Supporting information for

Synthesis of Bi_2WO_{6-x} nanodots with oxygen vacancies as all-in-one nanoagent for simultaneous CT/IR imaging and photothermal/photodynamic therapy of tumors

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1 Experimental section

1.1 Materials

Bismuth nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$), sodium tungstate dehydrate ($Na_2WO_4 \cdot 2H_2O$), citric acid monohydrate (CA), hexadecyl trimethyl ammonium bromide (CTAB), 1,3-diphenyl isobenzofuran (DPBF) were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Acetone and ammonia solution ($NH_3 \cdot H_2O$) were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. Calcein-AM, propidium iodide (PI), 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI), cell counting kit-8 (Cck-8) and phosphate buffer saline (PBS) were purchased from Beyotime Biotechnology Co., Ltd.

2.2 Characterization

Both black Bi_2WO_{6-x} and faint-yellow Bi_2WO_6 samples were analyzed by using high-

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resolution transmission electron microscopy (HR-TEM, FEI Talos F200S) equipped with energy dispersive spectroscopy (EDS), powder X-Ray diffractometer (XRD, Bruker D4), electron paramagnetic resonance spectroscopy (EPR, Bruker EMX-10/12) at 110 K, X-ray photoelectron spectroscopy (XPS, Escalab 250Xi), UV-vis-NIR absorption spectrophotometer (Shimadzu UV-3600). The Fourier transform infrared (FT-IR) spectrum was obtained from FT-IR spectrometer (Nicolet 8700). The concentration of Bi_2WO_{6-x} dispersion was measured by high dispersion inductively coupled plasma atomic emission spectroscopy (ICP-AES, Prodigy, USA). The diameter of nanodots in dispersion was acquired by using Particle Size & Zeta Potential Analyzer (nano ZS).

1.3 Cell experiments

Cytotoxicity test of Bi_2WO_{6-x}. The viability of 4T1 cell was used to evaluate the cytotoxicity of Bi_2WO_{6-x}-$CA_{1.0}$ nanodots by using standard CCK-8 assay. The 4T1 cells which density is 10^4 cells per well were seeded into 96-well culture plate. Subsequently, the 4T1 cells were incubated at standard situation (37 °C, 5% CO_2) in a humidified incubator for 6 h. Then Bi_2WO_{6-x}-$CA_{1.0}$ dispersions were injected into the 96-well culture plate at different Bi concentrations (0.34-0 g L^{-1}). After incubated 24 h and/or 48 h, 10 μL of cck-8 mixed with 100 μL of culture medium were added to each 96 wells. Then the cell viability was calculated by the absorbance at 450 and 650 nm of assay. All experiments were repeated 5 times independently.

Intercellular singlet oxygen detection. 350 μL of 1640 culture medium containing 4T1 cells were seeded to 24-well culture plate at a density of 10^4 cell/well. After incubated 24 h,

the medium was replaced by 200 μL of 1640 culture medium containing Saline and Bi_2WO_{6-x}-$CA_{1.0}$ dispersion. The 1640 cells were divided into four groups: (a) Saline; (b) Saline + laser; (c) Bi_2WO_{6-x}-$CA_{1.0}$; (d) Bi_2WO_{6-x}-$CA_{1.0}$ + laser. Then the cells were stained with ROS-sensitive probe (2′,7′-Dichlorofluorescin diacetate, DCFH-DA), irradiated by 808 nm laser (1.0 W cm^{-2}) for 10 min and incubated for 30 min. Subsequently, the cells were washed with the fresh 1640 culture medium without serum three times and stained with DPAI. Finally, the prepared 1640 cells were imaged by CFM to detect singlet oxygen ($^1O_2$).

Photothermal/photodynamic therapy in vitro. 350 μL of 1640 culture medium containing 4T1 cells were seeded to 24-well culture plate at a density of 10^4 cell/well. After incubated 24 h, the medium was replaced by 200 μl of 1640 culture medium containing Saline and Bi_2WO_{6-x} dispersion. The cells were divided into four groups according to the different medium containing materials with/without irradiation: (a) Saline; (b) Saline + laser; (c) Bi_2WO_{6-x}-$CA_{1.0}$; (d) Bi_2WO_{6-x}-$CA_{1.0}$ + laser. Then the cells of group (b) & (d) were irradiated by 808 nm laser at an output power density of 1.0 W cm^{-2} for 10 min. Subsequently, the cells were washed with PBS and incubated for 30 min after stained with Calcein-AM and propidium iodide (PI). Then the 4T1 cells were imaged by a confocal fluorescence microscope (CFM, Leica TCS SP8, Leica Microsystems).

