Supporting Information

# Interaction of Graphene Oxide with Human Serum Albumin and Its Mechanism

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## Experimental Section

*Preparation of GO nanosheet:* GO was prepared by Hummer's method with minor modification. Briefly, native graphite flake (1 g) was mixed with concentrated $H_2SO_4$ (1.5 mL), $K_2S_2O_8$ (0.5 g), and $P_2O_5$ (0.5 g), and then incubated at 80℃ for 6 h to preoxidize the graphite. The product was then dried in air at ambient temperature overnight, after washing with distilled water until neutral and filtering. This preoxidized graphite was then subjected to oxidation by Hammer's method. The preoxidized graphite powder (1 g) was placed in concentrated $H_2SO_4$ (23mL) at 0 ℃. $KMnO_4$ (3 g) was added gradually with stirring while keeping the temperature of the

mixture below 20 ℃. The mixture was then stirred at 35 ℃ for 2 h, followed by the addition of distilled water (46 mL), and stirring was continued for 15 min. Distilled water (140 mL) was then added to terminate the reaction. Subsequently, 30% $H_2O_2$ (2 mL) was added and the color of the mixture changed to bright yellow. The mixture was centrifuged and washed with 10% HCl solution to remove residual metal ions. The precipitate was then washed with distilled water and centrifuged repeatedly until the solution became neutral. To exfoliate the oxidized graphite, the product was treated with an ultrasonic probe at 400W for 30min, followed by centrifuging at 13 000 rpm for 30min. The exfoliated GO was obtained in the supernatant. The precipitate was exfoliated repeatedly. The product obtained was steadily dispersed in water and would not precipitate for several months.

*Polymers modification of GO:* Carboxylic GO (GO-COOH) was also prepared to get negative charged GO. The epoxy and hydroxyl groups on GO surface were oxidized into carboxy groups by chemical modification with sodium chloroacetate. Chitosan (CS, MW=10 KD), polyethylenimine (PEI, MW=25 KD) are common polymers used in GO modification to improve its solubility. A carboxyl activating regent (EDC) was ultilized to initiate the formation of an amide linkage between GO and polymers. 10 mg GO dispersed in DI water (10 ml) was first mixed with PEI/ CS (50 mg) and sonciated for 30min. 200 mg EDC was then added into the suspension. After reaction overnight under stirring, the suspension was filtrated through a MW=100 KD centrifugal filter (Millipore) several times to completely remove excess EDC and unreacted polymers. Finally, the prepared GO-PEI/ GO-CS were dispersed in DI water.

*Fluorescence quenching measurements:* Intrinsic tryptophan fluorescence quenching induced by GONS was recorded using SLM Aminco SPF-400 spectro-fluorimeter and 0.2 cm×1 cm×4 cm quartz cells. Tryptophan fluorescence of HSA was measured by exciting the protein solution at 298 nm. The fluorescence emission spectra were recorded from 300 to 450 nm containing various concentrations of GONS. Each spectrum was an average of three individual samples. HSA

concentration was fixed at 100 μg $mL^{-1}$. Nanosheets concentration range was 0 to 100 μg $mL^{-1}$ (GO), 0 to 100 μg $mL^{-1}$ (GO-COOH), 0 to 700 μg $mL^{-1}$ (GO-PEI) and 0 to 400 μg $mL^{-1}$ (GO-CS). Measurements were performed three times to calculate the binding constant. The pH influence on the interaction between GONS and HSA was also detected using fluorescence quenching measurements. HSA concentration was fixed at 100 μg $mL^{-1}$. Nanosheets concentration was 50 μg $mL^{-1}$ for GO and GO-COOH, 200 μg $mL^{-1}$ for GO-PEI and GO-CS.

*CD measurements:* CD spectra were recorded on a circular dichroism spectrometer model 410 (AVIV Biomedical Inc. Lakewood, NJ USA), over the range of 200–250 and 325–500 nm, using 0.2 and 1.0 cm cuvettes respectively. The spectra are expressed as molar ellipticity [θ] in deg $cm^2$ $dmol^{-1}$. The experiments were performed at 25℃ and three scans were averaged. For the comformational change detection, HSA with concentration of 100 μg $mL^{-1}$ were mixed with GONS with concentration of 40 μg $mL^{-1}$ for 2 hours reaction. For the functional change detection, GONS with concentration of 100 μg $mL^{-1}$ were firstly mixed with HSA with concentration of 1 mg $mL^{-1}$ for 2 hours, after that 4.4 μg $mL^{-1}$ bilirubin (the molar ration of HSA bilirubin was 1:1) was added to each mixture for 2 hours. No precipitate phenomenon occurred in the experiments.

