Electronic Supplementary Information

Rapid binary visual detection of oxalate in urine sample of urolithiasis patients *via* competitive recognition and distance reading test strips

Piaopiao Chen*[a,#], Lihang Cen[b,#], Yue Wang[a,#], Yunjin Bai[a,#], Tian Shi[a], Xianhua Chen[b]

[a] Department of Laboratory Medicine, Med+X Center for Manufacturing, West China Hospital, Sichuan University, Chengdu, Sichuan, 610041, China

[b] Department of Clinical Laboratory, Liuzhou Key Laboratory of Molecular Diagnosis, Guangxi Key Laboratory of Molecular Diagnosis and Application, Affiliated Liutie Central Hospital of Guangxi Medical University, Liuzhou, Guangxi, 545007, China

*Corresponding author. E-mail: chenpp0828@wchscu.cn.

[#] Piaopiao Chen, Lihang Cen, Yue Wang and Yunjin Bai contributed equally to this work.

Content

Reagents and Materials. Na_2TeO_3, 3-mercaptopropionic acid (MPA), $NaNO_3$, and $Mg(NO_3)_2$ were ordered from Aladdin Reagent Co. (Shanghai, China). $CdCl_2$, KBH_4, $Na_3C_6H_5O_7 \cdot 2H_2O$, HCl, NaOH, and nitric acid (HNO_3) were purchased from Kelong Chemical Reagents (Chengdu, China). CuCl and $CuCl_2$ were obtained from Shanghai Sangon Biotechnology Co., Ltd (Shanghai, China). Calcein (Fig. S1), ascorbic acid (AA), cysteine (Cys), glutathione (GSH), glucose, urea, NaCl, KCl, $MgCl_2$, $CaCl_2$, K_2HPO_4, KH_2PO_4, $BaCl_2$, $MnSO_4$, $AlCl_3$, $Fe(NO_3)_3$, $CdCl_2$, $Co(NO_3)_3$, $ZnCl_2$, and oxalate were purchased from Sigma Company (Shanghai, China). 3-(*N*-Morpholino) propanesulfonic acid (MOPS) was purchased from Solarbio Technology Co.; Ltd (Beijing, China). Human urine samples were donated from the West China Hospital of Sichuan University (Chengdu, China. approval number: 2018182). All work solutions were prepared with 10 mM pH 7.3 MOPS buffer (100 mM $NaNO_3$). High purity deionized water (18.2 MΩ-cm) was obtained from Milli-Q water system (Chengdu Ultrapure Technology Co., Ltd., Chengdu, China). All solutions were stored at 4 °C in a refrigerator until use.

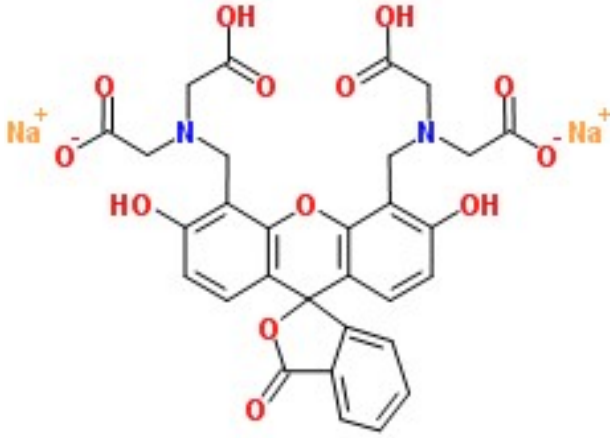

Fig. S1. The chemical structure of calcein disodium salt.

Instruments. The absorption and fluorescence spectrum of calcein and CdTe QDs were recorded using the Duetta Spectrophotometer (HORIBA Canada Inc). High-resolution transmission electron microscope (HR-TEM) measurements of CdTe QDs, Cu^{+} + QDs, and Cu^{2+} + QDs were carried out by a Tecnai G2F20 STWIN TEM at an accelerating voltage of 200 kV (FEI Co.; USA).

