Supporting Information

Highly green fluorescent Nb_2C MXene quantum dots

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Experimental

Materials. Nitric acid and sodium hydroxide were purchased from Beijing Chemical Works. Ethylenediamine was purchased from Aladdin Technology Co., Ltd. Sulphuric acid was purchased from Tianjin Guangfu Fine Chemical Co., Ltd. The Nb2C MXene nanosheets used for the prepared experiments were obtained from Jilin 11 Technology Co., Ltd. All chemicals are of analytical grade except for the superior grade sulfuric acid and used without further purification. Deionized water produced by BK-10B from Dongguanshi Qianjing environmental equipment Co., Ltd was used in all the experiments.

Synthesis of S,N-MQDs. In a typical procedure, 100 mg of Nb_2C MXene was suspended in concentrated sulfuric acid and nitric acid in an oil bath and held at a constant temperature of 100 ℃ for 12 h. The acid solution was cooled to room temperature and added to NaOH water solution slowly until the pH reached 7. Sulfuric and nitrogen co-doped Nb_2C quantum dots (S,N-MQDs) were synthesized using the following hydrothermal method. Initially, 15 mL of water, 0.05 g of L-Cysteine and prepared MXene dots were added to a 50 mL Teflon-lined stainless-steel autoclave. Then, the autoclave was kept at a temperature of 160 ℃ for 12 h. The reaction mixture was adequately separated and purified using the following procedures. The mixture was filtered through a 0.45 μm polytetrafluoroethylene membrane to remove impurities and the filtrate was dialyzed in a 1000 Da dialysis bag for 24 hours to obtain the final S,N-MQDs. Then, the filtrate was dialyzed in a 1000 Da dialysis bag against ultrapure water which was renewed every 10-12 h for 2 days, until no Na+ was detected in the ultrapure water. The N-MQDs were fabricated in the same way by replacing L-Cysteine with ethylenediamine, respectively.

Characterization. Fluorescence measurements were performed with a fluorescence spectrophotometer (FS5 from Techcomp (China Ltd). Each measurement was conducted three times to obtain an average value. The obtained PL decay curves were fitted using a single-exponential function: $R(t) = B1 \times e^{-(t/\tau)}$, where the fit parameter τ is the PL decay time. For all lifetime measurements, the systematic error was approximately ±0.1 ns. The quantum yield (QY) of MQDs was measured using an FS-30 quantum yield accessory with an integrating sphere and the ultraviolet-visible (UV-Vis) absorption spectra of the samples were measured using a UV-Vis spectrometer (Jasco V-570). The surface morphology of MQDs was studied by an atomic force microscope (AFM) after the drop mica substrate over the diluted solution was dried. The surface morphology of the materials was characterized with scanning electron microscopy (SEM) using a Hitachi SU8010 with an acceleration voltage of 15 kV. Scanning transmission electron microscopy (STEM) images and energy-dispersive X-ray spectroscopy (EDS), mapping profiles were collected on an FEI Talos F200X transmission electron microscope operated at 200 kV in order to study diameters of MQDs and elements. 1-2 drops of a dilute aqueous solution of S,N-MQDs were dropped onto carbon film on copper mesh for high-resolution TEM (HRTEM) measurements. Fourier transform infrared spectroscopy (FTIR, Nicolet IS10, USA) was used to characterize the functional groups on the surface of S,N-MQDs. A Thermo Fisher Escalab 250Xi X-ray powder photoelectron spectrometer was used to analyze the X-ray photoelectron spectroscopy (XPS) of the sample surface composition. The structure of the materials was investigated by X-ray diffraction analysis (XRD, Bruker D8 Advance, Germany) and was performed in an angle range of 5–90°. In this work, rigorous ab initio calculations were performed by the DMol3 program, using the double numerical basis set including d-polarization

functions (DND) with all-electron treatment, a global orbital cutoff of 6.0 Å, and the generalized gradient approximation (GGA) parameterized by Perdew, Burke and Ernzernhof (PBE) for the exchange-correlation functional. To obtain an accurate energy gap of MXene quantum dots, we employed the hybrid B3LYP functional for selected sized systems. Geometry optimization was performed with convergence criteria of 10^{-5} a.u. for the total energies, 2×10^{-3} a.u. for the maximum forces, and 5×10^{-3} Å for the maximum displacement of each atom.

THP-1 cell culture and cytotoxicity test. The monocytic THP-1 cells (ATCC) were maintained in RPMI1640 medium (Hyclone; GE Healthcare, Chicago, IL, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Waltham, MA, USA) and 1% Penicillin/Streptomycin Solution (Beyotime, Nantong, China). For cytotoxicity test, 3×10^5 per well THP-1 cells in 24-well plates were incubated with various concentrations of MQDs for 24 h, and then cell counting kit-8 (CCK-8) test was done by using commercial kits purchased from Beyotime Biotechnology (Nantong, China).

