Electronic supplementary information (ESI) for

Disclosing the nature of thermo-responsiveness of poly(*N*-isopropyl acrylamide)-based polymeric micelles: aggregation or fusion?

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Experimental Section

Materials. Polyethylene oxide monomethylether (PEO_{43}; M_n = 1900) was purchased from Alfa Aesar. 2-(Dodecylthiocarbonothioylthio)-2-methylpropanoic acid (DDMAT) was synthesized according to our previously reported method.[1] *N*-isopropyl acrylamide (NIPAM), 7-hydroxy-4-methylcoumarin, 2-bromoethanol, methacryloyl chloride were purchased from Aladdin Chemistry, Co. 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS) was synthesized according to a literature method.[2] Dicyclohexylcarbodiimide (DCC, 96%), 4-dimethylaminopyridine (DMAP, 99%), potassium carbonate, $MgSO_4$, triethylamine (TEA), acetone, dichloromethane, tetrahydrofuran (THF), ethanol and other solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China) and used as received. AIBN was recrystallized from MeOH and stored at 4 °C before use.

Characterization. ^{1}H NMR. ^{1}H NMR spectra were recorded using a Bruker AV 400 MHz spectrometer at room temperature with $CDCl_3$ and D_2O as solvents and tetramethylsilane (TMS) as standard.

DLS. The dynamic light scattering (DLS) was used to determine the hydrodynamic diameters (D_h) and polydispersity of self-assemblies formed from block copolymers by a ZetaSizer Nano series instrument (Malvern Instruments ZS 90).

UV-Vis Spectroscopy. UV-vis spectroscopy of the photo-cross-linking process was acquired using a UV759S UV-vis spectrophotometer (Shanghai Precision and Scientific Instrument Co., Ltd.). All samples were analysed using quartz cuvettes with a path length of 10 mm.

TEM. The copper grids were treated by plasma cleaner (HARRIK plasma, PDC-32G) to form a hydrophilic surface. The copper grids were pre-heated to 45 ^{o}C. Each sample (3 μL) was then dropped onto the copper grid and dried at 45 ^{o}C. Imaging was recorded on a JEOL JEM-2100F instrument at 200 kV equipped with a Gatan 894 Ultrascan 1k CCD camera. The TEM samples were prepared according to the following protocol: the micelles solution at 45 ^{o}C was cross-linked at the same temperature to fix the

structure at first. Then the solution (3 μL) was dropped to a pre-heated copper grid and dried in a drying oven at 45 °C. The key point of this sample preparation process is to keep the environmental temperature at 45 °C to maintain its original structure.

Synthesis of macro chain transfer agent (macro-CTA) of PEO-DDMAT. PEO-DDMAT was synthesized according to a previous method.[3] ^{1}H NMR was presented in Fig. S1 in ESI†.

Synthesis of monomer 7-(2-methacryloyloxyethoxy)-4-methylcoumarin (CMA). Based on our previous work,[4, 5] 7-hydroxy-4-methylcoumarin (5.00 g, 0.0300 mol) was first dissolved in 250 mL of acetone. K_2CO_3 (21.14 g, 0.1500 mol) and 2-bromoethanol (17.92 g, 0.1400 mol) were then added successively. The mixture was allowed to react at 60 °C in an oil bath with reflux under argon protection for 1 week. The reaction mixture was directly recrystallized from ethanol to yield white crystal. Yield: ~92%.

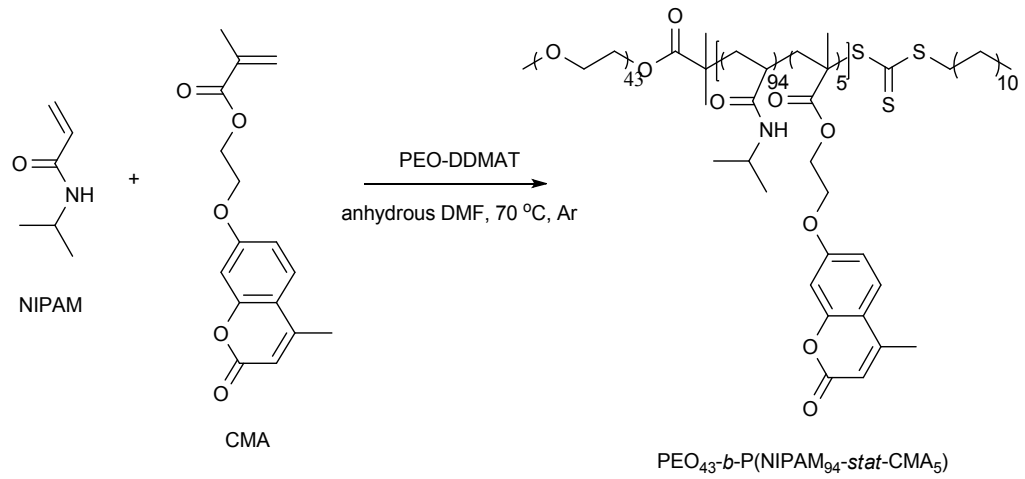
The resultant 7-(2-hydroxyethoxy)-4-methylcoumarin (2.00 g, 9.08 mmol) and TEA (3.30 g, 32.60 mmol) were dissolved in anhydrous THF (200 mL) with ice bath. Methacryloyl chloride (2.65 g, 25.28 mmol) was first dissolved in 50 mL anhydrous THF and then added dropwise to the mixture within 45 minutes with ice bath. Afterwards, the mixture was allowed to react at room temperature with stirring for 48 h and quenched by 5.0 mL of MeOH. After filtration, the solvent was removed by rotary evaporation. The residue was re-dissolved in DCM and extracted with DI water and saturated brine twice successively. The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to yield a yellowish crude product. Further purification was carried out with column chromatography (n-hexane/EtOAc, 1/4) to finally get the 7-(2-methacryloyloxyethoxy)-4-methylcoumarin monomer. Yield: ~60%. ^{1}H NMR was presented in Fig. S2†.

