Dual-Organelle-Targeted Near-Infrared Carbon Dots for Visualizing Glucose Dynamics in Cellular Energy Metabolism with Potential in Exercise Physiology

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Experiment

Characterization and instrument

Fluorescence spectrometer (Horiba, USA) was measured by record the fluorescence changes of B-OH-CDs. The microstructure and lattice information of the B-OH-CDs are obtained by the a Talos F200X electron microscope (FEI, Czech Republic). The X-ray powder diffraction (XRD) pattern of the B-OH-CDs was obtained by the Bruker D8 Advance X-ray diffractometer (Bruker, Germany). Fourier Transform infrared spectroscopy 5700 infrared spectrum analyzer (Bruker, Germany) was used to record Fourier transform infrared (FTIR) spectrum. X-ray photoelectron spectroscopy (XPS) was collected on a PHI 5000 Versa Probe III spectrometer (ULVAC-PHI, Japan). The fluorescence lifetime was measured by an Edinburgh FLS1000 fluorescence spectrophotometer (Edinburgh, UK).

Materials

1-Methylisoquinoline was purchased from Innochem Chemical Technology (Peking, China, 4-Formylbenzeneboronic acid was purchased from Leyan Chemical Technology (Shanghai, China). Ultrapure water from the Milli-Q system was used throughout the experiments. Fetal Bovine Serum, FBS (Umedium He Fei China). Other commercial reagents, unless otherwise specified, were purchased from Innochem, Sigma-Aldrich, and Beyotime and used without further purification.

Synthesis of B-OH-CDs

In a typical synthesis, 0.4 g 1-Methylisoquinoline and 1 g 4-Formylbenzeneboronic acid were dissolved in 15 mL DMF; after constant stirring, the resultant solution was successively transferred into a 25 mL poly(tetrafluoroethylene) liner stainless steel autoclave. After being heated at 150 °C for 8 hours and then Cooled down to room temperature. Subsequently, the B-OH-CDs crude product was centrifuged at 12000 rpm for 10 min to remove large particles. The solution dialyzed with a 1500 Da dialysis bag for 36 h. Finally, the purified solution freeze-dried to obtain a brown powder and store in refrigerator (4 °C) for further use.

Stability of B-OH-CDs

Different concentrations of NaCl: In the fluorescence stability section, the concentration of B-OH-CDs was uniformly set at 10 mg/L, while the NaCl concentrations were 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 40, 60, 90, 120, 150, 200 and 300 mM.

Detection of glucose

The detection of glucose was performed at room temperature in an aqueous solution of B-OH-CDs at pH 7.4 using PBS excited at 480 nm. Stock standard solutions of 0.1 mM glucose were prepared by dissolving an appropriate amount of glucose in aqueous and calibrating the volume to 150 mL in a volumetric flask. Other metal ion solutions were prepared in the same way before use. The fluorescent intensity of the B-OH-CDs was recorded after different concentrations of glucose aqueous solution was added to the same amount of B-OH-CDs (1 mg/L) respectively and the mixed solutions were reaction for 5 min. To evaluate the selectivity of B-OH-CDs, their optical responses to various metal ions and activity molecule. The fluorescence spectra were collected subsequently from 500 nm to 750 nm and the slit widths of the excitation and emission were both 5 nm.

Detection of glucose in real samples

Human serum samples were thawed and centrifuged at 12,000 rpm for 10 min to remove impurities. A 10 μL aliquot of the supernatant was diluted 20-fold with PBS to minimize matrix effects. The diluted serum (10 μL) was mixed with 190 μL of B-OH-CDs solution and incubated at 37 °C for 30 min. The fluorescence intensity was then recorded.

