Electronic Supplementary Information for:

A graphene oxide-peptide fluorescence sensor tailor-made for simple and sensitive detection of matrix metalloproteinase 2

Duan Feng, Yangyang Zhang, Tingting Feng, Wen Shi, Xiaohua Li and Huimin Ma*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. E-mail: mahm@iccas.ac.cn

Materials and apparatus

Matrix metalloproteinase 2 (MMP2), 4-aminophenylmercuric acetate, 1,10-phenanthroline, bovine serum albumin and human serum albumin were obtained from Sigma-Aldrich. Matrix metalloproteinase 1 was obtained from Sino Biological Inc. The FITC(fluorescein isothiocyanate)-labeled peptide (FITC-GPLGVRG-NH_2) was purchased from Beijing SBS Genetech Co., Ltd. Graphene oxide (GO) was prepared following the reported procedure (Zheng, et al., *Chem. Commun.*, 2010, **46**, 5728-5730). Tris(hydroxymethyl)-aminomethane (Tris) was purchased from J&K Chemical. $CaCl_2$, NaCl, KCl, $MgCl_2$, glucose, vitamin B1 and glutamine were obtained from Beijing Chemicals, Ltd. Other reagents employed were all of analytical grade, and were used without further purification. TCNB buffer (50 mM Tris with 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% Brij 35; pH 7.5) was employed in the experiments. Deionized and distilled water was used throughout.

A Hitachi F-2500 spectrofluorimeter was used for fluorescence measurements. pH values were recorded with a Delta 320 pH-meter [Mettler-Toledo Instruments (Shanghai) Co., China]. Scanning electron microscopy analysis was made on a Hitachi S-4300 field emission scanning electron microscope. Atomic force microscopy measurements were carried out with a Nanoscope IIIa instrument (Digital Instruments) operating in tapping mode. The density of cells was determined by cell counting chamber [Bio-system Medical

Technology (Shanghai) Co., Ltd]. The samples for atomic force microscopy images were prepared by depositing a diluted water dispersion of GO on a freshly cleaved mica surface and allowing it to dry under ambient conditions. The incubation was carried out in Shaker incubator (SKY-100C, Shanghai Sukun Industry & Commerce Co., Ltd).

Characterization of GO

The prepared GO was characterized by scanning electron microscopy (Fig. S1) and atomic force microscopy (Fig. S2). These images accord with those reported previously (Zheng, et al., *Chem. Commun*., 2010, **46**, 5728-5730), confirming the successful preparation of GO.

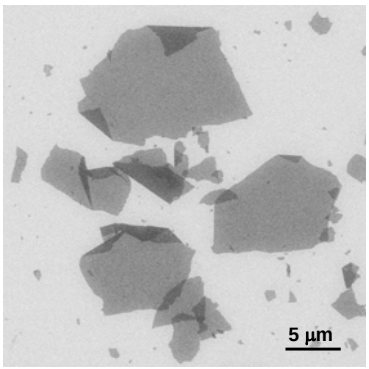

Fig. S1. The scanning electron microscopy image of GO.

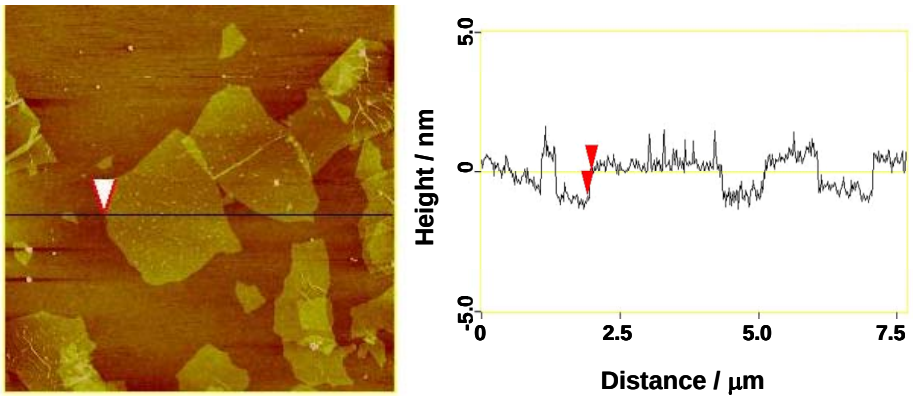

Fig. S2. The atomic force microscopy image of GO on mica.

