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Electronic Supplementary Information

Real-time Electrochemical Monitoring Covalent Bond Formation in Solution via Nanoparticle-Electrode Collisions

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Experimental details

Materials & Methods

Microperoxidase-11 (MP-11), Lomant's reagent, i.e. 3, 3′-Dithiodipropionic acid di(N-hydroxysuccinimide ester) and 3-mercaptopropanoic acid (MPA) 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%), hydrogen peroxide (30%) and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific. Millipore water (18 MΩ cm) was used to wash the electrodes and prepare buffer solutions in all experiments. All chemicals were of reagent grade and used as received.

Preparation, characterization of reduced graphene oxide (rGO) nanosheets and MP-11 functionalised rGO (MP-11/rGO) nanosheets

The rGO nanosheets were functionalised with MP-11 by non-covalent methods and formed a sandwich structure with MP-11 on both sides of rGO sheets.[1] The details about preparation and characterization of rGO nanosheets and self-assembly of MP-11 functionalised rGO sheets were reported in the previous publication.[1] The standard concentration of MP-11 and rGO in electrolyte solution is 0.018 and 0.005 mg mL^{-1}, respectively. The sizes of rGO nanosheets were measured by atomic force microscope (AFM) to be 40 ± 10 nm. The MP-11 functionalised rGO (MP-11/rGO) nanosheets were characterized by UV-Vis spectroscopy (CARY 300 Bio UV-Vis spectrometer), Raman spectrometer and Alpha FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany). Raman measurements were conducted using Renishaw Invia Raman Microspectrometer (Renishawplc, Gloucestershire, UK) equipped with a 514 nm laser and a thermo-electrical cooled CCD detector. Spectral data were acquired using 20 s exposure time and 100% power.

The immobilization of MP-11 on rGO nanosheets was verified by Raman, UV and AFM. From the Raman spectra, the characteristic peaks of MP-11 could be clearly observed and there were five sharp peaks at 1321, 1378, 1539, 1563 and 1614 cm^{-1}. (Figure S1A) For rGO, D band and G band is at 1350 and 1580 cm^{-1}, respectively. For the MP-11/rGO, two additional peaks were observed at 1354 and 1586 cm^{-1}, which are the typical D band and G band from the rGO. The D and G band exhibited a shift of 4-6 cm^{-1}. The D band indicates the disorder of graphene sheets while the G band stands for the structure of the in-plane sp^2 bond. Compared with the rGO, the I_D/I_G of MP-11/rGO increased from 0.94 to 0.98. This might be contributed to the increased disorder and the reduced in-plane sp^2 π conjugation that induced by the addition of MP-11 via non-covalent interaction.[2, 3] The peaks of MP-11 in MP-11/rGO exhibited a slight shift (3-4 cm^{-1}) which can be easily seen in the inset graph. All of these signals might be used to confirm the successful functionalization of rGO sheets by MP-11 molecules. Further, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was also applied to examine the MP-11/rGO nanosheets. Figure S1B shows the FTIR spectra of rGO, MP-11 and MP-11/rGO, which indicated three strong absorbation peaks from MP-11 including a NH_2 stretching peak around 1647 cm^{-1}, a strong C=N peak at 1530 cm^{-1} and a CH_3 stretching peak 1392 cm^{-1}, respectively. When the MP-11 was coated on rGO, we observed the peak around 1647 cm^{-1} red-shifted to 1586 cm^{-1} and the C=N peak moved to 1508 cm^{-1}. Meanwhile, the intensity of peak of CH_3 at 1391 cm^{-1} became stronger than that of CH_3 on rGO sheets. In the previous work,[1] we have also characterized MP-11/rGO using UV-Vis spectroscopy and AFM. UV-Vis spectra shows that the MP-11 remains their characteristic peak at in the presence of rGO, as shown in Figure S1C, the peak located at 409 nm is enhanced with the increased concentration of MP-11, which probably due to the non-covalent interaction between MP-11 molecules and rGO nanosheets. AFM topographic heights of MP-11/rGO sheets are in the range of 3-4 nm, suggesting that the MP-11 has successfully combined on the rGO sheets.