*Surface zeta potential measurements:* Zeta potential of GONS were measured in a Malvern ZEN3600 (Malvern Instruments, UK). GONS were filtrated through centrifugal filters (Millipore) several times to completely remove impurities and finally dispersed in DI water (pH=7.4). And the measurements were performed in triplicate.

*Operation of bilirubin:* Solutions of bilirubin were prepared daily. About 10 mg of bilirubin were dissolved in 10 ml of 0.02 M aqueous NaOH and diluted to 100 ml with distilled water. The solutions were protected from light during preparation and storage at 4 ℃. All procedures involving bilirubin were carried out under minimal light.

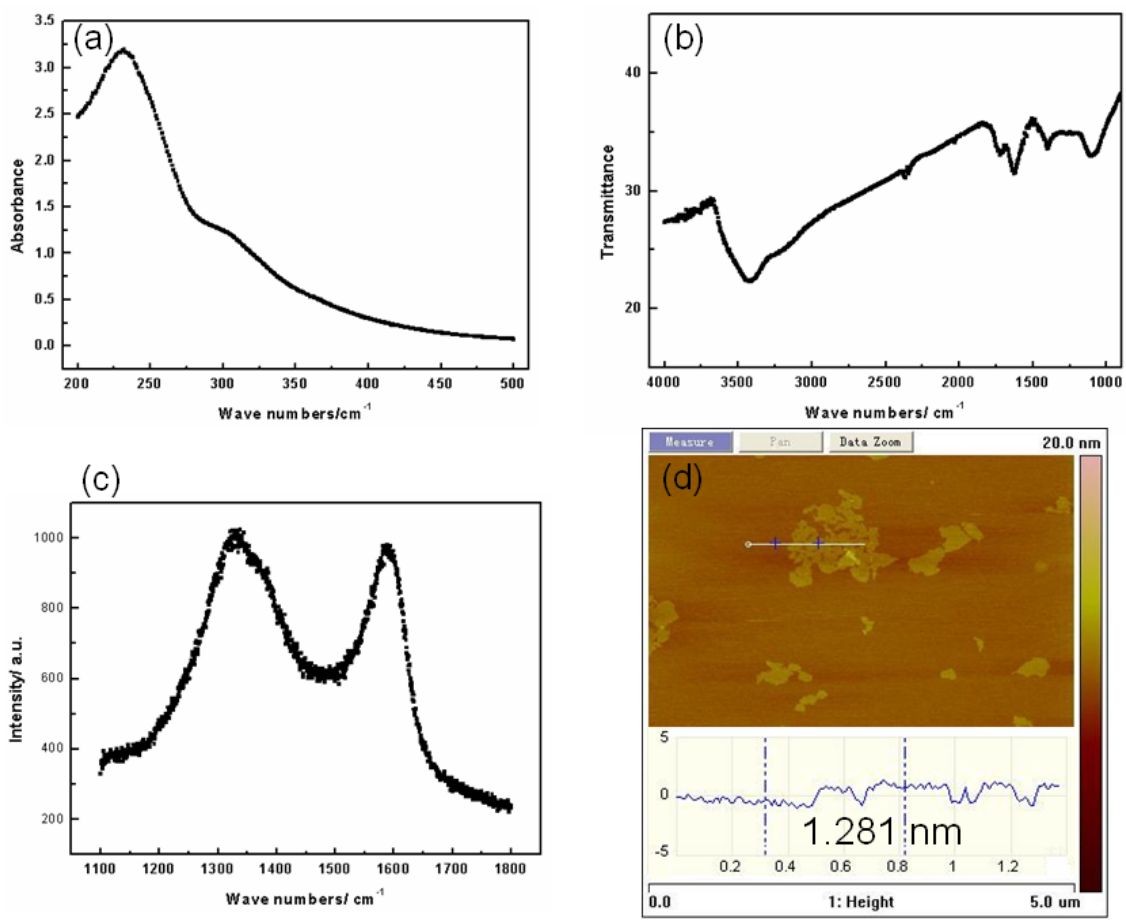


Figure S1. Characterizations of GO: (a) UV-vis spectrum of GO; (b) FT-IR spectrum of GO; (c) Raman spectrum of GO; (d) Atomic force microscopy height image of GO.

