Electronic Supplementary Information

Photo and acid dual degradable polymeric nanoparticles from an o-nitrobenzyl dithiol with thiol-ene click polymerization

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Experimental section

1.1 Materials.

Unless otherwise stated, all chemicals were obtained from Aladdin (Shanghai, China) and used as received. 1,4-bis(bromomethyl)benzene was purchased from J&K Scientific (Shanghai, China).

1.2 Methods.

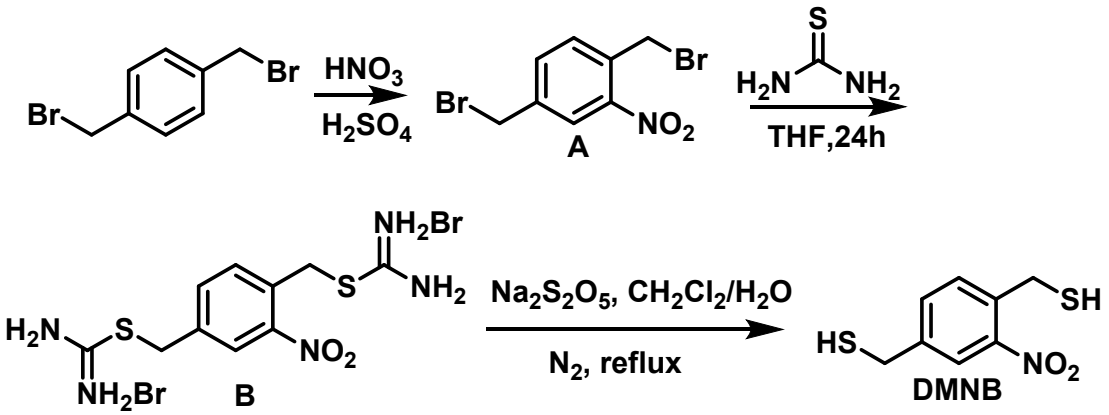
The Bruker Avance-III 500 instrument (500 MHz) was used to measure the ^{1}H and ^{13}C NMR spectra. Gel permeation chromatography (GPC) data was obtained using a Waters Alliance HPLC e2695 with THF eluents (35 ^{o}C) at a rate of 1 mL/min through a 7.8 × 300 mm column (with guard column) and a 2414 Refractive Index Detector. Polystyrene was used as the standard for THF GPC. The PerkinElmer FT-IR Spectrometer Frontier^{TM} was used to

record infrared absorption spectra, while the HORIBA JOBIN YVON fluorescence spectrophotometer Fluoromax-4 was used to record fluorescence spectra. TGA was characterized using a Perkin Elmer instrument (Diamond TG Spectrum GX, USA). DLS measurements were carried out using a Malvern instrument (Zetasizer Nano ZS90) with a 50 mW linearly polarized laser tuned at 532 nm. SEM was performed on a Nova Nanosem 200 system operated at an acceleration voltage of 10 kV. Photo-irradiation was performed using a UV-Vis spot curing system (FUTANSI, UVSF80, Shanghai).

1.3 Synthesis of monomers and polymers

1.3.1 Synthesis of 1,4-bis(methanethiol)-2-Nitrobenzene (monomer 1, M1)

Synthesis of 1,4-bis(methanethiol)-2-Nitrobenzene was performed according to a method adapted from our previous report[1].



Scheme S1 synthesis of 1,4-bis(methanethiol)-2-Nitrobenzene (**DMNB**).

In this experiment, a solution of 1,4-bis(bromomethyl)benzene (10.5 g, 0.038 mol) in concentrated aqueous H_2SO_4 (98%, 20 mL) was placed in an ice bath. Then, 10 mL Nitric acid (69%) was slowly added drop by drop at 0 ^oC. After allowing the reaction to warm to room temperature and stirring it overnight, the resulting mixture was poured into 500 mL ice-water and filtered. The yellow solid obtained was washed three times with water (3*200 mL). Crystallization of the product from ethanol yielded 10.8 g (yield: 98.9%) of yellow needle-shaped 1,4-bis(bromomethyl)-2-nitrobenzene (compound A).

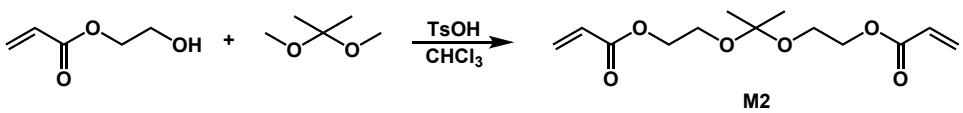
^{1}H NMR ($CDCl_3$, 500MHz): δ8.070 (s, 1H), δ7.642 (d, 1H), δ7.571 (d, 1H), δ4.814 (s, 2H), δ4.494(s, 2H).

Compound B was obtained by adding 2.4 equivalents of thiourea (4.22 g, 0.0554 mol) to a solution of 1,4-bis(bromomethyl)-2-nitrobenzene (7.13 g, 0.023 mol) in dry THF (100 mL). The mixture was stirred overnight at room temperature, resulting in the formation of white precipitates. After being filtered and washed with ethyl acetate, the white solid was dried under vacuum, yielding 12.27 g of compound B (77.25% yield). ^{1}H NMR (CD_3OD: δ8.25 (s, 1H), δ7.825 (d, 1H), δ7.738 (s, 1H), δ4.766 (s, 2H), δ4.578 (s, 2H).

