Supporting Information

An Activatable Two-Photon Fluorescence Nanoprobe for Bioimaging of Glutathione in Living Cells and Tissues

Hong-Min Meng[†], Zhen Jin[‡], Yifan Lv[†], Chan Yang[†], Xiao-Bing Zhang*[†], Weihong Tan[†] and Ru-Qin Yu[†]

†Molecular Sciences and Biomedicine Laboratory, State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Collaborative Innovation Center for Chemistry and Molecular Medicine, Hunan University, Changsha 410082, China

‡College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China

* To whom correspondence should be addressed.

xbzhang@hnu.edu.cn

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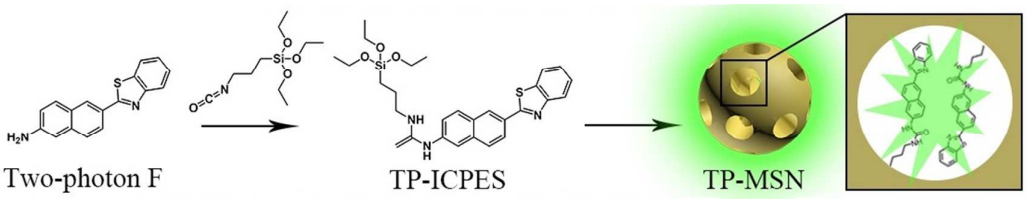

Scheme S1. Synthesis of TP-ICPES and TP-MSN.

Scheme S1 showed that two-photon MSNs were first synthesized by a base-catalyzed sol-gel procedure using a two-photon fluorophore-conjugated organic silane monomer.

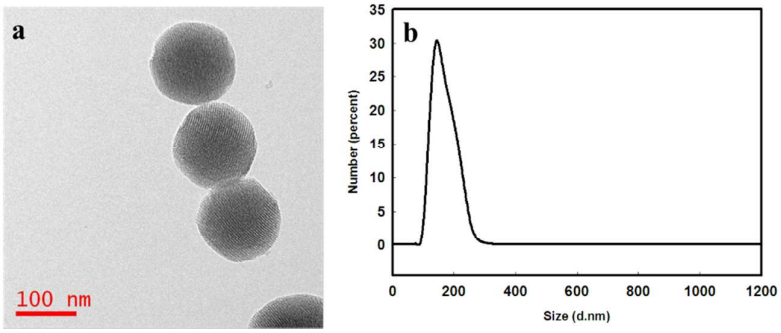

Figure S1. (a) TEM image of two-photon MSN; (b) DLS data of two-photon MSN.

As seen from Figure S1, the two-photon MSN has good monodispersity and that the size (diameter) was measured at about 140 nm.

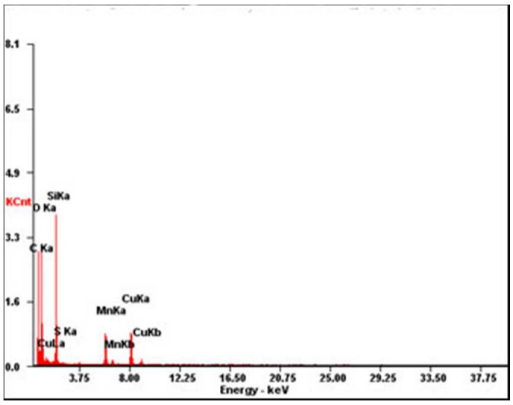

Figure S2. Energy dispersive spectroscopy analysis of TP-MSNs@MnO_2.

As seen from Figure S2, the presence of amino groups on the surface of the two-photon MSNs, negatively charged MnO_2 nanosheets can be easily adsorbed onto positively charged MSNs through electrostatic interaction.

