Supporting Information

A Two-Photon Ratiometric Fluorescence Probe for Cupric Ions in Live Cells and Tissues

Anwei Zhu, Changqin Ding, Yang Tian*

Department of Chemistry, Tongji University, Siping Road 1239, Shanghai 200092, China.

Materials and General Instrument. Citric acid, superoxide dismutase from bovine liver (SOD), Nile Blue chloride, ascorbic acid (AA), diethyldithiocarbamate (DDC), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), propidium iodide (PI), cytochrome c (Cyt. *c*), potassium phosphate dibasic trihydrate, ethylenediaminetetraacetic acid (EDTA), methyl thiazolyl tetrazolium (MTT) and dimethylsulphoxide (DMSO) were obtained from Sigma. Cell culture reagents were purchased from Gibco. Annexin V-FITC Apoptosis Assay Detection Kit was purchased from KeyGEN Biotech. Triton X-100 and sodium hydroxide were obtained from Aladdin regent company. Other chemicals were analytically pure and used as received. Solutions of metal ions were all prepared from their chloride salts. Ultrapure water was used from a Millipore water purification system. Transmission electron microscopy (TEM) images were obtained with a JEOL JEM-2100 microscope. X-ray photoelectron spectroscopy (XPS) (model PHI 5000 ESCA, Perkin Elmer, USA) equipped with Al Kα source (1486.6 eV photons) was used to track the assembly process of the probe. All binding energies (BEs) were referred to as the C 1s peak (284.6 eV) arising from surface hydrocarbons (or adventitious hydrocarbon). Atomic force microscope (AFM) images were recorded by tapping mode on the Multimode 8 AFM from Bruker. Fourier transform infrared spectroscopy (FT-IR) was recorded on a Nicolet model 6700 Fourier transform infrared spectrometer. Ultracentrifugal experiments were performed on Beckman Allegra 64R ultracentrifuge.

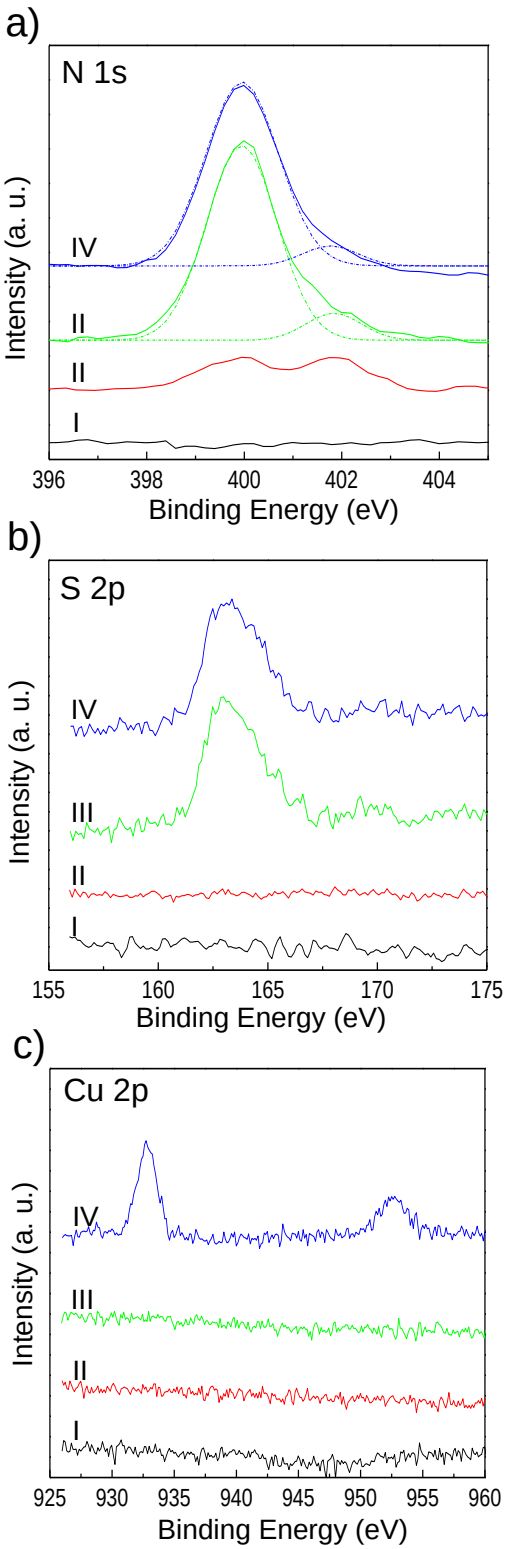

Figure S1 XPS spectra for (a) N 1s, (b) S 2p, (c) Cu 2p obtained at (I) GQDs, (II) GQD@Nile, (III) GQD@Nile-E_2Zn_2SOD, and (IV) the reconstituted GQD@Nile-E_2Zn_2SOD with Cu^{2+}.