Synthesis of CdTe QDs. CdTe QDs were synthesized referring to a previously reported method.[1-3] First, a 50 mL solution of $CdCl_2$ (0.5 mmol) and trisodium citrate (0.2 g) was prepared. MPA (52 μL) was then instantly added to the solution, and the solution pH was adjusted to 10.5 using NaOH. Na_2TeO_3 (0.1 mmol) and KBH_4 (50 mg) were added to the prepared solution and refluxed for 1 h to obtain CdTe QDs. Subsequently, CdTe QDs of high purity was obtained by precipitating with *n*-propanol and centrifuging (11000 rpm). The purified red CdTe QDs were dispersed in ultrapure water before use.

Analysis of oxalate.

QDs as signal reporters: First, 50 μL of 10 mM MOPS buffer (100 mM $NaNO_3$, pH 7.3), 5 μL $CuCl_2$ solution (80 μM), and 40 μL different concentrations of oxalate (1 nM-10 mM) were mixed at room temperature for 1.5 min for the reduction reaction. CdTe QDs solution (stock solution, 0.3 μL) was added to the solution at room temperature for 2.5 min to complete the cation exchange reaction. Finally,

fluorescence emission spectra were obtained by scanning from 500 to 750 nm at 365 nm.

Calcein as signal reporters: First, 50 μL of 10 mM MOPS buffer (100 mM $NaNO_3$, pH 7.3), 5 μL $CuCl_2$ solution (80 μM), and 40 μL different concentrations of oxalate (1 nM-10 mM) were mixed at room temperature for 1.5 min for the reduction reaction. Approximately 16 μL calcein (25 μM) was added to the solution at room temperature for 30 s to complete the complexation reaction. Finally, fluorescence emission spectra were obtained by scanning from 500 to 650 nm at 486 nm.

Optimization of conditions for oxalate analysis

We have optimized the conditions affecting the analytical performance to obtain better experimental results (Fig. S2). We found that as the reaction time of Cu^{2+} and oxalate increased, the fluorescence values of calcein and QDs gradually decreased, and the reaction was completed after 90 s (Fig. S2A). As the volume of Cu^{2+} increased, the difference between the fluorescence signals of the oxalate-containing solution (oxalate + Cu^{2+}+ calcein + QDs) and the blank solution (lower content of oxalate + Cu^{2+} + calcein + QDs) initially increased and then decreased. The highest difference value was observed when the volume of Cu^{2+} was 7 μL. When the volune of Cu^{2+} was 7 μL 50 μM, Cu^{2+} was maximally reduced to Cu^{+} by oxalate (Fig. S2B and S2C). When the volumes of calcein (10 μM) and QDs (five times diluted) were 4.8 μL and 3 μL, respectively, the difference in fluorescence signal values between

blank solution and oxalate solution was the highest (Fig. S2D and S2E). Furthermore, the Cu^{+} reduction product of oxalate and Cu^{2+} had a fast-quenching effect on calcein and QDs, which can be completed within 90 s (Fig. S2F).

