Fluorescence Sensor based on Cascade Signal Amplification Strategy for Ultra-sensitive detection of Cu²⁺

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Figure S1. FDTD simulation of (a) AgNI and (b) AuNR.

2. SEM image of AgNIs.



Figure S2. SEM image of AgNIs.

3. TEM images of CTAB coated AuNRs and polymer coated AuNRs.



Figure S3. TEM image of (a) CTAB-coated AuNRs and (b) polymer-coated AuNRs

4. UV-Vis spectrums and Zeta-potential of CTAB-coated AuNRs and polymercoated AuNRs.



Figure S4. (a) UV-Vis spectrums and (b) Zeta-potential of CTAB-coated AuNRs and polymer-coated AuNRs.

5. Optimization of AuNRs coating times for the fabrication of plasmonic substrate.



Figure S5. Fluorescence intensity on the plasmonic substrate with different AuNRs coating times.

6. SEM image and EDS mappings of AuNRs/AgNIs substrate.



Figure S6. (a) SEM image of AuNRs/AgNIs substrate and EDS mapping (b) Au element (green) (c) Ag element (red) and (d) merge.

7. Fluorescence curves with the concentrations of alkynyl added.



Figure S7. (a) Fluorescence curve with 0, 2, 5, 10, 20, 50, 100, 200 μ M of alkynyl added and (b) the endpoint intensity for 30-min-reaction.

8. Fluorescence curves under different pH.



Figure S8. Fluorescence curve under pH 7, 8, 9, 10, 11, and 12.

9. Click-chemistry-based fluorescence probes for Cu²⁺ detection.



Figure S9. (a) Fluorescence curve for different concentrations of Cu^{2+} in clickchemistry-based sensing system. **(b)** The fitted line was based on the fluorescence intensity at 680 nm and the corresponding concentrations of Cu^{2+} .

10. Drying Temperature.



Figure S10. Time demand for drying under 50 °C, 65 °C and 80 °C.

11. Correlation analysis.



Figure S11. Correlation analysis for results of urinary copper quantification by our platform and ICP-MS.

12. Competitive effect for adsorbing Cu^{2+} by the *E. coli*.



Premixture Time

Figure S12. Intensity obtained using our platform of 10 μ M Cu²⁺ premixed with 5×10⁷ CFU/mL *E. coli* for 1, 5, and 10 min.

13. Influence of different concentrations of Cef-susceptible E. coli and Cef-resistant E.



coli for sensing system containing different amounts of copper ions.

Figure S13. Calibration curves obtained by intensity change received by our platform for (**a**,**b**) 1 μ M, (**c**,**d**) 5 μ M and (**e**,**f**) 10 μ M Cu²⁺ as a catalyst with different concentrations of added (**a**,**c**,**e**) Cef-susceptible *E. coli* and (**b**,**d**,**f**) Cef-resistant *E. coli*.

	Our sensor (µM)	ICP-MS (µM)
Volunteer 1	0.89	1.03
Volunteer 2	0.75	0.93
Volunteer 3	0.88	1.02
Volunteer 4	0.85	1.08
Volunteer 5	1.09	1.28
Volunteer 6	1.09	1.29
Patient 1	2.05	2.32
Patient 2	1.82	2.14
Patient 3	2.04	2.22
Patient 4	2.08	2.22
Patient 5	2.23	2.47
Patient 6	2.04	2.41

 Table S1. Urinary copper detection by our sensor and ICP-MS.

The test result of each subject was expressed as the average of more than three

parallel experiments.

Table S2. Clinical UTI samples used in this study. "+" = positive, "-" = negative, GER = department of geriatrics, NEP = department of nephrology, SUR = department of pain clinical, CHI = department of traditional Chinese medicine, END = department of endocrinology, ORT = department of joint branch, DER = department of dermatology, ECU = department of emergency, ICU = intensive care unit, PED = department of pediatrics, URS = department of urology, ONC = department medical oncology, NEH = department of rehabilitation.

Samula	Unin a ID	Donortmont	Service	Ceftriaxone	
Sample	Urine ID	Department	Species	MIC (µg/mL)	
1	210716021	GER	E. coli	>32	
2	210908008	NEP	E. coli	>32	
3	211205001	SUR	E. coli	>32	
4	211006004	CHI	E. coli	>32	
5	210925002	END	E. coli	>32	
6	211015013	ORT	E. coli	>32	
7	210711016	ORT	E. coli	>32	
8	211206010	NEP	E. coli	>32	
9	210703003	DER	E. coli	>32	
10	210627025	ECU	E. coli	>32	
11	210826013	PED	E. coli	>32	
12	210713043	PED	E. coli	>32	
13	211207002	END	E. coli	<=1	
14	210305004	NEP	E. coli	<=1	
15	210411008	GER	E. coli	<=1	
16	210103002	END	E. coli	<=1	
17	211027001	CHI	E. coli	<=1	
18	210206017	CAR	E. coli	<=1	

19	210120036	ECU	E. coli	<=1
20	210112003	SUR	E. coli	<=1
21	210417027	URS	E. coli	<=1
22	210305006	END	E. coli	<=1

All	the	UTI	samples	were	collected	from	Huazhong	University	of	Science	and
Tec	hnol	ogy L	Jnion She	nzhen	Hospital.						

Bacteria	AUC	Cutoff	Sensitivity	Specificity	Accuracy
Daeteria	nee	Cuton	(%)	(%)	(%)
	0.9861	3231	100	90.91	95.45
E. coli				(58.72-	(77.16-
			(71.31-100)	99.77)	99.88)

Table S3. Sensitivity and Specificity of AST for UTI samples using our sensor

Ninety-five percent confidence intervals are indicated in parentheses.