Compound B (4.98 g, 0.0108 mol) was suspended in a mixture of dichloromethane (58.8 mL) and water (44.1 mL). Eight equivalents of sodium metabisulfite ($Na_2S_2O_5$) (16.43 g, 0.0864 mmol) were added to the mixture, resulting in a heterogeneous mixture. The mixture was then refluxed for 4 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was partitioned and separated. The aqueous layer was washed once with 20 mL of dichloromethane (DCM). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated, yielding 2.44 g of compound DMNB with a 98% yield.^{1}H NMR ($CDCl_3$): δ7.973 (s, 1H), δ7.560 (s, 1H), δ7.435 (s, 1H), δ3.990 (d, 2H), δ3.789 (d, 2H), 2.130 (t, 1H), δ1.844 (t, 1H); ^{13}C NMR (125MHz, $CDCl_3$): δ147.56, 141.89, 135.63, 133.24, 131.95, 124.58, 27.93, 25.78.

The overall yield of DMNB from 1,4-bis(bromomethyl)benzene is about 74.9%.

1.3.2 Synthesis of ketal containing diacrylate (KDA)



Scheme S2. Synthesis of ketal containing diacrylate (KDA).

KDA was synthesized through ketal exchange reaction of 2-hydroxyethyl acrylate and 2,2-dimethoxypropane in dry chloroform in the presence of p-toluene sulfonic acid (p-TSA) catalyst. To achieve this, a mixture of 20.1g

(172.8 mmol) of 2-hydroxyethyl acrylate, 6.1g (57.6 mmol) of 2,2-dimethoxypropane, 13.1mg of p-toluenesulfonic acid, and 200mg of hydroquinone in dry chloroform was stirred at 80 ^{o}C for 6 h. The solution was then distilled to remove methanol-chloroform azeotrope (53.4 ^{o}C and the remaining chloroform under vacuum. The resulting colorless liquid (M2) was purified using rapid column chromatography with a volume ratio of 1:4 of ethyl acetate to petroleum ether. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.49 – 6.41 (m, 2H), 6.18 (dd, *J* = 17.3, 10.4 Hz, 2H), 5.91 – 5.83 (m, 2H), 4.35 – 4.29 (m, 4H), 3.77 – 3.70 (m, 4H), 1.42 (s, 6H).

1.3.3 Synthesis of polymer mPEG1.9K-SH

In brief, a solution was prepared by dissolving mPEG1.9 K (3.82 g, 2 mmol), 3-mercaptopropionic acid (0.42 g, 4 mmol), and hafnium (IV) chloride (5 mg) in 20 mL of toluene. The mixture was stirred at 120 ^{o}C under nitrogen for 24 h. The solution was then distilled to remove the toluene-water azeotrope, and the remaining toluene was removed by vacuum at 110 ^{o}C for 2 h. The crude product was dissolved in CH_2Cl_2, dried over anhydrous $MgSO_4$, filtered, and concentrated. The polymer was precipitated twice from an excess volume of diethyl ether to yield a white powder (3.69 g, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 – 4.26 (m, 2H), 3.85 – 3.81 (m, 1H), 3.66 (s, 255H), 3.50 – 3.46 (m, 1H), 3.40 (s, 3H), 2.80 (dd, *J* = 14.2, 7.6 Hz, 2H), 2.70 (t, *J* = 6.6 Hz, 2H), 1.70 (t, *J* = 8.3 Hz, 1H). GPC: M_n=2.13 × 10^3 g/mol, PDI= 1.15.

1.3.4 Synthesis of photo and acid dual degradable polymer (PKNB)

Briefly, a solution was prepared at room temperature by dissolving 1.08 g (5 mmol) of BMNB and 2 drops of TEA in 15 mL THF. Slowly and dropwise, 1.50 g (5.5 mmol) of KDA in THF was added, and the reaction proceeded for 2 h. To stop the reaction, 0.1 mL acetic acid was added. The resulting solution was concentrated under vacuum and precipitated in cold ether three times, yielding yellowish solid polymer PKNB (a poly(β-thioester)). PKNB was analyzed using various techniques, including ^{1}H NMR, FT-IR, GPC, and TGA. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 4.28 – 4.21 (m, 4H), 4.08 (s, 2H), 3.80 (s, 2H), 3.66 (s, 4H),

2.77 – 2.69 (m, 4H), 2.63 (dd, *J* = 10.4, 6.5 Hz, 4H), 1.37 (d, *J* = 9.9 Hz, 6H). GPC: M_n=3.42 × 10^3 g/mol, PDI= 2.07.

1.3.5 Synthesis of triblock copolymer mPEG-PKNB-mPEG

Typically, at room temperature, a solution was prepared by dissolving 0.5 g (0.2 mmol) of PKNB, 1.0 g (0.5 mmol) of mPEG-SH (2.1K) and 1 drop of TEA in 8 mL THF. The reaction was allowed to proceed for 2 h before being quenched with 0.1 mL acetic acid. The resulting solution was concentrated under vacuum and precipitated in cold ether, yielding a yellowish crude product. The crude product was then subjected to dialysis against water using a dialysis bag (MWCO 3000) to remove excess mPEG-SH. The aqueous solution was filtered and lyophilized to obtain mPEG-PKNB-mPEG, which was characterized using 1H NMR, FT-IR, GPC and TGA techniques. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 6.43 (d, *J* = 17.0 Hz, 1H), 4.34 – 4.20 (m, 6H), 4.08 (s, 2H), 3.82 (d, *J* = 16.3 Hz, 3H), 3.66 (s, 61H), 3.40 (s, 1H), 2.73 (dd, *J* = 13.7, 6.8 Hz, 4H), 2.67 – 2.59 (m, 5H), 1.38 (s, 6H). GPC: M_n=7.66 × 10^3 g/mol, PDI= 2.01.

1.4 Preparation of mPEG-PKNB-mPEG NPs

Under vigorous stirring, 10 mg of polymer mPEG-PKNB-mPEG was added drop by drop to 0.5 mL of DMF, followed by 5 mL of deionized water. The resulting mixture was then placed in a dialysis bag with a MWCO of 1000 and submerged in deionized water. The size and morphology of these polymeric nanoparticles were analyzed by DLS and SEM.

