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Supporting Information

Spiropyran in Nanoassemblies as a Photosensitizer for Photoswitchable ROS Generation in Living Cells

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Experimental section

1. Materials and Methods

All reagents were commercially available and used as received without further purification unless otherwise stated. Poly(ethylene glycol) methacrylate (PEGMA, Mn ~ 360 Da), branched polyethylenimine (bPEI, 25 kDa) were all from Sigma Aldrich, PEGMA was passed through a basic alumina column to remove inhibitor and then stored at –20 °C before use. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from absolute ethanol and stored at –20 °C before use. N,N-Dimethylformamide (DMF) was distilled over phosphorus pentoxide (P_2O_5) and then distilled just before use. Fetal bovine serum (FBS) was purchased from Biological Industries. The Luciferase reporter plasmid DNA (pDNA) was amplified in DH5α strain of *Escherichia coli* DH5α and prepared by EndoFree Plasmid Mega Kit (5) (QIAGEN, Germany). HeLa cells were obtained from American Type Culture Collection.

NMR spectra were recorded on a Bruker AMX 400 spectrophotometer with use of the residual solvent or TMS as the internal reference. The molecular weight and molecular distribution measurements were performed using GPC device with DMF as eluent at a flow rate of 1.0 mL min^{-1} at 35 °C and with a Waters 244 equipped with internal refractive index (RI) detector and a PL1110-6504 column, calibrated in relation to polyethylene glycol standards. Atomic force microscopy (AFM) images were collected on a Bruker Dimension ICON (Bruker, USA). Particle sizes and ζ-potential values of polyplexes were measured on Zetasizer nanoseries (Nano zs90, Malvern Instruments Ltd., U.K.). Ultraviolet-visible (UV-vis) spectra and fluorescent emission spectra were taken on a Shimadzu UV-2600 spectrophotometer and Hitachi F-7000 fluorimeter respectively. *In vitro* cell images were acquired by an inverted fluorescence microscope (Nikon TE2000-U, Japan) or confocal laser scanning microscopy (CLSM, LSM 710, Zeiss, Germany). 300 W Xe lamp (CEAULIGHT, China) and LED lamp (pE-4000, CoolLED Ltd., U.K.) were used as light sources. Flow cytometric analysis was performed on BD FACS Calibur (BD Biosciences) and

results were analyzed using FlowJo software (Tree Star, Ashland, OR).

2. Synthesis of Monomer and Polymer

2.1 Synthesis of hydrophobic monomer 1'-(2-methacryloxyethyl)-3',3'-dimethyl-6-nitro-spiro-(2H-1-benzopyran-2',2'-indoline) (SPMA)

Monomer SPMA was synthesized according to a previously reported literature.[1,2] A typical synthetic route to SPMA is shown in Figure S1. In brief, SPOH (2.0 g, 5.68 mmol) was dissolved in cold anhydrous dichloromethane (DCM, 30 mL) under argon, triethylamine (Et_3N, 1.27 g, 12.6 mmol) was added and the mixture was protected against exposure to visible light. Methacryloyl chloride (1.19 g, 11.3 mmol) in anhydrous DCM (10 mL) was then added dropwise over 0.5 h. The mixture was allowed to stir at room temperature overnight. After removal of solvent under vacuum, the residue was purified through flash silica gel column chromatography with hexane-DCM (v/v, 1/2) as eluent to afford compound SPMA as a pale yellow solid (1.08 g, 45% yield).

SPMA, ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.98 – 8.06 (m, 2H), 7.17 – 7.24 (m, 1H), 7.10 (d, 1H), 6.87 (q, 2H), 6.68 – 6.77 (m, 2H), 6.07 and 5.57 (d, 2H), 5.86 (d, 1H), 4.3 (t, 2H), 3.28 – 3.51 (m, 2H), 1.90 (s, 3H), 1.30 (s, 3H), 1.16 (s, 3H).

2.2 Synthesis of 2-(4-tert-butyloxycarbonyl imidazolyl)ethyl methacrylamide (Boc-HMA)

A typical synthetic route to Boc-HMA is shown in Figure S2. Histamine dihydrochloride (1.84 g, 10.0 mmol) and NaOH (1.2 g, 30.0 mmol) was dissolved in 20 mL of distilled water in a single-necked 100 mL flask equipped with a stirrer and a 25 mLdropping funnel and cooled in an ice bath. A solution of methacryloyl chloride (1.05 g, 10.0 mmol) in DCM (10 mL) was added dropwise under vigorous stirring over a period of 1 h. The reaction mixture was warmed to room temperature and stirred vigorously for additional 4 h. After removal of DCM, the aqueous phase was collected and freeze-dried. The residue was re-dissolved in 40 mL of absolute isopropanol and filtered. The filtrate was concentrated under reduced pressure, added

dropwise to a large volume of pre-cooled hexane, and stored at 0 ~ 4 °C overnight. A white crystal precipitate was collected by filtration, washed with a cold mixed solvent of isopropanol and hexane, dried under vacuum, and afforded the white solid (2-(4-imidazolyl)ethyl methacrylamide, HMA, 0.99 g, 55% yield).

HMA, ^{1}H NMR (400 MHz, D_2O), δ (ppm): 7.72 (s, 1H), 6.92 (s, 1H), 5.57 and 5.38 (d, 2H), 3.49 (t, 2H), 2.82 (t, 2H), 1.86 (s, 3H).

HMA, ^{13}C NMR (101 MHz, D_2O), δ (ppm): 168.4, 135.69, 134.6, 129.83, 127.01, 116.76, 39.04, 25.81, 23.6.

HMA (0.9 g, 5 mmol) and Et_3N (0.6 g, 6 mmol) were dissolved in DMF (15 mL) and cooled in an ice bath. Di-*tert*-butyl dicarbonate ($(Boc)_2O$, 1.2 g, 5.5 mmol) was added and the solution was stirred overnight at room temperature. Pre-cooled water (45 mL) was added slowly and the solution was extracted with chloroform ($CHCl_3$, 3 × 15 mL). The combined organic phases were dried over anhydrous sodium sulfate and evaporated. The crude product was purified by flash silica gel column chromatography eluted with ethyl acetate-hexane (v/v, gradient 1:1 to 1:0) to afford Boc-HMA as clear oil (Boc-HMA, 0.98 g, 70% yield).

Boc-HMA, ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.03 (s, 1H), 7.16 (s, 1H), 5.73 and 5.31 (d, 2H), 3.59 (dt, 2H), 2.78 (t, 2H), 1.97 (s, 3H), 1.62 (s, 9H).

Boc-HMA, ^{13}C NMR (101 MHz, $CDCl_3$), δ (ppm): 168.3, 146.94, 141.1, 139.97, 136.77, 119.53, 113.73, 85.67, 39.19, 27.89, 26.40, 18.64.

Boc-HMA, HRMS, calculated for $[M+H]^+$: 280.1661, found $[M+H]^+$: 280.1656.

2.3 Synthesis of copolymer poly(HMA-st-PEGMA-st-SPMA) (P1 ~ P3)

P1 ~ **P3** precursors were synthesized by traditional free radical copolymerization. A typical procedure for the synthesis of **P3** precursor using AIBN as initiator was described below. To a 5-mL single-necked flask equipped with a magnetic stir bar, Boc-HMA (23 mg, 0.082 mmol), PEGMA (49.5 mg, 0.137 mmol), and SPMA (23.1 mg, 0.055 mmol) were dissolved in anhydrous DMF (0.5 mL) and then AIBN (0.76 mg, 0.005 mmol) was added to the mixture. The reaction mixture was allowed to stir until it became homogeneous. The solution was purged with argon for 45 min. The

flask was sealed and heated at 70 °C. The polymerization was conducted for 24 h in the dark, and quenched by immersion of the reaction flask into frozen liquid (–30 °C). The mixture was precipitated three times into pre-cooled ether (25 mL), and centrifuged at 8000 rpm for 15 min. The copolymer was dried in high vacuum at 40 °C and obtained as a brown solid (51 mg, 53.4%). **P1** and **P2** precursors were synthesized in the same procedure.

poly(Boc-HMA-*st*-PEGMA-*st*-SPMA), ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.10 – 7.90 (NC*H*N, C*H*C(NO_2)C*H*), 7.25 – 6.60 (NC*H*CCH_2, NCC*H*C*H*C*H*C*H*C, C*H*C(O)CC*H*), 5.95 – 5.80 (NC(O)C*H*CH), 4.20 – 3.80 (OCOC*H*$_2$CH_2), 3.75 – 3.50 (OCOCH_2C*H*$_2$O(C*H*$_2$C*H*$_2$)n, CH_2C*H*$_2$N), 2.75 – 2.55 (OCNHC*H*$_2$C*H*$_2$CH), 2.15 – 1.70 (C*H*$_2$ of the polymer backbone), 1.70 – 1.55 (C*H*$_3$ of the Boc group), 1.45 – 0.70 (C*H*$_3$ of the polymer backbone and spiropyran group).

