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Electronic Supplementary Information (ESI †)

Fluorescence Detection and Imaging of Intracellular Sulphite Using A Remote Light Activatable Photochromic Nanoprobe

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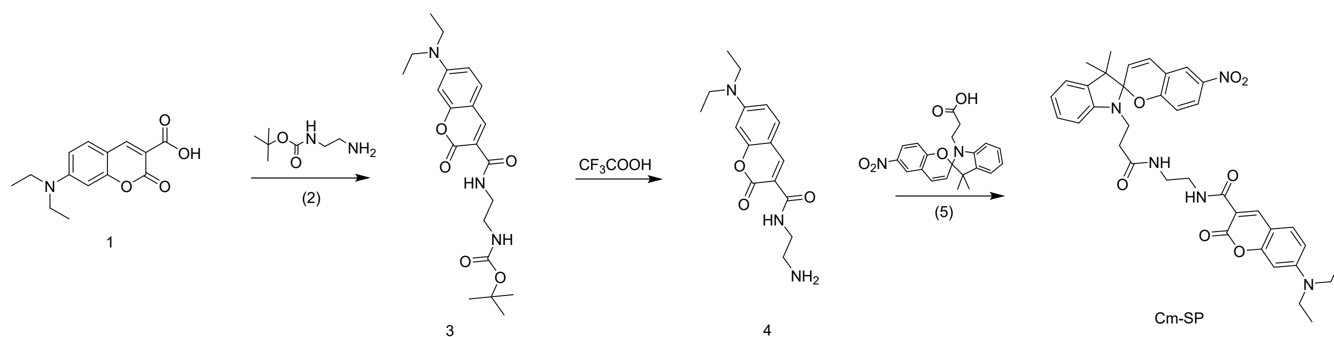
S1. General Information

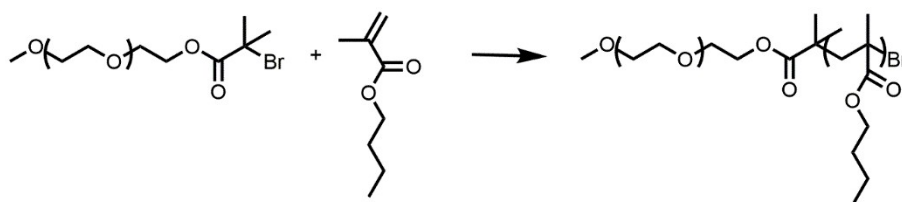
Materials and physical measurements

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), *L*-glutamine, penicillin, and streptomycin sulphate were purchased from Life Technologies. The PBS buffer consisting of 137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4 and 1.8 mM KH_2PO_4 was prepared in our laboratory. Solvents used are of analytical grade, except those for recrystallization and optical tests, which were distilled prior to use. Thin-layer chromatography (TLC) was carried out on aluminium sheets coated with silica gel 60 F254 (MERCK). All other chemicals were used as received unless otherwise indicated. Deionized distilled water was used throughout. All oxygen or moisture sensitive reactions were performed under argon atmosphere using the standard Schleck method.

^{1}H NMR Spectra were recorded using Bruker AM-400 Spectrometers. DMSO-d_6, $CDCl_3$ were used as solvent. Absorption and fluorescence Spectra were recorded using Varian Cary 500 and SHIMADZU RF-5301 PC Fluorescence Spectrometer, respectively. The UV (365 nm, 2.6 mW cm^{-2}) and visible light (550 nm, 150 mW) were used as light sources for UV and visible light irradiation, respectively. The size of polymer nanoparticles was measured on a Nano Zeta-Sizer (Malvern Instruments). The morphology of the nanoparticles was characterized using transmission electron microscope (TEM) (Hitachi HT7700). Confocal fluorescence imaging in live cells were carried out on a Leica SP8 laser-scanning microscope. The images were analysis by ImageJ software version 1.44p, and colocalization analysis was performed by a Co-localization Analysis Plugin. Flow cytometry analysis was performed on an Accuri C6 flow cytometer with a 405 nm laser excitation and emission filter of 460 ± 20 nm and 610 ± 20 nm. The data were analysed with CytExpert software. All data were presented as the mean ± SD for each group of three experiments.



Scheme S1. Synthesis procedure of **Cm-SP**.



Scheme S2. Design and Synthesis of **mPEG-b-PBMA**

Photochromic test of Poly-Cm-SP in solution

Solution of **Poly-Cm-SP** (10 μM) was prepared by dispersing as-prepared nanoparticles in PBS (0.01 M, pH 7.4). The light-controlled reversible fluorescence switches for nanoprobe was carried out in a cuvette with a path length of 5 mm. With the excitation wavelength at 405 nm, the emission spectra between 420 nm and 750 nm were recorded. The slits for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded at 298 K. For a photochromic cycling, the solution was irradiated with UV light (365 nm, 2.6 mW cm^{-2}) in darkroom, and the fluorescence spectroscopy was tested every 2 s until fluorescence intensity unchanged. Then, the solution was reversibly irradiated with Vis-light (550 nm, 150 mW) in darkroom, and the fluorescence spectroscopy was tested every 5 s until the fluorescence intensity unchanged.

Fluorescence spectroscopy for sulphite detection

Solution of **Poly-Cm-SP** (10 μM) was prepared by dispersing as-prepared nanoparticles in PBS (0.01 M, pH 7.4). Conversion of **Poly-Cm-SP** to **Poly-Cm-MR** was conducted in PBS buffer by UV light (365 nm, 2.6 mW cm^{-2}) irradiation for 30 s. Stock solution of 1 mM of SO_3^{2-} was prepared by dissolving Na_2SO_3 in PBS (0.01 M, pH 7.4). The fluorescence measurements for nanoprobe were carried out in a cuvette with a path length of 5 mm. The emission spectra between 420 nm and 750 nm were recorded with the excitation wavelength at 405 nm. The slits for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded at 298 K.

Cell culture

Murine macrophage cell line, RAW 264.7 (ATCC® TIB-71™) was obtained from American Type Cell Collection. RAW 264.7 cells were cultured in DMEM media, supplemented with 10% fetal bovine serum and penicillin (10 U/mL)/streptomycin (10 μg/mL). All cells were cultured in a humidified incubator at 37 °C and 5% CO_2. The cell growth medium was replaced every two days. RAW 264.7 cells were routinely detached with trypsin-EDTA solution and then seeded in a 25 mL cell culture bottle. The cells were reached about 80% confluence before experiments.

Fluorescence imaging and photochromic test in cells

RAW 264.7 macrophage cells were typically seeded at a density of 5 x 10^4 cells/mL in a cover glass-bottomed culture dish (d = 22 mm). After 24 h growth in incubator, the culture medium was carefully replaced with freshly prepared medium containing **Poly-Cm-SP** (10 μM). Then, the cells were incubated at 37 °C for another 2 h. RAW 264.7 cells were washed with PBS for three times before confocal microscopy imaging. Sequential irradiation by UV light (365 nm) for 10 s and Vis-light (550 nm) for 20 s in darkroom was performed, followed by fluorescence imaging of both green and red channels after each irradiation. The excitation wavelength was 405 nm and 550 nm and emission channel 440-480 nm and 590-630 nm for blue and red channel, respectively.