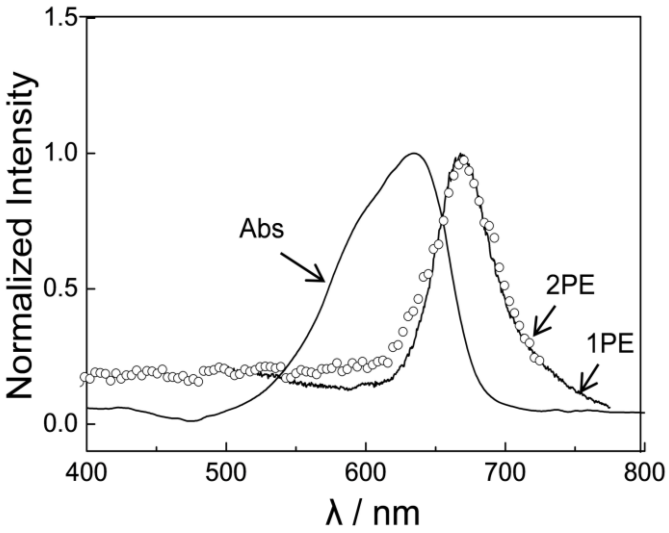

Figure S2 UV-vis absorption spectrum, one-photon (400 nm excitation) and two-photon (800 nm excitation) fluorescence spectra of the Nile Blue solution.

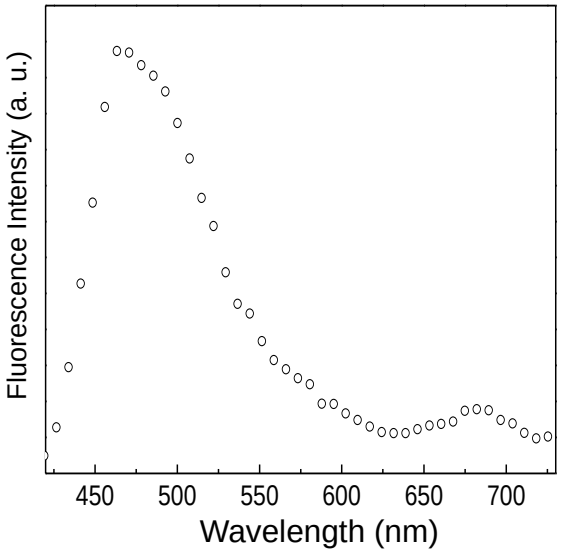

Figure S3 Two-photon (800 nm excitation) fluorescence spectrum of GQD@Nile in 20 mM HEPES at pH 7.4.

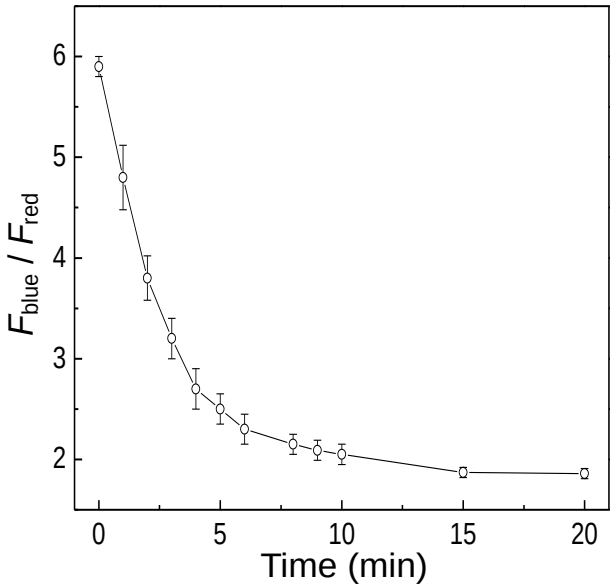

Figure S4 Time dependence of the fluorescence ratio changes for GQD@Nile-E_2Zn_2SOD (0.1 mg mL^{-1} in 20 mM HEPES buffer, pH 7.4) after the addition of 3 μM Cu^{2+}.

