Electronic Supplementary Information

Hydrogen Peroxide Activatable Nanoprobe for Light-controlled "Double-Check" Multi-colour Fluorescence Imaging

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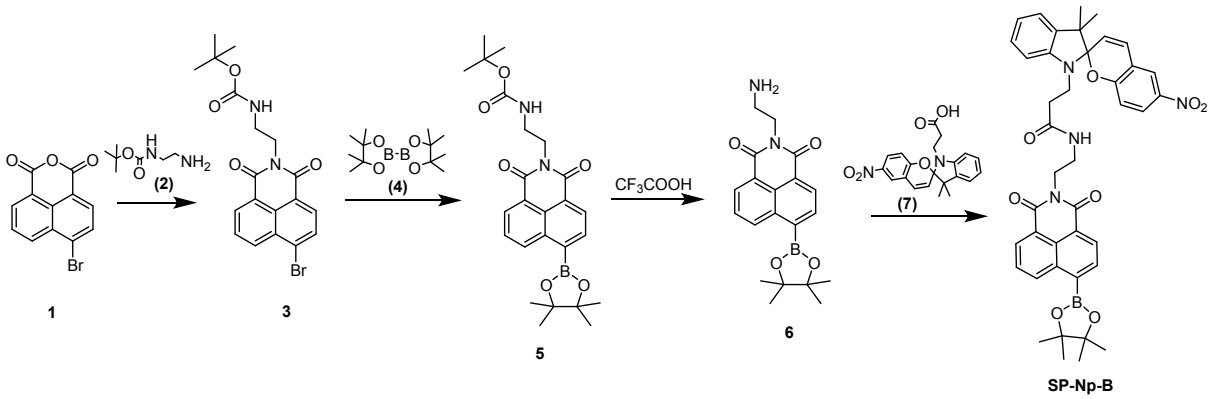
S1. General Information

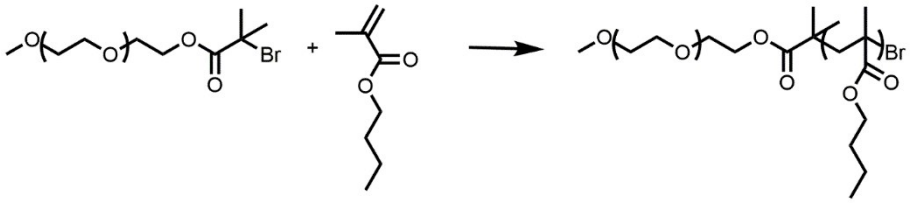
Materials and physical measurements

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lipopolysaccharide (LPS), were 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 were measured on a Nano Zeta-Sizer (Malvern Instruments). The morphology of the nanoparticles were 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 colocalisation analysis was performed by a Co-localization Analysis Plugin. Flow cytometry analysis was performed on an Accuri C6 flow cytometer with a 488 nm laser excitation and emission filter of 525 ± 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 SP-Np-B.



Scheme S2. Design and Synthesis of **mPEG-b-PBMA**[1-3]

General information for Ploy-SP-Np-B reacting with H_2O_2

Solution of **Poly-SP-Np-B** (10 μM) was prepared by dispersing as-prepared nanoparticles in PBS (0.01 M, pH 7.4). Stock solution of 1 mM of H_2O_2 was prepared in PBS (0.01 M, pH 7.4). The fluorescence spectra of nanoprobe for H_2O_2 activation was measured using a quartz cuvette of path length of 5 mm. The excitation wavelength was performed at 440 nm and emission between 450 and 750 nm was recorded. The slit for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded at 298 K.

Photochromic test in solution

Solution of **Poly-SP-Np-B** (10 μM) was prepared by dispersing as-prepared nanoparticle in PBS (0.01 M, pH 7.4). **Poly-SP-Np** was prepared in-situ by adding 100 μM H_2O_2 into 10 μM **Poly-SP-Np-B** in PBS (0.01 M, pH 7.4). The solution was irradiated with UV light (365 nm)

in darkroom. Fluorescence spectra were recorded every 2 s irradiation until the maximum spectrum changes obtained. Then the solution was irradiated with visible light (550 nm) in darkroom. The fluorescence spectra were tested every 2 s irradiation until the spectra retained unchanged. The light-controlled reversible fluorescence switch measurements for **Poly-SP-Np-B/Poly-SP-Np** nanoparticles were carried out with a quartz cuvette of a path length of 1 cm. For the green channel fluorescence (λ_{em} = 450-750 nm) and red channel fluorescence (λ_{em} = 560-750 nm), excitations were performed at 440 nm and 550 nm, respectively. The slit for both excitation and emission spectra was 5 nm. Unless otherwise mentioned, all the spectra were recorded at 298 K.

Cell line and 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

Three group experiments were performed to evaluate the feasibility of light-controlled "double-check" fluorescence imaging in live cells. For each group experiment, sequential irradiation by UV light (365 nm, 2.6 mW cm^{-2}) for 10 s and Vis-light (550 nm, 150 mW) for 20 s in a darkroom was performed, followed by fluorescence imaging of both green and red channels after each irradiation. The excitation wavelength was 405 nm, 442 nm, and 550 nm and emission channel 450-470 nm, 520-540 nm, and 600-620 nm for DAPI, green channel and red channel, respectively.[4-5]

Group 1: for "double-check" fluorescence imaging, 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 (ϕ = 22 mm). After 24 h growth in incubator, the culture medium was carefully replaced with freshly prepared medium containing **Poly-SP-Np-B** (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.

Group 2: for exogenous H_2O_2 activation and multiple colour fluorescence imaging, **Poly-SP-Np-B** internalized RAW 264.7 cells were incubated with 100 μM H_2O_2 in PBS for 30 min. Cells were washed with PBS for three times before microscope imaging.

Group 3: for endogenous H_2O_2 activation and multiple colour fluorescence imaging, RAW 264.7 macrophage cells were pre-treated with lipopolysaccharide (LPS, 200 ng/mL) for 24 h, and then incubated with **Poly-SP-Np-B** (10 μM) for another 2 h. The cells were then washed with PBS prior to the confocal microscope imaging.[6]

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. Three group experiments were then conducted similar to above fluorescence imaging.

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-SP-Np-B** (10 μM) at 37 °C for another 2 h.

Group 2: Group 1 cells were further treated with 100 μM H_2O_2 in PBS for 30 min.

Group 3: After 24 h incubation at 37 °C, the cells were treated with LPS (200 ng/mL) for 24 h. The culture medium was discard and then the cells were further incubated with **Poly-SP-Np-B** (10 μM) at 37 °C for another 2 h.

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 20 s in darkroom were performed and the flow cytometry analysis were obtained by measuring fluorescence of 1 x 10^4 cells from each cell population.

Lysosome colocalisation

For lysosome colocalisation analysis, 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 (ϕ = 22 mm). After incubation at 37 °C for 24 h, the cell culture medium was replaced with freshly prepared medium containing **Poly-SP-Np-B** (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. 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 plates in growth medium at the density of 5 x 10^4 cells per well. After 24 h, the culture medium was replaced with a fresh medium containing **Poly-SP-Np-B** of different concentrations (0, 10, 20, 40, 80, 100, 200 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 x 10^4 cells per well. After 24 h, the cells were incubated with **Poly-SP-Np-B** (10 μM) for 2 h. After washing with PBS for three times, irradiation with UV/Vis light (365 nm/550 nm) for different cycles (0-7) 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.

2. Characterization of Poly-SP-Np-B and intermediates

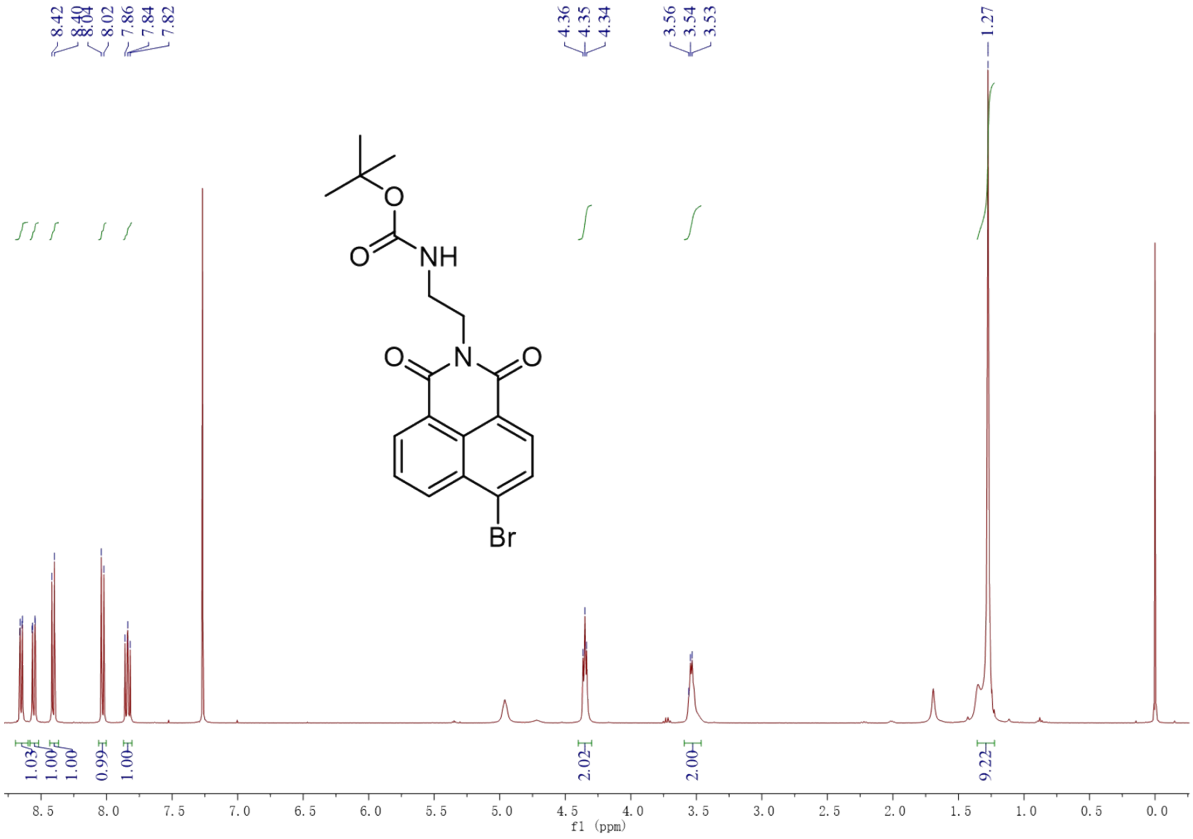


Fig. S1. ^{1}H NMR of Compound **3**.

