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Supporting Information

Aggregation morphology is a key factor determining protein adsorption on graphene oxide and reduced graphene oxide nanomaterials

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Methods and Materials

Preparation of RGOs

Graphene oxide (> 99%) was purchased from Nano Materials Tech Co. (Tianjin, China). Based on the information provided by the supplier, the product was synthesized using a modified Hummers method.

The procedures of preparing RGO materials were reported in our previous work.[S1] Briefly, to prepare the RGO materials, 1 mL of N_2H_4 (> 80%, Fuyu Fine Chemical Co., Ltd, China), 1 g of $NaBH_4$ (Victor Co., Ltd, China) or 2 g of L-ascorbic Acid (Victor Co., Ltd, China) was slowly added to 400 mL of a 250 mg/L GO suspension. For N_2H_4-reduced GO, the mixture was heated to 80 ºC under vigorous stirring for 24 h or for $NaBH_4$-reduced GO, the mixture was vigorous stirring for 2 h under 80 ºC. For L-ascorbic acid-reduced GO, the mixture was vigorously stirred for 24 h at room temperature. Afterward, the suspension was cooled to room temperature (if applicable), and filtered through 0.22-μm membrane filters. The retained RGO material on the filter was collected and added to approximately 200 mL DI water, then ultrasonicated at 100 W for 30 min. The rinsing procedure was repeated several (at least three) times to remove extra reductant. Finally, the filtrate was collected and re-ultrasonicated in DI water to make a 1 mg/ml stork solutions. The stock solutions (~1000 mg/L) were stored in borosilicate glass containers at 4 ºC in dark. Moreover, a fraction of each filtrate was vacuum-dried for material characterization. The RGO materials prepared using the three different reducing agents are referred to as N_2H_4-RGO, $NaBH_4$-RGO, and VC-RGO, respectively.

Characterization of GO and RGOs

Surface elemental composition of the GO/RGO materials were determined with X-ray

photoelectron spectroscopy (XPS) (PHI 5000 VersaProbe, Japan). Fourier transform infrared (FTIR) transmission spectra were obtained using a 110 Bruker TENSOR 27 apparatus (Bruker Optics Inc., Germany). Physical dimensions and morphologies of the materials were characterized by transmission electron microscopy (TEM) (JEOL, JEM-2100, Japan). Hydrodynamic diameter (D_h) and zeta potential of the GO/RGO suspensions were measured at 25 ºC using a ZetaSizer Nano ZS (Malvern Instruments, Worcestershire, U.K.). Relative hydrophobicity of GO/RGOs was estimated based on the partition coefficient, K_{DW}, of the materials using a method reported in the literature.[S2] The contact angle measurements were carried out in the air using the sessile drop method on a contact angle system OCA 20 (DataPhysics Instruments GmbH, Germany). The contact angle values reported are mean values measured for 4 μL water droplets at five positions of each material.

Incubation of GO/RGOs with Protein Mixtures

The GO/RGO suspension (~250 mg/L) was sonicated (100 W) in a water bath at 25 ºC for 30 min before incubating in phosphate buffer saline (PBS) with 10% FBS (v/v) (Genview, China) at 37 ºC. After 4 h, the GO/RGO-protein complexes were separated from the supernatant plasma by centrifugation at 16000 rpm (4 ºC) for 15 min. For further SDS-PAGE and LC-MS/MS analysis, the pellet was washed with PBS three times to remove the proteins with low affinity for the surface of GO/RGOs (i.e., the soft protein corona). The GO/RGO-protein complexes were re-suspended in protein loading buffer (Genview, China) and was then boiled for 5 minutes at 100 °C and centrifuged at 12000 rpm (4 ºC) for 5 min prior to further analysis. To determine the amount of FBS proteins adsorbed on GO and RGOs, Bradford Protein Assay Kit (Sangon Biotech, China) was used. Briefly, supernatant plasma after adsorption was taken for protein

content determination following the manufacturer's instructions.

Characterization of Protein Corona on GO/RGOs

The composition of the hard protein corona formed on the surface of GO/RGOs were characterized using SDS-PAGE and LC-MS/MS. For SDS-PAGE analysis, the protein-GO/RGO samples were separated on a 10% SDS–PAGE gel and stained using Protein Stains Q. Kit (Sangon Biotech, China) following the manufacturer's instructions. For LC-MS/MS analysis, protein bands were excised from the gels, destained and in-gel digested. After digestion, the peptide mixtures were analyzed and separate by LC-MS/MS using an ultimate 3000 nanoLC system (Dionex Inc., USA) and a tandem mass spectrometry (MS/MS) in a Q Exactive (Thermo scientific Inc., USA), and a C_{18} trap column (Thermo scientific Inc., USA). The raw data files were converted to Mascot generic format (mgf) files and searched in National Center for Biotechnology Information (NCBInr) database via Mascot Search (version 2.3.01, Matrix Science, http://www.matrixscience.com/).