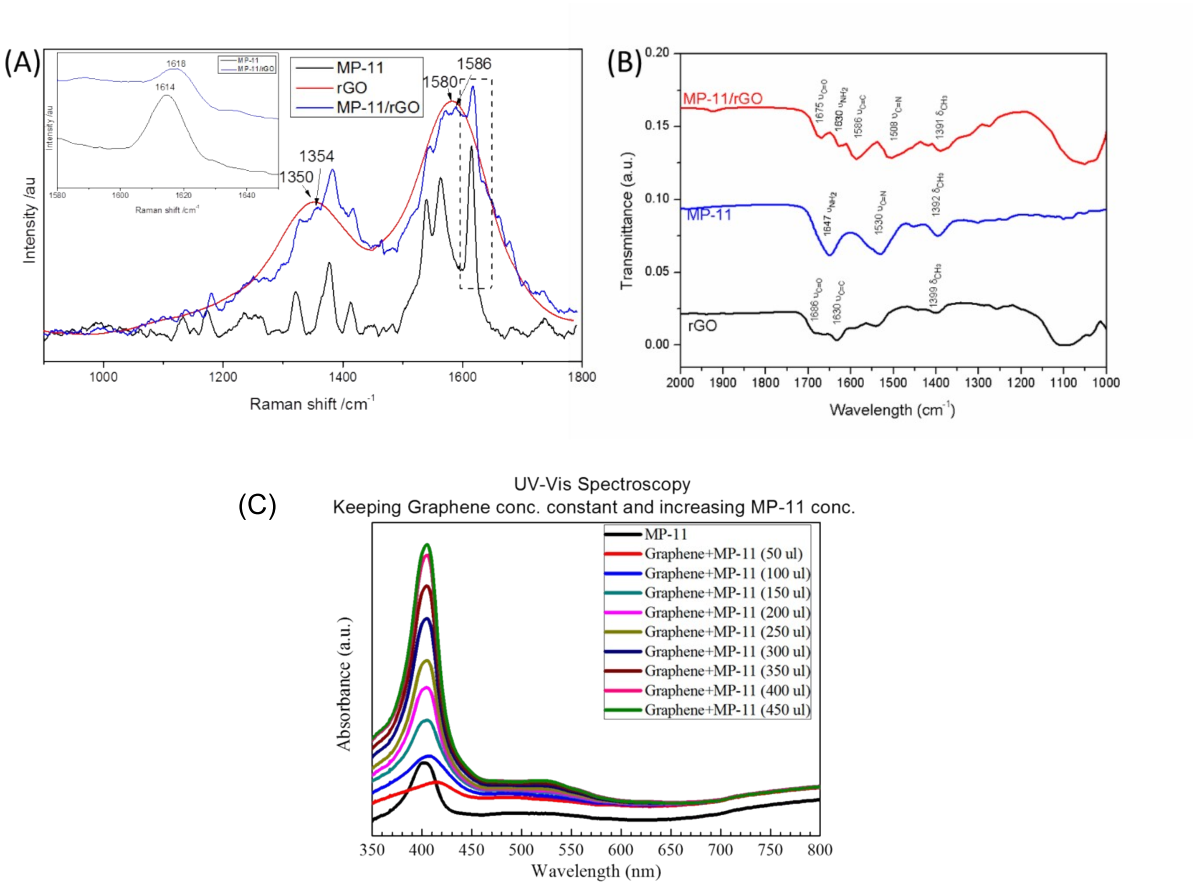

Figure S1 (A) Raman spectra and (B) FTIR spectra of MP-11, rGO and MP-11/ rGO nanosheet. (C) UV-Vis spectra of MP-11 and MP-11/rGO composites.