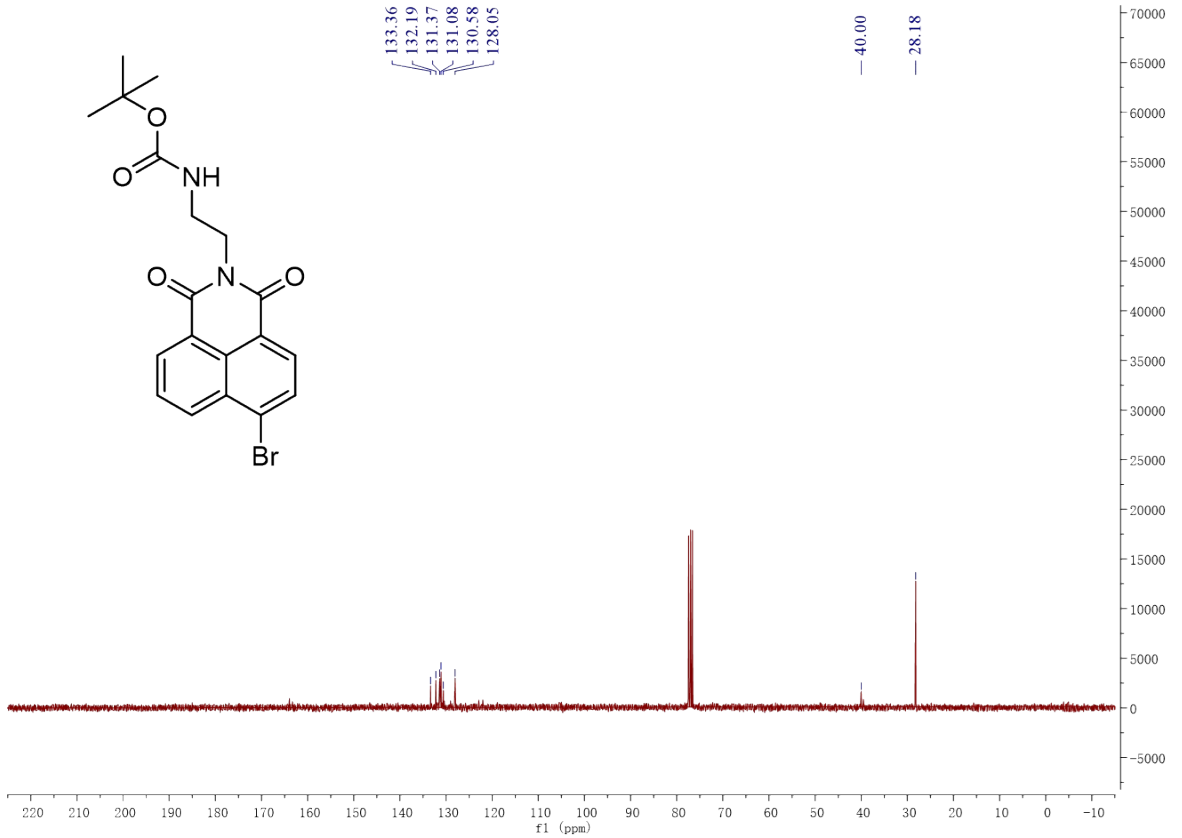


Fig. S2. ^{13}C NMR of Compound **3**.

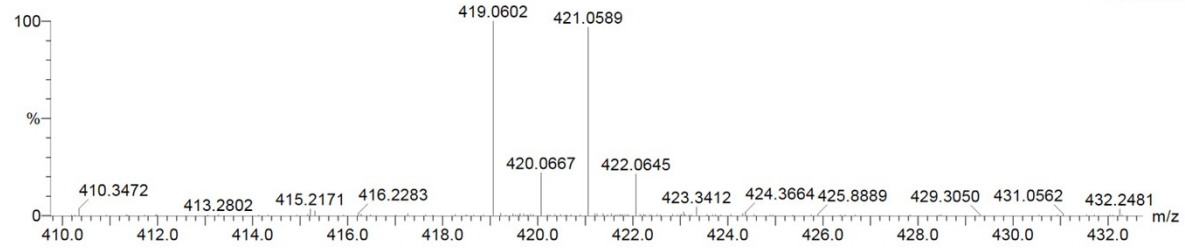
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
34 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-19 H: 0-99 N: 0-2 O: 0-4 Br: 0-1

H-TIAN
TH-ZYD-102 60 (0.673) Cm (60:61)

1: TOF MS ES+
2.79e+003



Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
419.0602	419.0606	-0.4	-1.0	10.5	87.7	0.0	C19 H20 N2 O4 Br

Fig. S3. MS of Compound **3**.

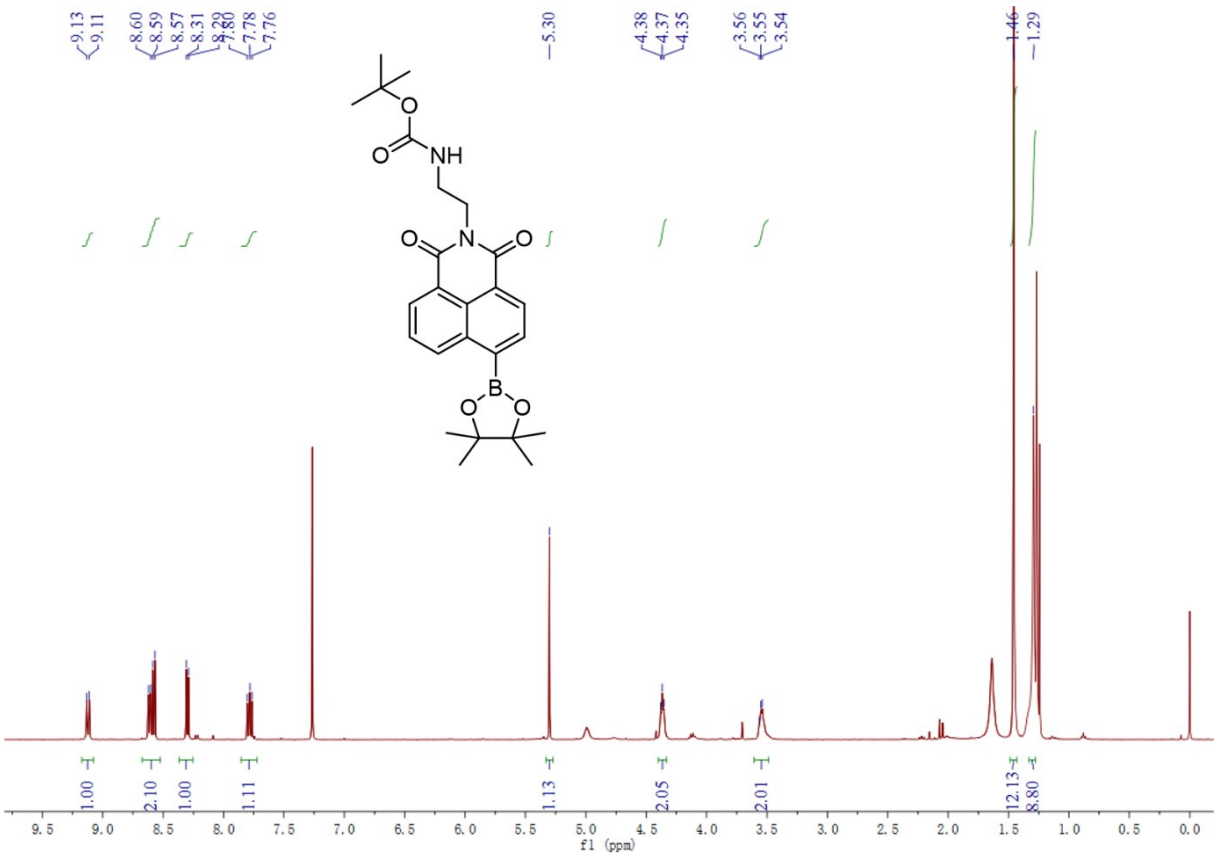


Fig. S4. ^{1}H NMR of Compound **5**.

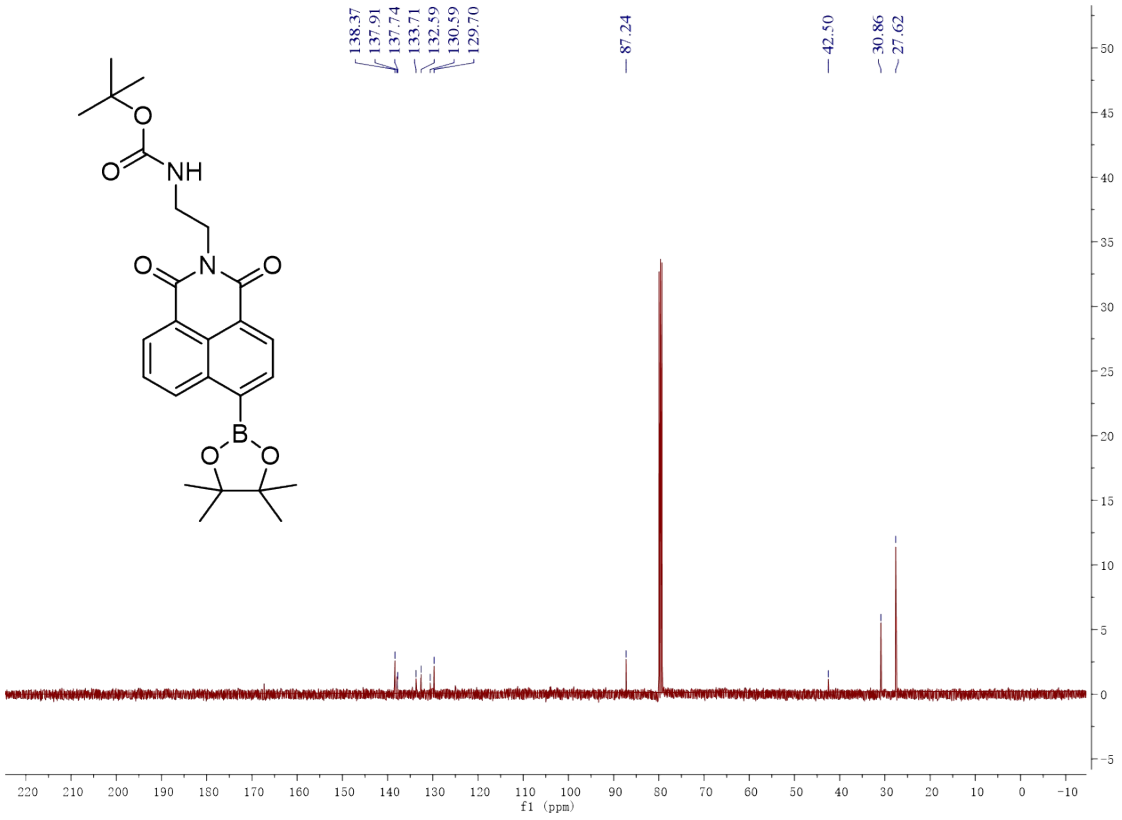


Fig. S5. ^{13}C NMR of Compound **5**.

