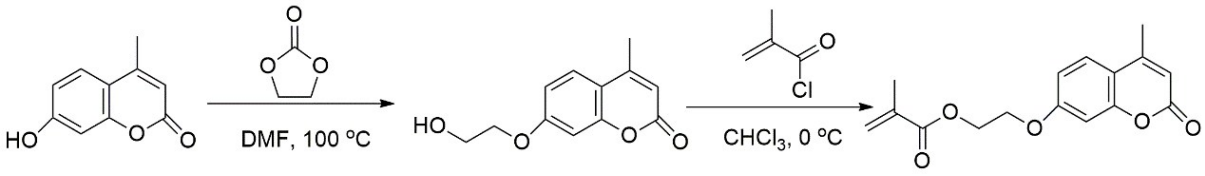
Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information

A General Method to Greatly Enhance Ultrasound-Responsiveness for Common Polymeric Assemblies

Jinkang Dou, Ruiqi Yang, Kun Du, Li Jiang, Xiayun Huang, and Daoyong Chen*

The State Key Laboratory of Molecular Engineering of Polymers and Department of Macromolecular Science Fudan University, 2005 Songhu Road, Shanghai 200438, People's Republic of China. E-mail: chendy@fudan.edu.cn



Scheme S1. Chemical Structures Showing the Synthesis of CMA.

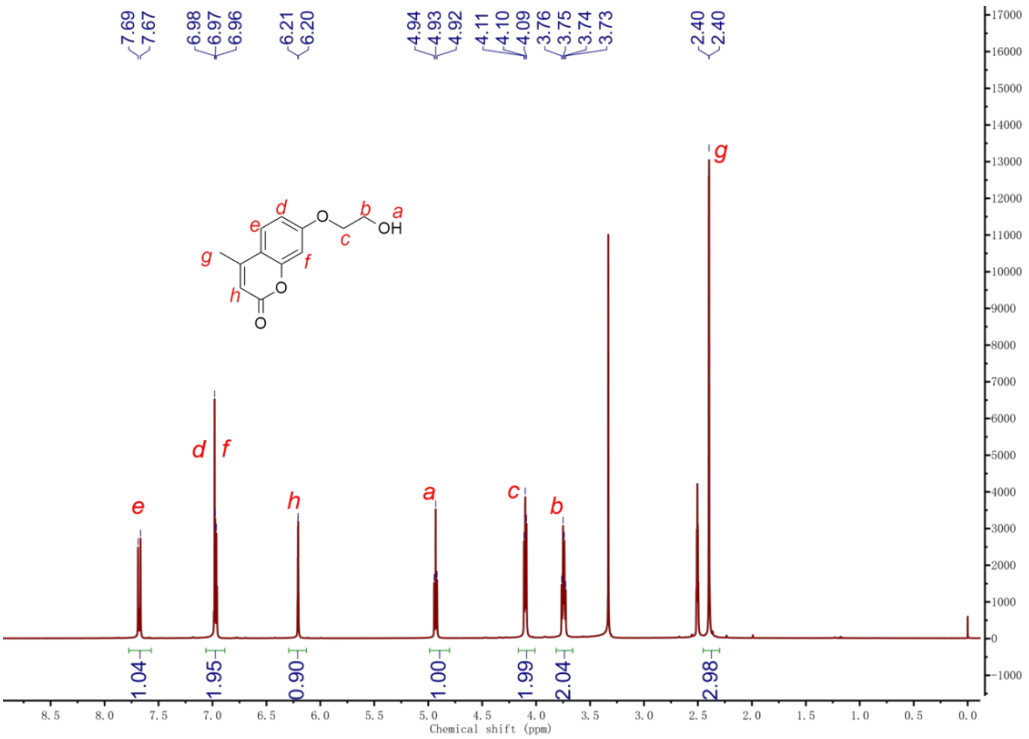
1. Synthesis of 7-(2-hydroxyethoxy)-4-methylcoumarin (HMC).[1]

