

## Electronic Supplementary Information

### Probing the tunable surface chemistry of graphene oxide

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### Materials & Methods

**Synthesis of graphene oxide and chemically reduced graphene oxide.** Graphene oxide dispersed in aqueous solution was synthesized using a modified Hummers' Method.<sup>1</sup> In a typical experiment, 2 g of graphite flakes (from Sigma-Aldrich) was mixed with 12 mL of concentrated H<sub>2</sub>SO<sub>4</sub> (Merck, 98% conc.) and kept stirring at 80°C for 5 h on a heating plate. Then the solution was cooled at room temperature and ultrasonicated using a water bath sonicator (VWR industries, GRANXUBA3) for another 5 h to break the larger flakes into smaller flakes. The mixture was diluted with 500 mL of distilled water (dH<sub>2</sub>O) and left overnight. The settled preoxidized graphite flakes were obtained by filtering the solution with porous filters (200 nm pore size). The residue was dried at 80°C in a drying oven to remove water quickly. To further transform the preoxidized graphite into graphite oxide, the resultant powder was put

into 120 mL conc.  $\text{H}_2\text{SO}_4$ . Next,  $\text{KMnO}_4$  (15 g) was added slowly (within 1 h) and the mixture was then stirred at room temperature for at least 2 h. The solution was diluted with 250 mL of  $\text{dH}_2\text{O}$  very carefully and stirred for a further 2 h, and then 700 mL  $\text{dH}_2\text{O}$  was added. Within a short period of time, 20 mL of  $\text{H}_2\text{O}_2$  (Sigma-Aldrich, 30%) was added to the mixture until the color turned bright yellow. Ultrasonication was conducted for 4 h in order to exfoliate graphene oxide sheets from the oxidized product. The resultant dispersion was divided into 15 mL batches and centrifuged at 10,000 rpm for 30 min (Eppendorf centrifuge 5810R). Pellets were re-dissolved in 1:10 HCl (Chem Supply, 32% w/w) with vigorous shaking and centrifuged for 10 min to remove unwanted metal ions. This was repeated another two times. Then pellets were collected and dissolved in 10 mL  $\text{dH}_2\text{O}$  and centrifuged for 10 min at 10,000 rpm to remove acid. Centrifugation was performed repeatedly with  $\text{dH}_2\text{O}$  until the light yellow supernatant was obtained which were GO sheets. The obtained GO was characterized using UV-Vis spectroscopy and atomic force microscopy.

Chemically reduced graphene oxide (CRGO) was tuned for surface hydrophobicity using a modified literature method.<sup>2</sup> Briefly, 200 mg of L-ascorbic acid (Sigma Aldrich, Reagent Grade) was added to 20 mL of GO dispersion (1 mg/mL) and stirred vigorously for different time intervals namely 1, 6, 12, 24 and 48 h. Once the reduction was carried out for the desired period of time, the reaction was stopped by centrifuging (Eppendorf centrifuge 5810R) at 11,000 rpm for 15 min and washing it thrice with  $\text{dH}_2\text{O}$ . The pH of the as prepared CRGO was adjusted to 9.5 with the aid of sodium hydroxide (1 mM). The reduction reaction and surface charge was monitored using UV-Vis and Raman spectroscopy.

**CRGO/[Ru(bpy)<sub>3</sub>]<sup>2+</sup> thin film preparation.** In a typical film formation, 2 mL of CRGO (1 mg/mL), namely 1, 6, 12, 24 and 48 h were vacuum filtered on an isoporous membrane (polycarbonate, hydrophilic, 0.22  $\mu\text{m}$ , 25 mm, white, plain, Millipore Corporation, Australia) using a vacuum filtration unit (RZ6, Vacuubrand

Inc., U.S.A.). Additional 4 mL dH<sub>2</sub>O was added to wash the film surface. Free standing films were peeled off from the membrane after drying. For the case of composite films, 2 mL of CRGO dispersion was mixed with 0.2 mL of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> solution (1 mM, Sigma-Aldrich) with vigorous stirring. Then same vacuum filtration was carried out to obtain a thin composite film. CRGO and composite films were characterized using X-Ray Powder Diffraction (XRD) and Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR).

