Electronic Supplementary Information for

A highly sensitive colorimetric metalloimmunoassay based on copper-mediated etching of gold nanorods

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1.1 Experimental section

1.1.1 Materials and reagents

Human IgG, goat anti-human IgG antibody and bovine serum albumin (BSA) were purchased from Beijing Dingguo changsheng biotechnology Co., Ltd (Beijing, China). Sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 4H_2O$), HBr, H_2O_2, Na_2CO_3, $NaHCO_3$, $Na_2HPO_4 \cdot 12H_2O$, hexadecyl xanthate (HDX), hexadecylamine (HDA), dithiolthreitol (DTT), 1,1-carbonyl diimidazole (CDI), anhydrous dioxane, toluene and Tween 20 were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other reagents were of analyticcal grade or better. Solutions were prepared with deionized water (18.2 MΩ, Pall Cascada).

Transmission electron microscopy (TEM) analyses were performed on a JEM-1230 electron microscope (Japan) operating at 100 kV. Extinction spectra were recorded on a Thermo Scientific NanoDrop 2000/2000C spectrophotometer.

1.1.2 Preparation of CuS nanoparticles and CuS-antibody conjugates

CuS nanoparticles and CuS-antibody conjugates were prepared according to the procedure of Liu and co-workers.[1]

Preparation of HDX potassium salts. The mixture of 9.69 g of hexadecanol and 2.24 g of potassium hydroxide was melted at 150 °C. The obtained solution was suspended with homogeneous stirring in 25 mL of toluene at 100 °C. To the solution, carbon disulfide (4.41 g) was added dropwise at 25 °C. The thick yellowish suspension was cooled and vigorously stirred for 1 h, and the solution was diluted with 100 mL of petroleum ether and stirred for additional 2 h. The product was filtered, and the obtained solid was washed with petroleum ether. The crude xanthate was then dried, filtered again with 20 mL cold deionized water, followed by drying in a vacuum oven and washing with petroleum ether. Finally, the HDX was vacuum-dried again.

Preparation of Cu-HDX. HDX (3.56 g) in 5 mL of methanol was mixed with equimolar $CuCl_2$ for 2 min. The mixture was centrifuged. The resulting Cu-HDX was washed twice with methanol and then dried under vacuum.

Preparation of CuS nanocrystals with a capping agent. 0.5 g of HDA, heated to 120 °C and cooled to 50 °C, was homogeneously mixed (by stirring) with 0.05 g of Cu-HDX. The mixture was then incubated at 100 °C for 30 min. The temperature was gradually increased to 120 °C for 1.5 h. The annealed metal sulfide nanocrystals were slowly cooled to 70 °C. The resulting brown CuS powders were washed twice with absolute methanol, and dried at room temperature followed by storage in the dark. TEM image (Fig. S1) shows that the spherical CuS nanocrystals is about 7 nm and the X-ray diffraction pattern (Fig. S2) indicates the synthesized CuS NPs is high quality.

Preparation of CuS-Antibody conjugates. 40 mg of synthesized CuS nanocrystals was dispersed in anhydrous dioxane with 1 g of DTT. The mixture was refluxed for 12 h at 70 °C. After centrifugation and washing three times with anhydrous dioxane, the nanocrystals were redispersed in anhydrous dioxane. The hydroxyl-modified nanocrystals were activated with CDI for 2 h at room temperature. Following the precipitation of the CDI-activated hydroxylated nanocrystals, the precipitate was washed twice with anhydioxane, and the products were dried under vacuum. The resulting powder was dissolved in dioxane. Then, the CDI-activated CuS nanocrystals were mixed with 100 μL of goat anti-humanIgG and the pH of the solution was adjusted to 8.5 by addition of NaOH. The resulting solution was stirred for 24 h and then centrifuged for 15 min at 14 000 rpm. The resulting antibodiy−nanocrystals were washed twice with dioxane and with PBS. The resulting conjugates were dispersed in 0.1 M phosphate buffer.

1.1.3 Preparation of gold nanorods

Gold nanorods were prepared according to the reported seed-mediated approach with some minor modifications by changing the amount of $AgNO_3$.[2] (1) *Seed preparation:* To 7.5 mL of CTAB (0.10 M) solution, 0.25 mL of $HAuCl_4 \cdot 3H_2O$ (0.01 M) and 0.60 mL of ice-cold $NaBH_4$ (0.01 M) were added in sequence. The mixed solution was kept in a 26 °C water bath for 2 h. *(2) AuNRs Growth:* 1.2 mL of 0.05 M $HAuCl_4 \cdot 3H_2O$, 0.3 mL $AgNO_3$ (0.01 M) and 0.96 mL ascorbic acid (0.1 M) were

added to 100 mL of CTAB (0.10 M) in sequence with stirring. Finally, 0.2 mL seed solution prepared in step (1) was added at room temperature. The color of the solution gradually changed to dark blue within 20 min. The solution was further left for 2 days without stirring.