1.5. Determination of the CMC

The pyrene fluorescence method[2] was utilized to determine the CMC of the mPEG-PKNB-mPEG NPs. To do so, acetone solution containing 50.00 μL of pyrene (0.06 mM) was added to 5.00 mL of various concentrations of polymeric micelle solutions. The mixtures were left to stir overnight at room temperature to eliminate the acetone. The polymeric micelle solutions of different concentrations were then subjected to excitation spectra measurement (emission wavelength: 395 nm). The fluorescence intensities at 384 nm and 373 nm were recorded, and the ratios were calculated.

The intersection point on the diagram of the ratios and the decadic logarithm (log10) of the concentrations represents the CMC.

1.6. Preparation of NR-loaded mPEG-PKNB-mPEG NPs

The method for preparing the NR-loaded micelles was previously described[3]. In brief, 20.00 mg of polymer mPEG-PKNB-mPEG and 6.00 mg of NR were dissolved in 2.00 mL of DMF, and the resulting mixture was slowly dripped into 10.00 mL of deionized water under vigorous stirring. The solution was stirred for 12 h and then transferred to a dialysis bag with a molecular weight cutoff (MWCO) of 2000, and dialyzed against deionized water overnight to remove the DMF. The resulting solution was filtered through a 0.45 μm membrane to obtain the NR-loaded micelles. To determine the drug loading capacity, the NR-loaded micelle solution was freeze-dried, dissolved in DMSO, and analyzed by fluorescence spectroscopy. Calibration curves were obtained by preparing a series of NR solutions with different concentrations in DMSO and measuring their fluorescence values. The drug loading content (DLC) and drug loading efficiency (DLE) were calculated using the following formulas:

$$\text{DLC (wt\%)} = (\text{weight of loaded drug/total weight of the polymer and loaded drug}) \times 100\% \quad (1)$$

$$\text{DLE (\%)} = (\text{weight of loaded drug/weight of drug in feed}) \times 100\% \quad (2)$$

1.7. The stability of the mPEG-PKNB-mPEG NPs

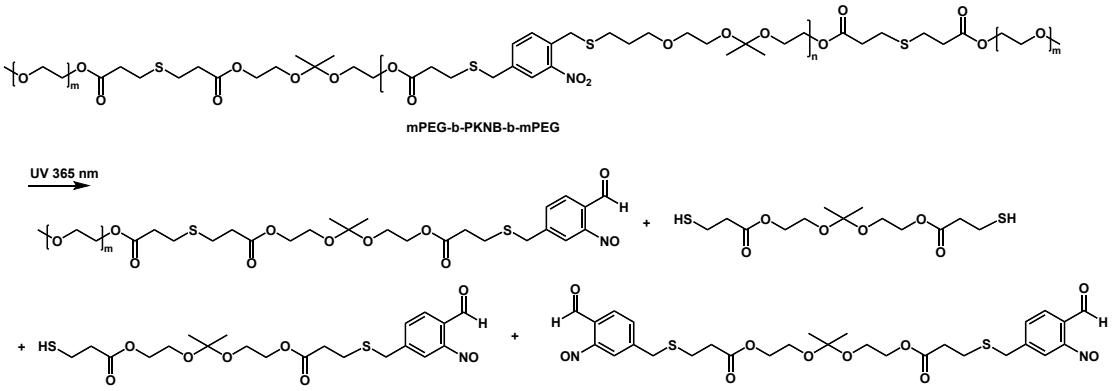
DLS was utilized to examine the stability of nanoparticles in PBS at pH 5.00 and 7.40. To achieve this, 5.00 mL of mPEG-PKNB-mPEG NPs (2.00 mg/mL) were prepared in PBS at both pH levels. The solutions were then placed in a 37°C water bath oscillator for either 24 or 48 h. The sizes of the polymeric nanoparticles were tracked using DLS.

1.8. Drug release *in vitro*

To assess the release of drugs from mPEG-PKNB-mPEG NPs loaded with NR, in vitro testing was conducted in PBS solutions with pH values of 5.00 or 7.40, with or without H_2O_2. The process involved dissolving 20.00 mg of

polymer and 6.00 mg of NR in 2.00 mL of DMF, which was stirred for 12 h. The solution was then dialyzed using deionized water with a MWCO of 2000 to eliminate DMF, and filtered through a 0.45 μm membrane to obtain NR-loaded micelles. Next, 1.00 mL of the NR-loaded micelles (2.00 mg/mL) was combined with varying concentrations of H_2O_2 solutions (1 mM, 5 mM, and 10 mM) and 8.00 mL of PBS (pH 5.0). In contrast, NR-loaded micelles were also prepared without H_2O_2 treatment in PBS solutions with pH values of 5.00 and 7.40. All solutions were incubated at 37 °C, and fluorescence data were collected at predetermined intervals with λ_{ex}=550 nm and λ_{em} = 570 nm - 850 nm, with a slit width of 5 nm.

1.9 Light responsiveness.



Scheme S3 Possible photo degradation of mPEG-PKNB-mPEG.