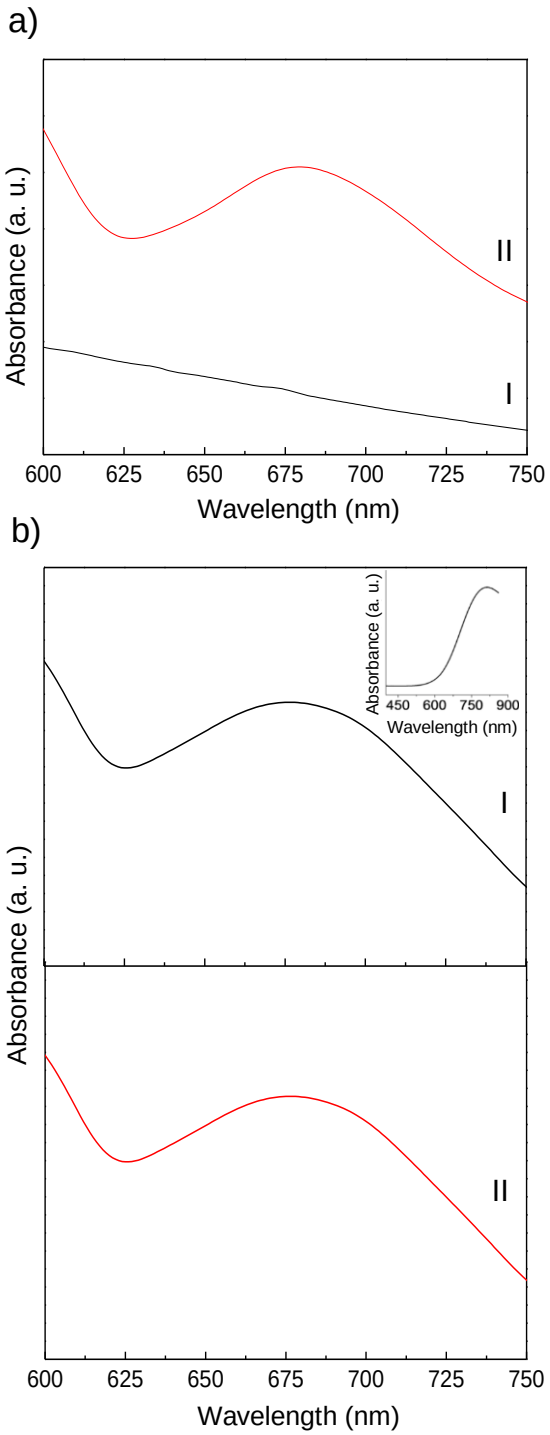

Figure S5 (a) UV-vis absorption spectra of (I) E_2Zn_2SOD and (II) the reconstituted SOD through the interaction of E_2Zn_2SOD with a stoichiometric amount of Cu^{2+}. As the extinction coefficient of the E_2Zn_2SOD-Cu^{2+} is extremely low (<2000 M^{-1} cm^{-1}) and the molar ratio of Nile Blue and E_2Zn_2SOD-Cu^{2+} is ~5, very limited number of Nile Blue molecule would decay by the resonance energy transfer and most of the Nile Blue molecules decay by the usual radiative and non-radiative rates. (b) UV-vis absorption spectra of (I) the native SOD aqueous solution (10 mg mL^{-1}) and (II) its mixture with Cu^{2+} (1 mM). Inset: UV-vis absorption spectrum of the $CuCl_2$ aqueous solution (1 M).

