Electronic Supplementary Information

A selective cascade reaction-based probe for colorimetric and ratiometric fluorescence detection of benzoyl peroxide in food and living cells

Xiaoli Wu [a], Lintao Zeng [a],* Bao-Quan Chen [a], Ming Zhang[a,]*, João Rodrigues[b,c], Ruilong Sheng[b,]*, Guang-Ming Bao[d,]*

[a.] *Tianjin Key Laboratory of Organic Solar Cells and Photochemical Conversion, Tianjin University of Technology, Tianjin 300384, P. R. China. E-mail: zlt1981@126.com (L. Zeng); zm2404@tjut.edu.cn (M. Zhang).*

[b.] *Department of Chemsitry and Materials Science, Hubei Engineering University, Hubei Xiaogan 432100, P. R. China.*

[c.] *CQM-Centro de Química da Madeira, Universidade da Madeira, Campus da Penteada, 9000-390, Funchal, Madeira, Portugal. E-mail: ruilong.sheng@ staff.uma.pt (R. Sheng)*

[d.] *Institute of Veterinary Drug/Jiangxi Provincial Key Laboratory for Animal Health, Jiangxi Agricultural University, Nanchang, 330045, P.R. China. E-mail: bycb2005@gmail.com (G. M. Bao).*

Table of Contents

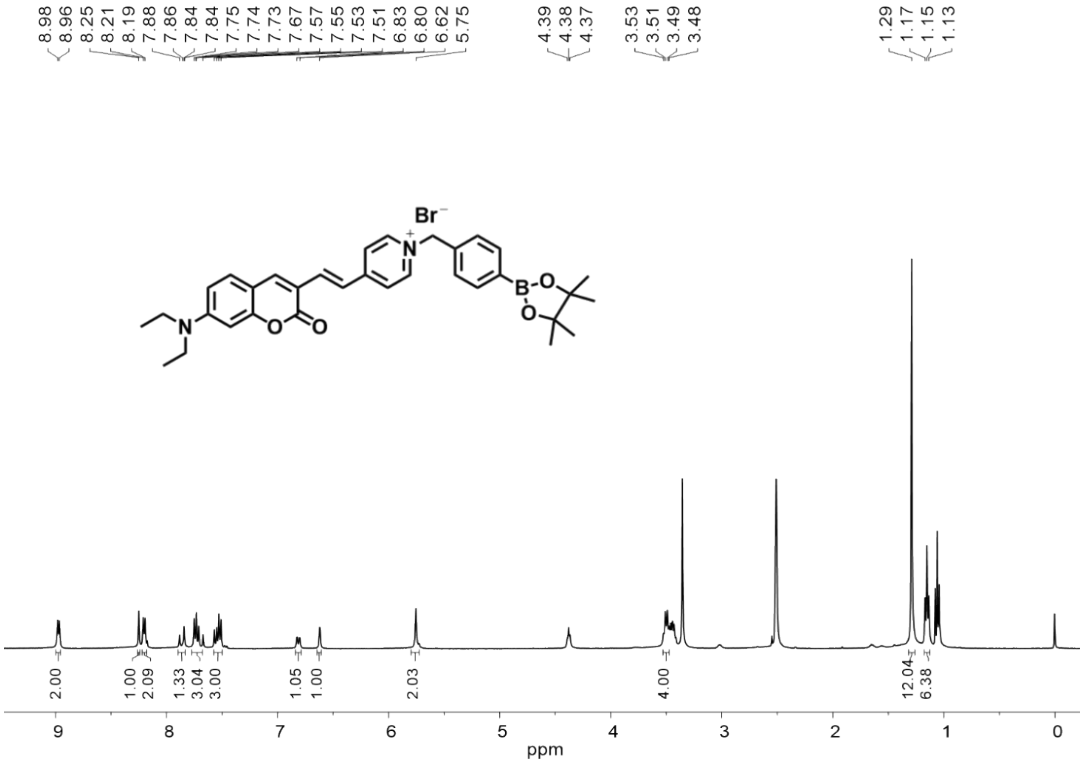

Fig. S1. [1]H NMR spectrum of probe in DMSO-d6 (400 MHZ)