A semiquantitative assessment of the protein amounts according to spectral counting (SpC) reported in Monopoli's literature,[S3] which represents the total number of the LC-MS/MS spectra for all peptides attributed to a matched protein. The SpC of each protein identity was calculated as the relative protein quantity using equation 1:

$$NpSpC_k = \left(\frac{\left(SpC/M_w\right)_k}{\sum_{i=1}^{n} \left(SpC/M_w\right)_i} \right) \times 100 \qquad \text{(eq 1)}$$

where $NpSpC_k$ is the percentage normalized spectral count for protein k, SpC is the spectral count identified by LC-MS/MS analysis, and M_w is the molecular weight (kD) of protein k.

Adsorption Experiments with Model Protein

For adsorption experiments with the model protein, bovine serum albumin (BSA, Genview, China), GO/RGOs stock suspension were first added to PBS in 20-ml vials, and then a Pluronic F-127 stock solution was added and the mixture was homogenized. Next, BSA protein stock solution was added to the vials and the total volume was adjusted to 10 ml with PBS. The vials were shaken at 150 rpm for 4 h to reach adsorption equilibrium. Afterward, same procedures for determining adsorption amount of FBS were carried out by using Bradford Protein Assay Kit (Sangon Biotech, China). The blank (i.e., samples without GO/RGOs) showed no adsorption of proteins to the vials. The adsorbed mass at each equilibrium concentration was calculated based on a mass balance approach.

Table S1. Analysis of the deconvoluted C1s peak from X-ray photoelectron spectroscopy and relative atomic percentage of GO and RGOs.

GO/ RGO [a]	C [b] (wt%)				total C[b] (wt%)	total O [b] (wt%)
	aromatic rings	epoxy/ hydroxyl	carbonyl	carboxyl		
GO	29.95	21.83	10.12	6.41	68.31	31.15
VC-RGO	35.04	18.09	7.79	11.34	72.26	26.73
$NaBH_4$-RGO	40.63	28.86	-	4.65	74.14	20.82
N_2H_4-RGO	48.99	22.79	-	7.91	79.7	14.05

[a] RGO represents reduced GO; the terms "VC", "$NaBH_4$" and "N_2H_4" indicate the reductant used to reduce GO.
[b] Analyzed with X-ray photoelectron spectroscopy.

