

Electronic Supplementary Information for

A visualized method for Cu²⁺ ions detection by self-assembling azide functionalized free graphene oxide using click chemistry

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The supplementary data includes all the additional information as noted in the manuscript.

1. Materials

Graphene oxide (GO) was purchased from Nanoon (Hebei, China), which was prepared by oxidizing graphite based on a modified Hummers method. Qdot 625 CdSe/ZnS amino [poly(ethylene glycol), PEG] quantum dots (QDs-NH₂, 625 nm) were purchased from Jia Yuan Quantum Dots Corporation., Ltd (WuHan, China). 1-Pyrenebutyric acid succinimidyl ester (95%) was purchased from Sigma-Aldrich (St. Louis, MO). N-hydroxysuccinimide (NHS, 99%) and 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC, 99%) were obtained from GL Biochem Ltd. (Shanghai,

China). 4-Pentynoic acid (97%) was purchased from Meryer (Shanghai, China). 3-Azido-1-propanamine (95%) was obtained from TCI (Shanghai, China). Cupric sulfate (CuSO_4 , 99%) and sodium ascorbate (99%) were obtained from Aladdin (Shanghai, China). All solvents and other chemicals were purchased from local commercial suppliers and were of analytical reagent grade unless otherwise specified. Deionized (DI) water (Milli-Q, Millipore, Bedford, MA) was used to prepare aqueous solutions.

2. General Methods

Preparation of azide functionalized graphene oxide (GO- N_3). As illustrated in Scheme 1, GO- N_3 was prepared by surface adsorption of 1-pyrenebutyric acid succinimidyl ester on GO via π - π stacking followed by subsequent treatment with 3-azido-1-propanamine.¹ To be specific, 100 mg of 1-pyrenebutyric acid succinimidyl ester was added to 1 mL (5 mg/mL) of GO dispersed in THF and incubated for 2 h at room temperature. Next, excess 1-pyrenebutyric acid succinimidyl ester was removed by repeated washing with THF. The heavily 1-pyrenebutyric acid succinimidyl ester - functionalized GO (5 mg) was further incubated with 10 mL of 3-azido-1-propanamine in tetrahydrofuran (THF) for 24 h to synthesis the GO- N_3 . Finally, the obtained GO- N_3 was washed three times with THF to remove excess 3-azido-1-propanamine.

Preparation of alkyne functionalized quantum dots (QDs-alkyne). As illustrated in Scheme 1, QDs-alkyne was prepared by following a standard procedure² through conjugating the 4-pentynoic acid to QDs- NH_2 . To 2 mL of 4-pentynoic acid (0.035 g in the tris-borate buffer), EDC (0.10 g) and NHS (0.30 g) were added and stirred for half an hour. Finally, 20 μL of QDs (8 μM solution in tris-borate buffer) was added and stirred for 2 h. The unreacted materials were removed with a desalting NAP-5 column (GE Healthcare). The resulting QDs-alkyne conjugates were stored in a refrigerator before use.

Determination of Cu²⁺ and calculation of the detection limit

To determine the Cu²⁺ ions and evaluate the minimum concentration of Cu²⁺ ions in aqueous solution detectable by the formation of GO precipitates, we added Cu²⁺ into the mixture of GO-N₃ and QDs-alkyne to obtain a Cu²⁺ concentration of 10 μM, 1 μM, 0.1 μM, 10 nM, 1 nM, and 0.1 nM, respectively. Sodium ascorbate at five times the concentration of Cu²⁺ was also added. After adding the sodium ascorbate, we observe the formation of GO-N₃ precipitates.

Selectivity of the Cu²⁺

To evaluate the selectivity of this assay, we prepared other environmentally relevant metal ions in place of copper, including Fe³⁺, Cr³⁺, Zn²⁺, Mn²⁺, Fe²⁺, Ca²⁺ and K⁺ at concentrations of 1 μM. The test procedures were similar to those described above. Also, we increased the concentrations of these ions to 500 times the concentration of Cu²⁺, that is, at 500 μM. Then we observe the reaction phenomenon.

Meanwhile, to test whether the detection of Cu²⁺ is interference by mixtures of other cations, we combined Cu²⁺ with the following different types of cations, Fe²⁺, Zn²⁺, Ca²⁺, Mn²⁺ and K⁺, in the assay. The test procedures were similar to those described above.