The process of photodegradation of polymers

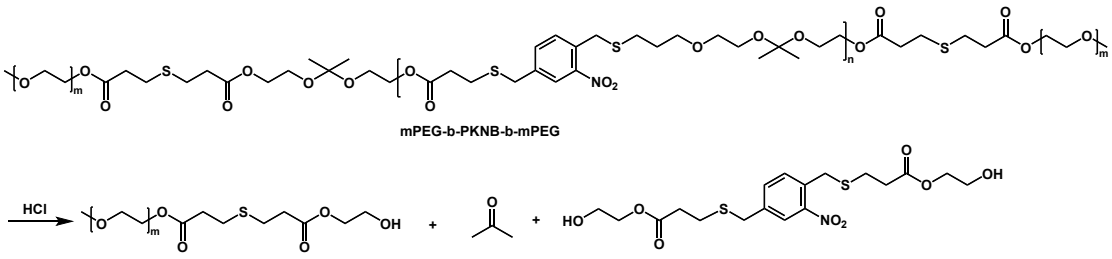
In instances where oxygen was absent, 10 mg of mPEG-PKNB-mPEG was dissolved in 0.7 mL of $CDCl_3$ and sealed in a nuclear magnetic tube. The tube was then exposed to 200 Mw/cm^2 UV light at 365 nm (FUTANSI, UVSF80) for a specified duration and analyzed directly using ^{1}H NMR. Following the ^{1}H NMR analysis, the solution in the nuclear magnetic tube was concentrated using reduced pressure, and subjected to FT-IR and GPC characterization.

DLS was used to assess the degradation of polymer mPEG-PKNB-mPEG nanoparticles when exposed to UV radiation. The changes in size of the nanoparticles were monitored as they were irradiated with 200 mW/cm^2

UV light at 365 nm for varying time intervals. The solutions containing 2 mg/mL of mPEG-PKNB-mPEG nanoparticles were analyzed by DLS to record the alterations in micellar size.

To investigate the light sensitivity of mPEG-PKNB-mPEG nanoparticles, fluorescence analysis was conducted. The procedure involved creating 2 mg/L polymer mPEG-PKNB-mPEG nanoparticles loaded with Nile red (NR), according to the aforementioned method. The solutions were then exposed to UV radiation for predetermined durations and analyzed for fluorescence. The measurements were taken with λ_{ex} = 550 nm and λ_{em} ranging from 570 nm to 850 nm, with a slit width of 5 nm.

1.10 Acid-degradable behaviors.



Scheme S4 Possible acid degradation of mPEG-PKNB-mPEG.

The pH-responsiveness of polymers was investigated using ^{1}H NMR to analyze changes in polymer structure under acidic conditions. The polymer mPEG-PKNB-mPEG (20 mg) was dissolved in $CDCl_3$ (0.7 mL) in a nuclear magnetic tube, and 1 M HCl (10 μL) was added. The solution was oscillated for 1 h before being investigated by ^{1}H NMR. $CDCl_3$ was then evaporated under reduced pressure, and the residual was analyzed by GPC.

To monitor variations in the micellar size of mPEG-PKNB-mPEG nanoparticles, DLS was used after treatment with different pH PBS. mPEG-PKNB-mPEG nanoparticles (20 mg/L) were prepared using the aforementioned method. A 1 mL solution of 20 mg/L mPEG-PKNB-mPEG nanoparticles was mixed with 9 mL of PBS (pH 5.5, and 7.4) to obtain a 2 mg/L polymeric nanoparticles solution. The solutions were then incubated at 25 ^{o}C, and the micellar size was recorded by DLS at predetermined intervals.

Fluorescence was utilized to investigate the acid-degradable behavior. mPEG-PKNB-mPEG nanoparticles containing 20 mg/L of Nile Red (NR) were created. A 1 mL solution of 20 mg/L NR-loaded mPEG-PKNB-mPEG nanoparticles was combined with 9 mL of PBS at pH 5.5 and 7.4, resulting in a solution of 2 mg/L NR-loaded mPEG-PKNB-mPEG nanoparticles. The solutions were then left to incubate at 25 ^{o}C and fluorescence data was recorded at predetermined intervals. The parameters used were λ_{ex} = 550 nm, λ_{em} ranging from 570 to 750 nm, and a slit width of 5 nm.

1.11 Cell viability assay of mPEG-PKNB-mPEG.

The mouse NIH 3T3 cells were cultured in DMEM supplemented with 10% FBS at 37 ^{o}C in a 5% CO_2 humidified atmosphere. Then cells were seeded in 96-well plates at a density of 5.0 × 10^3 cells per well, and various concentrations (0-10 mg/mL) of mPEG-PKNB-mPEG was added and incubated for 24, 48, 72 and 96 h at 37 ^{o}C. The MTS-based CellTiter 96® Aqueous One Solution Cell Proliferation Assay Reagent (Promega Corp., WI, USA) was used to treat cells at 37 ^{o}C for 3 h as per the manufacturer's instructions. Cell viability was calculated by measuring absorbance at a wavelength of 490 nm using a SpectraMax Plus 384 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

1.12 Statistical analysis.

The investigation involved conducting each experiment a minimum of three times, and the resulting data was presented as mean±standard deviation (SD). Statistical analysis was carried out using t-test and analysis of variance (ANOVA).

