# Electronic Supplementary Information

## A simple and sensitive fluorescence assay for biothiols and acetylcholinesterase activity detection based on HSA-AuNCs@$Cu^{2+}$ complex

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**Chemicals and Materials**

Acetylcholinesterase (AChE), glucose oxidase (GOD), alkaline phosphatase (ALP), acetylthiocholine (ATCh), N-ethylmaleimide, thrombin, exonuclease I (Exo I), protein kinase (PKA), lipase, and lysozyme were purchased from Sigma-Aldrich (Shanghai, China). $HAuCl_4$, glutathione (GSH), cysteine (Cys), homocysteine (Hcy), phenylalanine (Phe), glycine (Gly), threonine (Thr), histidine (His), alanine (Ala), glutamine (Gln), arginine (Arg), tyrosine (Tyr), valine (Val), serine (Ser), proline (Pro), lysine (Lys), and human serum albumin (HSA) were purchased from Sangon Biotech Group Co., Ltd. (Shanghai, China). All reagents of analytical grade were directly used without additional purification. All reagents were used as received without further purification. All solutions were prepared with ultrapure water.

**Instruments**

UV-visible (UV-vis) absorption spectra were taken using a Cary Bio-100 UV/Vis spectrometer (Varian). Fluorescence experiments measurement was carried on F-4600 spectrophotometer (Hitachi, Japan). A BRUKER TENSOR 27 spectrophotometer (Bruker, Germany) was employed to collect the Fourier-transform infrared spectrum (FT-IR) in the range of 4000-400 $cm^{-1}$. Transmission electron microscopy images (TEM) were obtained by transmission electron microscope (JEOL Model JEM-2010F) at an accelerating voltage of 200 kV.

**Real sample assay**

In order to prove the viability of our proposed sensors for practical applications, human serum was adopted as a model matrix. Human serum samples were treated by

centrifugation at 10,000 rpm for 10 min. Next, the supernatant was diluted by ultrapure so that original GSH concentration could fall in the standard calibration curve of this assay. The supernatant was spiked with additional known concentrations of GSH (1, 2, and 5 μM). The detection procedure was the same as that described in the aforementioned experiment for GSH detection in buffer. To detect AChE, the obtained human serum samples were diluted 500 times followed by adding N-ethylmaleimide to eliminate the interferences of biothiols. AChE with different concentrations (1, 2 and 5 mU/mL) was respectively added to prepare the spiked samples. The prepared samples were detected according to the aforementioned steps. The serum from volunteers was collected by the First Affiliated Hospital of Zhengzhou University, and informed consent was obtained. All experiments were performed in compliance with the relevant laws and institutional guidelines and approved by the Life Science Ethics Review Committee of Zhengzhou University.

**Table S1.** Comparison of different methods for the determination of GSH and Cys.

| Detection method | Cys | | GSH | | Ref. |
|---|---|---|---|---|---|
| | Linear range (μM) | LOD (μM) | Linear range (μM) | LOD (μM) | |
| Fluorescence / silver nanoclusters | 0.1-1.5 | 0.045 | — | — | 1 |
| Fluorescence / silver nanoclusters | 0.025-0.2 | 0.0021 | 0.025-0.2 | 0.0062 | 2 |
| Fluorescence / silver nanoclusters | 0.1–10 | 0.042 | 0.5–6 | 0.38 | 3 |
| Fluorescence/ CdTe/CdSe quantum dots | 0.2–100 | 0.131 | 0.2–100 | 0.02 | 4 |
| Fluorescence/ g-$C_3N_4$ nanosheet–$MnO_2$ nanocomposite | — | — | 0–2000 | 0.2 | 5 |
| Fluorescence/ CdTe quantum dots (QDs)−Hg(II) system | 2–20 | 0.6 | 0.6–20 | 0.1 | 6 |
| Fluorescence/ organic fluorescence probe | 0–0.35 | 0.05 | 0–0.35 | 0.053 | 7 |
| Colorimetry / $Fe_3O_4$ magnetic nanoparticles | — | — | 3–30 | 3 | 8 |
| Colorimetry / carbon nanodots | — | — | 0–7 | 0.3 | 9 |
| Fluorescence/ DNA-Ag NCs | 0.5–4.5 | 0.134 | 0–5 | 0.172 | 10 |
| Fluorescence/ HSA-AuNCs@$Cu^{2+}$ complex | 0.02–0.4 | 0.016 | 0.02–0.5 | 0.012 | This work |