De-protection of the polymer precursors was conducted following a literature procedure.[3] Typically the polymer precursor (40 mg) was dissolved in DCM (0.5 mL) while cooling in an ice bath. Trifluoroacetic acid (TFA, 0.5 mL) was added dropwise into the solution. After 3 h of reaction at room temperature, solvent was removed by bubbling with nitrogen and dialyzed (MWCO = 7000 Da) against HEPES buffer solution (10 mM, pH 7.0) to give cationic polymer.

poly(HMA-*st*-PEGMA-*st*-SPMA), ^{1}H NMR (400 MHz, D_2O), δ (ppm): 8.75 – 8.50 (NC*H*N, C*H*C(NO_2)C*H*), 7.50 – 6.00 (NC*H*CCH_2, NCC*H*C*H*C*H*C*H*C, C*H*C(O)CC*H*, NC(O)C*H*CH), 4.65 – 2.80 (OCNHC*H*$_2$C*H*$_2$CH, OCOC*H*$_2$CH_2, OCOCH_2C*H*$_2$N, OCOCH_2C*H*$_2$O(C*H*$_2$C*H*$_2$)n), 2.05 – 1.70 (C*H*$_2$ of the polymer backbone), 1.20 – 0.50 (C*H*$_3$ of the polymer backbone and spiropyran group).

2.4 Synthesis of copolymer poly(HMA-st-PEGMA) (P4)

A typical procedure for the synthesis of **P4** precursor using AIBN as initiator was described below. To a 5-mL single-necked flask equipped with a magnetic stir bar, Boc-HMA (33 mg, 0.118 mmol), PEGMA (42.6 mg, 0.118 mmol) were dissolved in anhydrous DMF (0.5 mL) and then AIBN (0.65 mg, 0.004 mmol) was added to the mixture. The reaction mixture was allowed to stir until it became homogeneous. The

solution was purged with argon for 45 min. The flask was sealed and heated at 70 °C. The polymerization was conducted for 24 h in the dark, and quenched by immersion of the reaction flask into frozen liquid (–30 °C). The mixture was precipitated three times into pre-cooled ether (25 mL), and centrifuged at 8000 rpm for 15 min. The copolymer was dried in high vacuum at 40 °C and obtained as a white solid (45 mg, 59.5%).

poly(Boc-HMA-*st*-PEGMA), ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.05 – 7.97 (NC*H*N), 7.24 – 7.10 (NC*H*CCH_2), 4.20 – 3.55 (OCH_2CH_2O of the PEG side chain), 2.75 – 2.60 (OCNHCH_2CH_2CH), 2.20 – 1.75 (CH_2 of the polymer backbone), 1.68 – 1.55 (CH_3 of the Boc group), 1.20 – 0.77 (CH_3 of the polymer backbone).

De-protection of the polymer precursors was conducted following a literature procedure.[3] Typically the polymer precursor (40 mg) was dissolved in DCM (0.5 mL) while cooling in an ice bath. Trifluoroacetic acid (TFA, 0.5 mL) was added dropwise into the solution. After 3 h of reaction at room temperature, solvent was removed by bubbling with nitrogen and dialyzed (MWCO = 7000 Da) against HEPES buffer solution (10 mM, pH 7.0) to give cationic polymer.

poly(HMA-*st*-PEGMA-*st*-SPMA), ^{1}H NMR (400 MHz, D_2O), δ (ppm): 8.60 – 8.50 (NC*H*N), 7.25 – 7.20 (NC*H*CCH_2), 4.25 – 3.50 (CH_2 of the PEG side chain), 2.90 – 2.75 (OCNHCH_2CH_2CH), 2.00 – 1.50 (CH_2 of the polymer backbone), 1.20 – 0.50 (CH_3 of the polymer backbone).

3. Preparation and Characterization of Polyplexes Nanoparticles

3.1 Preparation of polyplexes nanoparticles (NPs)

Luciferase reporter plasmid DNA (pDNA) was complexed with cationic polymer **P1**-**P4** in HEPES buffer solution (10 mM, pH 7.0) by self-assembling at *r* 30 (the mass ratio of **P1**-**P4** to pDNA) to form the **P1**-**P4** polyplexes nanoparticles (**NP**s). **P1**-**P3** were pre-irradiated with UV light (365 nm, 2 mW cm^{-2}). Then, a solution of pDNA in HEPES buffer was mixed gently with a same volume of polymer solution and **NP**s dispersion was allowed to stand at room temperature for 20 min.

3.2 Dynamic light scattering (DLS) and ζ-potential measurements of NPs

The hydrodynamic diameters and surface charge of **P1**-**P4 NP**s were measured at 25 °C using a Zetasizer nanoseries (Nano zs90, Malvern Instruments Ltd., U.K.) equipped with dynamic light scattering (unless stated otherwise, analysis was performed at an angle of 90°) and a standard capillary cell, respectively. For investigation of polyplex stability, **P1**-**P4 NP**s were irradiated under UV light (365 nm, 90 mW cm^{-2}, 1 min) and subsequent visible light (>500 nm, 250 mW cm^{-2}, 3 min) prior to the DLS tests.

The hydrodynamic diameters of **P1**-**P4 NP**s (*r* 30) that are prepared by assembling pure **P1**-**P4** with complexes (*r* 1 and *r* 5) are utilized to compare with the results of one-step assembly between **P1**-**P4** and pDNA. The above measurements were performed in triplicate (3 × 30 times).

For data analysis, the viscosity (0.8905 mPas) and refractive index (1.333) of pure water at 25 °C were used. The hydrodynamic diameters were calculated from the Stokes-Einstein equation and calibrated with polymer microspheres as nanosphere size standards. The ζ-potentials of **NP**s were measured in a disposable folded capillary cell (DTS 1070, Malvern Instruments Ltd., U.K.) containing 1 mM KCl, and calculated from the obtained electrophoretic mobility by applying the Smoluchowski equation.

3.3 Imaging of NPs by atomic force microscopy (AFM)

The **P1**-**P4 NP**s prepared at *r* 30 were treated under UV light (365 nm, 90 mW cm^{-2}, 1 min) and subsequent visible light (>500 nm, 250 mW cm^{-2}, 3 min). The **P1**-**P4 NP**s solutions (50 μL, the concentration of pDNA is 10 μg mL^{-1}) were deposited on a freshly cleaved mica substrate for 30 min. The solutions were then dried under a gentle flow of nitrogen gas. AFM imaging was performed in the ScanAsyst mode with Pyrex-Nitride probes (NanoWorld, Switzerland) on a Bruker Dimension ICON (Bruker, Germany) controlled by Nanoscope 8.10 software (Bruker, USA). The cantilever oscillation frequency was tuned to the resonance frequency of the cantilever with software self-calibration. The 256 × 256 images were recorded at a scan rate of 1.0 - 1.5 Hz with a sampling density of 15 - 380 nm^2 per pixel. The raw

AFM images were processed only for background removal (flattening) using the software.

3.4 Imaging of NPs by transmission electron microscopy (TEM)

For investigation of polyplex stability, **P1**-**P4 NP**s prepared at *r* 30 in one step were irradiated under UV light (365 nm, 90 mW cm^{-2}, 1 min) and subsequent visible light (>500 nm, 250 mW cm^{-2}, 3 min) prior to the TEM tests.

P1-**P4 NP**s (*r* 30) were also prepared by assembling pure **P1**-**P4** with complexes (*r* 1 and *r* 5), and the morphologies were compared with the above results under TEM.

Uranyl acetate (Beijing Zhongjingkeyi Technology Co., Ltd, China) solution should be filtrated with centrifugal filter devices (VIVASPIN 500, 3000 MWCO, PES, Sartorius-Stedim Biotech) before use. The **NP**s solutions were dropped on 150-mesh copper grids with carbon-coated formvar support (Beijing Zhongjingkeyi Technology Co., Ltd, China). After 5 min, the excess solutions were wicked off by using filter paper. Then a drop of 0.8 % purified uranyl acetate solution was placed on the grid for 4 min. The grid was blotted with filter paper and washed with ethanol, and left it air dried under ambient conditions.

