An rGQDs / chitosan nanocomposite-based pH-sensitive probe: Application to sensing in urease activity assays

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Experimental Section

Preparation and purification of rGQDs

GQDs were synthesized by cutting down graphene oxide (GO) through the hydrothermal approach as described in previous reports [1]. According to the reports in the literature [2], GO was prepared using graphite powder based on a modified Hummer's method. The resultant GO powder (0.05 g) was treated in concentrated H_2SO_4 (10 mL) and concentrated HNO_3 (3.3 mL). The mixture was subjected to ultrasonic treatment for 4 h and then transferred to a high-pressure reactor at 100 °C for 24 h. The mixture was naturally cooled to room temperature when the reaction was finished and was transferred into 130 mL of deionized water. The pH of the solution was adjusted to neutral by continuous addition of sodium bicarbonate. Dialysis was performed for 3 days to remove salt ions completely using a dialysis membrane (retained molecular weight: 1000 Da)

$NaBH_4$ can selectively reduce the carbonyl and epoxy moieties on the surface and edge of GQDs to hydroxyl groups in order to obtain rGQDs. Therefore, $NaBH_4$ (1 g) was added to the as-prepared GQDs solution (30 mL) and stirred for 4 h at room temperature. After the reduction, the product was transferred to a dialysis tube for complete elimination of the ions for 3 days. The final product solution was obtained with the concentration of 0.5 mg mL^{-1}.

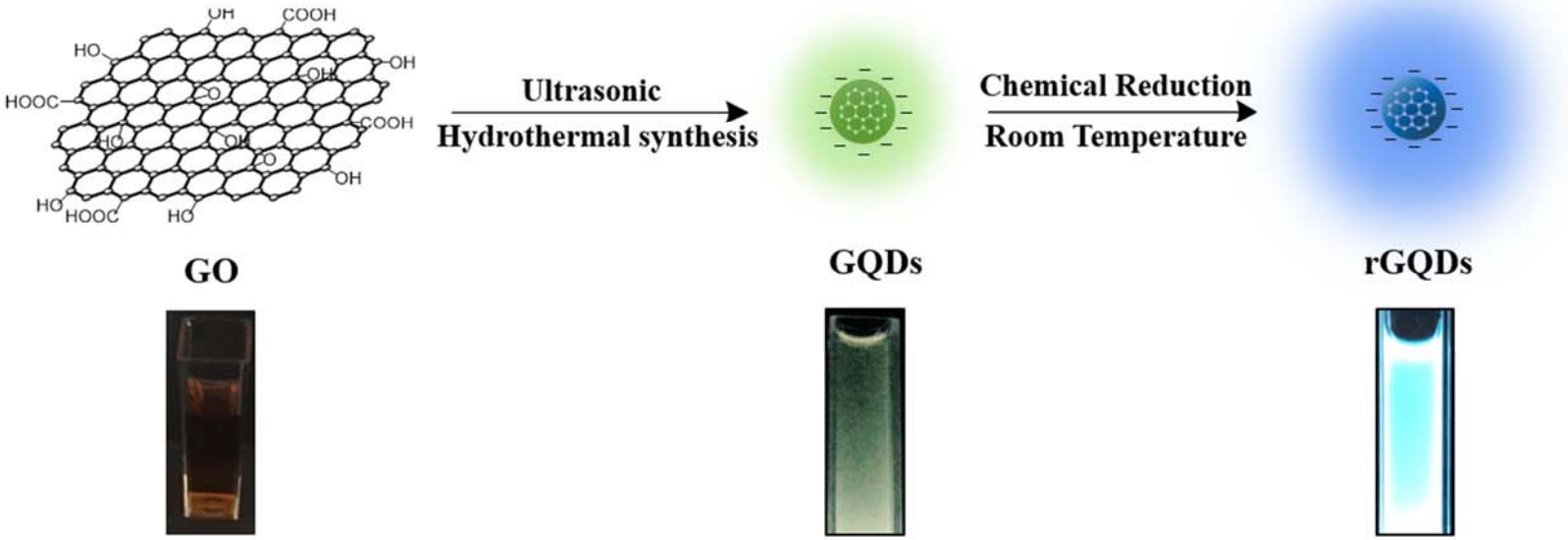

Fig. S1 Synthesis process of GQDs and rGQDs and the photographs of GO, GQDs and rGQDs solutions taken under 365 nm UV light.