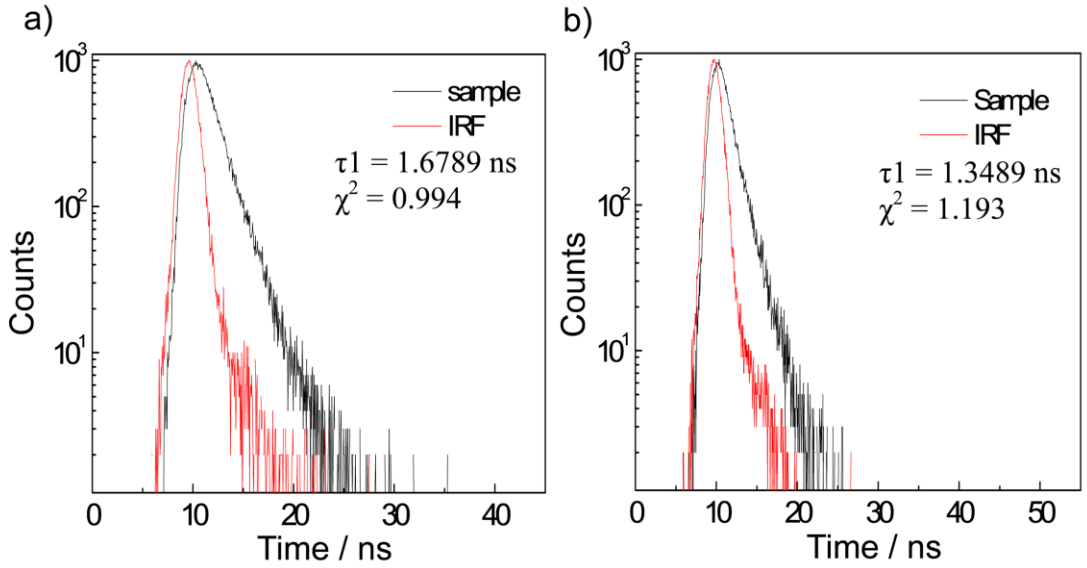

Figure S6 Time-resolved fluorescence of 0.1 mg mL^{-1} solution of nanohybrids in the (a) absence and (b) presence of a stoichiometric amount of Cu^{2+}. λ_{ex} = 400 nm, λ_{em} = 465 nm.