3.5 Determination of fluorescence quantum yields (Φ_f) of cationic polymers and NPs

For measurement of the fluorescence quantum yields (Φ_f) of polymers and **NP**s, the emission spectra were recorded using the maximum excitation wavelengths and the integrated area of the fluorescence spectra were acquired (EX Slit: 5.0 nm, EM Slit: 5.0 nm, PMT Voltage: 600 V, Scan speed: 12000 nm min^{-1}) in HEPES buffer solution (10 mM, pH 7.0) at 25 °C. The quantum yields were then calculated with Rhodamine B in acetonitrile (Φ_f = 0.89) as standard[4] according to equation (1):[5]

$$\boldsymbol{\Phi}_{unk} = \boldsymbol{\Phi}_{std} \left(\frac{\boldsymbol{A}_{std}}{\boldsymbol{I}_{std}}\right)\left(\frac{\boldsymbol{I}_{unk}}{\boldsymbol{A}_{unk}}\right)\left(\frac{\boldsymbol{\eta}_{unk}}{\boldsymbol{\eta}_{std}}\right)^2 \quad (1)$$

where Φ is the quantum yield, A is the absorption intensity, I is the integrated area of fluorescence spectra, and η is the refractive index of the solvent.

3.6 Determination of singlet oxygen ($^1O_2$) generation efficiencies (Φ_Δ) of cationic

polymers and NPs

Water-soluble tetrasodium α,α′-(anthracene-9,10-diyl)bis(methylmalonate) (ABMM) was used as $^1O_2$ trapping agent[6] and Rose Bengal (RB) was employed as the standard photosensitizer (Φ_{RB} = 0.75 in water). The absorption spectra were measured at various irradiation times and a green light (520 nm, 20 mW cm^{-2}) was employed as the irradiation source. The Φ_Δ was calculated by the following Equation (2):[7]

$$\boldsymbol{\Phi_\Delta} = \boldsymbol{\Phi_{\Delta,RB}} \left(\frac{\boldsymbol{K_{unk}}}{\boldsymbol{K_{RB}}}\right)\left(\frac{\boldsymbol{I_{RB}}}{\boldsymbol{I_{unk}}}\right) \quad (2)$$

where Φ_Δ represents the $^1O_2$ quantum yield, K stands for the decomposition rate constants of ABMM, and I represents the rate of light absorption at 520 nm.

3.7 Photothermal experiments of NPs

To study the photothermal effect of **P3 NP**s, 200 μL solutions with a final **P3** (in a form of **NP**s) concentration of 0.4 mg/mL were irradiated with various photoswitching cycles of alternating UV light (365 nm, 90 mW cm^{-2}, 1 min) and visible light (>500 nm, 250 mW cm^{-2}, 3 min). An IR thermal camera (Fotric 225-1, S/N 225-1607A-14150) was used to record the temperature of the solution at each time point. DI water and free polymer **P3** (0.4 mg/mL) solution were used as control.

4. Cellular imaging by confocal laser scanning microscopy (CLSM)

HeLa cells were seeded at a density of 7 × 10^4 cells per well for CLSM in 4-well glass-bottomed plates (Φ = 24 mm) and incubated in complete DMEM containing 10% fetal bovine serum in the 37 °C incubator containing 5% CO_2 for 24 h. The cells were incubated with **P3** and **P3 NP**s, respectively, for additional 4 h. After washing twice with PBS, the cells were imaged by a Zeiss laser scanning microscope 710 with a 63× oil-immersion objective lens, using Zen 2008 software (Carl Zeiss) for data analysis. **P3** was excited at 405 nm or 543 nm and emission signal was collected at 600 ~ 750 nm ranges. **P3 NP**s were excited at 543 nm and emission signal was collected at 600 ~ 750 nm ranges.

5. Intracellular reactive oxygen species (ROS) assay

5.1 Cell viability assay by MTT

The cytotoxicity of **P3 NP**s was evaluated by MTT assay toward HeLa cells. The cells were seeded into black 96-well plates (1×10^4 per well) and incubated in complete DMEM containing 10% fetal bovine serum at 37 °C under 5% CO_2 for 24 h. The medium was replaced with fresh growth medium containing varying amount of **P3 NP**s for 4 h. The cells were washed twice with PBS buffer, and subjected to light irradiation treatment. After additional incubation for 24 h, the cells were washed with PBS twice. Then, the fresh culture medium contained MTT (100 μL, 0.5 mg mL^{-1}) was added into each well and incubated for 4 h at 37 °C. The supernatant was removed and the MTT formazan product was washed with PBS buffer. Then DMSO (150 μL) was added and the plates were shaken for 20 min. Cell viability was expressed as the ratio of OD values measured at 490 nm and 720 nm relative to the control group on a multimode microplate reader. The cytotoxicity of **P4 NP**s was assayed by the same procedure.

5.2 Measurement of intracellular ROS

The oxidant-sensitive dye (2, 7-dichlordihydrofluorescein diacetate, DCFH-DA) was used as a probe for intracellular ROS detection.[8] HeLa cells were seeded into black 96-well plates (Thermo Nunc, product number: 165305, Thermo Fisher Scientific) (1×10^4 per well) and incubated in complete DMEM containing 10% fetal bovine serum at 37 °C under 5% CO_2 for 24 h. After incubation with **P3 NP**s over a period of 4 h, HeLa cells were subjected to LED UV light irradiation (365 nm, 90 mW cm^{-2}, 1 min) to accumulate MC state. The MC-abundant cells were then subjected to photoswitching cycles before going into ROS detection procedure, or directly went into ROS detection procedure by incubation with DCFH-DA for 20 min in dark, washing with PBS buffer, treatment with LED visible light (>500 nm, 250 mW cm^{-2}, 10 min) and imaging under inverted fluorescent microscope.

5.3 Cell apoptosis analysis by flow cytometry

For the cell apoptosis analysis, HeLa cells were seeded into 12-well plates (1×10^6 per well) and incubated in complete DMEM containing 10% fetal bovine serum at 37 °C under 5% CO_2 for 24 h. Then the cells were incubated with **P3 NP**s for 4 h.

After washing twice with PBS buffer, cells were subjected to alternating light irradiation treatment, cultured for another 24 h, and digested by trypsinization, centrifuged, stained by the Annexin V-FITC apoptosis detection kit (KeyGen Biotech, Nanjing, China), and analyzed through flow cytometry.

Table S1. Comparison of this work with previous reports in spiropyran-focused **NP**s.[a]

Preparation Method	Aggregation Type	Diameter /nm	$\lambda_{max, UV}$	$\lambda_{max, Flu.}$	Φ_f [b]	ROS	Particles Function	Reference
Emulsion Polymerization	N.A.	40 ~ 400	588	660	0.24	/	Imaging	Zhu, etal [9]
Emulsion polymerization	N.A.	50 ~ 110	588	670	0.18	/	Imaging	Zhu, etal [10]
Self-assembly	N.A.	59	470 (Vis), 570 (UV)	530 (Vis), 640 (UV)	/	/	Labelling and sensing	Chen, etal [11]
Emulsion Polymerization	N.A.	60 ~ 80	570	665	0.28	/	Imaging	Tian, etal [12]
Self-assembly	N.A.	50 (25 °C) 36 (35 °C)	≈ 550	645	/	/	Probes	Li, etal [13]
Covalently attached to iron oxide NPs	N.A.	70/540 (UV), 520 (Vis)	563	/	/	/	Modulation of T_2 relaxation time of magnetic NPs	Osborne, etal [14]
Emulsion polymerization	N.A.	61 ~ 80	570	665	0.28	/	Imaging	Zhu, etal [15]
Self-assembly	N.A.	37	522 (BODIPY)	544 (BODIPY)	0.44 (BODIPY)	/	Imaging	Yildiz, etal [16]
Self-assembly, gold NPs	N.A.	24 (Vis), 300 (UV)	520	/	/	/	Logic gates	Liu, etal [17]
Self-assembly	N.A.	150 (Vis), 50 (UV)	551	672	/	/	Imaging,	Tong, etal [18]
HF etching, aggregate	H-type	260 ± 44	549	566	/	/	Nanoscale capsules for biomedical applications	Achilleos, etal [19]

Preparation Method	Aggregation Type	Diameters /nm	$\lambda_{max, UV}$	$\lambda_{max, Flu.}$	Φ_f [b]	ROS	Particles Function	Reference
Self-assembly, gold NPs	N.A.	30 ~ 500	531	/	/	/	Size control of AuNPs	Shiraishi, etal [20]
Covalently MSNs	N.A.	300	550	/	/	/	Controlled release platform	Chen, etal [21]
Emulsion polymerization	N.A.	60 ~ 700	≈ 550	/	/	/	Stimuli-responsive latex NPs	Abdollahi, etal [22]
Engineered MSNs	N.A.	80	400 ~ 460 (Rhod-SP) 550 (Rhod-SP, Cu^{2+})	590 (Rhod-SP, Cu^{2+}) 575 (Rhod-SP, Cu^{2+})	/	/	Probes	Li, etal [23]
SP-UCNP	N.A.	60 × 80	560	/	/	/	Cell adhesion and detachment	Li, etal [24]
Self-assembly	N.A.	27.5 ~ 45.9	550	637	/	/	Drug delivery system	Aibani, etal [25]
Self-assembly	N.A.	100	535	532 (FRET)	0.14	/	Probes	Zhang, etal [26]
Self-assembly	J-type	126 ± 25	549	644	0.27	Controlled generation of $^1O_2$	Cell imaging and killing	This work

a "/" means "not available"; *b* determined using Rhodamine B as standard.9 Spiropyrans have been reported as a component in nanosystems in cell imaging (ref: 9, 10, 12, 15, 16, 18) or biosensors (ref: 11, 13, 23, 26), drug delivery (ref: 21, 25) and cell biology (ref: 24). Recent researches are focused on the preparation of SP-containing NPs using emulsion polymerization method (ref: 22), self-assembly technology and surface modification of inorganic nanoparticles (ref: 14, 17, 19, 20) by functionalizing the material surface with SPs. However, the study of oxygen photosensitization by spiropyrans, likely dependent on the aggregation state, need be well investigated.

