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Ionic strength and pH reversible response of visible and near-infrared fluorescence of graphene oxide nanosheets for monitoring extracellular pH

Jin-Long Chen and Xiu-Ping Yan*

Research Center for Analytical Sciences, College of Chemistry, Nankai University, 94 Weijin Road, Tianjin 300071, China

***To whom correspondence should be addressed. E-mail: xpyan@nankai.edu.cn**

Preparation of vis-NIR Fluorescent Graphene Oxide Nanosheets (GO). Graphite flakes were obtained from Qingdao Henlide graphite Co., Ltd. with a particle size of 20 μm. Water dispersions and solid of graphite oxide were prepared from natural graphite powder using a modified Hummers and Offeman's method (Hummers, W. S.; Offeman, R. E. *J. Am. Chem. Soc.* 1958, *80*, 1339; Cote, L. J.; Kim, F.; Huang, J. X. *J. Am. Chem. Soc.* 2009, *131*, 1043–1049). In a typical reaction, to a glass beaker 0.5 g of graphite flakes, 0.5 g of $NaNO_3$ and 23 mL of 98% (w/w) H_2SO_4 were added, and mixed under stirring in an ice bath. Then, 3g of $KMnO_4$ was slowly added to the mixture in an ice bath, and thoroughly mixed. The beaker placed in a 35 ^oC water bath, and the solution was stirred for about 1 h to form a thick paste. 40 mL of high-purity water was added to the formed paste, and stirred at 90 ^oC for 35 min.

Finally, 100 mL of water was added, followed by the slow addition of 3 mL of H_2O_2 (30%), meanwhile the color of the solution turned from dark brown to yellow. The warm solution was then filtered and washed with 100 mL high-purity water.

For preparation of GO, the filter cake was then dispersed in water by mild sonication for 1 h. The suspension was centrifuged at 1000 rpm for 5 min. The supernatant was collected for centrifugation at 1000 rpm for 5 min (3-5 times) to remove all visible particles. The supernatant was collected and then centrifuged at 10,000 rpm for 15 min twice. The supernatant containing small GO pieces and water-soluble byproducts was discarded, while the sediment was collected. The solid of GO was obtained from the sediment under air-dry. The slurry or solution of GO was obtained by redispersion of the as-made GO in water under mild sonication using a table-top ultrasonic cleaner or mechanical agitation.

Instrumentation and Characterization. The UV-vis absorption spectra were performed on a Shimazu UV-3600 UV-vis-NIR spectrophotometer at room temperature. The Fourier transform IR (FT-IR) spectra of (4,000-400 cm^{-1}) were measured on Nicolet IR magna-560 spectrometer with pure KBr as the background. X-ray photoelectron spectroscopy (XPS) measurements were recorded using a Kratos Axis Ultra DLD spectrometer employing a monochromated Al-Ka X-ray source ($h\nu$=1486.6 eV), hybrid (magnetic/electrostatic) optics and a multi-channel plate and delay line detector (DLD). All XPS spectra were recorded using an aperture slot of 300 × 700 microns, survey spectra were recorded with a pass energy of 160 eV, and high resolution spectra with a pass energy of 40 eV. The structure and morphology of

GO was characterized by Transmission electron microscopy (TEM) on JEOL-100CXII (JEOL, Japan) with an accelerating voltage of 100 kV. Tapping mode Atomic Force Microscopy (AFM) measurements were performed using a Multimode SPM from Digital Instruments with a Nanoscope IIIa Controller. The samples for AFM images were prepared by depositing a diluted water dispersion of GO on a freshly cleaved mica surface and allowing it to dry under ambient conditions.

All fluorescence measurements were performed on a Hitachi F-4500 Fluorescence Spectrophotometer (Tokyo, Japan) equipped with a plotter unit and a 1-cm quartz cell. The operation conditions were described as followings: PMT voltage: 950 V, Ex and Em slit : 10 nm, Response: 2 s.

Cell Culture and Extracelullar pH Monitoring. Chronic granulocytic leukemia (CGL) cancer cell (32D-BA), and normal mouse cell (32Dc-13) were cultured and provided by Institute of Zoology of the Chinese Academy of Sciences (CAS). Cells were seeded at a density of 1×10^6 cells mL^{-1} for culture in RPMI 1640 Medium (GIBCO company) at 37 °C under a humidified atmosphere containing 5% CO_2 for three days. Aliquots of the live cell suspension after a certain period of culture were incubated with the GO for fluorescence measurements and extracellular pH monitoring. The extracellular pH changes measured by using the GO as a vis-NIR fluorescence sensor were also proved by a pH meter on the live cell suspension.