7-hydroxy-4-methylcoumarin (13.2 g, 0.075 mol) and ethylene carbonate (6.6 g, 0.075 mol) were dissolved in 60 mL of DMF in a 100 mL round-bottomed flask. Potassium carbonate (20.7 g, 0.15 mol) was added to the mixture. After being purged with Argon for 60 min, the flask was placed in a preheated oil bath at 100 ℃ for 12 h, then cooled the bath down to room temperature. The product was precipitated in cold water and recrystallized twice from ethyl

acetate. ^{1}H NMR (DMSO-d_6, δ, ppm) 7.67 (d, aromatic, 1H), 7.00–6.90 (m, aromatic, 2H), 6.20 (d, C=C–H, 1H), 4.93 (t,–OH, 1H), 4.10 (t, CH_2, 2H,), 3.75 (dd, CH_2, 2H), 2.40 (d, CH_3, 3H).

2. Synthesis of 7-(2-methacryloyloxyethoxy)-4-methylcoumarin (CMA).[2]

HMC (5 g, 22.7 mmol) and TEA (6.9 mL, 49.4 mmol) were dissolved in 80 mL of $CHCl_3$ and cooled in ice bath. Then, methacryloyl chloride (4.6 mL, 47.8 mmol) was added dropwise to this cold mixture for 1 h. After being stirred for 24 h at room temperature, the reaction mixture was treated with dichloromethane and the organic layer was washed with brine (60 mL × 2), followed by drying with magnesium sulfate. Finally, the solvent was removed by rotary evaporation and the residue was recrystallized in ethanol twice to obtain white powdery crystals. ^{1}H NMR ($CDCl_3$, δ, ppm) 1.96 (s, CH_3, 3H), 2.40 (s, CH_3, 3H), 4.29 (t, CH_2OR, 2H), 4.53 (t, CH_2OAr, 2H), 5.61 (s, C=CH_2, 1H), 6.15 (s, C=CH_2, 1H), 6.15 (d, C=C–H, 1H), 6.8–6.9 (m, aromatic, 2H), 7.52 (d, aromatic, 1H).



