Supporting Information

A Selective and Sensitive Fluorescent Probe for Glucose on the Basis of Boronic Acid Functionalized Graphene Quantum Dots

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Experimental Section:

Reagents. 3-Aminobenzeneboronic acid (APBA) monohydrate, 97% was purchased from Alpha Aesar (Tianjin, China), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Sigma-Adrich (USA). Graphite powder (spectral grade) and other chemicals (analytical grade) were provided by Sinopharm Group Chemical Regent Co., Ltd. (Shanghai, China). All solvents and chemicals in this work were used without further purification unless stated. Double-distilled water was used throughout the experiments.

Apparatus. The fluorescence and the absorption spectra were recorded with a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) and a Shimadzu UV-1800 spectrophotometer (Tokyo, Japan), respectively. JEM-2010 transmission electron microscope (TEM) (JEOL Ltd. Japan) and BioScope atomic force microscope (AFM) (NanoScope IIIa SPM System, Digital Instruments, Inc., U.S.A) were used to study the morphology of the prepared GQD and APBA-GQD. Standard solutions or brain microdialysates were delivered from gas-impermeable syringes (BAS) and pumped through a liquid switch (CMA 110, Solna, Sweden) by a microdialysis pump (CMA 402, Solna, Sweden).

Synthesis of graphene oxide (GO). Graphene oxide was synthesized from graphite powders by a modified Hummers method[1]. Typically, 0.5 g of graphite, 0.5 g of $NaNO_3$, and 23 mL of H_2SO_4 were mixed in an ice bath. 3 g of $KMnO_4$ was carefully added into the mixture in 10 min. The suspension was transferred into 35 °C water bath and stirred for 2 h. 40 mL of water was then slowly added by a dropping funnel. The solution was refluxed for 30 min at 95 °C. 100 mL of

water was continuously added. Subsequently, 3 mL of H_2O_2 (30%) was dropped in to change the color of the solution from dark brown to yellow. The resulting suspension was filtered with a 0.22 μm microporous membrane, and further washed with 200 mL HCl (1 M) and 200 mL deionized water for twice. The brown solid was vacuum-dried at 50 °C for 24 h to obtain GO.

Preparation of graphene quantum dots (GQDs)[2]. GO (0.05g) was added into a mixture of concentrated H_2SO_4 (10 mL) and HNO_3 (30 mL). The solution was treated with mild ultrasonication for 24h (500 W, 40 KHz). The mixture was then diluted with distilled water (100 mL) and filtered through a 0.22 μm microporous membrane to remove the acids. Purified GO were re-dispersed in distilled water (40 mL) and the pH was adjusted to 8.0 with NaOH. The suspension was transferred to a poly(tetrafluoroethylene) (Teflon)-lined autoclave and heated at 200°C for 24 h. After cooling to room temperature, the solution was further dialyzed in a dialysis bag (retained molecular weight: 3500 Da) for 48 h to obtain the pristine GQDs.

Synthesis of 3-aminobenzeneboronic acid functionalized graphene quantum dots (APBA-GQDs). Firstly, 2 mL GQD solution was mixed with pH 7.4 phosphate buffered solution (PBS) (0.2 M, 1:1). Subsequently, 12 mg APBA and 16 mg 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was added into the solution. The mixture was incubated for 3 h at room temperature under shaking and free of light. The suspension was further dialyzed in a dialysis bag (retained molecular weight: 3500 Da) for 48 h to obtain APBA-GQDs.

Quantum yields (QYs) measurements. Rhodamine B (QY=0.31 in water) was chosen as a standard. The quantum yields of pristine GQDs and APBA-GQDs were calculated as follows[3, 4]:

$$\Phi_i = \Phi_0 \times \frac{I_i}{I_0} \times \frac{A_0}{A_i} \times \frac{n_i^2}{n_0^2}$$

where Φ is the quantum yield, I is the measured integrated emission intensity, n is the refractive index of the solvent (1.33 for water), and A is the optical density for each sample. The subscript "0" refers to the reference standard with known quantum yield, while the subscript "i" refers to each sample. The quantum yields were all measured at 320 nm wavelength excitation.

