Electronic Supplementary Information

Dual-Responsive Amphiphilic Cysteine Block Copolypeptide: Self-assembled Vesicles for Dye Encapsulation and Photo-Triggered Release

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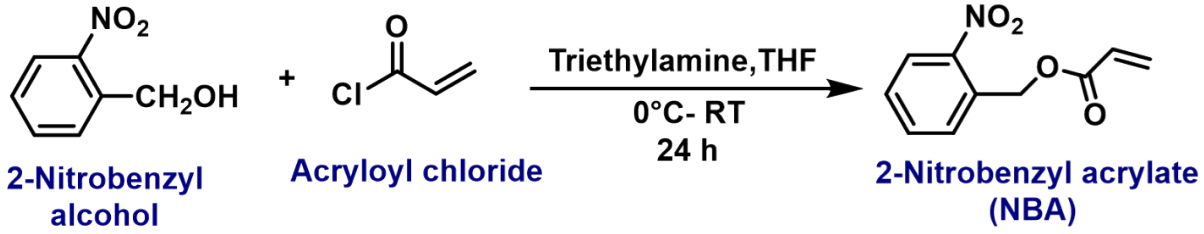
Synthesis of 2-nitrobenzyl acrylate (NBA) monomer

'2-Nitrobenzyl alcohol (5g; 32.6 mmol) was dissolved in 27 mL of dry THF in a two necked RB flask, equipped with a pressure-equalizing stopcock under argon gas atmosphere. Et_3N (4.5 mL; 32.6 mmol) was then added to the reaction mixture and was placed in an ice bath at 0 °C with constant stirring for at least 15 min. At this temperature, acryloyl chloride (2.5 mL;31.9 mmol) was added dropwise over a period of 30 min with constant magnetic stirring and allowed to raise the temperature to 25°C. The mixture was stirred magnetically for overnight. After completion of the reaction, THF was evaporated in rotary evaporator. The crude reaction mixture was dissolved in 60 mL of ethylacetate and was washed with 4×water and 2× brine to purify. The organic part was collected from separating funnel, followed by drying over anhydrous Na_2SO_4. After filtration, the solvent was removed in a rotary evaporator. Finally, the yellow viscous liquid, NBA monomer was isolated from by silica gel column chromatography with 1:6 ethylacetate/hexane mixture. (yield = 90%). The obtained NBA monomer was characterized by FTIR and ^1H NMR spectroscopy and as can be seen in Figure S4, and Figure S6, respectively



Scheme S1. Synthesis of 2-Nitrobenzyl acrylate (NBA) monomer.

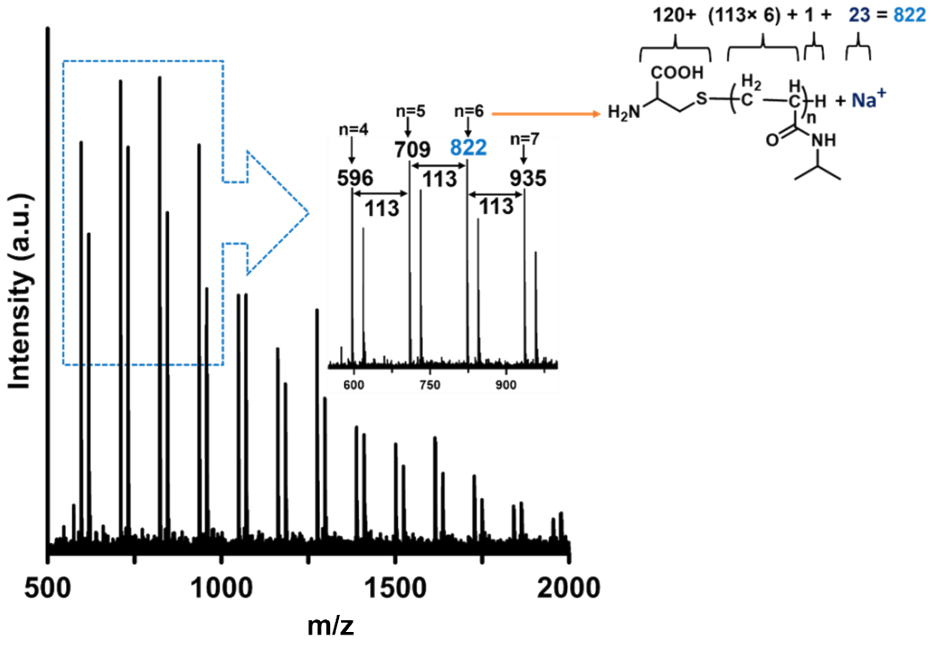


Figure S1. MALDI-TOF-MS spectrum of Cys-PNIPAM

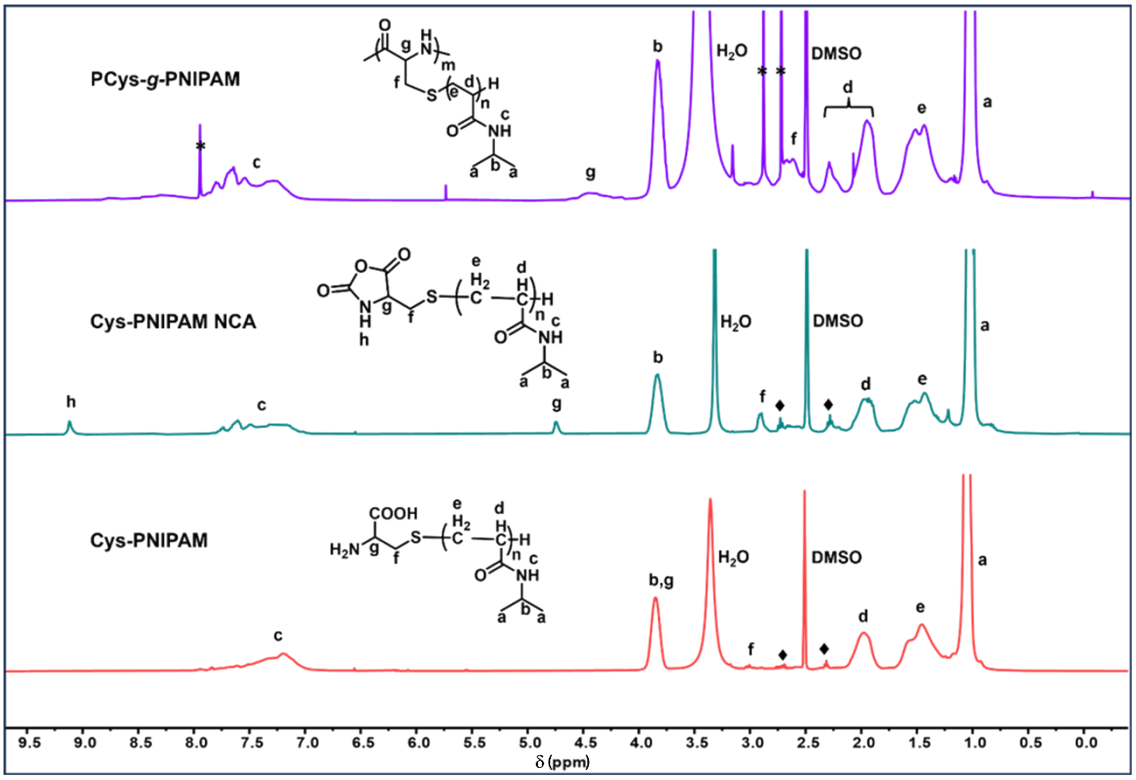


Figure S2. ^{1}H-NMR spectra of Cys-PNIPAM, Cys-PNIPAM NCA and PCys-*g*-PNIPAM in DMSO-d_6

^{1}H NMR spectrum of Cys-PNIPAM (500 MHz, DMSO-d_6, TMS) (ppm): 0.85-1.20 (-CH_3 of PNIPAM backbone at position a), 1.24-1.71 (-CH_2 of PNIPAM backbone at position e), 1.78-2.15 (-CH of PNIPAM backbone at position d), 2.97-3.03 (-methylene proton of cysteine residue at position f), 3.76-3.92 (-CH of PNIPAM backbone at position b and methine proton of cysteine residue at position g), 6.82-8.00 (-CH of PNIPAM at position c). [♦ The signals were possibly due to the traces of unreacted cysteine residues present in the system]