3D brain organoid labelling. The 3D brain organoids were purchased from HopStem Biotech (Hangzhou, China) and maintained according to supplier's instructions. The 3D brain organoids were stained with 5 mg/mL S,N-MQDs for 24 h. After staining, the organoids were embedded in Richard-Allan Scientific™ Neg-50™ (purchased from Thermo-Fisher, USA), and then frozen-sectioned at 20 m by using Thermo NX50. After stained by DAPI (to lable nuclei), the samples were imaged by Axio Observer3 (Carl Zeiss) at excitation of 350 nm (for DAPI) and 488 nm (for S,N-MQDs).

Immunohistochemistry (IHC). The 3D brain organoids were fixed by 4% paraformaldehyde (PFA) at room temperature for 3 h and then rinsed for 3 times by PBS. The samples were dehydrated at 4 ℃ by 30% sucrose solution, and embedded in Richard-Allan Scientific™ Neg-50™ (Thermo-Fisher, USA). The frozen sections were taken at 20 m by using Thermo NX50 and blocked in 10% NDS (Jackson Immuno Researh) at room temperature for 30 min. After that, the frozen sections were incubated with first antibody (1:500 goat BRN2 antibody, Santa; 1:500 rat CTIP2 antibody, 1:500 SATB2 mouse antibody, 1:300 rabbit TBR1 antibody, Abcam) overnight at 4 ℃, rinsed with PBS for 3 times, and incubated with second antibody (1:1000 Alexa Flour 488 Donkey anti-rat lgG or Alexa Flour 546 Donkey anti-mouse lgG, Invitrogen) for 1 h at dark. Finally, the samples were stained by DAPI (to visualize nuclei) and observed under Axio Observer3 microscope (Carl Zeiss) at the excitation of 488 nm or 546 nm.

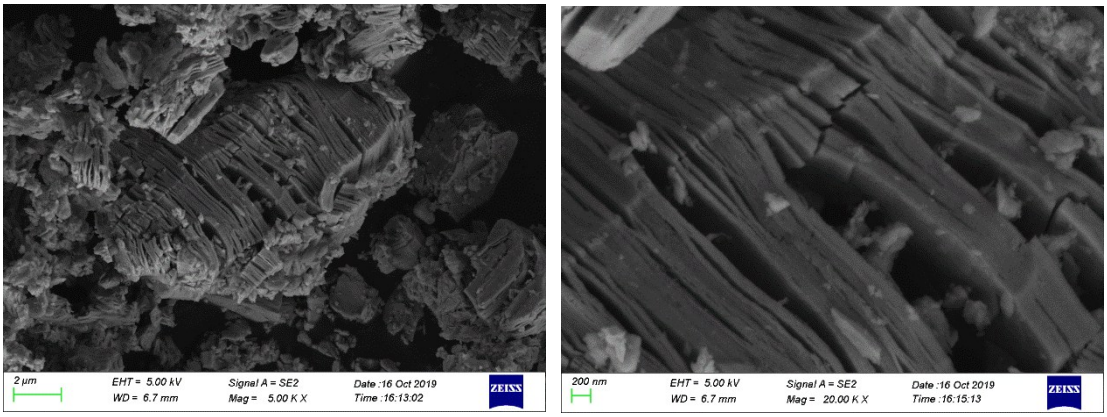

Figure S1 SEM images of as-obtained layered Nb_2C MXene.

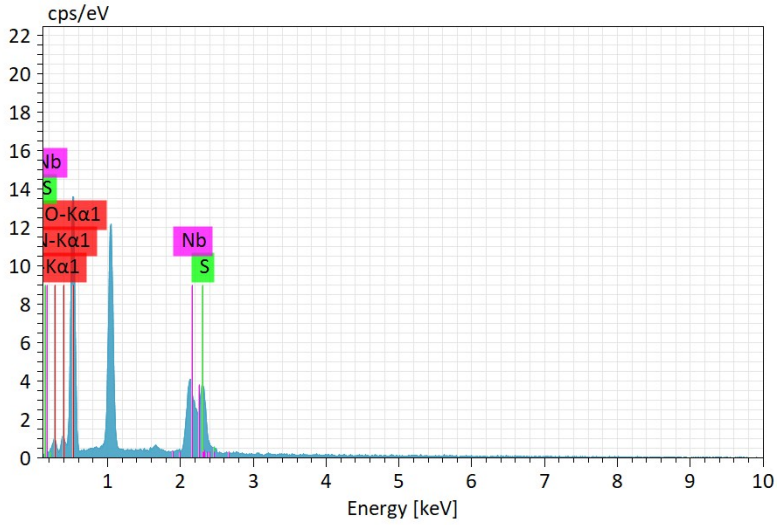

Figure S2 SEM-EDX (Energy-dispersive X-ray spectroscopy) of pristine Nb_2C.