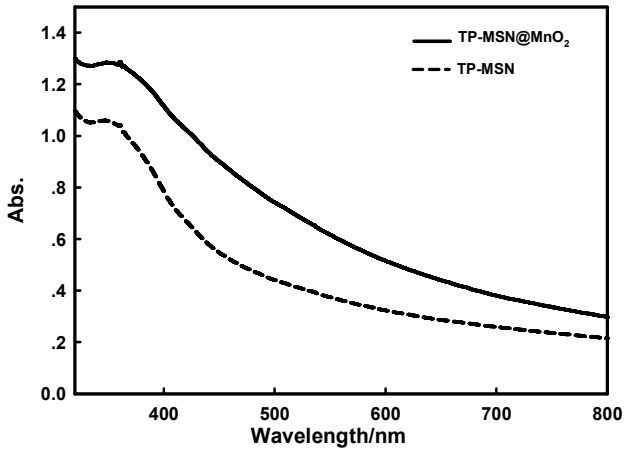

Figure S3. The UV-vis absorption spectrum of TP-MSN and TP-MSN@MnO_2.

As seen from Figure S3, the UV-vis absorption spectra of TP-MSN@MnO_2 nanocomposite indicate the formation of the MnO_2 adsorbed onto the TP-MSNs with corresponding peaks at 380 nm.

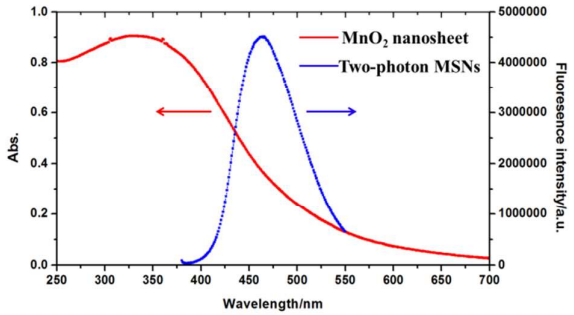

Figure S4. Spectral overlap showing the fluorescence emission spectrum of the two-photon MSNs (blue) and the UV-vis absorption spectrum of MnO_2 nanosheet (red).

As shown in Figure S4, the absorbance spectrum of MnO_2 nanosheet overlaps well with the fluorescence emission of the TP-MSNs, thereby leading to ET from the TP-MSNs to the MnO_2.

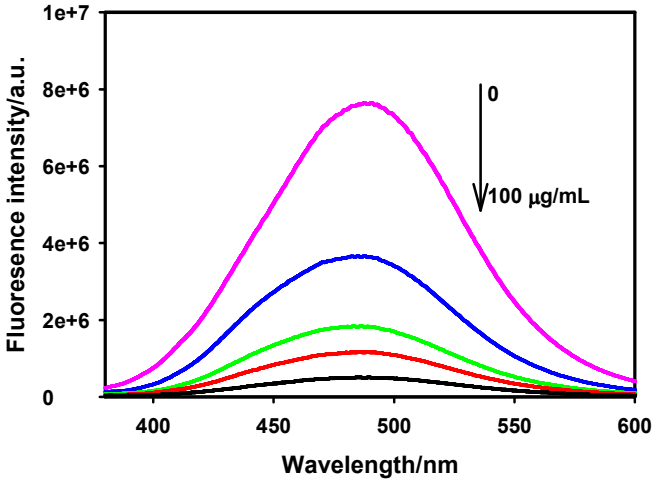

Figure S5. Fluorescence quenching of TP-MSN (100 μg mL^{-1}) by varying amounts of MnO_2 nanosheets.

It is clear from Figure S5 that the fluorescence quenching degree was dependent on the concentration of MnO_2 nanaosheet and a maximum quenching degree up to 98.1% was achieved with 100 μg/mL quencher.