Measurement of pKa of GO

To a sonicated suspension of GO, a solution of 0.01 M NaOH was added dropwise, and the pH of the reaction solution was monitored by a pH-meter. As shown in Fig. S3, the apparent pKa of GO is found to be about 5.2.

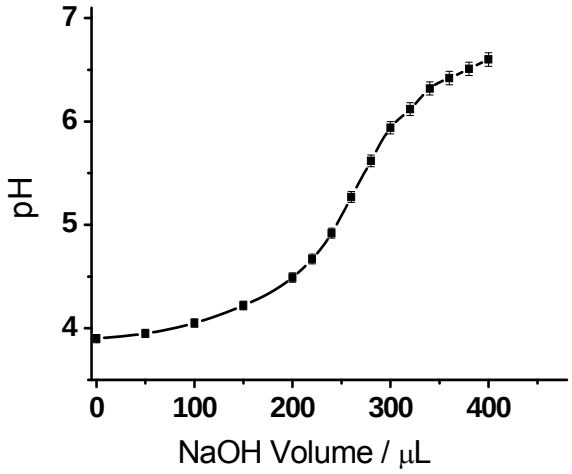

Fig. S3. Acid-base titration curve of GO with NaOH (0.01 M).

Preparation of the GO-peptide complex

The size of the GO-flakes was controlled with a fixed ultrasonic time of 15 min. Unless otherwise noted, the GO-peptide complex was prepared by treating the FITC-labeled peptide (100 nM) with the sonicated GO (30 μg/mL) in the TCNB buffer (pH 7.5).

Effect of pH on the fluorescence of the GO-peptide complex

The fluorescence of FITC and the GO-peptide complex at different pH values was studied. The pH values of the solution were adjusted with 1 M HCl or 1 M NaOH and measured with pH-meter. The results are shown in Fig. S4.

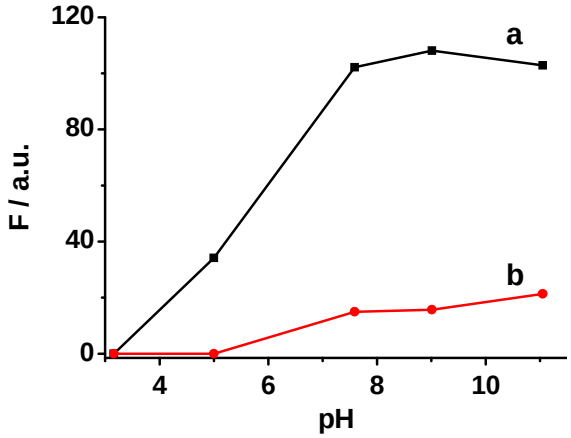

Fig. S4. The change in fluorescence intensity ($\lambda_{ex/em}$ = 470/516 nm) of (a) FITC (100 nM) and (b) the GO-peptide complex (in which the peptide concentration was 100 nM) at different pH values: 3.2, 5.0, 7.5, 9.0 and 11.1.

Optimization of the concentration of GO

Different volumes of GO solution (0.1 mg/mL) were added into the TCNB buffer containing 100 nM of the FITC-labeled peptide. After mixing and then standing for 10 min, the fluorescence spectra of the GO-peptide complex were recorded (Fig. S5).