Preparation of modified electrode (Lomant/Au and MPA/Au electrode) and electrochemical measurements

All electrochemical measurements were performed using a potentiostat (BASi, USA) with a conventional three-electrode system comprising a bare or modified working electrode, an Ag/AgCl reference and a Pt wire counter electrode. All the electrodes were purchased from CH Instruments (Austin, USA). 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. Disk gold electrodes (diameter of 12.5 μm and 2 mm) were polished with 1.0 μm, followed by 0.3 and 0.05 μm alumina slurry to produce a mirror-like surface. The electrodes were ultrasonicated respectively in water and ethanol for 10 min to remove the trace alumina. Then the electrodes were cleaned electrochemically by potential cycling between -0.3 and 1.7 V at a scan rate of 200 mV s^{-1} in 1 M sulfuric acid until reproducible scans were recorded.[4] After that the clean electrodes were washed with ultrapure water and used for electrochemical test. 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.

The Lomant's reagent modified gold electrode was prepared by immersing the gold electrode into a solution of Lomant's reagent in DMSO with a concentration of 5 mg mL^{-1} for 6 hours.[5] The MPA self-assembled monolayer (SAM) modified gold electrode was prepared by immersing the gold UME in MPA solution in ethanol with a concentration of 10 mM for 6 hours at room temperature.[6] The modified electrodes were denoted as Lomant/Au and MPA/Au electrode, respectively. To confirm the covalent bond formation, the Lomant/Au and MPA/Au electrode were further immersed in MP-11/rGO solution for 4 hours in the presence of EDC at room temperature, which were denoted as MP-11/rGO/Lomant/Au and MP-11/rGO/MPA/Au electrode. The electrochemical characterization of the modified electrode was done by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) by using an Au electrode (diameter 2 mm) as shown in Figure S2 and Figure S3.

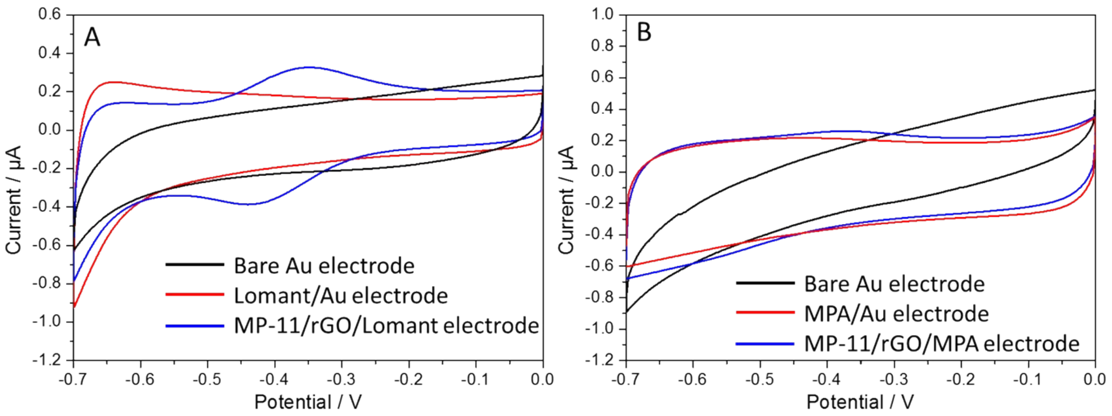

Figure S2. Cyclic voltammograms (CVs) at a scan rate of 50 mV s^{-1} in 0.05 M phosphate buffer (pH = 7.4).

It is noted that MP-11 is a redox species that undergoes redox reaction via a one electron transfer process.[7] Therefore, the attachment of MP-11/rGO nanosheets with the Lomant reagents could be evidenced by CVs. As shown in Figure S2 (A), we have not seen no obvious redox peaks in bare Au electrode and Lomant/Au electrode in phosphate buffer solution (pH = 7.4). However, after immersing the Lomant/Au electrode in MP-11/rGO solution, a well-defined redox peaks centered at -0.4 V were observed, indicated the attachment of MP-11/rGO nanosheets. In Figure S2 B, no obvious peaks after immersing the MPA/Au electrode in MP-11/rGO solution proves that no MP-11/rGO nanosheet is immobilized on the electrode surface.

