Supporting Information

Detection and discrimination of glutathione among biological thiols based on the oxalyl dihydrazide decorated sulfur nanodots

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Experimental Section

Materials:

Sublimation sulfur powder, polyethylene glycol (PEG-400), L-Glutathione reduced (GSH) Homocysteine (Hcy), and Cysteamine (CA) were bought from Aladdin. Iron nitrate nonahydrate (Fe(NO3)3·9H2O), anhydrous magnesium chloride (MgCl2), zinc nitrate hexahydrate (Zn(NO3)2),L-glutamine (Gln), and L (+)-ascorbic acid (Vc) were obtained from Macklin (Shanghai). Sodium chloride (NaCl), potassium chloride (KCl), ethylene glycol, and copper acetate monohydrate were bought from Tianjin Bei chen Founder Reagent Factory. Sodium hydroxide (NaOH), Oxalyldihydrazide (ODH), L-histidine (His), L-alanine (Ala), and L(+)-glutamic acid (Glu) were obtained from Innochem. Human blood samples were taken from the Affiliated Hospital of Hebei University. The ELISA test kit for GSH was obtained from ADANTI (Wuhan, China) , with an item No. of AD20773.

Characterization:

The device transmission electron microscope images were acquired from a transmission electron microscope (JEOL-2100Plus, JEOL, Japan). X-ray photoelectron spectroscopy (XPS) measurements were performed on a photoelectron spectrometer (ESCALAB-MK II 250, Thermo, USA). The fluorescence spectra were carried out on a photoluminescence spectrophotometer (F-7000, Hitachi, Japan). Time-resolved fluorescence decay curves were measured from a time-resolved fluorescence spectrometer (FLS980, Edinburgh, UK). The fluorescence quantum yield was measured through an absolute method by directly comparing the absorbed light and the emitted light, aided by a spectrometer (FLS980, Edinburgh, UK), equipped with integrating sphere. Fourier transform infrared spectroscopy (FTIR) were obtained on a Fourier transform infrared spectrometer (Nicolet iS10, Thermo, USA).

Synthesis of S-dots:

S-dots were synthesized by previous reported methods.[1, 2] Powder of sublimed sulfur (1.4 g), polyethylene glycol (3.0 mL), sodium hydroxide (4.0 g) and deionized water (50.0 mL) were in turn added into a 100 mL round bottom flask. After heating the mixture at 90 ℃ for 72h, the sublimated sulfur gradually dissolved into a dark-yellow solution, which gradually transferred into a light-yellow solution. Then, 3.0 mL of the mixture was mixed with 4.0 mL of H2O2 solution (5.5 wt.%) in a 15 mL centrifuge tube under vigorous stirring. The mixture showed intensive blue emission, indicating the formation of luminescent S-dots. The as-obtained products were centrifuged and stored at 4 ℃ for further use.

Detection of GSH:

S-dots (50 μL) were mixed with 30 mM of ODH (600 μL, dissolved in PBS buffer with a pH of 8.0), which was incubated for 9 h at room temperature. Then, various concentrations of GSH solutions (200 μL) were added into the above mixture solutions. After reacting for 30 min, the fluorescence spectra were collected, and the data was used to bult a calibration curve. For the detection of GSH in human serum, fresh blood samples were collected from healthy volunteers. Human serum samples were obtained according to standard processing methods. The samples were diluted by 20 times for

direct detection. Then, the human serum samples were treated as the GSH for the subsequent detection. A calibration curve was obtained by adding different concentrations of GSH into the diluted human serum samples, followed by recording the fluorescence response of ODH@S-dots. The LOD was calculated based on the triple standard deviation of blank samples divided by slope of the linear regression equation.

Specificity and salt tolerance test:

In order to verify the specificity of the system for GSH, Na+, K+, Mg2+, Zn2+, Fe3+, Ala, His, Glu, Gln, Vc, and Cys were added to the system for specificity test. Other conditions were the same as for GSH detection. The concentrations of these reagents are the same as those in human serum, as listed in Table S3. To evaluate the salt tolerance of the detection system, a series of concentrations of NaCl (0-100 mM) were added to the detection system before conducting GSH testing. We also tested the specificity of several thiol reagents (Cys, CA ,Hcy, 2-Me) on the system.

