Supporting information for

Synthesis of Bi$_2$WO$_{6-x}$ 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$_3$)$_3$·5H$_2$O), sodium tungstate dehydrate (Na$_2$WO$_4$·2H$_2$O), 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$_3$·H$_2$O) 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$_2$WO$_{6-x}$ and faint-yellow Bi$_2$WO$_6$ samples were analyzed by using high-
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$_2$WO$_{6-x}$ 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$_2$WO$_{6-x}$. The viability of 4T1 cell was used to evaluate the cytotoxicity of Bi$_2$WO$_{6-x}$-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$_2$WO$_{6-x}$-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$_2$WO$_6$-$\cdot$CA$_{1.0}$ dispersion. The 1640 cells were divided into four groups: (a) Saline; (b) Saline + laser; (c) Bi$_2$WO$_{6-x}$-CA$_{1.0}$; (d) Bi$_2$WO$_{6-x}$-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 ($^1$O$_2$).

**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$_2$WO$_{6-x}$ 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$_2$WO$_{6-x}$-CA$_{1.0}$; (d) Bi$_2$WO$_{6-x}$-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_{2}WO_{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_{2}WO_{6-x} nanodots (0.17 g L^{-1}) and Iopromide solutions. CT imaging of mice were measured before and after the injection of Bi_{2}WO_{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_{2}WO_{6-x}-CA_{1.0} injection, and (IV) Bi_{2}WO_{6-x}-CA_{1.0} injection + Laser. Then mice of each group were intratumorally injected with saline (100 µL) and saline solution containing Bi_{2}WO_{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 observed by a digital microscope.

Figures

**Fig.S1** EDS pattern of Bi$_2$WO$_{6-x}$CA$_{1.0}$ sample.
Fig. S2 Mapping images of Bi$_2$WO$_{6-x}$-CA$_{1.0}$ sample.

![W 4f spectrum of both Bi$_2$WO$_{6-x}$ and Bi$_2$WO$_6$ nanodot samples.](image)

Fig. S3 W4f spectrum of both Bi$_2$WO$_{6-x}$ and Bi$_2$WO$_6$ nanodot samples.

Fig. S4 Photos of Bi$_2$WO$_{6-x}$-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$_2$WO$_6$-x injection group, after H&E staining treatment for 24h under a microscope at 100x and 400x magnification. The scale bars are 200 µm and 50µm, respectively.