Synthesis of PEO_{43}-*b*-P($NIPAM_{94}$-*stat*-CMA_5) block copolymer by RAFT polymerization. In a typical process (Scheme S1), macro-CTA PEO-DDMAT (0.20 g,

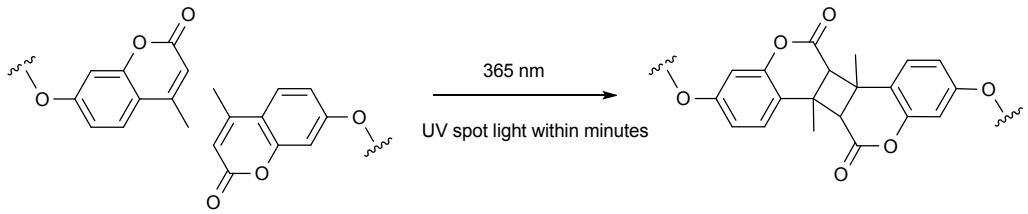
0.09 mmol), NIPAM (1.03 g, 8.90 mmol) and CMA (0.16 g, 0.53 mmol) were dissolved in a 25 mL flask. The mixture was flushed with argon for 15 min for deoxygenation. AIBN radical initiator (2.20 mg, 0.01 mmol) was then added followed by argon flushing for another 15 min. The flask was then sealed and immerged in an oil bath at 70 ^{o}C. The molar ratio of [PEO-DDMAT]/[NIPAM]/[CMA]/[AIBN] was 1:100:6:0.15. After 24 h of polymerization, the reaction was terminated by exposure to air. Following the evaporation of solvent, the residue was dissolved in DCM, precipitated from n-Hexane three times and dried in a vacuum at 25 ^{o}C. Yield: ~65%. Conversion was *ca.* 92 % for NIPAM and *ca.* 83% for CMA from ^{1}H NMR (Fig. 1).

Self-assembly and photo-cross-linking of block copolymer micelles. PEO_{43}-*b*-P($NIPAM_{94}$-*stat*-CMA_5) was dissolved to DI water directly to form polymer micelles (1.0 mg/mL). Then the micelles were placed under a UV spot curing system (8000 mw/cm^2) at a λ of 365 nm for 3 min to immobilize the structure.

Thermally responsive behaviour of micelles. The thermal responsive behaviour of micelles was studied by DLS, NMR and TEM. In a typical experiment, the D_h and PDI of micelles aqueous solution was measured by DLS from 25 to 45 ^{o}C, every 2 ^{o}C equilibrated for 20 min. The resulted micelle solution was then photo-cross-linked at 45 ^{o}C to fix the structure for TEM analysis.



Scheme S1. Synthesis route of PEO_{43}-*b*-P($NIPAM_{94}$-*stat*-CMA_5).



Scheme S2. Photo-cross-linking reaction in the micelles.

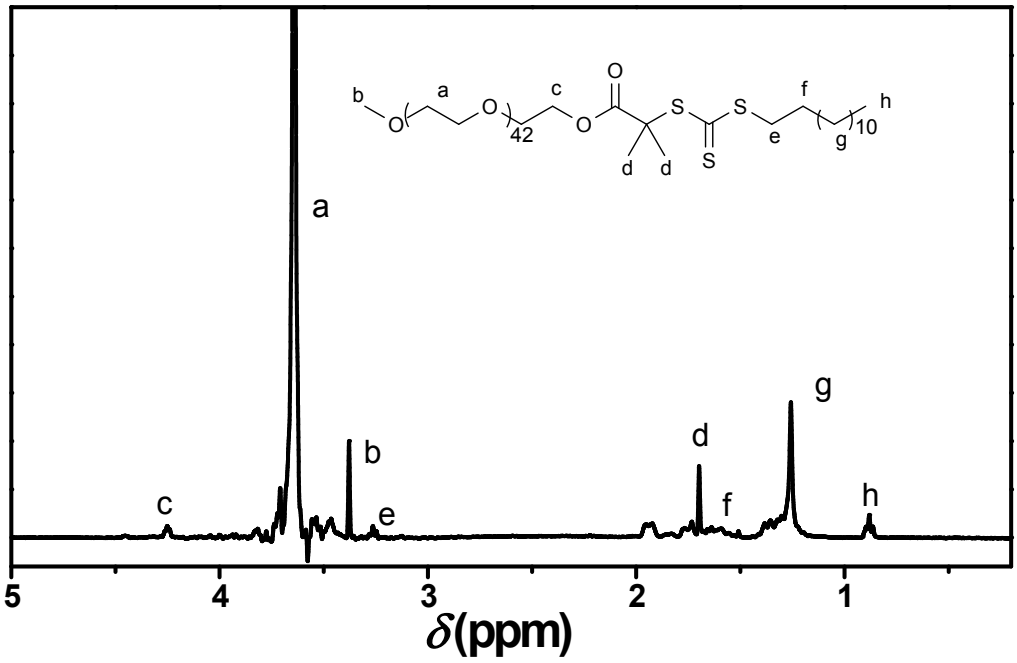


Fig. S1 ^{1}H NMR spectrum of macro-CTA PEO-DDMAT in $CDCl_3$.

