Electronic Supplementary Material (ESI) for Analyst.
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Electronic Supplementary Material for

Tracing Molecular Dynamics of living mitochondria under Phototherapy *via* Surface-Enhanced Raman Scattering Spectroscopy

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1. Materials and reagents

Tetrachloroauric acid trihydrate ($HAuCl4•3H_2O$), cetyltrimethylammonium bromide (CTAB) and indocyanine green (ICG) were purchased from Aladdin Industrial Corporation. Ascorbic acid was obtained from Beijing Chemical Company. $AgNO_3$ was purchased from Shanghai Chemical Company. $NaBH_4$ was obtained from Tianjin Fuchen Chemical Reagents Factory. 2',7'-dichloro-fluorescein diacetate (DCFH-DA) was purchased from Sigma. Dulbecco' modified Eagle's medium WST-1 was obtained from Hoffmann-La Roche LTD. Culture medium Dulbecco's Modification of Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific. Na_2HPO_4, NaH_2PO_4, NaOH and HCl were obtained from Beijing Chemical Factory. Hepes buffer solution was purchased from Gentihold (Beijing, China). Propidium Iodide (PI) and Calcein-AM were obtained from Thermo Fisher Scientific. Mitochondrial Membrane Potential Assay Kit with JC-1 was obtained from BestBio biotechnologies Co. Ltd. (Shanghai, China). Mitochondrial Isolation Kit was purchased from Invent Biotechnologies, Inc. (Beijing, China). MCF-7 (human breast cancer cell line) was bought from Shanghai ATCC cell bank who have been issued the permission of the Human Research Ethics Committee of the country for manipulations of human's cells.

2. Instruments

JEM-2100F field emission transmission electron microscope (TEM, JEOL, Tokyo, Japan) was used for characterizing the morphology of the prepared gold nanorods (AuNRs). Ultraviolet-visible (UV−vis) spectra were collected using an Ocean Optics USB4000 spectrometer. SERS spectra were obtained using a confocal Raman system (LabRAM Aramis, Horiba JobinYvon, USA) with a He-Ne (632.8 nm) laser as the excitation source. Confocal fluorescence images were collected using a FV1000 confocal fluorescence microscope (Olympus).

3. SERS spectra of living mitochondria and ICG

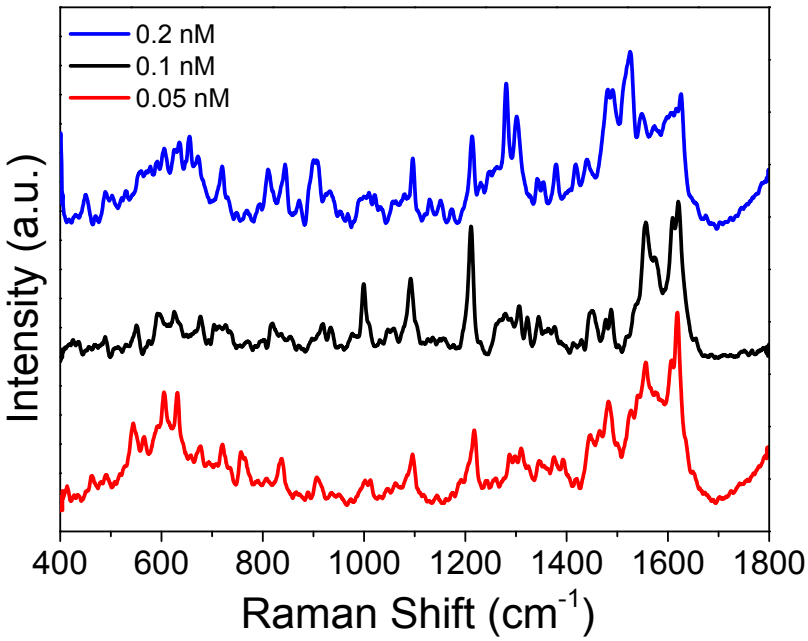

Fig. S1 SERS spectra of mitochondria mixed with different concentrations (0.05, 0.1, and 0.2 nM) of AuNRs.