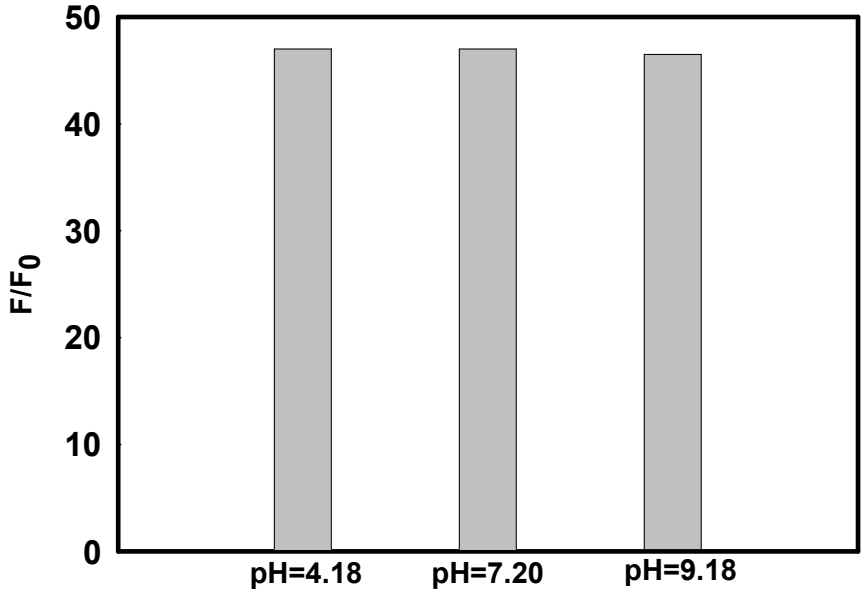

Figure S6. Effect of pH on the one-photon excited fluorescence intensity of TP-MSN@MnO_2 nanocomposite in the presence of GSH (1 mM).

This result indicates that these is no effect of pH on the fluorescent response of the probe.

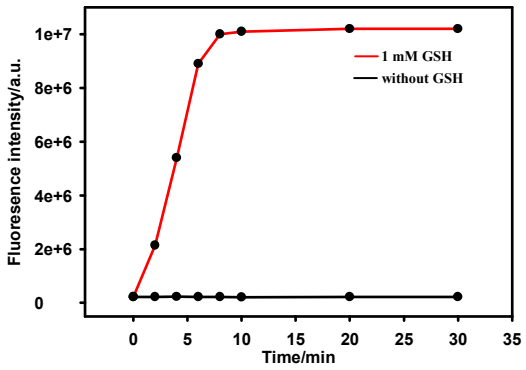

Figure S7. Fluorescence response of TP-MSN@MnO_2 nanocomposite in the absence of GSH (black) and in the presence of GSH (1 mM) (red),as a function of time.

As seen from Figure S7, the fluorescence intensity of TP-MSNs gradually increased with the elongation of time and reached equilibrium after a few minutes, revealing a rapid decomposition of the nanoscale MnO_2 by GSH at room temperature. Because of the GSH-mediated reduction of MnO_2, the FRET process is inhibited.

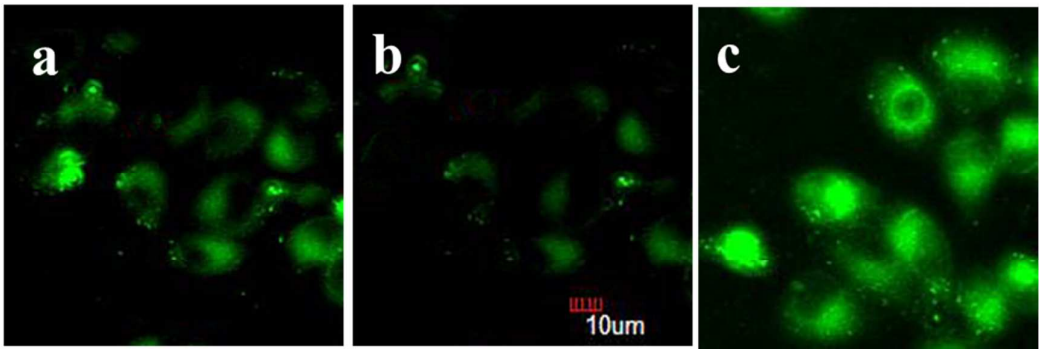

Figure S8. TP confocal microscopy images of GSH detection in live Hela cells. (a) TP image of cells incubated with the TP-MSN@MnO_2 nanocomposite; (b)TP image of Hela cells pretreated with LPA for 24 h and then NMM (500 μM) for 30 min, followed by incubation with the TP-MSN@MnO_2 nanocomposite; (c) TP image of Hela cells pretreated with LPA (500 μM) for 24 h, followed by incubation with the TP-MSN@MnO_2 nanocomposite.