3. Characterization

X-ray photoelectron spectroscopy (XPS) analyses were performed on an Axis Ultra X-ray photoelectron spectrometer with an Al X-ray source operating at 150 W (15 kV, 13 mA). The vacuum in the main chamber was kept above 3×10^{-6} Pa during XPS data acquisition. All the GO samples (GO and GO-N₃) were attached to the stainless steel substrate surface using a double-sided tape. Specimens were analyzed at an electron takeoff angle of 90°. For the GO samples, general survey scans (binding energy range 0-1100 eV, pass energy 160 eV) and high-resolution spectra (pass energy 80 eV) in the regions of C1s and N1s were recorded. Binding energies were referenced to the C1s binding energy at 284.6 eV. Elemental analysis were performed on an Elementar (Vario III) system. Raman spectra were recorded from 200 to 2500 cm⁻¹ on a Renishaw Invia Raman Microprobe using a 514.5 nm argon ion laser. Fourier-transform infrared spectroscopy (FT-IR) was recorded on a Nicolet 5700 spectrometer at wavelengths of 400-4000 cm⁻¹ and a resolution of 3 cm⁻¹ over 32 scans. The UV-vis absorption spectra

were obtained using a Shimadzu UV-1700 system at wavelengths ranging from 200 cm^{-1} to 700 cm^{-1} . Fluorescence spectra were measured on a Shimadzu RF-5301 system wavelengths ranging from 400 cm^{-1} to 800 cm^{-1} with a xenon lamp excitation source. The morphology of the GO-N₃ before/after adding Cu²⁺ was observed with transmission electron microscopy (TEM) (JEM-2000EX, JEOL, Japan). The surface charges of the samples were analyzed by ζ potential measurements using a Zetasizer Nano ZS (Malvern Instruments, Malvern, U.K.).

4. References

1. W. Zhang, H. Han, H. Bai, W. Tong, Y. Zhang, W. Ying and Weijie Qin, and Xiaohong Qian, *Anal. Chem.*, 2013, **85**, 2703–2709.
2. J. Hao, L. L. Huang, R. Zhang, H. Z. Wang and H. Y. Xie, *Anal. Chem.*, 2012, **84**, 8364–8370.

Table S1 The carbon, oxygen, nitrogen element analysis of GO and GO-N₃.