Cytotoxicity measurements

4T1 cells were seeded in 96-well plates at a density of 5×10^2 cells/mL, with 200 μL of culture medium per well and cultured at 37°C in a 5% CO_2 incubator for 24 h. After 24 hours of incubation, the cells were treated with various concentrations of B-OH-CDs (0, 10, 20, 40, 80, 120, 160, 200 μM) for an additional 24 hours. Following treatment, the culture medium was carefully aspirated, and 200 μL of MTT solution (500 μg/mL) was added to each well. The plates were then incubated for 4 hours at 37 °C. After incubation, the MTT solution was removed, and 150 μL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The plates were gently shaken at 80 rpm for 10 min, and the absorbance was measured at 490 nm using a microplate reader. Cell viability = (OD value in dosing group /OD value in control group) ×100% The absorbance of every well at 490 nm was measured with a microplate reader.

Glucose imaging

Fluorescent imaging in 4T1 Cells were performed in the similar procedures, 4T1 Cells were inoculated in a confocal dish at a density of 2×10^4 cells/mL and cultured at 37°C in a 5% CO_2 incubator for 24 h. After the cells adhered, 40 μM B-OH-CDs were added, and incubated in a 37 ℃ for 15 min. After the incubation, the cells were carefully washed 3 times with PBS. In the glucose detection positive group, then the cells were

supplemented with 50 uM glucose in the growth medium for 30 min at 37°C and imaged. In the negative control group, metformin (100 μM) was pre-incubated for 2 hours as a modulator of cellular glucose metabolism prior to incubation with the B-OH-CDs solution.

Co-localization imaging

4T1 cells were co-stained with 40 μM B-OH-CDs and an organelle dye (100 nM Mito-Tracker Green (Beyotime, C1048), 50 nM Lyso-Tracker Green (Beyotime) for 15min (37°C). After washing with PBS, fresh DMEM was added, and the cells were imaged with a confocal laser scanning microscope (CLSM). For fluorescence imaging of Mito-Tracker Green (MTG), Lyso-Tracker Green (LTG), $\lambda_{ex}/\lambda_{em}$ = 488/500−530 nm; B-OH-CDs: $\lambda_{ex}/\lambda_{em}$ = 514/580−650 nm.

Statistical methods

All experiments were conducted in triplicate, and data are presented as mean ± standard deviation (SD).

Quenching constant

Additionally, as suggested by the reviewers, we calculated the quenching constant of B-OH-CDs for glucose. According to the formula below, Q was calculated as: 12.39 $M^{-1}s^{-1}$.

$$\frac{F_0}{F} = 1 + [Q]K$$

note: F_0 represents the fluorescence intensity without quencher, F represents the fluorescence intensity with quencher added, K represents the quenching constant, and $[Q]$ represents the quencher concentration.

Table S1. Comparison of this B-OH-CQDs for the detection of glucose with other reported methods

Method	Sensing mode	LOD ($\mu mol \cdot L^{-1}$)	Wavelength (nm)	Applications	Ref
CDs	Fluorescence Enhancement	0.12	526	Real sample	1
QD-AuNP MS	Fluorescence off	2.8	563	Cell imaging	2
CDs/MnO_2 nanosheet	Fluorescence on	0.83	430	Real sample	3
CDs/Ti_3C_2 nanosheet	Fluorescence on	50	620	Real sample	4
CDs	Fluorescence Enhancement	30	440	-	5

CDs/gold nanoparticles	Fluorescence on	0.65	530	-	6
PY-POFs	Fluorescence off	5.6	472	Zebrafish imaging,	7
PDA-(-S-)NPs	Fluorescence off	0.6	450	-	8
MOFs	Colorimetry	0.0428	565	Real sample	9
Ln CPs	Colorimetry	0.0328	549	-	10
This article	**Switch off**	**0.085**	**615**	**Cell imaging, Real Sapmle**	