Elemental Composition Report **Page 1**

Single Mass Analysis
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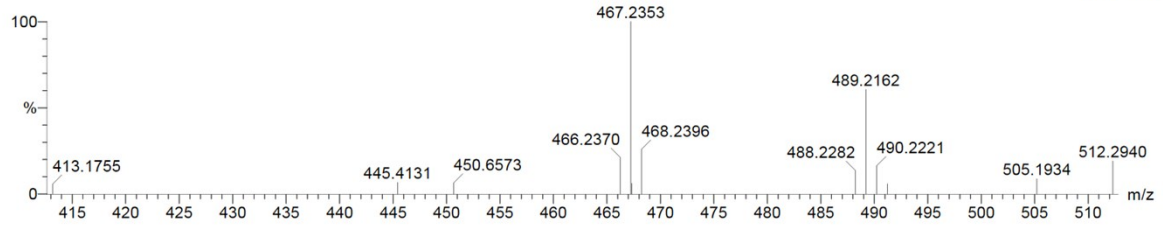
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
32 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-25 H: 0-32 N: 0-2 O: 0-6 B: 1-2

WH-ZHU ECUST institute of Fine Chem 13-Jun-2017
14:46:14
TH-ZX-001 28 (0.445) Cm (28) 1: TOF MS ES+
7.57e+002



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
467.2353	467.2353	0.0	0.0	11.5	14.8	0.0	C25 H32 N2 O6 B

Fig. S6. MS of Compound **5**.

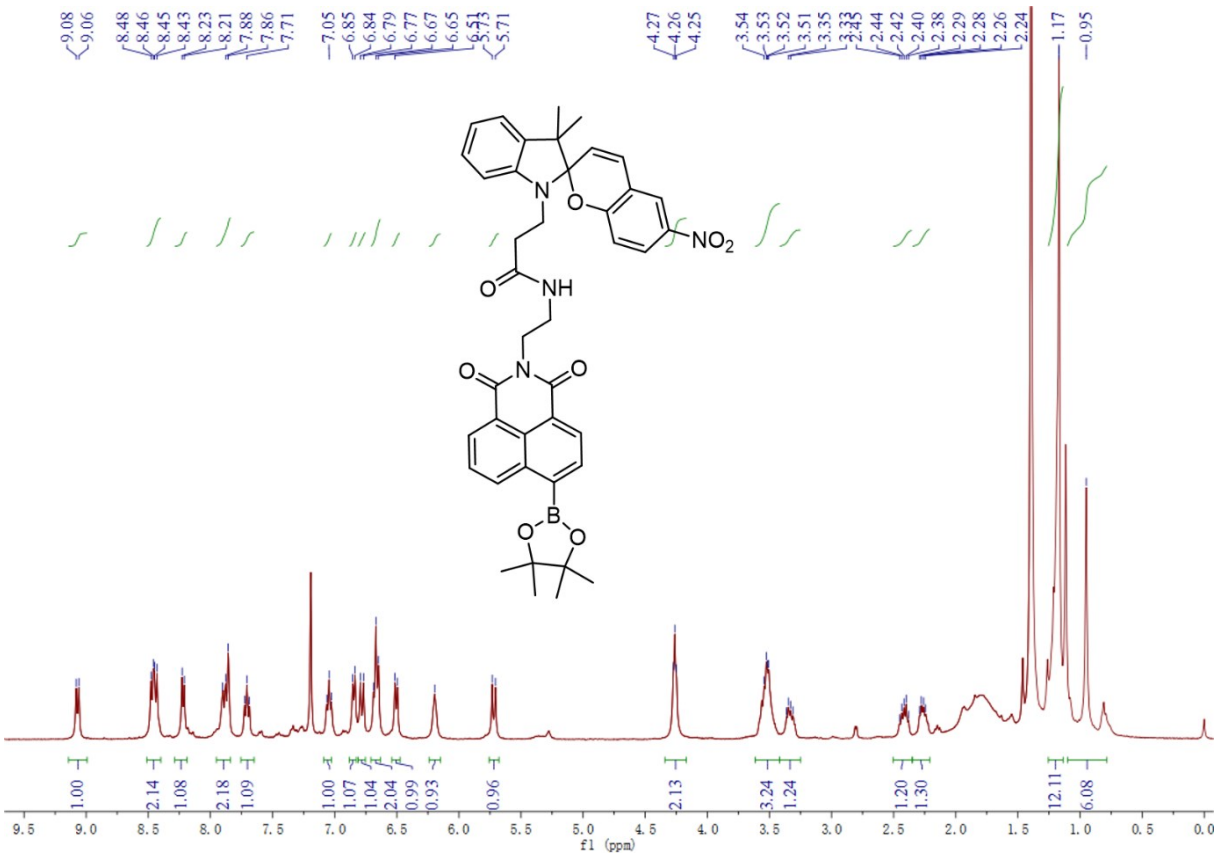


Fig. S7. ^{1}H NMR of Compound **SP-Np-B**.

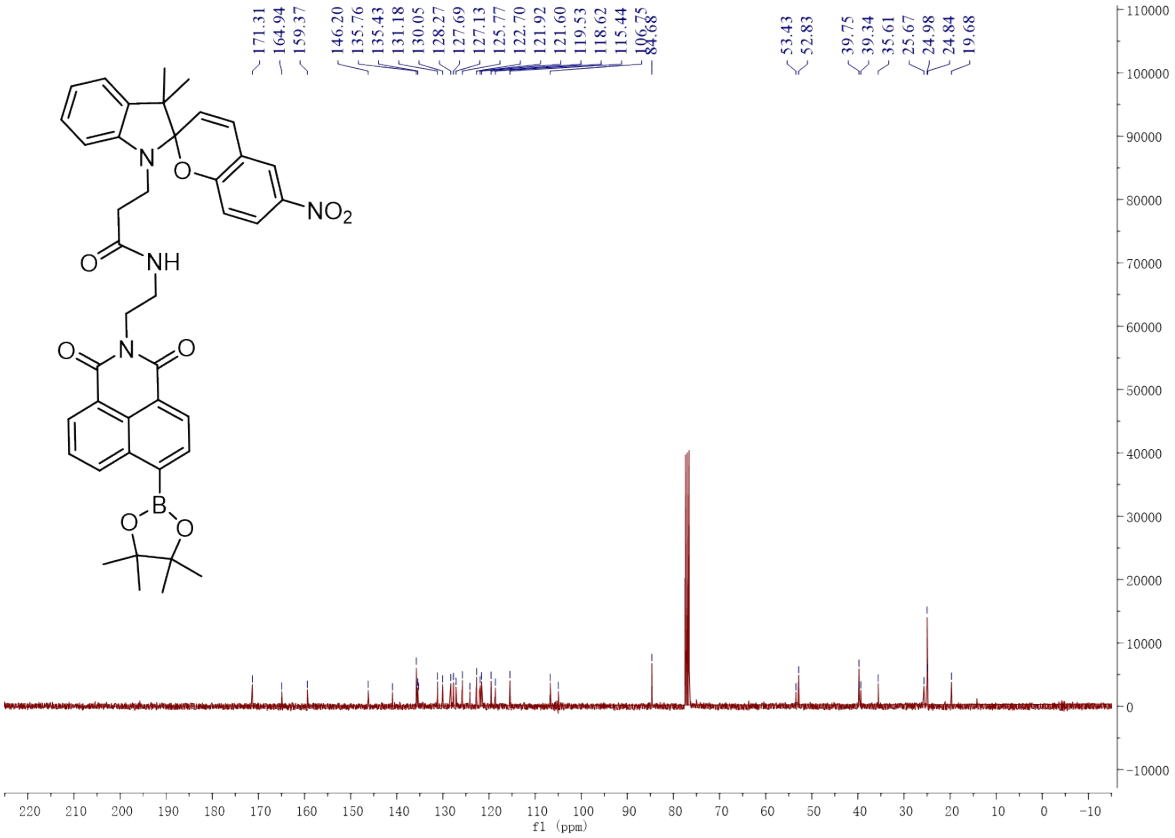


Fig. S8. ^{13}C NMR of Compound **SP-Np-B**.

Analysis Info

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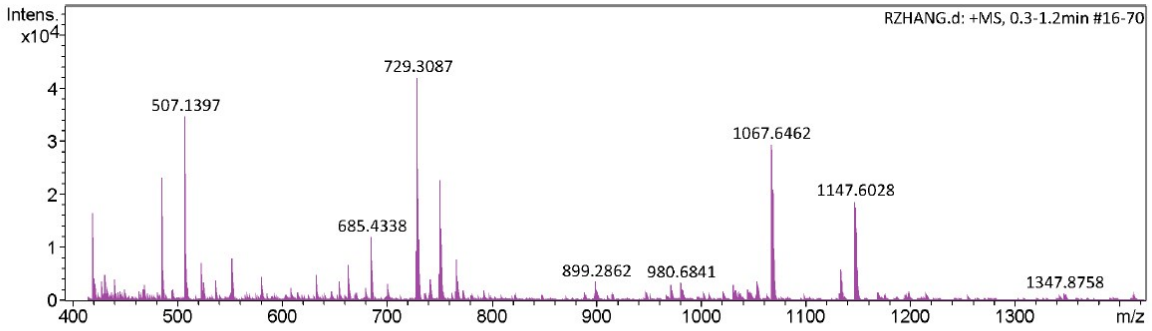
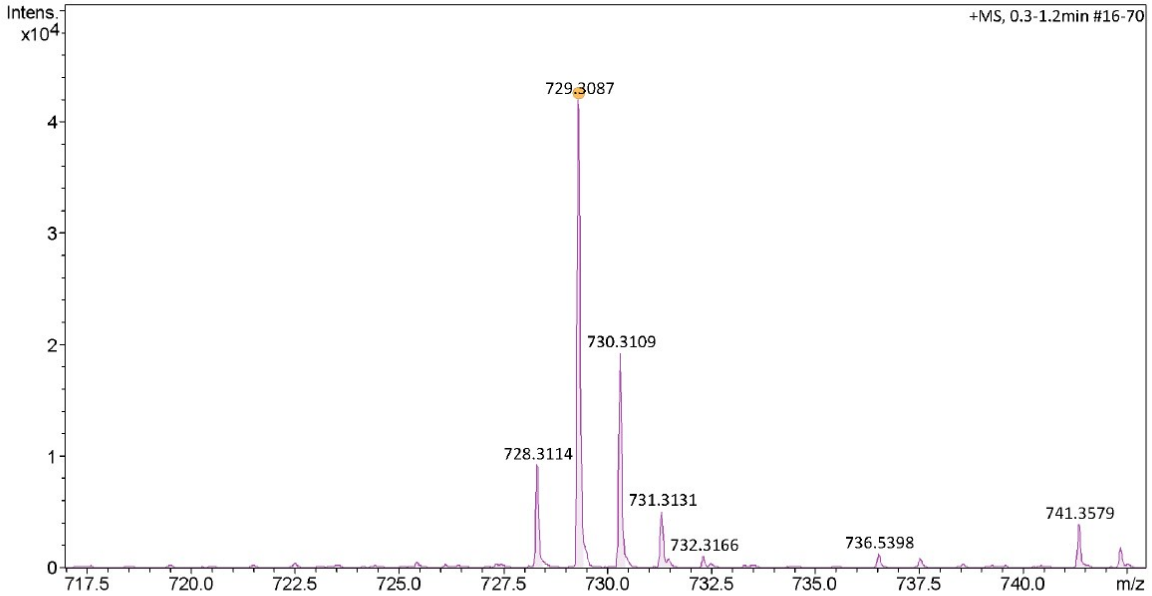
Method	Tune_Wide_Pos_2018_20180528.m	Operator	BDAL@DE
Sample Name		Instrument	micrOTOF-Q 228888.00070
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	3200 V	Set Dry Heater	180 °C
Scan Begin	420 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	550.0 Vpp	Set Divert Valve	Source





Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
729.3087	1	C41H42BN4O8	729.3090	1.4	7.0	1	100.00	23.5	even	ok
	1	C41H42BN4O8	729.3090	1.4	7.0	1	100.00	23.5	even	ok

Fig. S9. MS of Compound **SP-Np-B**.

