Supplementary Material for Chemical Communications
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Supplementary Data

Label-Free Fluorescent Detection of Cu(II) Ions Based on the DNA Cleavage-Dependent Graphene-Quenched DNAzymes

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Reagents and Instruments

Cu(II)-dependent DNAzymes (Cu-Sub: 5'-T_{10}AGCTTCTTTCTAATACGGCTTACC-3' and Cu-Enz: 5'-GGTAAGCCTGGGCCTCTTTCTTTTTAAGAAAGAAC-3') were purchased from Takara Biotechnology Co. (Dalian, China) purified by high-performance liquid chromatography (HPLC). GelRed (10000×) in water was purchased from Biotium. NaCl, $CuCl_2$, $BaCl_2$, $CaCl_2$, $CdCl_2$, $FeCl_3$, $MnCl_2$, $Pb(NO_3)_2$ and $ZnCl_2$ were of analytical reagent grade and purchased from the Kemiou Agent Co., Tianjin (China). Other reagents such as HCl, HNO_3, and H_2O_2 (30%) and anhydrous ethanol were used as received without any treatment. Sodium ascorbate was obtained from Sinopharm Chemical Reagent Co., Ltd (China). Ultrapure water obtained from a Millipore water purification system (resistivity > 18.0 MΩ cm^{-1}, Laikie Instrument Co., Ltd, Shanghai, China) was used throughout the experiments. Phosphate buffer solution (PBS, 20 mM) with pH 7.4 was prepared by mixing the stock solution of Na_2HPO_4 and NaH_2PO_4. FL measurements were performed using a Hitachi F-4500 spectrofluorimeter with a scan rate at 2400 nm/min. The excitation wavelength was at 530 nm. The slits for excitation and emission were set at 5 nm/10 nm.

Preparation of Graphene Oxide and Graphene

Graphene oxide was prepared by our reported method.[1] An environment-friendly hydrothermal route was employed to convert graphene oxide to graphene. The resultant solution of graphene (0.1 mg/mL) was stored at room temperature and employed in the following experiments.

Preparation of DNAzymes

In brief, 1.8 μM Cu-Sub and 1.8 μM Cu-Enz were firstly mixed in a buffer (pH 7.4) containing 20 mM PBS, 0.5 M NaCl. Then the mixture was warmed to 95 ℃ for 2 min in a water bath and subsequently allowed to cool naturally to room temperature (25 ℃). The obtained DNAzymes were stored at 4 ℃.

Fluorescent Assay for Cu(II)

In a typical experiment, 200 μL of GelRed (100×) and 800 μL of DNAzymes were incubated for 20 min at 25 ℃. The final concentration of Cu(II)-dependent DNAzymes was 1.45 μM. Subsequently, 340 μL of PBS buffer (20 mM, 50 mM NaCl, pH 7.4), 20 μL of DNAzymes-GelRed, 120 μL of graphene solution (0.1 mg/mL) were sequentially added into a microcentrifuge tube. After 15 min reaction, the solution was transferred to a quartz cell. Finally, 10 μL of ascorbate (10 mM) and 10 μL of different concentrations of Cu(II) were added for time-dependent FL measurement at $\lambda_{ex}/\lambda_{em}$ = 530/613 nm. The final volume of the solution was fixed at 500 μL. The control experiment was carried out under the same condition without the addition of Cu(II).

Figures

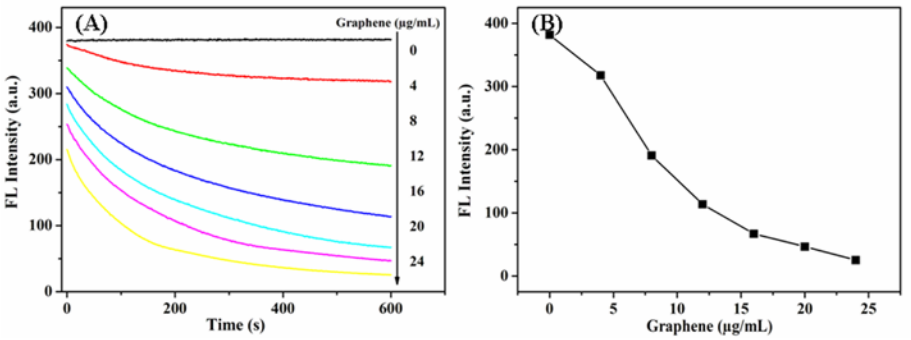

Fig. S1 (A) Kinetics study for the FL changes of the DNAzymes-GelRed complex with different concentrations of graphene. (B) FL changes of DNAzymes-GelRed complex as a function of graphene with a series of concentrations. Experimental conditions: 20 mM PBS, 50 mM NaCl, pH 7.4, 58 nM DNAzymes, GelRed (20×), reaction time: 10 min (25 °C), excitation wavelength: 530 nm, emission wavelength: 613 nm.

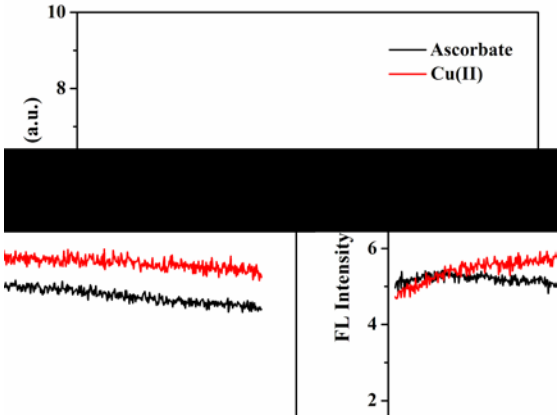

Fig. S2 Kinetics study for FL changes of the label-free graphene-quenched DNAzymes in the absence of Cu(II) or ascorbate. Experimental conditions: 20 mM PBS, 50 mM NaCl, pH 7.4, 58 nM DNAzymes, 24 μg/mL graphene, GelRed (20×), 0.2 mM ascorbate or 0.5 mM Cu(II), reaction time: 10 min (25 °C), excitation wavelength: 530 nm, emission wavelength: 613 nm.

Reference

1 M. Liu, H. M. Zhao, X. Quan, S. Chen and X. F. Fan, *Chem. Commun.*, 2010, **46**, 7909-7911.