### **Instruments and sample preparation**

**UV-Visible Spectroscopy (UV-Vis).** All the scans were performed in a continuous mode from 800 nm to 200 nm using quartz cuvette of path length 1 mm, with a scan rate of 500 nm/min and data interval of 1 nm using Varian Cary 300. For the preparation of GO/[Ru(bpy)<sub>3</sub>]<sup>2+</sup> samples used in Figure 2a, the concentration of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> was kept as 0.02 mM and that of GO increased gradually (5, 10, 25, 50 and 100 µg mL<sup>-1</sup>). The pH value of all graphene solution (including GO and CRGO) is adjusted to 9.5 using sodium hydroxide. For the samples in Figure S4a and Figure 3a, all of the CRGO samples were diluted to a concentration of 40 µg mL<sup>-1</sup>. And the [Ru(bpy)<sub>3</sub>]<sup>2+</sup> added into different CRGO in Figure 3a had the same concentration – 40 µM. All of the samples were mixed in centrifuge tubes (1.5 mL) with strong shaking and few minutes sonication in order to obtain well-dispersed solution.

**Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR).** ATR-FTIR was performed using Alpha FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector and a single reflection diamond ATR sampling module (Platinum ATR Quick- Snap™).<sup>3</sup> Spectral resolution 4 cm<sup>-1</sup> with 256 co-added scans were used. Background measurements were obtained before scanning each sample. Secondary derivative and curve fitting was done using OPUS 6.0 software suite. All of the FTIR samples were fabricated into thin films by vacuum filtration. Each film contained 1

mg graphene and had a diameter of 2 cm.

**Raman spectroscopy.** Raman measurements were conducted using Renishaw Invia Raman Microspectrometer (Renishaw plc, Gloucestershire, UK), equipped with a 457 nm laser, 1,800 grating and a thermo-electrical cooled CCD detector. Spectral data was acquired using 20 s exposure time, 5% power together with 4 cm<sup>-1</sup> spectral resolution. The sample preparation method was to put one drop of diluted solution on the flat aluminum-foil paper. After liquid evaporated, the residues were used for Raman tests.

**Zetasizer.** The particle size and surface charge of CRGO and its composites were measured using Zetasizer nano ZS. Normal two-way cuvette was used for particle size and disposable capillary cell DTS 1061 (Malvern Instruments, Worcestershire, UK) was used specially for zeta potentials. All measurements were carried out at room temperature with refractive index 2.4 and equilibration time of 2 min. Before any test, the pH value of graphene samples was tuned to 9.5 to prevent the sample from aggregation. The concentration of GO used in the tests was 0.1 mg mL<sup>-1</sup> and the usage of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> increased gradually (0, 0.02, 0.04, 0.08 and 0.16 mM).

**Photoluminescence spectra.** The photoluminescence spectra were measured using a fluorescence spectrophotometer (Cary, Eclipse). A quartz cuvette (pathlength 10 mm, purchased from Starna Pty. Ltd.) with 4 transparent sides was used in all the measurement. For the sample preparation in Figure 2b, the concentration of photoluminescent substance – [Ru(bpy)<sub>3</sub>]<sup>2+</sup> was adjusted to 0.05 mM with different mass of GO (0, 0.03, 0.06, 0.15, 0.30 mg mL<sup>-1</sup>). And in Figure 3b, the concentrations of CRGO were all 0.1 mg mL<sup>-1</sup> and that of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> was kept as 40 μM. The excitation wavelengths used in all of the experiments was 286 nm.

**X-Ray Powder Diffraction (XRD).** XRD results were tested using X'Pert Powder

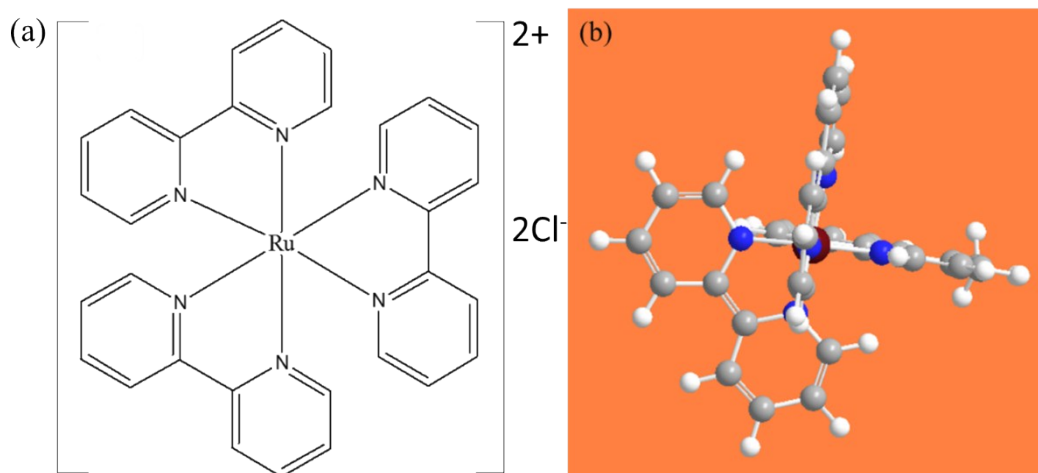
Instrument (The Analytical X-ray Company). The operating voltage was set as 40 mV and current was 30 mA. The diffraction angle started with 6° and ended at 70°. Sample preparation was totally the same with that used in ATR-FTIR and the films were fixed on a glass slide using tape.

