Electronic Supplementary Information

Protein Electrochemistry Using Graphene-Based Nano-Assembly: An Ultrasensitive Electrochemical Detection of Protein Molecules via Nanoparticle-Electrode Collisions

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When the molecules adhere to the surface, the steady-state diffusion-controlled flux of molecules to the ultromicroelectrode (UME) surface, J, is given by [1]

$$J = 4DCr \qquad \text{(S1)}$$

where *D* is the diffusion coefficient，*C* is the concentration, and r is the radius of the UME. Usually, the only one or few electrons would transfer between the molecules and the electrode to yield a current which is too small to be detected. Only when the molecules can catalyze another reaction, the current can be greatly amplified to be detected. The amplitude of the current steps at the mass transfer limiting current generated at molecules in contact with a planar electrode is given by[1]

$$I = 4\pi(\ln 2)\, nFDCr \qquad \text{(S2)}$$

where n is the number of electrons transferred, F is the Faraday constant, D is diffusion coefficient, C is the bulk concentration of electroactive species, and r is the electrode radius. So we design to immobilize a bunch of MP-11 molecules on the surface of graphene. Then the number of molecules can be increased and the spike current can increase obviously.

Experimental details

Reagents

Microperoxidase-11, Graphite nanofiber and hydrazine hydrate were purchased from Sigma-Aldrich. Sodium phosphate dibasic anhydrous (Na_2HPO_4, 99.5%), sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$, 99.5%), sodium chloride (NaCl, 99.6%), Sulfuric acid (98%), potassium permanganate (99.5%), potassium persulfate (99.5%), phosphorus (V) oxide (98%) and hydrochloric acid (37%) were purchased from Fisher Scitific. Millipore water (18 MΩ) was used in all experiments. All chemicals were of reagent grade and used as received.

Preparation of graphene nanosheets

Graphene sheets were synthesized with the reduction of graphite oxide (GO) which was synthesized from graphite nanofibers by modified Hummer's Method.[2-4] Graphite nanofiber (2 g) was oxidized by concentrated H_2SO_4 (10 mL) and P_2O_5 (1 g) at 80 °C. The resultant dark blue mixture was thermally isolated and allowed to cool to room temperature in a period of 6 h. Then the mixture was diluted, filtered and washed with distilled water till the filtrate became neutral. The preoxidized graphite was then dried and mixed with cold concentrated H_2SO_4 (50 mL), followed by slow addition of $KMnO_4$ (6 g) under stirring and cooling condition to keep the temperature below 20 °C. The solution was then stirred at 35 °C for 2 h, followed by the addition of distilled water (400 mL). The reaction was stopped with the addition of a mixture of 280 mL of distilled water and 5 mL of 30 % H_2O_2. Then the mixture changed to bright yellow. The product was dispersed in water, filtered, washed with aqueous 1:10 HCl and then dried under vacuum to afford GO powder for preparation of graphene. The GO powder (15 mg) was dissolved into ultrapure water (45 mL) through sonication. The graphene was produced by the reduction of GO with PH=10 using hydrazine (60%, 20 μL) at 95 °C for 1 h. The graphene was washed by distilled water to remove the residuary hydrazine and ammonia.

Preparation of MP-11 conjugated graphene

The MP-11/Graphene was prepared by a self-assembly method.[5-7] 1 mL graphene (0.05 mg/mL) and 1 mL MP-11 (0.186 mg/mL) were added into a vial. The mixture was diluted to 10 mL and stirred for 12 h. The MP-11 can immobilize with the graphene. Then the MP-11 will self-assembly on the surface of graphene. The MP-11/graphene solutions should be washed to remove the excess MP-11 molecules. After washing the volume was adjusted to 10 mL. It can be used for UV, Raman, AFM and electrochemical tests. Fig. S1 shows the schematics of the self-assembly of MP-11 with graphene. Fig. S2 shows the digital photograph of graphene (a) and MP-11/ graphene.