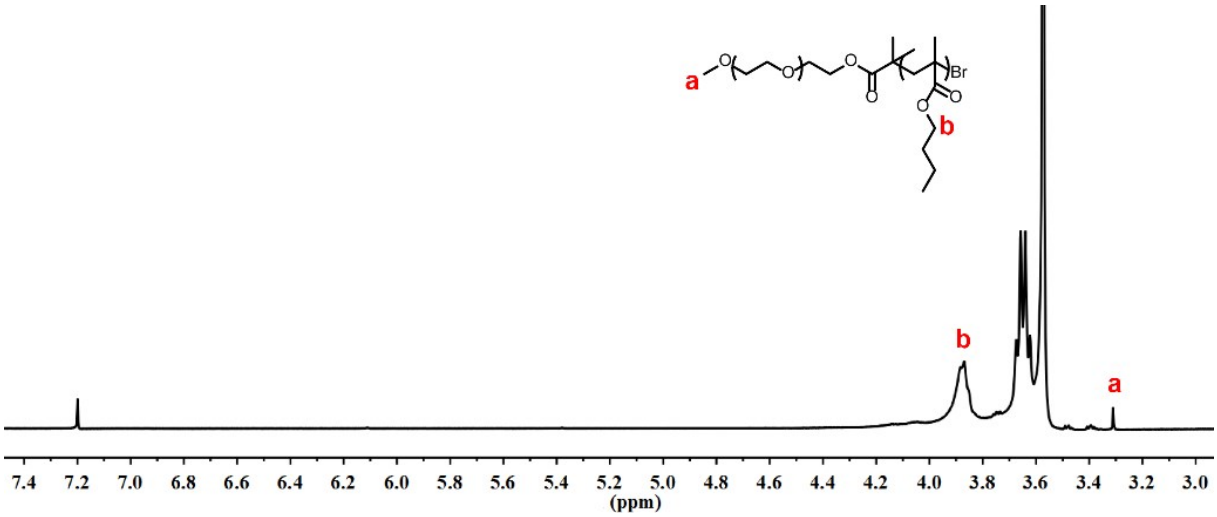


Fig. S10. ^{1}H NMR of mPEG-b-PBMA.

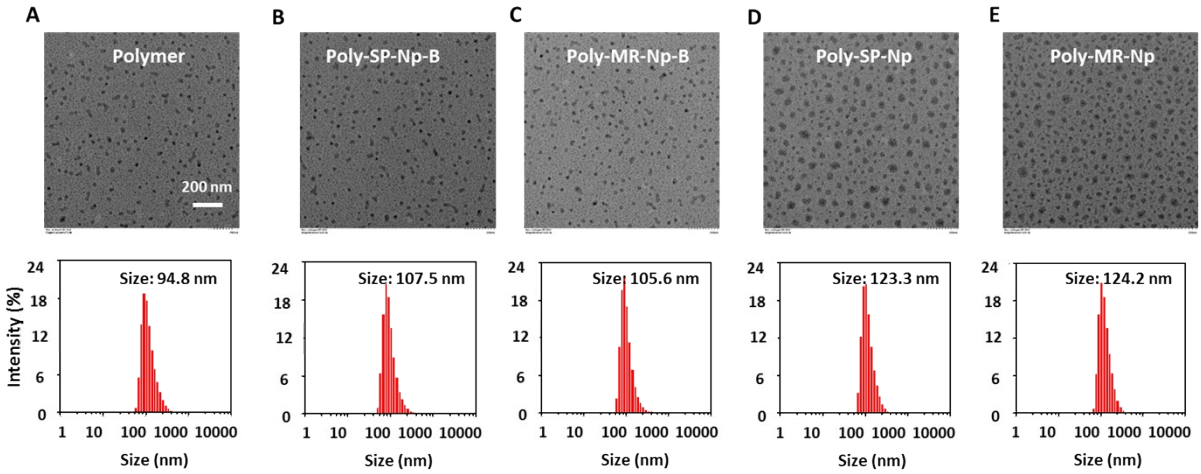


Fig. S11. TEM image and DLS for different states of nanoprobe. Polymer (A); Poly-SP-Np-B (B); Poly-MR-Np-B (C); Poly-SP-Np (D); Poly-MR-Np (E). The scale bar of TEM images is 200 nm.

S3. Light-controlled multiple colour fluorescence response in solution

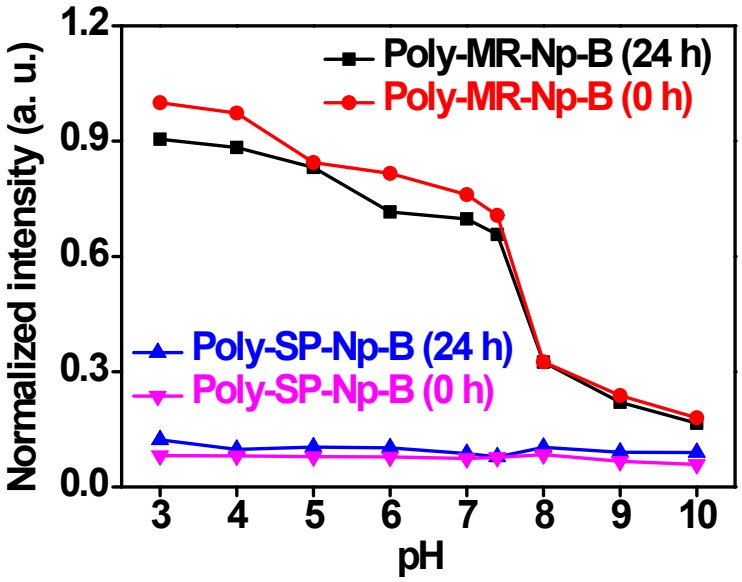


Fig. S12. pH stability of **Poly-SP-Np-B** and **Poly-MR-Np-B** (10 μM) with different pH and different hours at room temperature. All measurements were carried out in PBS buffer (λ_{ex} = 440 nm, λ_{em} = 605 nm).

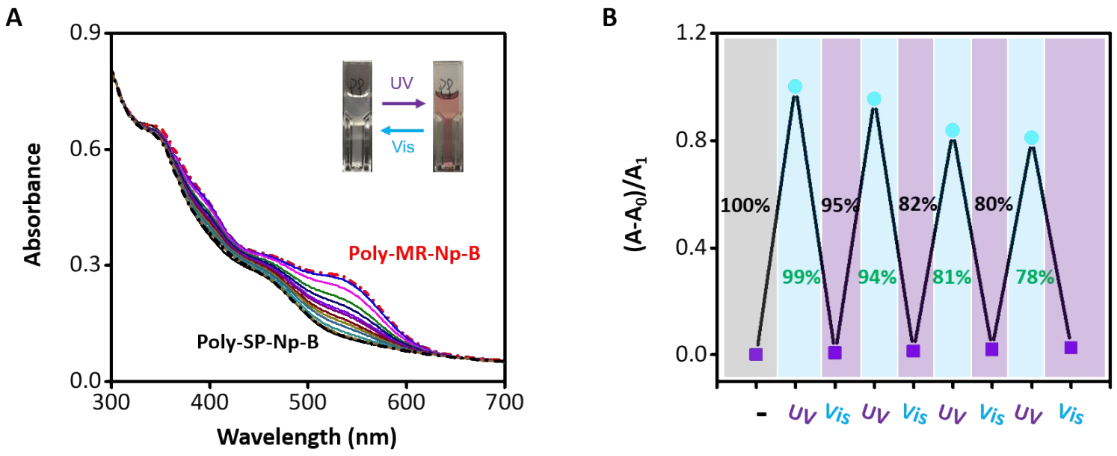
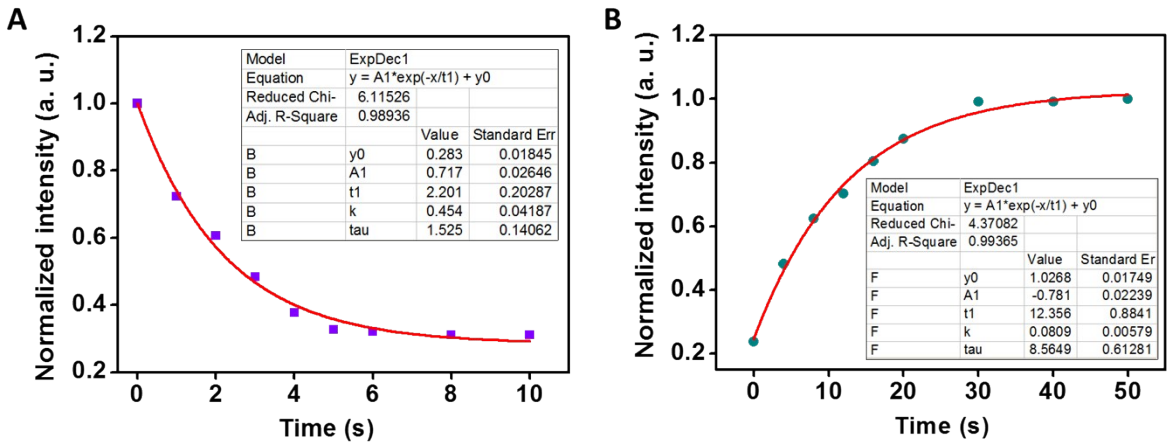


Fig. S13. UV-Vis absorption spectra of (A) **Poly-SP-Np-B** (10 μM) upon UV irradiation (365 nm) with time (0-10 s, interval: 1 s), and **Poly-MR-Np-B** (10 μM) upon visible (Vis) irradiation (550 nm) with time (0-20 s, interval: 2 s), and (B) UV-vis absorbance photo-switching (λ_{abs} = 540 nm) of **Poly-SP-Np-B** (A_0)/**Poly-MR-Np-B** (A_1) (10 μM). All measurements were carried out in 0.01 M PBS buffer of pH 7.4.