Comparison of GO with Activated Carbon and Expanded Graphite for Fluorescent Sensing. We have tried to compare the other carbon materials such as activated carbon and expanded graphite with the GO for fluorescence measurement, but failed because of no fluorescence signals observed for those aqueous dispersions

of activated carbon and expanded graphite and difficulty to disperse the sort carbon samples in water even under high power sonication.

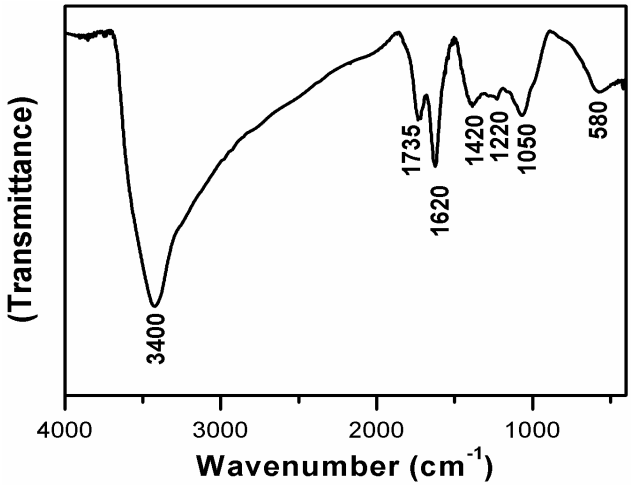

Fig. S1 FTIR spectra of the as-prepared GO. The characteristic vibrations are the broad and intense peak of O–H groups at 3400 cm^{-1}, strong C=O peak at 1735 cm^{-1}, the O–H deformation peak at 1420 cm^{-1}, the C–OH stretching peak at 1220 cm^{-1}, and the C–O stretching peak at 1050 cm^{-1}. The peak at 1620 cm^{-1} was assigned to the vibrations of the adsorbed water molecules and also the contributions from the skeletal vibrations of unoxidized graphitic domains.

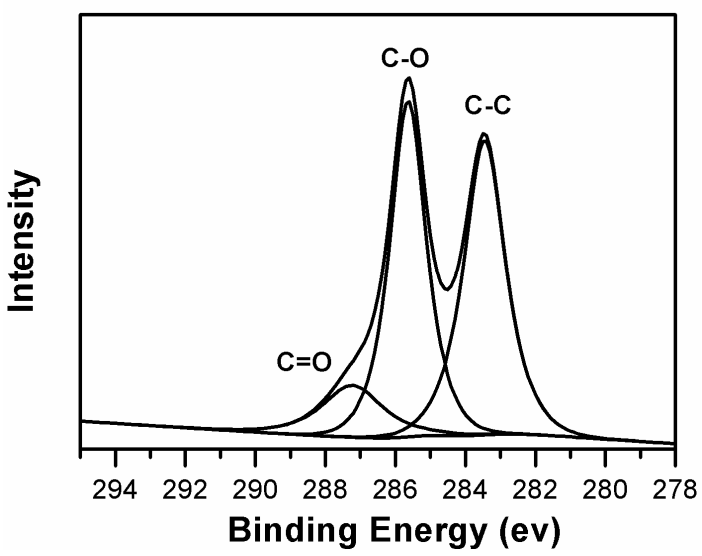

Fig. S2 X-ray photoelectron spectra for the C_{1s} of the as-prepared graphene oxide, showing the presence of three types of carbon bonds: C-C (284.5 eV), C-O (286.6 eV), C=O (287.8 eV).