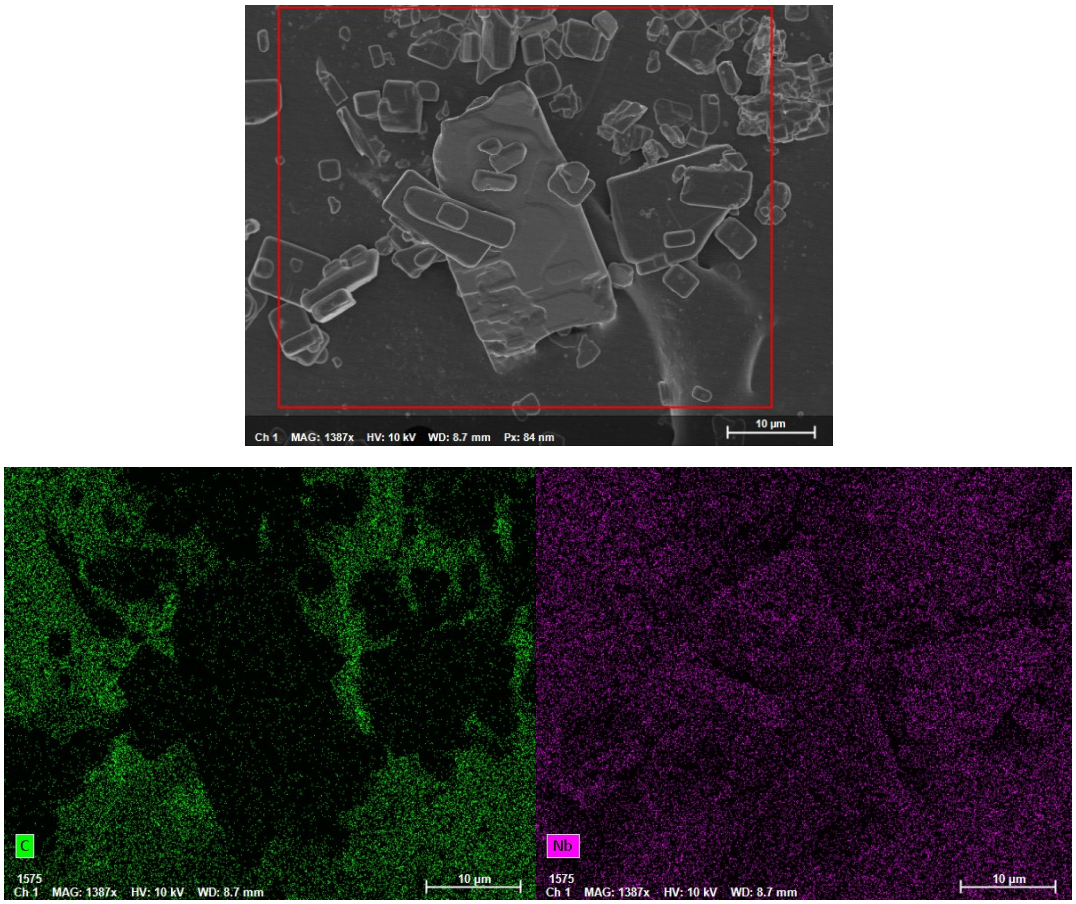

Figure S3 SEM-EDS elemental mapping images of the pristine Nb_2C sheet.