C

State	k (s^{-1})	R^2
SP → MR isomerisation	0.4543	0.9894
MR → SP isomerisation	0.0809	0.9937

Fig. S14. Isomerization kinetics of **Poly-SP-Np-B** to **Poly-MR-Np-B** (SP → MR) and **Poly-MR-Np-B** to **Poly-SP-Np-B** (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} = 440 nm, λ_{em} = 605 nm).

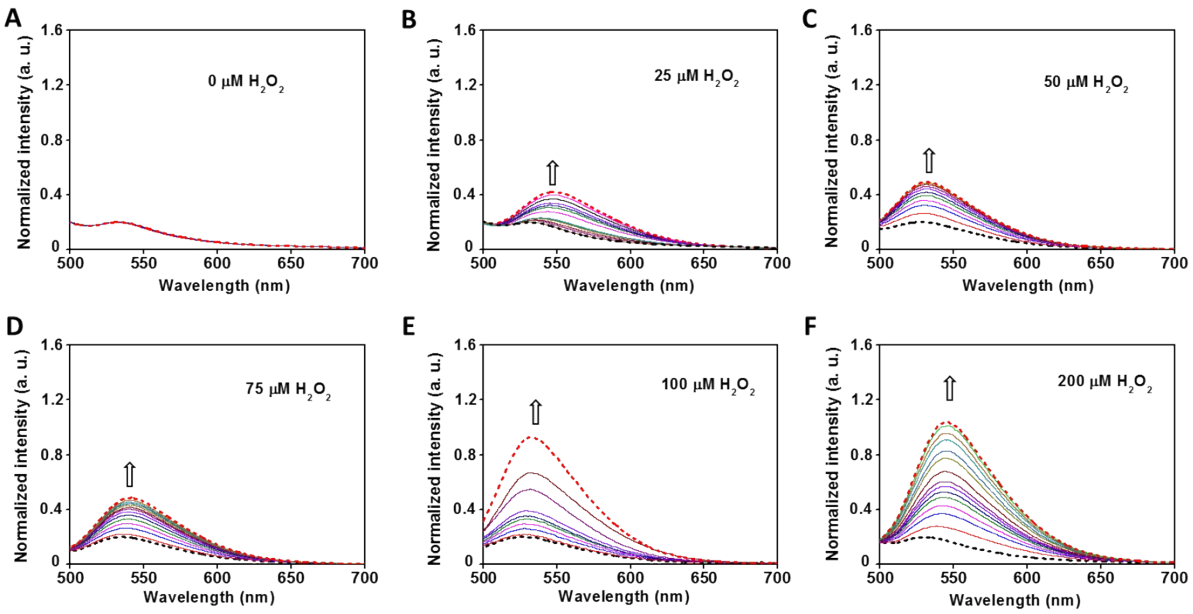


Fig. S15. Fluorescence spectra of **Poly-SP-Np-B** (10 μM) in the absence and presence of different concentration of H_2O_2 in 0.01 M PBS buffer of pH 7.4. The concentrations of H_2O_2 are: (A) 0 μM, (B) 25 μM, (C) 50 μM, (D) 75 μM, (E) 100 μM, and (F) 200 μM. All spectra were measured within 30 min with 2 min interval (λ_{ex} = 440 nm).

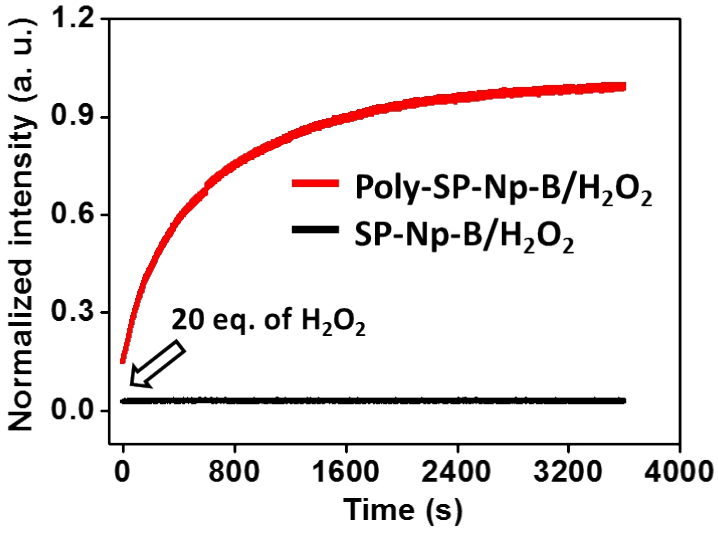


Fig. S16. Fluorescence response of probe **Poly-SP-Np-B** (conc. of SP-Np-B, 10 μM) and **SP-Np-B** (10 μM) with 20 equiv. of H_2O_2 in PBS buffer of pH 7.4.

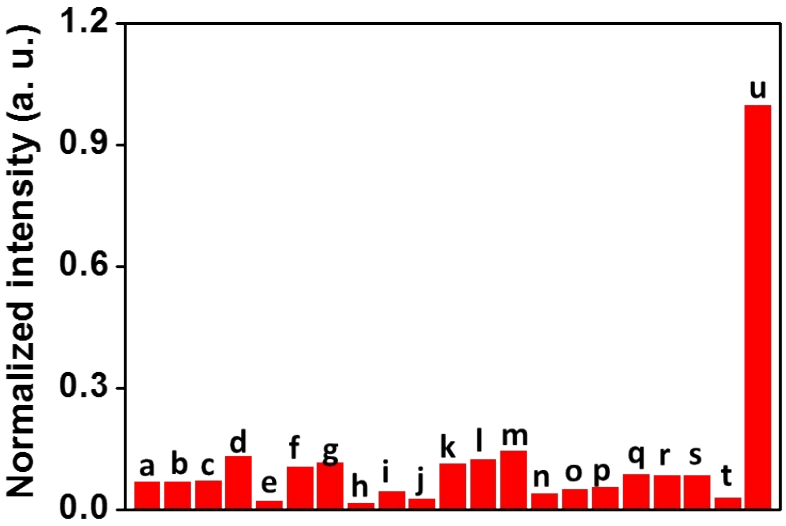


Fig. S17. Selective fluorescence response of **Poly-SP-Np-B** (10 μM) towards H_2O_2 over other species (500 μM), including probe only (a), Cys (b), Hcy (c), GSH (d), NaF (e), NaCl (f), NaBr (g), NaI (h), $NaHCO_3$ (i), NaHS (j), Na_2CO_3 (k), Na_2SO_4 (l), $ONOO^-$ (m), $Na_2S_2O_3$ (n), NaOCl (o), CH_3COONa (p), $NaNO_3$ (q), $NaNO_2$ (r), Na_2HPO_4 (s), NaH_2PO_4 (t) and 100 μM H_2O_2 (u). All measurements were performed in 0.01 M PBS buffer of pH 7.4 (λ_{ex} = 440 nm, λ_{em} = 531 nm).

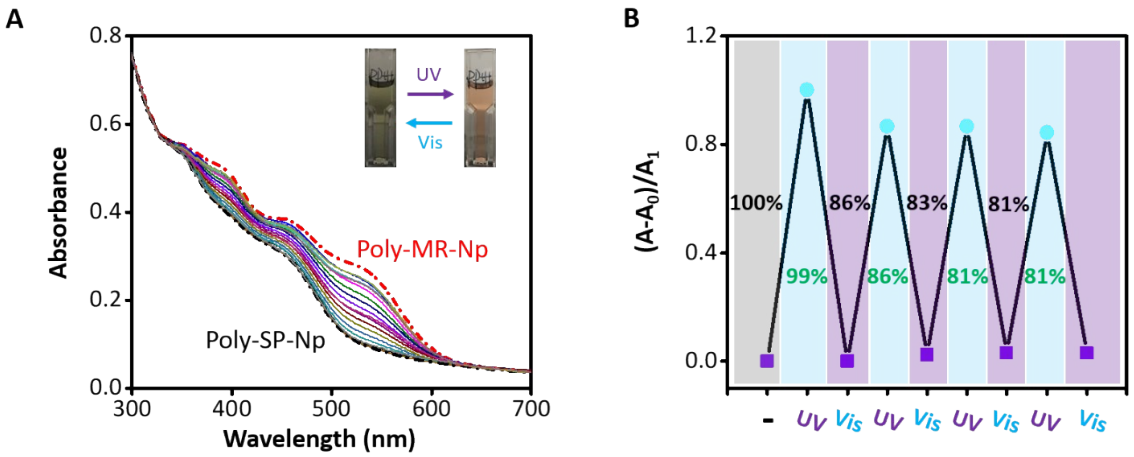


Fig. S18. UV-Vis absorption spectra of **Poly-SP-Np** (10 μM) upon UV irradiation (365 nm) with time (0-10 s, interval: 1 s) and **Poly-MR-Np** (10 μM) upon Vis irradiation (550 nm) with time (0-20 s, interval: 2 s) (A), and UV-Vis absorbance photo-switching (at 540 nm) of **Poly-SP-Np** (A_0)/**Poly-MR-Np** (A_1) (10 μM) (B). All measurements were carried out in 0.01 M PBS buffer of pH 7.4.