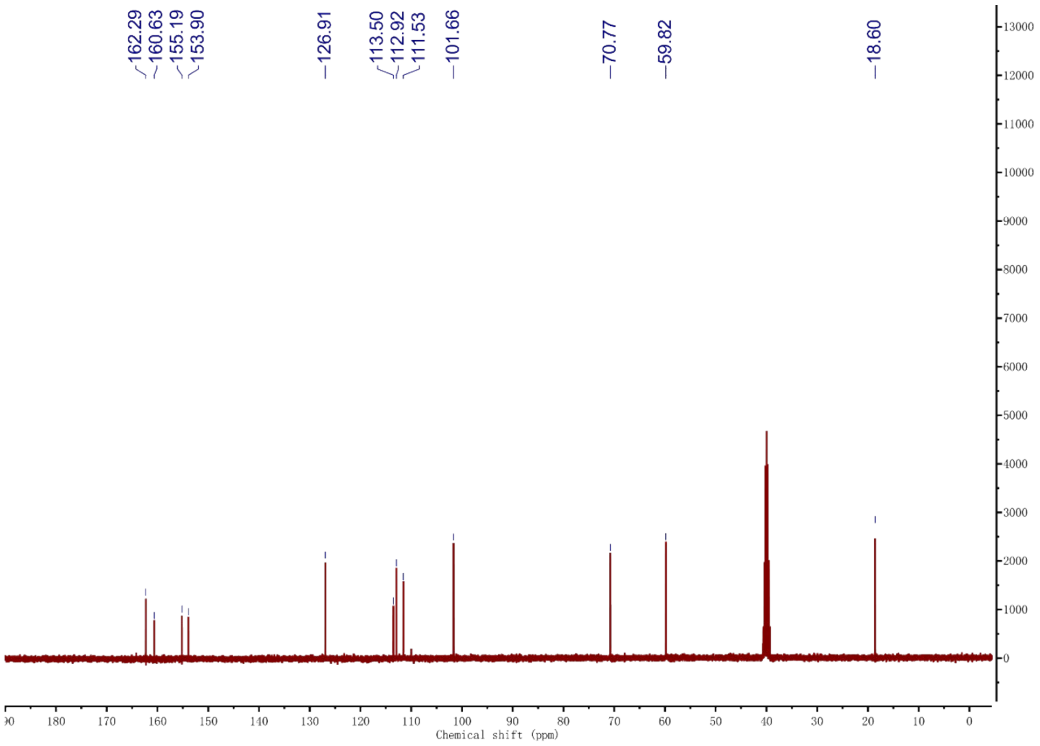
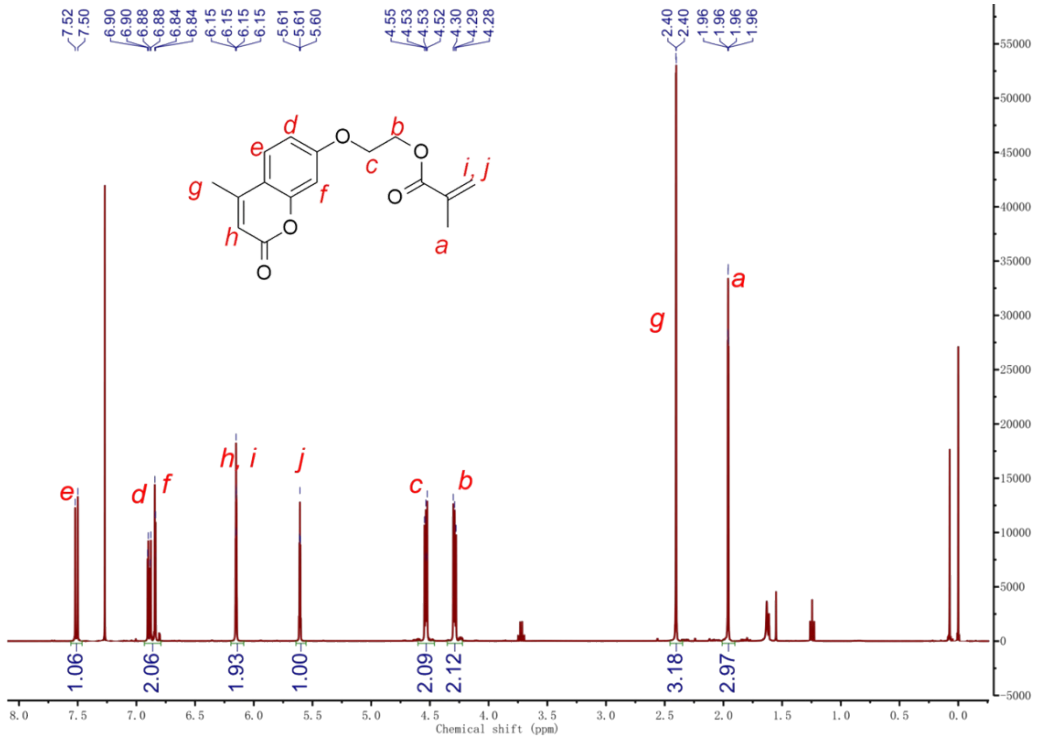


Figure S1. ^{1}H NMR and ^{13}C NMR spectra of **HMC** in DMSO-d_6.