Sample	C %	O %	N %
GO	42.90	48.00	/
GO-N ₃	52.15	36.38	8.80

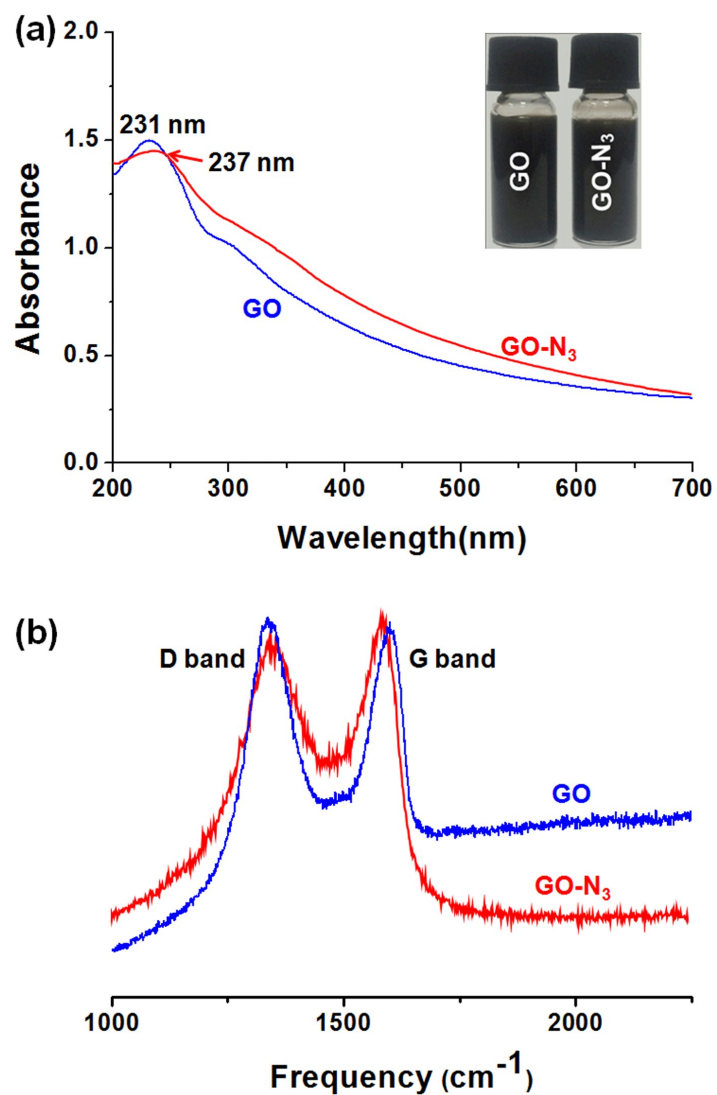


Figure S1 (a) The UV-Vis spectra of GO (blue line) and GO-N₃ (red line), inset: the disperse images of GO and GO-N₃ in the water (5 mg/mL); (b) the Raman spectra of GO (blue line) and GO-N₃ (red line).

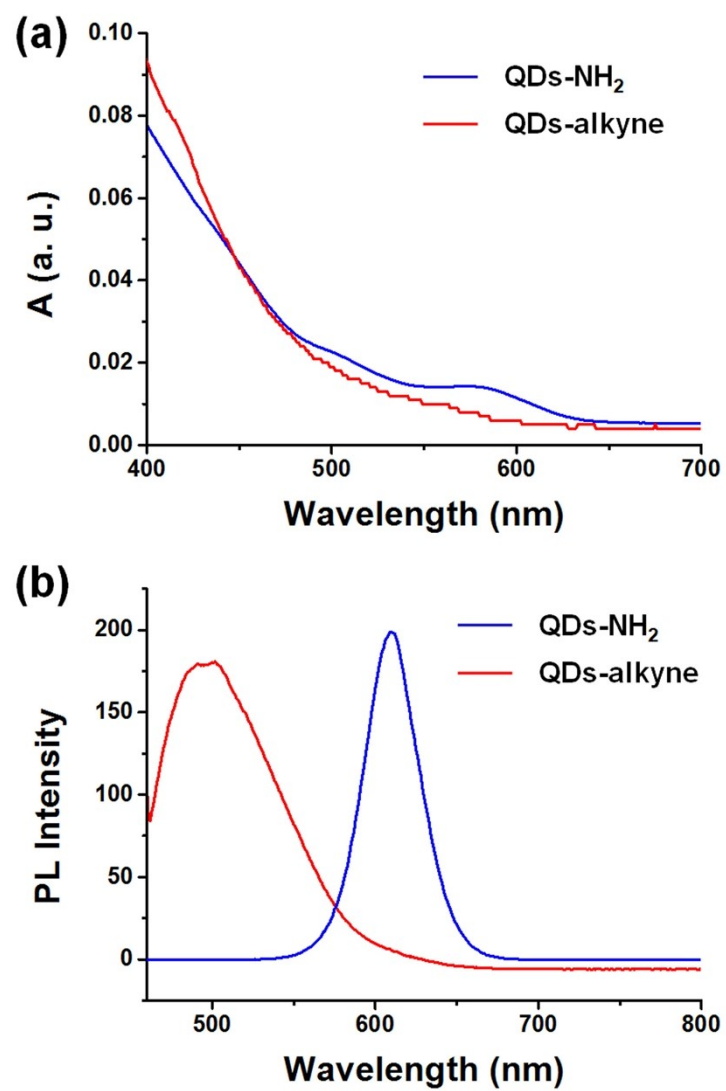


Figure S2. Absorption (a) and emission (b) spectra of QDs-NH₂ and QDs-alkyne.