**Atomic Force Microscopy (AFM).** For AFM imaging purpose, multimode 8 from Bruker biosciences corporation (USA) was used in peak force quantitative nano-mechanical imaging mode. All the high resolution images were obtained at a scan rate of 0.977 Hz, 512 scans/lines and aspect ratio of 1 at room temperature. The image processing was carried out using Nanoscope Analysis (Version 8.1) provided with the instrument and the height profile was obtained by WsXM (Nanotech Electronica, S.L., Spain). The probes used for scanning was also obtained from Bruker (Scanasyst Air). For sample preparation, highly diluted solution was drop casted on freshly cleaved mica surface and allowed to dry at room temperature with help of spin coater (WS-650MZ-23NPP).

**Cyclic voltammetry (CV).** All voltammetric experiments were carried out using a Biologic SAS, model 1. A three-electrode system was used for CV constituting of a counter electrode (platinum mesh), a reference electrode (Ag/AgCl) and a working electrode. The working electrode was obtained by drop casting samples on a glassy carbon electrode (GCE).

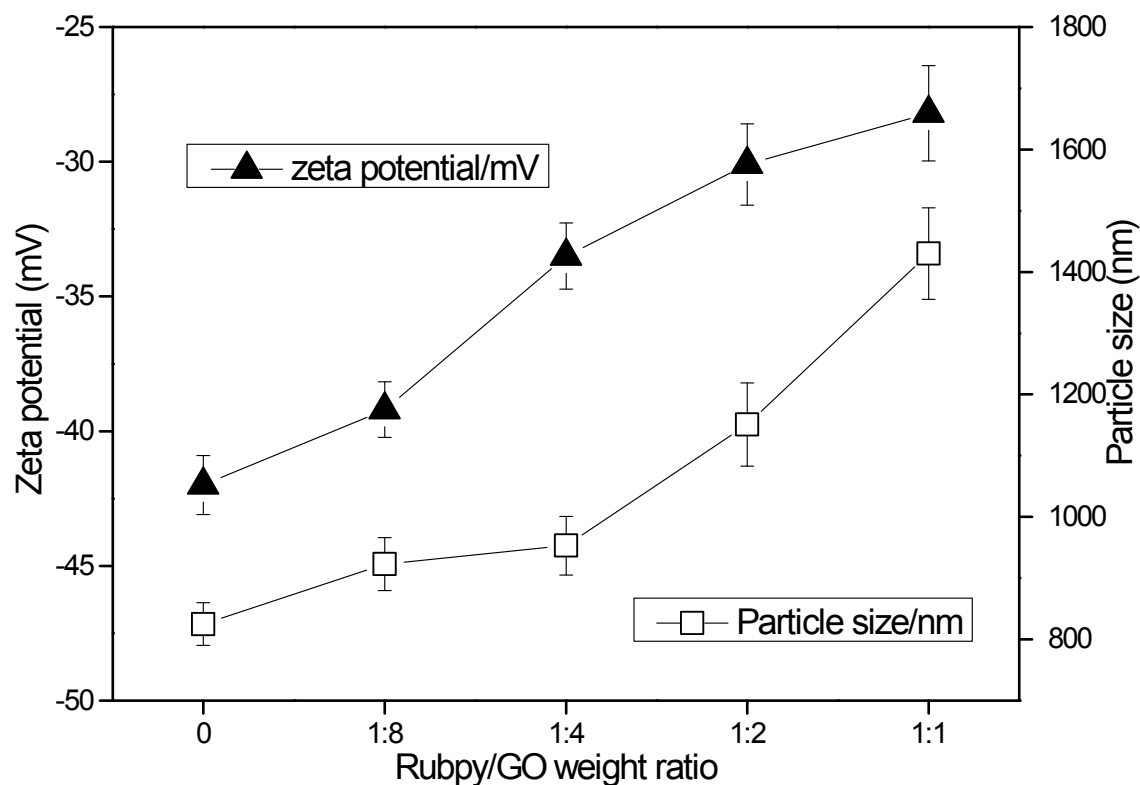
## **Characterizations**

In Figure S1a, we show the chemical structure of  $[\text{Ru}(\text{bpy})_3]^{2+}$  molecule. Figure S1b is the spatial structure of  $[\text{Ru}(\text{bpy})_3]^{2+}$  molecule generated by the software (Chem3D Ultra 8.0), which was the lowest-energy form. It shows the three ligands extended to three different orientations. We used the coordinate figures to estimate its size to be around 1.0 nm across.