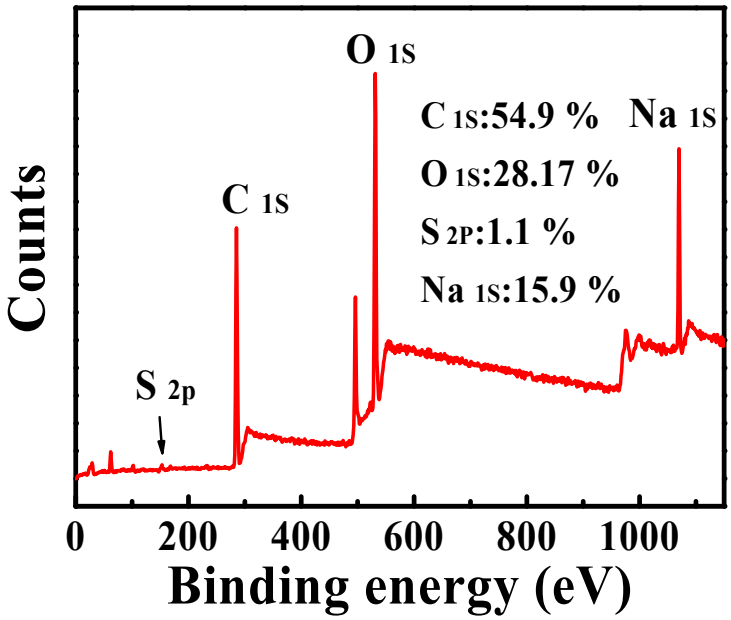

Figure S1. Full scan XPS spectrum of S-dots.

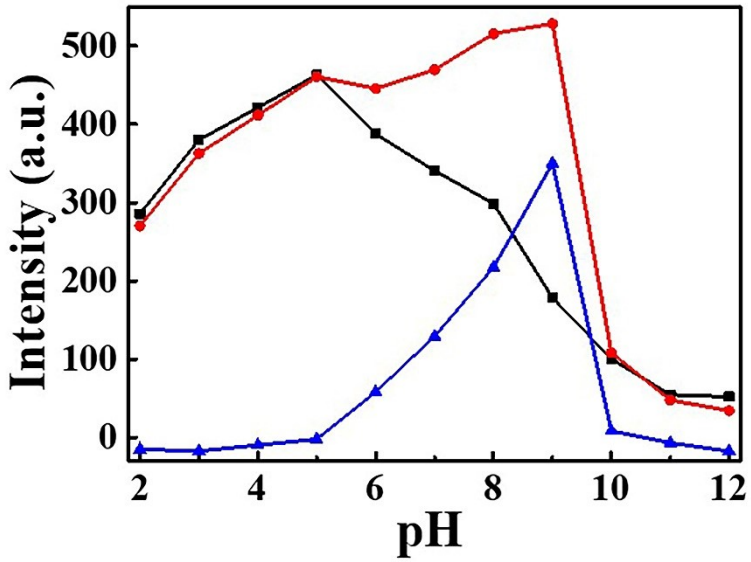

Figure S2. Evolution of fluorescence intensities as a function of pH for ODH@S-dots in the presence (red line) and absence (black line) of GSH, and the recovered fluorescence intensities (blue line). All the spectra were recorded under the excitation of 350 nm.

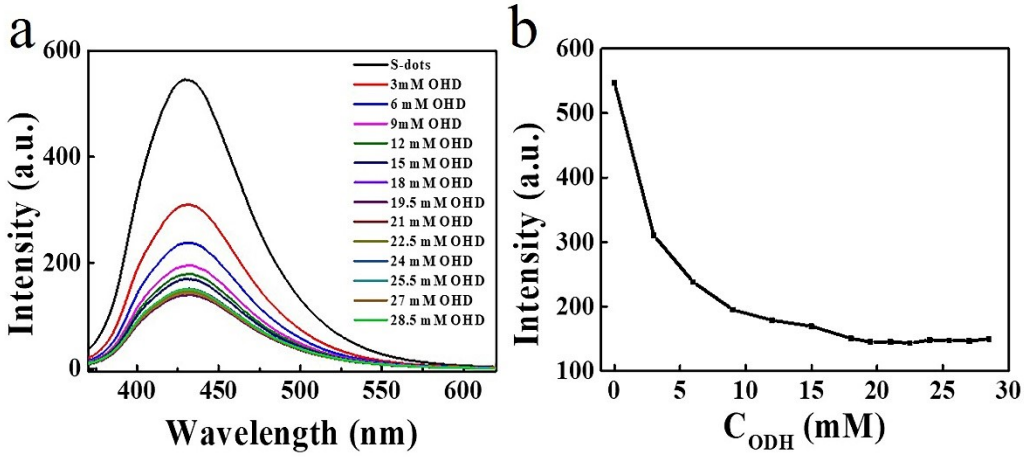

Figure S3. Fluorescence spectra of the detection system after adding different concentrations of ODH (a). Revolution of fluorescence intensities as a function of ODH concentration (b). All the spectra were recorded under the excitation of 350 nm.