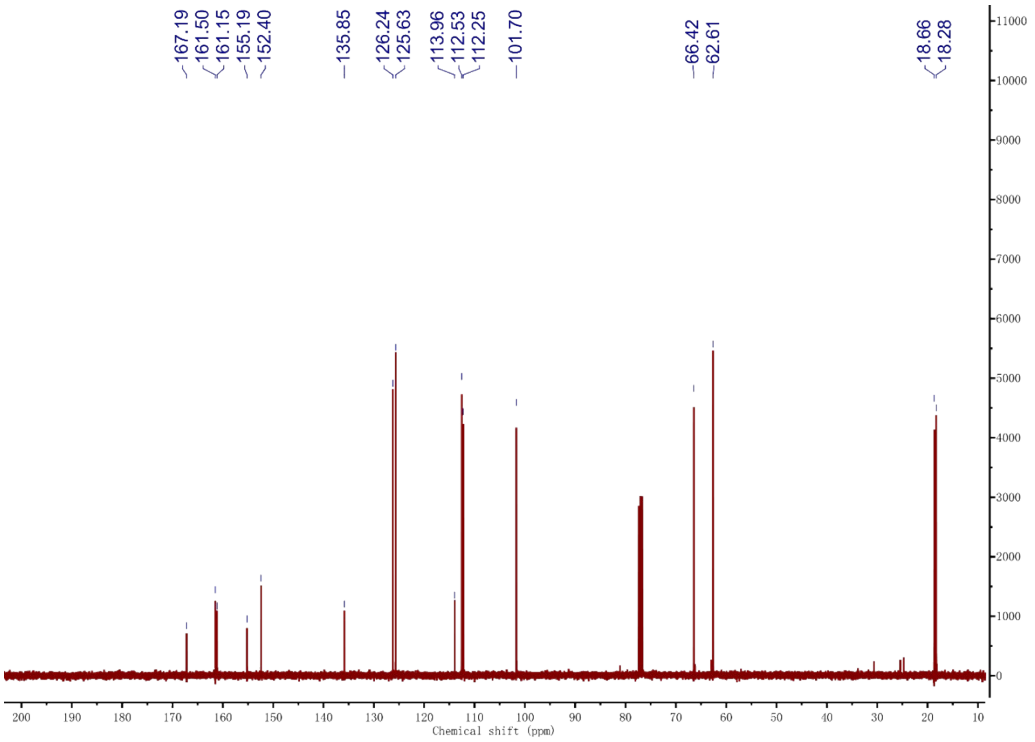
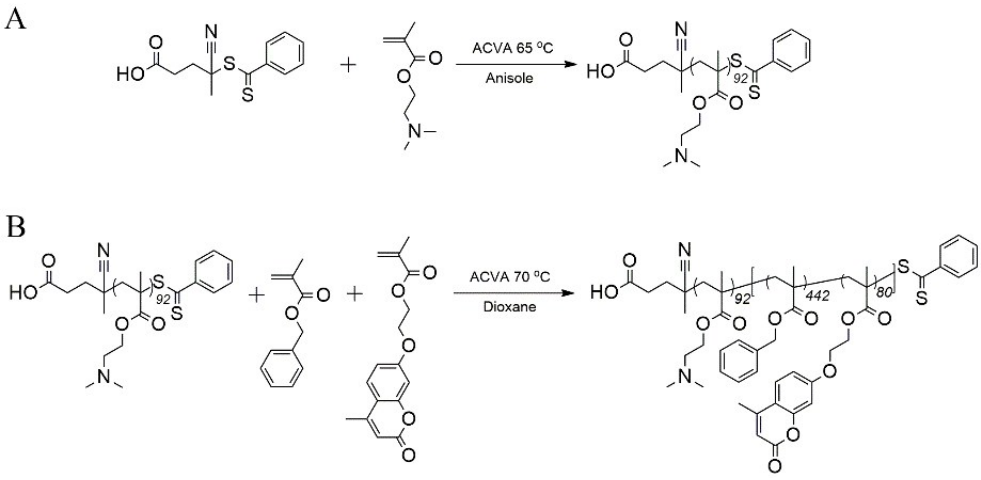


Figure S2. ^{1}H NMR and ^{13}C NMR spectra of **CMA** in $CDCl_3$.



Scheme S2. Synthesis of the Diblock Copolymer by RAFT Polymerization.