This result indicates that this TP-MSN@MnO_2 nanocomposite can be rapidly delivered into the cytoplasm and a high level of GSH is expressed in HeLa cells.

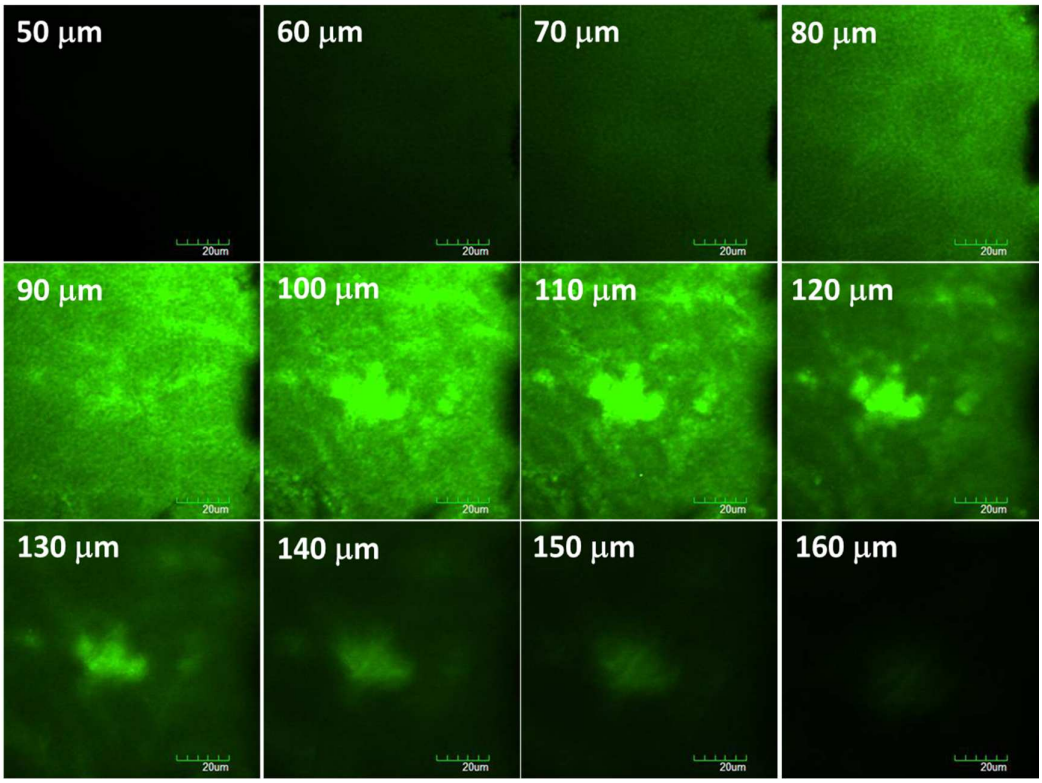

Figure S9. Depth fluorescence images of TP-MSNs in tissues were obtained with spectral confocal multiphoton microscopy (Olympus, FV1000) with a high-performance mode-locked titanium-sapphire laser source (MaiTai, Spectra-Physics, USA). Next, the changes of fluorescence intensity with scan depth were determined by spectral confocal multiphoton microscopy (Olympus, FV1000) in the z-scan mode (from 0 to 400 μm; step size: 1 μm). The images were collected at 450-530 nm (green channel). Scale bars: 30 μm.

The results clearly demonstrate that TP-MSNs@MnO_2 has increased light penetration depth in tissue.