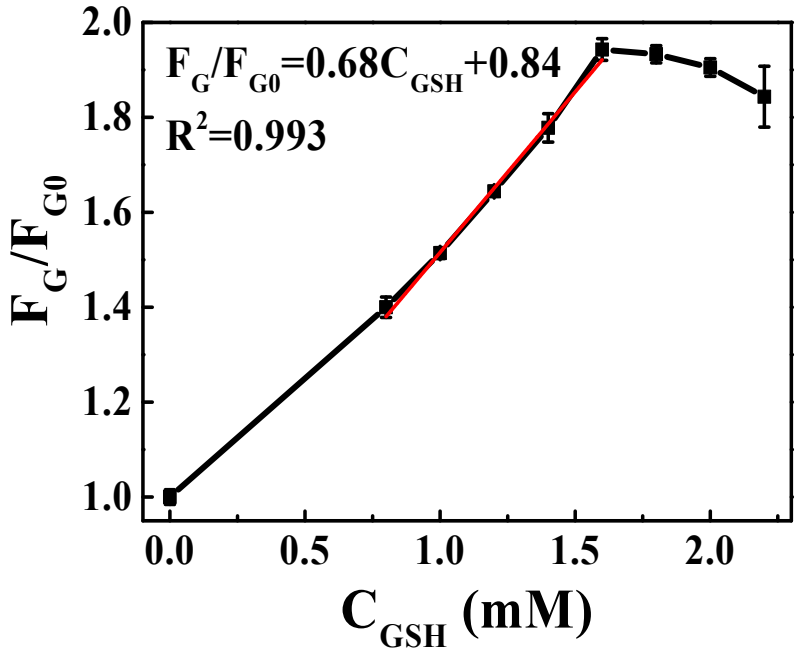

Figure S4. The relationship between the fluorescence ratio of F_G/F_{G0} and the concentration of GSH in the matrix of human blood samples. The intensities are recorded at 425 nm.

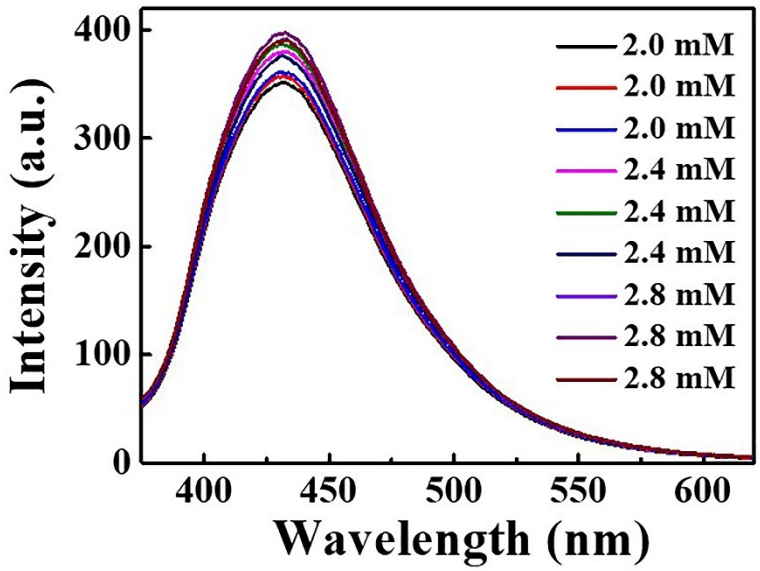

Figure S5. Fluorescence spectra of recovery test after adding different concentrations of GSH.

Table S1. Fluorescence lifetimes ($\tau_{1,2,3}$, ns) and the fraction of the emission intensity ($f_{1,2,3}$ %) obtained from fittings of experimental fluorescence decay curves of S-dots, ODH@S-dots and ODH@S-dots with GSH.

Sample	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	τ_{avg} (ns)
S-dots	1.37 (46.79 %)	2.21 (47.66 %)	14.30 (5.55 %)	2.49
ODH@S-dots	0.94 (35.20 %)	2.19 (58.06 %)	12.84 (6.75 %)	2.47
ODH@S-dots+GSH	0.99 (44.12%)	2.45 (49.59%)	13.63 (62.9%)	2.51

Table S2. Comparison of the detection performance of our assay with recently reported works.

Probes	LOD	Linear range	Ref
CR-POM	510 μM	500-1000 μM	ACS Applied Materials&Interfaces 2019,11,31,27558-27567
UiO-67-sbdc	97.5 μM	0.5-10 mM	Journal of Solid State Chemistry 2019, 270, 317-323
CQDs-MnO_2 nanocomposites	15 μM	50-200 μM	ACS Applied Nano Materials 3 (2020) 5955-5964
cG-Nanopore	100 μM	0.5-10 mM	Anal. Chemistry 2021, 93, 4240-4245
Cou-Br	90 μM	0-15 mM	ACS Sens. 2020, 5, 242−249
ODH@S-dots	119.6 μM	800-3600 μM	This work

Table S3. Concentrations of the interferant used during robustness test.

Interferent	Concentration
Na^+	1.6 mM
K^+	2.0 mM
Mg^{2+}	0.29 mM
Zn^{2+}	50 μM
Fe^{3+}	10 μM
Alanine(Ala)	50 μM
Histidine(His)	50 μM
Glutamic acid(Glu)	50 μM
Glutamine(Gln)	50 μM
Vitamine(Vc)	50 μM
Cysteine(Cys)	250 μM

References

1. Y.-e. Shi, P. Zhang, D. Yang and Z. Wang, ***Chem. Commun.***, 2020, 56, 10982-10988.

2. H. Wang, Z. Wang, Y. Xiong, S. V. Kershaw, T. Li, Y. Wang, Y. Zhai and A. L. Rogach, ***Angew. Chem. Int. Ed.*** , 2019, 58, 7040-7044.