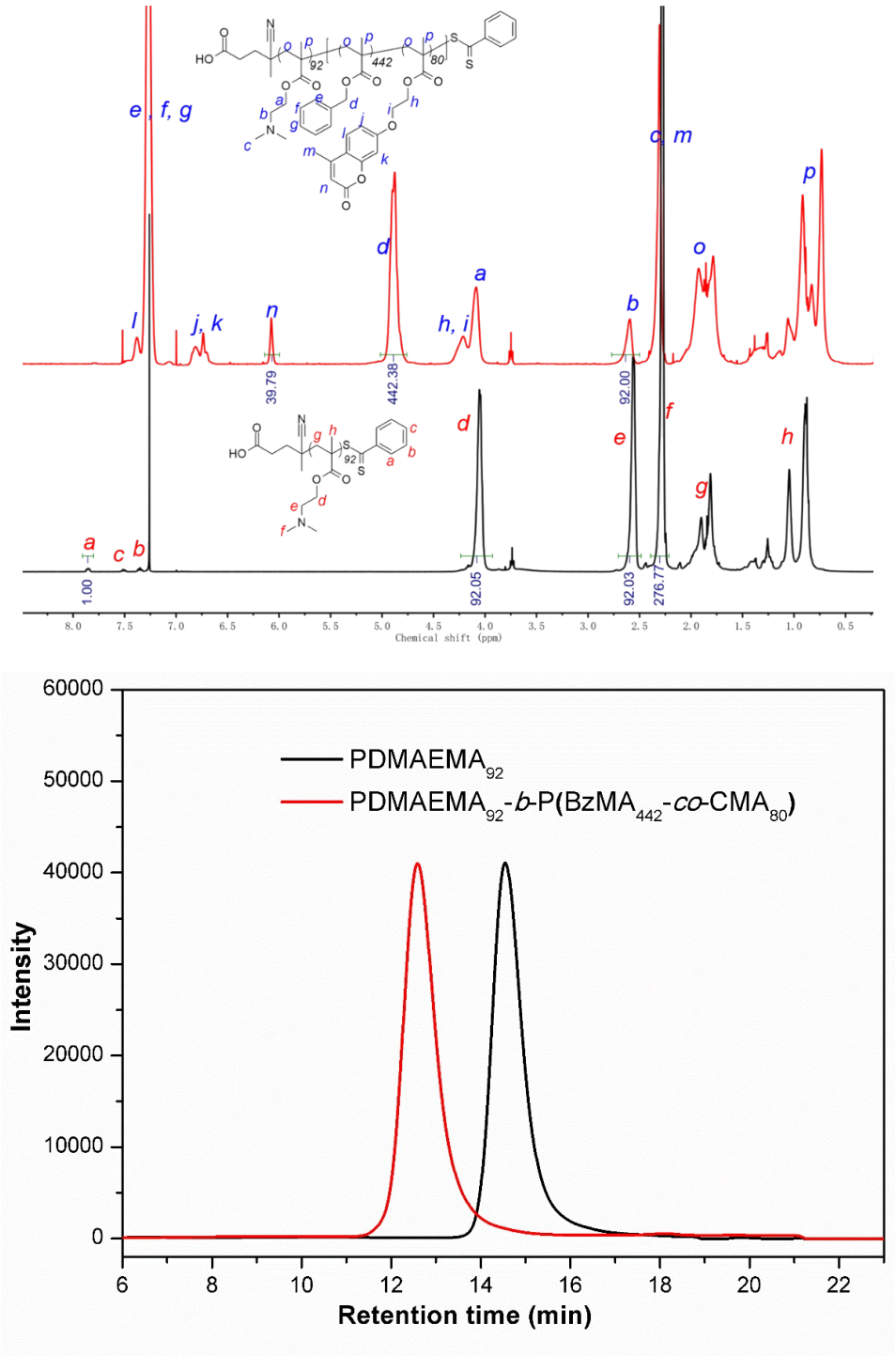


Figure S3. ^{1}H NMR spectra in $CDCl_3$ (the upper panel) and GPC curves of $PDMAEMA_{92}$ macro-chain transfer agent and $PDMAEMA_{92}$-*b*-P($BzMA_{442}$-*co*-CMA_{80}) precursor block copolymers.