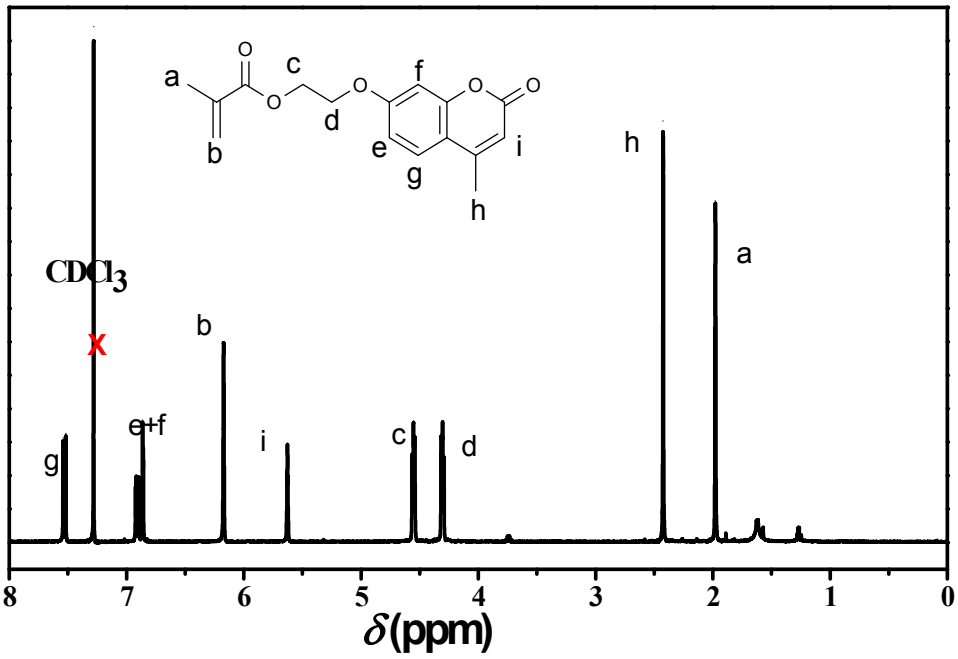


Fig. S2 ^{1}H NMR spectrum of monomer 7-(2-methacryloyloxyethoxy)-4-methylcoumarin (CMA) in $CDCl_3$.

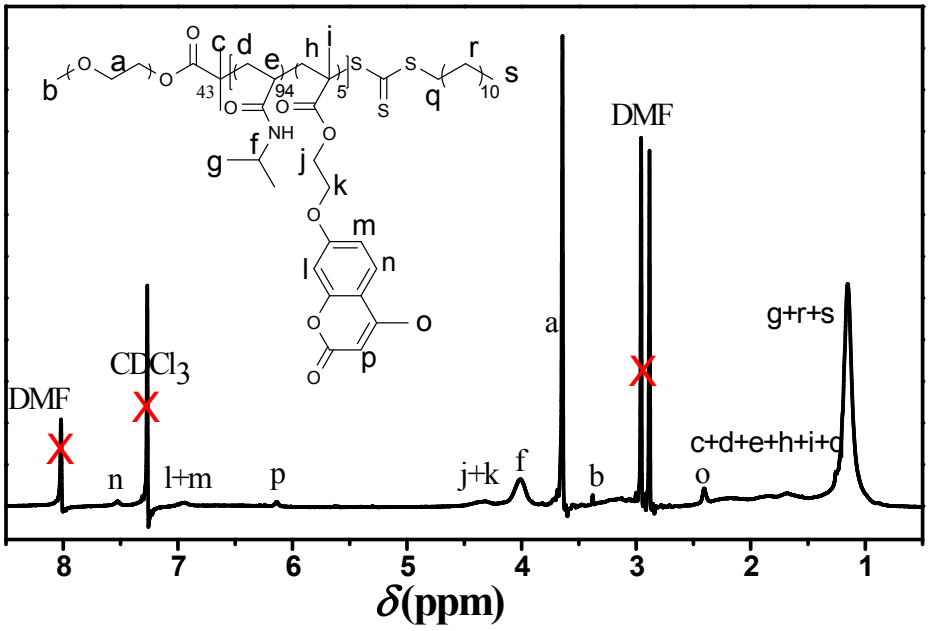


Fig. S3 ^{1}H NMR spectrum of PEO_{43}-*b*-P($NIPAM_{94}$-*stat*-CMA_5) in $CDCl_3$.