^{1}H NMR spectrum of Cys-PNIPAM NCA (500 MHz, DMSO-d_6, TMS) (ppm): 0.95-1.16 (-CH_3 of PNIPAM backbone at position a), 1.28-1.69 (-CH_2 of PNIPAM backbone at position e), 1.80-2.17 (-CH of PNIPAM backbone at position d), 2.82-2.98 (-methylene proton of cysteine residue at position f), 3.73-3.96 (-CH of PNIPAM backbone at position b), 4.66-4.84 (methine proton of cysteine residue at position g), 6.98-7.88 (-CH of PNIPAM at position c**).** [♦ The signals were possibly due to the traces of unreacted cysteine residues present in the system]

^{1}H NMR spectrum of PCys-*g*-PNIPAM (500 MHz, DMSO-d_6, TMS) (ppm): 0.83-1.14 (-CH_3 of PNIPAM backbone at position a), 1.24-1.74 (-CH_2 of PNIPAM backbone at position e), 1.78-2.38 (-CH of PNIPAM backbone at position d), 2.57-2.73 (-methylene proton of cysteine residue at position f), 3.71-3.94 (-CH of PNIPAM backbone at position b), 4.10-4.65 (methine proton of cysteine residue at position g), 7.00-7.90 (-CH of PNIPAM at position c). [∗ Signals due to DMF]

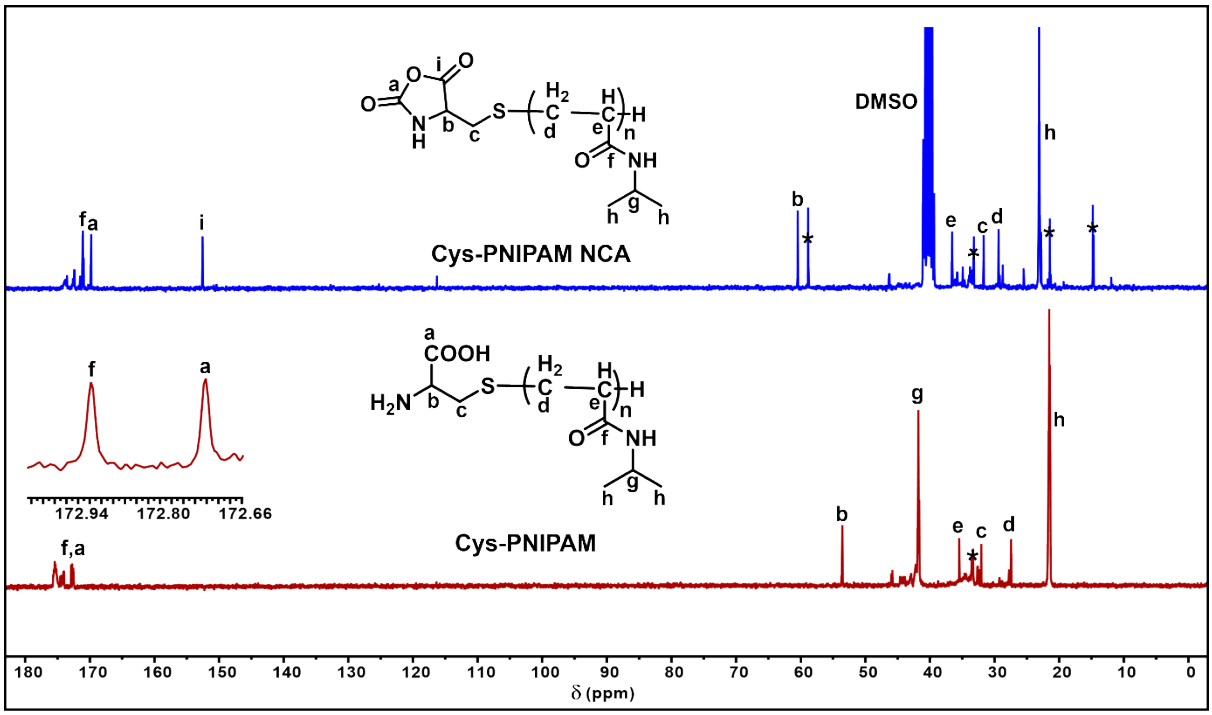


Figure S3. ^{13}C-NMR spectra of Cys-PNIPAM in D_2O and Cys-PNIPAM NCA in DMSO-d_6.

^{13}C-NMR spectrum of Cys-PNIPAM (75 MHz, D_2O, TMS) (ppm):

171.2 (>C=O of amide group at position f), 169.6 (>C=O of carboxylic group at position a), 53.4, 33.4 (carbons of cysteine residue at position b and c), 42.8, 35.1,27.8,22.01 (carbons of PNIPAM residue at position g, e,d,h).[* The signals were possibly due to the traces of unreacted cysteine residues present in the system]

^{13}C NMR spectrum of Cys-PNIPAM NCA (75 MHz, DMSO-d6, TMS) (ppm):

171.2 (>C=O of amide group at position f), 169.6, 152.6 (>C=O of two anhydride group at position a and i respectively), 60.3, 31.4 (carbons of cysteine residue at position b and c), 36.4,29.1,23.01 (carbons of PNIPAM residue at position e,d,h).[* The signals were possibly due to the traces of unreacted cysteine residues and EtOAC present in the system].