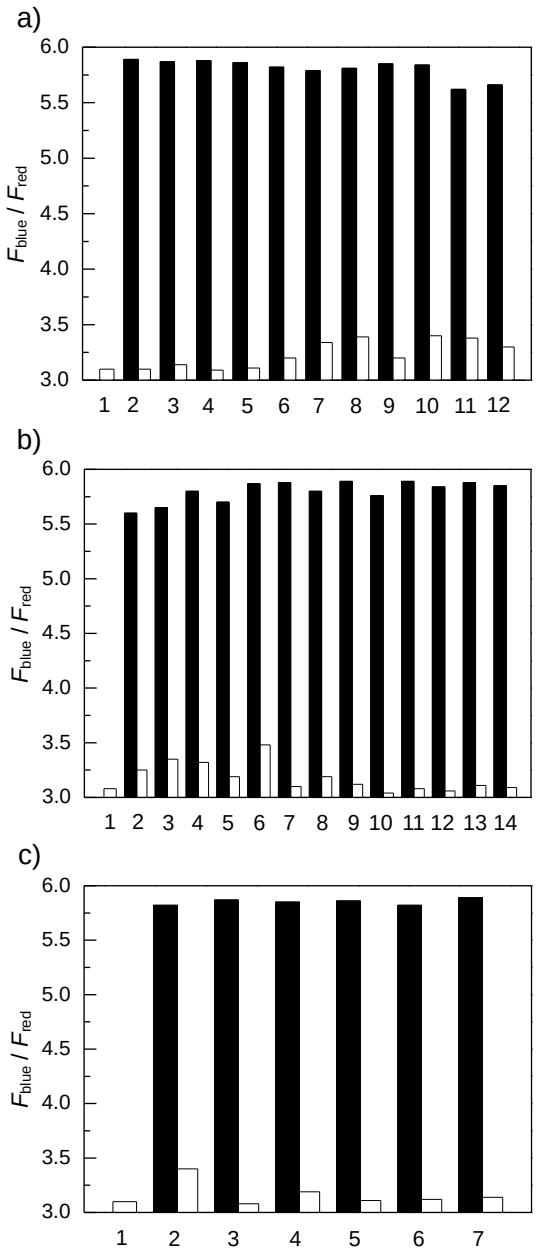

Figure S7 a) Fluorescence responses of GQD@Nile-E_2Zn_2SOD toward various metal ions. From 1 to 12 are: Cu^{2+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Cu^{+}. Black bars represent the addition of an excess of metal ions (1 mM for Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}; 10 μM for other cations) to a 0.1 mg mL^{-1} solution of GQD@Nile-E_2Zn_2SOD (pH=7.4). White bars represent the subsequent addition of 2 μM Cu^{2+} to the solution. b) Fluorescence responses of GQD@Nile-E_2Zn_2SOD toward various amino acids. From 1 to 14 are: Cu^{2+}, Arg, Cys, Gly, Glu, His, Ile, Lys, Leu, Met, Phe, Ser, Thr and Val. Black bars represent the addition of 10 μM amino acid to a 0.1 mg mL^{-1} solution of GQD@Nile-E_2Zn_2SOD (pH=7.4). White bars represent the subsequent addition of 2 μM Cu^{2+} to the solution. c) Fluorescence responses of GQD@Nile-E_2Zn_2SOD towards other biological species. From 1 to 7 are: Cu^{2+}, GSH, H_2O_2, Glucose, cytochrome *c*, myoglobin, hemoglobin. Black bars represent the addition of biological species (1 mM for GSH, Glucose, 0.01 μM for H_2O_2, 1 μM for cytochrome *c*, myoglobin and hemoglobin) to a 0.1 mg mL^{-1} solution of GQD@Nile-E_2Zn_2SOD (pH=7.4). White bars represent the subsequent addition of 2 μM Cu^{2+} to the solution. Excitation was provided at 800 nm.

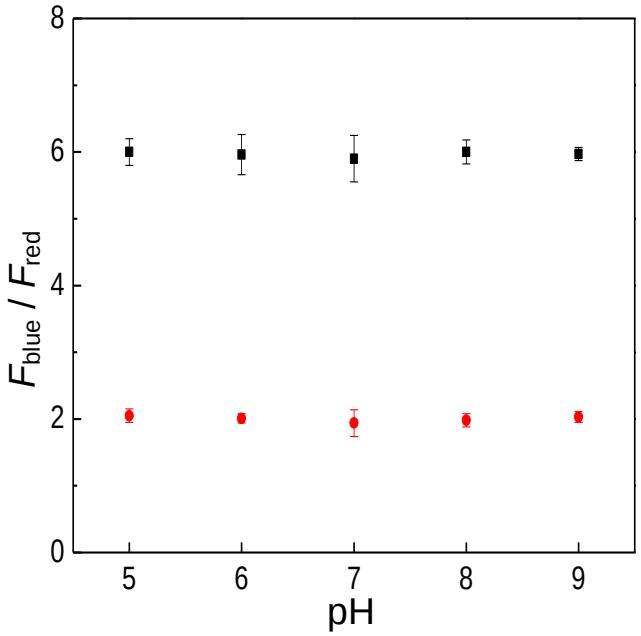

Figure S8 Effect of pH on the two-photon fluorescence ratio of GQD@Nile-E_2Zn_2SOD in the absence of Cu^{2+} (black squares) and in the presence of 3 μM Cu^{2+}. (red circles).