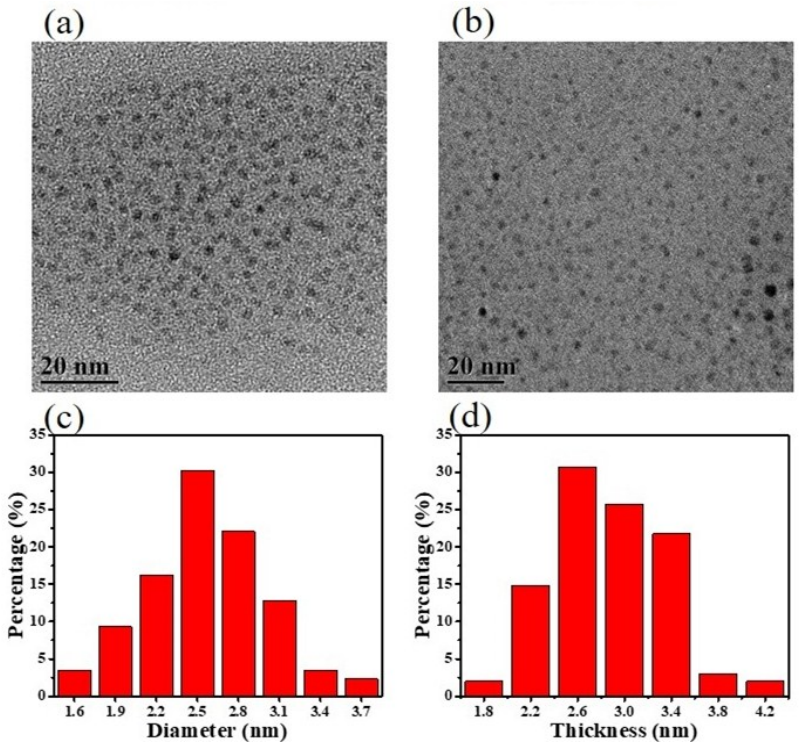

Figure S4 TEM images and diameter size distribution of (a, c) MQDs, (b, d) N-MQDs.

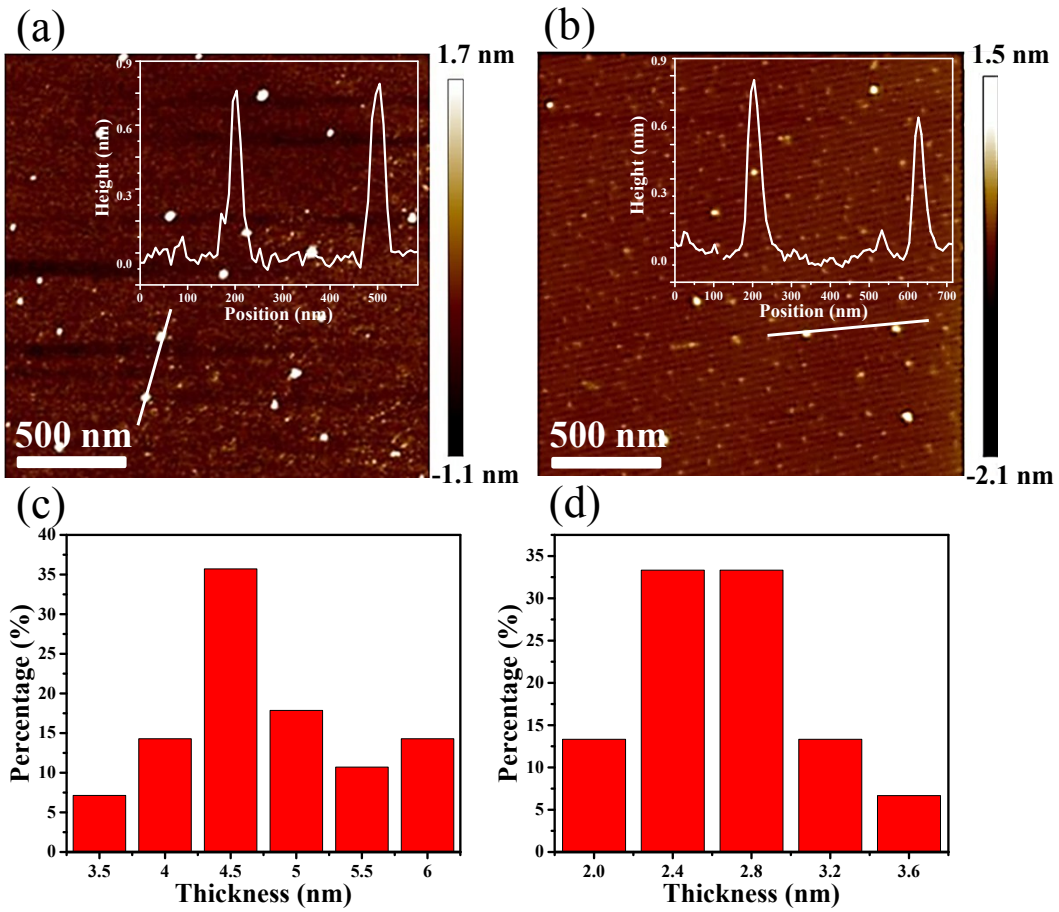

Figure S5 AFM images and thickness distribution of the prepared (a, c) MQDs and (b, d) N-MQDs. Insets are height profiles of corresponding lines.

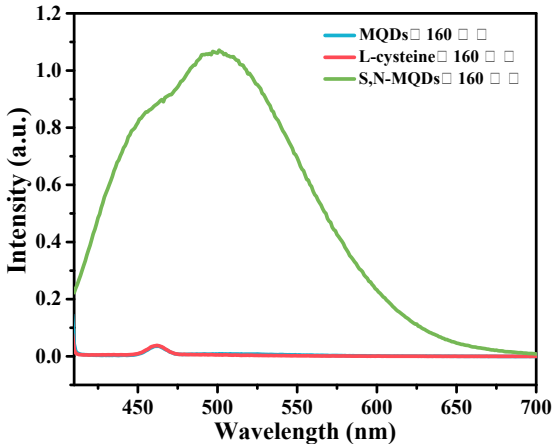

Figure S6 Photoluminescence spectra of (a) Photoluminescence spectra (UV light 400 nm) of S,N-MQDs, L-cysteine (160 °C, 12 h) and Nb2C (160 °C, 12 h, after acid treated).