Pretreatment of urine, saliva, and soil samples

Urine sample: Drug-free human urine samples were collected from the school hospital of Jilin University. Centrifugal treatments (10,000 rpm for 10 mins at room temperature) were carried out to eliminate the precipitated urine protein and improve the recovery according to previous reports [3]. The supernatant was separated. Then the samples were then diluted 10-folds with deionized water and were adjusted to pH=7.4 by HCl or NaOH solution. Different concentrations of urease were added to the diluted urine samples to prepare the spiked samples. The urease activity in real samples were determined according to the procedure mentioned in the main body.

Saliva sample: Saliva sample was obtained from the volunteer with informed consent. Then the samples were diluted 10-folds with deionized water and were adjusted to pH=7.4 by HCl or NaOH solution. Different concentrations of urease were added to the diluted saliva samples to prepare the spiked samples. The detection procedure was the same as that of urease detection.

Soil sample: Soil sample was collected from Jilin University campus. After sift out the large chunks of gravel from the soil through a 2 mm sieve, the soil sample were dried in air for 24 h. The treated soil samples (0.1 g) were suspended with 10 mL ultrapure water and adjusted to pH=7.4 by HCl or NaOH solution. The mixture was centrifuged and purified through ultra-filtration. The soil suspension was obtained (10.0 mg mL^{-1}). Different concentrations of urease were added to the soil suspension samples to prepare the spiked samples. The detection procedure was the same as that of urease detection.

All experiments were performed in compliance with the relevant laws and institutional guidelines, and the relevant institutional committees have approved the experiments.