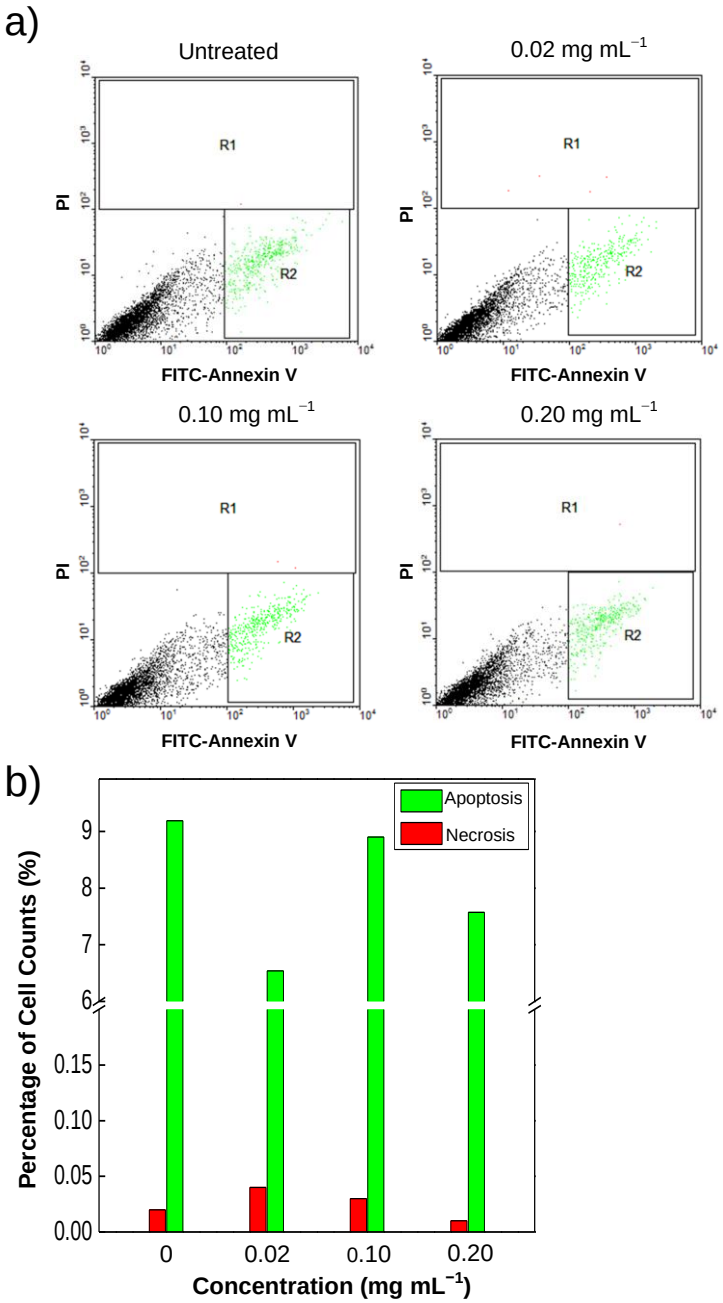

Figure S9 Apoptosis assay of A549 cells after GQD@Nile-E_2Zn_2SOD treatment. A549 cells were incubated with GQD@Nile-E_2Zn_2SOD at 0.02, 0.10, and 0.20 mg mL^{-1} for 24 h. Cells were stained by FITC-annexin V and Propidium iodide (PI) to label the apoptotic cells and necrotic cells, respectively, for flow cytometry (FACS) measurement. (a) FACS data of A549 cells after different amount of GQD@Nile-E_2Zn_2SOD treatment. R1 (red) and R2 (green) represent the regions of dead cells and apoptotic cells, respectively. (b) Percentage of apoptotic cells and dead cells after different GQD@Nile-E_2Zn_2SOD treatment.