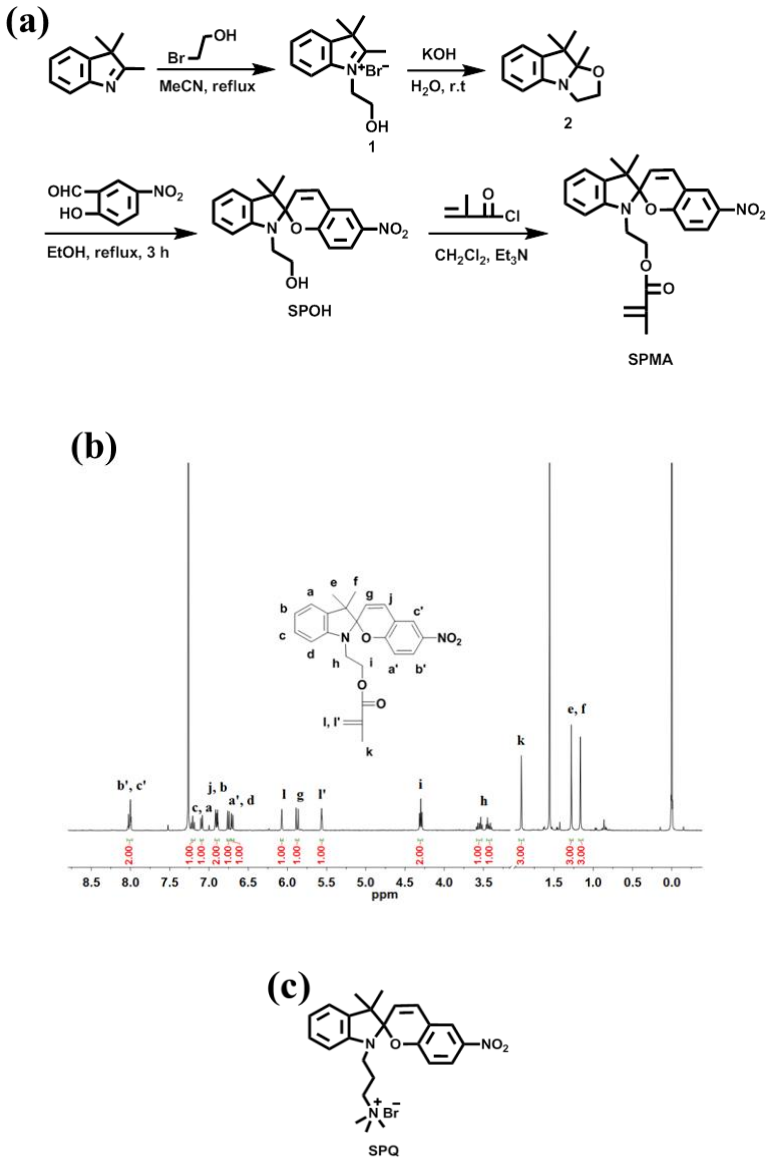
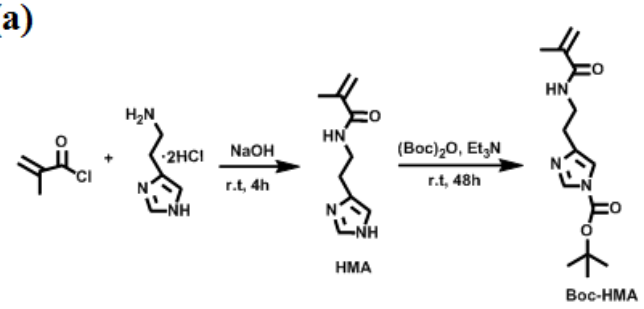


Fig. S1 (a) Synthetic route of SPMA, (b) ^{1}H NMR spectrum of SPMA (400 MHz, $CDCl_3$, 298K), and (c) chemical structure of SPQ.

(a)



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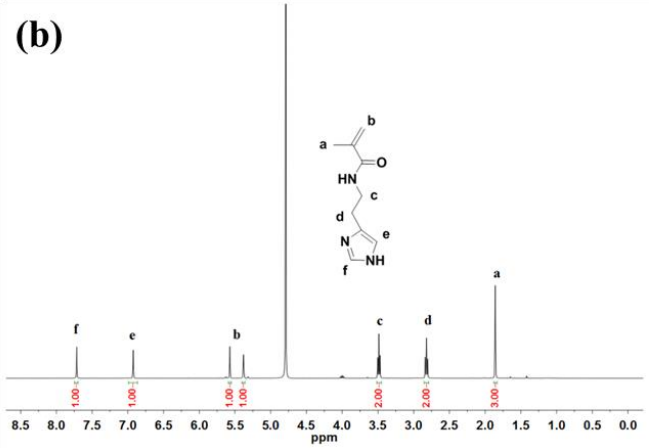

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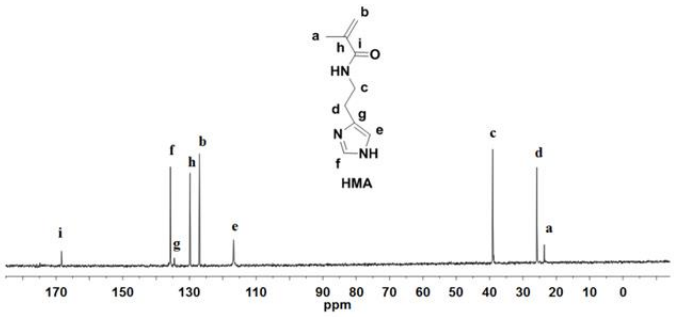

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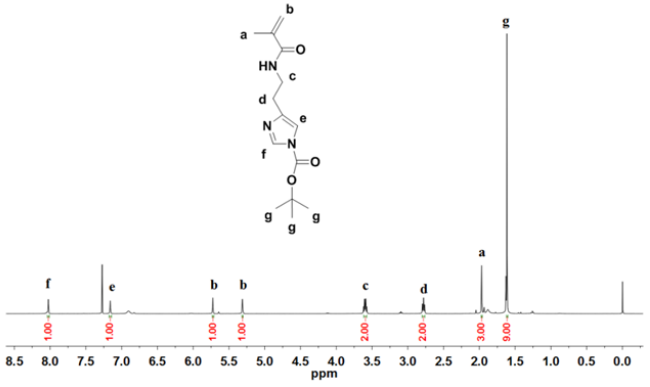