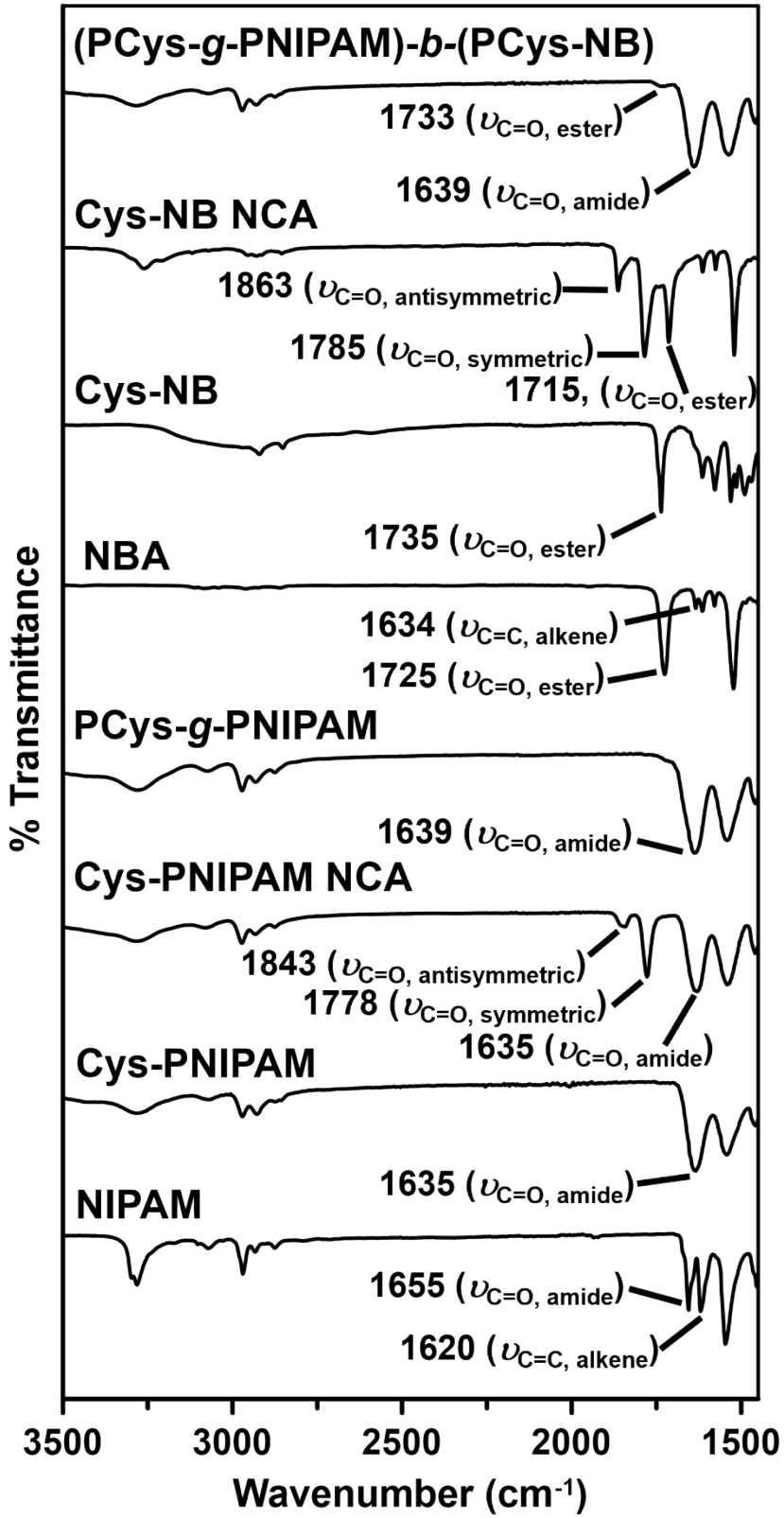


Figure S4. FTIR spectra of all the as-synthesized compounds

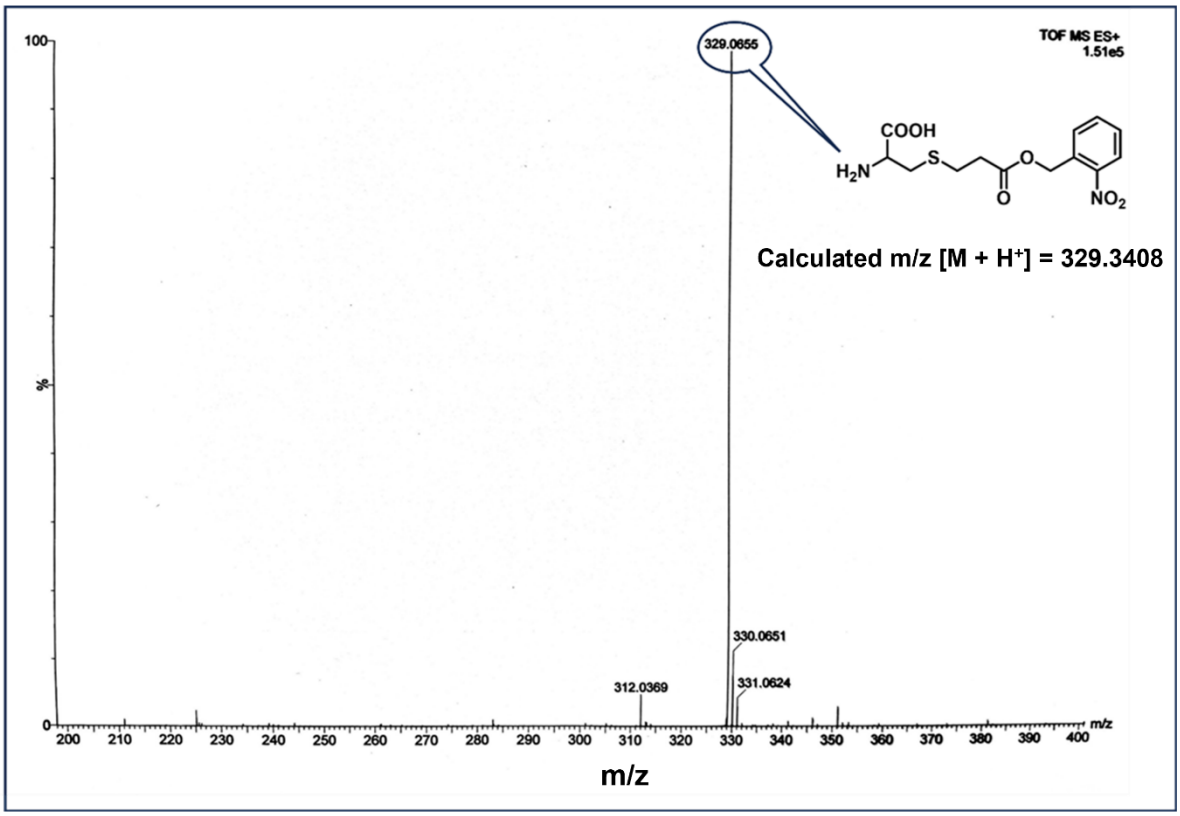


Figure S5. ESI-MS spectrum of Cys-NB in mixture of CH_3CN: H_2O (1:1).

ESI-MS spectrum of Cys-NB [m/z $(Cys\text{-}NB\text{-}H)^+$=329.0656 (observed); 329.3408 (calculated)]

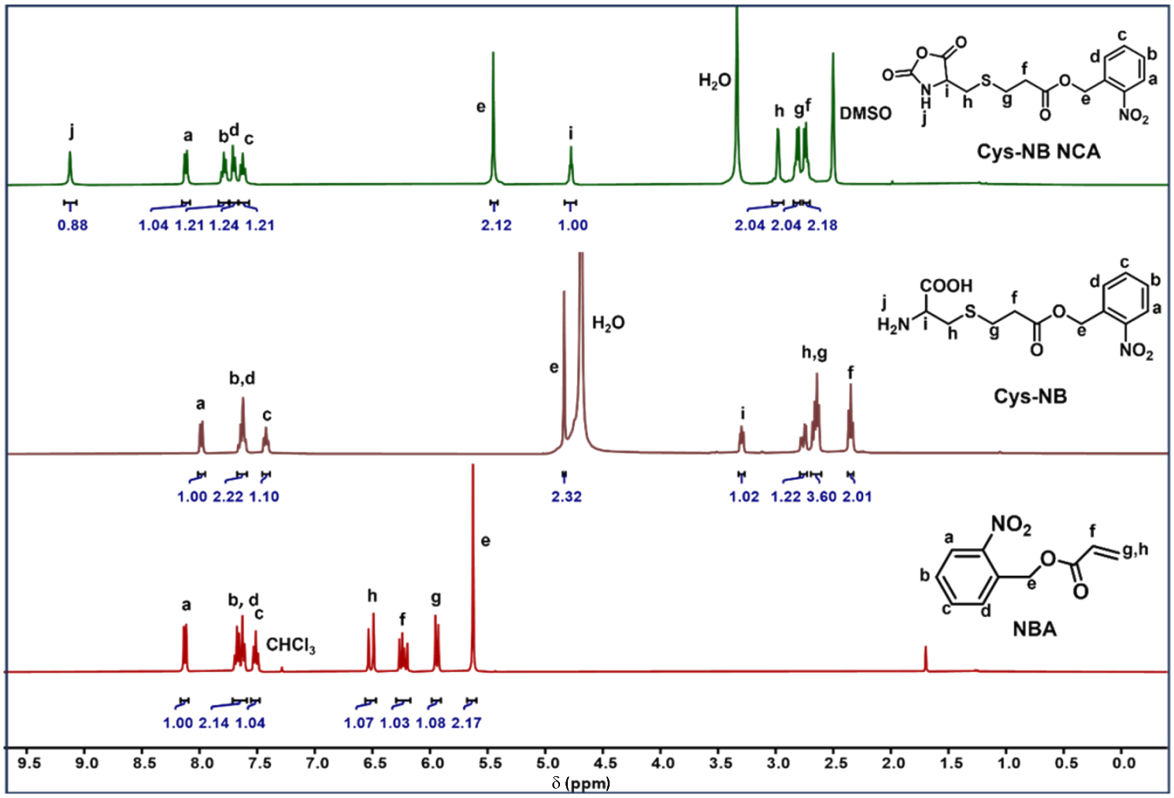


Figure S6. ^{1}H-NMR spectra of NBA in $CDCl_3$, Cys-NB in D_2O and Cys-NB NCA in DMSO-d_6.

^{1}H NMR spectrum of NBA (500 MHz, $CDCl_3$, TMS) (ppm):
5.62 (2H, -O-CH2-Ar at position e), 5.95 (1H, vinylic proton at position g), 6.5 (1H, vinylic proton at position h), 6.25 (1H, vinylic proton at position f), 7.5 (1H, Ar at position c), 7.65 (2H, Ar at position b,d), 8.13 (1H,Ar at position a).

^{1}H NMR spectrum of Cys-NB (500 MHz, D_2O, TMS) (ppm):
2.36 (2H, at position f), 2.66 (2H, at position g), 2.77 (2H, at position h), 3.30 (1H, at position i), 4.85(2H, at position e),7.44 (1H, Ar at position c), 7.63 (2H, Ar at position b,d), 7.99 (1H,Ar at position a).

^{1}H NMR spectrum of Cys-NB NCA (500 MHz, DMSO-d_6, TMS) (ppm):
2.74 (2H, at position g), 2.80 (2H, at position g), 2.98 (2H, at position h), 4.78(1H, at position i), 5.45(2H, at position e),7.63 (1H, Ar at position c), 7.71 (1H, Ar at position d), 7.80 (1H, Ar at position b), 8.13 (1H, Ar at position a), 9.14 (1H, Ar at position j).