The electrochemistry of the electrode with different modification stages was also studied in $Fe(CN)_6^{3-/4-}$ solution as shown in Figure S3. The pronounced ferricyanide electrochemistry observed on the bare Au electrode was suppressed after modification with Lomant reagent and MPA reagent (Figure S3 A and C). The incomplete current suppression and diversity between Lomant/Au and MPA/Au electrode is due to the steric hindrance of Lomant and MPA molecules, leaving un-densed monolayer on the electrode surface. Further immobilization of MP-11/rGO nanosheets on Lomant/Au electrode surface via covalent bonds could “switch on” the electrical communication that could be evidenced from the regained electrochemistry in Figure S3 (A) and the decreased charge transfer resistance in Figure S3 (B). However, no obvious regained electrochemistry in Figure S3C and S3D after incubating the MPA/Au electrode in MP-11/rGO solution indirectly demonstrated the un-attachment of MP-11/rGO nanosheets. All these signals indicate the stepwise modification of Lomant/Au and MPA/Au electrode as well as the Hit-and-Stand model and Hit-and-Run model, respectively.

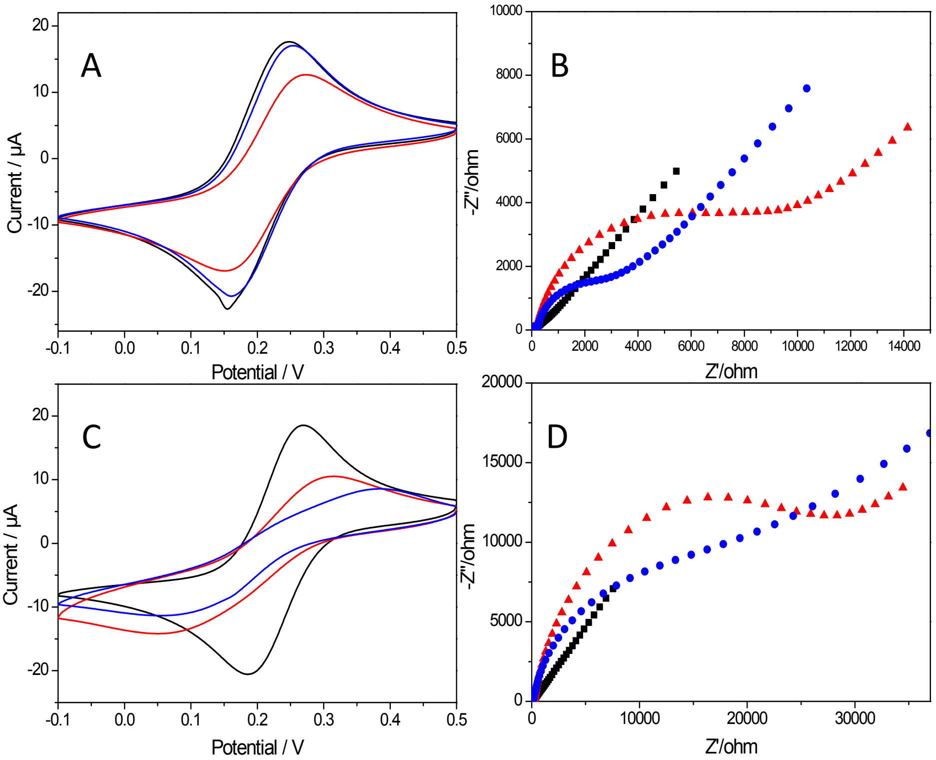

Figure S3. The CVs (A) and electrochemical impedance spectroscopy (EIS) (B) spectra of MP-11/rGO /Lomant/Au electrode fabrication process. (Dark line: bare Au electrode, red line: Lomant/Au electrode, Blue line: MP-11/rGO/Lomant/Au electrode). The CV (C) and EIS (D) spectra of MP-11/rGO/MPA/Au electrode fabrication process. (Dark line: bare Au electrode, red line: MPA/Au electrode, Blue line: MP-11/rGO/MPA/Au electrode)

