Supplementary Material for Chemical Communications

Supplementary Data

Controllable oxidative DNA cleavage-dependent regulation of graphene-DNA interaction

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Reagents and Instruments

DNA oligomers (15 mer: 5'-FAM-TCTCTCTCGTTTGTG-3'; 23 mer: 5'-FAM-TCTCTCTCTCTCTCTCGTTTGTG-3' and 35 mer: 5'-FAM-AATGTTCGATGCTGACGGTCCATATGGACCGTCAA-3') were purchased from Takara Biotechnology Co. (Dalian, China). All of the DNA oligomers were purified by high-performance liquid chromatography (HPLC). All other reagents were of analytical reagent grade and purchased from the Kemiou Agent Co., Tianjin (China). Sodium ascorbate was obtained from Sinopharm Chemical Reagent Co., Ltd (China). Other reagents such as HCl, HNO_3, and H_2O_2 (30%) were used as received without any further treatment. 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. All the glassware was firstly cleaned with a mixture of HCl and HNO_3 (ratio of HCl/HNO_3 = 3:1 in volume) and thoroughly rinsed with ultrapure water. 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 1200 nm/min. The excitation wavelength was at 494 nm, and the photomultiplier tube (PMT) voltage was 700 V. The slits for excitation and emission were set at 5 nm/10 nm. Atomic force microscopy (AFM) measurements were carried out on Agilent PicoPlus II.

Preparation of Graphene Sheet

Graphene sheet was prepared by our reported method.[1] In brief, graphite powder (1.0 g) was firstly dispersed in concentrated H_2SO_4 (23 mL) and stirred 12 h at room temperature. $KMnO_4$ (3 g) was then added gradually under stirring at 0 °C. Successively, the mixture was sonicated for 6 h to give a dark green solution. Then, ultrapure water (46 mL) was slowly transferred to the mixture. After keeping boiling for 30 min, the reaction was then terminated by the addition of ultrapure water (140 mL) and H_2O_2 (30%, 10 mL). Finally, the yellow product was separated by centrifugation, and washed with 5% HCl and ultrapure water, sequently. An environment-friendly hydrothermal route was used to convert graphene oxide to graphene. The resultant graphene (0.1 mg/mL) was stored at room temperature and employed in the following experiments. A typical AFM image showed a graphene flake was with the lateral width of hundreds nanometers (Fig. S1a). The cross-section of a AFM image indicated that graphene flake was with an average height of 10 Å (Fig. S1b), which was consistent with the single-layer graphene of 0.8-1.2 nm reported by other groups.[2]

Fluorescent Assay for DNA Cleavage

In a typical experiment, 290 μL of buffer (20 mM PBS, 50 mM NaCl, pH 7.4), 10 μL of 35 mer ssDNA (2.9 μM), 100 μL of graphene solution (0.1 mg/mL) were sequentially added into a microcentrifuge tube. After 30 min reaction, the solution was transferred to a quartz cell at room temperature. Subsequently, different volumes of ascorbate (25 mM) and 10 μL of Cu(II) or Fe(III) stock solution (25 mM) were added for time-dependent FL measurement at $\lambda_{ex}/\lambda_{em}$ = 494/518 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) (the final concentration of ascorbate was 3 mM). All experiments were performed at 25°C and repeated three times. For ssDNA-1 (15 mer) and ssDNA-2 (23 mer)-mediated cleavage reaction, the concentrations of graphene were 12 μg/mL and 15 μg/mL, respectively.

DNA Cleavage Inhibition Assay

The inhibition experiments were the same as the above assay for DNA (35 mer) cleavage in a microcentrifuge tube, except for the first addition of different concentrations of thiourea before the addition of ascorbate (1.5 mM). Time-dependent FL measurement was then performed at $\lambda_{ex}/\lambda_{em}$ = 494/518 nm.

Electrophoresis Analysis

35 mer ssDNA before and after the cleavage reaction were respectively loaded onto loading buffer containing 30 mM EDTA, 36% (v/v) Glycerol, 0.05% (w/v) Xylene Cyanol FF, and 0.05% (w/v) Bromophenol blue, pH 7.0. The samples were then put on a 20% polyacrylamide gel in a 1×TAE buffer (40 mM Tris acetate, 2 mM EDTA, pH 8.5) followed by electrophoresis for 240 min at 60 V. After ethidium bromide staining, the gel was imaged using a G: BOX HR system (Gene Co., Ltd).