Animal experiments and real sample measurements. All procedures involving animals were conducted with the approval of the Animal Ethics Committee in ECNU, China. Male

Sprague–Dawley rat (weight 200-250 g) were purchased from Shanghai SLAC Laboratory animal Co. Ltd and acclimatized for 4 days. The rat was anesthetized with chloral hydrate (initial dose of 300 mg/kg (i.p.) with additional injection of 50 mg/kg (i.p.) as needed to maintain anesthesia) and wrapped in a homeothermic blanket (Beijing Tide-Gene Biotechnology Development Center). The rat was placed in a stereotaxic frame (Beijing Tide-Gene Biotechnology Development Center) with the incisor bar set at 5 mm above the interaural line and appropriately placed hole was drilled through the skull. A microdialysis probe (CMA/110/111 Tub) was implanted in the striatum at the site of 2.5 mm anterior to bregma, 2.5 mm lateral from midline, and 7.0 mm below dura. In order to reduce the injury to the rat, the microdialysis probe was carefully implanted into the striatum of rats within 30 min. After continuous perfusion for 30 min with aCSF solution, the microdialysis system started to collect microdialysates at a rate of 2.0 μL/min for 3 hours as the real sample for glucose determination. All the measurement were reproduced for at least three times, presented as mean ±S.D. (n=3).

For the determination of relative recovery, the probe was immersed in a vial filled with a known concentration of glucose and then the probe was perfused with aCSF solution at the rate of 2.0 μL/min. The relative recovery is just the ratio between the concentration of glucose which can be calculated from its calibration curve and the known concentration of glucose in the vial. It can be calculated from the following equation:

$$R(\%) = \frac{C_{in}}{C_{out}} \times 100$$

where $R(\%)$ is the relative recovery in percentage, C_{out} is the concentration of the solution calculated from the calibration curve, C_{in} is the known concentration of the solution in the vial. In this work, the $R(\%)$ was calculated to be 10.4%.

Table S1. QYs of the pristine GQDs and APBA-GQDs

Sample	Integrated emission intensity (*I*)	Absorption at 320nm (*A*)	Refractive Index (*n*)	Quantum yield (Φ)
Rhodamine B	127833.9	0.102	1.33	0.31
GQDs	31953.802	0.274	1.33	0.029±0.002
APBA-GQDs	56273.0	0.028	1.33	0.497±0.030

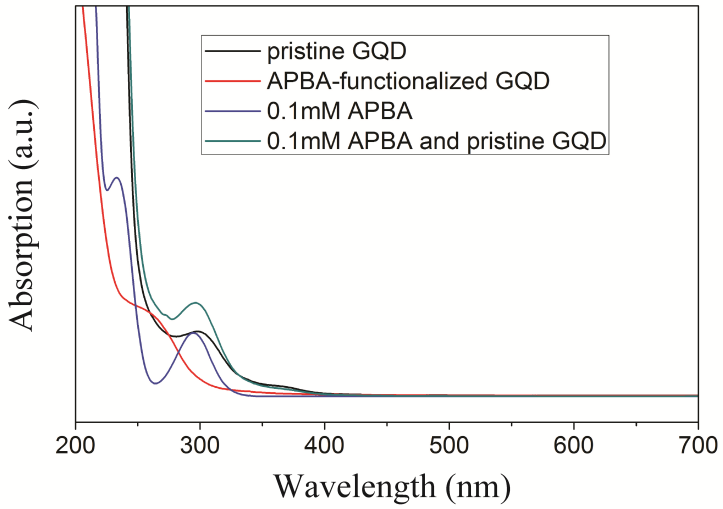


Figure S1. UV-vis spectra of pristine GQD, APBA-functionalized GQD, free APBA and mixture of free APBA and GQD.