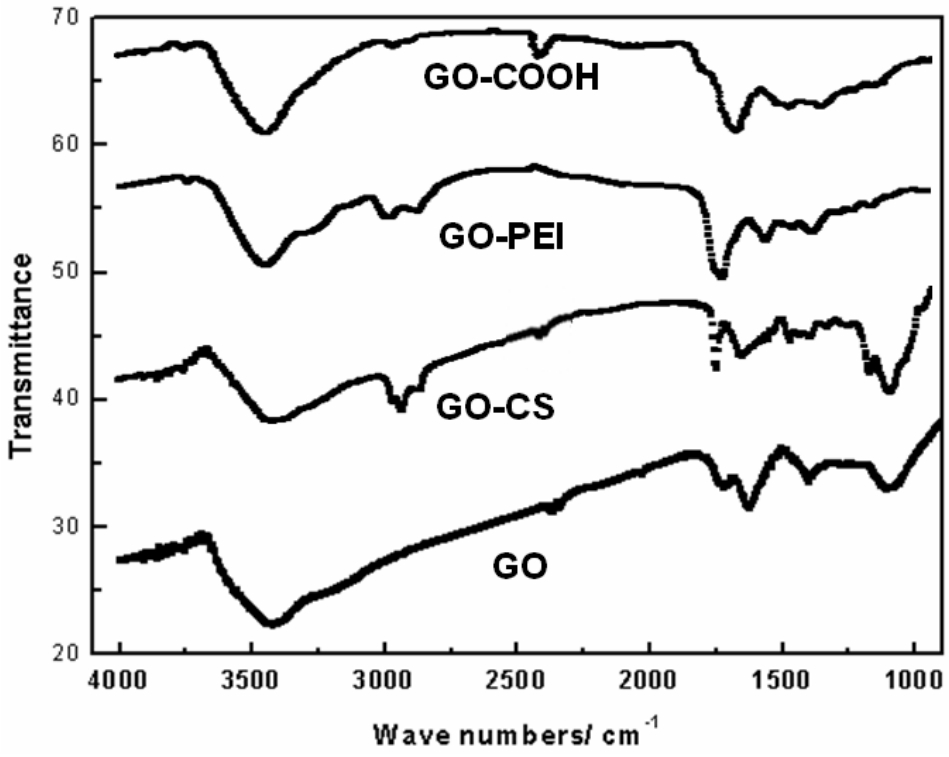


Figure S2. FT-IR spectroscopy of GO modification: The peaks at 1720, 1640 and 1100 $cm^{-1}$ in the FT-IR spectra are characteristic of the C=O and C-O stretches of the ketone groups and epoxy groups, respectively, on GO. Compared with GO，the FT-IR spectrum of GO-COOH shows a stronger -COOH peak and a bigger peak width. This implies that partial epoxy and hydroxyl are activated into carboxyl. In the GO-CS spectrum, the peak at 1100 $cm^{-1}$ almost disappears, and a strong band emerges at 1735 $cm^{-1}$, corresponding to the C=O characteristic stretching band of the amide groups. A peak at 1458 $cm^{-1}$ presumably confirms the C–N stretching bonds of the amide group. Additionally, the characteristic band of the glucopyranose rings of the GO-CS appears at 1150 $cm^{-1}$, respectively, implying that CS is attached. In the spectrum of the GO-PEI, also a strong band emerged at 1735 $cm^{-1}$, corresponding to the C=O characteristic stretching band of the amide groups, a -NH2 bending peak at 1560 $cm^{-1}$ and a C-N stretching vibration at 1460 $cm^{-1}$ from PEI are also observed.