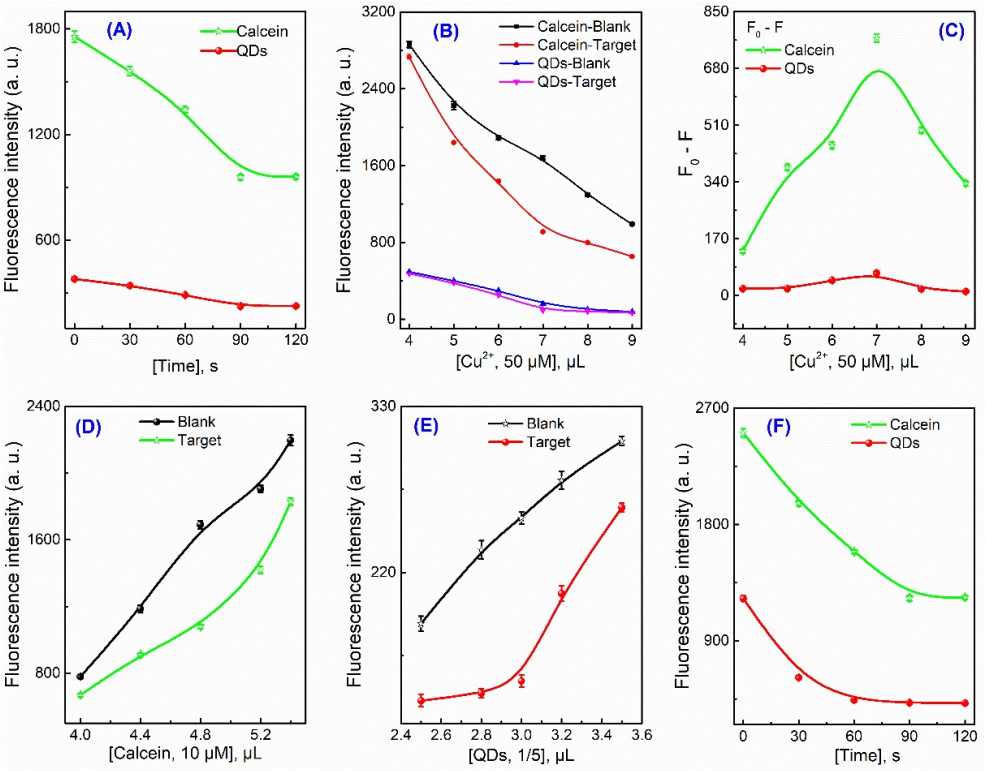

Fig. S2. Optimization of conditions for oxalate analysis. (A) Time for oxalate to reduce Cu^{2+}. (B, C) Concentration of Cu^{2+}. (D) Amount of calcein. (E) Amount of QDs. (F) Time of complexation and cation exchange reaction between calcein and QDs with Cu^{2+}. The error bars resulted from three repeated measurements.

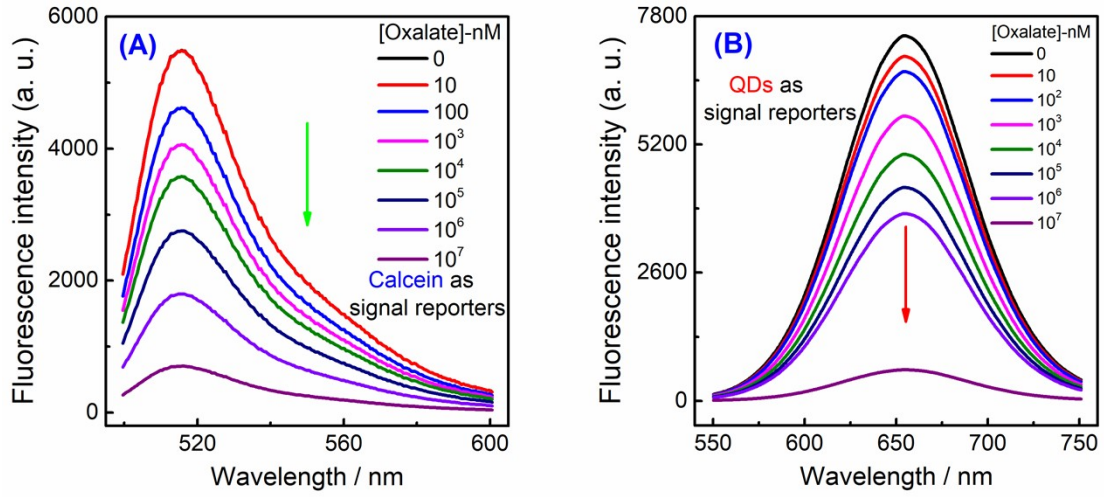

Fig. S3. Analytical performance of oxalate. (A and B) Spectrum and fluorescence signal variation of quantitative analysis of oxalate used calcein and QDs as signal reporters, respectively. Error bars originated from at least three repeated measurements.