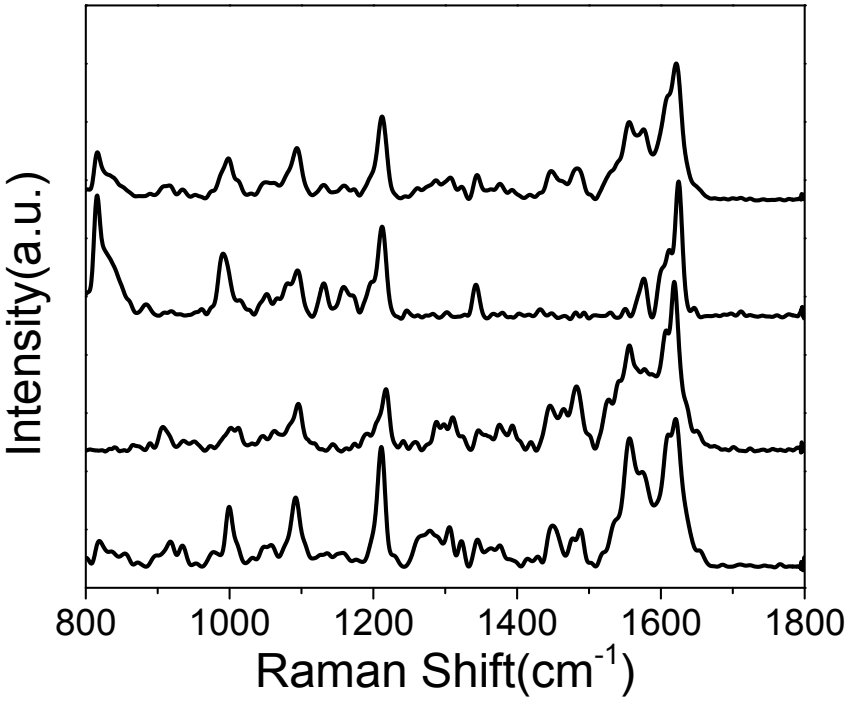

Fig. S2 The SERS spectra of four isolated MCF-7 mitochondria with the assistance of AuNRs (0.3 mL, 0.1 nM) at a volume ratio of 1:1. λ_{ex}=632.8 nm, t=10 s and accumulations=2 times.

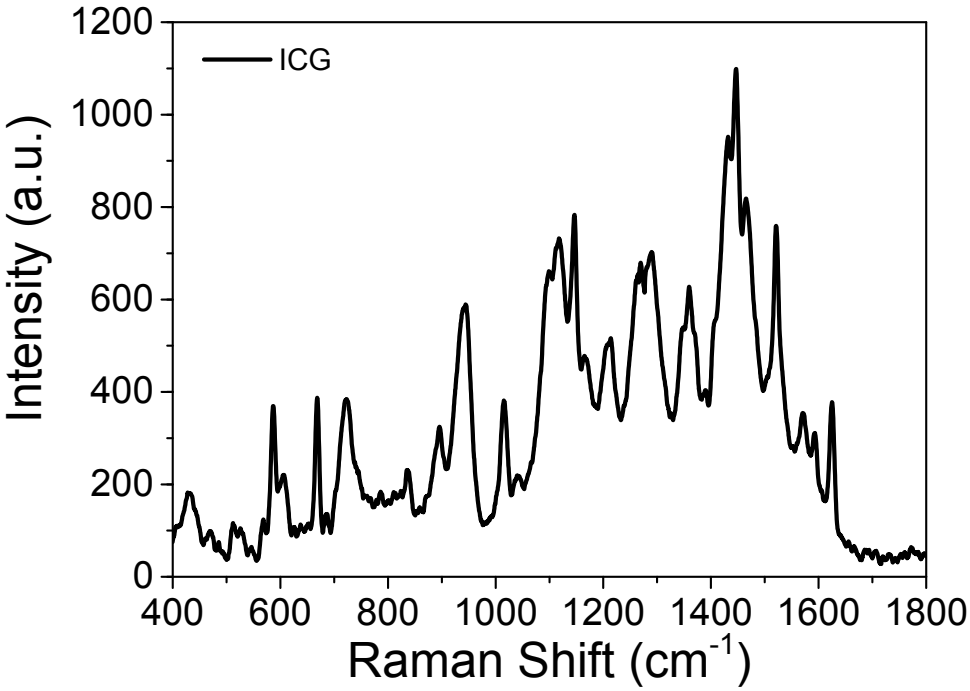

Fig. S3 The SERS spectra of 0.25 mg/mL of ICG with the assistance of AuNRs (0.3 mL, 0.1 nM) at a volume ratio of 1:1. λex=632.8 nm, t=10 s and accumulations=2 times.