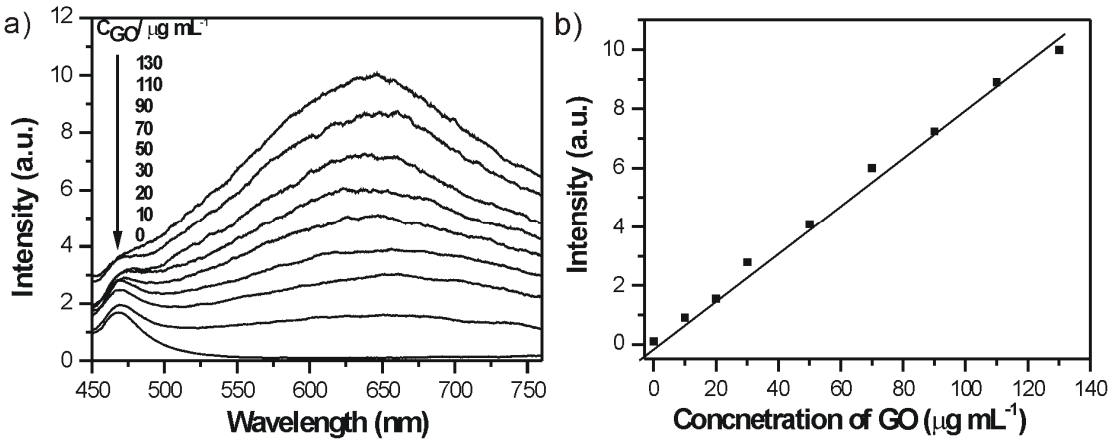

Fig. S3 (a) The fluorescence spectra of GO at various concentrations; (b) Plots of the NIR fluorescence intensity of GO at 650 nm against its concentration.

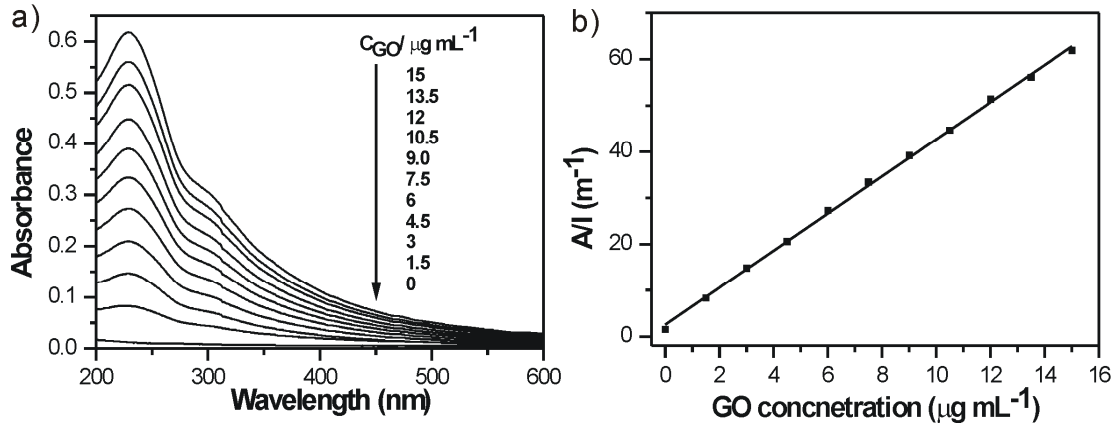

Fig. S4 (a) The absorption spectra of GO at various concentrations; (b) Plots of the absorbance of GO at 230 nm against its concentration. Note that the dispersions are almost transparent due to the low concentration of GO. A large absorption coefficient of 3967 L g^{-1} m^{-1} was obtained for GO at 230 nm.

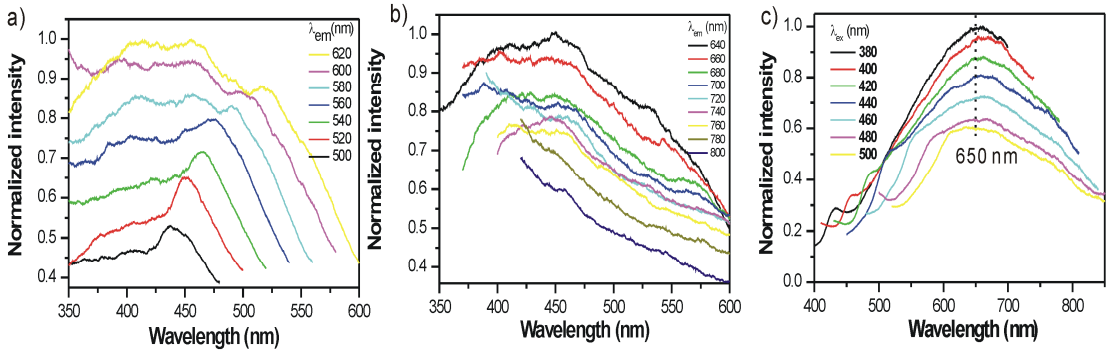

Fig. S5 (a) and (b) Emission-dependent excitation wavelength, (c) Excitation-independent maximum emission wavelength.