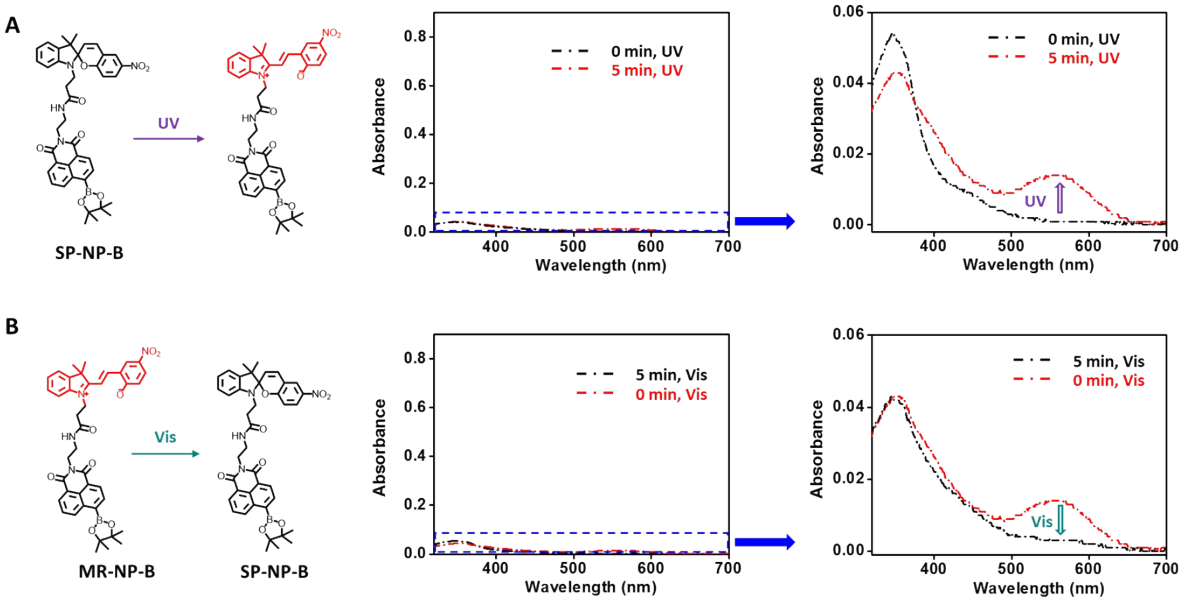


Fig. S19. UV-Vis absorption spectra of **SP-Np-B** (10 μM) upon UV irradiation (365 nm) with time (0-300 s,) (A), and **SP-Np-B** (10 μM) upon visible light irradiation (530 nm) with time (0-300 s) (B). All measurements were carried out in 0.01 M PBS buffer of pH 7.4 (containing 1% DMSO (v/v) as the co-solvent).

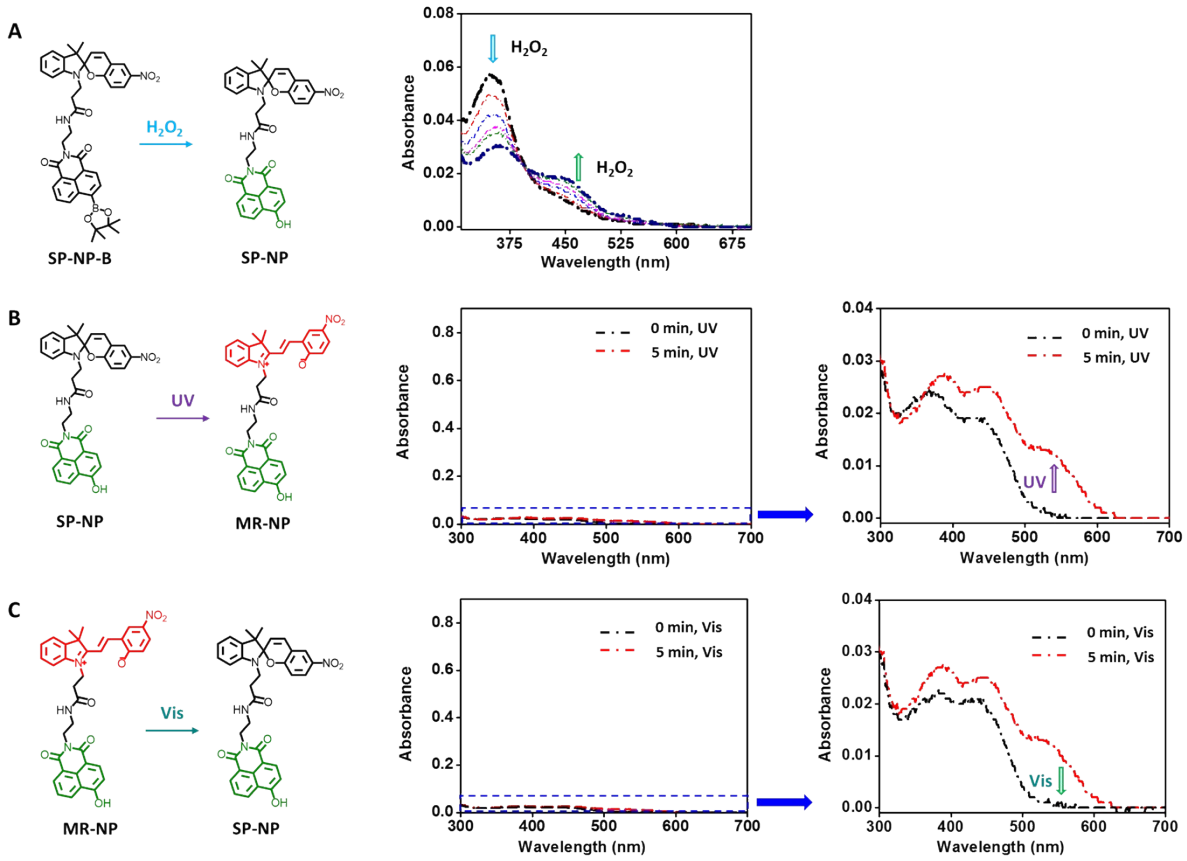


Fig. S20. UV-Vis absorption spectra of **SP-Np-B** (10 μM) with 500 μM H_2O_2 (A). The spectra were measured within 600 s (interval: 120 s). **SP-Np** (10 μM) upon UV irradiation (365 nm) with time (0-300 s) (B) and **MR-Np** (10 μM) upon visible light irradiation (530 nm) with time (0-300 s) (C). All measurements were carried out in 0.01 M PBS buffer of pH 7.4 (containing 1% DMSO (v/v) as co-solvent).

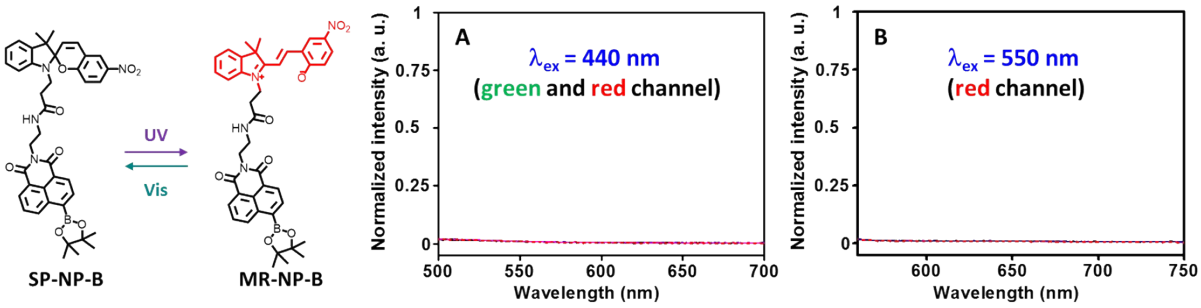


Fig. S21. Fluorescence spectra of **SP-Np-B** (10 μM) upon UV light irradiation (365 nm, UV irradiation time: 0-300 s, interval: 30 s) and visible light irradiation (550 nm, Vis irradiation time: 0-300 s; interval: 60 s). Excitation was performed at 440 nm (A) for spectra of both green and red channel and at 550 nm (B) for red channel emission spectra. All measurements were carried out in 0.01 M PBS buffer of pH 7.4 (containing 1% DMSO (v/v) as the co-solvent).

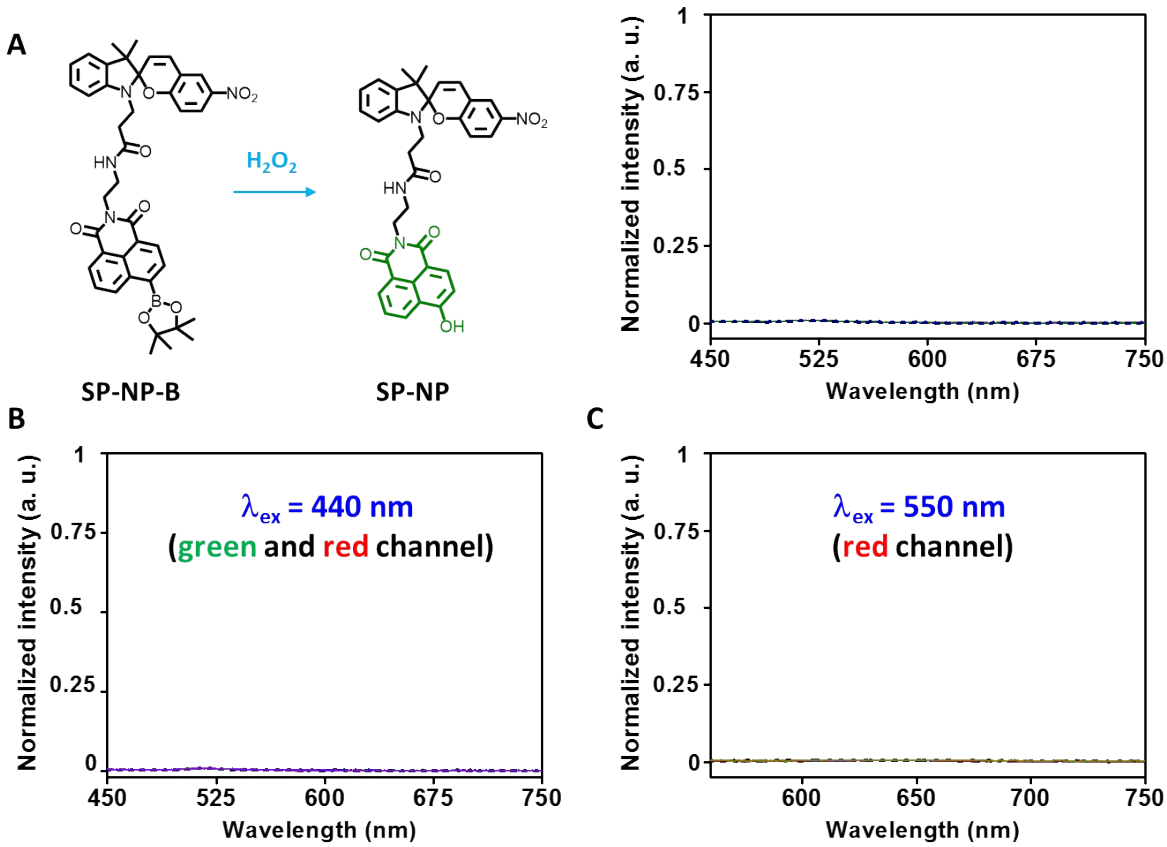


Fig. S22. Fluorescence spectra of **SP-Np-B** (10 μM) with 500 μM H_2O_2, with time (0-600 s; interval: 60 s) (λ_{ex} = 440 nm) (A). UV light irradiation (365 nm, UV irradiation time: 0-300 s, interval: 30 s) and visible light irradiation (550 nm, Vis irradiation time: 0-300 s; interval: 60 s) were then conducted on the product of **SP-Np-B** (10 μM) reacting with H_2O_2. Excitation was performed at 440 nm (B) for spectra of both green and red channel and at 550 nm (C) for red channel emission spectra. All measurements were carried out in 0.01 M PBS buffer of pH 7.4 (containing 1% DMSO (v/v) as the co-solvent).