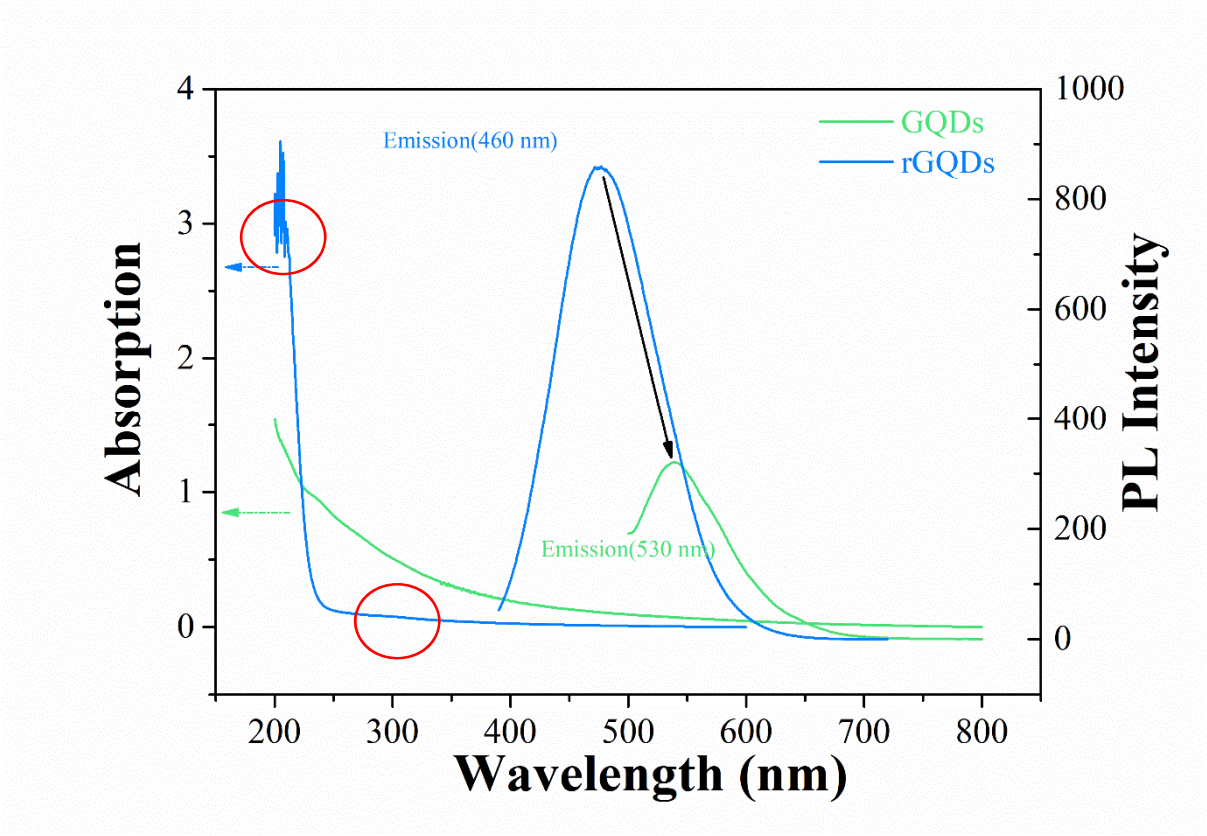

Fig. S2 UV-vis absorption and fluorescence emission spectra of the GQDs (green line) and rGQDs (blue line) solution.