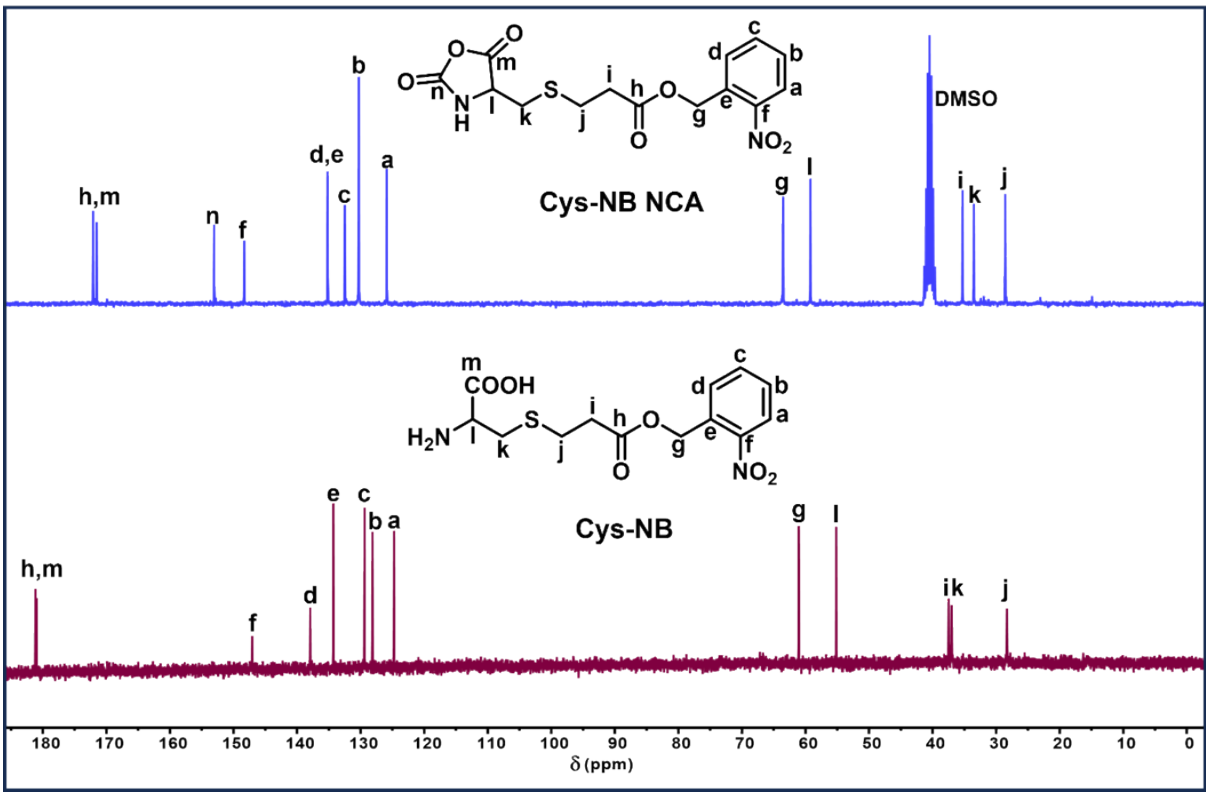


Figure S7. ^{13}C-NMR spectra of Cys-NB in D_2O and Cys-NB NCA in DMSO-d_6

^{13}C-NMR spectrum of Cys-NB (75 MHz, D_2O, TMS) (ppm):

181.1 (>C=O of ester group at position h), 180.9 (>C=O of carboxylic group at position m), 147.0, 137.9,134.2, 129.3, 128.1, 124.7 (aromatic carbons at position f,d,e,c,b,a respectively), 60.8 (-CH_2 at position g), 55.2 (-CH at position l), 37.4, 37.0, 28.3 (-CH_2 at position i,k,j respectively).

^{13}C-NMR spectrum of Cys-NB NCA (75 MHz, DMSO-d_6, TMS) (ppm):

172.0 (>C=O of ester group at position h), 171.5,152.9 (>C=O of two anhydride group at position m and n respectively), 148.4, 135.3,135.2,132.4, 130.3, 125.7, 124.7 (aromatic carbons at position f,d,e,c,b,a respectively), 63.5 (-CH2 at position g), 59.2 (-CH at position l), 35.1, 33.0, 28.8 (-CH2 at position i,k,j respectively).

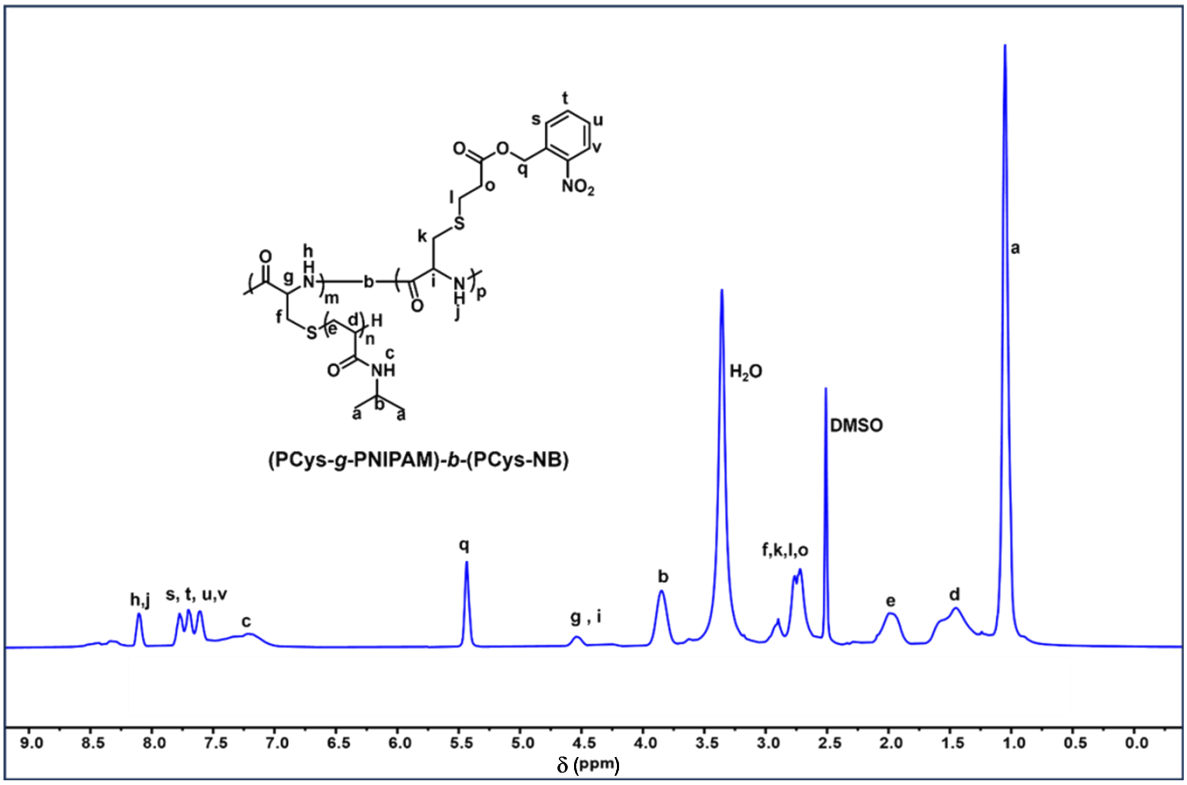


Figure S8. ^{1}H-NMR spectrum of (PCys-*g*-PNIPAM)-*b*-(PCys-NB) (**P1**) in DMSO-d_6

^{1}H NMR spectrum of (PCys-*g*-PNIPAM)-*b*-(PCys-NB) (500 MHz, DMSO-d_6, TMS) (ppm): 0.85-1.16 (-CH_3 of PNIPAM backbone at position a), 1.24-1.71 (-CH_2 of PNIPAM backbone at position d), 1.78-2.15 (-CH of PNIPAM backbone at position e), 2.59-3.01 (-methylene proton of cysteine residue and PCys-NB at position f,k,l,o), 3.69-3.98 (-CH of PNIPAM backbone at position b), 4.14-4.64 (methine proton of PNIPAM and PCys-NB residue at position g,i), 5.35-5.50 (2H at position q), 6.96-7.50 (-CH of PNIPAM at position c), 7.54-7.84 (4H Ar at position s,t,u,v), 8.02-8.15 (-NH protons of PCys-*g*-PNIPAM and PCys-NB at position h,j).

