Supporting information for

Fabrication and Multifunctional Properties of Ultrasmall Water-Soluble Tungsten Oxide Quantum Dots

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Experimental Section

Materials: Bulk tungsten sulfide (WS₂), 3,3,5,5-Tetramethylbenzidine (TMB), 2,2'-azino-bis(3ethylbenzothiazoline-6-sulfonicacid)dia-mmonium salt (ABTS) and o-phenylenediamine (OPD) were purchased from Aladdin Reagent Company (Shanghai, China). K₂S₂O₈, Na₂HPO₄, KH₂PO₄, concentrated H₂SO₄, BaCl₂ and 30 wt% H₂O₂ were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Roswell Park Memorial Institute (RPMI-1640), fetal bovine serum (FBS), penicillin–streptomycin (10,000 unit/mL penicillin and streptomycin in 0.83% saline), and 0.025% trypsin solution containing 0.53 mM sodium ethylenediaminetetraacetate (Na₂EDTA) were obtained from Gibco. Hepg2 liver cancer cells were obtained from Beijing Ding Guo Chang Sheng Biotechnology Co. Ltd (Beijing, China). Other reagents and chemicals were of at least analytical reagent grade and used without further purification. Solutions were prepared with water purified by a Milli-Q purification system (Millipore, USA).

Apparatus: High resolution transmission electron microscopy (HRTEM) images were collected with a JEM-2100 TEM instrument (JEOL, Japan). The energy dispersive X-ray spectrometer (EDS) fitted to a transmission electron microscope operating on a nanoprobe mode was used for elemental analysis. Atomic force microscope (AFM) image was carried out on Bruker NanoScope V (Germany). The fluorescence and the absorption spectra were recorded with an Eclipse spectrofluorometer (Varian) and Hitachi UV-2450 spectrophotometer (Shimadzu, Japan), respectively. Absorbance at 652 nm was monitored for quantitative analysis. Fourier transform infrared spectra (FTIR) of the products were recorded on a Bruker Vertex 70 FIIR spectrometer. X-ray photoelectron spectroscopy (XPS) was performed using an ESCALAB 250XI electron spectrometer (Thermo, USA) with monochromatic Al K α radiation for analysis of the surface composition and chemical states of the product. X-Ray powder diffraction (XRD) patterns of the products were recorded with a Rigaku/Max-3A diffractometer with Cu K α radiation (λ =0.15418 nm). The electrogenerated chemiluminescence (ECL) signals were obtained by a MPI-E multifunctional chemiluminescent analyzer (Xi'an Rimax Electronics Co. Ltd, China) with a

three-electrode system where the modified glassy carbon electrode was used as the working electrode, Pt wire as the counter electrode, and an Ag/AgCl as the reference electrode. The photomultiplier tube (PMT) was biased at 800 V in the experiments. The ECL spectrum was detected by a series of highenergy optical filters (400, 412,423, 440, 460, 505, 520, 535, 555, 575, 590, 605, 620 nm). Hepg2 liver cancer cells were observed with inverted fluorescence microscope (Dmi3000B, Leica, German).

Preparation of WO_x QDs: WO_x QDs were synthesized through a hydrothermal method using WS₂ as the precursor. In brief, 30 mg of commercial WS₂ powder was dispersed in 200 mL water (65 °C). After ultrasonication for 17 h, the suspension was filtered through a 0.22 μ m microporous membrane resulting in a silver gray filter cake. Then, the resulting filter cake was suspended in 30 mL water and transferred into a 50 mL Teflon-lined stainless steel autoclave and reacted at 200 °C for 12 h. After the solution cooled naturally, the suspension was filtered through a 0.22 μ m membrane to remove large stands of the products, yielding a colorless solution. The supernatant containing WO_x QDs was collected after distillation, ethanol dispersion and centrifugation. Finally, the resulting WO_x QDs were re-suspended in ultrapure water and stored in room temperature.