Figures

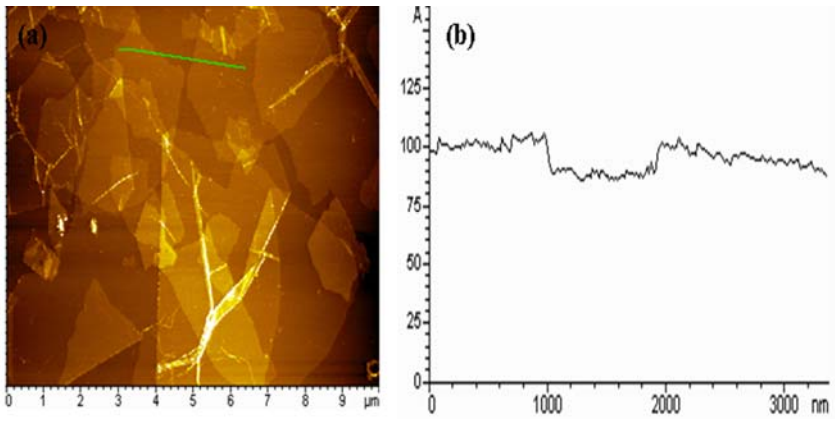

Fig. S1 Typical AFM image (A) and height profile (B) of as-prepared graphene on freshly cleaved mica substrate.

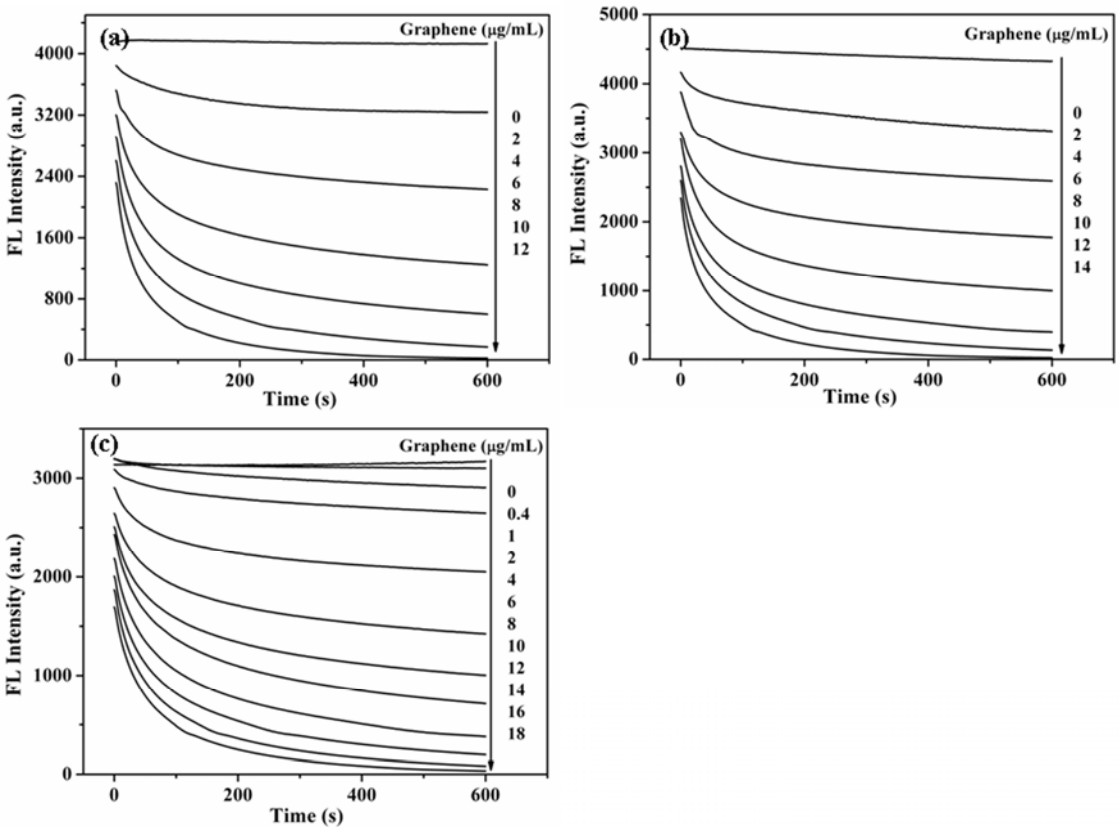

Fig.S2 Kinetics study for the FAM-labeled ssDNA with different lengths as a function of graphene with a series of concentrations: 15 mer (a); 23 mer (b) and 35 mer (c). The buffer contained 20 mM PBS (pH 7.4), 50 mM NaCl and 58 nM ssDNA. $\lambda_{ex}/\lambda_{em}$ = 494/518 nm.