S4. Light-controlled multiple colour fluorescence imaging

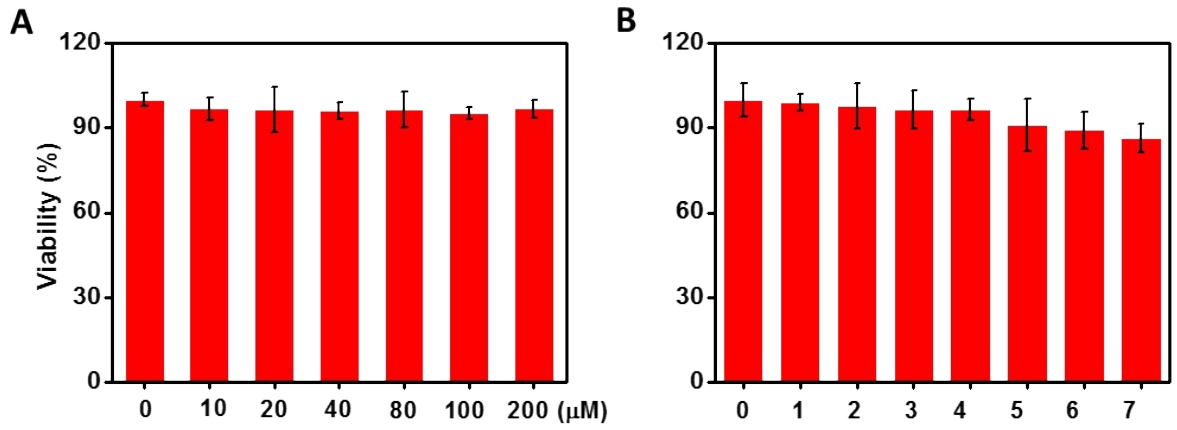


Fig. S23. MTT cytotoxicity assay. Viability of Raw 264.7 cell in the presence of increasing **Poly-SP-Np-B** (A). Viability of Raw 264.7 cell with **Poly-SP-Np-B** (10 μM) under 1-7 alternate UV/Vis irradiation cycles (B).

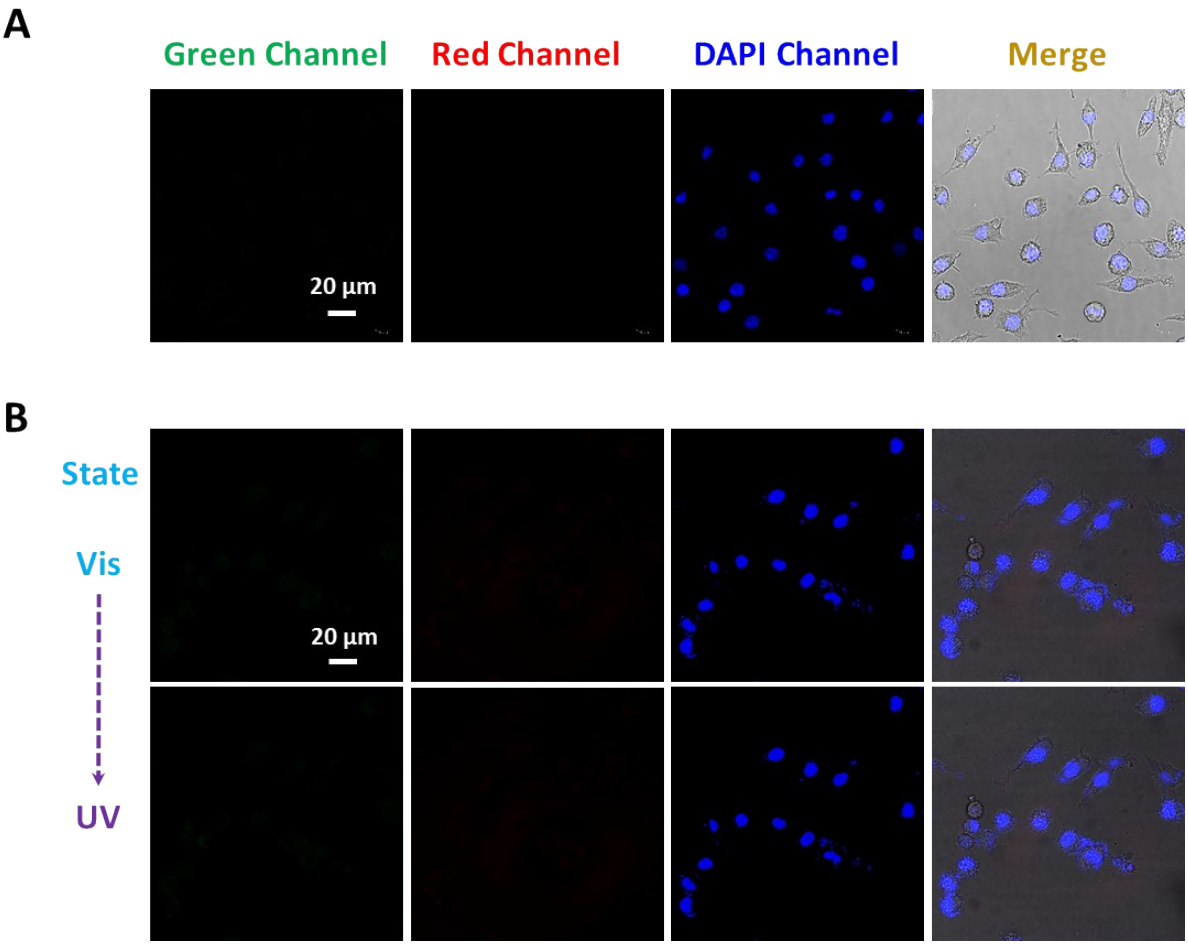


Fig. S24. Fluorescent imaging of RAW 264.7 macrophage cells incubated with polymer nanoparticle (A) and SP-Np-B (10 μM) only (B). The SP-Np-B internalized RAW 264.7 macrophage cells were then irradiated by UV (365 nm) and visible (550 nm) light alternatively. Scale bar is 20 μm.

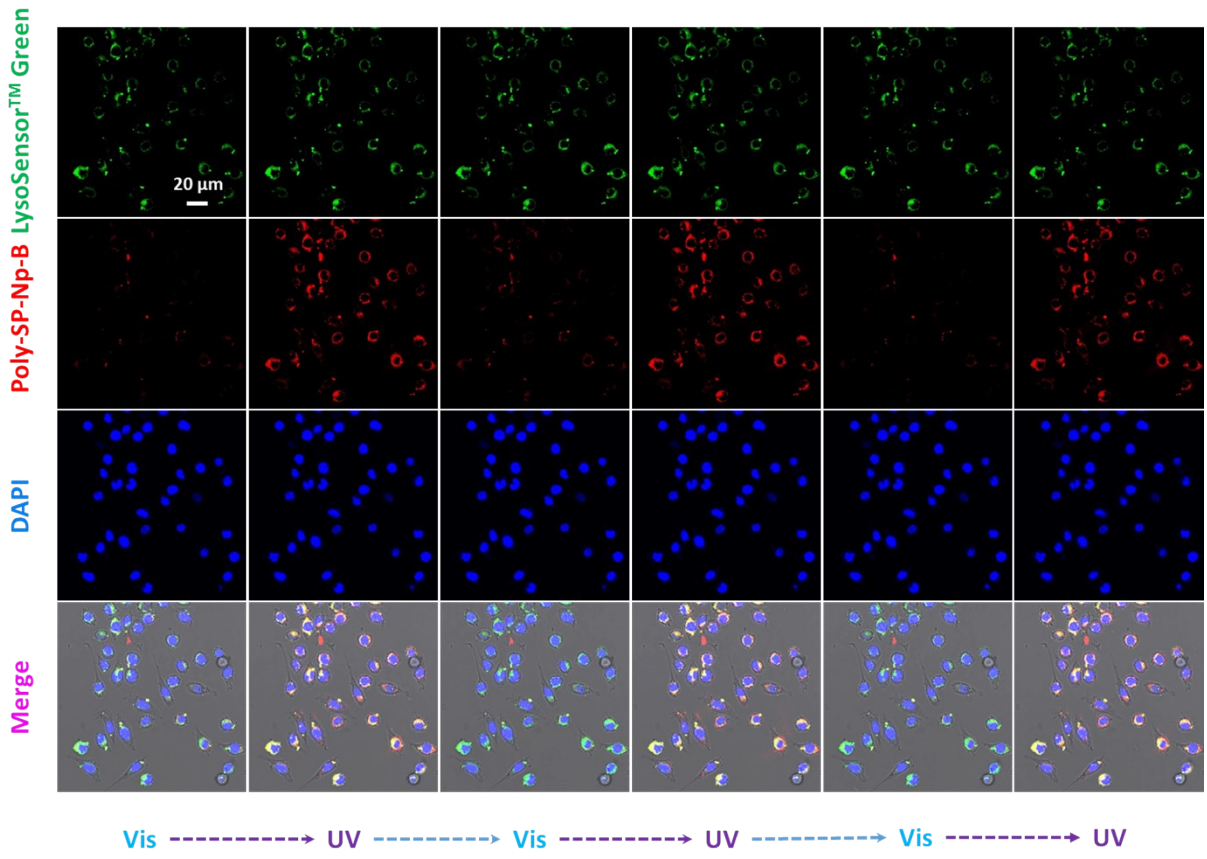


Fig. S25. UV/Vis cycling and colocalisation of **Poly-SP-Np-B** with LysoSensor™ Green in RAW 264.7 macrophage cells. The **Poly-SP-Np-B** and LysoSensor™ Green loaded RAW 264.7 macrophage cells were then irradiated by alternate UV/vis light irradiation for 10 s and 20 s, respectively. Scale bar is 20 μm.

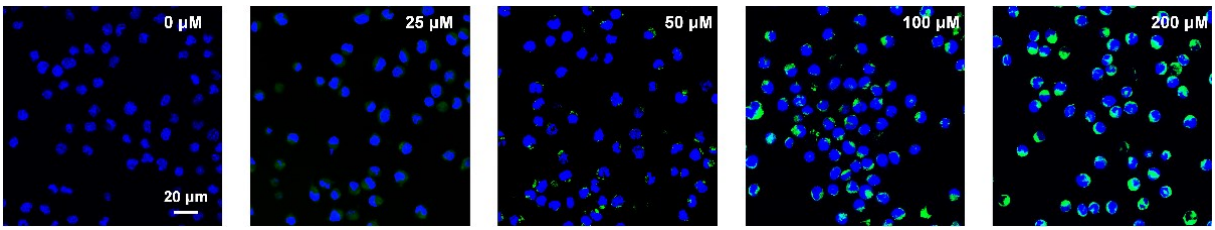


Fig. S26. Fluorescence imaging of RAW 264.7 macrophage cells incubated with **Poly-SP-Np-B** (10 μM) and treated with H_2O_2 (0, 25, 50, 100, 200 μM). The cells were then stained with DAPI prior to confocal fluorescence imaging. Scale bar is 20 μm.