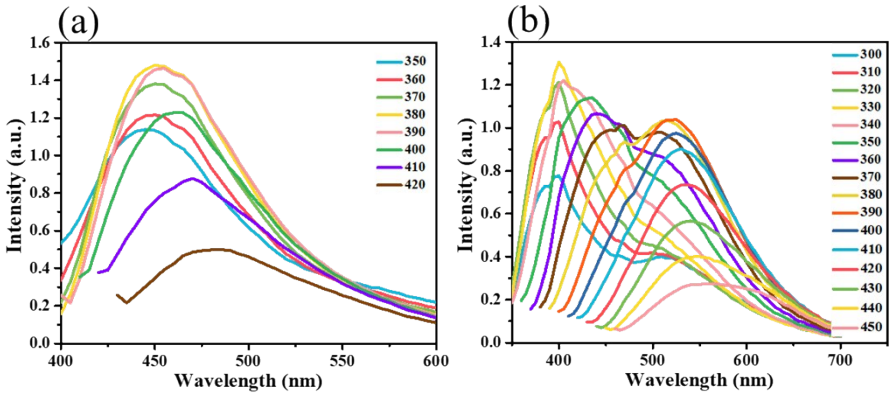

Figure S7 Photoluminescence spectra of (a) N-MQDs; (b) S,N-MQDs (0.2 g).

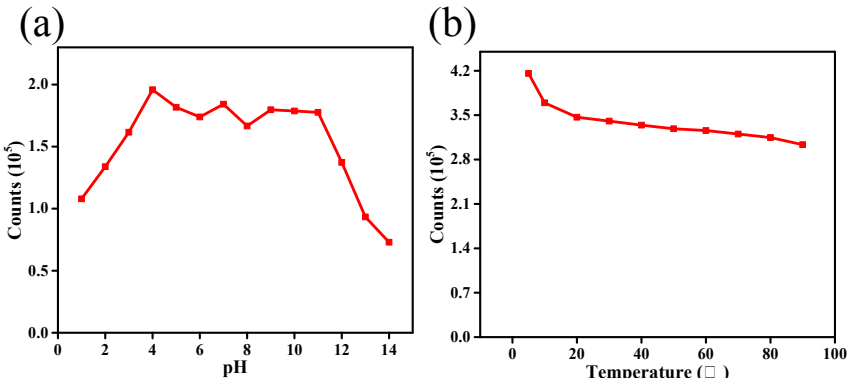

Figure S8 Fluorescence intensity variation of the S,N-MQDs as a function of (a) pH, and (b) temperature under 380 nm light illumination.