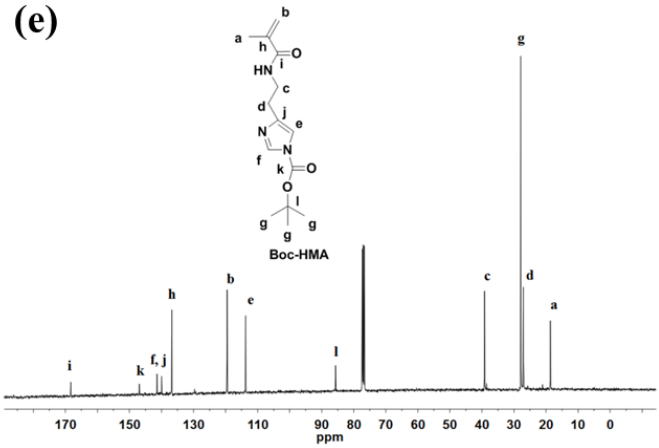
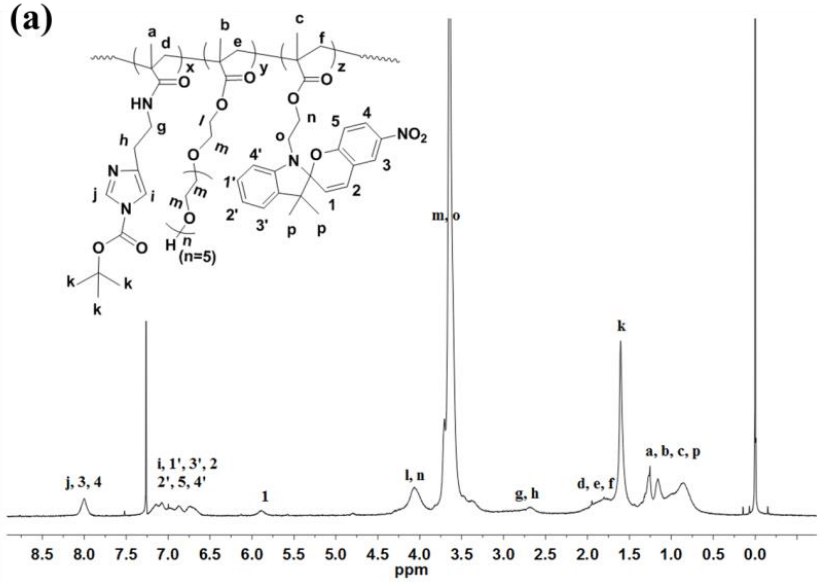
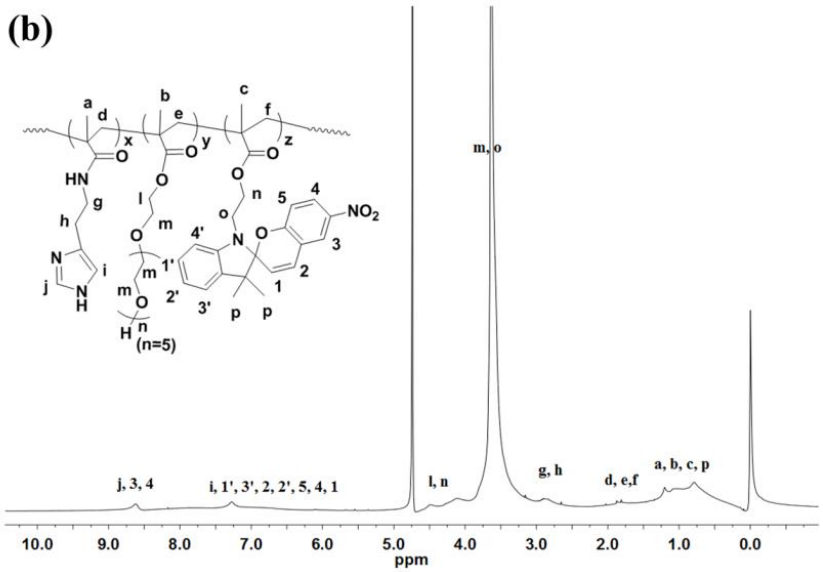


Fig. S2 (a) Synthetic route of Boc-HMA, (b) ^{1}H NMR spectrum of HMA (400 MHz, D_2O, 298K), (c) ^{13}C NMR spectrum of HMA (100 MHz, D_2O, 298K), (d) ^{1}H NMR spectrum of Boc-HMA (400 MHz, $CDCl_3$, 298K), and (e) ^{13}C NMR spectrum of Boc-HMA (100 MHz, $CDCl_3$, 298K).


(a)
j, 3, 4
i, 1', 3', 2
2', 5, 4'
1
l, n
m, o
g, h
d, e, f
k
a, b, c, p
ppm


(b)
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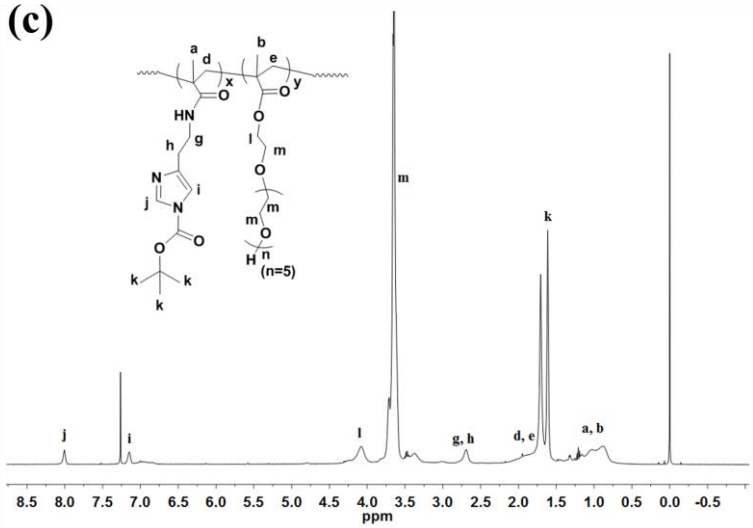
i, 1', 3', 2, 2', 5, 4, 1
l, n
m, o
g, h
d, e,f
a, b, c, p
ppm


(c)
j
i
l
m
g, h
d, e
k
a, b
ppm

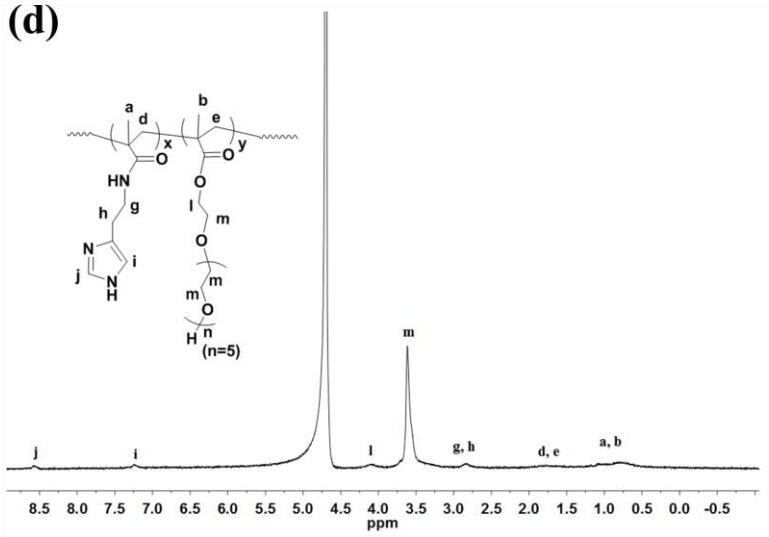


Fig. S3 ^{1}H NMR spectrum of (a) **P3** precursor in $CDCl_3$, (b) **P3** in D_2O, (c) **P4** precursor in $CDCl_3$, (d) **P4** in D_2O.

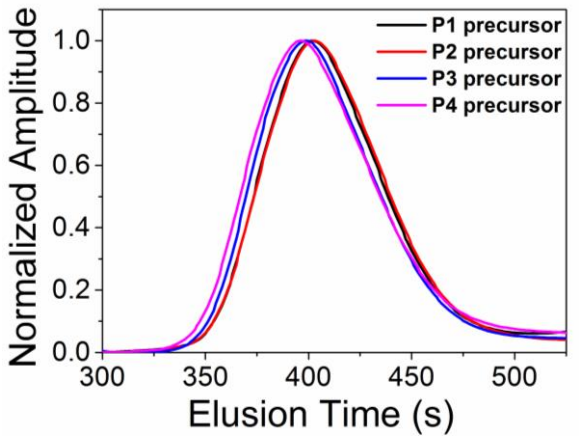


Fig. S4 GPC traces of **P1**-**P4** precursors in DMF.

Table S2. Polymerization of **P1**-**P4** precursors in DMF at 70 °C.

Sample	Theoretical x:y:z[a]			Experimental x:y:z[b]			$M_{n,GPC}$[c] g/mol	$M_{w,GPC}$[c] g/mol	PDI
	Boc-HMA	PEGMA	SPMA	Boc-HMA	PEGMA	SPMA			
P1 precursor	45%	50%	5%	37.5%	58.3%	4.2%	12600	21200	1.68
P2 precursor	40%	50%	10%	35.6%	52.7%	11.7%	11700	21000	1.79
P3 precursor	30%	50%	20%	29.6%	48.8%	21.6%	13100	23600	1.80
P4 precursor	50%	50%	0%	44.5%	55.5%	0%	13400	25300	1.89

[a] Theoretical molar ratio of Boc-HMA : PEGMA : SPMA.