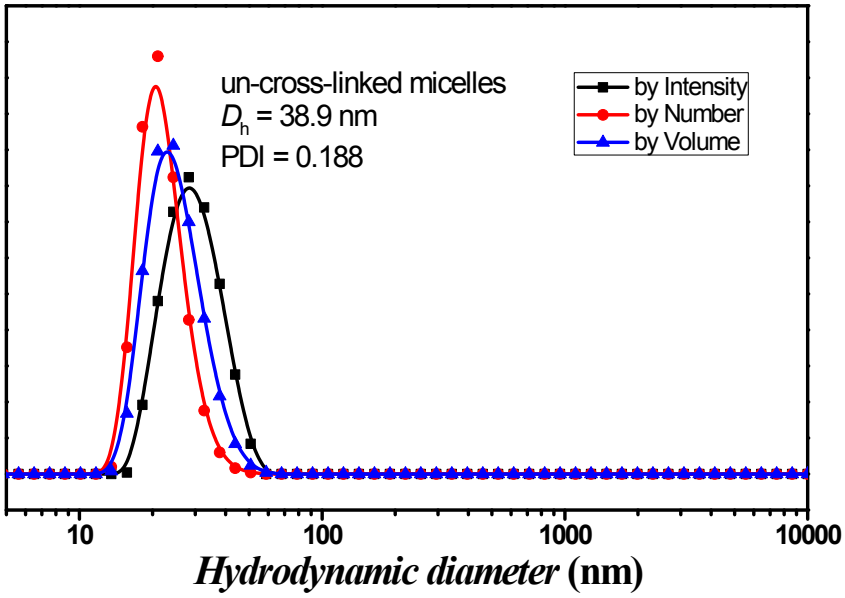


Fig. S4 DLS study of un-cross-linked micelles by directly dissolved the copolymer in DI water (C_{ini} = 1.0 mg/mL).

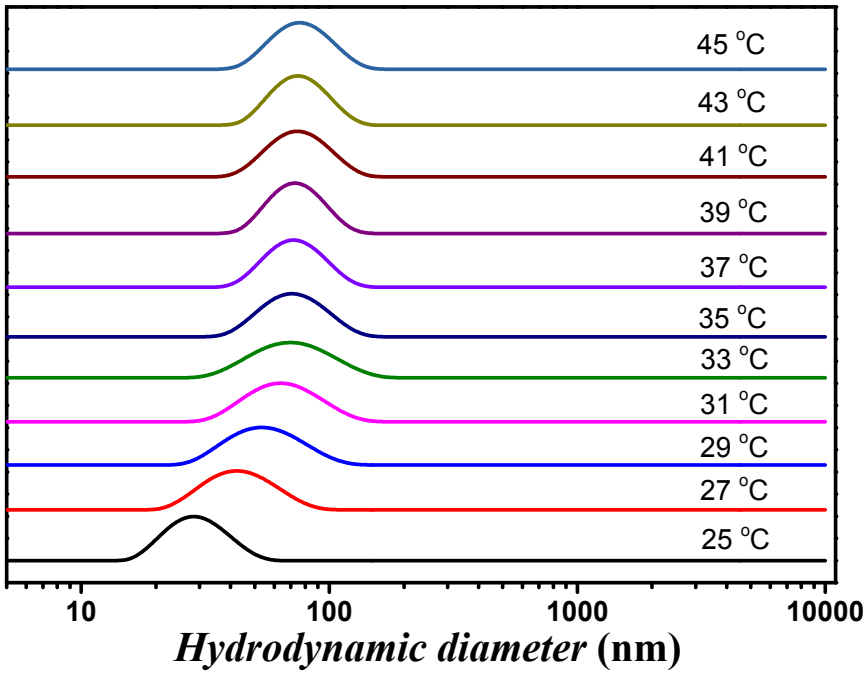


Fig. S5 Size distributions by intensity at selected temperatures corresponding to Fig. 2 from DLS study.