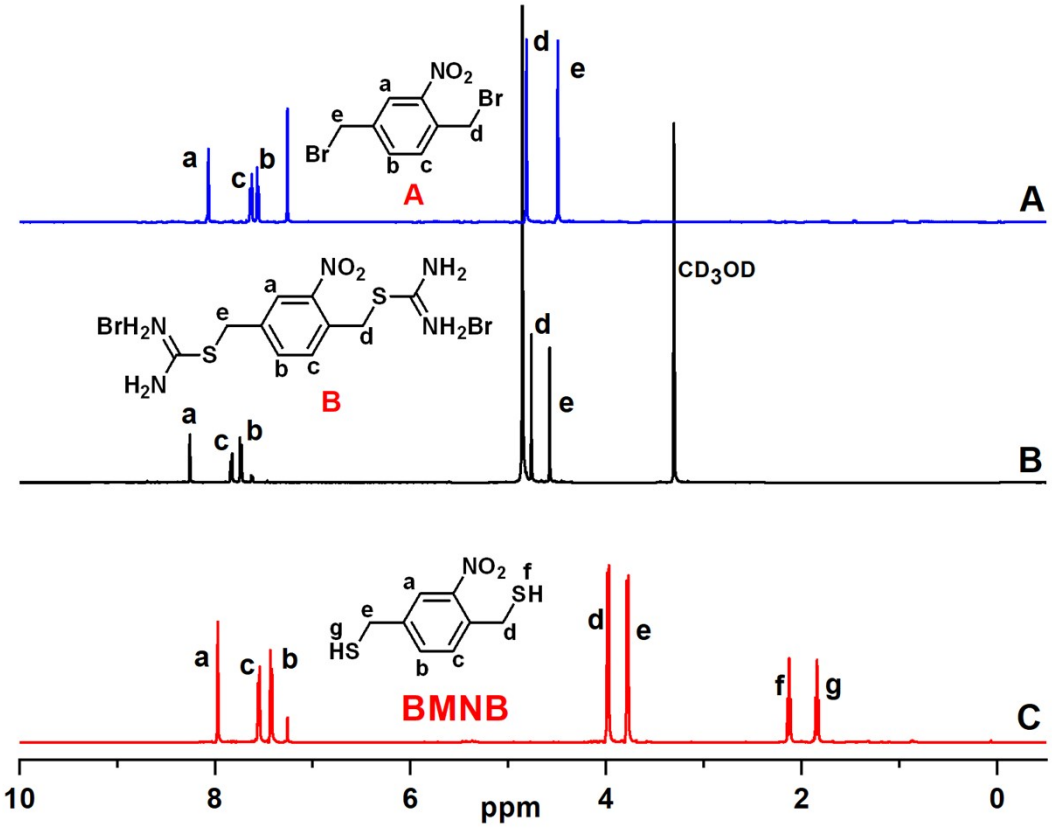


Fig. S1 ^{1}H NMR ($CDCl_3$) spectra of 1,4-bis(methanethiol)-2-Nitrobenzene (BMNB, C) and its precursors (A and B).

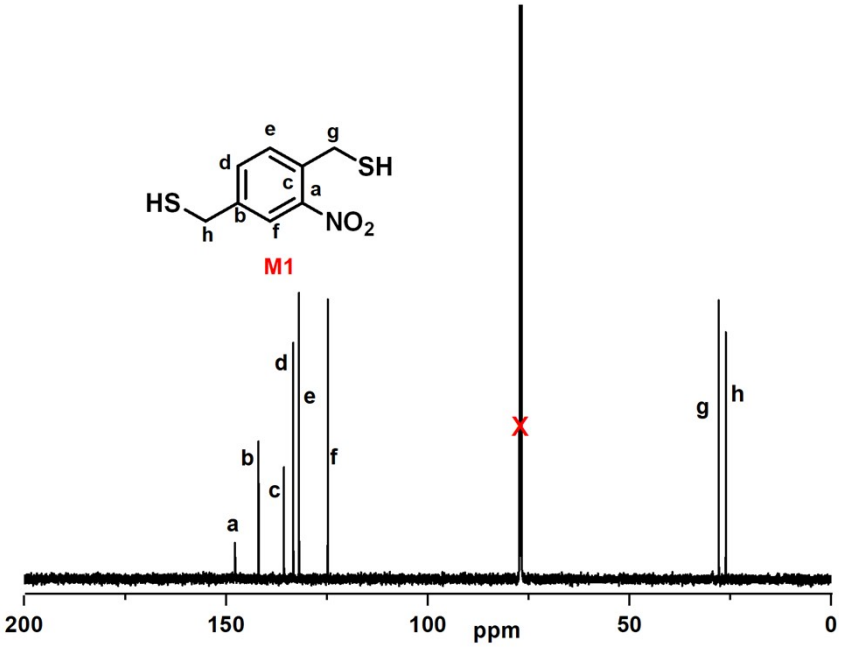


Fig. S2 ^{13}C NMR ($CDCl_3$) spectrum of compound BMNB.