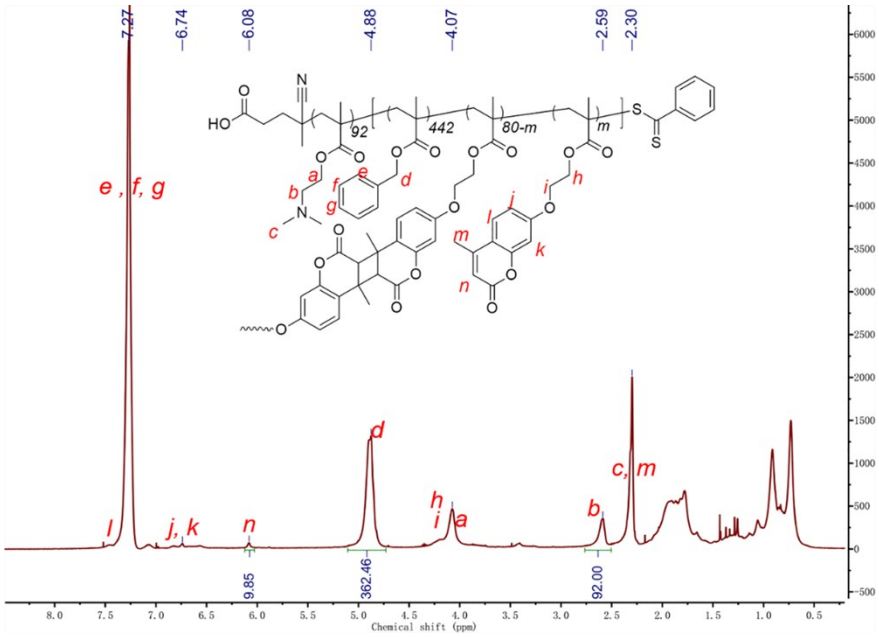


Figure S4. ^{1}H NMR spectra of ICSCs prepared from PDMAEMA$_{92}$-*b*-P(BzMA$_{442}$-*co*-CMA$_{80}$) precursor block copolymers in $CDCl_3$.