Fluorescence imaging of sulphite in cells

Poly-Cm-SP was incubated with RAW 264.7 for 2 h (in dark) and washed with PBS for three times. The cell then was treated with UV irradiation for 30 s, and then incubated with 100 μM sulphite for another 20 min. The cells were then washed with PBS for three times before subjecting to fluorescence imaging. For fluorescence imaging, excitation wavelength was 405 nm and 550 nm and emission channel 440-480 nm and 590-630 nm for blue and red channel, respectively.

Flow cytometry analysis

Flow cytometry analysis was performed to evaluate the fluorescence responses of photochromic nanoprobes in live cells. RAW 264.7 cells were seeded in a 6-well plate at a density of 1×10^6 cells/well. Similar to above fluorescence imaging, two group experiments were then conducted for flow cytometry analysis in live cells.

Group 1: After 24 h incubation at 37 °C, the culture medium was discard and the cells were washed with PBS for three time. Cells were then incubated with freshly prepared medium containing **Poly-Cm-SP** (10 μM) at 37 °C for another 2 h.

Group 2: After 24 h incubation at 37 °C, the cells were treated **Poly-Cm-SP** (10 μM) at 37 °C for another 2 h. The culture medium was discarded and then the cells were treated by UV light for 10 s, followed by the incubation with sulphite (100 μM) at 37 °C for another 20 min.

For flow cytometry analysis, the cells were suspended by treating with trypsin and collected after washing with PBS. For each group of experiment, alternate irradiations by UV light (365 nm) for 10 s and Vis-light (550 nm) for 30 s in darkroom were performed and the flow cytometry analysis were obtained by measuring fluorescence of 1×10^4 cells from each cell population.

Lysosome colocalization.

For lysosome colocalization analysis, RAW 264.7 macrophage cells were typically seeded at a density of 5×10^4 cells/mL in a cover glass-bottomed culture dish (d = 22 mm). After incubation at 37 °C for 24 h,

the cell culture medium was replaced with freshly prepared medium containing **Poly-Cm-SP** (10 μM) and the cells were further incubated at 37 °C for 2 h. Then, the cells were stained with LysoSensor™ Green following protocol from Life Technologies. The cells were then rinsed with PBS before subjecting to microscope imaging. Cells was irradiated by alternate UV/Vis light and fluorescence images were recorded with a confocal laser-scanning microscope.

Cell viability assay

Cell cytotoxicity was assessed by the typical 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. RAW 264.7 macrophage cells were seeded in a 96-well plate in growth medium at the density of 5×10^4 cells per well. After 24 h, the culture medium was replaced with a fresh medium containing **Poly-Cm-SP** of different concentrations (0, 10, 20, 40, 80, 100 μM). The group with the addition of culture medium only was employed as the control, and the wells containing culture media without cells were used as blanks. The cells were then incubated in a humidified incubator for another 24 h. MTT solution in PBS buffer was then added into each well after the removal of culture media, followed by further incubation for 4 h in a dark at 37 °C. Finally, excess MTT solution was carefully removed and the formed formazan was dissolved by adding of dimethyl sulfoxide (DMSO, 100 μL) to each well. The absorbance at 490 nm was measured in an Infinite M200 Pro Microplate Reader. The results from the five individual experiments were averaged. The following formula was used to calculate the viability of cell growth: Viability (%) = (mean of absorbance value of treatment group - blank)/(mean absorbance value of control - blank) × 100.

Photocytotoxicity

Photocytotoxicity was assessed by the typical 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. RAW 264.7 macrophage cells were seeded in a 96-well plates in growth medium at the density of 5×10^4 cells per well. After 24 h, the cells were incubated with **Poly-Cm-SP** (10 μM) for 2 h. After washing with PBS for three times, irradiation with UV/Vis light (365 nm/550 nm)

for different cycles (0-6) was performed. MTT solution in PBS buffer was then added into each well after the removal of culture media, followed by further incubation for 4 h in a dark at 37 °C. Finally, excess MTT solution was carefully removed and the formed formazan was dissolved by adding of dimethyl sulfoxide (DMSO, 100 μL) to each well. The absorbance at 490 nm was measured in an Infinite M200 Pro Microplate Reader. The results from the five individual experiments were averaged. The following formula was used to calculate the viability of cell growth: Viability (%) = (mean of absorbance value of treatment group - blank)/(mean absorbance value of control - blank) × 100.

S2. Characterization of Poly-Cm-SP and intermediates

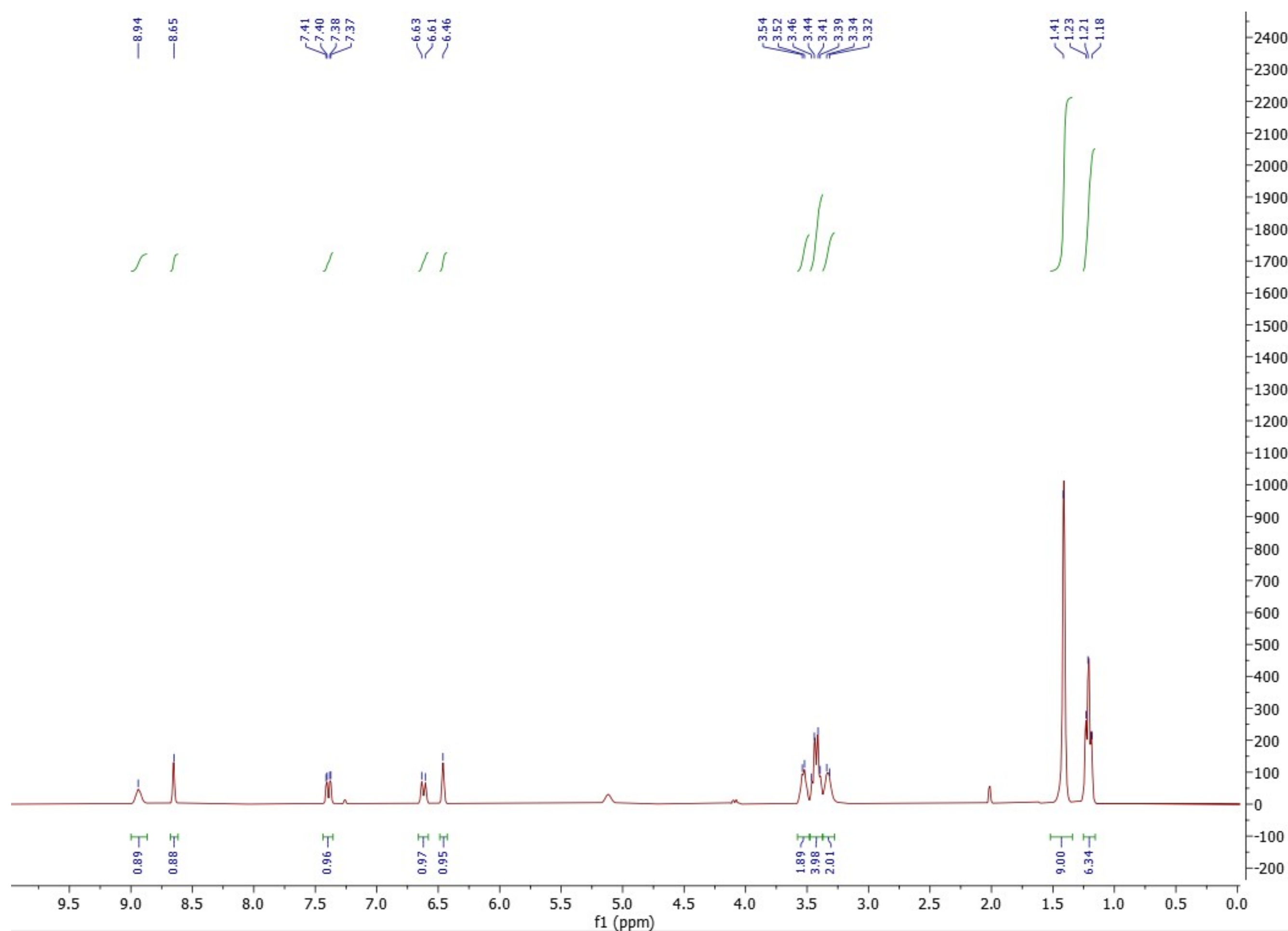


Fig. S1. ^{1}H NMR of Compound **3**.