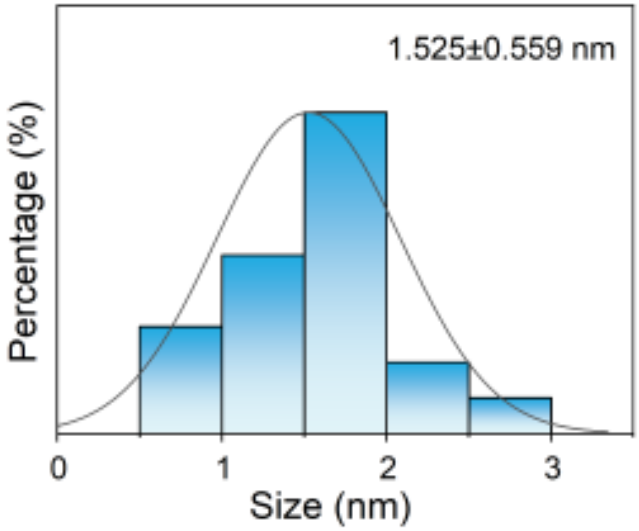

Fig. S1 The size distribution of B-OH-CDs.

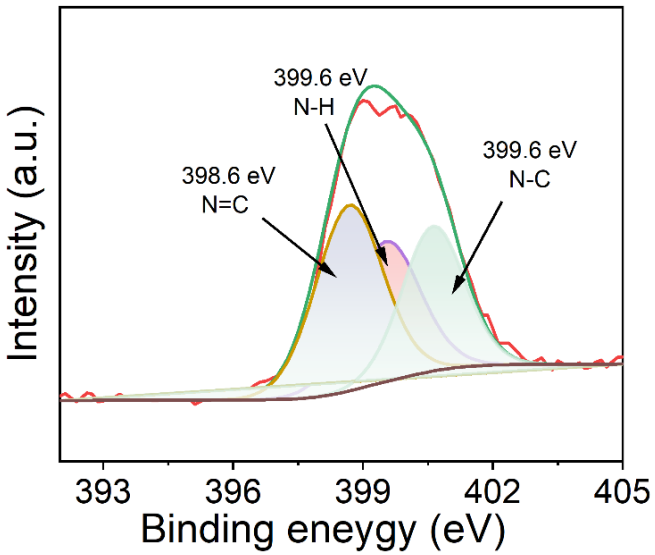

Fig. S2 The high resolution XPS spectra of N 1s for B-OH-CDs.

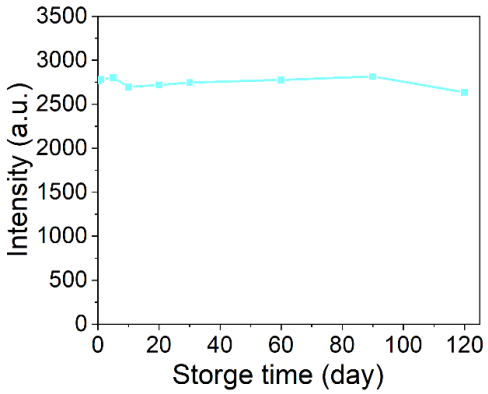

Fig. S3. The fluorescence stability of storge time for B-OH-CDs.

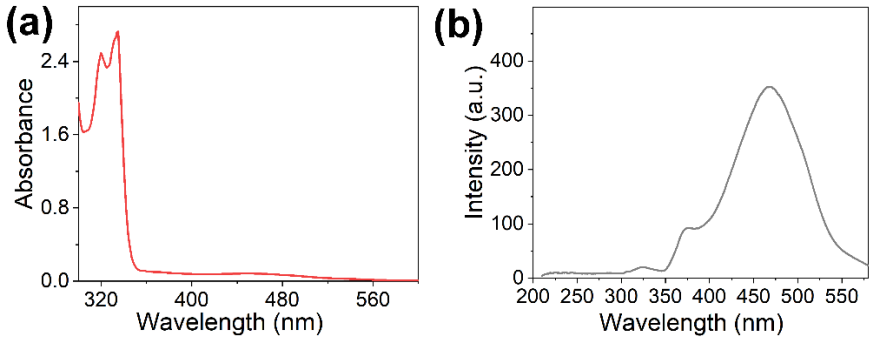

Fig. S4 (a-b) UV-Vis absorption spectrum and the excitation spectrum of the B-OH-CDs