1.4 Animal experiments

Animals and tumor model. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of the U.S. National Institutes of Health (NIH Publication no. 86-23, revised 1985) and approved by the Animal Ethics

Committee of Donghua University. BALB/c nude mice (15-20 g, male) were purchased from Shanghai Laboratory Animal Center (SLAC, Shanghai, China). 4T1 cells (3.0×10^6 per mouse) were injected subcutaneously into the backside of each mouse to prepare tumor-bearing mice.

CT imaging in vivo. X-ray attenuation coefficient of Bi_2WO_{6-x}-$CA_{1.0}$ nanodot dispersions and commercial Iobitridol solutions with various concentrations were studied with a GE LightSpeed VCT imaging system with 120 kV. When the diameter of tumors reached at 6 mm, the mice were hocused by pentobarbital (10 mg kg^{-1}) through intraperitoneal injection method. Then the mice were intratumorally injected different volume of PBS (0 and 100 μl) containing Bi_2WO_{6-x} nanodots (0.17 g L^{-1}) and Iopromide solutions. CT imaging of mice were measured before and after the injection of Bi_2WO_{6-x} nanodots dispersion for 30 min.

Photothermal/photodynamic therapy in vivo. 4T1 tumor-bearing mice were assigned to four groups randomly: (I) Saline injection, (II) Saline injection + Laser, (III) Bi_2WO_{6-x}-$CA_{1.0}$ injection, and (IV) Bi_2WO_{6-x}-$CA_{1.0}$ injection + Laser. Then mice of each group were intratumorally injected with saline (100 μL) and saline solution containing Bi_2WO_{6-x} nanodots (100 μL, 0.17 g L^{-1}). After 1 h, the tumor of mice in group (b,d) were irradiated by 808 nm laser (1.0 W cm^{-2}) for 10 min. The infrared thermal imaging was real-time recorded by IR-thermal imaging camera.

Histology analysis. After mouse sacrificed, major organs and tumors were obtained. The concentration of Bi ions in main organs were measured by Inductively coupled plasma mass spectrometry (ICP-MS); Finally, major organs were sectioned into slices, stained using by

hematoxylin and eosin (H&E) staining, and then and observed by a digital microscope.

2 Figures

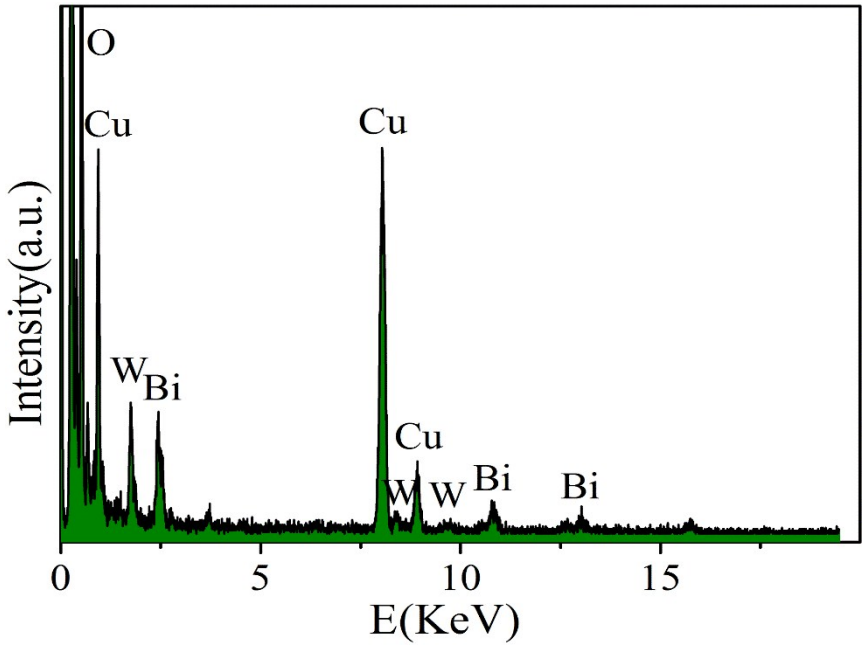

Fig.S1 EDS pattern of Bi_2WO_{6-x}-$CA_{1.0}$ sample.