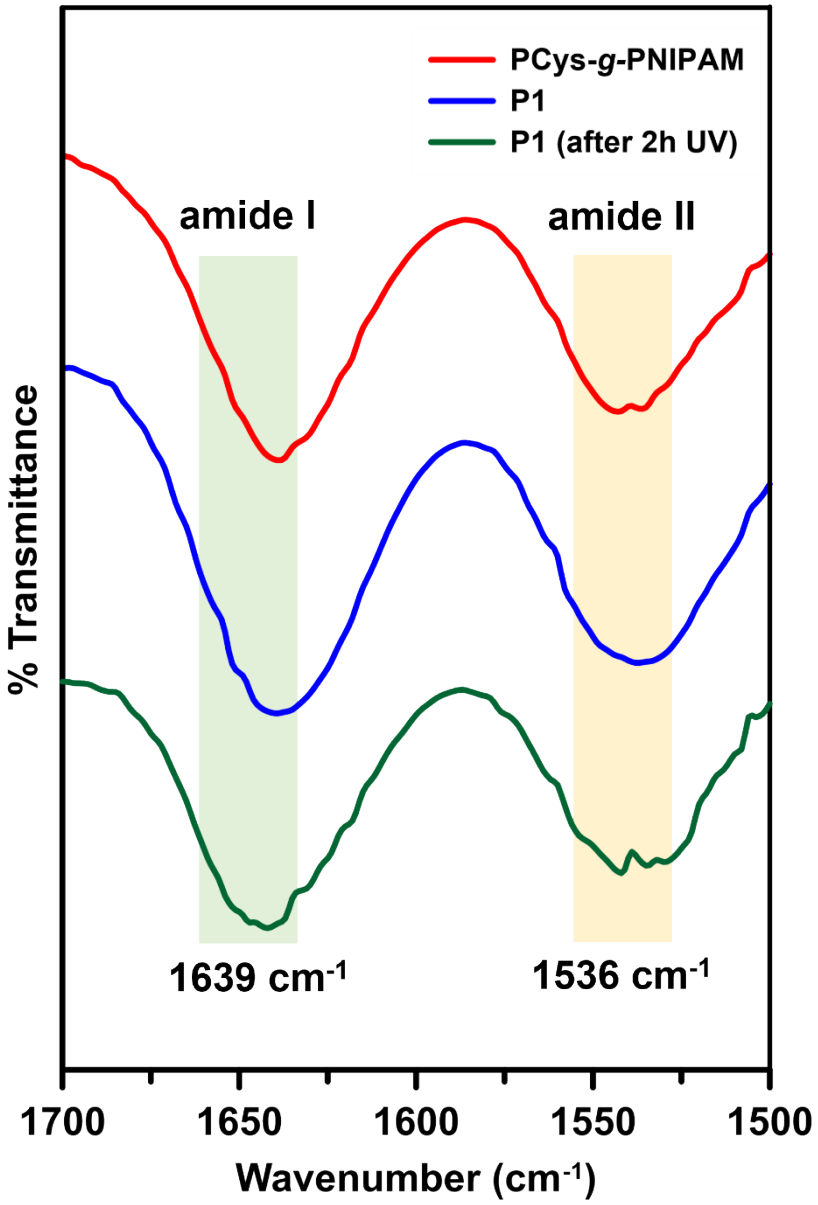


Figure S9. FTIR spectra of PCys-*g*-PNIPAM, (PCys-*g*-PNIPAM)-*b*-(PCys-NB) (**P1**) and **P1** after UV irradiation showing the vibration bands related to their secondary structure.

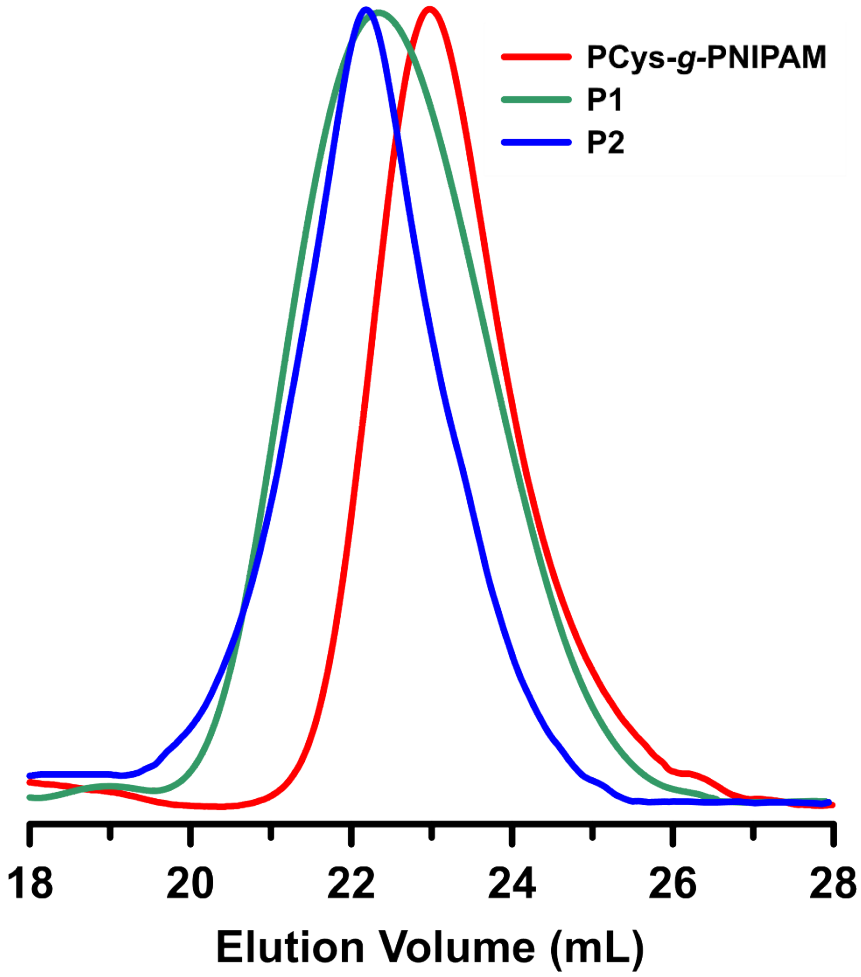


Figure S10. SEC chromatograms of all the polypeptides.

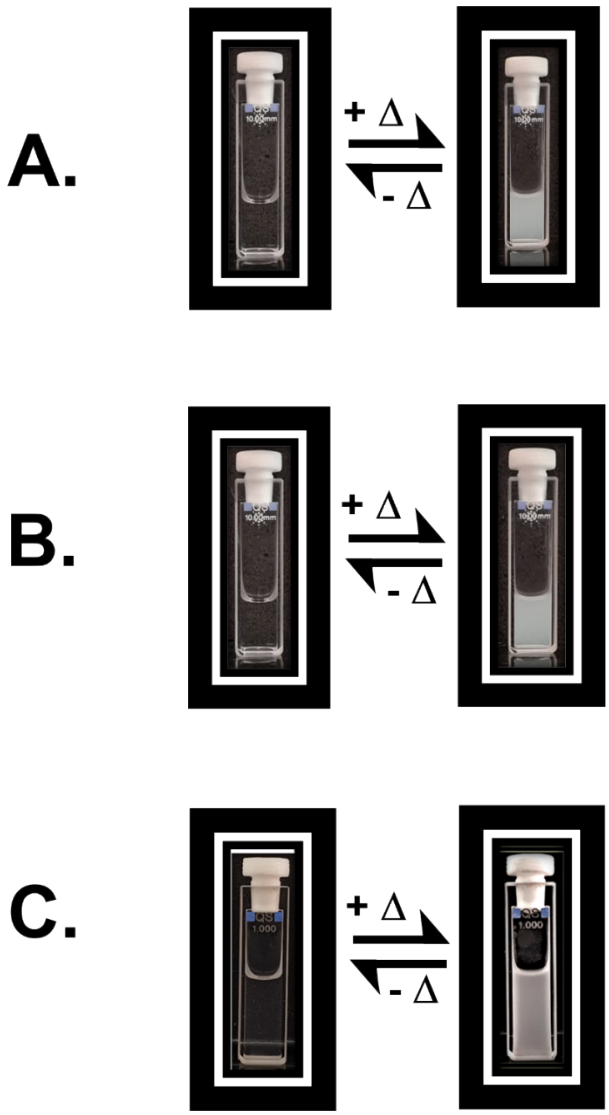


Figure S11. Photographs of A. (Cys-PNIPAM), B. (PCys-g-PNIPAM), and C. ((PCys-g-PNIPAM)-*b*-(PCys-NB) in water (0.2 wt %) showing reversible soluble-insoluble-soluble LCST-type phase-transition.