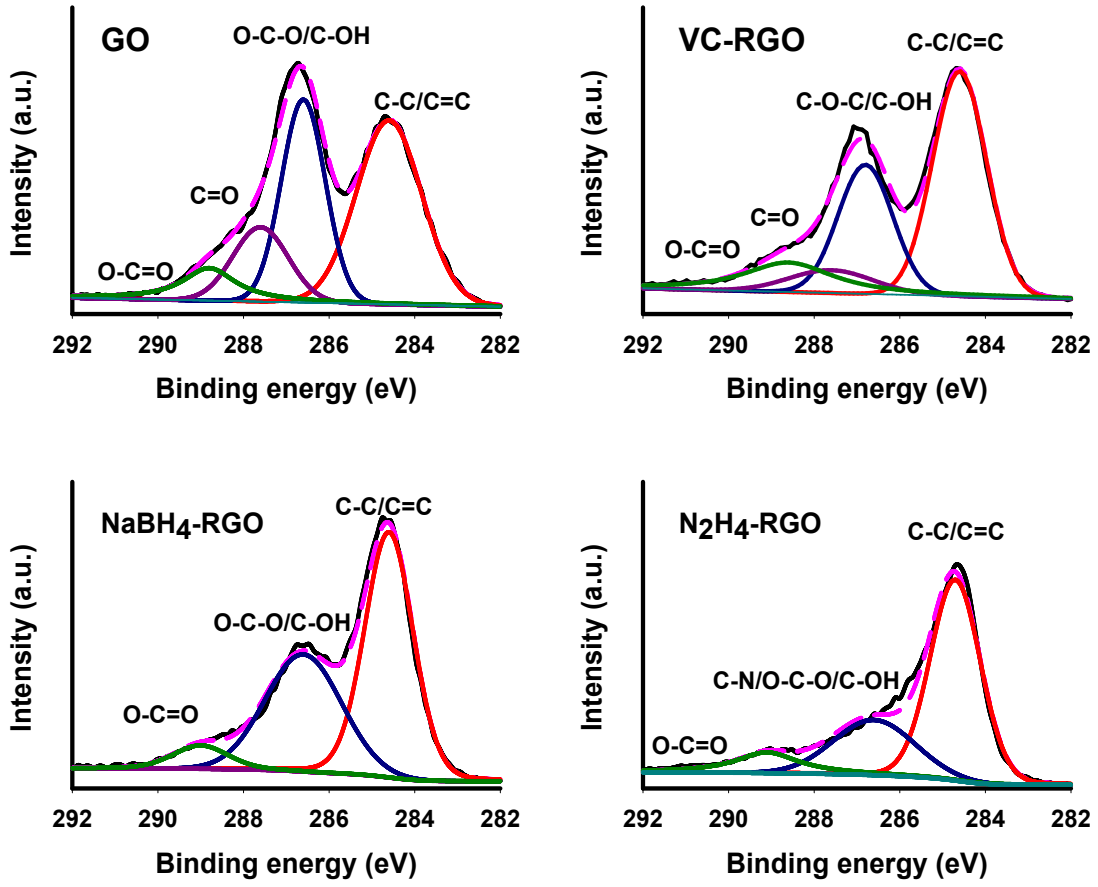

Fig. S1. X-ray photoelectron spectroscopy (XPS) spectra with C1 peaks deconvoluted for GO (A), VC-RGO (B), $NaBH_4$-RGO (C) and N_2H_4-RGO (D). The peaks with the binding energy of 284.6, 286.6, 287.8 eV and 288.8 eV were assigned to the carbon atoms in aromatic rings (C-C/C=C), epoxy/hydroxyl (C-O-C/C-OH), carbonyl (C=O) and carboxyl (O-C=O), respectively.

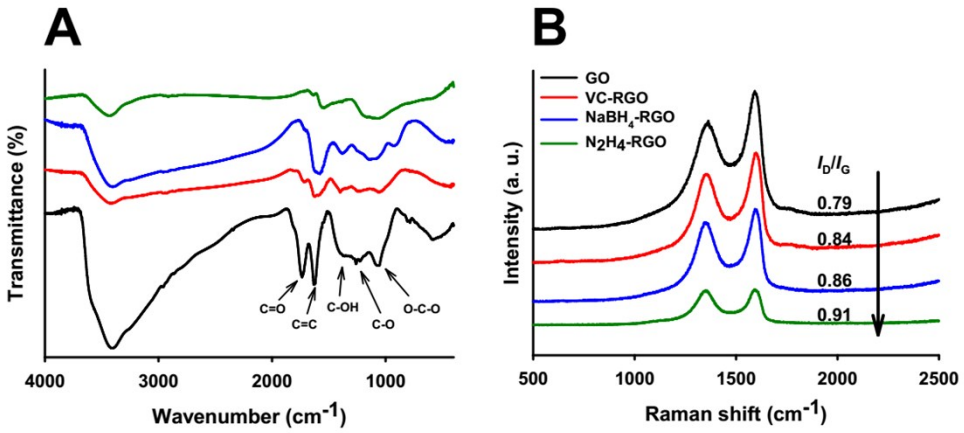

Fig. S2. Fourier transform infrared transmission (FT-IR) spectra (A) and Raman spectra (B) of GO and RGO materials, which showed the loss of surface O-functional groups and formation of new graphitic domains after reduction.

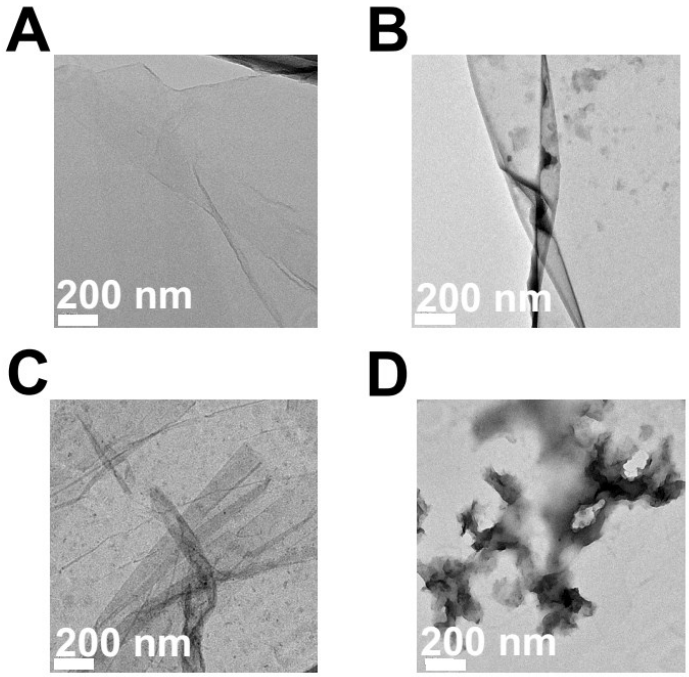

Fig. S3. Transmission electronic microscopy (TEM) images of GO (A), VC-RGO (B), $NaBH_4$-RGO (C) and N_2H_4-RGO (D) in deionized water.

Supporting Dataset.

Supporting Dataset provides the complete results of the LC-MS/MS, which include all the information about the compositions of hard corona (Mascot score > 500), properties of proteins and calculation of spectral counting (SpC).

References

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