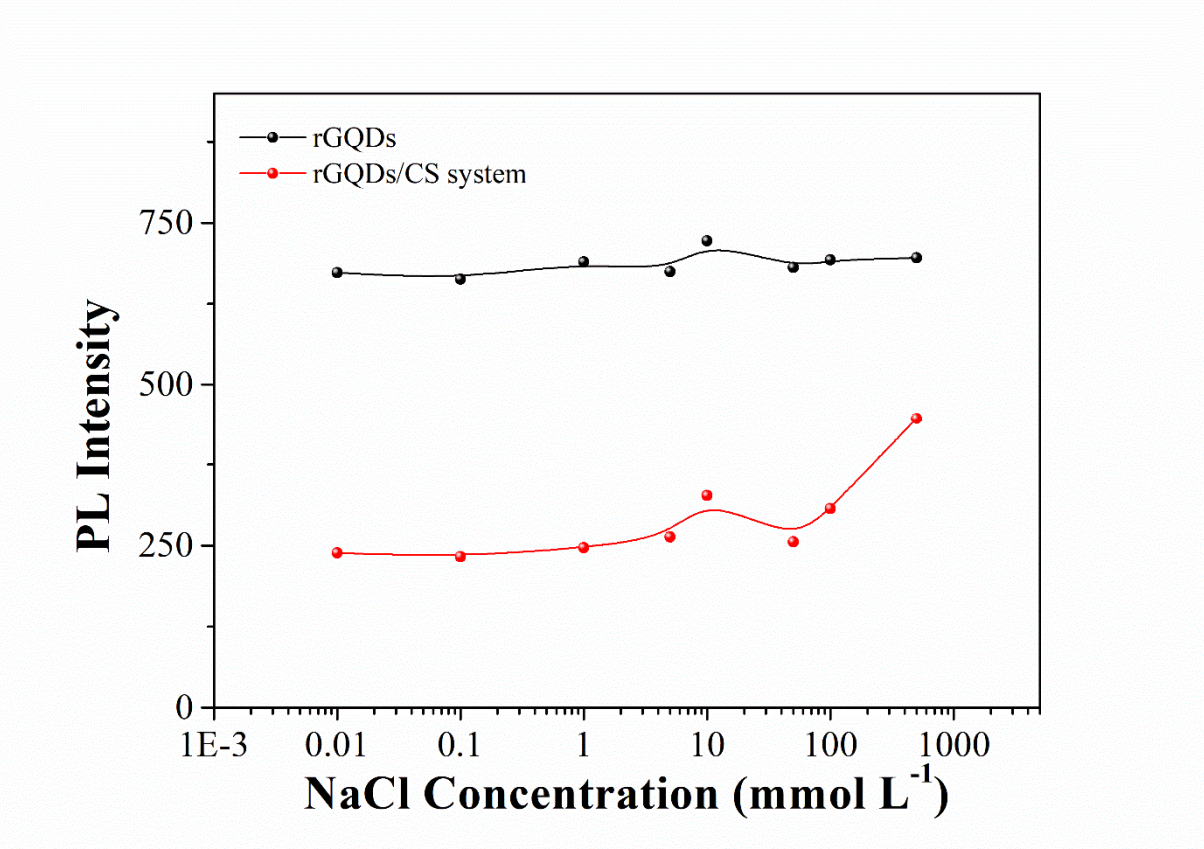

Fig.S3 The effect of salt concentration (1 μM~1 M) on the PL intensity of rGQDs in the absence and presence of CS (35 ng mL^{-1}); (c) The temporal evolution on the PL intensity of rGQDs/CS system;

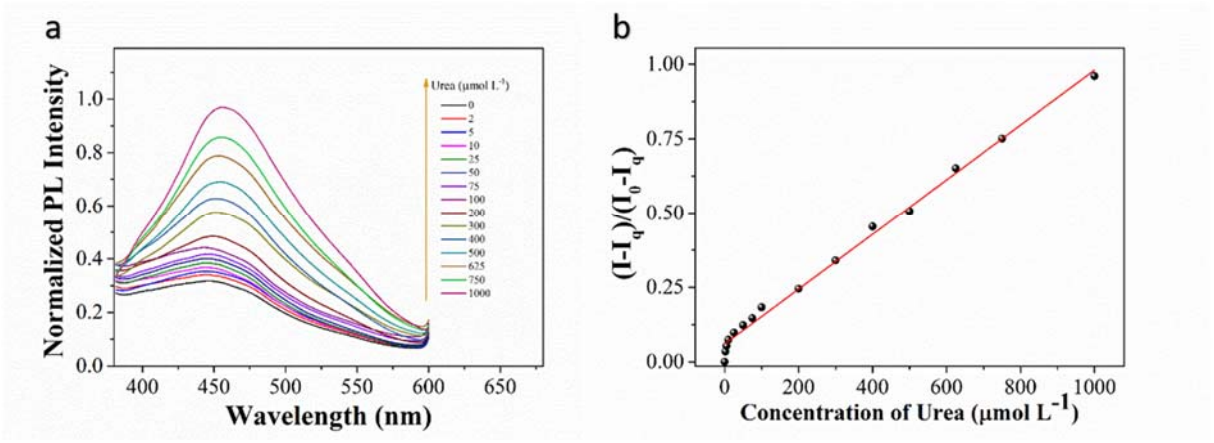

Fig. S4 (a) Fluorescence emission spectra of the rGQDs/CS system in the presence of the enzymatic hydrolysate which contained urea at various concentrations from 2 to1000 μmol L^{-1} and a fixed concentration of urease. Each measurement was taken after incubation of the sample mixture at 37°C for 20 min. (b) The linear plot of recovery effectivity versus urea concentrations in the range of 2-1000 μmol L^{-1}. The concentration of rGQDs, CS and urease were 50 μg mL^{-1}, 35 ng $mL^{-1}$and 1 U mL^{-1}, respectively.