Table S1. Comparison of methods for the determination of oxalate

Method	Linear range; Limit of detection;	Sample	Reference
Colorimetric (ZnS QDs)	1 -10 nM; 0.2 nM	NM[a]	4
Colorimetric (TMB-MnO_2)	7.8-250 μM; 0.91 μM	Artificial urine	5
Fluorescence (Carbon dots-Cu^{2+})	10–70 mM; 1 mM	NM	6
IELC-MS[b]	10 -500 μM; 2 μM	Mouse urine and primary mouse hepatocytes	7
LC-MS[c]	53 nM-10 μM	Soil	8
HPLC[d] ($Ru(phen)_2$, $Ce(SO_4)_2$, H_2SO_4)	10 μM-4 mM; 6.2 μM	Spinach	9
Electrochemical (Nano-g-C_3N_4)[e]	1-1000 μM; 0.75 μM	Urine; recovery	10
Electrochemical (SiO_2-Pt NPs/GCE)[f]	0-45 μM; 25 nM	Tomato; Spinach	11
Electrochemical; (Graphite/Ag/AgCl nanocomposite)	0.01-0.75 mM; 3.7 μM	Urine; Spinach; recovery	12
Electrochemical (Pt/CB-Ni-rGO)[g]	20 μM-60 mM; 2.35 μM	Artificial urine; recovery	13
Electrochemical; (NH_2-GQD-GO)[h]	0.5-2.0 and 2-55 mM; 50 μM	Spiked urine	14
Electrochemical; (GNPs-MWCNT/Au)[i]	1-800 μM; 1 μM	Urine, serum, fruit, vegetables	15
Electrochemical (Graphene-Ag NRs)	3-30 mM; 0.04 mM	Tap water; recovery	16
Electrochemical; (Pd/SBA-15/CPE)[l]	10-140 μM; 0.4 μM	Tomato; onion	17
Electrochemical; (WC NTs-PtNPs)[j]	0-125 nM; 12 nM	Tomato	18
Fluorescence (QDs-Cu^{2+})	100 nM-10 mM; 120 nM	Urine; patients with urinary stones	19
Fluorescence (calcein; QDs; Cu^{2+})	10 pM-10 nM；3 pM	Urine; patients with urinary stones	This work

[a] Not mentioned; [b] ion exclusion chromatography-mass spectrometry; [c] liquid chromatography-mass spectrometry: [d] high performance liquid chromatography; [e] graphite carbon nitride; [f] glassy carbon electrode; [g] platinum/carbon black-nickel-reduced graphene oxide; [h] amino-functionalized graphene quantum dots graphene oxide; [i] multi-walled carbon nanotubes.