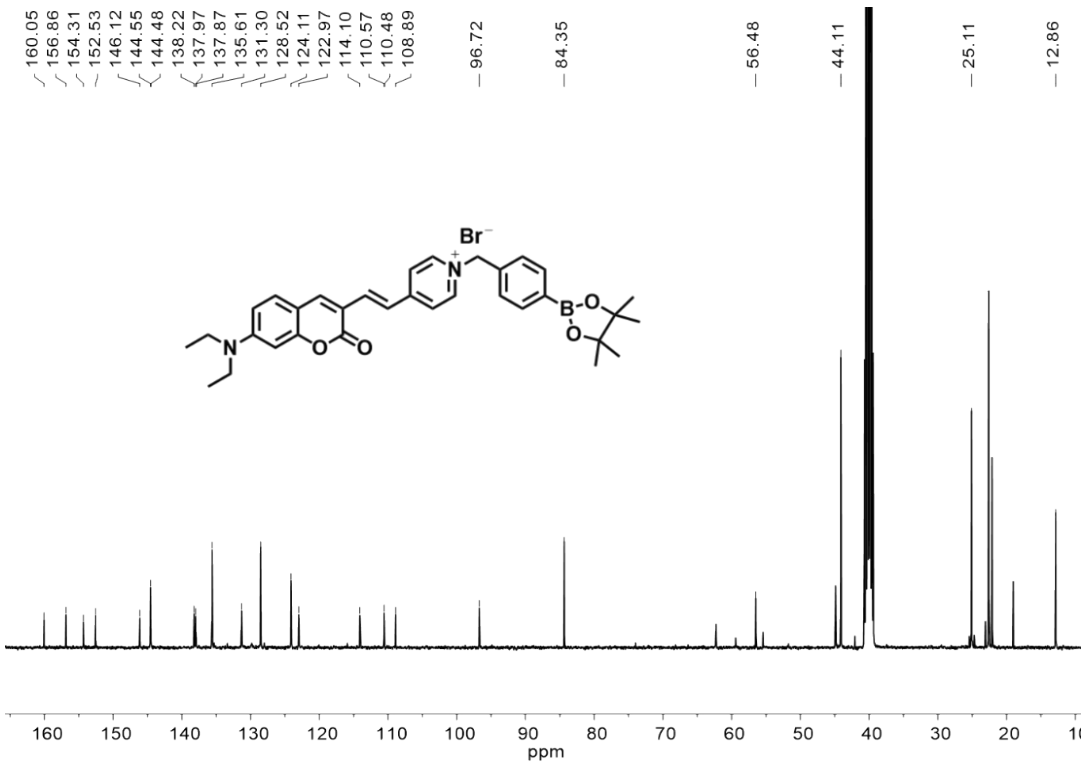

Fig. S2. [13]C NMR spectrum of probe in DMSO-d6 (100MHZ)