Preparation of pyridine-WO_x QDs: The pyridine-WO_x QDs (Pyr-WO_x QDs) were synthesized according to a previous report of Lin et al¹ with a slightly modification. In brief, 10 mL (350 μ g mL⁻¹) of the as-prepared WO_x QDs were mixed with 10 mL pyridine and heated in an oil bath at 70 °C for 3 h, and then a subsequent extended exchange reaction was performed at room temperature while keeping stirring. After 12 h, the excessive pyridine solution was isolated by addition of hexane in separatory funnel. The pyridine-WO_x QDs were finally collected by centrifugation.

Quantum yield measurements: The quantum yield (QY) of fluorescent WO_x QDs was obtained by the following steps. 2-Aminopyridine dissolved in 0.1 M H_2SO_4 (quantum yield: 0.6 at 285 nm) was chosen as reference². Then UV-vis absorption and PL emission spectra (with 285 nm excitation) of WO_x QDs and reference were measured respectively. The accurate QY value was calculated according to the given

equation:

$$\phi_X = \phi_{ST} \left(\frac{Grad_X}{Grad_{ST}} \right) \left(\frac{\eta_X^2}{\eta_{ST}^2} \right)$$

Where the subscripts ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, *Grad* is the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η the refractive index of the solvent.

Cellular imaging: Hepg2 liver cancer cells (6×10^6 cell mL⁻¹) were maintained in RPMI-1640 media supplemented with 10% FBS, and 0.5% p01202 enicillin-streptomycin solution in a humidified atmosphere with 5% CO₂ at 37 °C. The medium was replaced every 2 days throughout the lifetime of all cultures. When Hepg2 cells nearly take up the culture bottle (about 80%), where medium was about 4 mL, the cells were incubated with WO_x QDs (350 µg mL⁻¹, 200 µL) for 24 h. Then, the cells were washed with PBS for three times to remove the excess QDs. Immediately after the incubation and washing steps, the images were taken by aninverted fluorescence microscope. The photographs of the colorimetric assays were imported into Adobe Photoshop and converted into grayscale mode. The mean grayscale intensities were quantified using the histogram function of Adobe Photoshop. The ultimate mean intensity value of each detection zone was obtained by subtracting the measured average detection zone intensity from the mean intensity of the blank control.

Cell viability test under UV light: First, after incubating the Hepg2 liver cancer cells $(1 \times 10^4 \text{ cell mL}^{-1})$ for 48 h, the medium was replaced with 200 µL of fresh medium (as the control experiment) and 200 µL of fresh medium containing a concentration of WO_x QDs (70 µg mL⁻¹), and the cells were exposed to the UV irradiation with 0 s, 10 s, 30 s, 60 s, 120 s, and 300 s. Then, the medium was removed, and fresh medium containing MTT (20 µL, 5 mg mL⁻¹) was added into each well. After incubating the cells for 4 h, the absorbance of the solution was measured to assess the relative viability of the cells using a Bio-Rad 680 microplate reader. Optical density (OD) was read at a wavelength of 490 nm. The cell viability was estimated according to the following equation.

Cell viability(%) =
$$\frac{OD_{Treated}}{OD_{Control}} \times 100\%$$

where $OD_{Control}$ is the optical density in the absence of UV light, and $OD_{Treated}$ is the optical density in the presence of UV light.

Cellular toxicity test: The cytotoxicity study of as-prepared WO_x QDs was carried out using the MTT assay on Hepg2 liver cancer cells and endothelial cells (the culture procedure is similar to that of Hepg2 liver cancer cells as the above mentioned) by following standard protocols. Briefly, endothelial cells and Hepg2 liver cancer cells were seeded in 96-well plates at 1×10^4 cells per well in Dulbecco's Modified Eagle's Medium (DMEM) medium with 10% fetal bovine serum and 100 µg mL⁻¹ penicillin/streptomycin and incubated at 37 °C in a humidified atmosphere with 5% CO₂. After incubating the cells for 24 h, the medium was replaced with 200 µL of fresh medium containing a concentration of WO_x QDs (70 µg mL⁻¹, pH 7.0). At certain times (0, 12, 24, 36, 48, 60, 72 and 84 h), the medium was removed, and fresh medium (200 µL) containing MTT (20 µL, 5 mg mL⁻¹) was added into each well. After incubating the cells for 4 h, the absorbance of the solution was measured to assess the relative viability of the cells using a Bio-Rad 680 microplate reader. Optical density (OD) was read at a wavelength of 490 nm. The cell viability was estimated according to the following equation.

