mixture antibiotics samples in river water.

#	Independent variable						Identity	Verified
	AuNCs (L-H_2O_2)	AuNCs-Cu^{2+}	AuNCs-Fe^{3+}	AuNCs-Fe^{2+}	AuNCs (H-H_2O_2)	AuNCs-Ag^{+}		
1	1.619	0.643	2.128	1.533	1.474	1.216	TC + CTC	TC + CTC
2	1.171	0.757	1.837	1.347	1.846	1.130	TC + CM	TC + CM
3	2.039	0.698	2.550	1.530	0.924	1.128	OTC + CTC	OTC + CTC
4	2.287	1.171	3.412	2.179	1.348	1.514	OTC + Cip	OTC + Cip
5	1.228	0.679	1.868	1.406	1.187	1.688	OTC + GM	OTC + GM
6	1.689	0.671	2.039	1.369	1.050	1.337	CTC + Van	CTC + Van
7	2.328	1.296	2.387	2.141	1.298	1.664	Cip + Van	Cip + Van
8	0.593	0.758	0.506	0.707	1.242	1.161	Van + GM	Van + GM
9	1.689	0.524	1.945	1.736	1.035	1.067	TC + OTC + CTC	TC + OTC + CTC
10	1.411	0.784	1.591	1.324	1.284	1.269	TC + Van + GM	TC + Van + GM
11	1.529	0.486	3.473	1.526	2.006	2.704	Six antibiotics	Six antibiotics