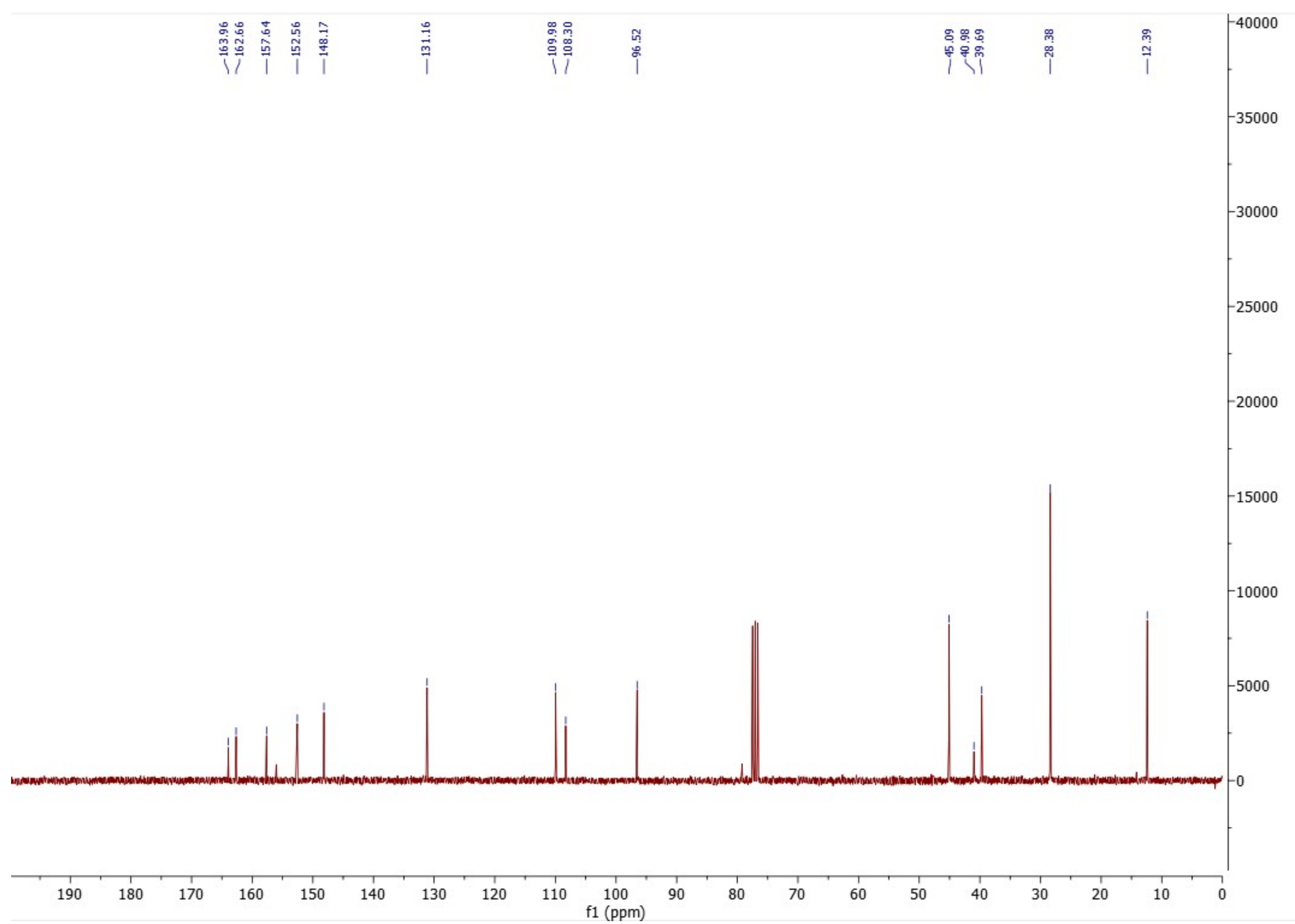


Fig. S2. ^{13}C NMR of Compound **3**.