Cell viability(%) =
$$\frac{OD_{Treated}}{OD_{Control}} \times 100\%$$

Where $OD_{Control}$ is the optical density in the absence of WO_x QDs, and $OD_{Treated}$ is the optical density in the presence of WO_x QDs.

Peroxidase-like activities of WO_x QDs: To investigate the peroxidase-like activity of the WO_x QDs, the catalytic oxidation of the peroxidase substrate TMB and ABTS in the presence of H_2O_2 was tested. A typical experiment was carried out as follows: 20 µL WO_x QDs (10 µg mL⁻¹), 40 µL TMB or ABTS (4mM), 40 µL H_2O_2 (3%) and 100 µL HAc-NaAc buffer (0.2 M, pH 3.6) were mixed thoroughly, and further incubated at 37 °C for different time. The UV–vis absorption spectra were recorded after

reaction for different time at 37 °C. The control experiments were performed in the absence of H_2O_2 or WO_x QDs.

Fabrication of the modified electrode for ECL: Glassy carbon electrode (GCE, 3 mm diameter, CH Instruments, Inc.) was wet polished carefully with 1.0, 0.3 and 0.05 μ m alumina slurry, followed by washing thoroughly with ultrapure water. The electrode was then successively sonicated in 1:1 nitric acid, ethanol and doubly distilled water, and then allowed to dry at room temperature. Then, 5 μ L of the WO_x QDs solution (350 μ g mL⁻¹) was dripped onto the surface of well-polished GCE, and then drying slowly in air at room temperature. The modified electrode was donated as WO_x QDs/GCE.

References

- (1) S. Cong, Y. Y. Tian, Q. W. Li, Z. G. Zhao, F. X. Geng, Adv. Mater., 2014, 26, 4260-4267.
- (2) R. Rusakowicz, A. C. Testa, J. Phys. Chem., 1968, 72, 2680–2681.



Fig. S1 AFM images of WO_x QDs on Mica substrate.



Fig. S2 XRD pattern of the WO_x QDs with the peak positions in the JCPDS card.



Fig. S3 The photographs for proofing the formation of H_2SO_4 of (A) the product after hydrothermal reaction in the present work and (B) take the Na_2CO_3 as the control test.



Fig. S4 FT-IR spectra for the WO_x QDs.



Fig. S5 FT-IR spectra (A) and EDS (B) of the pyridine-WO_x QDs. The appearance of a peak characteristic of C-N-C bonds for pyridine molecules at 1460 cm⁻¹ in the FT-IR spectrum and the appearance of W, O, C and N elements in the EDS data indicated the successful synthesis of pyridine-WO_x QDs.



Fig. S6 The photographs of the WO_x QDs aqueous solution (left) and pyridine- WO_x QDs aqueous solution (right) after being stored for 10 days and 100 days.



Fig. S7 UV-Vis absorbance spectrum of the WO_x QDs solution. Inset: plot of $(\alpha hv)^{0.5}$ against *hv*.



Fig. S8 Luminescence stability of WO_x QDs. Fluorescence intensity of WO_x QDs at different storage time (A), pH values (B), and ionic strengths (ionic strengths were controlled by various concentrations of NaCl in aqueous solution), (D) Fluorescence intensity variation of WO_x QDs as a function of time under 254 nm excitation.

The luminescence stability of the WO_x QDs has been investigated under different conditions (Fig. S8). The luminescence intensity of the WO_x QDs didn't decrease in the 30 days (Shown in Fig. S8A) and even 12 months (Shown in inset of Fig. S8(A)) at room temperature. Meanwhile, the pH values were studied in the range from 3 to 11. As shown in Fig. S8 (B), the luminescence intensity of the WO_x QDs was strongest at pH 3. When the pH value range was from 4 to 11, the luminescence intensity of the WO_x QDs kept stable basically. Moreover, Fig. S8 (C) showed that there were no significant changes of the luminescence intensity of the WO_x QDs adding different NaCl concentration (from 0 to 1 M), indicating that the luminescence intensity of the WO_x QDs exhibited highly stable in the presence of different ionic strengths. Besides, the dependence of fluorescence intensity on the exposure time under 254 nm UV irradiation was investigated as well. From Fig. S8 (D), the results showed that the WO_x QDs are highly resistant to photobleaching in the 60 min. Therefore, the WOx QDs showed high luminescence stability.