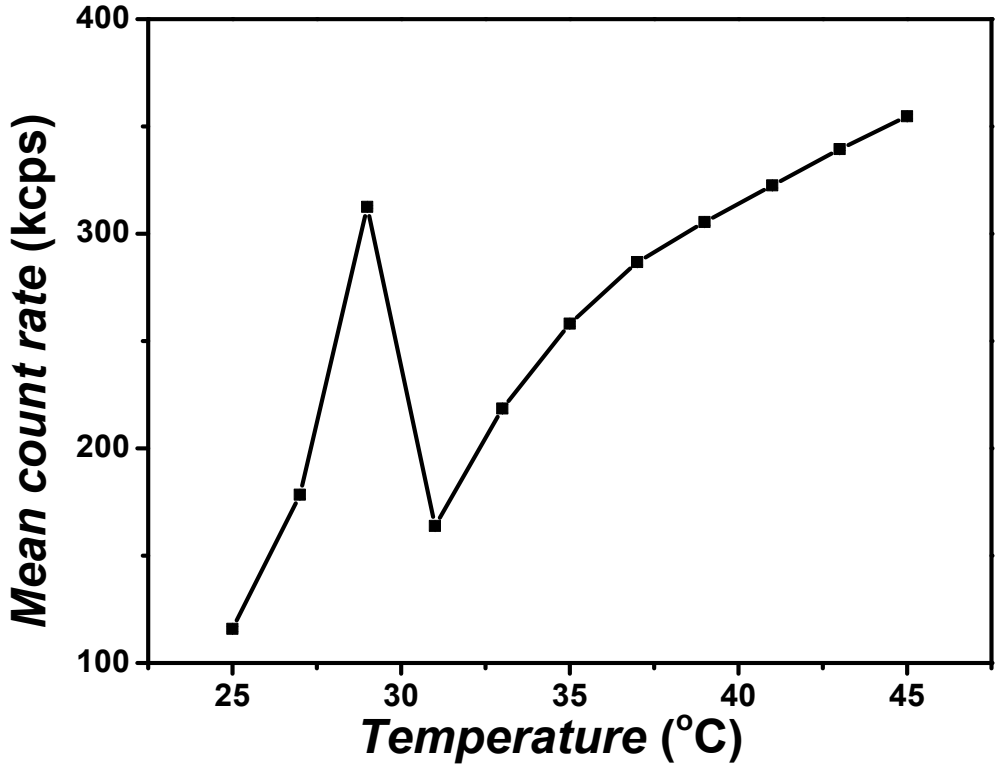


Fig. S6 Evolution of the scattered intensity at selected temperatures corresponding to Figure 2 by DLS study. The increase in the mean count rate below 29 °C indicates a micelles collision or aggregation state. The sharp drop between 29 and 31 °C corresponds to the imbalanced state during a volume phase transition process. After that, the solution becomes stable gradually.

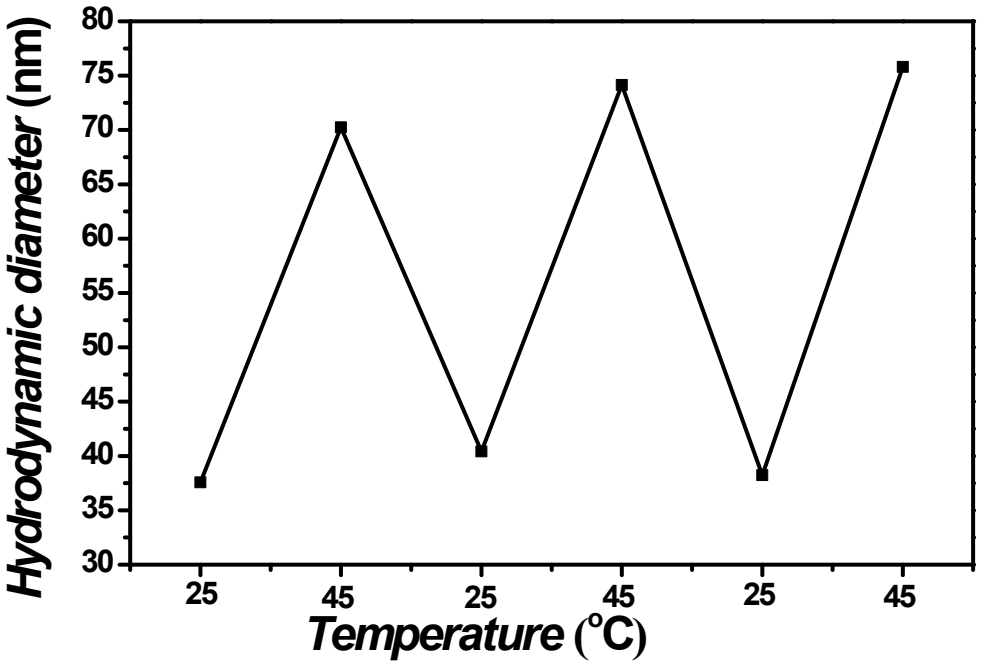


Fig. S7 Variation of hydrodynamic diameters of un-cross-linked micelles during heating-cooling cycles ($C_{polymer}$ = 1.0 mg/mL).