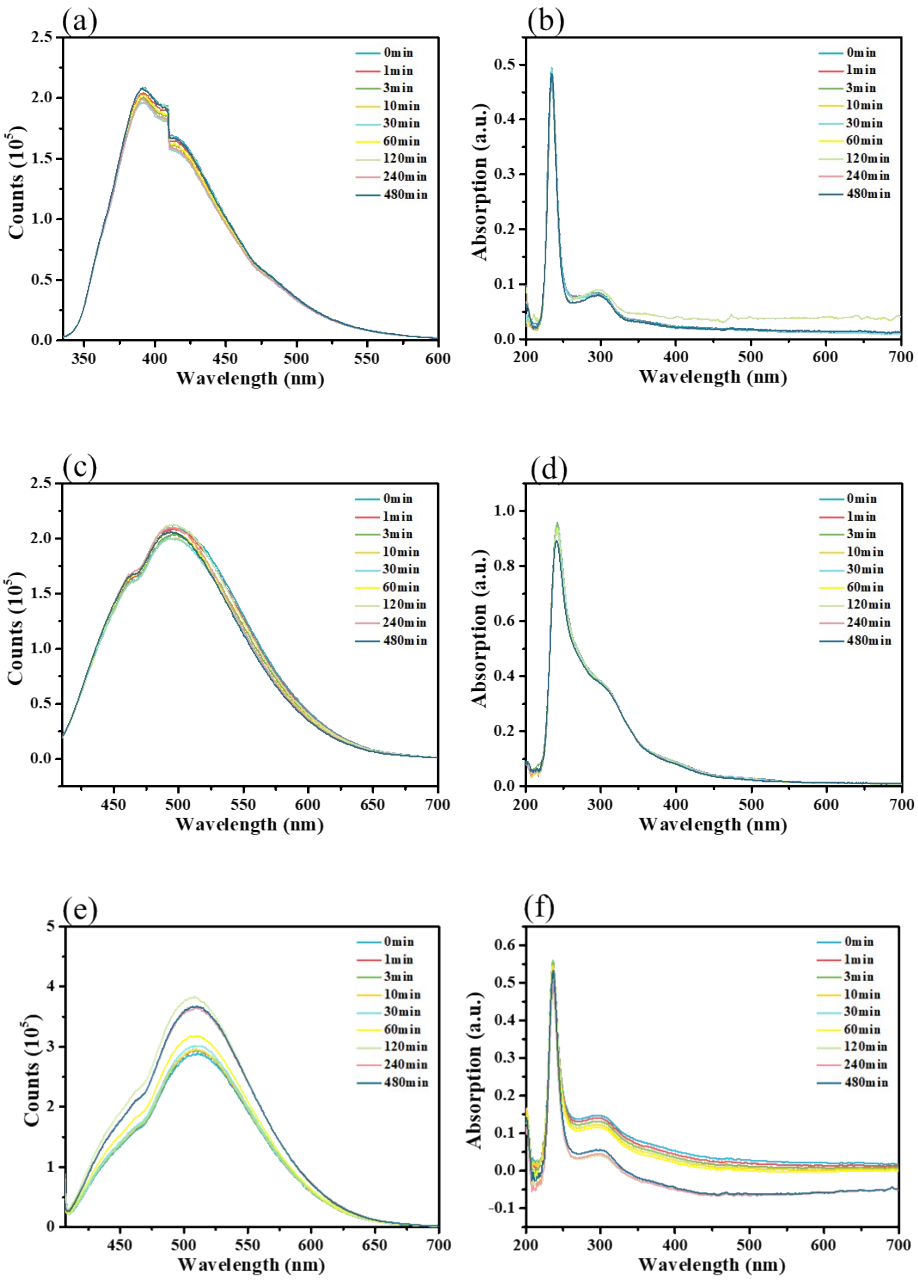

Figure S9 (a, c, e) Time-dependent fluorescence intensity and (b, d, f) absorption changes of the N-MQDs, S,N-MQDs (0.2 g), S,N-MQDs (0.05 g).

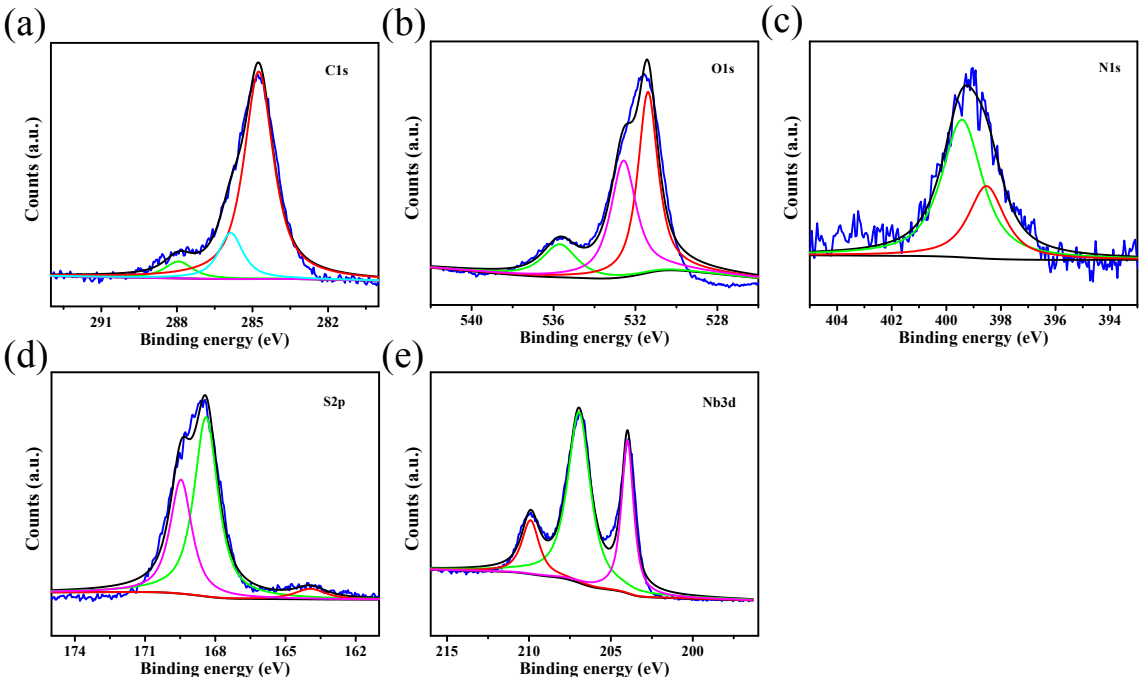

Figure S10 High-resolution XPS spectra of (a) C1s, (b) O1s, (c) N1s, (d) S2p and (e) Nb3d for the S,N-MQDs.