Fig. S9 Cytotoxicity testing results via a MTT assay. Effect of WO_x QDs on Hepg2 cells (A) and endothelial cells (B) viability with different time.



Fig. S10 Effect of UV irradiation on Hepg2 cells in the absent of WO_x QDs (left) and in the present of WOx QDs (right) with different time.

The cell cytotoxicity experiments of the WO_x QDs were evaluated using both cancer cell (Hepg2 cells) and healthy cell (endothelial cells) line by the MTT viability assay. The results showed that the WO_x QDs showed no apparent toxicity to the cells even to the healthy cells incubated for 84 h with WO_x QDs (Fig. S9). Thus, the WO_x QDs showed the low cytotoxicity and the good biocompatibility. On the other hand, as shown in Fig. S10, cell damage was hardly observed when the Hepg2 cells in the absent and present of WO_x QDs were exposed to the 254 nm UV irradiation for 300 s. And, the WO_x QDs has also been demonstrated highly resistant to photobleaching in the 60 min (Fig. S8 (D)). Therefore, according to the results, the WO_x QDs exhibited excellent photoluminescent property, low toxicity, high luminescence stability against photobleaching, which make them promising luminescence probes and suitable for bio-imaging applications.



Fig. S11 (A) A bright field microphotograph of Hepg2 cells labeled with the WO_x QDs, (B) A fluorescence microphotograph of the cells.



Fig. S12 Color change of reaction solution (35 μ g mL⁻¹ WO_x QDs + 0.8 mM TMB + 0.2 M H₂O₂) as the increasing of reaction time.



Fig. S13 The UV–visible absorption spectra changes of the reaction solution of 35 μ g mL⁻¹ WO_x QDs + 0.8 mM TMB + 0.2 M H₂O₂ with the increasing of reaction time.



Fig. S14 (A) Time-dependent absorbance change at 414 nm in the different reaction systems: (a) 0.8 mM ABTS + 0.2 M H₂O₂, (b) (a) + 35 μ g mL⁻¹ WO_x QDs, (c) (a) + 35 μ g mL⁻¹ Pyr-WOx QDs. (B) Time-dependent absorbance change at 450 nm in the different reaction systems: (a) 0.8 mM OPD + 0.2 M H₂O₂, (b) (a) + 35 μ g mL⁻¹ WO_x QDs, (c) (a) + 35 μ g mL⁻¹ WO_x QDs. Solutions were prepared with phosphate-buffered saline (PBS, pH 3.6, 0.2 M).



Fig. S15 (A) Images of the colored products (35 μ g mL⁻¹ WO_x QDs + 0.8 mM ABTS) for different concentrations of H₂O₂: 0 (a), 0.002 (b), 0.01 (c), 0.02 (d), 0.04 (e), 0.1 (f), 0.2 (g), 0.4 (h), 0.6 (i), 0.8 (j), 1 (k), 1.2 (l), 1.4 (m), 1.6 (n), 1.8 (o), and 2 (p) mM. (B) The concentration calibration curve constructed using the grayscale intensity values converted from the images of the colored products.



Fig. S16 CVs of bare GCE (c) and WO_x QDs/GCE (d) in 0.1 M PBS (pH 5.0) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl.



Fig. S17 ECL of a WO_x QDs (a) and the Pyr-WO_x QDs modified (b) GCEs in 0.1 M PBS (pH 5.0) containing 0.1 M K₂S₂O₈ and 0.1 M KCl.



Fig. S18 ECL intensity–time profiles of the WO_x QDs/GCE in different concentrations of DA concentration: 0 (a), 1×10^{-15} (b), 1×10^{-14} (c), 1×10^{-13} (d), 1×10^{-12} (e), 1×10^{-11} (f), 1×10^{-10} (g), 1×10^{-9} (h), 1×10^{-8} (i), 1×10^{-7} (j), 1×10^{-6} (k), and 1×10^{-5} (l) M. Inset: Linear calibration plot for DA detection.