The CV was conducted using a gold electrode with a diameter of 2 mm to confirm the redox potential of MP-11/rGO nanosheets. The redox potential of MP-11/rGO nanosheets is used to determine the potential for the nanoparticle-electrode collision. Figure S4 shows the CV of MP-11/rGO nanosheet at a bare gold electrode and Lomant/Au electrode (2 mm in diameter) at a scan rate 0.2 Vs^{-1}. It shows that the reduction potential for MP-11/rGO sheet is -0.4V. Then we determined the threshold potential for spike onset is -0.4 V.

As control experiments, chronoamperograms were recorded injecting MP-11 (but no rGO) at Lomant/Au and MPA/Au electrodes. Figure S5 shows that there nearly appears no redox current except for a background current. The background current is so small that it can be neglected compared the current transient in Figure 3.

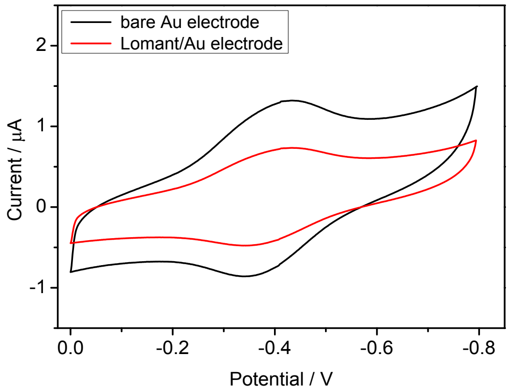

Figure S4. Cyclic voltammetry after injecting MP-11/rGO nanosheet solution at a bare gold electrode and Lomant/Au electrode (2mm in diameter) at a scan rate 0.2 Vs-1. The concentration of MP-11 and rGO in electrolyte solution is 0.018 and 0.005 mg mL^{-1}.

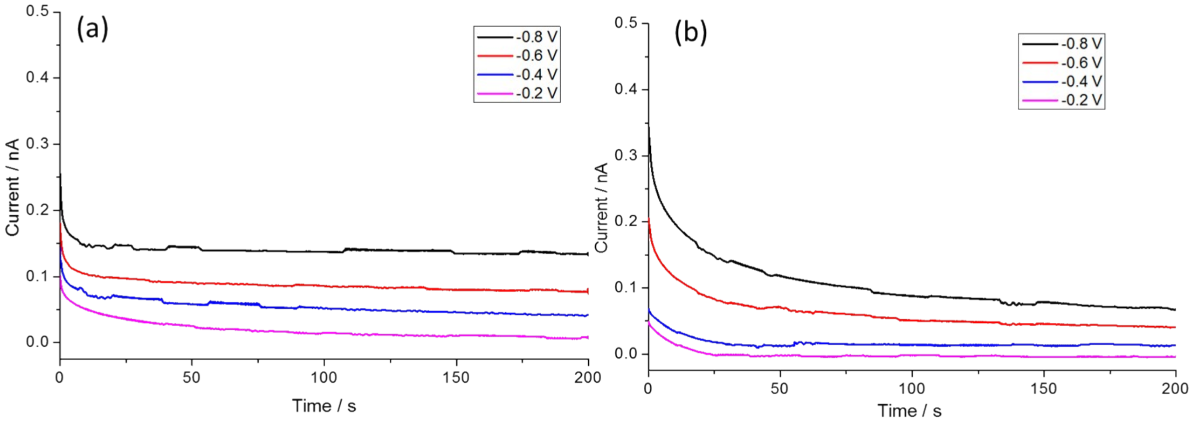

Figure S5. Current transient recorded after injection of MP-11 at Lomant/Au a) and MPA/Au b) at different potentials. No staircase or spike current was produced.

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