**Figure S1.** (a) Chemical structure of  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  and (b) the 3D structure of  $[\text{Ru}(\text{bpy})_3]^{2+}$  calculated by minimizing energy using the molecule mechanics method.

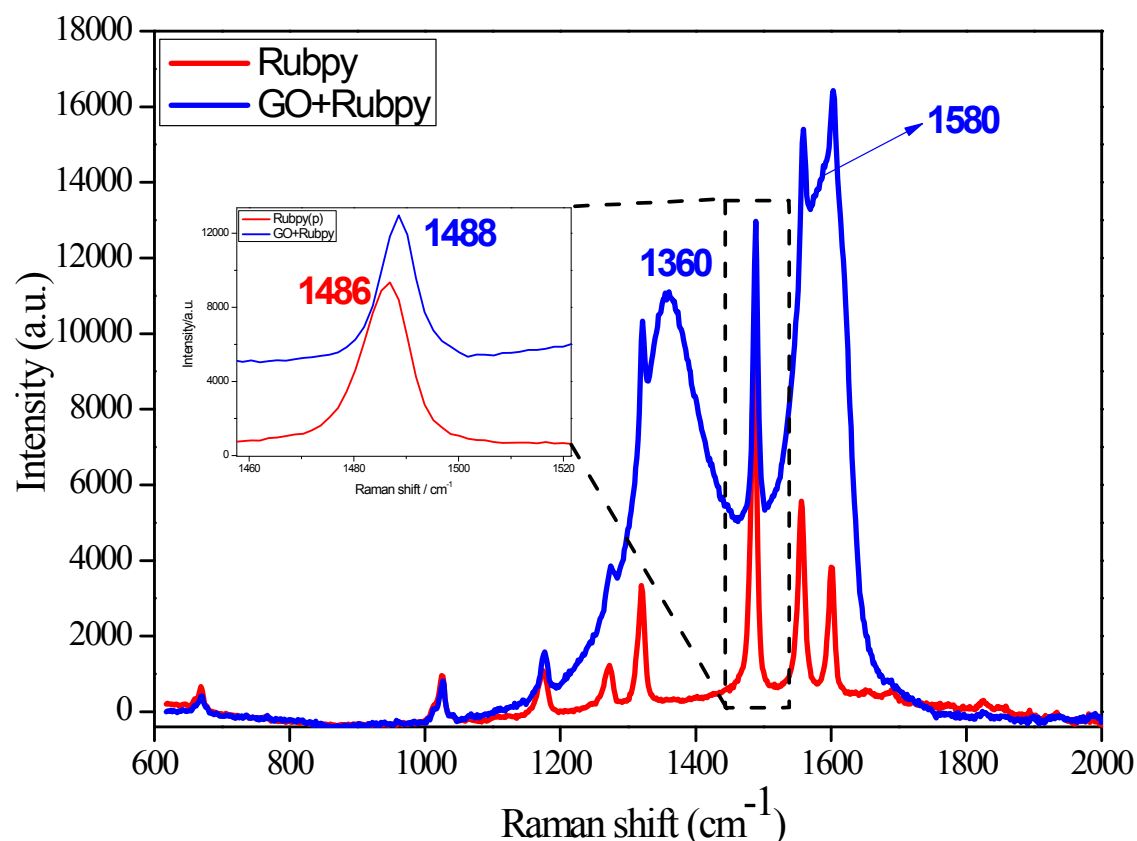
Zeta potential results for the detection of electrostatic attraction between GO and  $[\text{Ru}(\text{bpy})_3]^{2+}$  (Figure S2). Firstly, the pH value of GO solution was raised to approximately 9.5 by adding NaOH solution (1.0 mM) and a negative charge (-43 mV) was observed. Therefore, the strong repulsion forces between each GO sheet helped form single-layers in aqueous solution. The particle size was statistically detected to be 825 nm. With the gradual increase of  $[\text{Ru}(\text{bpy})_3]^{2+}$  solution (pH 5.5), which was positively charged (10 mV) in water, GO composites became less negatively charged due to charge neutralization. Meanwhile, loss of surface charge led to GO sheet aggregation, which was considered to be the reason for the observed increase in particle size.