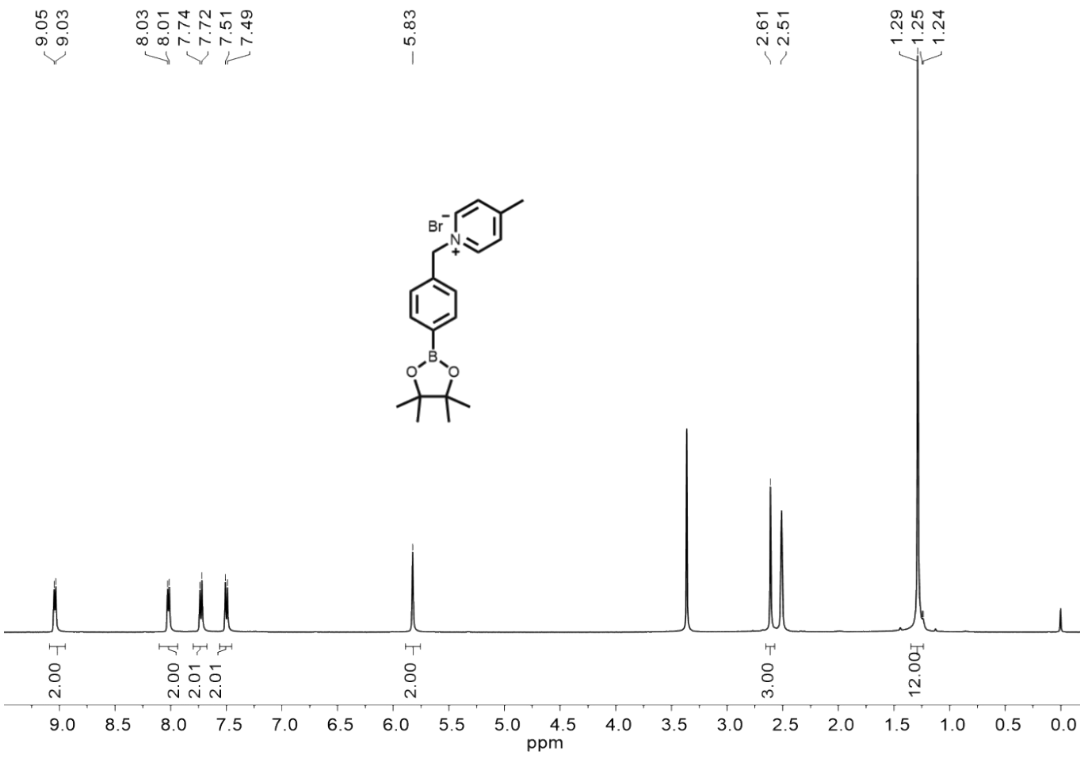

Fig. S3. [1]H NMR spectrum of compound 2 in DMSO-d6 (400 MHZ)

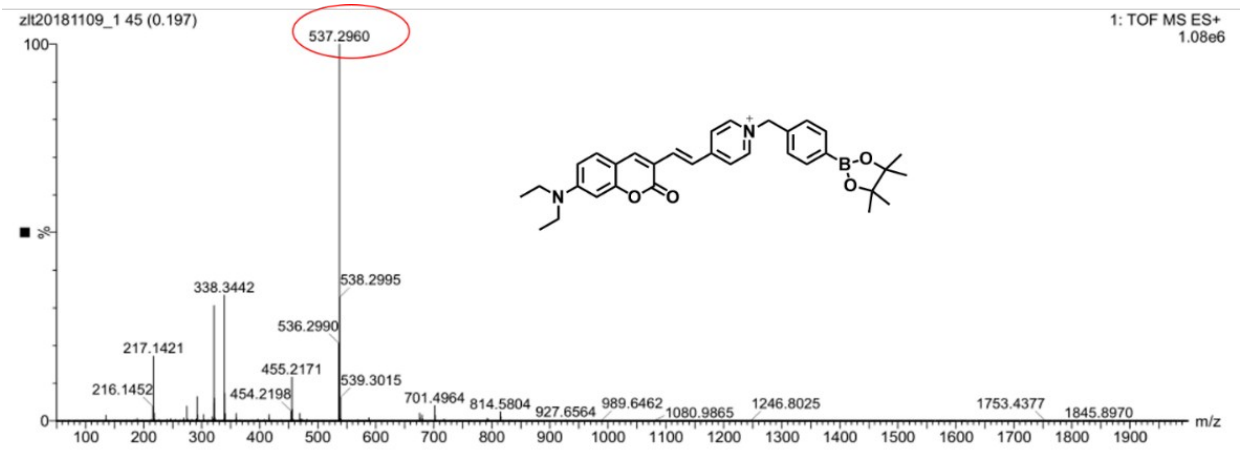

Fig. S4. HR-MS (ESI) spectrum of probe

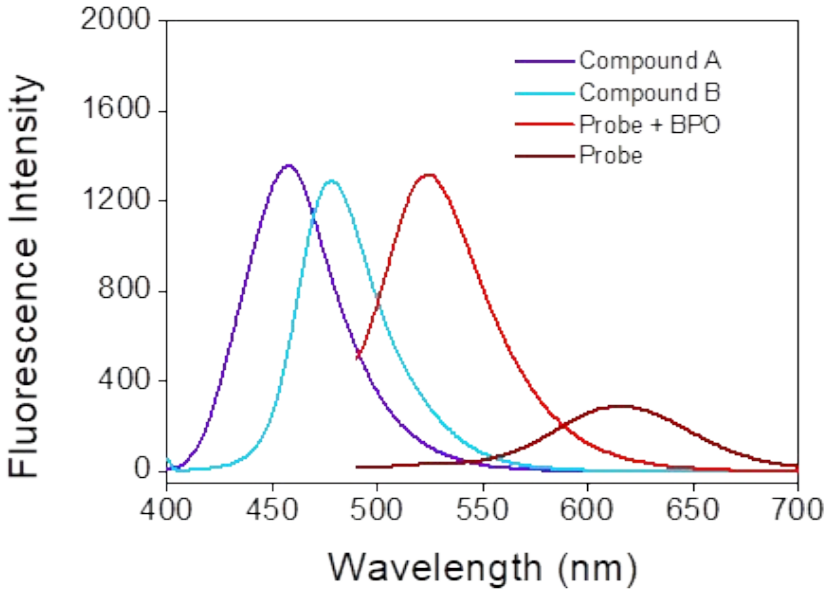

Fig. S5. Fluorescence emission spectra of compound A (7-(diethylamino)-2H-chromen-2-one), compound B (7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde), probe **Cou-BPO** and probe **Cou-BPO** in the presence of benzoyl peroxide(BPO).