**Table S2.** Comparison of different methods for the determination of AChE.

| Mode | Materials used | Linear range (mU/mL) | LOD (mU/mL) | Ref. |
|---|---|---|---|---|
| Fluorescence | DNA-Ag NCs | 0-4 | 0.071 | 10 |
| Fluorescence | carbon quantum dots | 14.2–121.8 | 4.25 | 11 |
| Fluorescence | conjugated polyelectrolytes | — | 50 | 12 |
| Fluorescence | organic fluorescence probe | 0–100 | 5 | 13 |
| Electrochemistry | silver electrode | 10–100 | 10 | 14 |
| Colorimetry | anionic polythiophene derivative | 200–1500 | 200 | 15 |
| Colorimetry | gold nanoparticles | 0.6–2 | 0.6 | 16 |
| Fluorescence | poly T-templated CuNPs | 0.11-2.78 | 0.05 | 17 |
| Fluorescence | DNA-templated silver nanoclusters | 0.1-1.25 | 0.05 | 18 |
| Fluorescence | HSA-AuNCs@$Cu^{2+}$ complex | 0.05-10 | 0.05 | This work |

**Fig. S1.** Effects of UV irradiation time on the fluorescence intensity variation of the HSA-AuNCs.

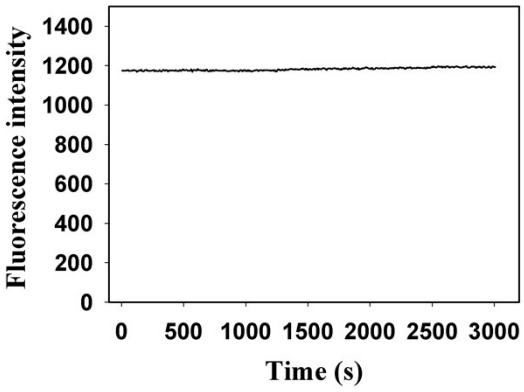


**Fig. S2.** Effect of the amount of $Cu^{2+}$ ions as quenchers on the fluorescence of the nanosystem. Error bars are standard deviation of three repetitive experiments.