**Figure S2.** Zeta potential and particle size results of GO/[Ru(bpy)<sub>3</sub>]<sup>2+</sup> composites, with increasing concentrations of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> from left to right, while the concentration GO was unchanged.

Raman spectra were measured with a laser excitation wavelength of 457 nm under ambient conditions. Raman is a sensitive method to characterize the change in the surface of graphene. The samples were prepared by dropping the solution on the flat aluminum foil, which did not show any signal, and then let it dry. The characteristic peaks of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> could be clearly seen in the red curve (Figure S3). Specifically, there were four sharp peaks at 1318, 1488, 1555 and 1600 cm<sup>-1</sup>. In the case of the GO/[Ru(bpy)<sub>3</sub>]<sup>2+</sup> composite, two additional peaks at 1360 and 1580 cm<sup>-1</sup>, the typical D band and G band from graphene materials, were observed. The peaks belonging to [Ru(bpy)<sub>3</sub>]<sup>2+</sup> exhibited a slight red-shift (2-3 cm<sup>-1</sup>) which can be more easily seen in the inset graph. This was evidence of the interaction of [Ru(bpy)<sub>3</sub>]<sup>2+</sup> and GO. The D band for GO indicates the disorder of carbon sheets while the G band stands for the

structure of the in-plane  $sp^2$  bond.  $I_D/I_G$  of GO was calculated to be 0.98. After mixed with  $[Ru(bpy)_3]^{2+}$ , this ratio decreased slightly to 0.93. The reason for this might be the remarkable increase in size and extended  $sp^2$   $\pi$  conjugation that was introduced by  $[Ru(bpy)_3]^{2+}$  *via*  $\pi$ - $\pi$  stacking interactions.

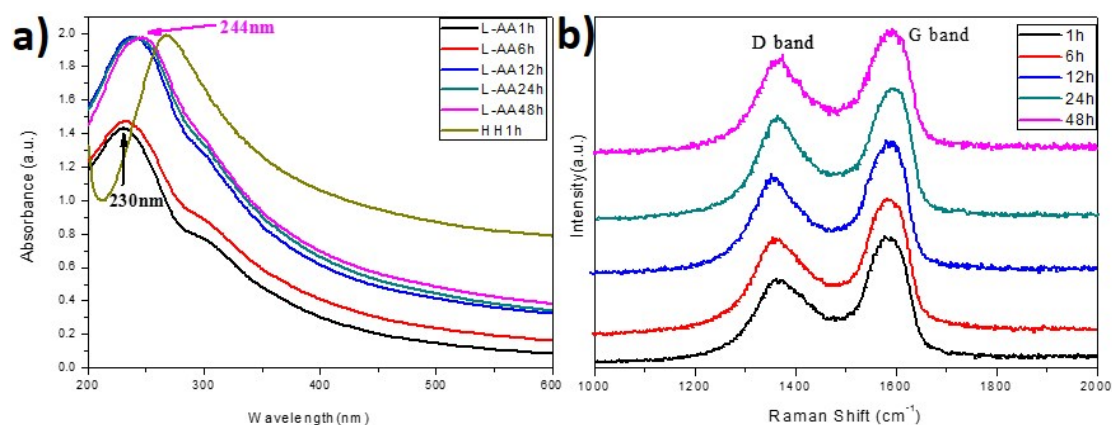


**Figure S3.** Raman spectra of  $[Ru(bpy)_3]^{2+}$  (red curve) and GO/ $[Ru(bpy)_3]^{2+}$  composite (blue curve) measured under the laser (457 nm) excitation.

UV-Vis spectra was utilized to monitor the chemical reduction as shown in Figure S4a. In case of the 1h sample, it showed a shoulder around 300 nm due to the  $n$ - $\pi^*$  transition of C=O bonds and a peak around 230 nm due to the  $\pi$ - $\pi^*$  transition of C-C bonds.<sup>4</sup> At 6 h, the changes were very small, with the peak center slightly shifted from 230 nm to 231 nm. By 12 h, this peak had moved to 238 nm and finally, it reached 244 nm after 48 h. Meanwhile, the broad peak around 300 nm gradually diminished due to removal of the C=O bands. Obviously the reduction reaction was halfway when comparing with those CRGO whose C-C bond center moved to 268 nm reduced