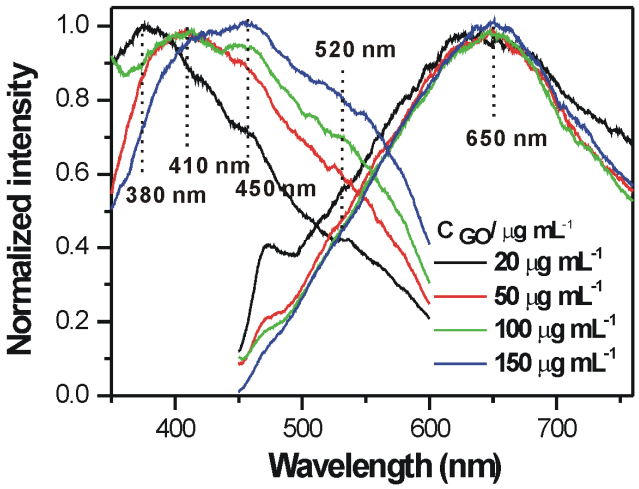

Fig. S6 Concentration-dependent excitation spectra (Right) and concentration-independent maximum emission wavelength (Left) of GO.

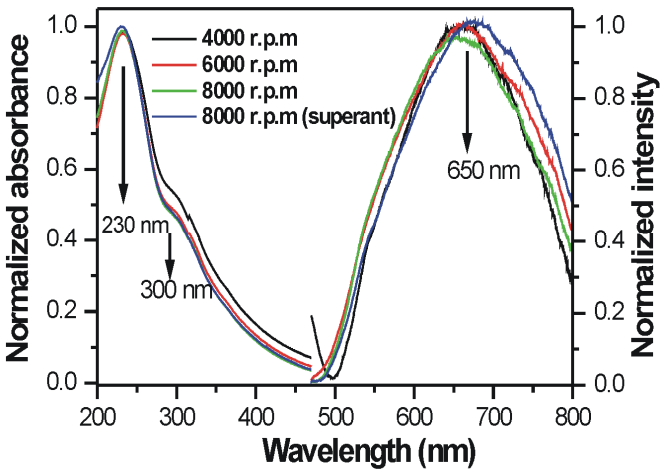

Fig. S7 Negligible spectra difference including absorption (Left) and emission spectra (Right) for the resultant GO samples collected under centrifugation at different speeds.