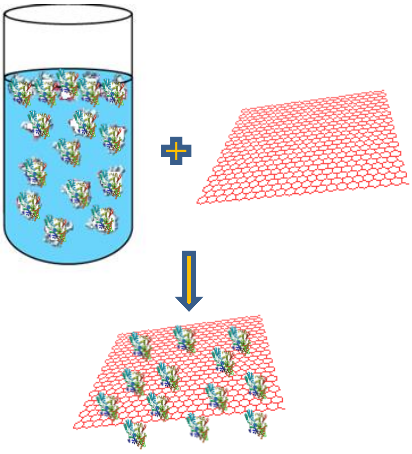
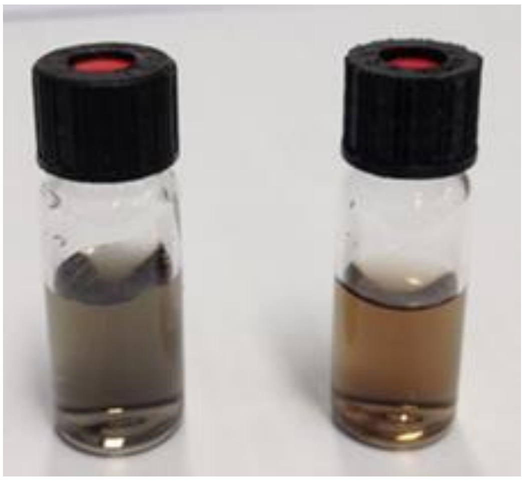


Fig. S1 Schematics of the self-assembly of MP-11 with graphene sheets.



(a) graphene **(b) MP-11/graphene**

Fig. S2 Digital photograph of graphene (a) and MP-11/ grapheme (b).

Characterization of MP-11/Graphene

Absorption spectra of MP-11/Graphene were recorded by UV-Vis spectroscopy by CARY 300 Bio UV-Vis spectrometer at room temperature. Raman spectra were conducted using a Renishaw InVia Raman Microspectrometer (Renishaw plc, Gloucestershire, UK), which was equipped with a 514-nm Ar laser (Renishaw plc, Gloucestershire, UK) and a thermo-electrical cooled CCD detector. The size of the graphene was performed by Dynamic Light Scattering (DLS) and Atomic Force Microscope (AFM) (Bruker, USA). The morphology and size of the MP-11/graphene were characterized using AFM.

Electrochemical Measurements.

Cyclic voltammetry and chronoamperometry were performed using a potentiostat (BASi, USA) with a three-electrode cell. A working electrode, a Ag/AgCl reference and a Pt wire counter electrode were used. Working electrodes used were a bare Au (2 mm in diameter) macroelectrode or a Au (12.5 μm in diameter) microelectrode. All the electrodes were purchased from CH Instruments (Austin, USA). The electrode surface was cleaned first physically by polishing with alumina slurries (starting from 0.3 down to 0.05 μm, CH Instruments), rinsing with ethanol and deionized water, and sonicating in water for 5 min, to remove all physically adsorbed species. Then the electrode was potentiostated between 0.0 to -1.5 V vs. Ag/AgCl in 0.5 M H_2SO_4 until a reproducible voltammogram was obtained. Experiments were conducted at (293 ± 2) K within a Faraday cage and all solutions were degassed thoroughly with N_2 and an atmosphere of N_2 was maintained during the experiment. Impact spikes were analyzed by using the program Origin v.8.6 for spike identification and integration. Electrical noise was removed by applying Fourier transform filtering at 50 Hz and multiples up to 200 Hz. Spikes were automatically identified by the same software at a threshold of 15% of the highest spike.

Experimental results

UV characterization of the MP-11 conjugated graphene nanosheets

It is important to examine conformational variation of MP-11 upon interaction with graphene. An absorbance band at 403 nm is present for MP-11. The absorption band for MP-11/graphene is at 409 nm. Their shapes are similar to each other with smaller red shift for MP-11/graphene. UV-Vis spectra show that the MP-11 remains their characteristic peak at in the presence of graphene, suggesting that the MP-11 has successfully combined on the graphene sheets.