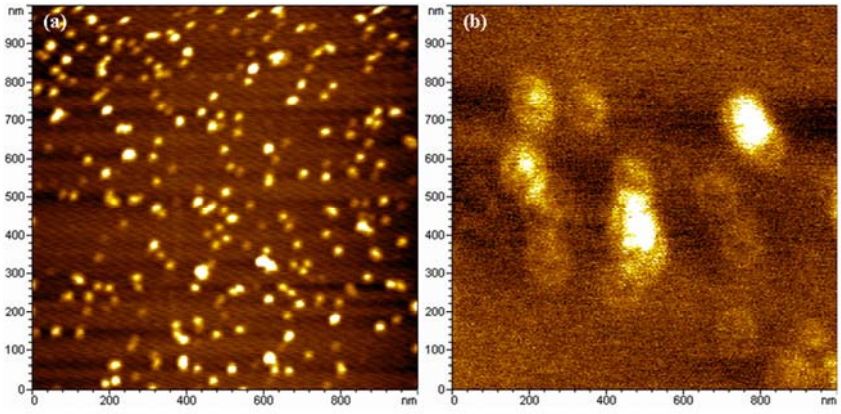

Fig. S3 Typical AFM image of ssDNA (35 mer) before (A) and after (B) the cleavage on freshly cleaved mica substrate.

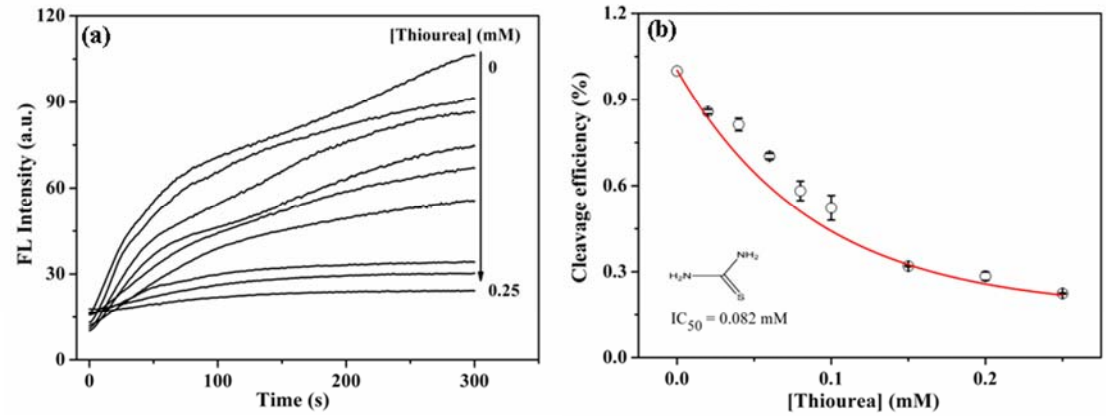

Fig. S4 Dose-dependent inhibition of DNA damage was carried out using graphene/ssDNA (35 mer) in Cu(II)/ascorbate-mediated Fenton reaction. (a) Kinetics study for FL changes with various amounts of thiourea. (b) Inhibition efficiency plotted as the function of thiourea concentration. The IC_{50} value was obtained from the curve.