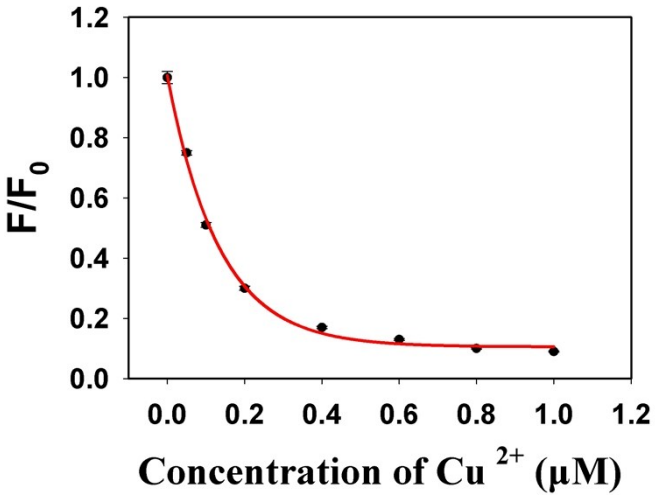


**Fig. S3.** Fluorescence intensity of HSA-AuNCs@ $Cu^{2+}$ complex after the addition of GSH as a function of time.

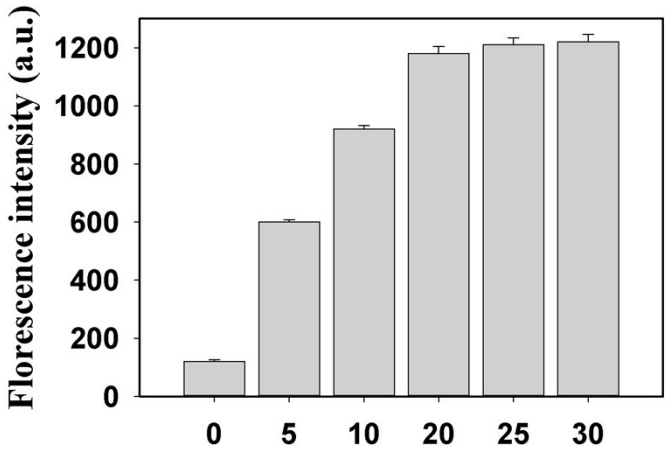


**Fig. S4.** (A) Fluorescence spectra of the HSA-AuNCs@$Cu^{2+}$ complex in the presence of increasing amounts of Hcy. The arrow indicates the signal changes with Hcy increasing (from bottom to top, 0, 50, 100, 200, 300, 400, and 600 nM). (B) Calibration curve of the assay system for Hcy detection.

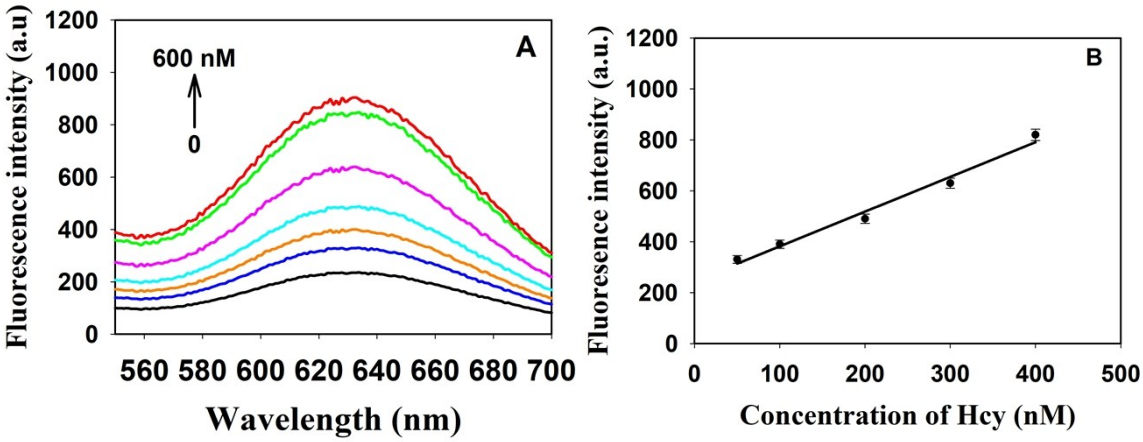
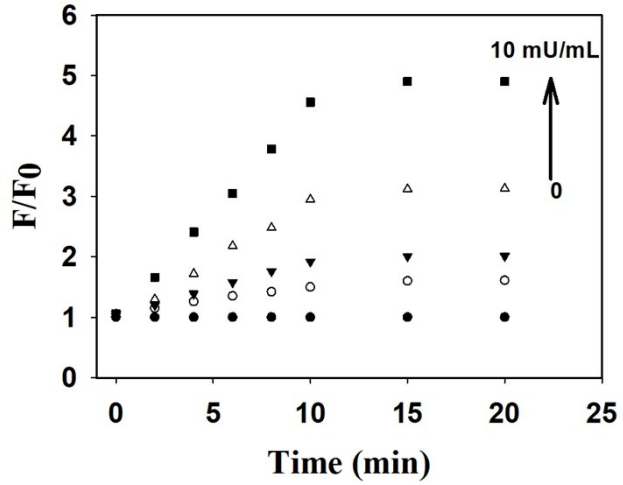


**Fig. S5** Kinetic detection of AChE activity: variation of fluorescence recovery efficiency versus the reaction time for the HSA-AuNCs@$Cu^{2+}$ complex solutions containing ATCh with different concentrations of AChE (0, 0.5, 1, 2.5, 5.0, and 10.0 mU/mL).



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