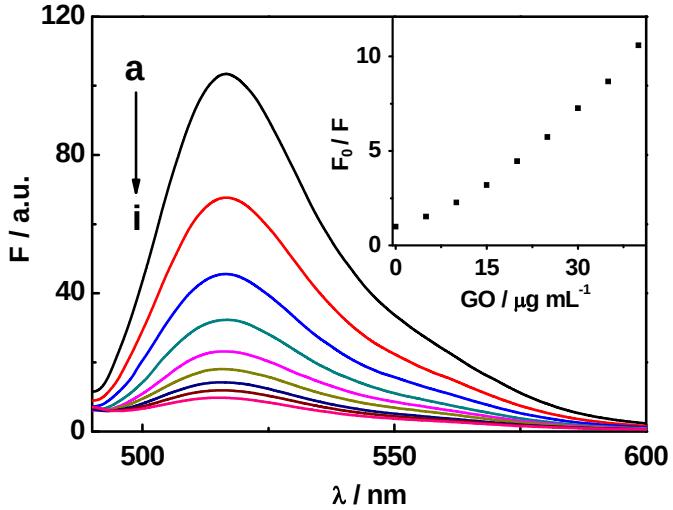

Fig. S5. Fluorescence spectra (λ_{ex} = 470 nm) of FITC-labeled peptide (100 nM) in the presence of varied concentrations of GO: (a) 0, (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30, (h) 35 and (i) 40 μg/mL. Inset shows the variation of the fluorescence intensity ratios (F_0/F) at 516 nm versus the concentration of GO, where F_0 and F are the fluorescence intensity of the FITC-labeled peptide before and after addition of GO, respectively.

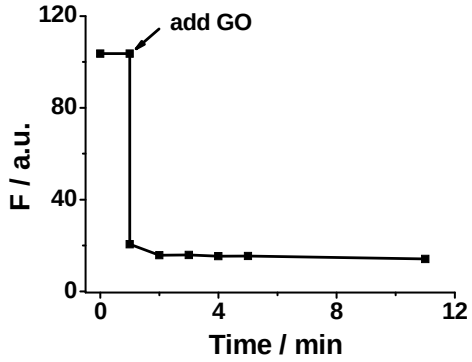

Fig. S6. Fluorescence quenching of the FITC-labeled peptide (100 nM) in TCNB buffer with 30 μg/mL GO as a function of incubation time at room temperature. $\lambda_{ex/em}$ = 470/516 nm.

Detection of MMP2 by the GO-peptide fluorescence sensor

Before detection, MMP2 (20 μL, 1.4 μM) was activated at 37 °C for 2 h with equal volume of 4-aminophenylmercuric acetate (2.5 mM) in the TCNB buffer (50 mM Tris with 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% Brij 35; pH 7.5) following the known procedure (Lee, et al., *Angew. Chem. Int. Ed*., 2008, **47**, 2804-2807).

Different concentrations of the activated MMP2 were incubated with the GO-peptide complex in the TCNB buffer at 37 °C for 2 h, and fluorescence intensity/spectrum of each sample was then recorded with $\lambda_{ex/em}$= 470/516 nm. The fluorescence responses of the reaction system to MMP2 at varied concentrations were shown in Fig. 1 and Fig. S7.

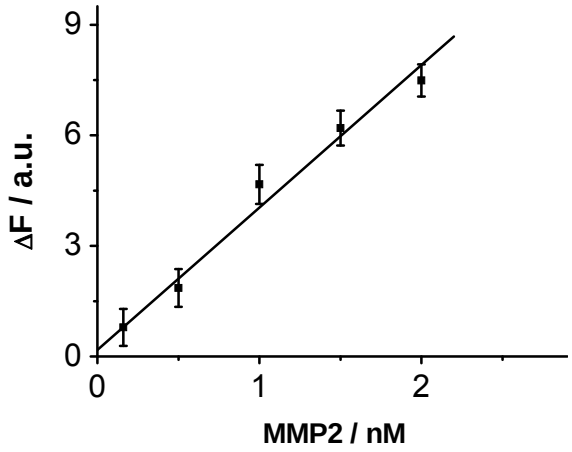

Fig. S7. Fluorescence intensity change (ΔF) of the GO-peptide as a function of MMP2 concentration. ΔF is the difference of fluorescence intensity of the GO-peptide complex in the presence and absence of MMP2. The data shown here represent the means and standard deviations of three independent experiments. $\lambda_{ex/em}$ = 470/516 nm.