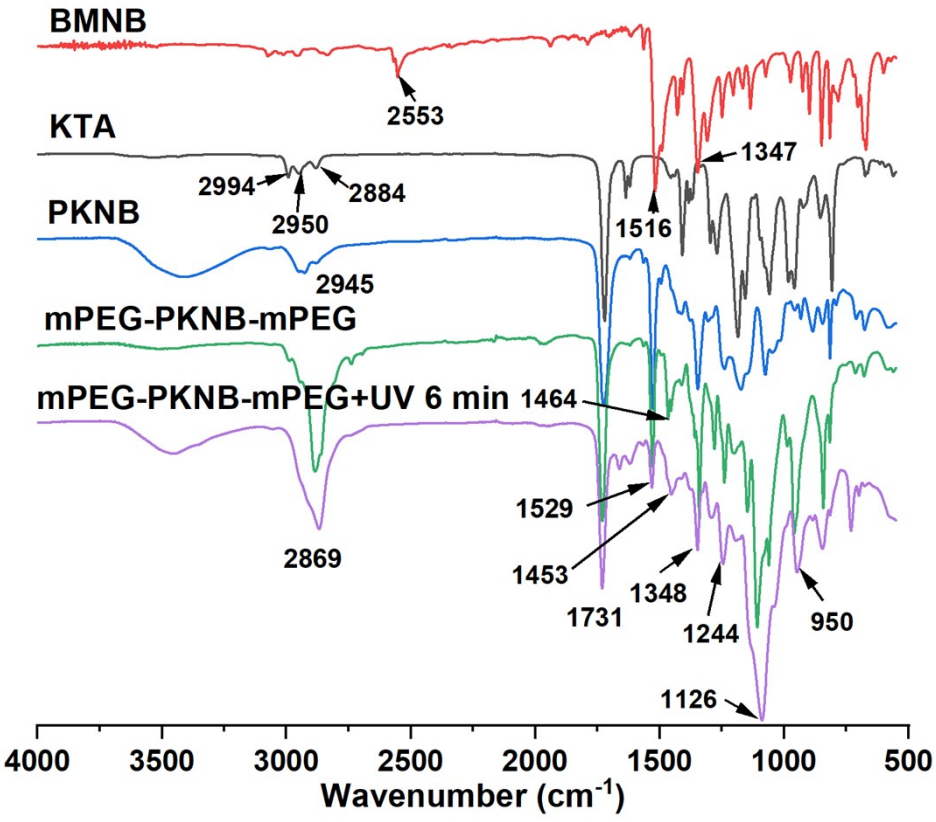


Fig. S3 FT-IR of monomers (BMNB and KDA), polymers (PKBN and mPEG-PKNB-mPEG) and the residual of mPEG-PKNB-mPEG after UV irradiation for 6 min.

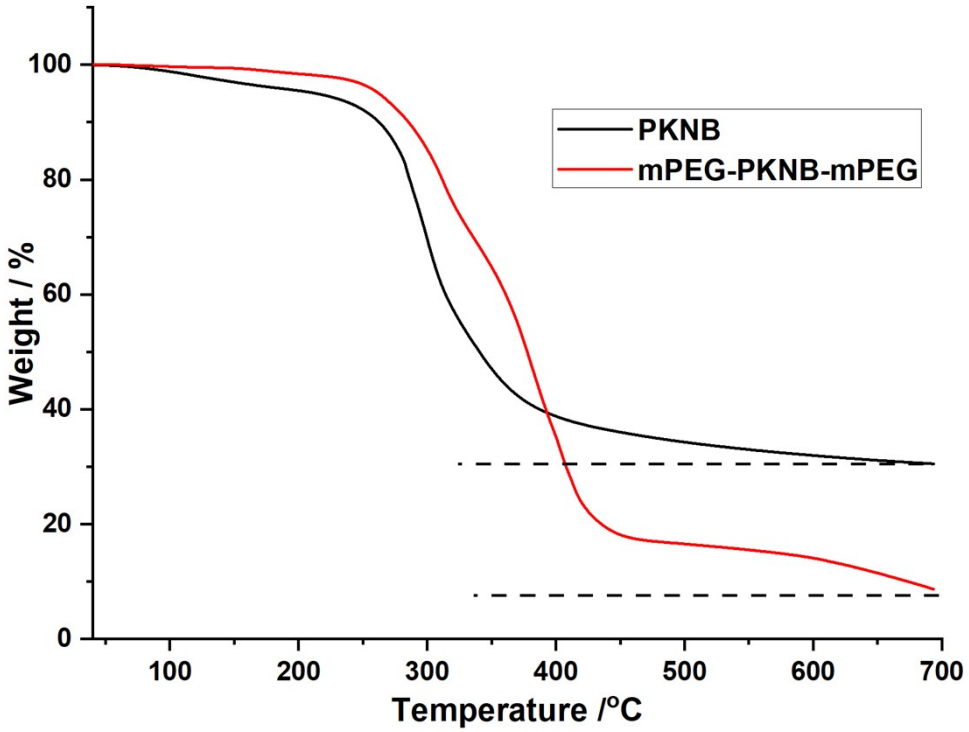


Fig. S4 TG curves of polymer PKNB and mPEG-PKNB-mPEG.

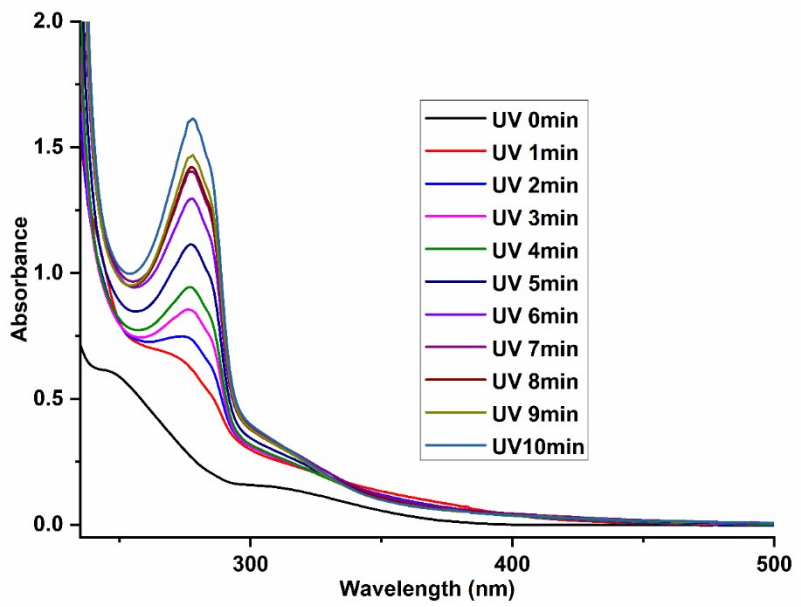


Fig. S5 The fluorescence of the residuals of mPEG-PKNB-mPEG after UV irradiation for different time.