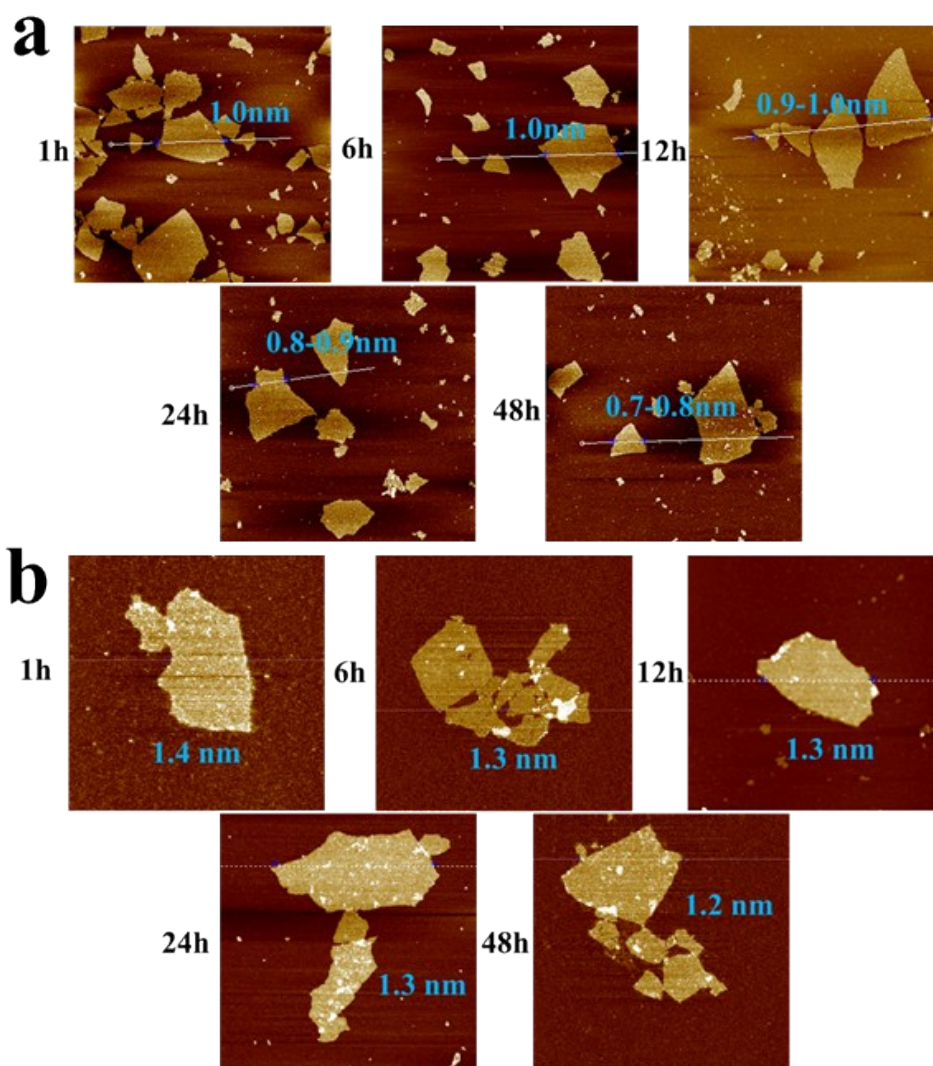


by hydrazine hydrate (HH) (black green curve).<sup>5</sup> Raman spectra was also used to characterize the changes that occurred during the reduction. The variation of the relative intensities of G (the  $E_{2g}$  mode of  $sp^2$  carbon atoms) and D (the symmetry  $A_{1g}$  mode) bands in Raman spectra revealed the change of the electronic conjugation state. As shown in Figure S4b,  $I_D/I_G$  grew with the increase of reaction time (from 0.95 at 1 h to 1.26 at 48 h). This agreed well with the results of GO reduced by hydrazine.<sup>6</sup> This method had the advantages of mild condition and lower degree of reduction, which was suitable for controllable adjustment of GO surface.



**Figure S4.** UV-Vis spectra (a) and Raman spectra (b) of CRGOs with different reduction time (1h, 6h, 12h, 24h and 48h).