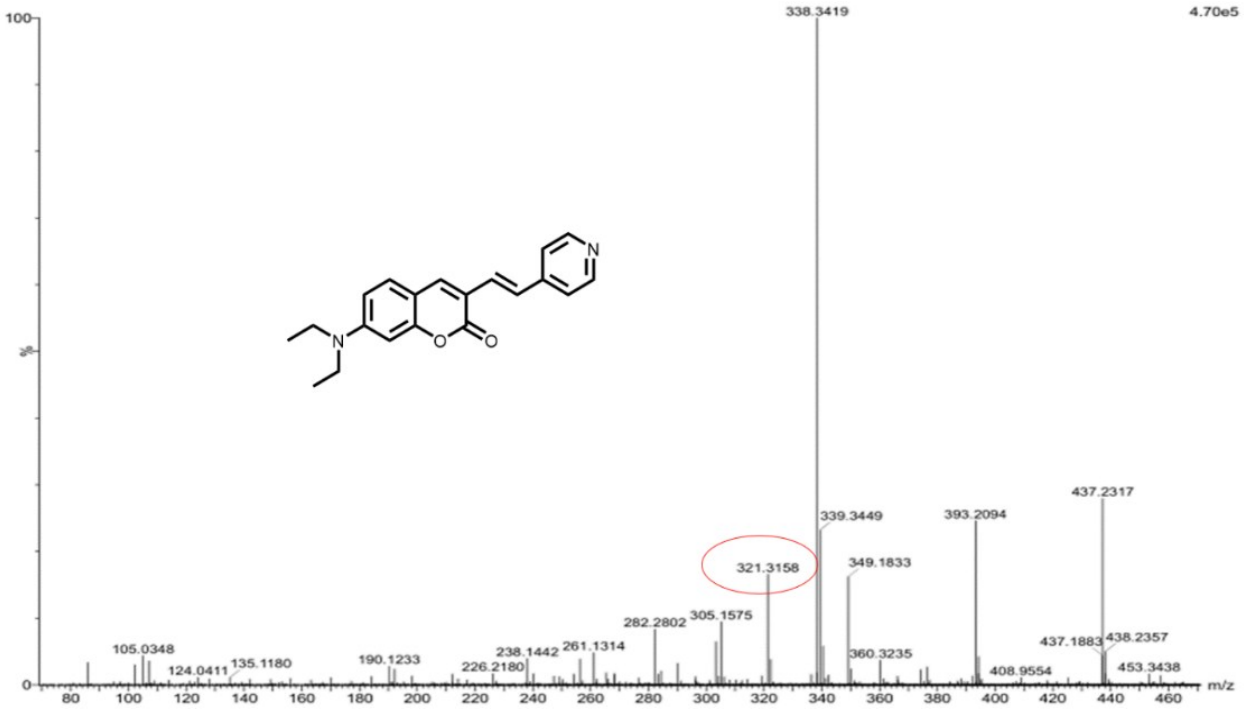

Fig. S6. HR-MS (ESI) spectrum of the probe in the presence of excessive benzoyl peroxide (BPO)