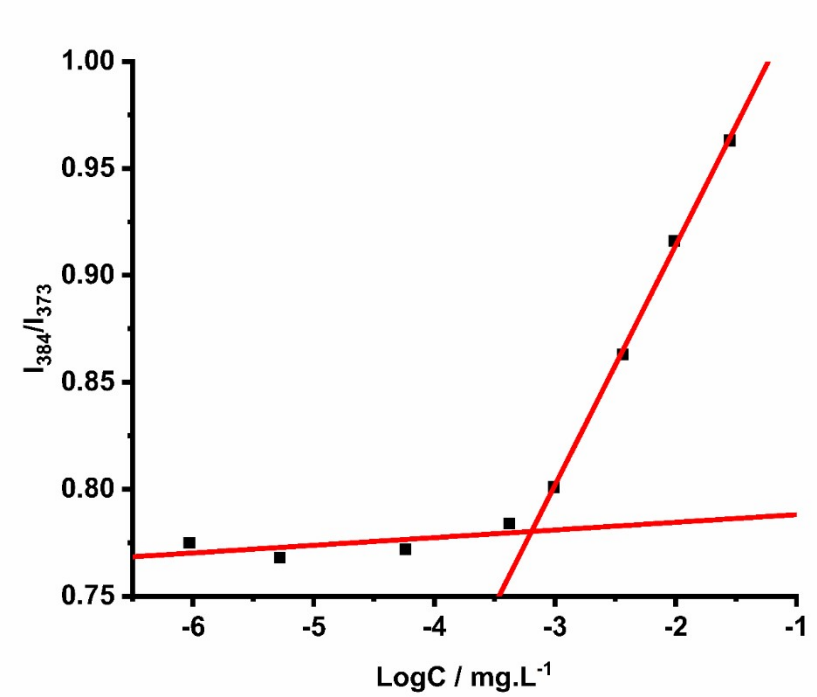


Fig. S6 The CMC of the mPEG-PKNB-mPEG nanoparticle.

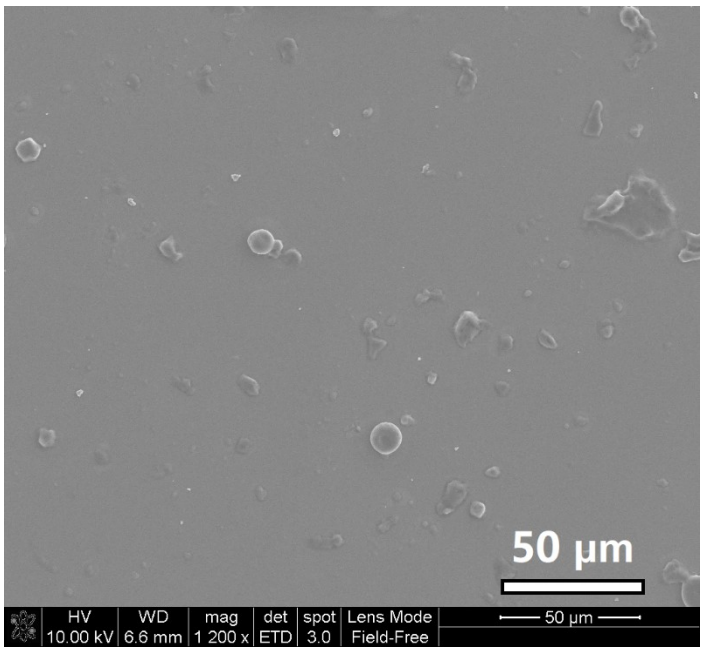


Fig. S7 SEM image of mPEG-PKNB-mPEG nanoparticles after HCl treatment for 3 min.

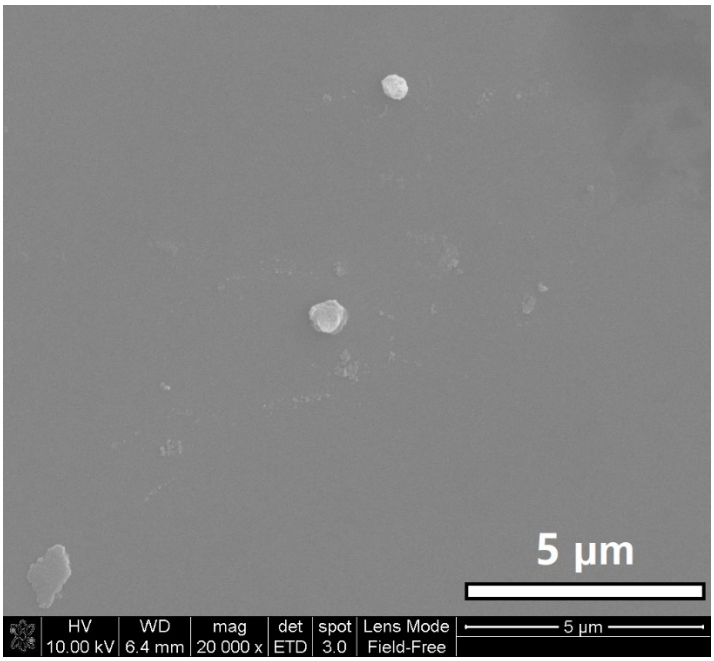


Fig. S8 SEM image of mPEG-PKNB-mPEG NPs after UV irradiation for 6 min.

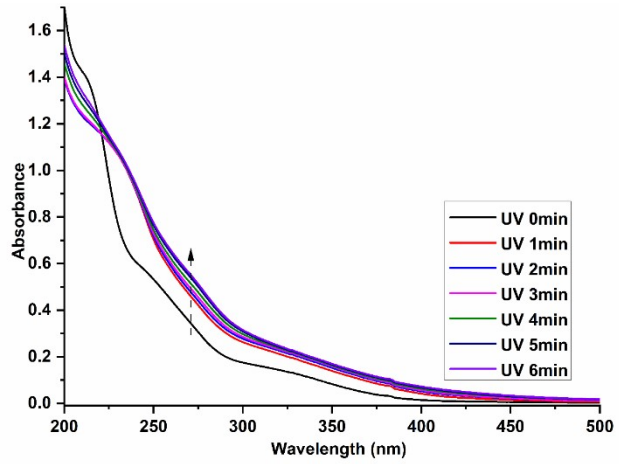


Fig. S9 The UV absorbance of the residuals of mPEG-PKNB-mPEG after UV irradiation for different time.