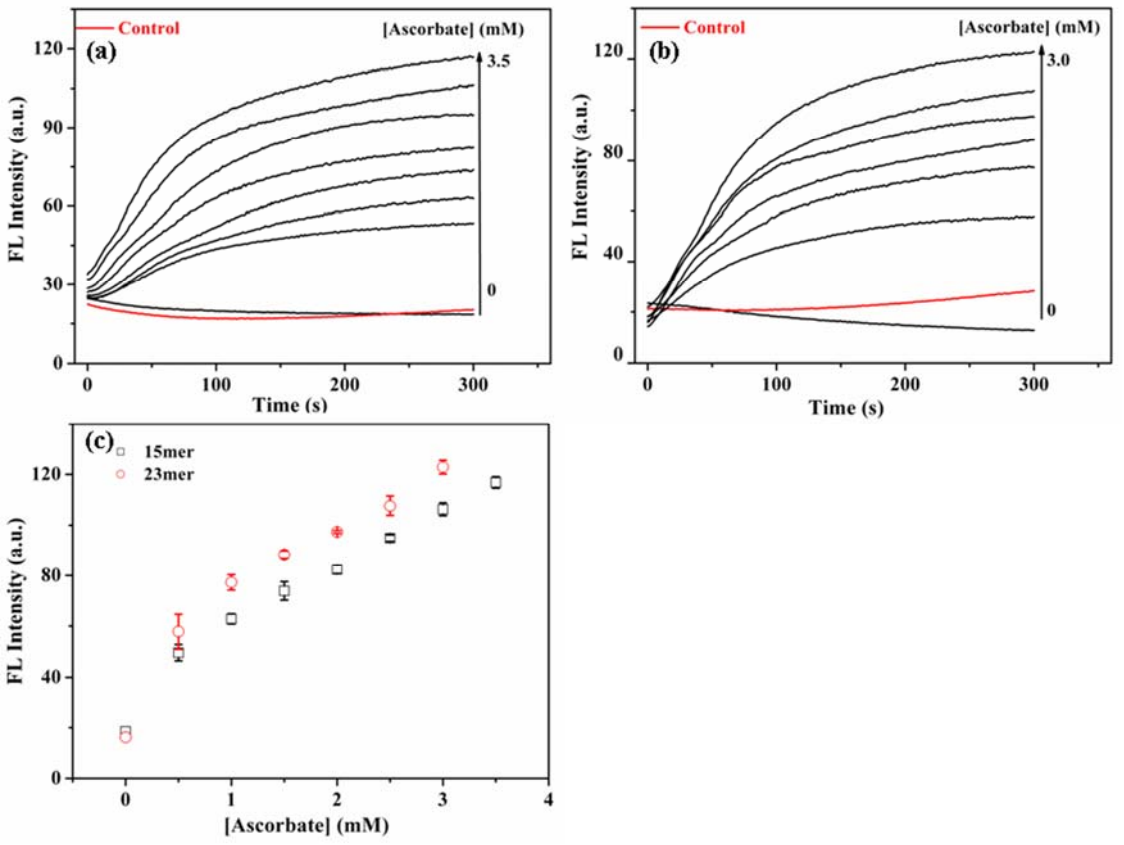

Fig. S5 Kinetics study for FL changes of the graphene/ssDNA in Cu(II)/ascorbate-mediated Fenton reaction with various amounts of ascorbate: (a) ssDNA-1 (15 mer); (b) ssDNA (23 mer). (c) FL signaling plotted as the function of ascorbate concentration. The buffer contained 20 mM PBS (pH 7.4), 50 mM NaCl, 58 nM ssDNA and 0.5 mM Cu(II). $\lambda_{ex}/\lambda_{em}$ = 494/518 nm.

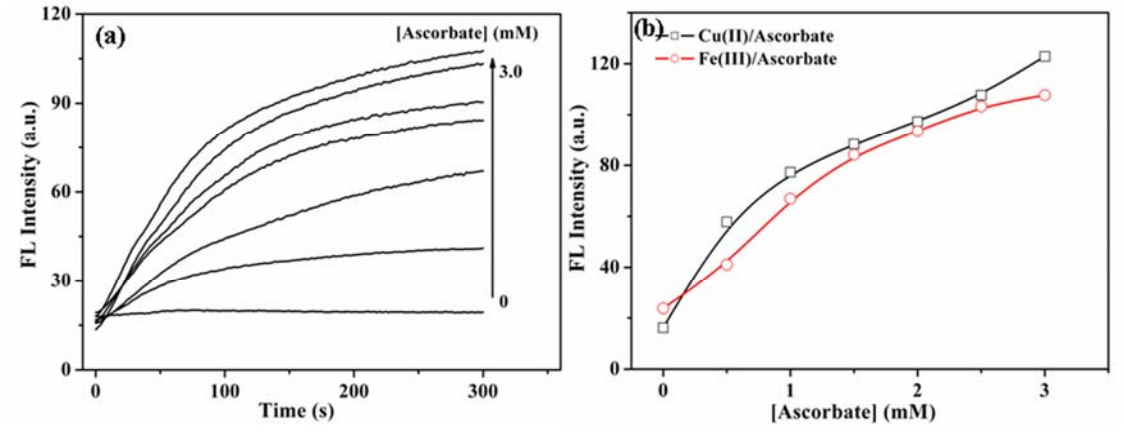

Fig. S6 Kinetics study for FL changes of the graphene/ssDNA (23 mer) in Fe(III)/ascorbate-mediated Fenton reaction with various amounts of ascorbate.(b) FL signaling plotted as the function of ascorbate concentration.

Reference

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

2 (a) H.-L. Guo, X.-F. Wang, Q.-Y. Qian, F.-B. Wang and X.-H. Xia, *ACS Nano*, 2009, **3**, 2653-2659; (b) L. J. Cote, F. Kim and J. X. Huang, *J. Am. Chem. Soc.*, 2009, **131**, 1043-1049; (c) J. Kim, L. J. Cote, F. Kim and J. X. Huang, *J. Am. Chem. Soc.*, 2010, **132**, 260-267.