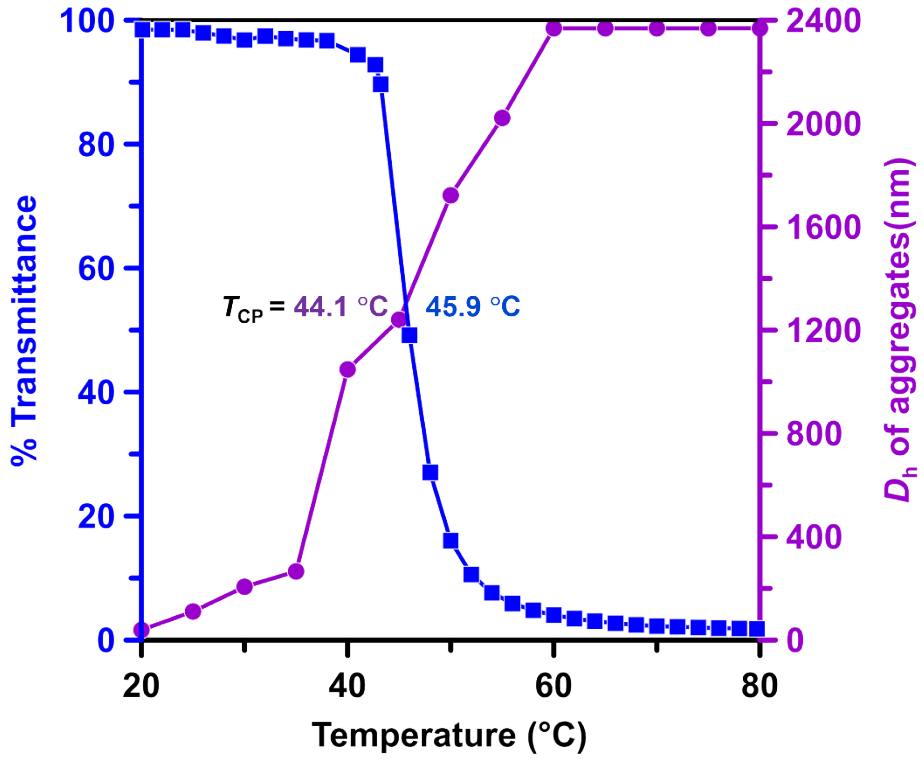


Figure S12. DLS was used to measure the average hydrodynamic diameter of P1 (0.1 wt% in water) as a function of temperature. The results are shown in the accompanying plot (violet).

The correlation between hydrodynamic diameter (D_h, ■) and transmittance at 500 nm (●) for a 0.1 wt% aqueous solution of **P1** at various temperatures was also shown for easy understanding. This data highlights the prominent cloud points of **P1**.

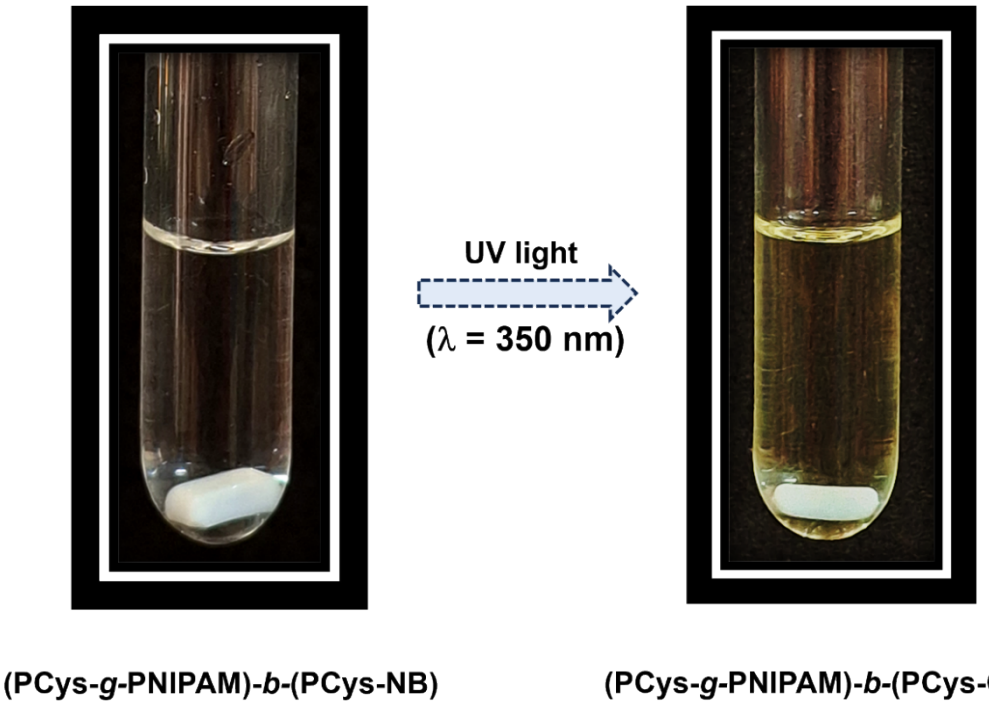


Figure S13. Photographs of (PCys-*g*-PNIPAM)-*b*-(PCys-NB) in water (0.025 wt %) before UV irradiation and after UV irradiation

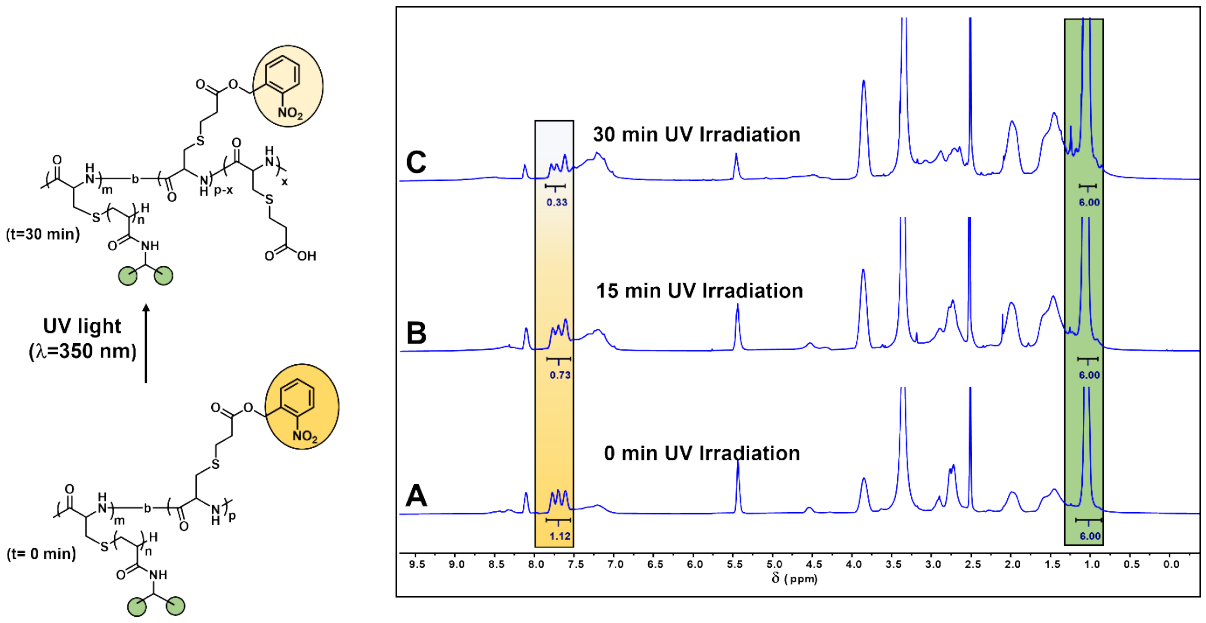


Figure S14. Quantitative ^{1}H-NMR spectra of (PCYS-*g*-PNIPAM)-*b*-(PCys-NB) (**P1**): (A) before; (B,C) after UV irradiation.

The comparison of the integration area (I) of the PCys-NB block protons of **P1** at ~7.54-7.84 ppm before and after UV irradiation.

Degree of photocleavage (%) after 30 min UV irradiation of **P1**

= [(I in before UV irradiation) – (I in after 30 min UV irradiation) / I in before UV irradiation] × 100

= [(1.12-0.33)/ 1.12] × 100 = 70.5

Determination of critical aggregation concentration (CAC).