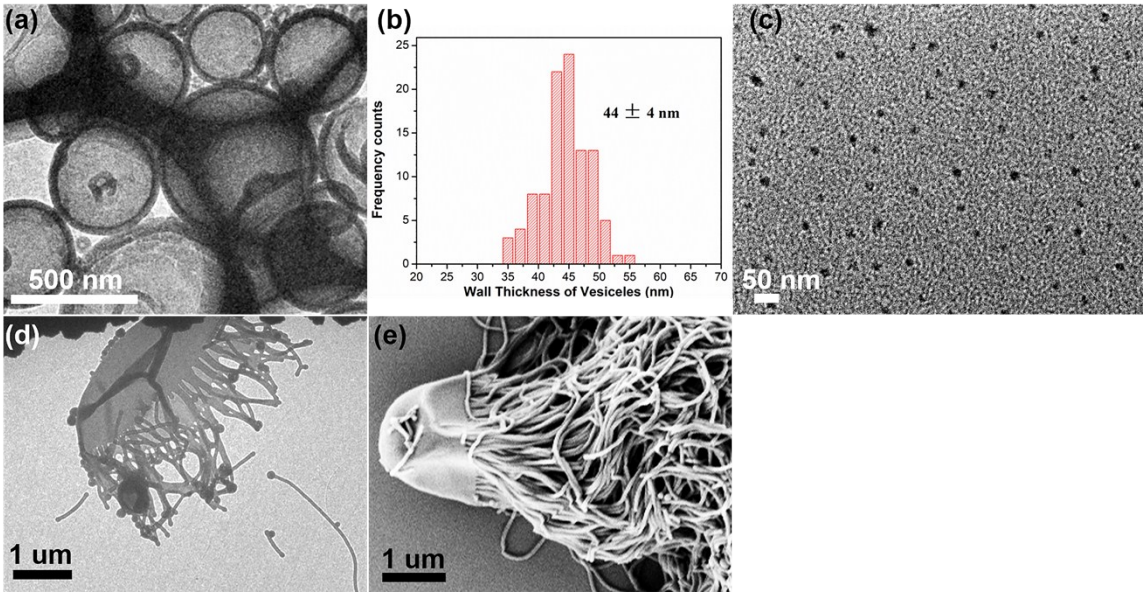


Figure S5. (a) TEM image of vesicles obtained by freeze-drying technique. (b) Wall thickness distribution of vesicles obtained by TEM analysis over randomly selected 100 vesicles. (c) TEM image of the spherical ICSCs micelles. (d, e) TEM and FE-SEM images of octopus-like structures that is in a small amount coexisting with the worm-like micelles formed by the ICSCs.

Text S1. TEM observation using the freeze-drying technique can further support the conclusion that the vesicles were formed by ICSCs in the suspension. This technique prepared the TEM samples by depositing a drop of the suspension onto a carbon-coated copper grid, rapidly freezing the sample in nitrogen, and drying sample using lyophilizer at -40 ^{o}C. In the as-obtained TEM images (Figure S5a), the assemblies are vesicles with the size and morphology close to those exhibited in the manuscript. In pure water, at the low temperature, the wall-forming component of the assemblies was in a glassy state, and thus any remarkably morphological change during the TEM-sample preparation should be prevented. Therefore, the TEM images obtained by the freeze-drying technique further confirmed that the vesicles were formed in the suspension.

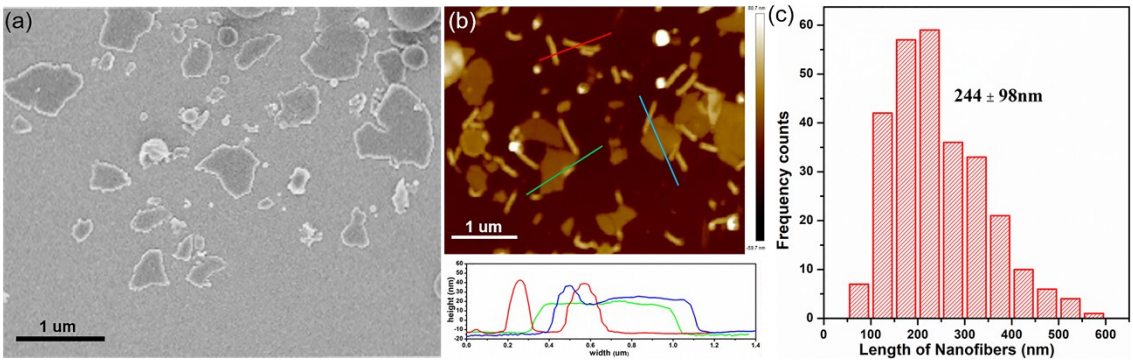


Figure S6. (a) FE-SEM image of the vesicle fragments. (b) AFM height images of the fragments containing both the short rods and the sheet-like particles which are the fragments of the octopus-like assemblies shown in Figure S5 c and d; it should be mentioned here that the assemblies in the suspension are mostly the worm-like micelles, and the octopus-like assemblies occupy only a very small fraction. (c) Length distribution of the short nanorods (the fragments of the worm-like micelles) obtained by unbiased statistics over 250 nanorods. Vesicles and worm-like micelles were formed by self-assembly of ICSCs and treated by ultrasound at 32.5 W for 5 min.

Text S2. Self-assembly of the Precursor Block Copolymers (PDMAEMA$_{92}$-*b*-P(BzMA$_{442}$-*co*-CMA$_{80}$)). The self-assembly of the precursor block copolymers was trigged by adding water at a certain pH to the polymer solution in THF at 1.0 mg/mL to the water/THF volume ratio of 2/1. The resultant mixture was then dialyzed against the water at the same pH to remove the organic solvent. Figure S7 shows that when the water at pH of 6.5 was added and then used for the dialysis, irregular vesicles with a diameter of 286 nm were obtained. The wall thickness of vesicles is ca. 72 ± 12 nm. When the pH value is 3.0, the resultant assemblies are the mixture of spherical micelles with a diameter of 62 nm and irregular aggregates with a larger size. When the pH value is 1.2, mixture of micelles and large compound micelles, and precipitates were obtained.