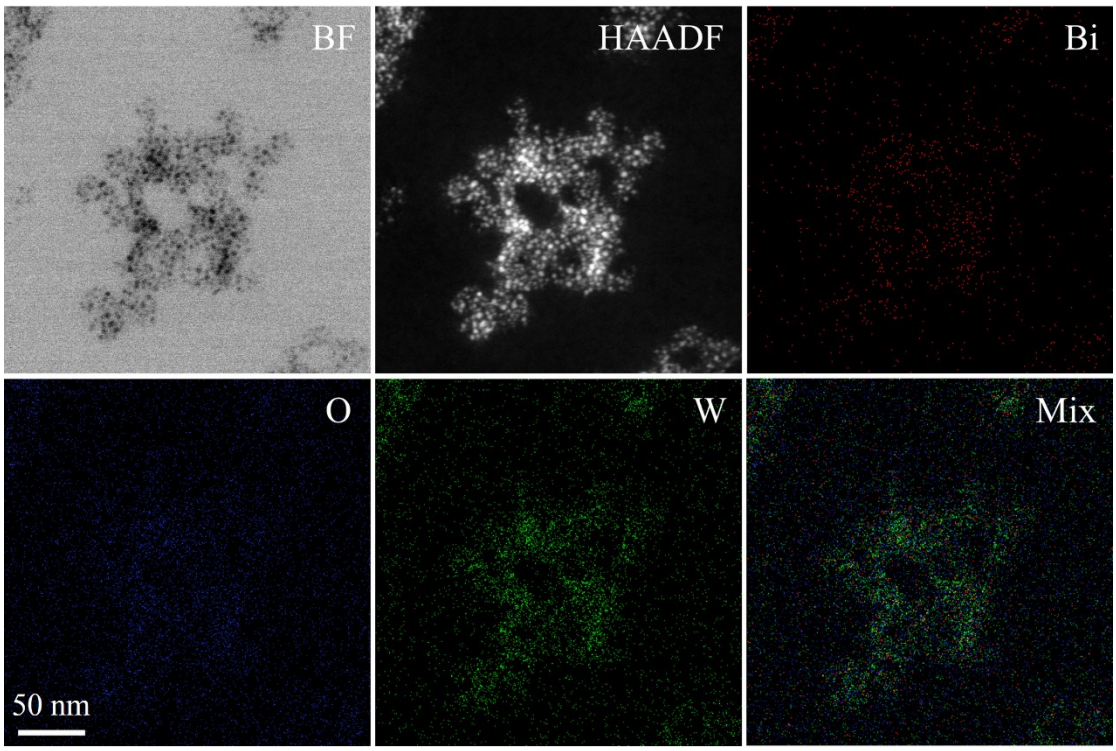

Fig.S2 Mapping images of Bi_2WO_{6-x}-$CA_{1.0}$ sample.

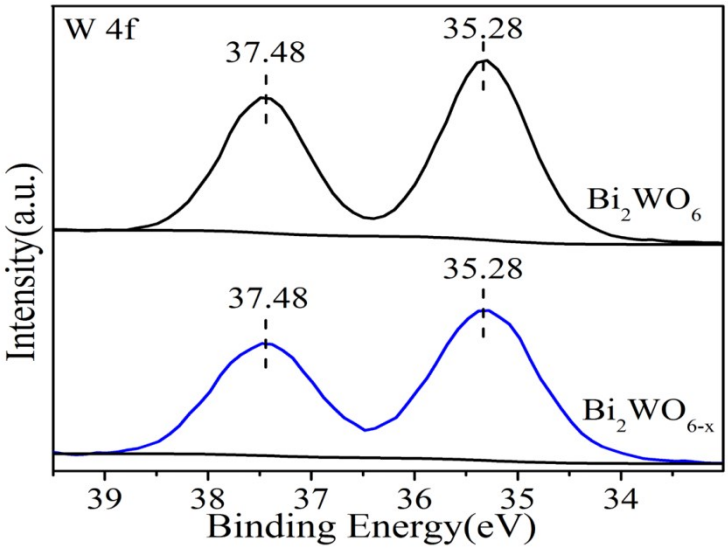

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Fig. S3 W4f spectrum of both Bi_2WO_{6-x} and Bi_2WO_6 nanodot samples.