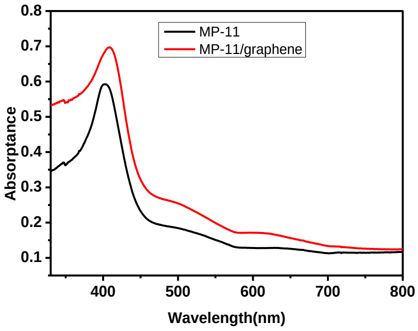


Fig.S3 UV-Vis spectroscopy of MP-11 (black line) and MP-11/graphene (red line).

Raman spectrums of graphene, MP-11 and MP-11/graphene

Fig.S4 is the Raman spectra of MP-11, graphene and MP-11/graphene. The MP-11/graphene keeps some peaks of MP-11 though the peak intensity is weaker than those of MP-11. The intensity ratio (I_D/I_G) is usually to indicate the level of chemical modification of graphitic carbon samples. The I_D/I_G ratio of MP-11/graphene and graphene is 0.98 and 0.94, respectively, which indicating the increase in disorder. The slight increase of I_D/I_G of MP-11/graphene could be attributed to an increase in the number of sp3 carbons on the graphene sheets which further verifies the successful immobilization of MP-11 molecules on graphene sheets. [8, 9]

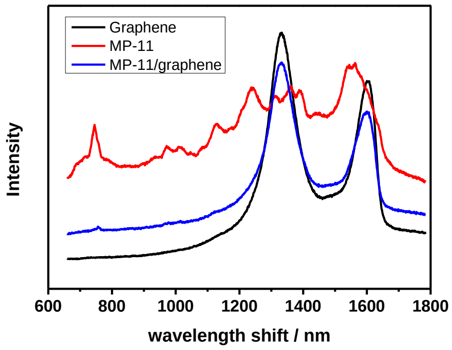


Fig.S4. Raman spectrums of graphene, MP-11 and MP-11/graphene using a laser with a wavelength of 514nm.

Size of MP-11/graphene

According to the MP-11/graphene hybrid AFM image, the average thickness is approximate 3 nm (Fig. S5). The concentration of MP-11 and graphene is 0.0186 mg/mL and 0.005 mg/mL, respectively. The average size of graphene sheet is 30±5 nm. The MP-11/graphene hybrid has a sandwich structure with graphene sheet in the middle and MP-11 on the surface and bottom of the graphene sheet. The 3 nm thickness of MP-11/graphene indicates that the MP-11 is monolayer on the surface of graphene sheet because the thickness of single layer graphene is 1 nm and single layer MP-11 is 1 nm. The increase of MP-11/graphene thickness could be attributed to the successful immobilization of the MP-11 on the surface of graphene sheets. The size of graphene sheets was also tested by Dynamic light scattering (DLS) experiments. The DLS data were obtained after the solution was ultrasonicated for 30 minutes. Fig. S6 shows that the graphene sheet with a concentration of 0.005 mg/mL has an average size of 49 ± 15 nm which is bigger than the size by AFM. DLS intensity depends with the sixth power of the diameter and then larger size graphenes are weighted more heavily in the measurement.[10] It is important to recall that DLS measurements assume 3D spherical particles, thus the numbers are to be taken more as the tumbling diameter of nanographene in solution, rather than actual size.[11, 12] The nanographene is not a real sphere though its diameter is much smaller than that of micrographene. The size distribution from DLS is just used as a reference. But the size distribution from DLS can give us the information of dispersity in an easy method. In our experiment, the graphene size we used is the one from AFM, not DLS.