The pyrene was used as a fluorescent probe to determine the CAC of the **P1** in water. It is known that the deciding parameter for CAC is the ratio of two characteristic emission bands of pyrene at λ= 392 nm (I_3) and λ= 372 nm (I_1), which is highly sensitive to the medium's polarity. A series of **P1** solution of varying concentrations (0.075 to 0.001 wt%) were prepared in DI water. A separate stock solution of pyrene in acetone was prepared at concentration of 10^{-4} M. From this stock, 20 µL aliquots were transferred to eight vials. The solvent was evaporated, and 4 mL of each **P1** solution was added to the respective vials. After 10 min of sonication, the solutions were left undisturbed overnight in dark. The final pyrene concentration was maintained at 5×10^{-7} M in all solutions. The emission spectra were recorded by exciting the solutions at λ= 334 nm using a slit of width of 5 nm. The ratio of I_{392} to I_{372} (I_3/I_1) was plotted against the logarithm of the **P1** concentration. The plot revealed a gradual increase of the I_3/I_1 value with increasing **P1** concentration, followed by a sudden jump after a particular concentration. The intersection point of the two resulting straight lines was identified as CAC and the value was 0.019 wt % for the **P1**.

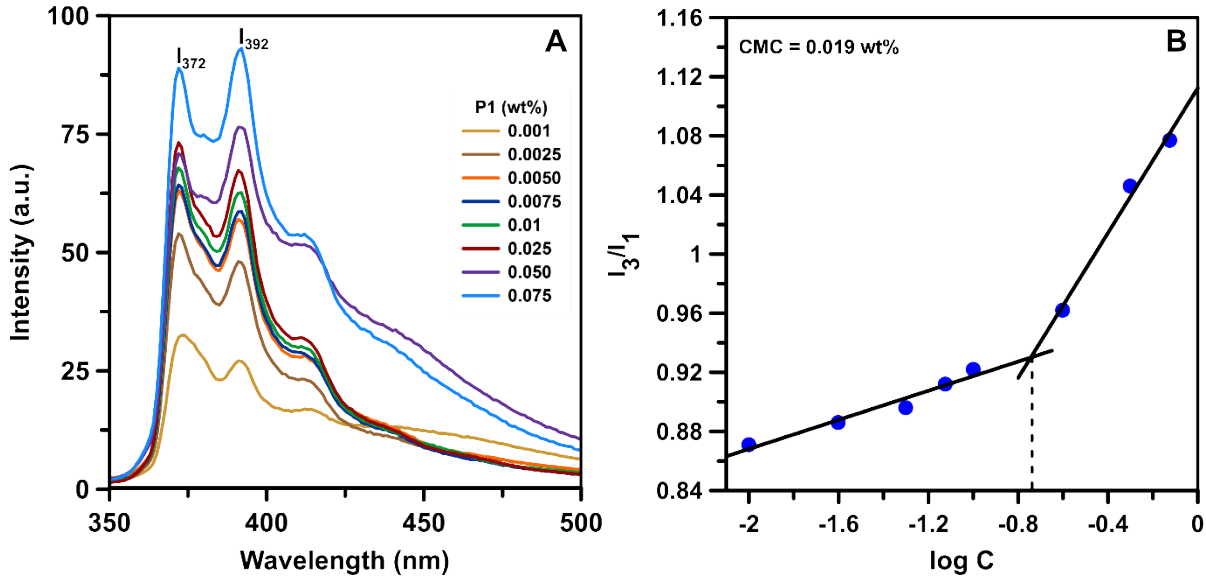


Figure S15. Emission spectra of pyrene (λ_{ex} = 334 nm) in the presence of aqueous **P1** solution of varying concentration (A). The plot of fluorescence vibronic intensities ratio (I_3/I_1) as a function of the **P1** concentration as measured from emission spectra (B).

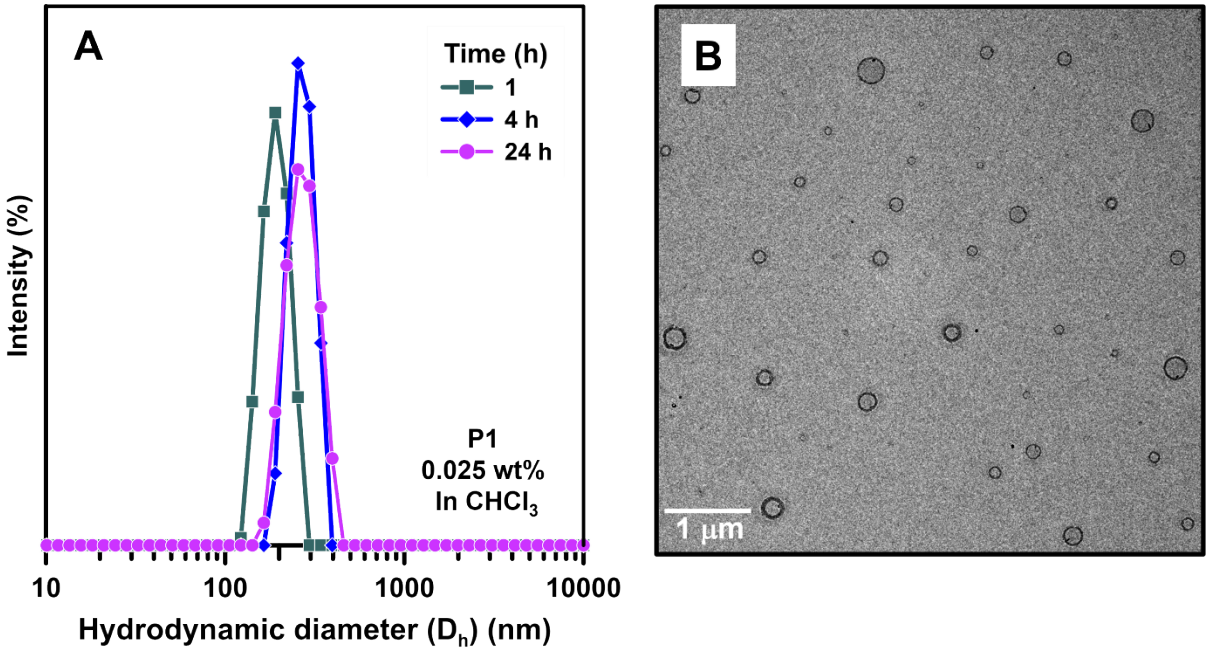


Figure S16. Time-dependent D_h measurement of aqueous **P1** at 25 °C (A) and FEG-TEM (B) images of **P1** vesicles in $CHCl_3$ (0.025 wt %).