1.1.4 Immunoassay procedure

The immunoassay was conducted by following a typical sandwich immunoreaction procedure. Initially, each polystyrene microwell was coated by 100 μL of 0.1 mg/ml goat anti-human IgG (0.05 M carbonate buffer, pH 9.6) and incubated at 4 °C overnight. The unbound antibody was washed away with 0.05 M phosphate buffer containing 0.1% Tween-20 (PBS-T, pH 7.4) six times. Then 120 μL of 1% BSA solution (PBS-B) was added to each well and the well was incubated at 37 °C for 1 h to block active sites. After washing, different concentrations of human IgG in PBS-B were added into the wells and incubated for 1 h at 37 °C. After washing with PBS-T, 100 μL of CuS-antibody conjugates were pipetted into each well followed by incubating at 37 °C for 1 h. The microwell was washed three times by water, followed by the addition of 120 μL of HBr solution (0.75M) to release Cu^{2+}. Then 40 μL of gold nanorods were added to the microwell. The microwell was incubated at 75 °C for 20 min. The resulting solution was then subjected to record the extinction spectrum.

2 Figures

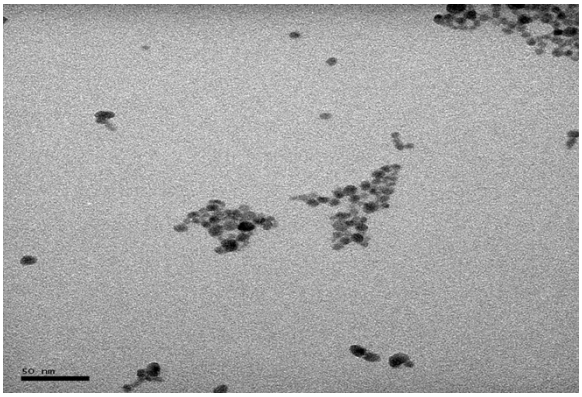

Fig. S1. TEM image of CuS nanocrystals obtained at the 70 °C reaction temperature

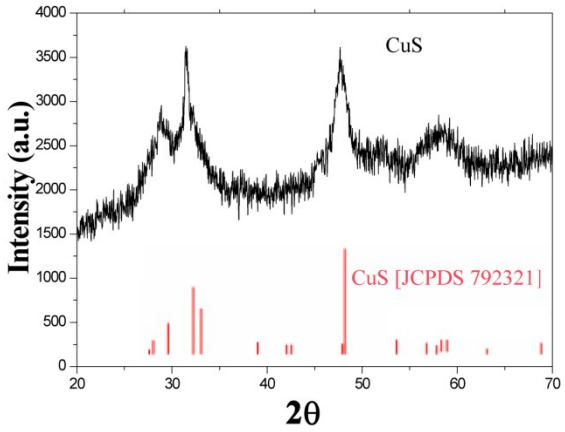

Fig. S2. X-ray diffraction pattern (XRD) of CuS nanocrystals. All diffraction peaks can be indexed as copper sulfide with lattice parameters similar to those of Joint Committee on Powder Diffraction Standards card 79-2321. The relatively broad diffraction peaks reflect the small size of copper sulfide crystals. No obvious impurity peaks were detected, indicating the acquirement of CuS nanocrystals with high quality.

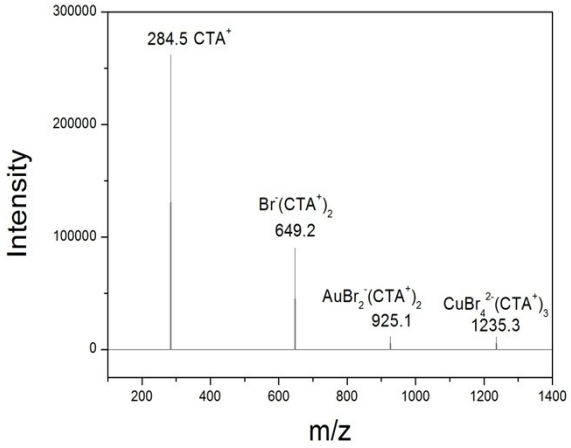

Fig. S3. Mass spectrum of gold nanorods solution (2.8 nM) after incubation with 1.0 mM Cu^{2+} in HBr buffer solution.

References

1. G. Liu, J. Wang, J. Kim, M. R. Jan and G. E. Collins, *Anal. Chem.*, 2004, **76**, 7126.

2. T. K. Sau and C. J. Murphy, *Langmuir*, 2004, **20**, 6414