[b] Measured molar ratio of Boc-HMA : PEGMA : SPMA by ^{1}H NMR. For **P1**-**P3** precursors, the relationship between x, y and z obeys to equation (1) and (2):

$$\frac{x+2z}{9x} = \frac{I(8.10\sim7.90)}{I(1.70\sim1.55)} \quad (1)$$

$$\frac{x+2z}{2z+21y} = \frac{I(8.10\sim7.90)}{I(3.75\sim3.50)} \quad (2)$$

For **P4** precursor, the relationship between x and y is available according to equation (3):

$$\frac{x}{y} = \frac{I(8.10\sim7.90)}{I(3.75\sim3.50)/21} \quad (3)$$

where I denotes the integral value of signals from proton resonance in the selected range.

[c] Measured by GPC using polyethylene glycol as standard in DMF.

Table S3. Zeta potentials of **P1-P4 NP**s prepared at *r* 30.

NPs	ζ potential (mV)
P1 NPs	8.3 ± 1.1
P2 NPs	7.4 ± 1.5
P3 NPs	6.1 ± 1.7
P4 NPs	7.5 ± 1.1

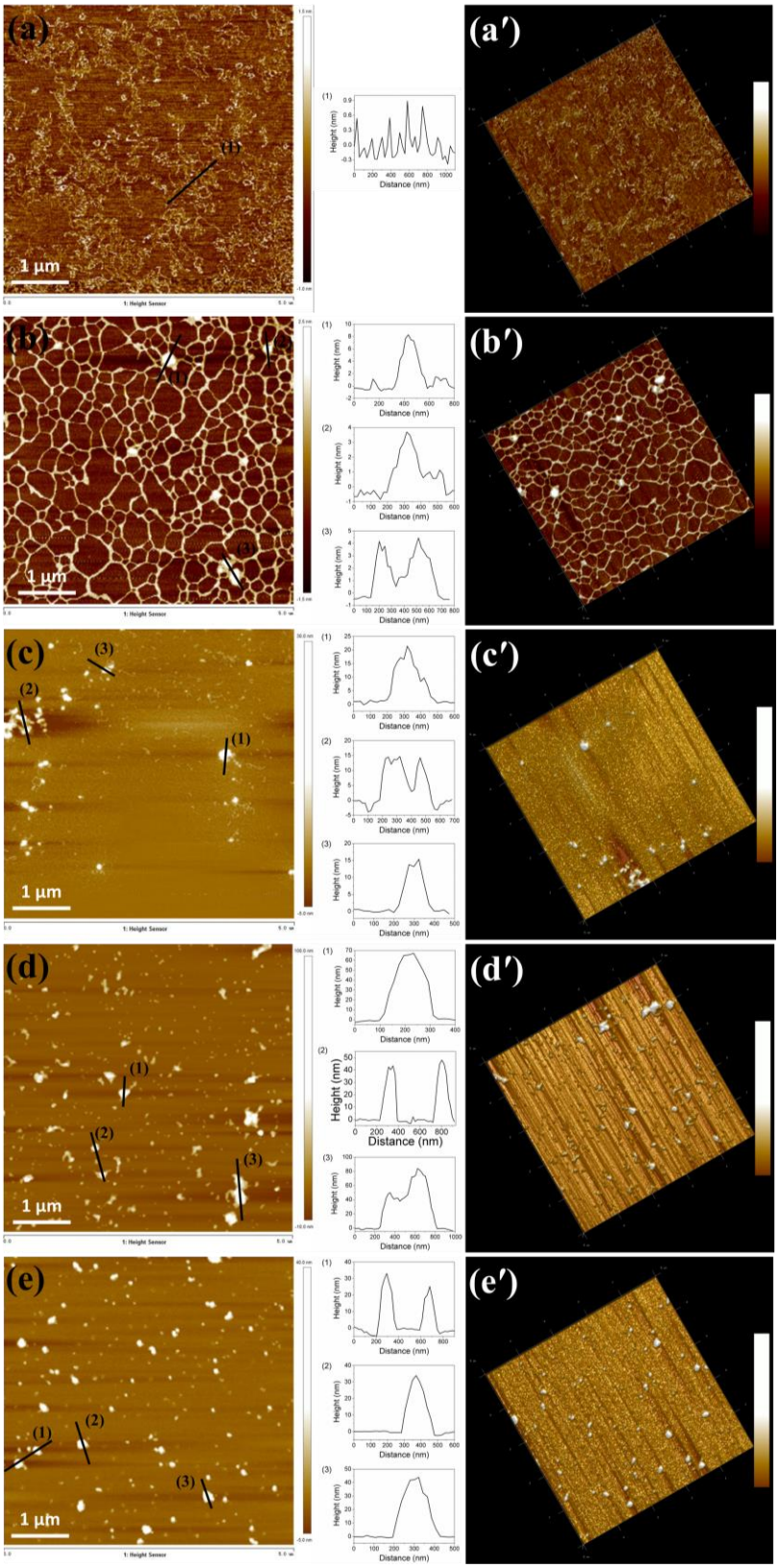


Fig. S5 AFM images (left: height; right: 3-D) of **P3 NP**s. (a-e) and (a′-e′) denote **P3 NP**s formed at *r* 0, 1, 5, 15, 30, respectively. The image size is 5 × 5 μm and scale bar is 1 μm.

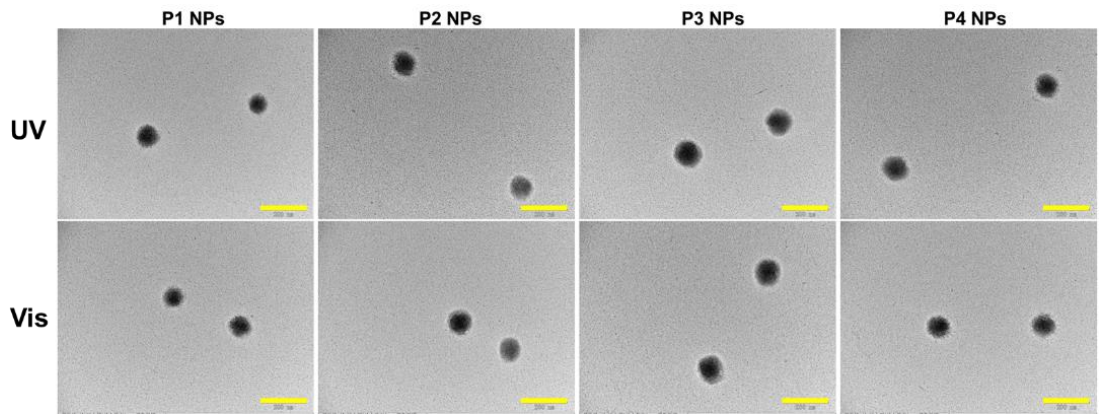


Fig. S6 TEM images of **P1-P4 NP**s (*r* 30) after UV light irradiation (365 nm, 90 mW cm^{-2}, 1 min) and subsequent visible light irradiation (> 500 nm, 250 mW cm^{-2}, 3 min). The scale bar indicates 200 nm.