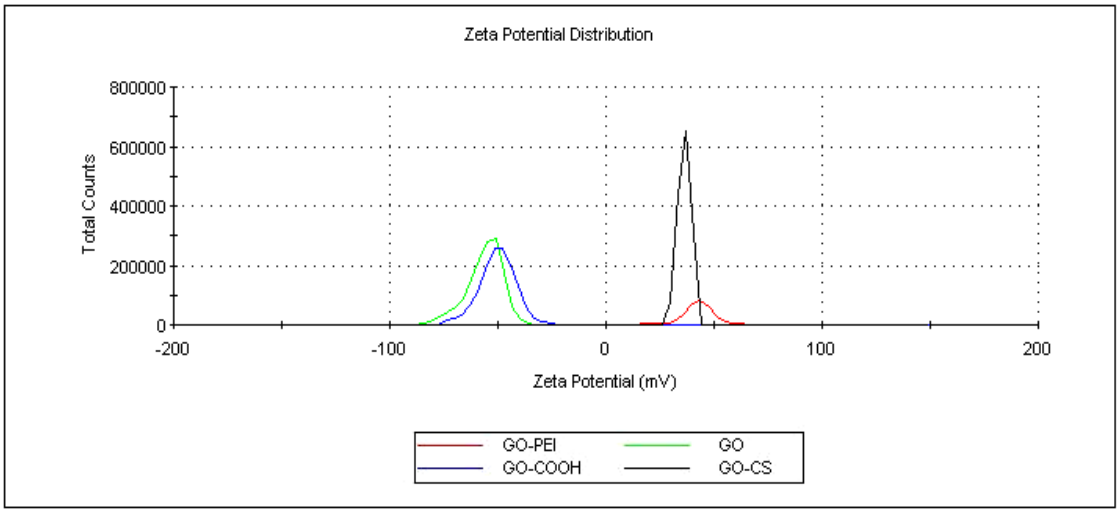


Figure S3. Zeta potential of GONS. The potential distribution curve of GO has a negatively charged peak with position centered at about -56.3 mV. For GO-COOH, the peak position of its zeta potential shifts to -50.3 mV. After modified with polymers, the peak position of the zeta potential for GO-PEI and GO-CS are shift to positively charged 42.4 and 36.3 mV respectively.

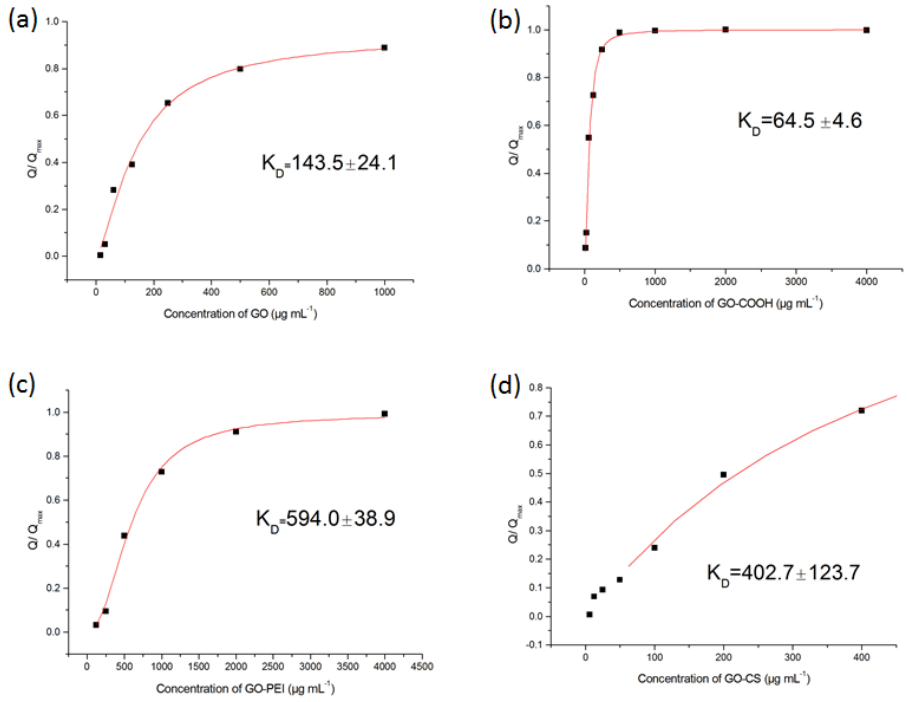


Figure S4. Binding constant calculated from fluorescence quenching measurements of GONS competitive binding bilirubin. (a) GO; (b) GO-COOH; (c) GO-PEI; (d) GO-CS. Bilirubin solution has the maximum emission at 525 nm with maximum excitation at 460 nm. GONS also has the ability to quench it. So the binding constant of GONS with bilirubin can be measured by fluorescence quenching spectroscopy as described in the maintext. After the data analysis, we find GO-COOH has the strongest binding affinity with bilirubin. However, in the system with GO-COOH, HSA and bilirubin present, HSA keeps about 96.3% bilirubin binding. This means GO-COOH competitive binding bilirubin can be ignored in HSA function study. GO-COOH shows the strongest binding with bilirubin and competitive binding bilirubin of other GONS could also be ignored.

Table S1 Zeta potential of GONS at PBS of different pH

| Zeta potential | pH=4.0 | pH=7.2 | pH=9.0 |
|---|---|---|---|
| GO | -25.7 mV | -26.6 mV | -31.0 mV |
| GO-COOH | -23.5 mV | -26.8 mV | -27.5 mV |
| GO-PEI | 23.7 mV | 19.9 mV | 18.6 mV |
| GO-CS | 24.8 mV | 10.0 mV | 4.0 mV |