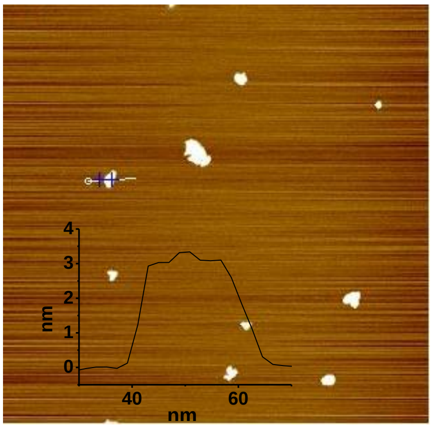


Fig. S5 AFM images of MP-11/graphene.（MP-11，0.0186 mg/mL, graphene, 0.005 mg/mL, scan size 1 μm）

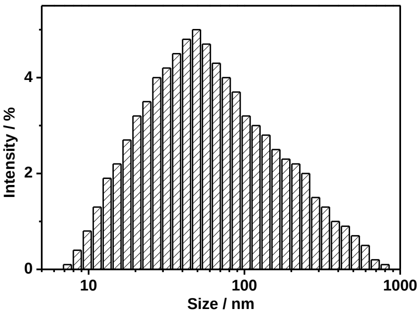


Fig.S6. Dynamic light scattering results for MP-11/graphene sheets. (MP-11，0.0186 mg/mL, graphene, 0.005 mg/mL）

Before we test the nanoparticle-electrode collision of MP-11/graphene, we should know the redox potential of MP-11. So we conducted cyclic voltammetry (CV) using a 2 mm diameter gold macroelectrode to confirm the redox potential of functionalizd graphene nanosheets. Fig. S7 shows that the reduction potential for MP-11 is about -400 mV, which is in well agreement with literature. [13] So we choose -400 mV as the threshold potential for MP-11 collision experiment.[14]

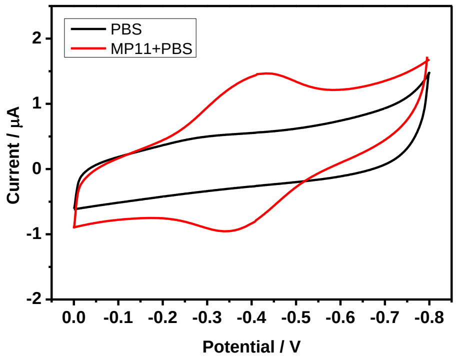


Fig. S7. Cyclic voltammetry performed using a gold electrode (2 mm in diameter) in a solution of 100 mM PBS (black) and MP-11 (0.0186 mg/mL) +100 mM PBS (red) at a scan rate 200 mV/s

A bare gold ultramicroelectrode with a diameter of 12.5 μ m was potentiostatted for the cyclic voltammetry in the PBS (10 mM, pH=7.0) and MP-11+PBS. Fig. S8 shows the cyclic voltammograms for the two groups in 10 mM PBS at a scan rate of 50 mV/ s. The curves show that there exist obvious redox peaks for MP-11 which is in well agreement with the literature.[15]

For a spike experiment at a given potential, the spike numbers were automatically identified at a threshold of 15% of the highest spike. The total charges were obtained by integration the spike current with time. The charge passed during each spike could be derived via dividing the total charges by the spike numbers.

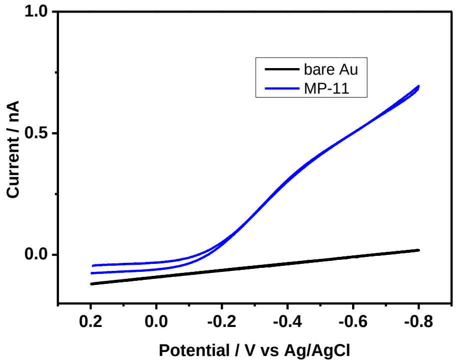


Fig. S8. Cyclic voltammograms of a bare Au electrode (black) and MP-11 (blue) in 10 mM PBS (pH 7.0). Scan rate: 50 mV/ s.

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