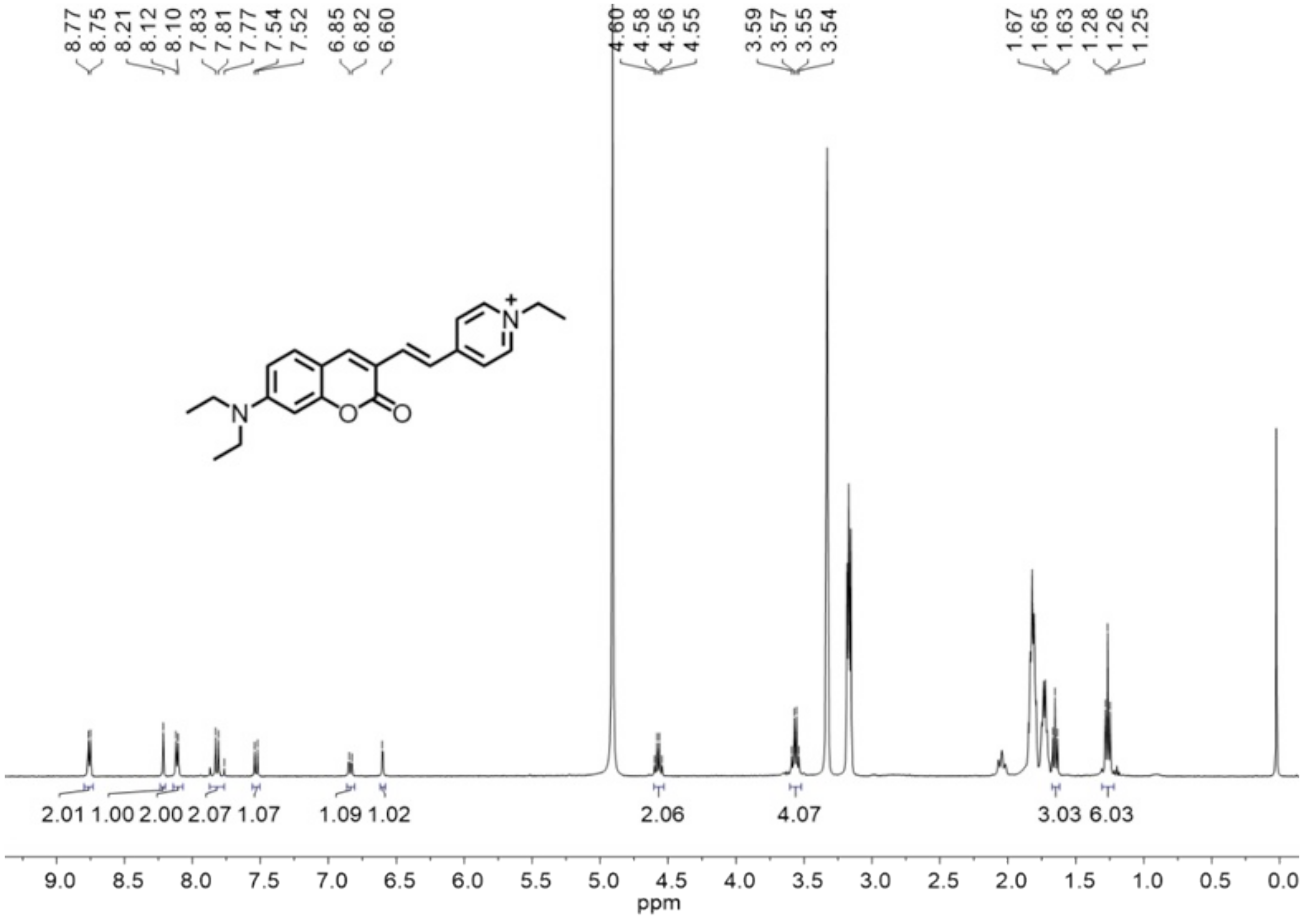

Fig. S7. [1]H NMR spectrum of **Cou-Pyr** in MeOD-d4 (400 MHZ)