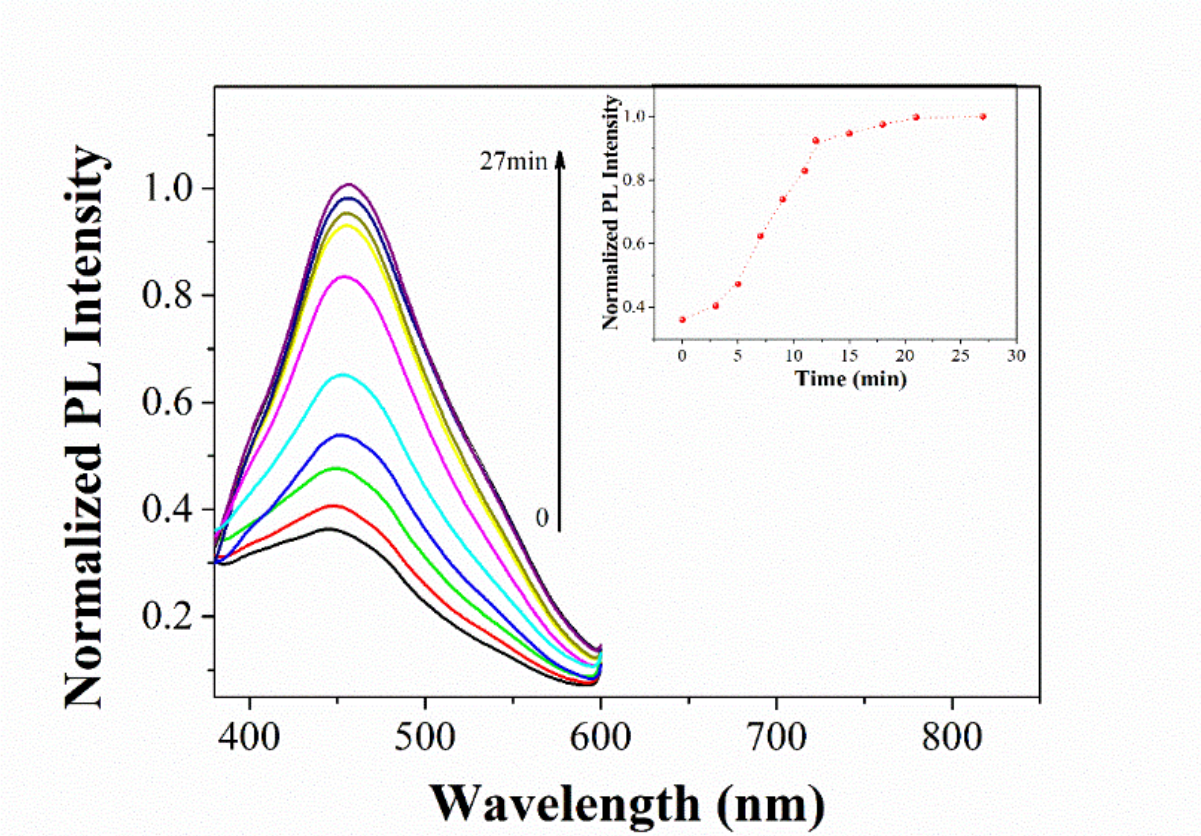

Fig. S5 Fluorescence emission spectra of the rGQDs/CS system in the presence of the enzymatic hydrolysate of urease under different hydrolysis time. Inset: Plots of the fluorescence intensity versus the hydrolysis time for the rGQDs/CS system in the presence of the enzymatic hydrolysate of urease. The concentration of rGQDs, CS, urea and urease were 50 μg mL^{-1}, 35 ng mL^{-1}, 1 mmol L^{-1} and 1 U mL^{-1}, respectively

Table S1. Comparison of performance of different methods for urease activity detection

Type	Sensing system	Linear range (mU mL^{-1})	Detection limit (mU mL^{-1})	Reference
Fluorometric	Polymer dot /pyrogallic acid	0.19~93	0.08	[4]
Fluorometric	Ni/Co layered double hydroxides-H_2O_2-homovanillic acid (HVA)	3.3~270	-	[5]
Fluorometric	NAC-gold nanoclusters	2.2~55	0.55	[6]
Fluorometric	CuO- H_2O_2- 3-(4-hydroxyphenyl)propionic acid (HPPA)	3~40	2.6	[7]
Liquid crystal (LC)	Surfactant-encapsulated phosphotungstate clusters (SECs) deposited onto octadecyltrichlorosilane-coated glass	0.02~3	0.03	[8]
Colorometric	Au NPs-H_2O_2 -TMB	1.8~90	1.8	[9]
Fluorometric	rGQDs/CS assembly system	50~750	36	This work

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