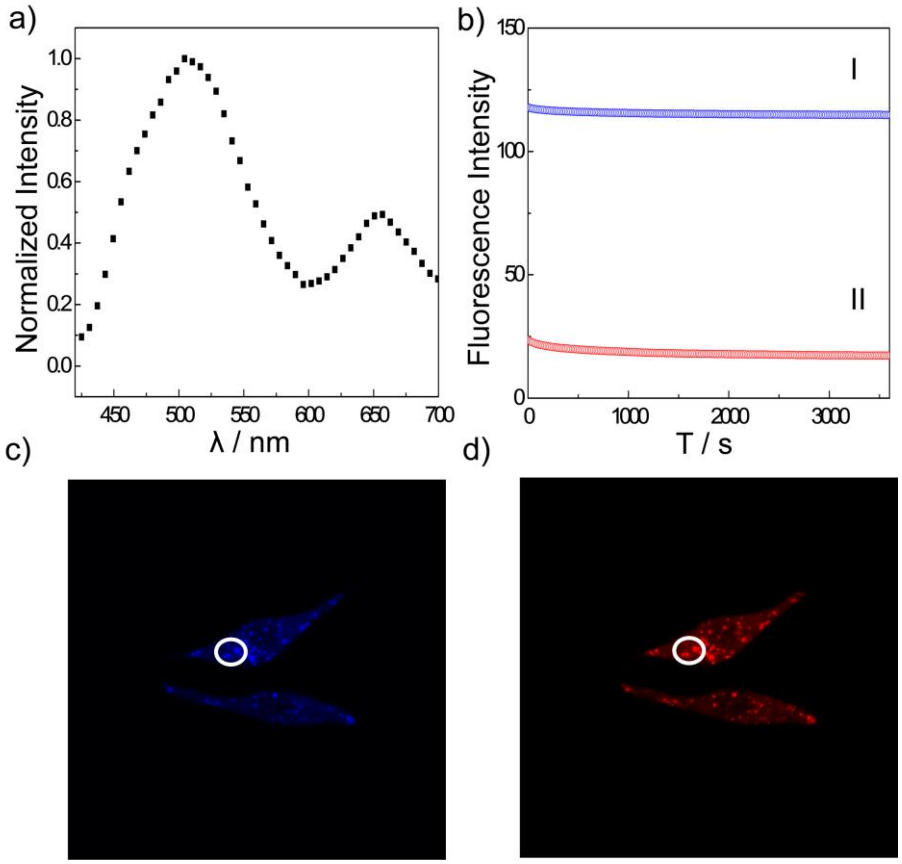

Figure S10 (a) Normalized two-photon fluorescence emission scan from GQD@Nile-E_2Zn_2SOD in A549 cells. (b) The relative two-photon fluorescence intensity from (I) the blue channel (Panel c, circled region) and (II) red channel (Panel d, circled region) as a function of time. The two-photon fluorescence response was collected at 450-600 nm for blue channel and at 620-700 nm for red channel upon fs-800 nm excitation. The digitized intensity was recorded with 8.14 sec intervals for the duration of one hour using xyt mode. (c, d) TPM images of GQD@Nile-E_2Zn_2SOD -labeled A549 cells collected at (c) blue channel (450-600 nm) and (d) red channel (620-700 nm).

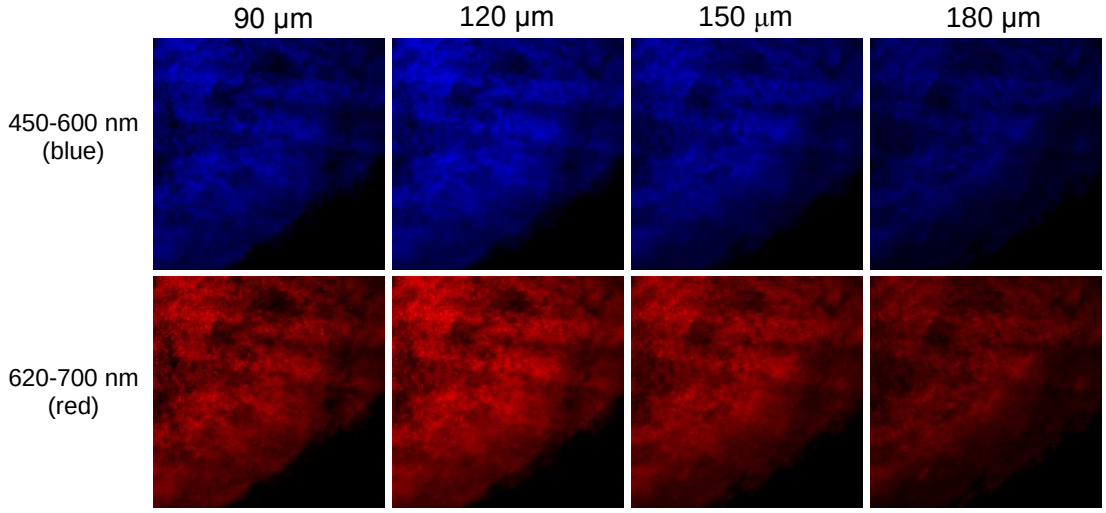

Figure S11 TPM images of lung cancer tissue slice labeling with GQD@Nile-E_2Zn_2SOD at the depth of approximately 90-180 μm with magnification at 10×. The TPM images were collected at two channels (blue = 450-600 nm, red = 620-700 nm) upon excitation at 800 nm with fs pulse.

Table S1 Cell viability values (%) versus the incubation concentration of GQD@Nile-E_2Zn_2SOD at 310 K for 24 and 48 h. The MTT proliferation test was repeated three times for the cell viability values.

concentration (mg mL^{-1})	24 h	48 h
0	100	100
0.02	98.4 ± 1.9	98.3 ± 3.9
0.04	97.6 ± 3.0	96.2 ± 3.1
0.06	96.8 ± 2.5	94.9 ± 1.8
0.08	95.8 ± 1.4	93.5 ± 2.5
0.1	94.0 ± 2.1	91.4 ± 4.9
0.2	94.1 ± 2.6	90.2 ± 2.3
0.5	92.5 ± 3.0	88.9 ± 1.4
1.0	90.3 ± 1.7	87.1 ± 2.8