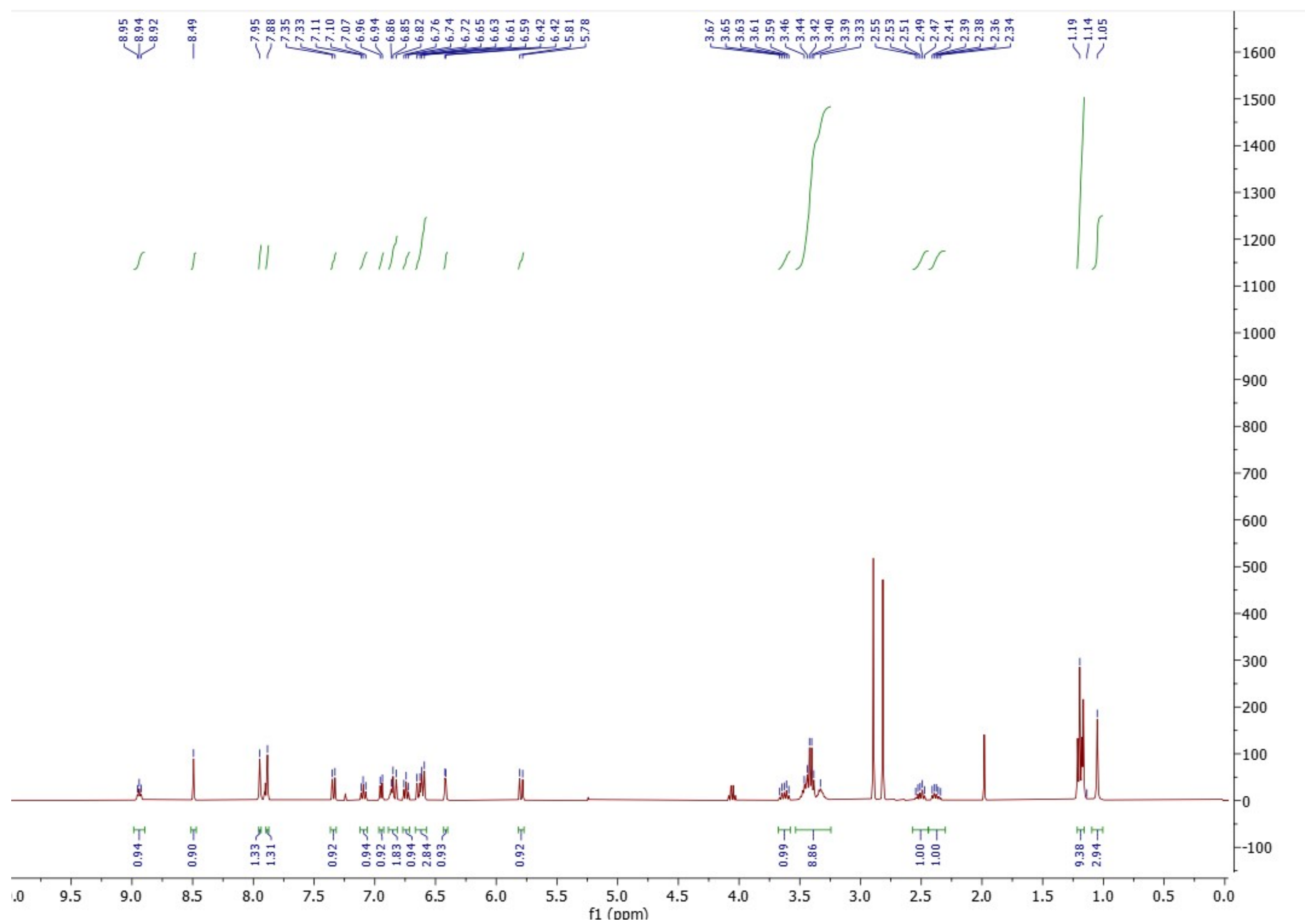


Fig. S3. ^{1}H NMR of Compound **Cm-SP**.

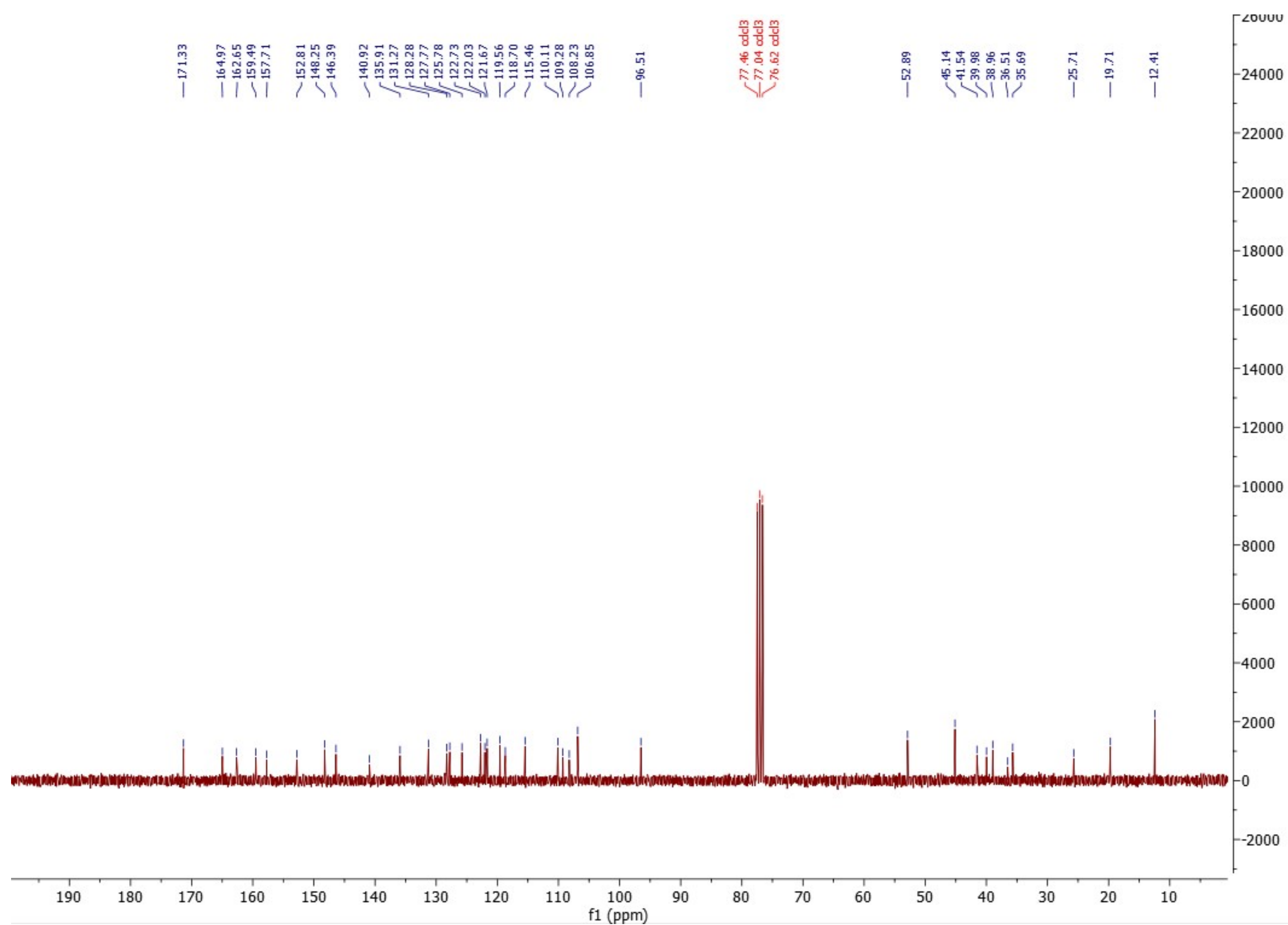


Fig. S4. ^{13}C NMR of Compound **Cm-SP**.

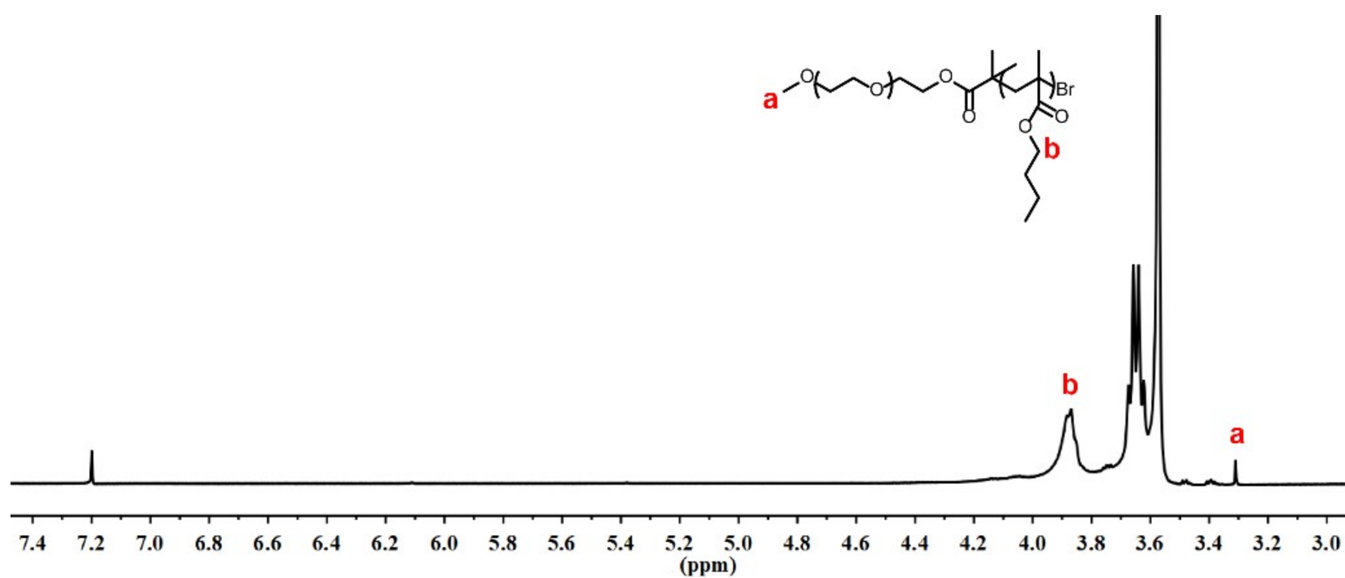


Fig. S5. ^{1}H NMR of mPEG-b-PBMA.