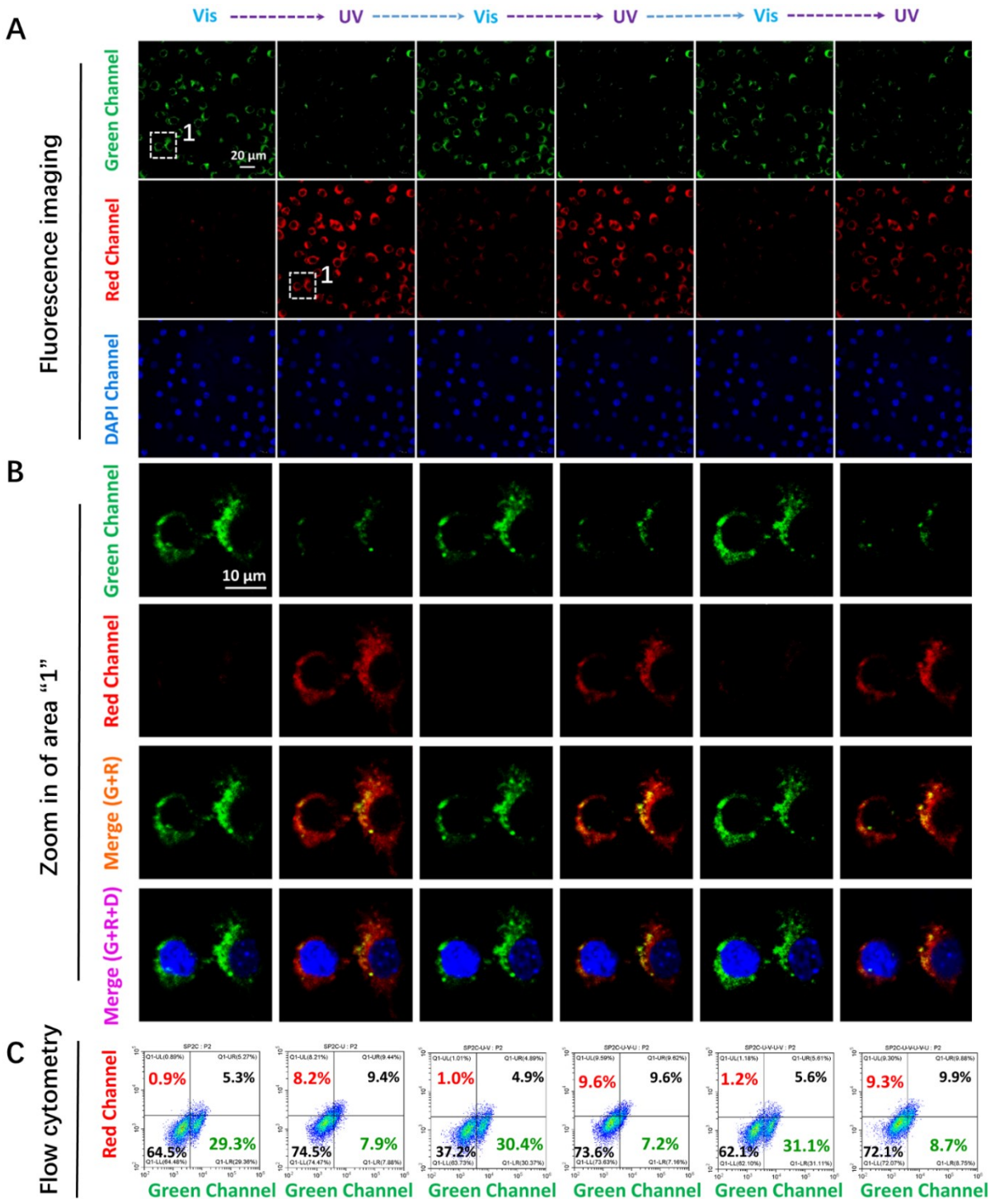


Fig. S27. UV/Vis cycling light-controlled dual colour fluorescence imaging and flow cytometry analysis of RAW 264.7 macrophage cells incubated with **Poly-SP-Np-B** followed by treatment with exogenous H_2O_2 (100 μM). RAW 264.7 macrophage cells were incubated with **Poly-SP-Np-B** (10 μM) for 2 h, and then treated with exogenous H_2O_2 (100 μM) for 30 min before alternate UV/vis light irradiation (A, B). The irradiation sequence is

Vis→UV→Vis→UV→Vis→UV. Scale bars are 20 μm (A) and 10 μm (B). The macrophage cells at each state were collected for flow cytometry analysis (C).

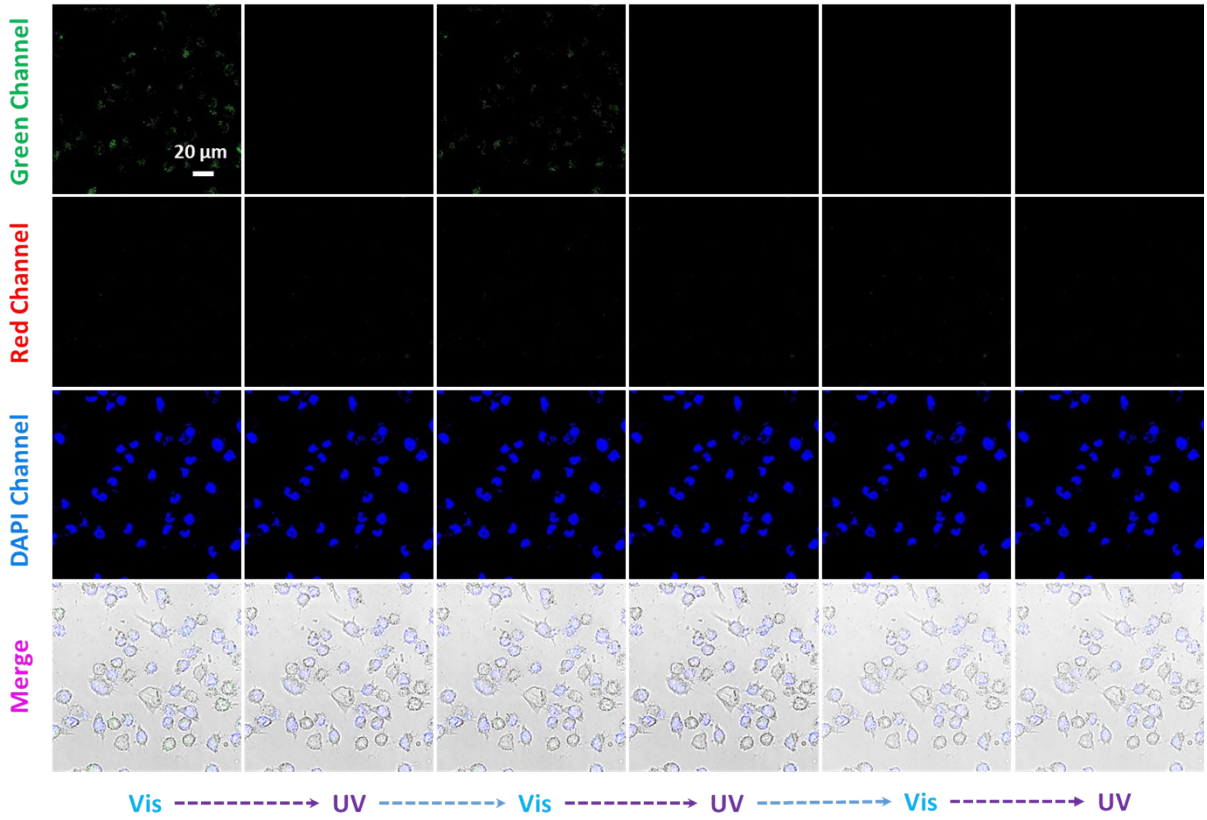


Fig. S28. UV/Vis cycling of RAW 264.7 macrophage cells incubated with SP-Np-B (10 μM) and then treated with exogenous H_2O_2 (100 μM). The cells were then subjected to UV/vis light irradiation alternatively. The irradiation sequence is Vis→UV→Vis→UV→Vis→UV. Scale bar is 20 μm.

S5. References

[1] Wang, T.; Wang, D.; Yu, H.; Wang, M.; Liu, J.; Feng, B.; Zhou, F.; Yin, Q.; Zhang, Z.; Huang, Y.; Li, Y., Intracellularly Acid-Switchable Multifunctional Micelles for Combinational Photo/Chemotherapy of the Drug-Resistant Tumor. *ACS Nano* **2016,** *10* (3), 3496-508.

[2] Yu, H.; Guo, C.; Feng, B.; Liu, J.; Chen, X.; Wang, D.; Teng, L.; Li, Y.; Yin, Q.; Zhang, Z.; Li, Y., Triple-Layered pH-Responsive Micelleplexes Loaded with siRNA and Cisplatin Prodrug for NF-Kappa B Targeted Treatment of Metastatic Breast Cancer. *Theranostics* **2016,** *6* (1), 14-27.

[3] Wang, D.; Wang, T.; Liu, J.; Yu, H.; Jiao, S.; Feng, B.; Zhou, F.; Fu, Y.; Yin, Q.; Zhang, P.; Zhang, Z.; Zhou, Z.; Li, Y., Acid-Activatable Versatile Micelleplexes for PD-L1 Blockade-Enhanced Cancer Photodynamic Immunotherapy. *Nano Lett.* **2016,** *16* (9), 5503-13.

[4] Fu, Y.; Han, H. H.; Zhang, J.; He, X. P.; Feringa, B. L.; Tian, H., Photocontrolled Fluorescence "Double-Check" Bioimaging Enabled by a Glycoprobe-Protein Hybrid. *J. Am. Chem. Soc.* **2018,** *140* (28), 8671-8674.

[5] Zhang, J.; Fu, Y.; Han, H. H.; Zang, Y.; Li, J.; He, X. P.; Feringa, B. L.; Tian, H., Remote light-controlled intracellular target recognition by photochromic fluorescent glycoprobes. *Nat. Commun.* **2017,** *8* (1), 987.

[6] Duan, Q.; Jia, P.; Zhuang, Z.; Liu, C.; Zhang, X.; Wang, Z.; Sheng, W.; Li, Z.; Zhu, H.; Zhu, B.; Zhang, X., Rational Design of a Hepatoma-Specific Fluorescent Probe for HOCl and Its Bioimaging Applications in Living HepG2 Cells. *Anal. Chem.* **2019,** *91* (3), 2163-2168.