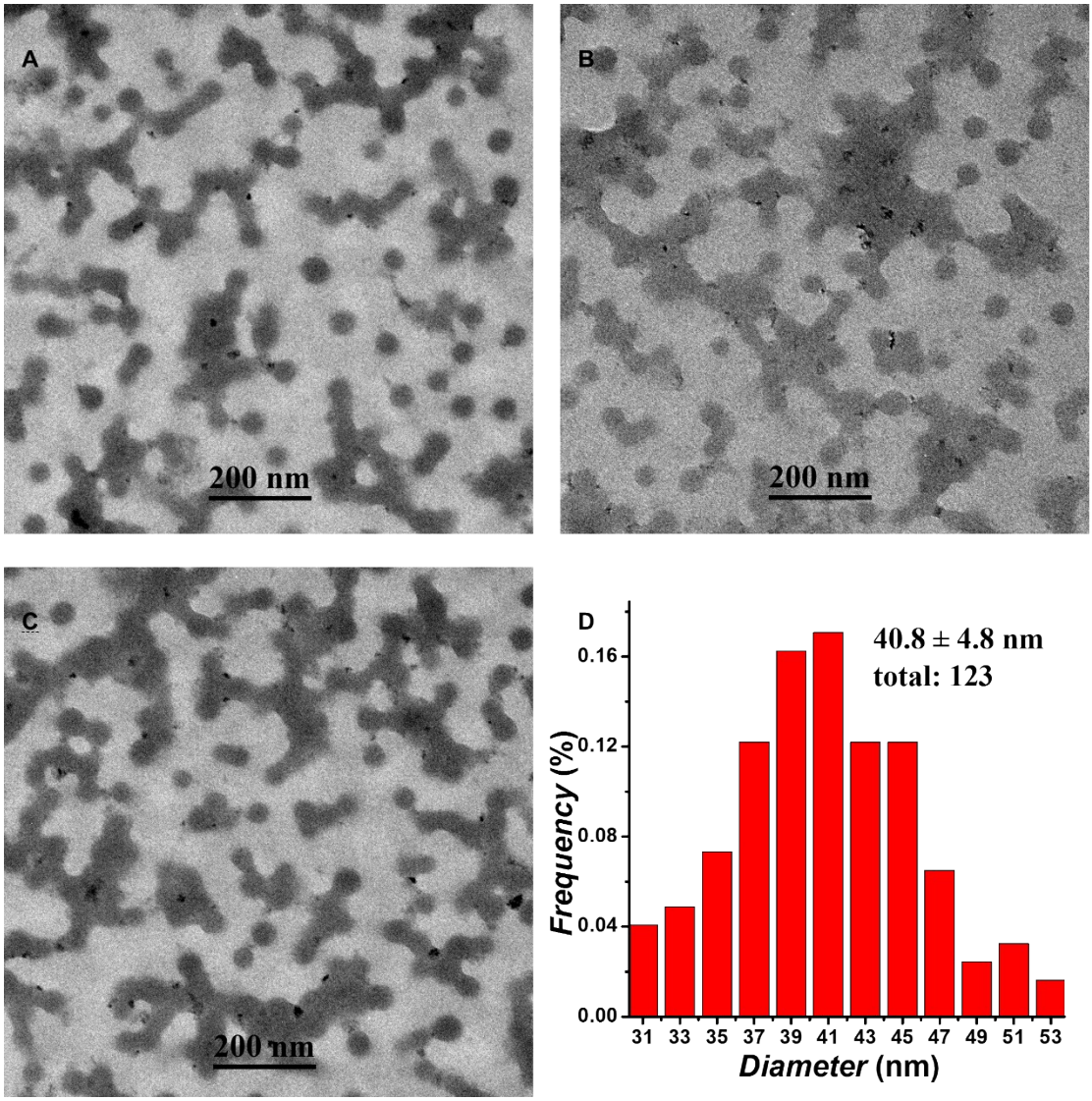


Fig. S8 TEM images of fused micelles after step-by-step heating un-cross-linked micelles to 45 °C and then UV-cross-linked for TEM analysis (A-C); Statistical mean diameter of isolated micelles calculated by TEM image (D). $C_{polymer}$ = 1.0 mg/mL. Both individual micelles and fusional structure coexisted and the size of the spherical micelles is *ca.* 40.8 ± 4.8 nm with a total number of 123 single micelles.

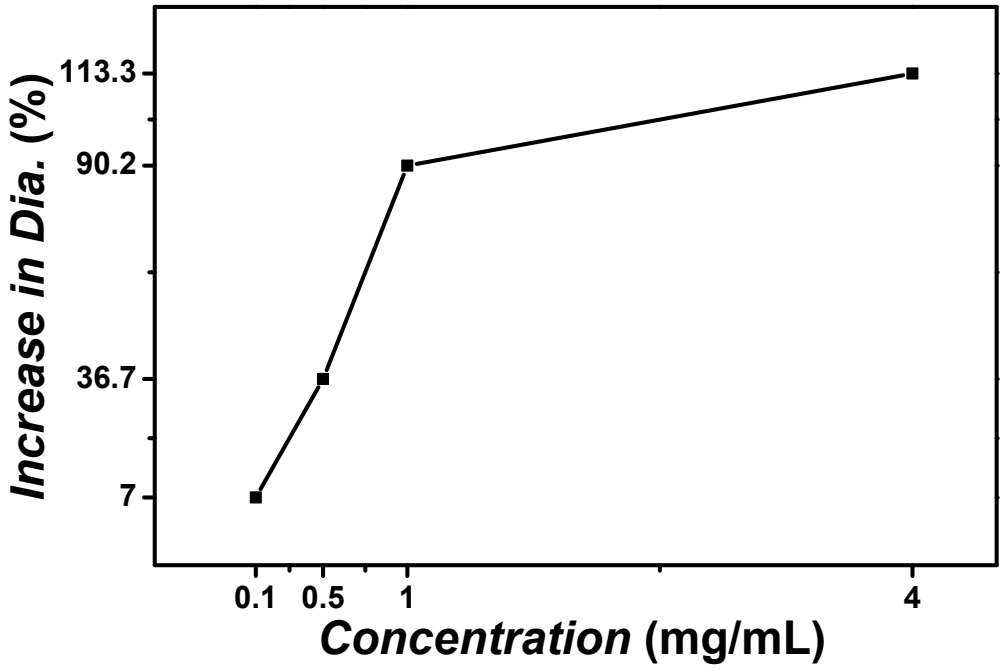


Fig. S9 Size increases of un-cross-linked micelles when heating from 25 °C to 45 °C at different initial concentrations.