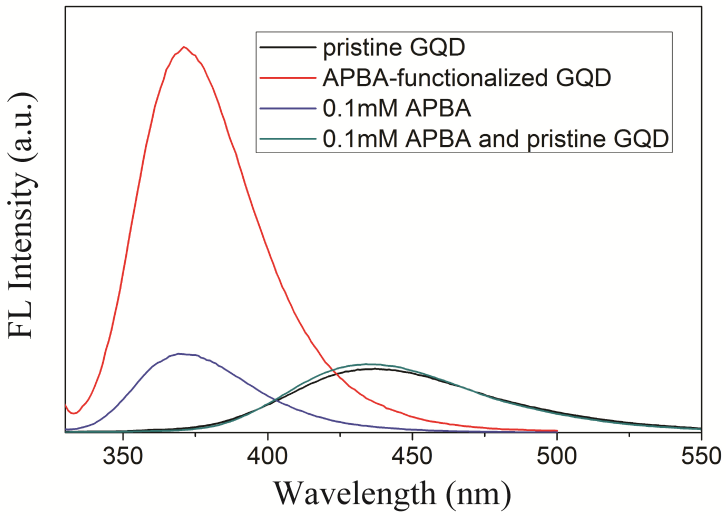
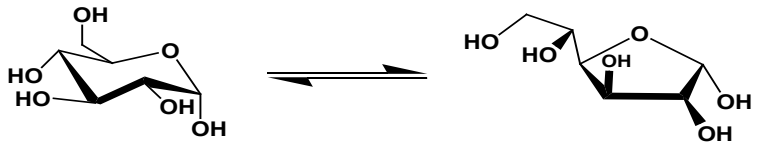
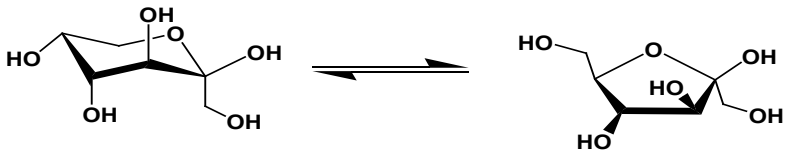
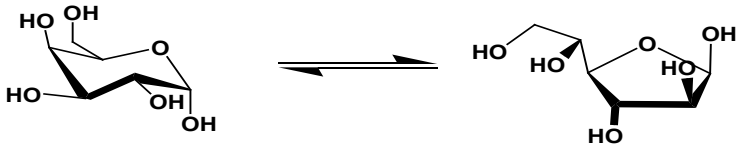
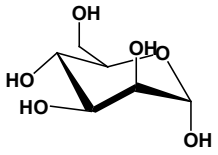
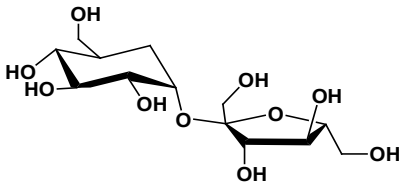
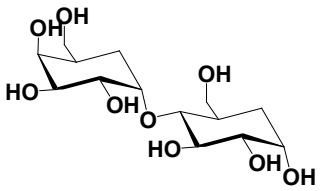
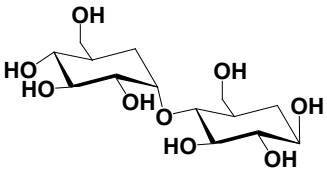


Figure S2. Fluorescent spectra of pristine GQD, APBA-functionalized GQD, free APBA and mixture of free APBA and GQD.

Table S2. The constitutional formula of D-glucose, fructose, mannose, galactose, sucrose, lactose and maltose[5].

Saccharide	Constitutional formula
D-glucose	
D-fructose	
D-galactose	
D-mannose	
Sucrose	
Lactose	
Maltose	

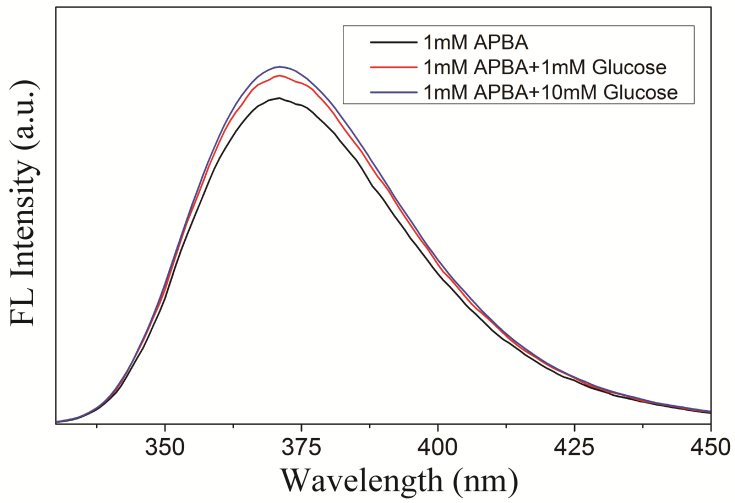


Figure S3. Fluorescent spectra of 1mM APBA in the absence and presence of 1mM, 10mM glucose.

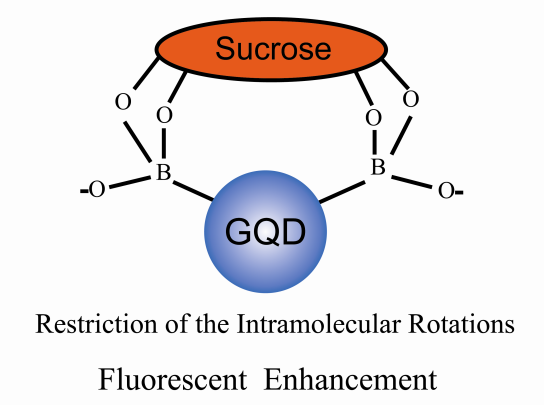


Figure S4. Proposed mechanism of fluorescent enhancement for the intramolecular rotations (RIR) effect of disaccharides.

Supporting References

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