4. Photothermal conversion efficiency of ICG

The aqueous solutions of ICG or their assemblies with different concentrations (0, 2.5 μg/mL, 25 μg/mL, 0.25 mg/mL, 2.5 mg/mL and 5 mg/mL in 3.5 mL quartz cell) were irradiated by an 808 nm laser (the power density is 4.0 W/cm^2) for 10 min. The temperatures of ICG contained solutions were recorded with the irradiation time by a thermocouple microprobe (ϕ = 0.5 mm) (STPC-510P, Xiamen Baidewo Technology Co., China) with an accuracy of ± 0.1 °C.

The photothermal conversion efficiency (η) of ICG was calculated according to Roper's report.[1] 1.0 mL of the ICG aqueous solution (5 mg/mL) was exposed to an 808 nm laser (4.0 W/cm^2) to increase its temperature to the maximum temperature. Then the light was shut off. The temperatures during the heating and cooling periods were recorded at an interval of 10 s.

The photothermal conversion efficiency (η) was calculated based on Equation (1).

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A_{780}})} \quad (1)$$

h (mW m^{-2} °C^{-1}) is heat transfer coefficient, S (m^2) is the surface area of the container, Tmax is the equilibrium temperature, and Tsurr is ambient temperature of the surroundings. In this experiment, Tmax−Tsurr is 40.6 °C. The Q_{Dis} (mW) expresses the heat from light absorbed by the cuvette

sample walls itself and it is measured to be 46.2 mW independently using a quartz cuvette cell containing the same volume water without ICG. I is the incident laser power (4000 mW) and A_{780} is the absorbance (12.51) of ICG at 780 nm.

To achieve hS, θ is introduced as an uncertain, as Equation (2).

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (2)$$

and a sample system time constant τ_s

$$\tau_s = \frac{\sum_i m_i C_{p,i}}{hS} \quad (3)$$

$$t = -\tau_s \ln(\theta) \quad (4)$$

τ_s is determined to be 215.783 s thus hS is deduced to be 19.5 mW/°C (substituted m = 1 g, C = 4.2 J g^{-1} K^{-1} in Equation (3). Finally, the η is calculated to be 18.64% from Equation (1).

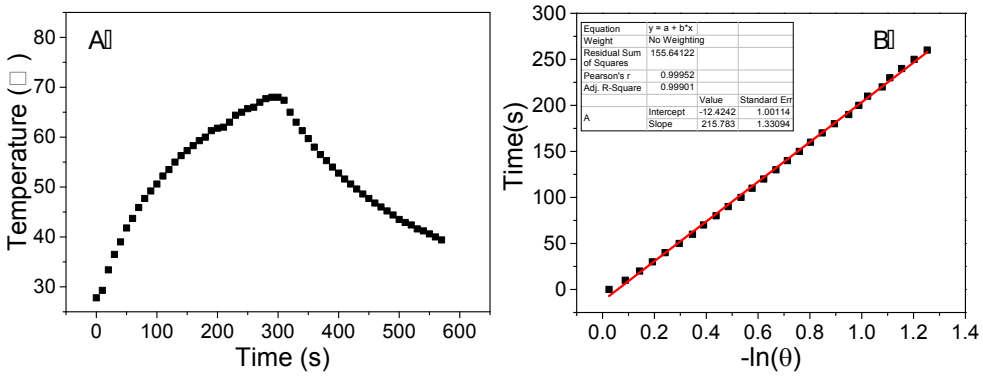

Figure S4. Temperature profiles (A) and the fitting curve (B) of 5.0 mg/mL of ICG solutions during and after they were irradiated with an 808 nm laser under a power density of 4.0 W/cm^2 for 9 min.