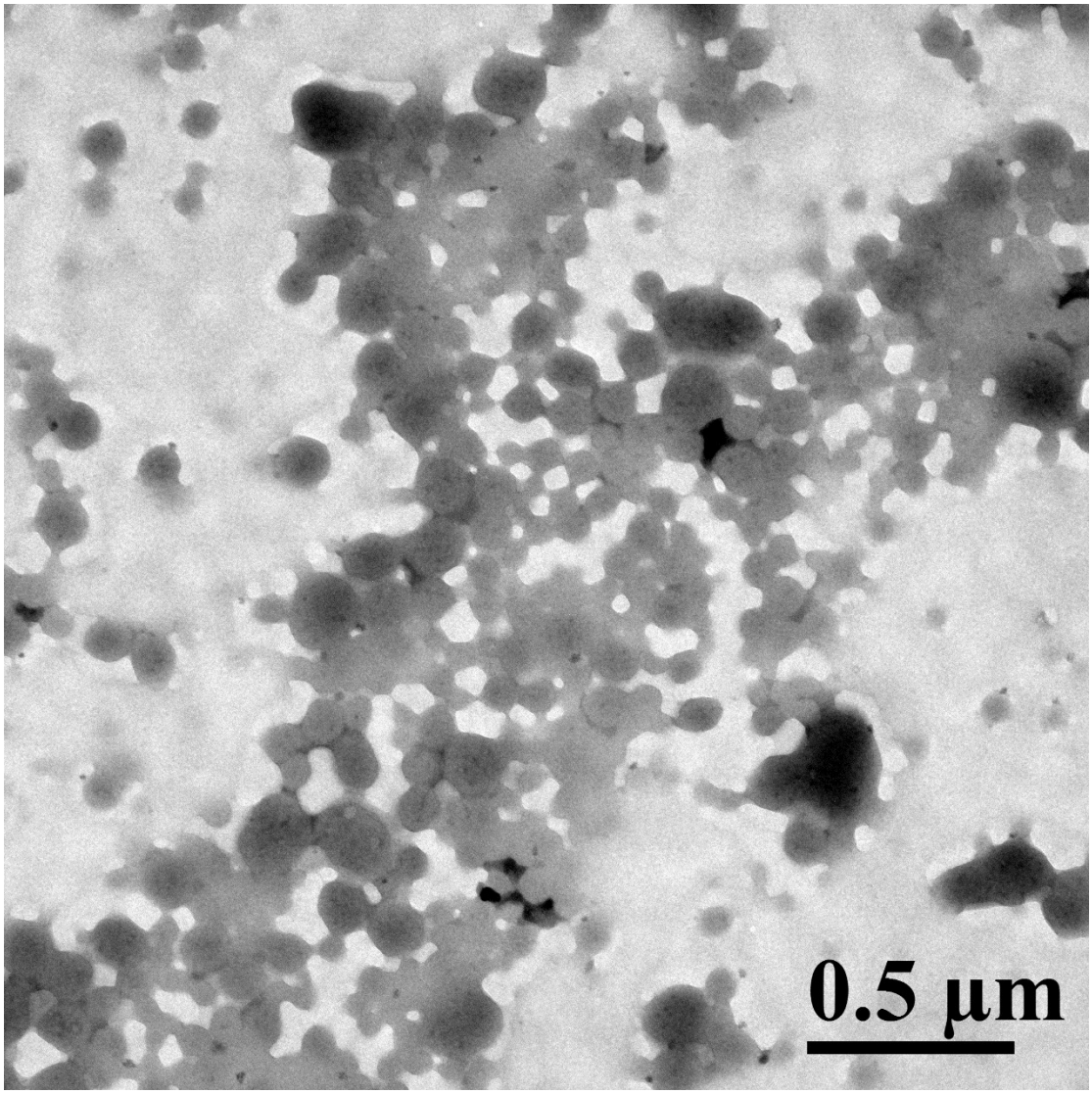


Fig. S10 TEM image of un-cross-linked micelles (25 °C) directly immersed in a water bath at 45 °C ($C_{polymer}$ = 1.0 mg/mL). This sample was finally cross-linked at 45 °C for fixing the morphology during the TEM study.

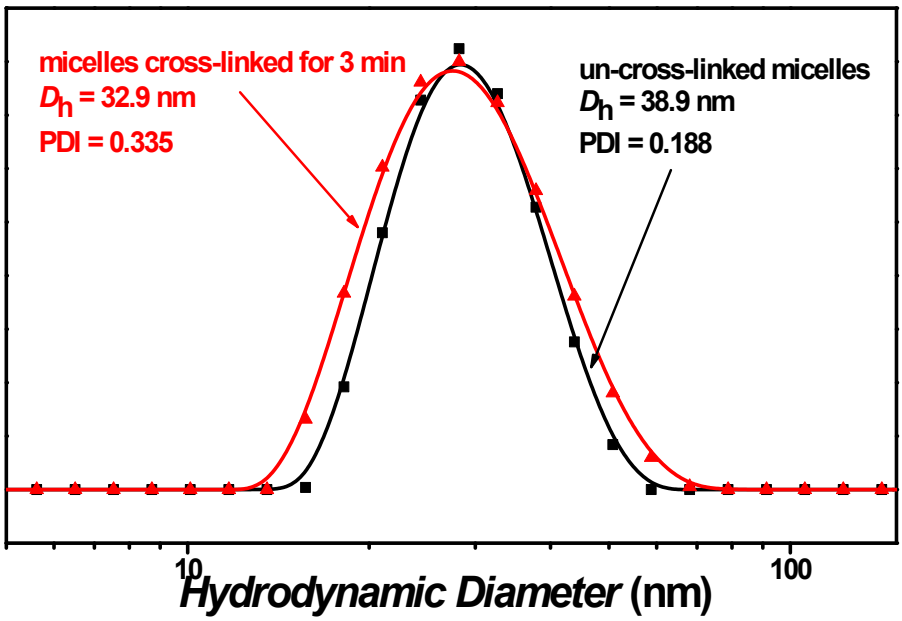


Fig. S11 Typical size distribution of micelles before and after cross-linking determined by DLS ($C_{polymer}$ = 1.0 mg/mL).