AFM images and corresponding height profiles were obtained to observe the morphology tuning of CRGOs. Figure S5a showed the change in height in CRGO sheets under different reduction reaction times. With step-by-step removal of surface oxygen groups, the thickness of monolayer CRGO dropped from 1.0 nm to 0.7 nm. And as mentioned in the main paper, Figure S5b was used to examine the heights of  $[Ru(bpy)_3]^{2+}$  on CRGO sheets, showing a decrease of average figures with longer reduction times. The measured thicknesses of monolayer  $[Ru(bpy)_3]^{2+}$  on CRGOs with different reduction time have been summarized in Table S1.

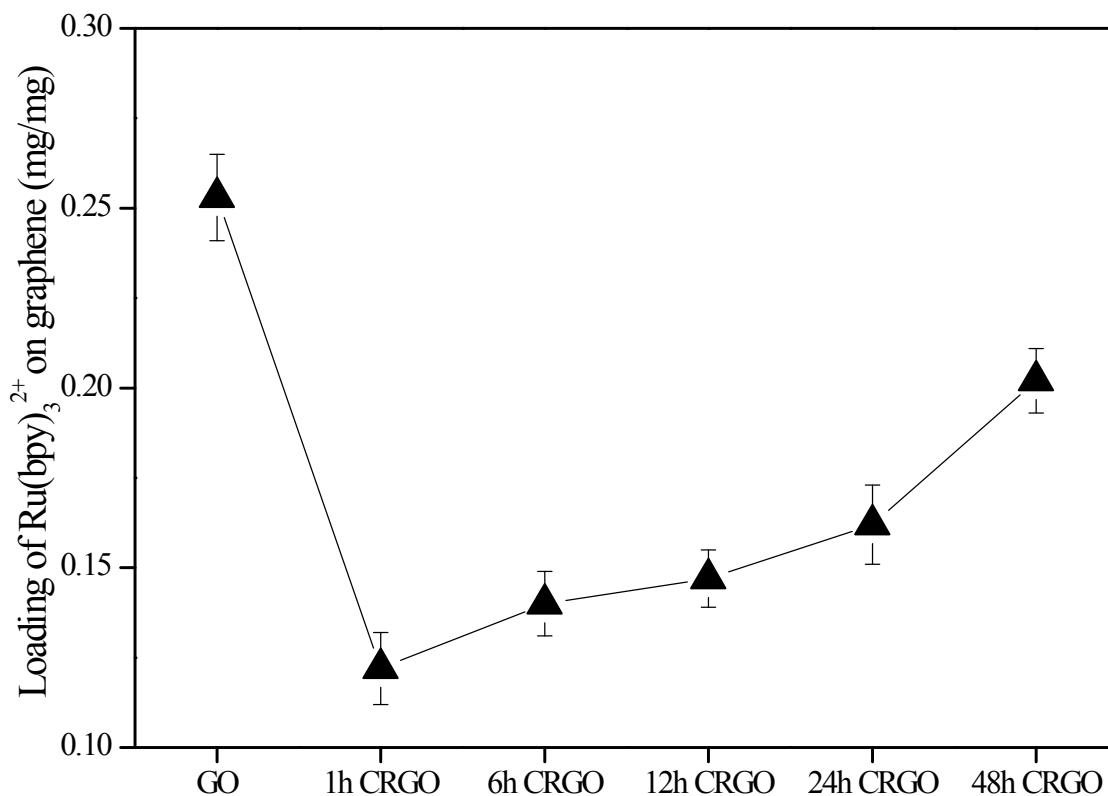


**Figure S5.** AFM images of (a) CRGOs with different reduction time (the digits in blue show the thicknesses of monolayer CRGOs) and (b) AFM images of monolayer  $[\text{Ru}(\text{bpy})_3]^{2+}$  on CRGOs (the digits in blue are the average thicknesses of monolayer  $[\text{Ru}(\text{bpy})_3]^{2+}$ ).

**Table S1.** Summary of the measured thicknesses of monolayer  $[\text{Ru}(\text{bpy})_3]^{2+}$  on different CRGOs.

Substrates	Average of the thickness of monolayer $[\text{Ru}(\text{bpy})_3]^{2+}$ on different CRGOs
CRGO 1h	$\approx 1.4$ nm
CRGO 6h	$\approx 1.3$ nm
CRGO 12h	$\approx 1.3$ nm
CRGO 24h	$\approx 1.3$ nm
CRGO 48h	$\approx 1.2$ nm