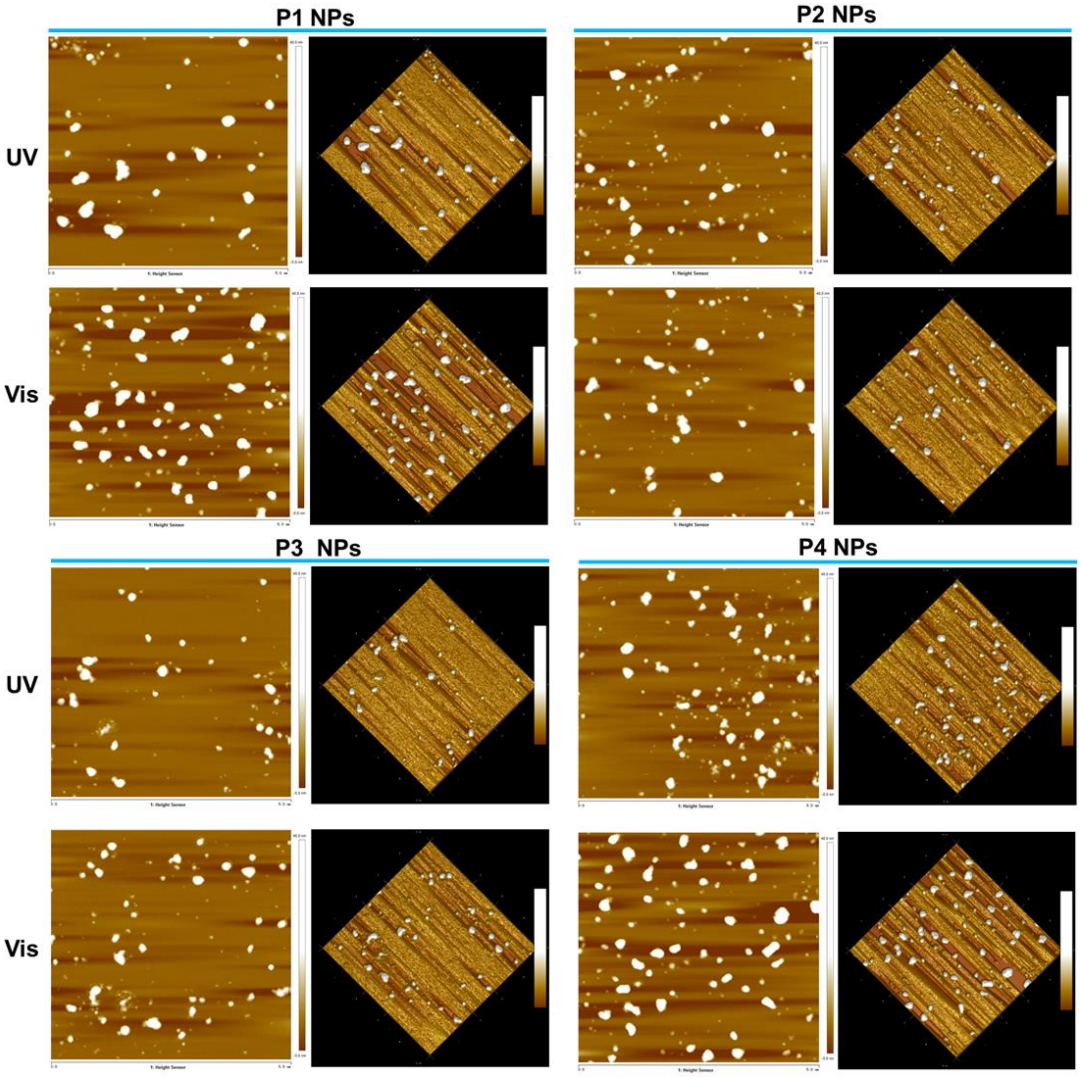


Fig. S7 AFM images (left: height; right: 3-D) of **P1**-**P4 NP**s (*r* 30) after UV light irradiation (365 nm, 90 mW cm^{-2}, 1 min) and subsequent visible light irradiation (> 500 nm, 250 mW cm^{-2}, 3 min). The image size is 5 × 5 μm.

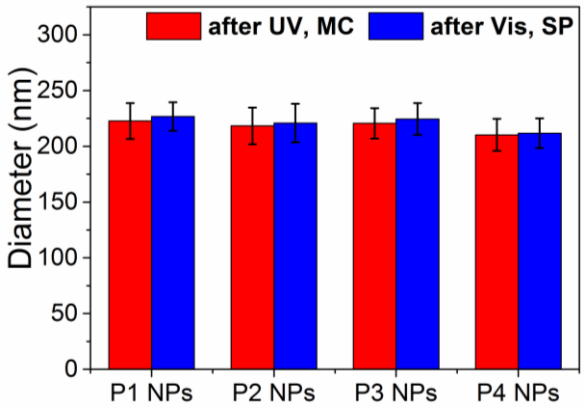


Fig. S8 Hydrodynamic diameters of **P1-P4 NP**s (*r* 30) after UV light irradiation (365 nm, 90 mW cm^{-2}, 1 min) and subsequent visible light irradiation (> 500 nm, 250 mW cm^{-2}, 3 min).

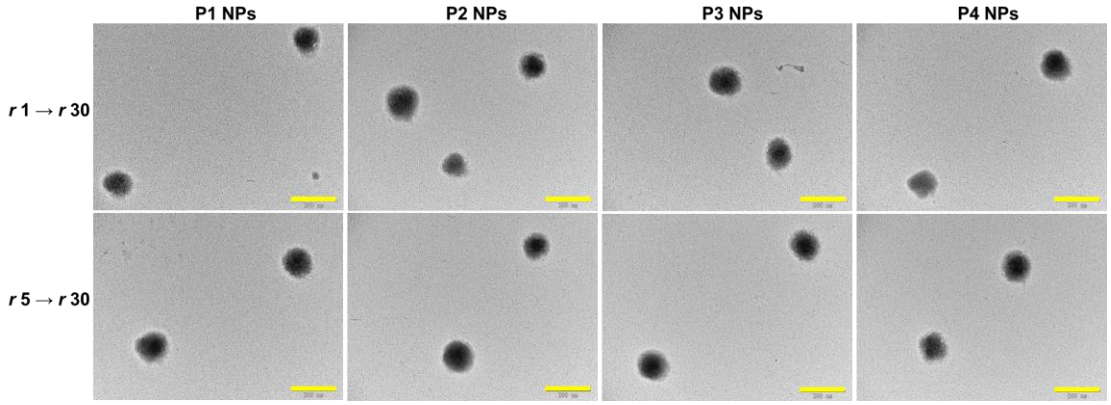


Fig. S9 TEM images of **P1-P4 NP**s (*r* 30) that are prepared by assembling pure **P1-P4** with complexes (*r* 1 and *r* 5). The scale bar indicates 200 nm.

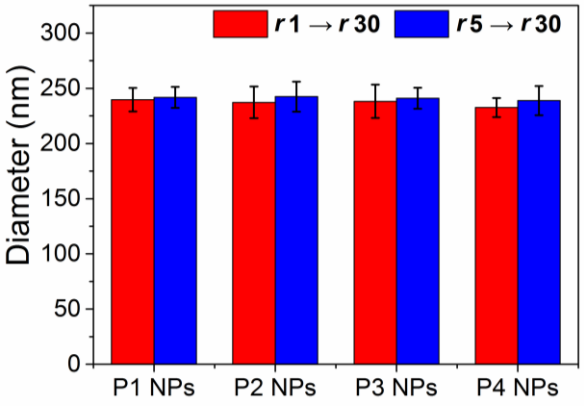


Fig. S10 Hydrodynamic diameters of **P1-P4 NP**s (*r* 30) that are prepared by assembling pure **P1-P4** with complexes (*r* 1 and *r* 5).

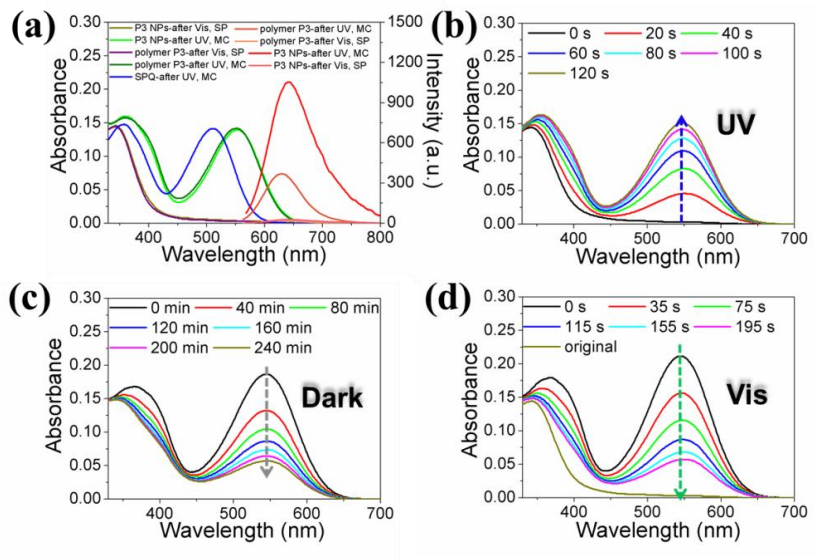


Fig. S11 (a) UV-Vis spectra (—) and fluorescence spectra (---) of polymer **P3** and **P3 NP**s. (b) SP-MC isomerization in **P3** by UV light. (c) MC-SP isomerization in dark. (d) MC-SP isomerization by visible light. UV (λ_1 365 nm: 2 mW cm^{-2}) and green light (λ_2 520 nm: 20 mW cm^{-2}) were applied for light treatment.

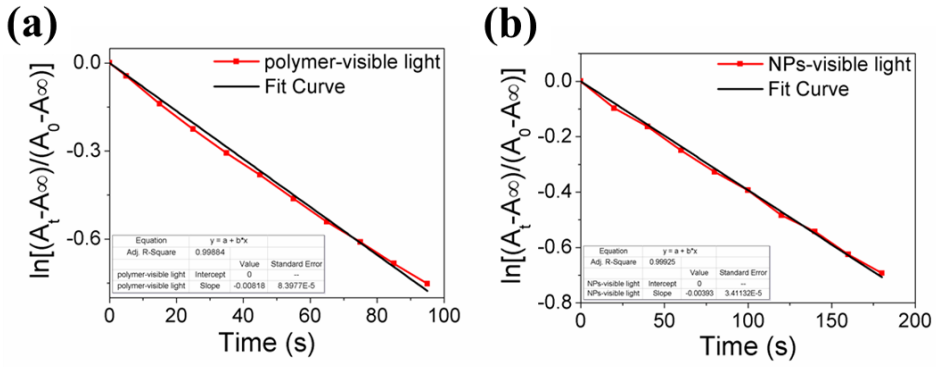


Fig. S12 First order kinetic plot of MC-SP photoisomerization reaction for (a) **P3** and (b) **P3 NP**s by plotting of ln[(A_t-A_∞)/(A_0-A_∞)] against irradiation time under visible light (λ_2 520 nm, 20 mW cm^{-2}) irradiation. A_0, A_t, and A_∞ represents the absorption at 549 nm at the beginning, at the time (t) of the reaction, and at the end, respectively.