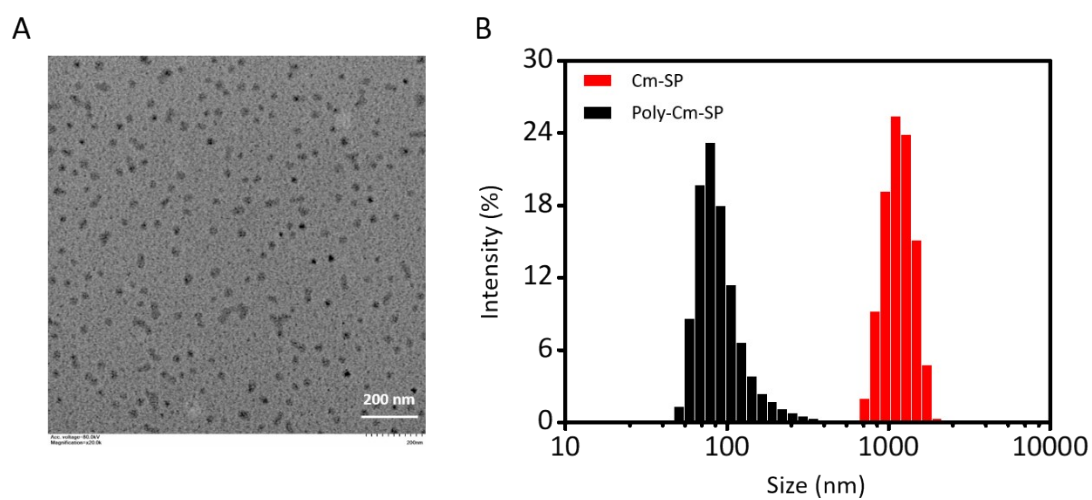


Fig. S6. TEM image (A) and DLS size distribution of **Poly-Cm-SP** (B) in water. The scale bar of TEM image is 200 nm.

S3. Light-controlled multiple colour fluorescence response in aqueous solution

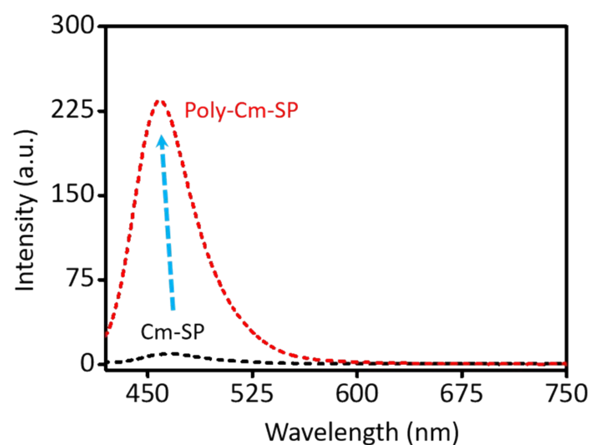


Fig. S7. Fluorescence spectra of **Cm-SP** (10 µM) and **Poly-Cm-SP** (10 µM) in 0.01 M PBS buffer of pH 7.4. (Blue channel, λ_{ex} = 405 nm).

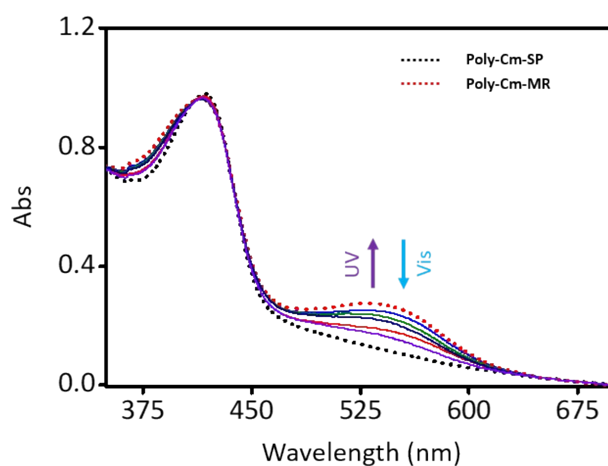


Fig. S8. Changes of UV-Vis absorption spectra of **Poly-Cm-SP** (10 µM) upon UV irradiation (365 nm) over the time (0-10 s, interval: 2 s), and **Poly-Cm-MR** (10 µM) upon visible (Vis) irradiation (550 nm) over the time (0-30 s, interval: 5 s). All measurements were carried out in 0.01 M PBS buffer of pH 7.4.