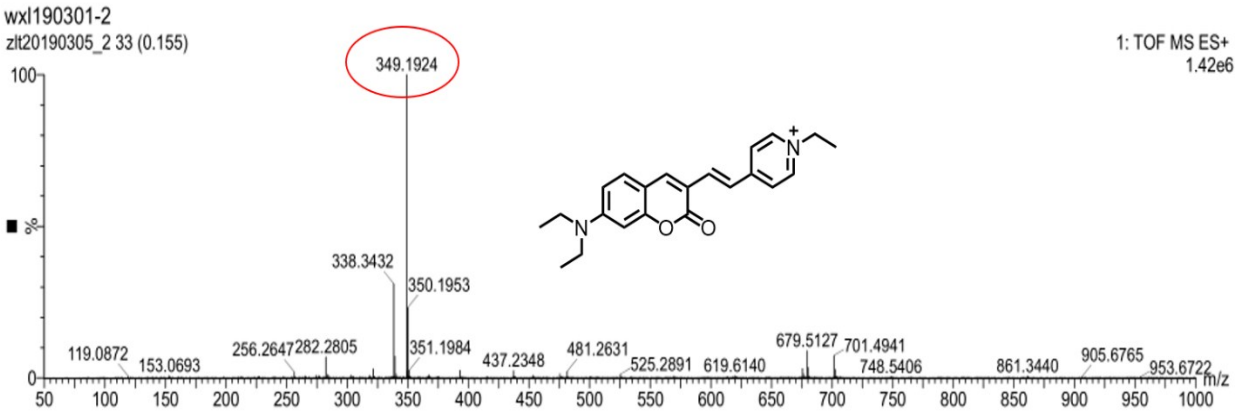

Fig. S8. HR-MS (ESI) spectrum of **Cou-Pyr**

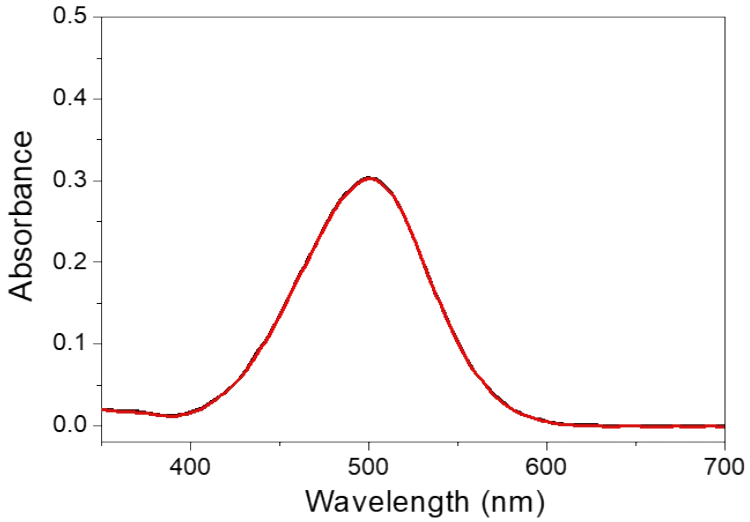

Fig. S9. Absorption spectra of **Cou-Pyr** (10 μM) after addition of benzoyl peroxide (BPO, 150 μM) in EtOH solution.