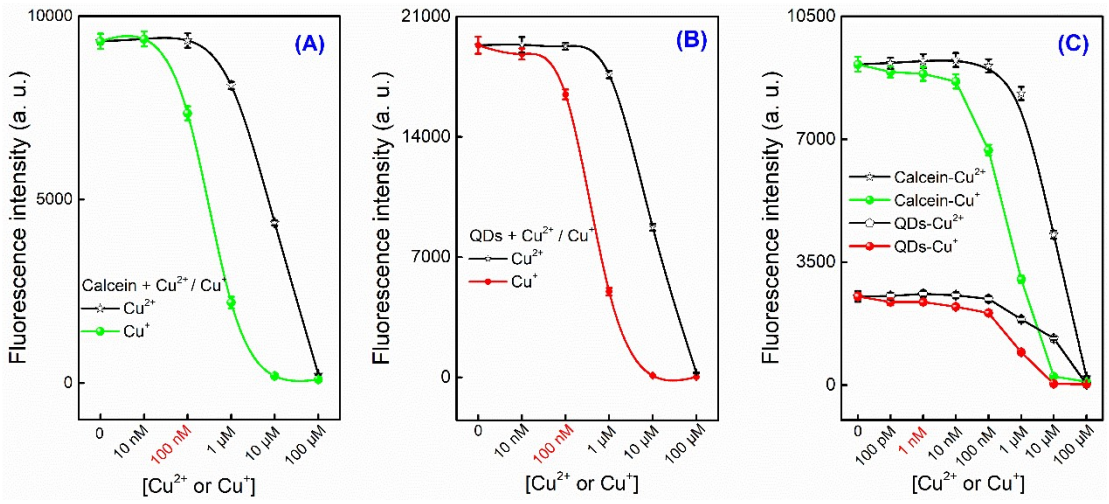

Fig. S4. The effect of different concentrations of Cu^{2+}/Cu^{+} on the fluorescence signals of calcein and QDs, respectively (A, B). The effect of different concentrations of Cu^{2+}/Cu^{+} on the fluorescence signal in the dual-signal mode of calcein and QDs (C). Error bars taken from three repeated tests.

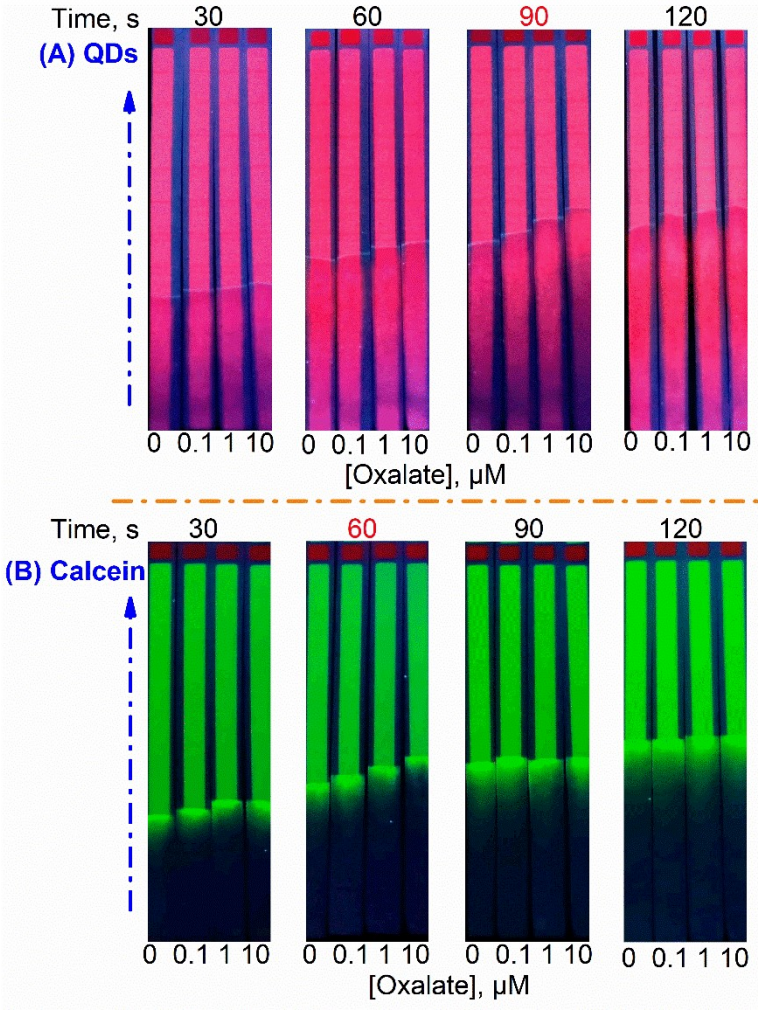

Fig. S5. Optimization of test strip detection time. (A) QDs, (B) Calcein.

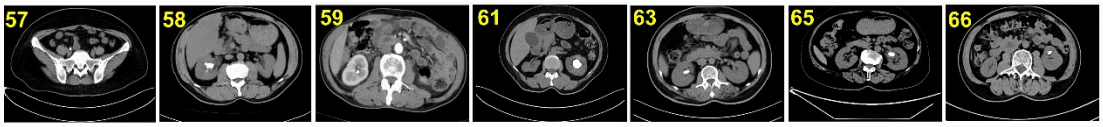

Fig. S6. Clinical CT images of uric acid stone patients.