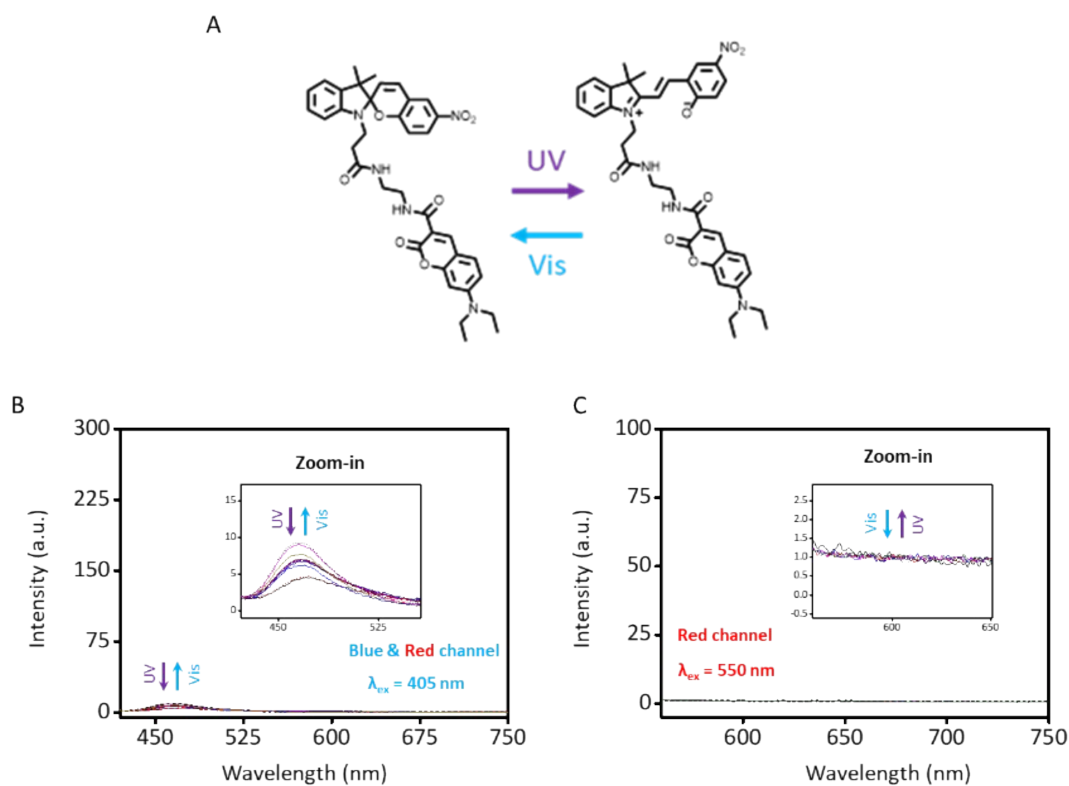
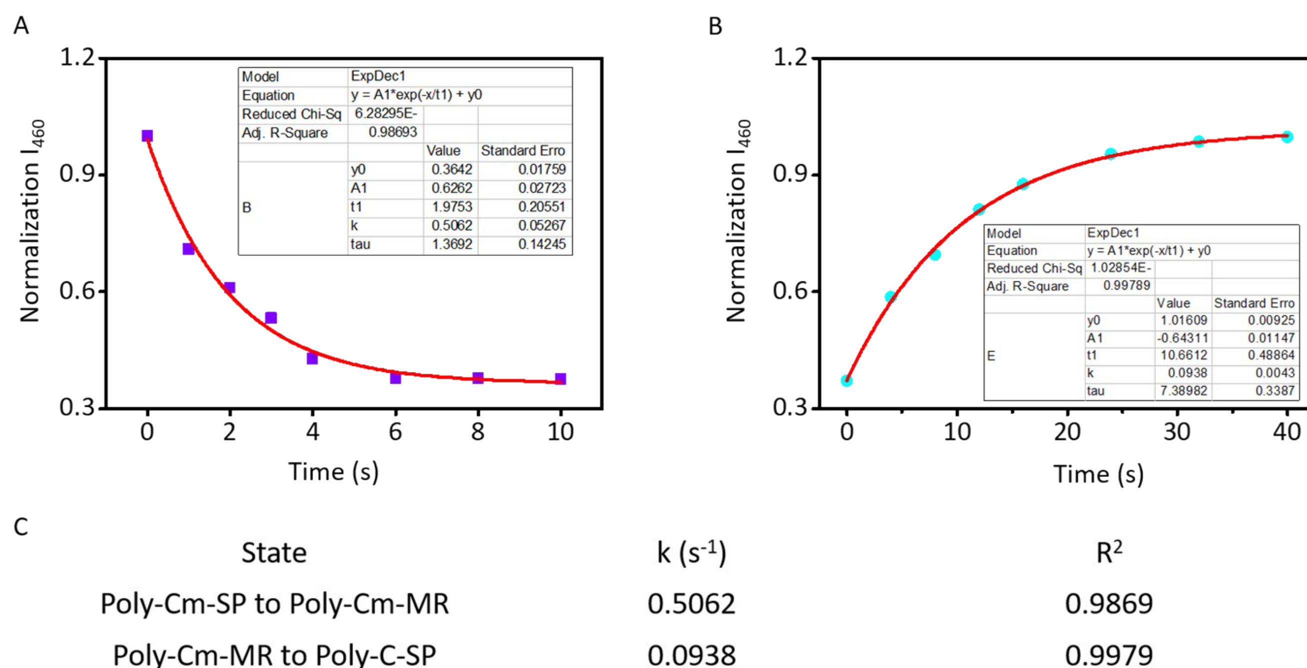


Fig. S9. Photoisomerizion process of Cm-SP (A), and fluorescence spectra changes of Cm-SP (10 μM) upon UV irradiation (365 nm) over the time (0-5 min, interval: 30 s), and Cm-MR (10 μM) upon visible (Vis) irradiation (550 nm) over the time (0-5 min, interval: 30 s), in blue channel (B) and red channel (C). Excitation wavelengths are 405 and 550 nm for blue and red channel, respectively. All measurements were carried out in 0.01 M PBS buffer of pH 7.4.



State	k (s^{-1})	R^2
Poly-Cm-SP to Poly-Cm-MR	0.5062	0.9869
Poly-Cm-MR to Poly-C-SP	0.0938	0.9979

Fig. S10. Isomerization kinetics of **Poly-Cm-SP** to **Poly-Cm-MR** (SP → MR) and **Poly-Cm-MR** to **Poly-Cm-SP** (MR → SP). Kinetic trace (A, B) and key parameters (C) of **Poly-SP-Np-B** (10 μM) in 0.01 M PBS Buffer of pH 7.4. The fluorescence intensity of the trace is normalized relative to the original fluorescence (λ_{ex} = 405 nm, λ_{em} = 460 nm).

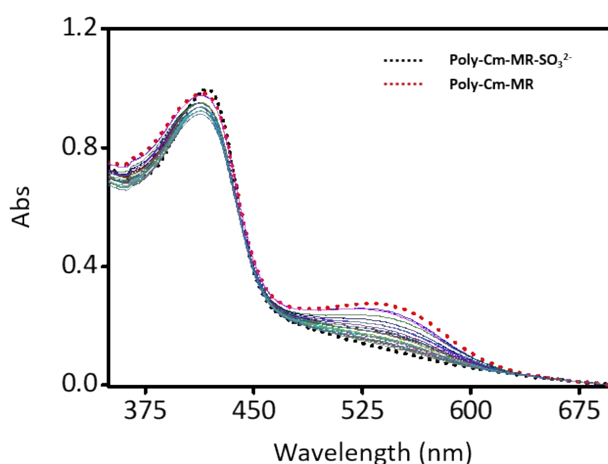


Fig. S11. Change of UV-Vis absorption spectra of **Poly-Cm-MR** (10 μM) with the addition of sulphite (0-100 μM, interval: 10 μM). All measurements were carried out in 0.01 M PBS buffer of pH 7.4.

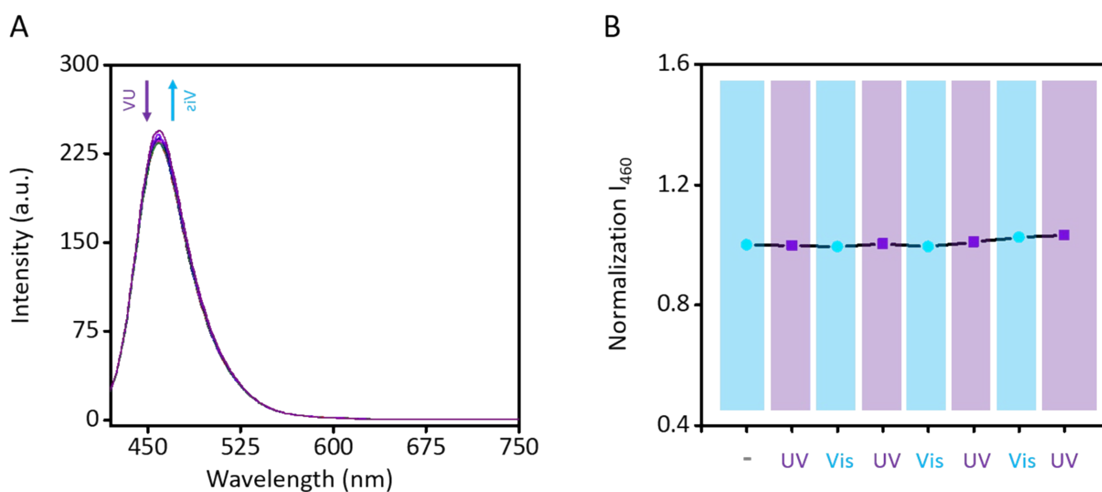


Fig. S12. Fluorescence response of nanoprobe **Poly-Cm-MR-SO_3^{2-}** (10 μM) with alternative UV/Vis light irradiation. All measurements were carried out in 0.01 M PBS buffer of pH 7.4.