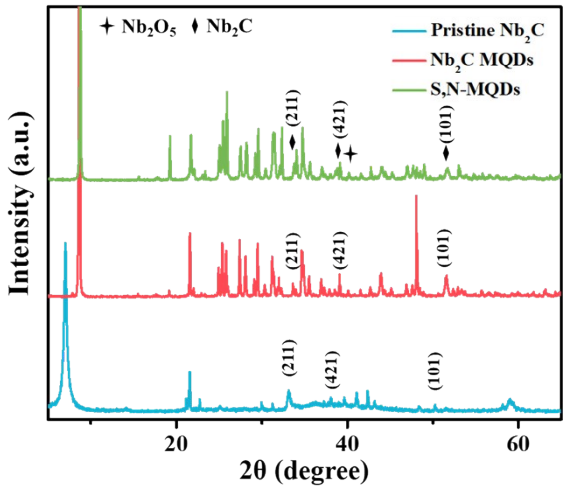

Figure S11 XRD spectra of pristine MXene, MQDs and S,N-MQDs.

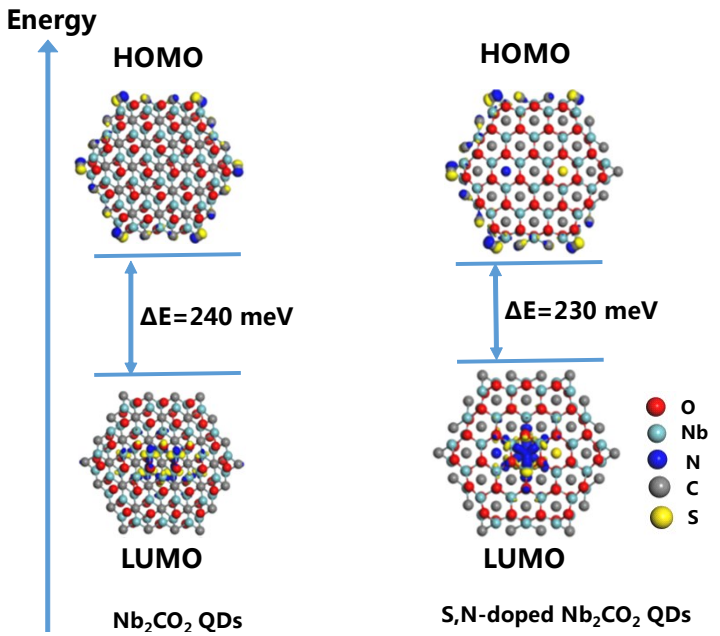

Figure S12 DFT calculated total and projected density of states of Nb_2CO_2 QDs and S,N-doped Nb_2CO_2 QDs.

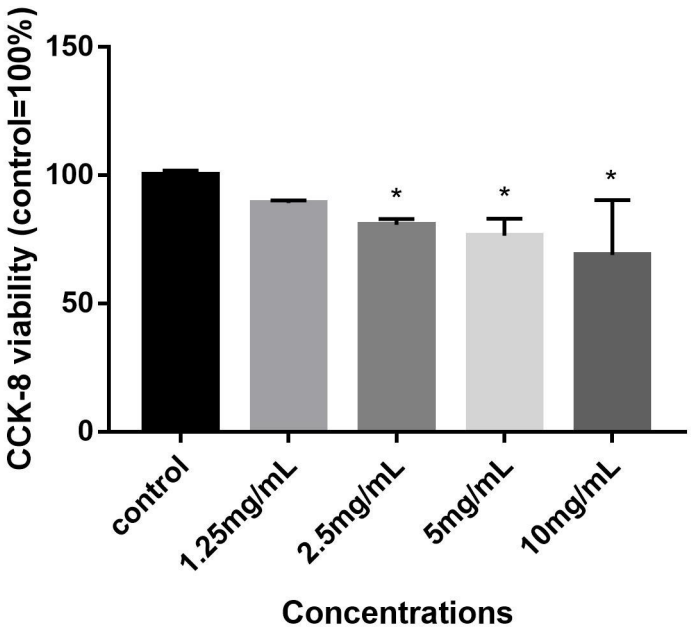

Figure S13 The cytotoxicity of QDs to THP-1 monocytes. THP-1 monocytes were exposed to various concentrations of S,N-MQDs for a period of 24 h. After exposure, the cellular viability was measured by using CCK-8 assay. *, $p<0.05$, compared with control. Data represent mean ± SD of six samples.