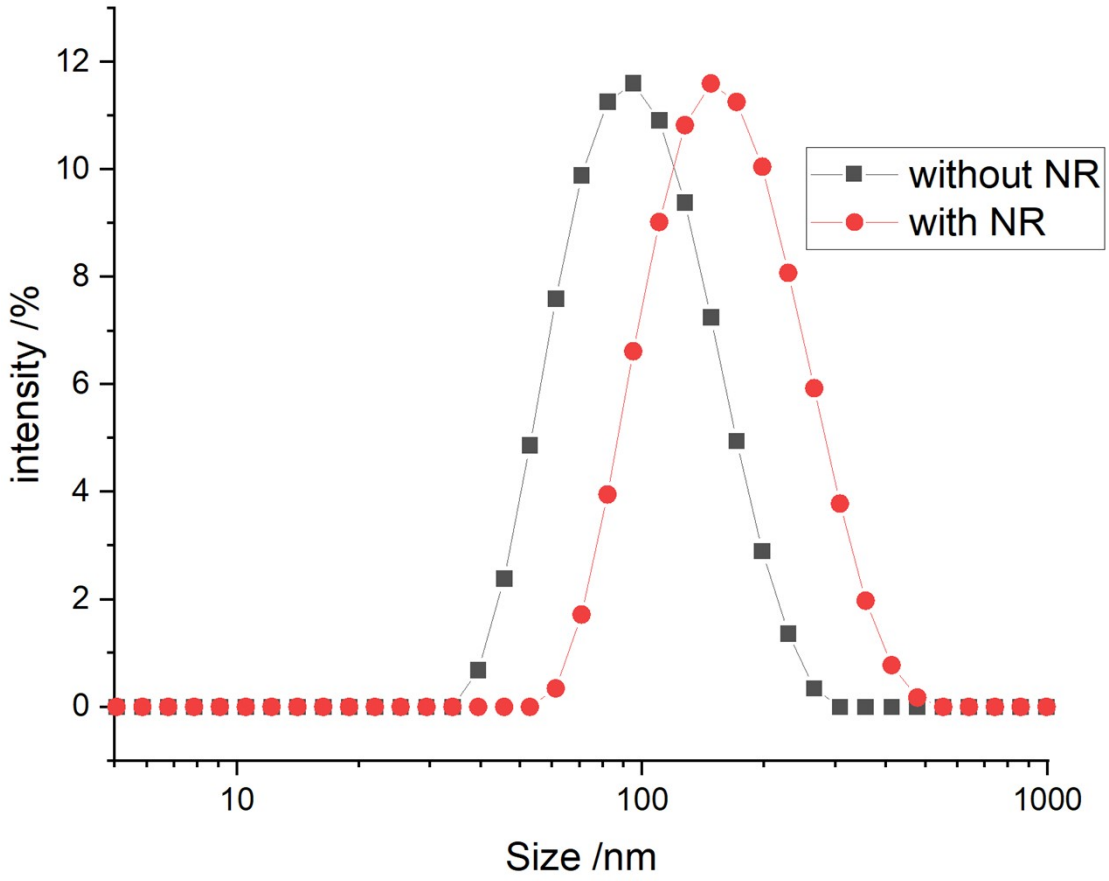


Fig. S10 DLS profiles of mPEG-PKNB-mPEG nanoparticles with (red) or without (black) Nile Red at pH 7.4.

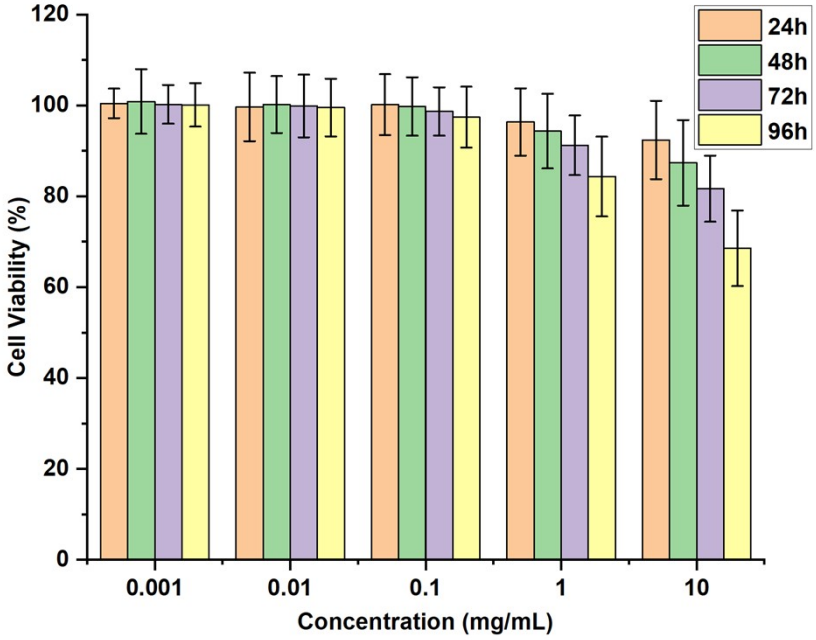


Fig. S11 Cell viability of NIH 3T3 cells incubated with different concentration of mPEG-PKNB-mPEG for different time.

References:

1. Z. Li, D. Li, L. Wang, C. Lu, P. Shan, X. Zou and Z. Li, *Polymer*, 2019, **168**, 16-20.
2. P. Shan, Y. Lu, W. Lu, X. Yin, H. Liu, D. Li, X. Lian, W. Wang, Z. Li and Z. Li, *Acs Applied Materials & Interfaces*, 2022, DOI: 10.1021/acsami.2c12106.
3. W. Wang and Y. Chau, *Chemistry of Materials*, 2012, **24**, 946-953.