To optimize the reaction time, varied concentrations of MMP2 were added into the solution of the GO-peptide complex (100 nM), and then the fluorescence was recorded with $\lambda_{ex/em}$= 470/516 nm as a function of time (Fig. S8).

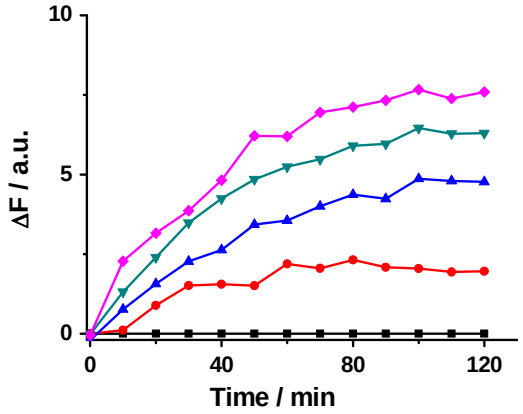

Fig. S8. The effect of reaction time on the fluorescence change of the GO-peptide complex reacting with varied concentrations of MMP2 (from bottom to top: 0.0, 0.5, 1.0, 1.5, and 2.0 nM) in TCNB buffer at 37 °C. $\lambda_{ex/em}$= 470/516 nm.

To confirm that the fluorescence enhancement was caused by MMP2, the effect of inhibitor (1,10-phenanthroline) on the activity of MMP2 was investigated. Briefly, the activated MMP2 (5 nM) was first mixed with the inhibitor (10 μM), and the resulting solution was then added to the solution of the GO-peptide complex (100 nM). After incubation at 37 °C for 2 h in the TCNB buffer, the reaction solution was subjected to fluorescence measurements. The results are shown in Fig. S9.

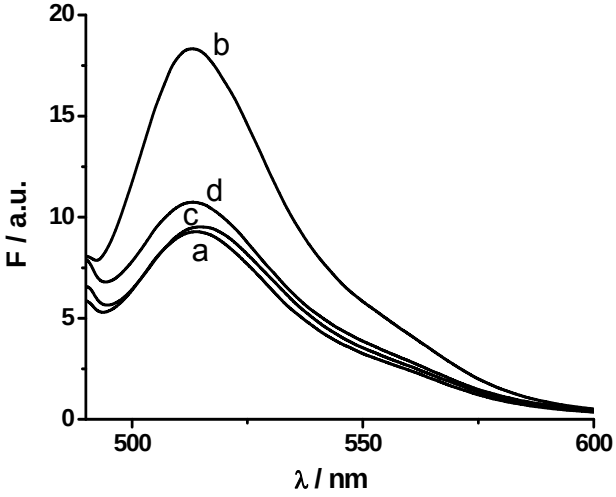

Fig. S9. Fluorescence spectra of different reaction systems. (a): the GO-peptide complex (100 nM) in the TCNB buffer (control); (b): (a) + MMP2 (5 nM); (c): (a) + 1,10-phenanthroline (10 μM); (d): (b) + 1,10-phenanthroline (10 μM). λ_{ex} = 470 nm.

Selectivity studies

To test the selectivity, the reaction of the GO-peptide complex (100 nM) with human serum albumin (100 nM), bovine serum albumin (100 nM), KCl (150 mM), $MgCl_2$ (2.5 mM), glucose (10 mM), vitamin B1 (1 mM), glutamine (1 mM), matrix metalloproteinase 1 (another member of MMPs, 5 nM), and MMP2 (5 nM), were examined in parallel under the same conditions (incubated at 37 °C for 2 h). The results are shown in Fig. S10.