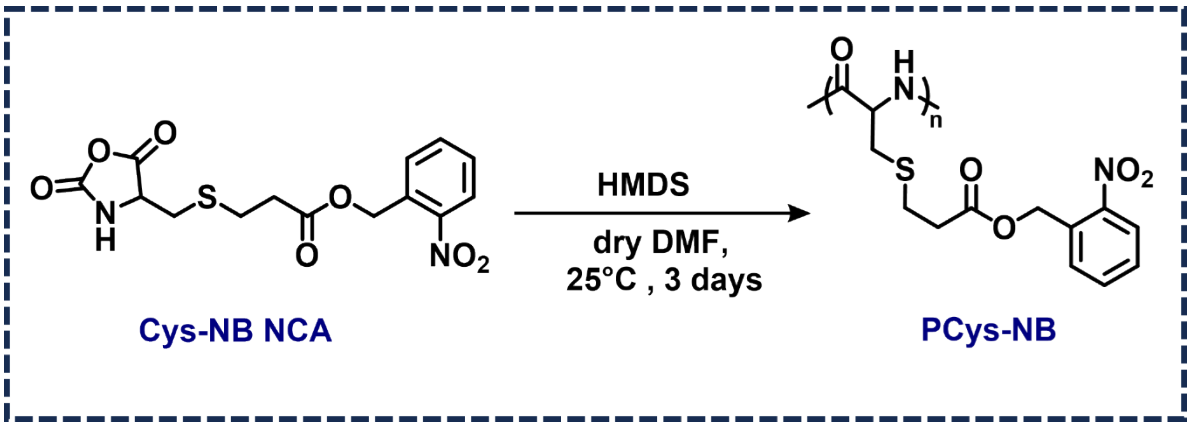


Figure S17 A. Synthesis of PCys-NB**.**

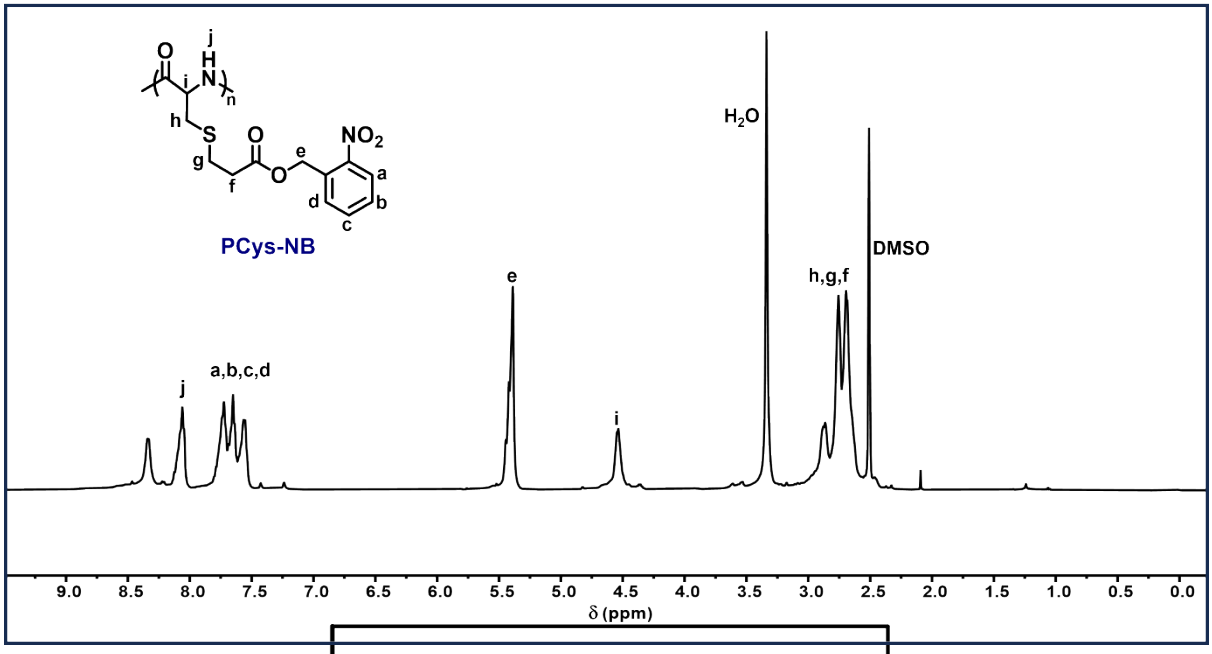


Figure S17 B. ^{1}H-NMR spectrum of PCys-NB.

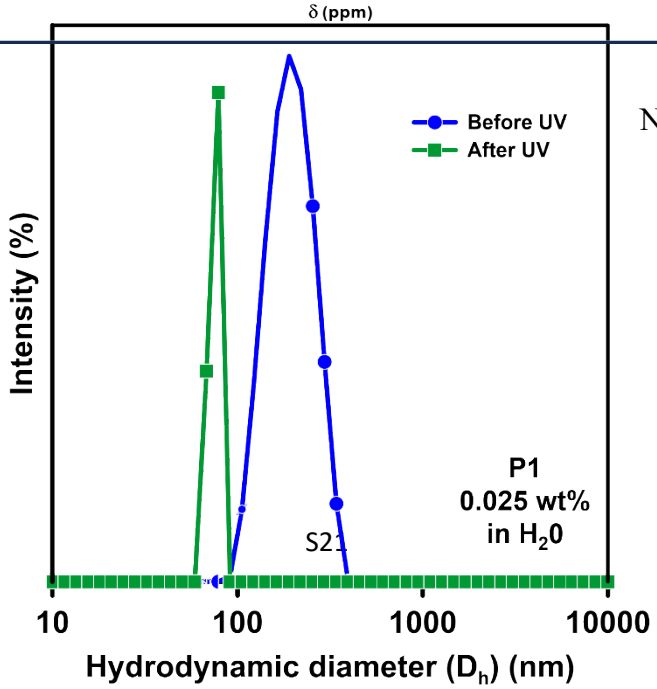


Figure S18. DLS curves of **P1** vesicles before and after UV light irradiation in H_2O (0.025 wt%).

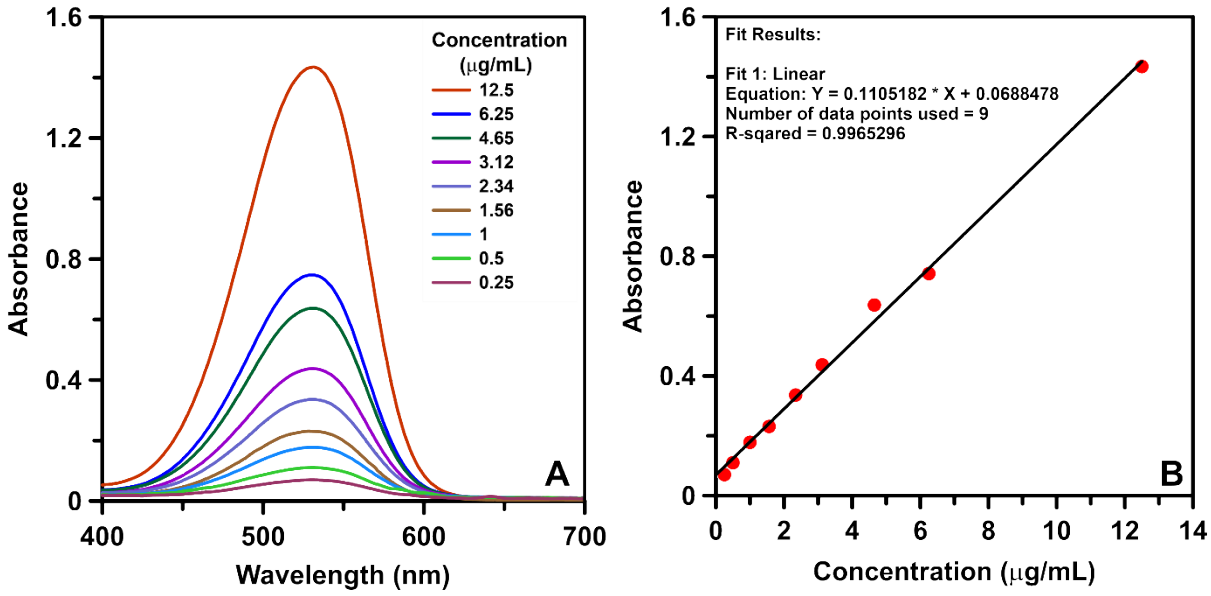


Figure S19. Concentration-dependent absorption spectra of Nile red (NR) in Acetone (A). A linear calibration plot was generated by measuring the absorbance at its maximum (531 nm) for different concentrations of NR (B).

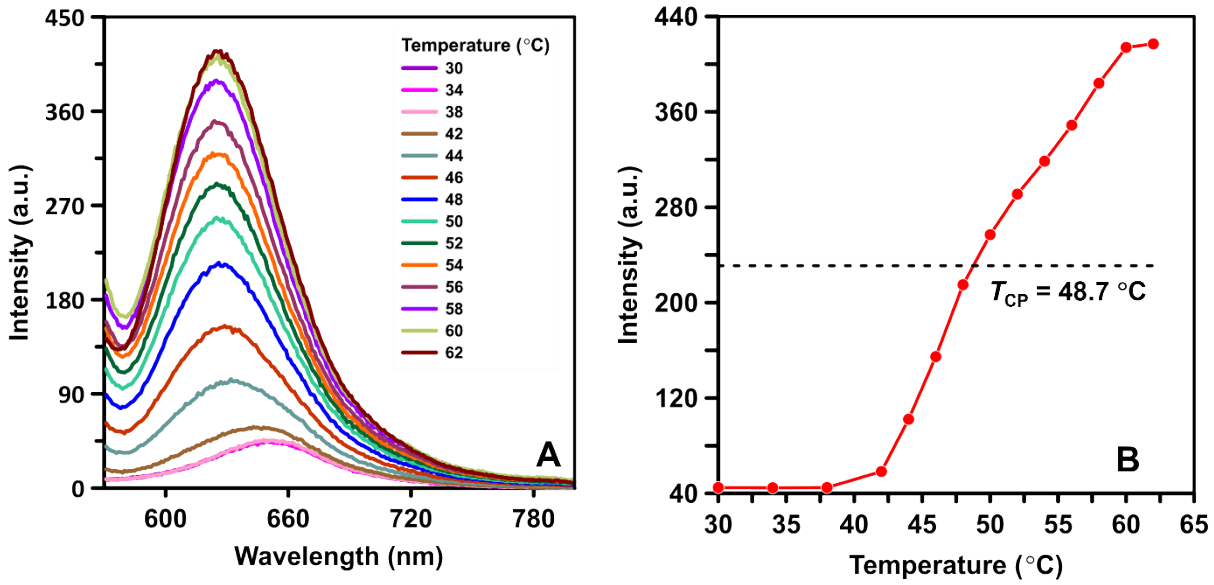


Figure S20. Temperature-dependent emission spectra of NR-loaded vesicles of **P1** (A). Plot of NR-loaded emission intensity against temperature of **P1** in water (B).