Supporting Information

Enzymatically synthesised MnO₂ nanoparticles for highly efficient near-infrared photothermal therapy and dual-responsive magnetic resonance imaging

Protein expression and purification

The harvested cells were suspended in equilibration buffer (100 mM Tris-HCl, pH = 8.0; 1.5 M NaCl) supplemented with 10 mM CaCl₂, 1 mM CuSO₄, and EDTA-free protease inhibitor (Sigma) and lysed using JY92-IID (Ningbo Dongnan Instrument Co., Ltd., China) for 1 min/ml cell lysate at 60% amplitude with 10-s on/off pulses on ice. The crude extract was clarified by 20 min of incubation at 70 °C. The debris was pelleted by centrifugation at 15,000 × g at 4 °C for 30 min, and the supernatant was filtered through a 0.4-μm-pore PVDF filter. The clarified lysate was then added to a 5-ml column volume (CV) of gravity flow Strep-Tactin@XT superflo® (IBA, Germany). The column was washed 5 × with 1 CV of buffer W (100 mM Tris/HCl, pH = 8.0; 150 mM NaCl; 1 mM EDTA), the wash fractions were collected, and 10 µl of the wash fraction was saved for analytical SDS-PAGE. Then, 6 × 0.5 CV of buffer BXT (100 mM Tris/HCl, pH = 8.0; 150 mM NaCl; 1 mM EDTA; 50 mM biotin) was added to obtain a high protein concentration in one fraction, followed by the addition of 0.6 CV as elution fraction 1 (E1), then 1.6 CV (E2) and finally 0.8 CV (E3). The main protein content should be in E2. A 10-µl sample of E2 can be used for SDS-PAGE analysis. All buffers up to this point were supplemented with 50 µM CuSO₄ to
avoid copper leaching by Tris. This as-isolated MnxEFG was then flash frozen in liquid nitrogen and stored at $-80\, ^\circ\text{C}$ until use. The protein was quantified by the Thermo Scientific Pierce bicinchoninic acid (BCA) protein assay.

**Content of protein in Bio-MnO$_2$ NPs**

The concentration of protein in Bio-MnO$_2$ NPs was determined as follows: 100 $\mu$L of Bio-MnO$_2$ NPs (final concentration was 150 $\mu$g mL$^{-1}$) was reacted with 10 mM GSH for 24 h. The protein in the product after Bio-MnO$_2$ NP decomposition was quantified by the Thermo Scientific Pierce bicinchoninic acid (BCA) protein assay, which was determined to be 7.5 $\mu$g mL$^{-1}$, and thus, the content of protein in Bio-MnO$_2$ NPs was 5%.

**Calculation of the photothermal conversion efficiency**

According to a previous report[1], the photothermal conversion efficiency ($\eta$) was calculated via equation 1:

$$\eta = \frac{hS(T_{\text{max}} - T_{\text{surr}}) - Q_s}{I(1 - 10^{-d})}$$  \hspace{1cm} (Eq 1)

where $h$ is the heat transfer coefficient, $S$ represents the surface area of the cell, $T_{\text{max}}$ is the maximum steady-state temperature, $T_{\text{surr}}$ is the ambient temperature of the surroundings, $Q_s$ is the heat dissipated from light absorbed by the cell itself, which is measured independently to be 0.27 mW, $I$ is the laser power (the area of the well was 3.14*(0.5)$^2$=0.785 cm$^2$, the power density was 1.5 W/cm$^2$, and the laser power was 0.785*1.5*1000=1177.5 mW), and $A$ is the absorbance of Bio-MnO$_2$ NPs at an
excitation wavelength of 808 nm, which was calculated to be 0.303 (Figure 3d).

We define $\theta$ as the following:

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}}$$  \hspace{1cm} (Eq 2)

where $(T - T_{surr})$ represents the temperature increase compared to the surroundings, and $(T_{max} - T_{surr})$ represents the temperature change at the maximum steady-state temperature.

$$\tau_s = -\frac{t}{\ln \theta}$$ \hspace{1cm} (Eq 3)

$\tau_s$ is the sample system time constant, which can be determined to be 157 s by the linear curve fitting of the temperature cooling time according to Figure 3f.

$$hS = \frac{m_s C_s}{\tau_s}$$ \hspace{1cm} (Eq 4)

where $m_s$ (0.2 g) and $C_s$ (4.2 J g$^{-1}$) are the mass and heat capacity of the solvent, respectively. Putting this value into equation (4), the final photothermal conversion efficiency ($\eta$) of Bio-MnO$_2$ NPs can be calculated to be 44%.

The photothermal conversion efficiency ($\eta$) of Chem-MnO$_2$ was calculated according to the same method. The mean absorbance at 808 nm and $\tau_s$ of Chem-MnO$_2$ was determined to be 0.3724 and 257 s, respectively. The final photothermal conversion efficiency ($\eta$) of Chem-MnO$_2$ was calculated to be 27.9%.

References


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Figure S1 (a) PCR amplification of *mnxEFG* gene fragments with homologous sequences from the vector pASG-IBA103 and linearization of pASG-IBA103 by PCR. (b) PCR amplification of the recombinant plasmid and the acceptor plasmid pASG-IBA103 (control) using sequencing primers. (c) BLAST results between the insert fragment and the *mnxEFG* gene. (d) SDS-PAGE results of purified protein.
Figure S2 TEM images of Bio-MnO$_2$ NPs formed by the biomineralization of Mnx at 30 °C for different time periods.

Figure S3 XRD profile of Chem-MnO$_2$.
Figure S4 Photos of Bio-MnO$_2$ NPs dispersed in deionized water, PBS and DMEM for 9 days and Chem-MnO$_2$ dispersed in deionized water for 1 h.

Figure S5 Hydrodynamic diameter of Bio-MnO$_2$ NPs as a function of incubation time.
Figure S6 EDS maps of Bio-MnO$_2$ NPs (30 min).

Figure S7 (a) Photothermal profile of Chem-MnO$_2$ (150 µg mL$^{-1}$) upon exposure to an 808-nm laser at 1.5 W cm$^{-2}$ for 10 min, after which the laser was shut off. (b) UV-vis-NIR spectra of Chem-MnO$_2$. (c) Time constant for heat transfer from the system ($\tau_s =257$ s) obtained by applying the linear time data from the cooling stage (1200 s)
versus the negative natural logarithm of the driving force temperature.

**Figure S8** Percentage of Mn$^{2+}$ released from Bio-MnO$_2$ NPs (150 μg mL$^{-1}$) as a function of incubation duration.
Figure S9 Relative viability of 4T1 cells after incubation with Chem-MnO$_2$ NPs for 24 h at different concentrations.
Figure S10 Photographs of representative mice after different treatments (16 days after the first treatment), excised 4T1 tumours from tumour-bearing mice and the results of TUNEL and antigen Ki-67 immunofluorescence staining of tumour tissues.