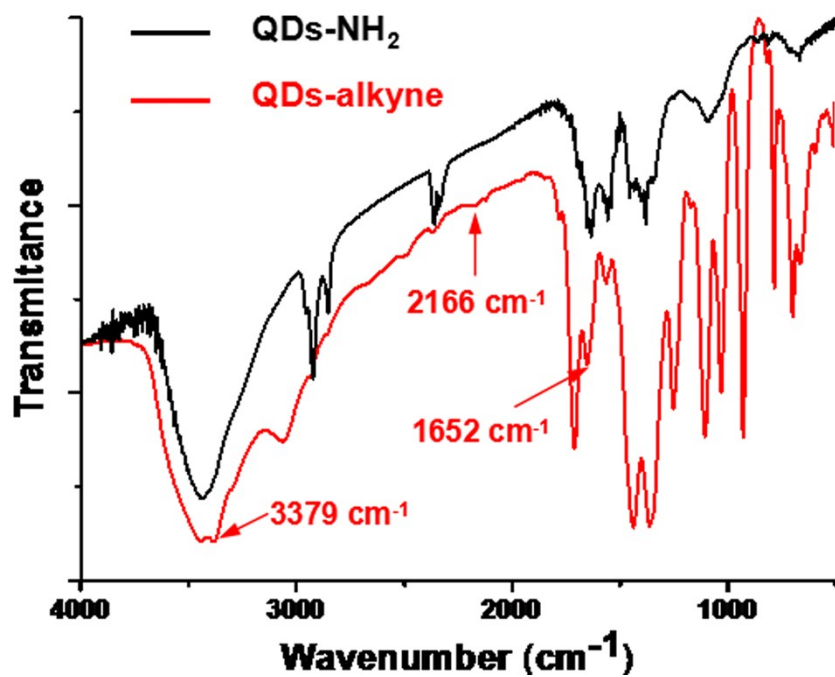


Figure S3. The FT-IR spectrum of QDs-NH₂ and QDs-alkyne.

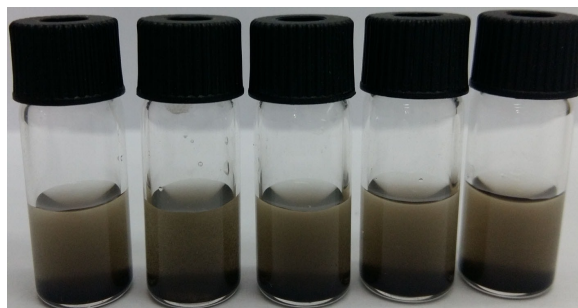


Figure S4. A photograph of the solutions containing the mixtures of GO-N₃ and QDs-alkyne with 1 μM Cu²⁺ in the present of sodium ascorbate for five replicates.

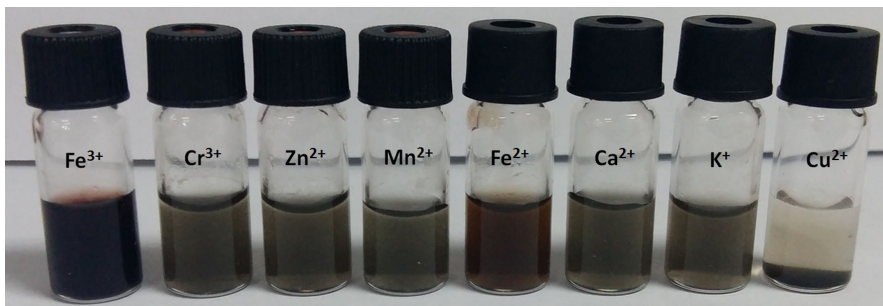


Figure S5. A photograph of the solutions containing the mixtures of GO-N₃ and QDs-alkyne with different metal cations in the present of sodium ascorbate. Ion concentration of metal cations is 500 μM .

$$[\text{Cu}^{2+}] = 1 \mu\text{M}.$$

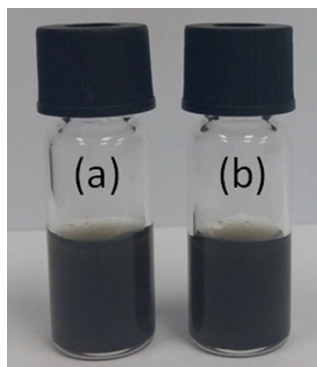


Figure S6. (a) A photograph of the solution containing the mixtures of GO-N₃ and QDs-alkyne with Cu²⁺ without sodium ascorbate; (b) a photograph of the solution containing the mixtures of GO-N₃ and QDs-alkyne with sodium ascorbate without Cu²⁺.