Supplementary explanation

The biocompatibility was tested by using THP-1 monocytes. After 24 h exposure, 2.5, 5, and 10 mg/mL S,N-MQDs significantly reduced cellular viability ($p<0.05$, compared with control). However, with the concentrations lower than 5 mg/mL, the cellular viability was higher than 75%, indicating that MQDs lower than 5 mg/mL were only modestly cytotoxic. Therefore, we used QDs at 5 mg/mL for organoid staining.

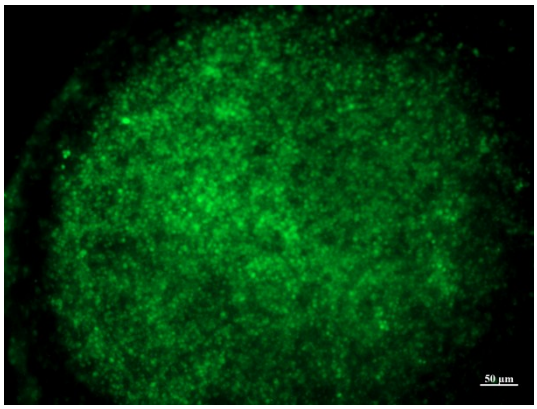

Figure S14 The staining of 3D brain organoids by QDs without DAPI counterstaining.

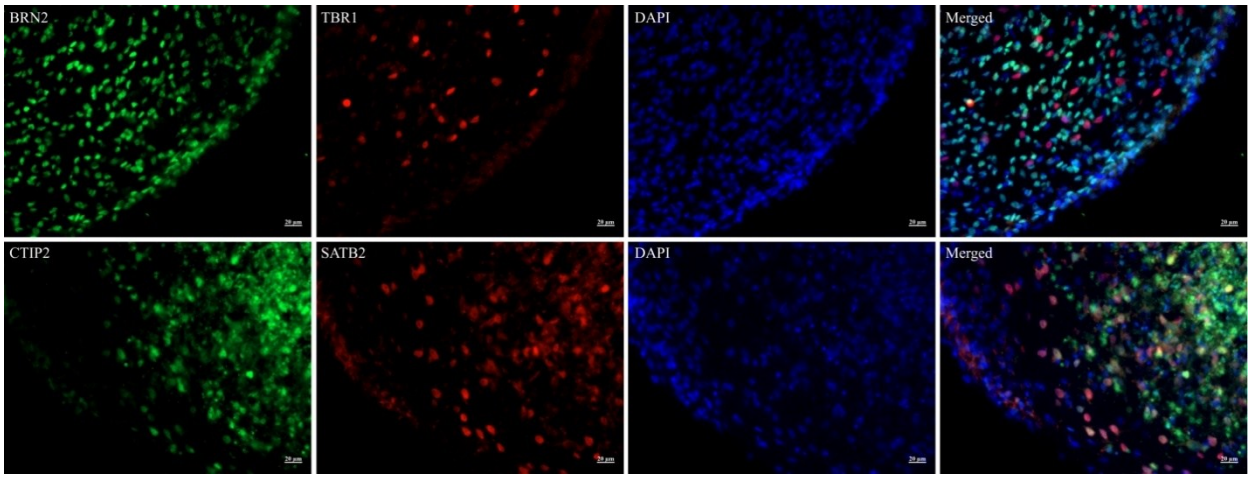

Figure S15 Immunohistochemical staining of the cortical layer markers (BRN2, TBR1, CTIP2, SATB2) of the 3D brain organoids.

Supplementary explanation

The 3D brain organoids expressed the cortical layer I, V, and VI marker T-box brain protein 1 (TBR1), the layer II to IV markers brain-2 (BRN2) and special AT-rich sequence-binding protein 2 (SATB2), as well as the layer V and VI marker chicken ovalbumin upstream promoter-transcription factor interacting protein 2 (CTIP2).

Table S1 The average diameter and thickness of the as-prepared MQDs, N-MQDs and S,N-MQDs.

Samples	Diameter(nm)	Thickness (nm)
MQDs	2.40	5.01
N-MQDs	2.66	2.80
S,N-MQDs	3.56	1.74

Table S2 The photoluminescence quantum yield (PLQY) and lifetimes of the as-prepared N-MQDs and S,N-MQDs.

Samples	PLQY	Lifetime(ns)
N-MQDs	8.97%	7.15
S,N-MQDs	18.8%	7.93

Table S3 Element content (Atomic%) of as-prepared Nb_2C, MQDs and S,N-MQDs.

Samples	Nb3d	C1s	O1S	N1s	S2p
Pristine Nb_2C	15.52	55.09	29.39	-	-
MQDs	0.06	31.45	60.7	-	-
S,N-MQDs	0.13	28	59.15	2.8	9.91