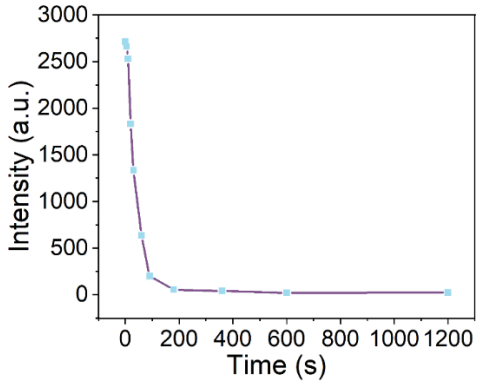

Fig. S5. The reaction time as the best conditions for recognizing glucose.

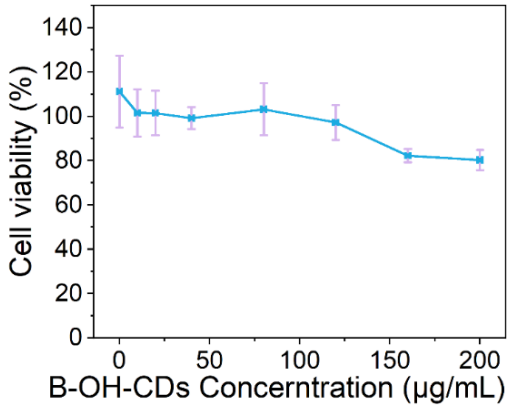

Fig. S6. The MTT Curve of B-OH-CDs.

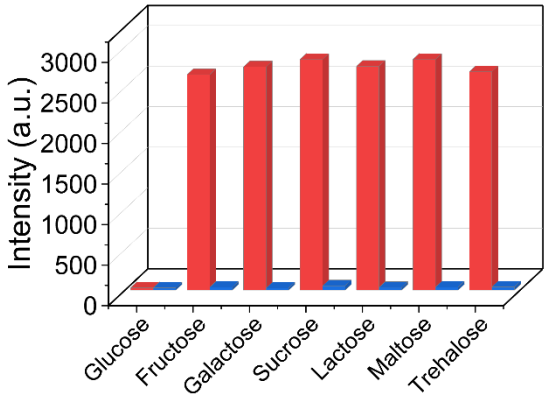

Fig. S7. Selectivity and competition for B-OH-CD with monosaccharides and disaccharides

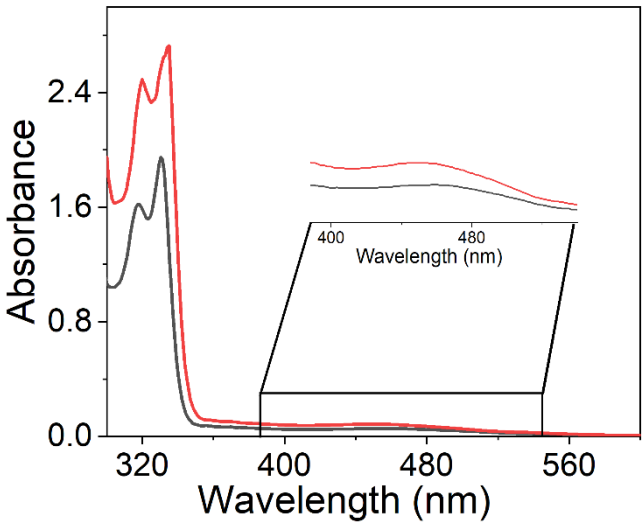

Fig. S8. UV-Vis absorption spectra of the B-OH-CDs before and after glucose addition

Reference

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