References

1 P. P. Chen, Y. J. Bai, S. Y. Tang, N. Wang, Y. Q. He, K. Huang, J. Huang, B. W. Ying and Y. Cao, *Nano Lett.*, 2022, **22**, 1710-1717.

2 P. P. Chen, Y. Q. He, T. Y. H. Liu, F. L. Li, K. Huang, D. Tang, P. J. Jiang, S. J. Wang, J. Zhou, J. Huang, Y. Xie, Y. G. Wei, J. Chen, W. Hu and B. W. Ying, *Biosens. Bioelectron.*, 2022, **202**, 114009.

3 P. P. Chen, Y. Wang, Y. Q. He, K. Huang, X. Wang, R. H. Zhou, T. Y. H. Liu, R. L. Qu, J. Zhou, W. Peng, M. Li, Y. J. Bai, J. Chen, J. Huang, J. Geng, Y. Xie, W. Hu and B. W. Ying, *ACS Nano*, 2021, **15**, 11634-11643.

4 R. N. Juine and A. Das, *ACS Sustainable Chem. Eng.*, 2020, **8**, 11579-11587.

5 Y. Gan, N. Hua, C. J. He, S. Q. Zhou, J. W. Tu, T. Liang, Y. X. Pan, D. Kirsanov, A. Legin, H. Wan and P. Wang, *Biosens. Bioelectron.*, 2019, **130**, 254-261.

6 S. R. Zhang, Q. Wang, G. H. Tian and H. G. Ge, *Mater. Lett.*, 2014, **115**, 233-236.

7 A. Schriewer, M. Brink, K. Gianmoena, C. Cadenas and H. Hayen, *J. Chromatogr. B.*, 2017, **1068**, 239-244.

8 L. Jaitz, B. Mueller, G. Koellensperger, D. Huber, E. Oburger, M. Puschenreiter and S. Hann, *Anal. Bioanal. Chem.*, 2011, **400**, 2587-2596.

9 F. W. Wu, Z. K. He, Q. Y. Luo and Y. E. Zeng, *Food Chem.*, 1999, **65**, 543-546.

10 T. Alizadeh, S. Nayeri and N. Hamidi, *RSC Adv.*, 2019, **9**, 13096-13103.

11 Y. S. Fang, X. Y. Xu, X. Q. Guo, B. Cui and L. S. Wang, *Anal. Bioanal. Chem.*, 2020, **412**, 5719-5727.

12 T. Alizadeh and S. Nayeri, *Mater. Sci. Eng. C.*, 2019, **100**, 826-836.

13 K. Income, N. Ratnarathorn, S. Themsirimongkon and W. Dungchai, *J. Electrochem. Sci. Technol.*, 2019, **10**, 416-423.

14 P. Mishra and B. R. Bhat, *Microchim Acat*, 2019, **186**, 646.

15 C. S. Pundir, N. Chauhan, Rajneesh, M. Verma and Ravi, *Sens. Actuators B Chem.*, 2011, **155**, 796-803.

16 R. D. Nagarajan and A. K. Sundramoorthy, *Sens. Actuators B Chem.*, 2019, **301**, 127132.

17 J. B. Raoof, F. Chekin and V. Ehsani, *Sens. Actuators B Chem.*, 2015, **207**, 291-296.

18 T. Maiyalagan, P. Kannan, M. Jönsson-Niedziolka and J. Niedziolka-Jönsson, *Anal. Chem.*, 2014, **86**, 7849-7857.

19 P. P. Chen, Y. J. Bai, Y. Tang, S. X. Yan, X. Y. Wang, W. R. Wei, J. Wang, M. Zhang, B. W. Ying and J. Geng, *J. Mater. Chem. B*, 2020, **8**, 7677-7684.