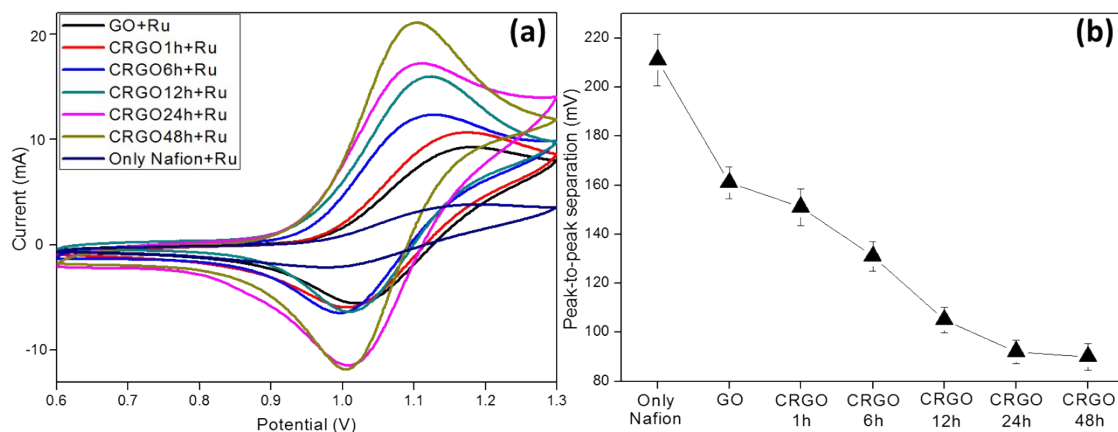
The loading of  $[\text{Ru}(\text{bpy})_3]^{2+}$  on GO was calculated using the intensities of the 286 nm absorbance peak of  $[\text{Ru}(\text{bpy})_3]^{2+}$ . Firstly, the standard curve for  $[\text{Ru}(\text{bpy})_3]^{2+}$  was plotted using a wide range of  $[\text{Ru}(\text{bpy})_3]^{2+}$  concentrations. Then, for each sample, 650  $\mu\text{L}$  of GO and 200  $\mu\text{L}$  of  $[\text{Ru}(\text{bpy})_3]^{2+}$  were mixed, shaken vigorously and centrifuged at 14,000 rpm for 1 h. The supernatant was used to measure the concentration of  $[\text{Ru}(\text{bpy})_3]^{2+}$  that was not bound to GO, enabling the loading of  $[\text{Ru}(\text{bpy})_3]^{2+}$  on the graphene material to be calculated. Obviously, GO has the best loading ability, which is due to the large quantities of oxygen groups on it. There is an increasing trend of  $[\text{Ru}(\text{bpy})_3]^{2+}$  immobilization from 1 h reduced sample to 48 h CRGO. Electrostatic and  $\pi$ - $\pi$  stacking interactions both contribute to  $[\text{Ru}(\text{bpy})_3]^{2+}$  binding, but chemical reduction brings opposite influences for these two related interactions.



**Figure S6.** Loading experiment of  $[Ru(bpy)_3]^{2+}$  on GO and CRGO with different reduction times.

Cyclic voltammetry (Figure S7) was used to investigate the electrochemistry of graphene/ $[Ru(bpy)_3]^{2+}$  composite. Here Nafion (Sigma Aldrich) was used to generate a protection film on the surface of glassy carbon electrode (GCE). Firstly, 5  $\mu$ L of graphene aqueous solution (0.1 mg mL<sup>-1</sup>) was dropped on GCE surface and dried in room temperature. Then 5  $\mu$ L of graphene (0.1 mg mL<sup>-1</sup>) / Nafion (0.5 wt. %) mixture was dropped on GCE surface and dried as well. This modified electrode was immersed in  $[Ru(bpy)_3]^{2+}$  solution (1 mM) and kept overnight. Next, its surface was carefully rinsed with distilled water and used for cyclic voltammetry tests. A three-electrode system was used for CV constituting of a counter electrode (platinum mesh), a reference electrode (Ag/AgCl) and a working electrode. For each sample, the CVs were scanned from 0.6 V to 1.3 V for 10 circles with a scan rate 100 mV s<sup>-1</sup> in the phosphate buffer solution (pH 7.5, 100 mM) in room temperature. The oxidation peak of  $[Ru(bpy)_3]^{2+}$  was found to undergo a negative shift while increasing the reduction

times of GO and there was a decreasing trend in the peak separation under controlled chemical reduction.



**Figure S7.** Cyclic voltammetry curves (a) and peak-to-peak separations (b) of graphene/[Ru(bpy)<sub>3</sub>]<sup>2+</sup> with different reduction times (these curves were obtained in aqueous 100 mM phosphate buffer at pH 7.5 with scan rate 100 mV s<sup>-1</sup> from 1.3 V to 0.6 V).

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