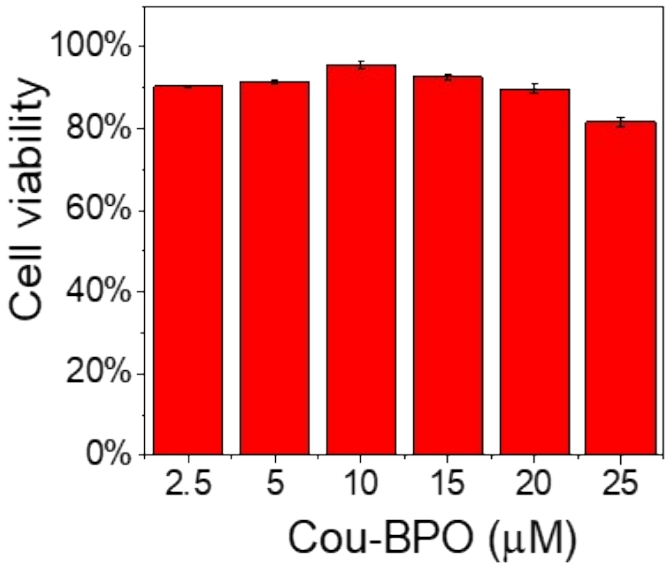

Fig. S10. Cytotoxicity of the **Cou-BPO** in living HeLa cells for 24 h

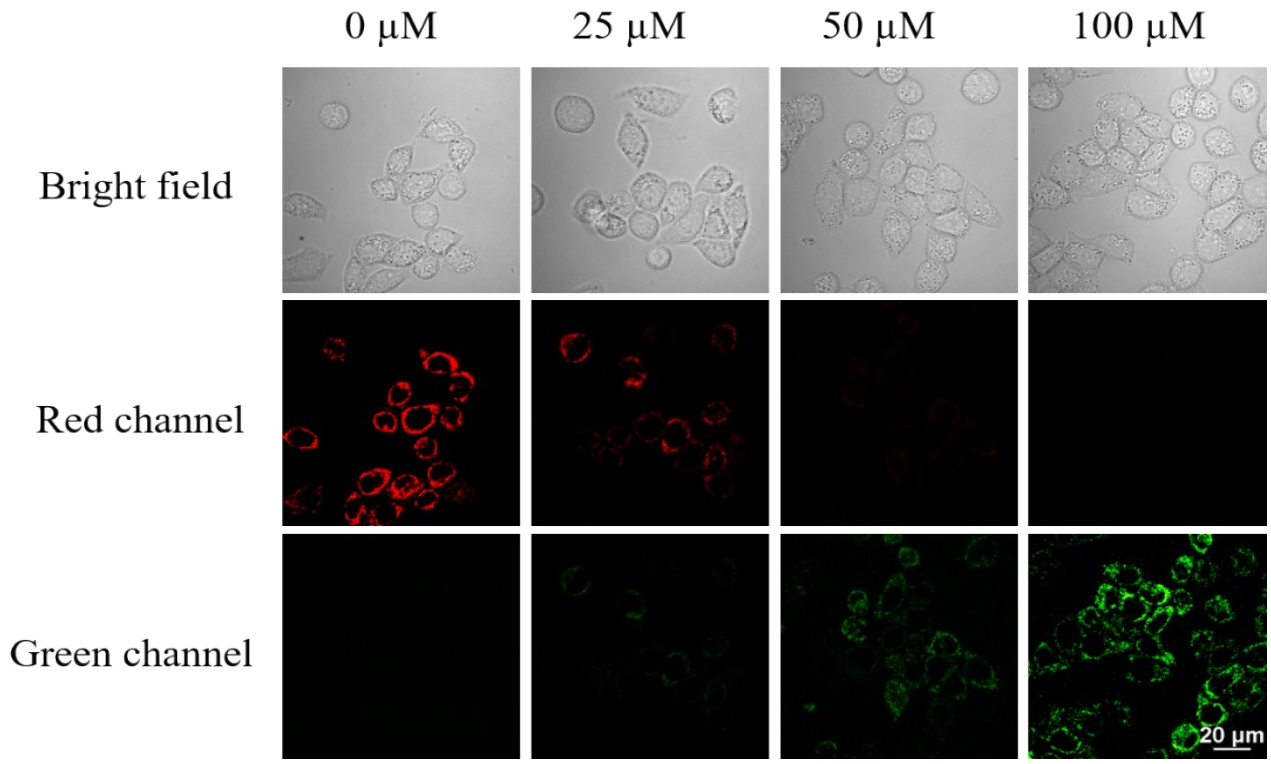

Fig. S11. Confocal fluorescence images of benzoyl peroxide in living HeLa cells.

S1. Experimental procedures for cytotoxicity measurement of Cou-BPO

Cytotoxicity of **Cou-BPO** was evaluated by Cell Counting Kit-8 (CCK-8) analysis in L929 cells. 5×103 cells/well were seeded in a 96-well plate (Corning) and incubated for 24 h at 37°C with 5% CO2. Cells were washed with Dulbecco's modified Eagle's medium (DMEM), and then incubated with Cou-BPO (2.5, 5, 10, 15 and 25 μM) for 24 h. Finally, they were washed three times with serum-free DMEM, then 100 μL serum-free DMEM containing 10% CCK-8 were added to each well and incubated for 1 h. The absorbance was measured at 450 nm on a plate reader. Cell viability rate was calculated according to the equation:

$$\text{Cell viability rate} = \frac{A - A_0}{A_S - A_0} \times 100\%$$

Where A is the absorbance of the experimental group, A_s is the absorbance of the control group (DMSO was used as the control), and A_0 is the absorbance of the blank group (no cells).

S2. Experimental procedures for fluorescence imaging of BPO in living cells

L929 cells (normal cell) were cultured in DMEM with 10% fetal bovine serum (FBS) and penicillin (100 units/mL)-streptomycin (100 units/mL) liquid (Invitrogen Corp., Carlsbad, CA) under 5% CO_2 at 37°C. The cells were seeded in uncoated 35 mm diameter glass-bottomed dishes and incubated for 24 h. Then the cells were incubated with **Cou-BPO** (10 μM) for 30 min, washed three times with 0.1 M PBS and mounted onto the microscope stage. For imaging of **BPO**, the above **Cou-BPO** treated cells were incubated with different concentrations of **Cou-BPO** (0, 25, 50, 100 μM) for 30 min and then used for confocal laser-scanning microscopy measurement. Fluorescence images were captured by a Nikon A1 confocal lasers canning microscope.