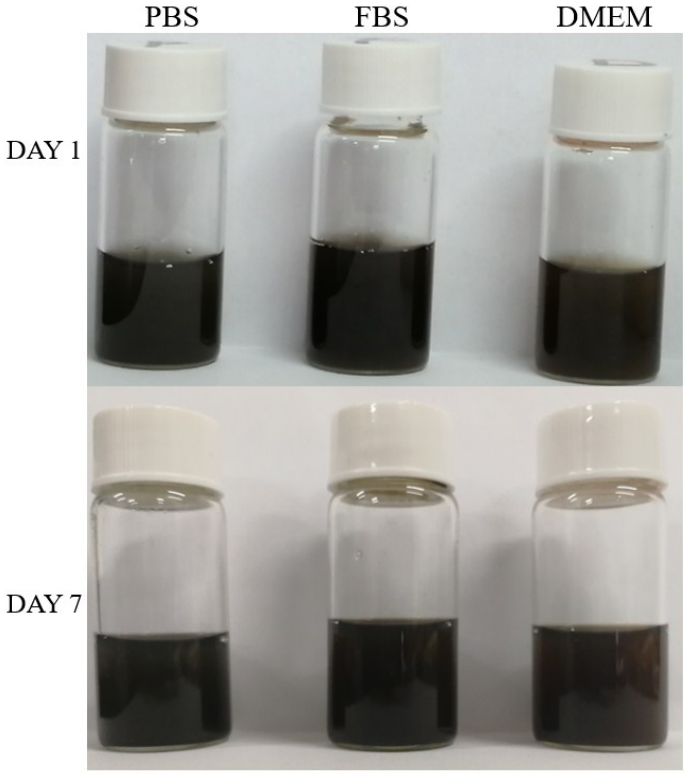

Fig. S4 Photos of Bi_2WO_{6-x}-$CA_{1.0}$ in PBS, FBS and DMEM dispersions for 1 and 7 day.

Fig.S5 Tumor photos after different treatment in four groups.

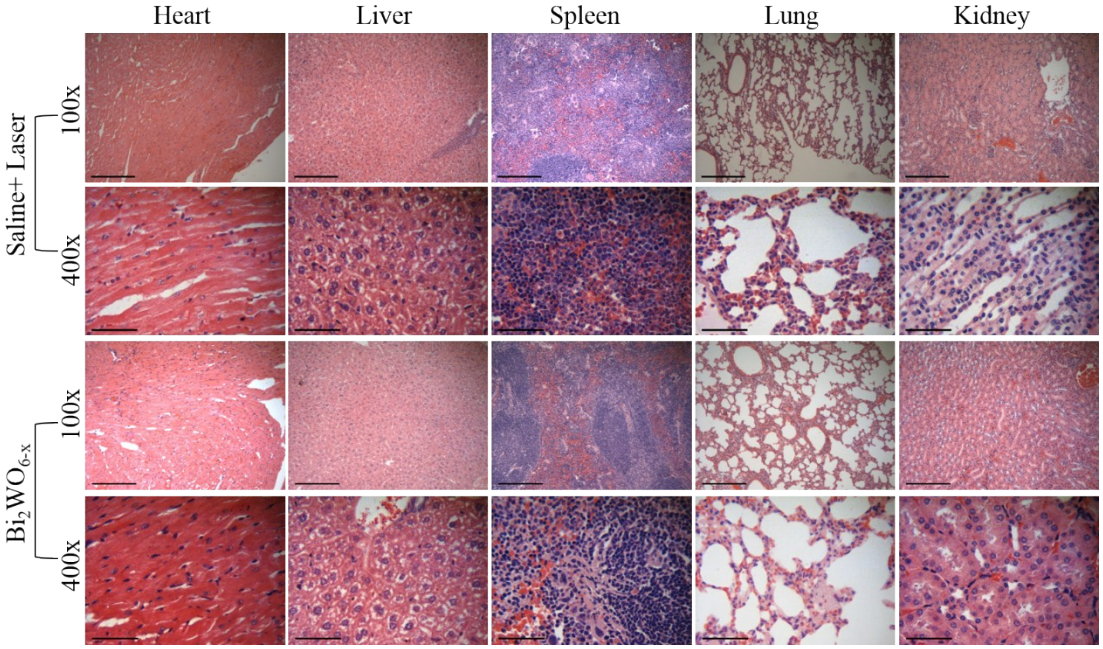

Fig.S6 Histological photographs of the major organ sections for saline injection + Laser group and Bi_2WO_{6-x} injection group, after H&E staining treatment for 24h under a microscope at 100x and 400xmagnification. The scale bars are 200 μm and 50μm, respectively.