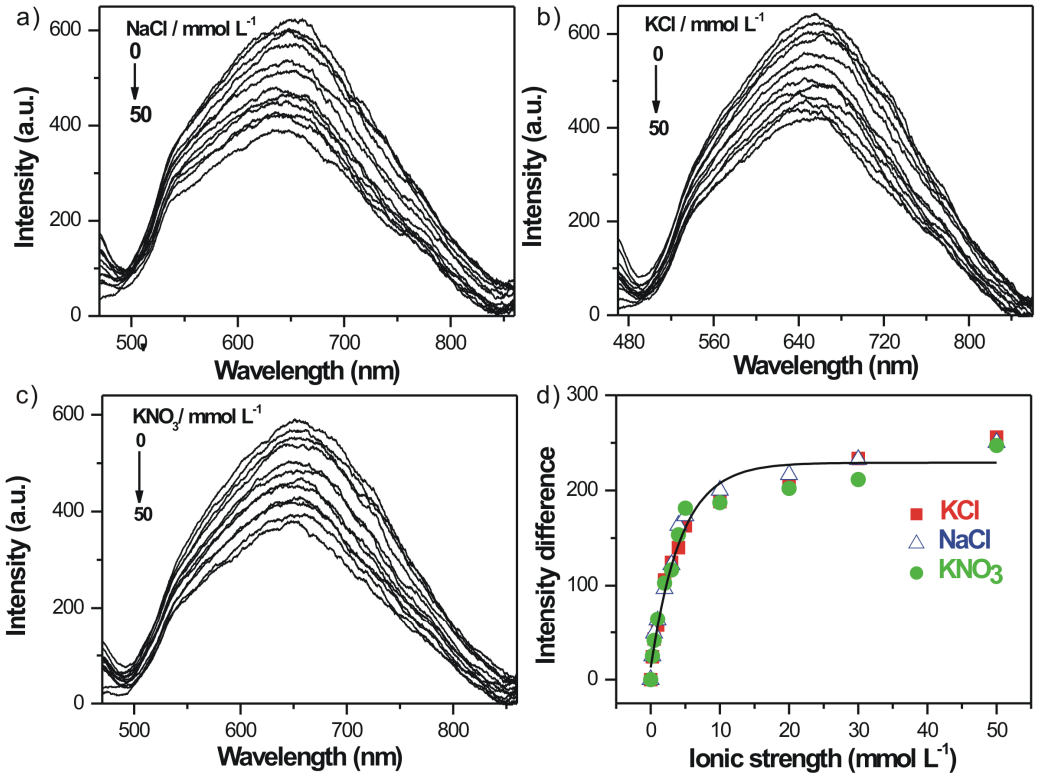

Fig. S8 The evolution of vis-NIR fluorescence spectra of GO colloids after adding NaCl (a), KCl (b), and KNO_3 (c) as a typical electrolyte. (d) Plots of NIR fluorescence intensity of GO at 650 nm against ionic strength.

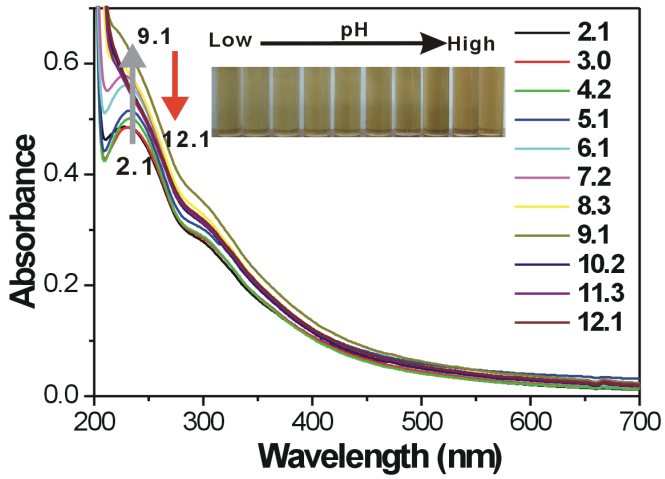

Fig. S9 pH dependent absorption spectra of the GO colloids (Inset: Photographs for the GO colloids at different pH values).

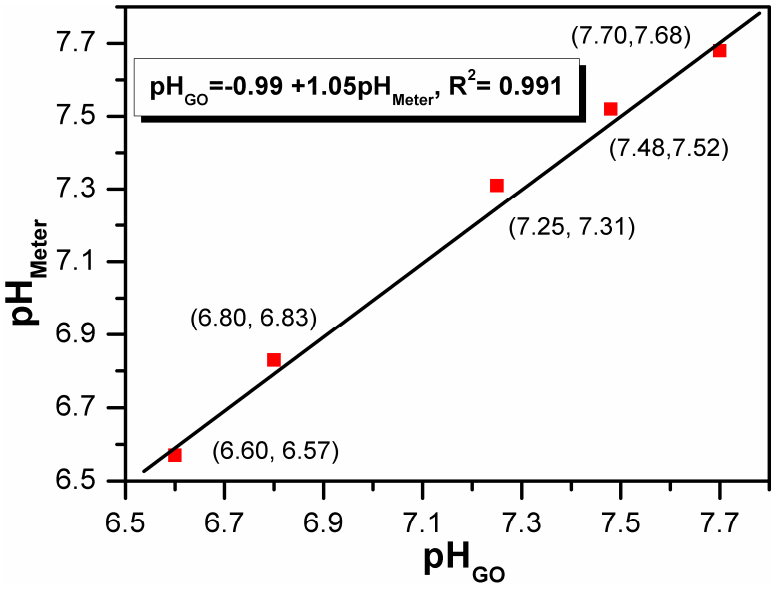

Fig. S10 Good agreement between the extracellular pH values measured by using the GO as a vis-NIR fluorescence sensor directly on the live cells and those measured by a pH meter on the live cell suspension, showing the accuracy of the developed GO vis-NIR fluorescence sensor for extracellular pH detection.