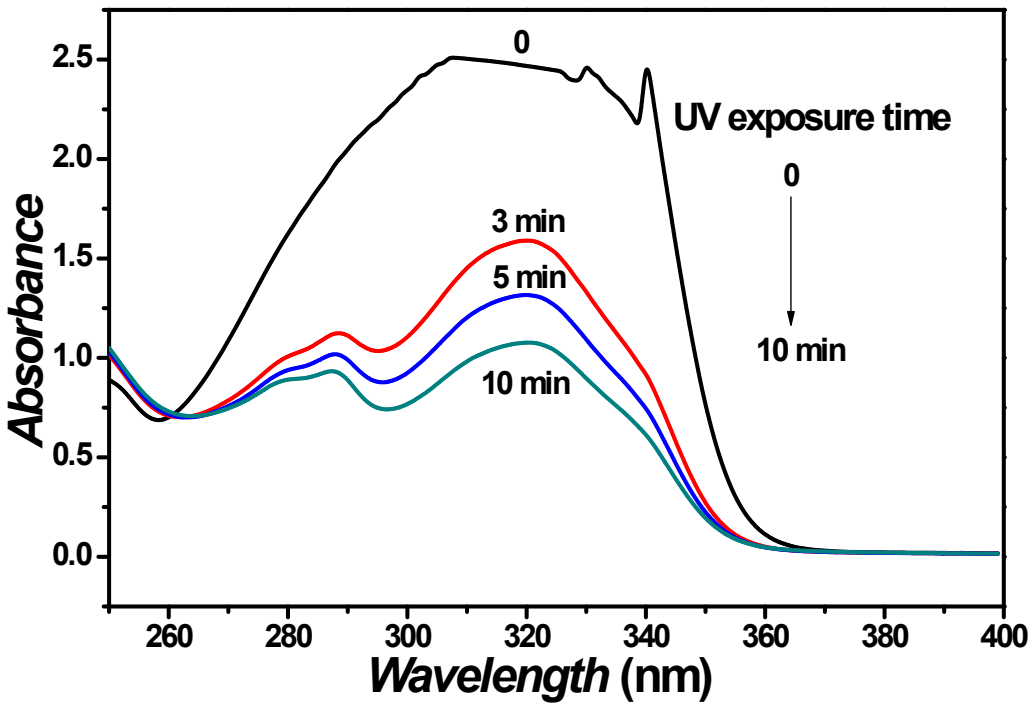


Fig. S12 UV-vis spectrum of the PEO_{43}-*b*-P($NIPAM_{94}$-*stat*-CMA_5) micelles under different UV exposure times.

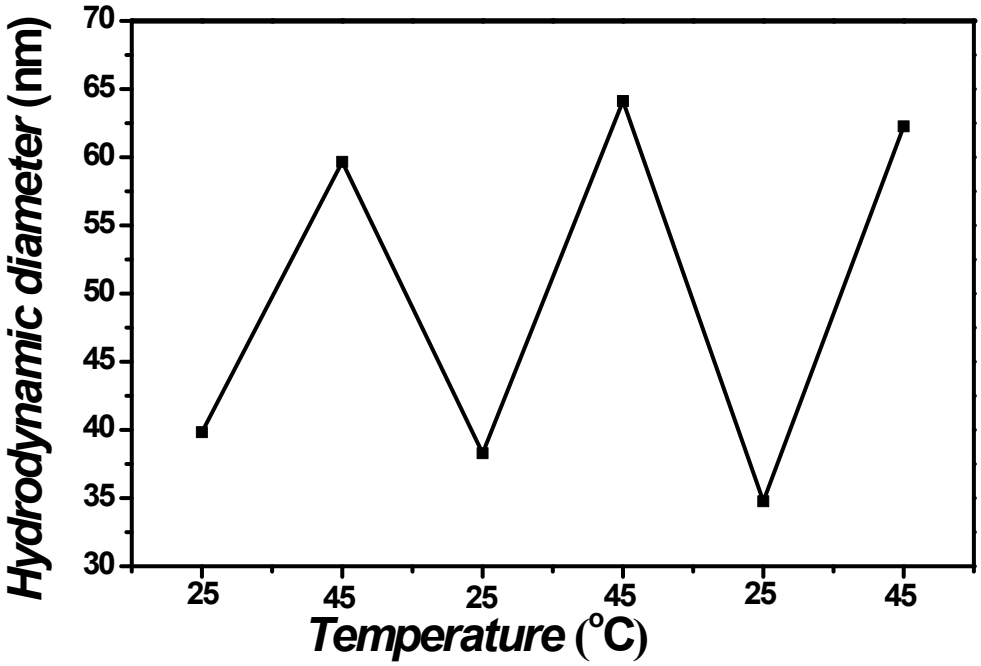


Fig. S53 Variation of hydrodynamic diameters of cross-linked micelles during heating-cooling cycles ($C_{polymer}$ = 1.0 mg/mL).

References

1. Y. Q. Zhu, L. Liu and J. Z. Du, *Macromolecules*, 2013, **46**, 194-203.
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4. Y. Q. Zhu, F. Y. K. Wang, C. Zhang and J. Z. Du, *ACS Nano*, 2014, **8**, 6644-6654.
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