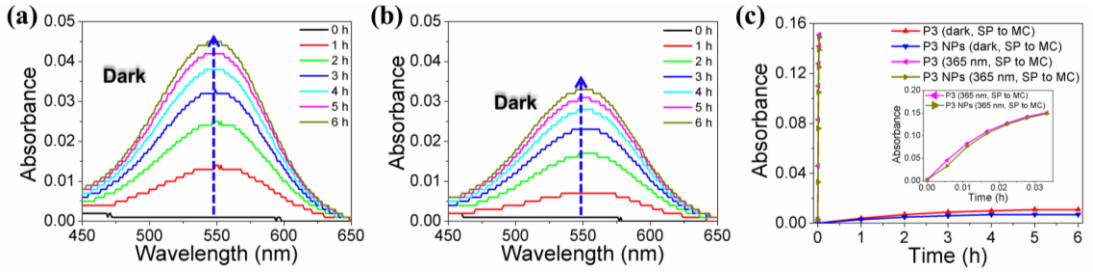


Fig. S13 Changes of absorption spectra for MC-SP self-isomerization of (a) **P3** and (b) **P3 NPs** over a period of 0 ~ 6 h in dark. (c) Changes of absorption intensity at 549 nm over a period of 0 ~ 6 h for monitoring MC-SP isomerization of **P3** and **P3 NPs** in dark or upon UV light (the inset graph, 365 nm: 2 mW cm^{-2}) irradiation.

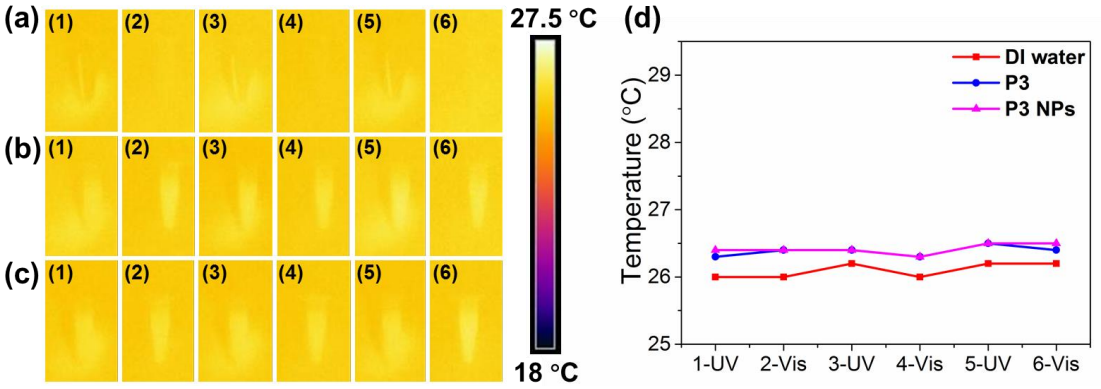


Fig. S14 IR thermal images of DI water (a), **P3** (0.4 mg mL^{-1}) solution (b) and **P3 NPs** suspension (c) with various photoswitching cycles of alternating UV light (365 nm, 90 mW cm^{-2}, 1 min) and visible light (>500 nm, 250 mW cm^{-2}, 3 min) treatments. The final concentration of **P3** (in a form of **NPs**) is 0.4 mg mL^{-1} applied in (c). The temperature changes (d) based on IR thermal imaging data in (a) ~ (c).

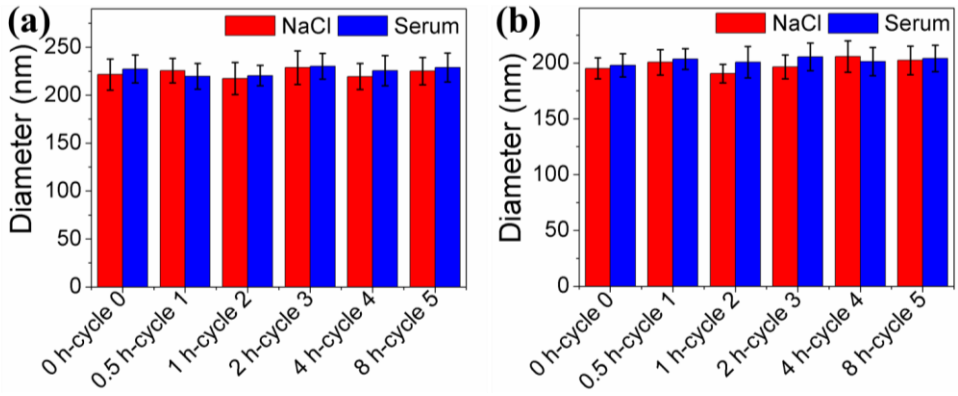


Fig. S15 Size changes of (a) **P3 NP**s and (b) **P4 NP**s in HEPES buffer containing 200 mM NaCl or in complete DMEM medium containing 10% fetal bovine serum. The hydrodynamic diameters were determined by DLS.

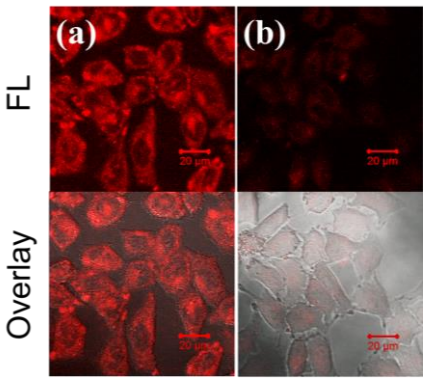


Fig. S16 CLSM imaging of HeLa cells treated by (a) **P3 NP**s and (b) **P3**. **P3** or **P3 NP**s were loaded at a polymer concentration of 0.2 mg mL^{-1}. Brief UV light irradiation (365 nm, 90 mW cm^{-2}, 1 min) was applied before CLSM imaging with excitation at 543 nm.

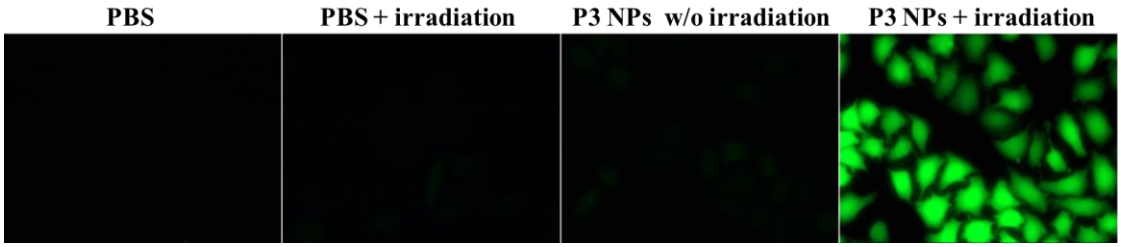


Fig. S17 Oxidized DCF fluorescence imaging in HeLa cells. For MC-abundant **P3 NP**s treatment, **P3 NP**s were loaded at a polymer concentration of 0.2 mg mL^{-1}. For irradiation treatment, LED visible light (> 500 nm, 250 mW cm^{-2}, 3 min) was applied.

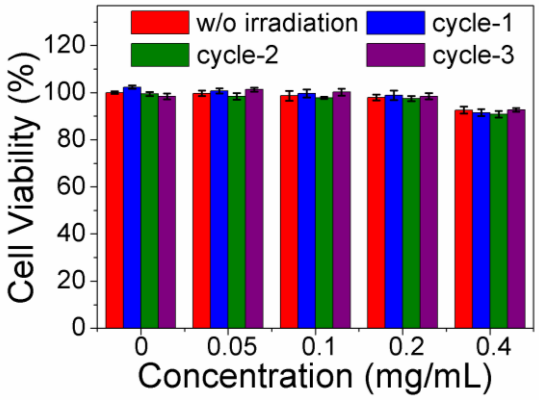


Fig. S18 MTT-based cell viability analysis of **P4 NP**s-treated HeLa cells after various photoswitching cycles of alternating UV-light (365 nm, 90 mW cm^{-2}, 1 min) and visible light (>500 nm, 250 mW cm^{-2}, 3 min) treatments.

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