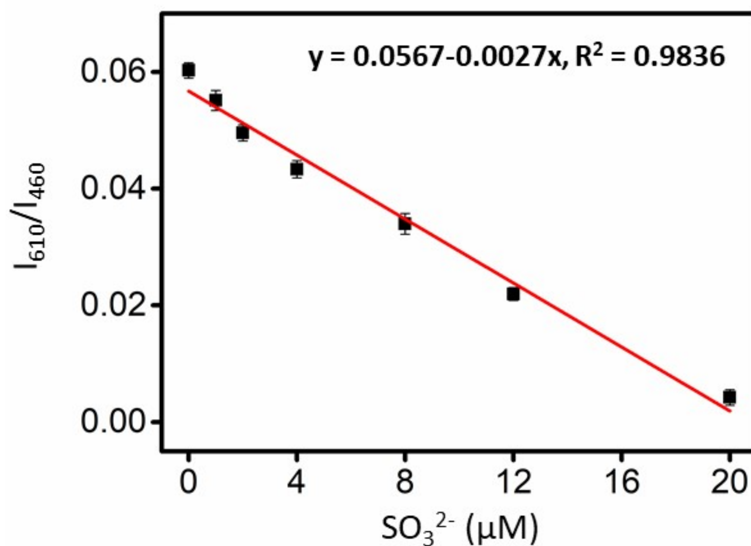


Fig. S13. Changes of ratio of I_{610}/I_{460} upon addition of different concentrations of sulfite (0, 1, 2, 4, 8, 12, 20 μM). All measurements were carried out in PBS buffer, pH 7.4, excitation wavelength: 405 nm. The error bar represents s.d. (n = 3).

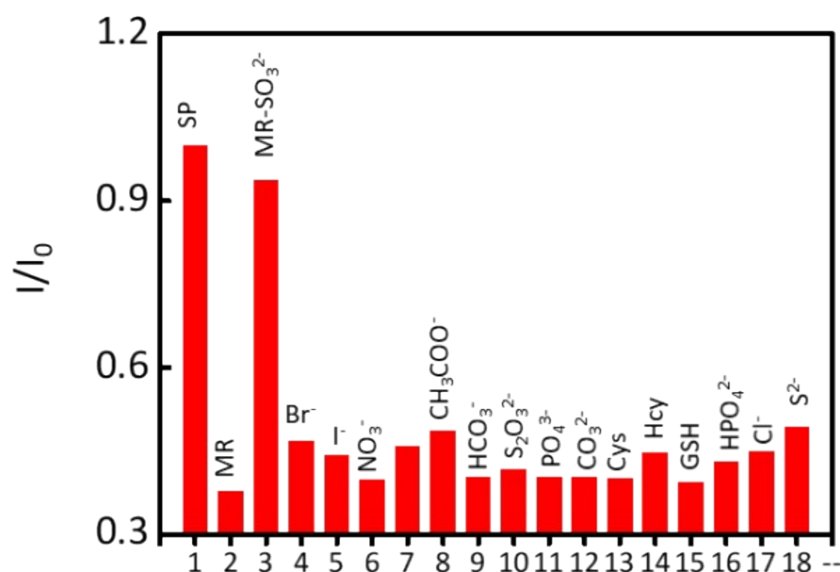


Fig. S14. Specificity of photochromic fluorescent nanoprobe. The fluorescence change of **Poly-Cm-SP** (10 μM, sample 1) and **Poly-Cm-MR** (10 μM, sample 2-17) with a range of analytes in phosphate buffered saline (PBS, 0.01 M, pH 7.4) at 298 K (excitation wavelength: 405 nm), where I_0 and I are the blue fluorescence (blue channel) of the initial spiropyran state and that of the UV-activated merocyanine state, respectively. Competing analytes 1-19: 1: Blank 1(nanoprobe alone, SP state), 2：Blank 2(nanoprobe alone, MR state), 3: SO_3^{2-}; 4: Br^-; 5: I^-; 6: NO_3^-; 7: NO_2^-; 8: CH_3COO^-; 9: HCO_3^-; 10: $S_2O_3^{2-}$; 11: PO_4^{3-}; 12: CO_3^{2-}; 13: Cys; 14: Hcy; 15: GSH; 16: HPO_4^{2-}; 17: Cl^-; and 18 S^{2-} , For SO_3^{2-}, the concentration is 50 μM, for GSH, the concentration is 5 mM, for other analytes, the concentration is 500 μM.

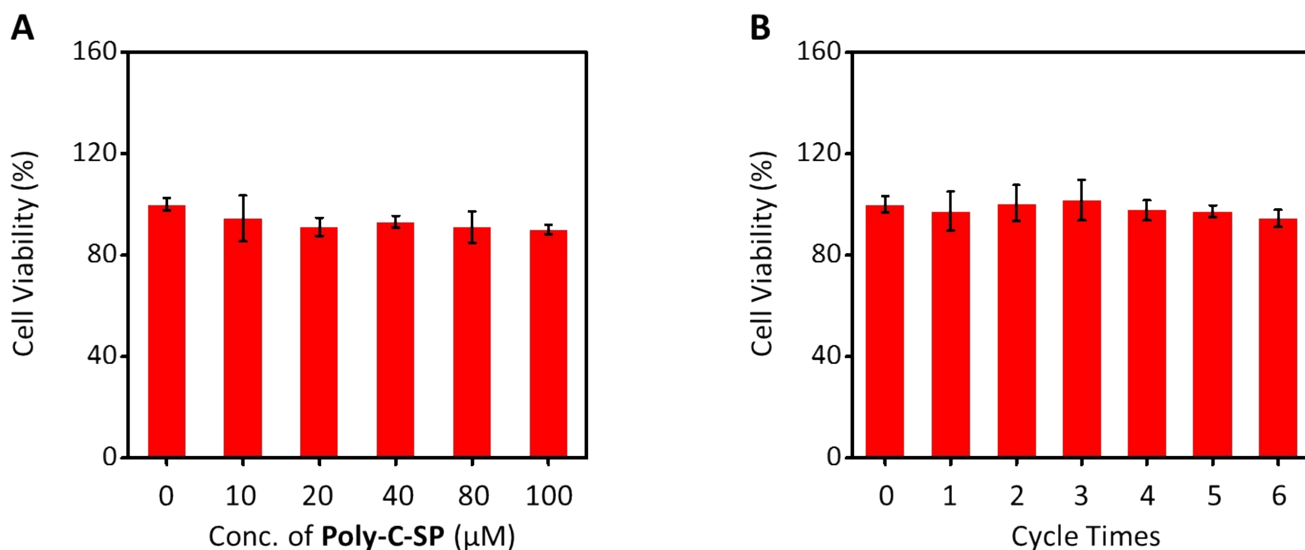


Fig. S15. MTT cytotoxicity assay. Viability of RAW 264.7 cell in the presence of increasing concentration of **Poly-Cm-SP** for 24 h (A). Viability of RAW 264.7 cell with **Poly-Cm-SP** (10 μM) under 1-6 times alternative UV/Vis irradiation cycles (B).