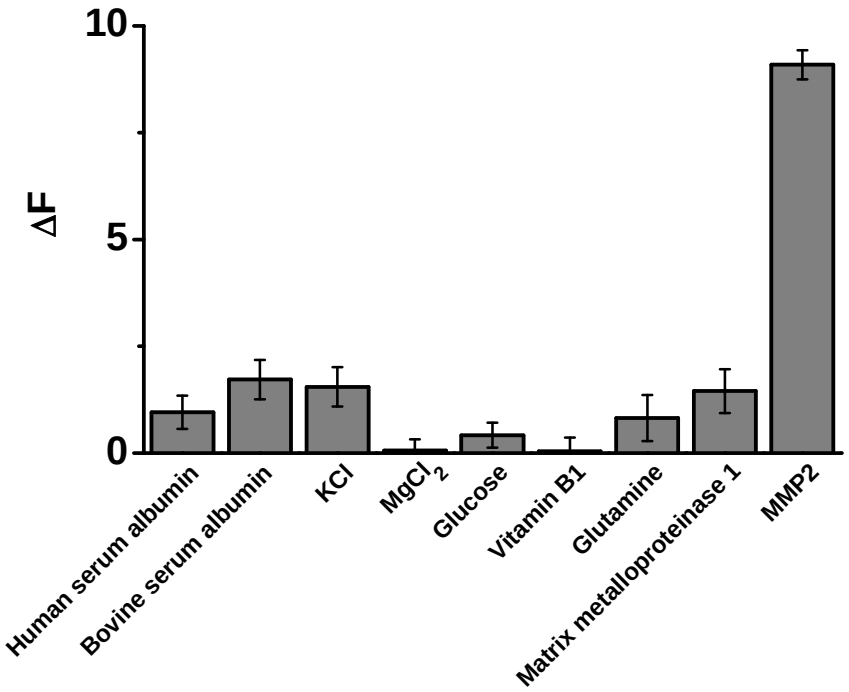

Fig. S10. Fluorescence responses of the GO-peptide complex (100 nM) to various substances: human serum albumin (100 nM), bovine serum albumin (100 nM), KCl (150 mM), $MgCl_2$ (2.5 mM), glucose (10 mM), vitamin B1 (1 mM), glutamine (1 mM), matrix metalloproteinase 1 (5 nM), or MMP2 (5 nM). ΔF is the difference of the fluorescence intensity of the GO-peptide complex in the presence and absence of a substance.

Determination of MMP2 concentration secreted by HeLa cells

HeLa cells were grown at 37 ºC for 24 h on glass-bottom culture dishes (MatTek Co.) in 1 mL of the cell culture media [Dulbecco's Modified Eagle Media supplemented with 10% (v/v) fetal bovine serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL)] in a

humidified atmosphere of 5% CO_2, whose densities in the culture media were measured by cell counting chamber. Then, an appropriate volume (typically 50 μL) of the culture media was collected and incubated with the GO-peptide complex (100 nM) in 1 mL of the TCNB buffer at 37 °C for 2 h. After that, the fluorescence of the reaction solutions was measured with $\lambda_{ex/em}$ = 470/516 nm, and according to the linear equation ($\Delta F = 3.86 \times$ [MMP2] (nM) + 0.18) the concentrations of MMP2 in the reaction solutions were determined. In this experiment, four different densities [0 (control, i.e., culture media), 5.48×10^5, 1.07×10^6, and 1.98×10^6 cells/mL, respectively] of HeLa cells were tested and compared (Table 1). The concentrations of MMP2 secreted by HeLa cells with the densities of 5.48×10^5 and 1.07×10^6 cells/mL were 1.1 and 2.0 nM in the reaction solutions (Table 1), which correspond to 22 and 40 nM MMP2 in the cell culture media, respectively.