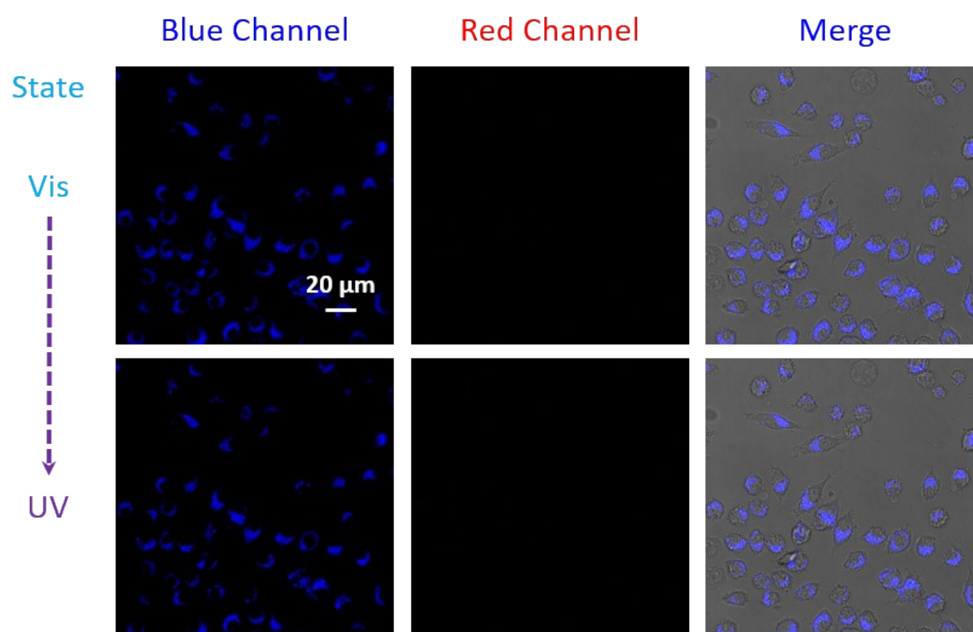


Fig. S16. Fluorescent imaging of RAW 264.7 macrophage cells incubated with **Cm-SP** (10 μM). The **Cm-SP** internalized RAW 264.7 macrophage cells were then irradiated with UV (365 nm) and visible (550 nm) light alternatively. Scale bar is 20 μm.

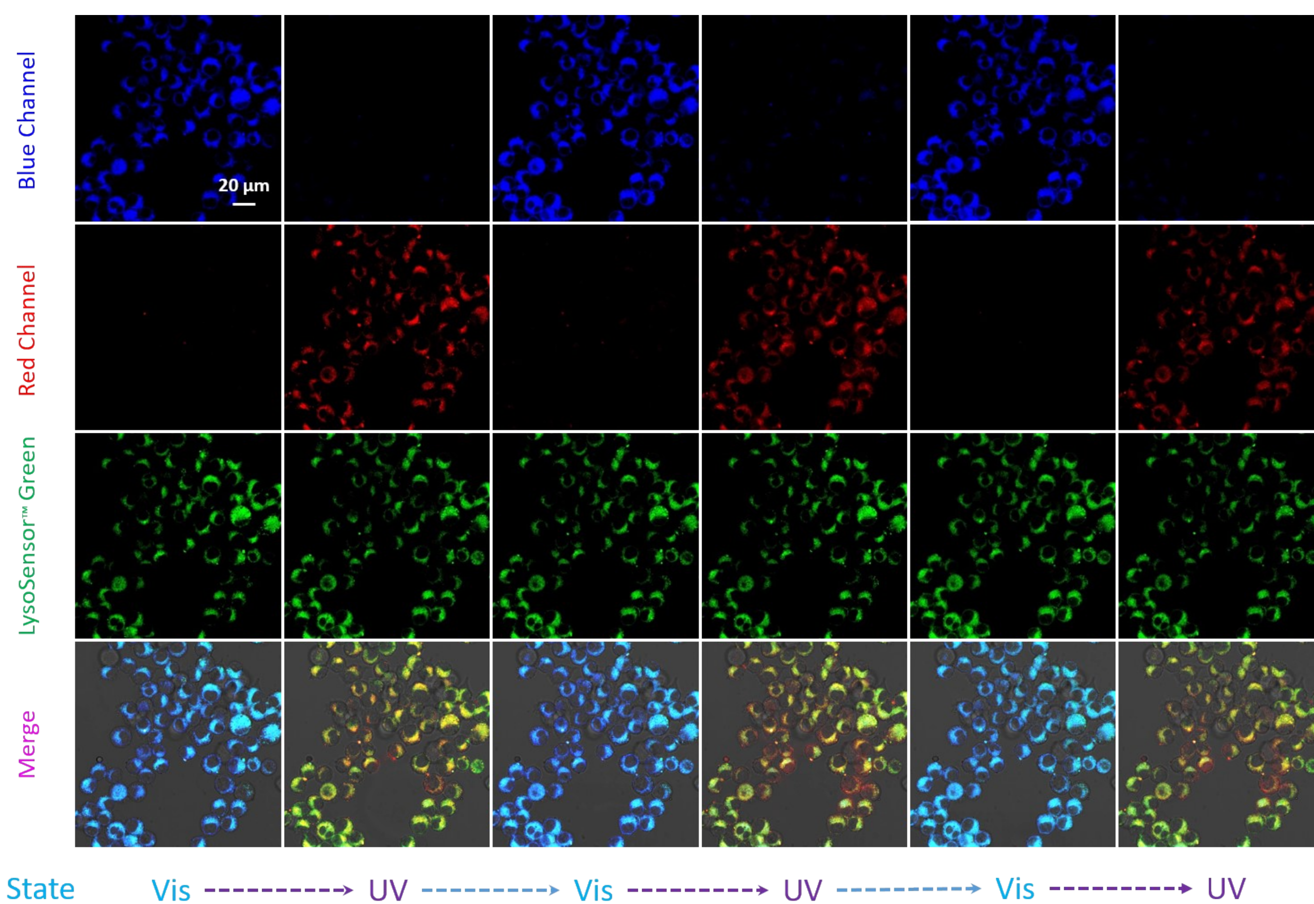


Fig. S17. UV/Vis cycling and colocalization images of **Poly-Cm-SP** with LysoSensor^{TM} Green in RAW 264.7 macrophage cells. The **Poly-Cm-SP** and LysoSensor^{TM} Green loaded RAW 264.7 macrophage cells were irradiated with alternate UV/vis light irradiation for 10 s and 20 s, respectively. Scale bar is 20 μm.