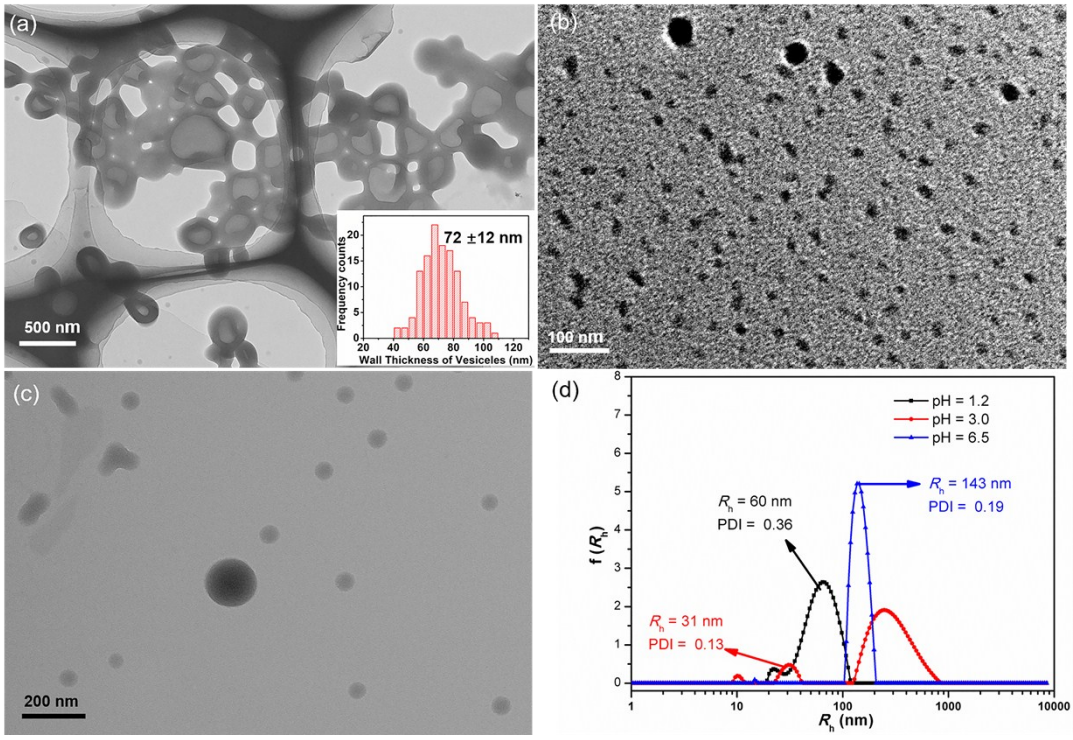


Figure S7. TEM images of assemblies formed by self-assembly of PDMAEMA$_{92}$-*b*-P(BzMA$_{442}$-*co*-CMA$_{80}$) precursor block copolymers by using the water at different pH values for both the water addition and the subsequent dialysis. (a) pH = 6.5, (b) pH = 3.0, (c) pH = 1.2. The inset in (a) is

the distribution of the wall thickness based on unbiased TEM observations over 100 vesicles. (d) DLS curves of the polymer assemblies formed at pH of 6.5 (R_h = 143 nm), 3.0 (R_h = 31 nm), and 1.2 (R_h = 60 nm), respectively.

Text S3. Ultrasound-Responsiveness of the Assemblies of Precursor Block Copolymers. The assemblies formed at the different pH values by the precursor block copolymer were treated by a highly enhanced ultrasound (260 W, 20-25 kHz) for a much longer time (15 min). No remarkable structural change was observed for each of the systems (Figure S8).

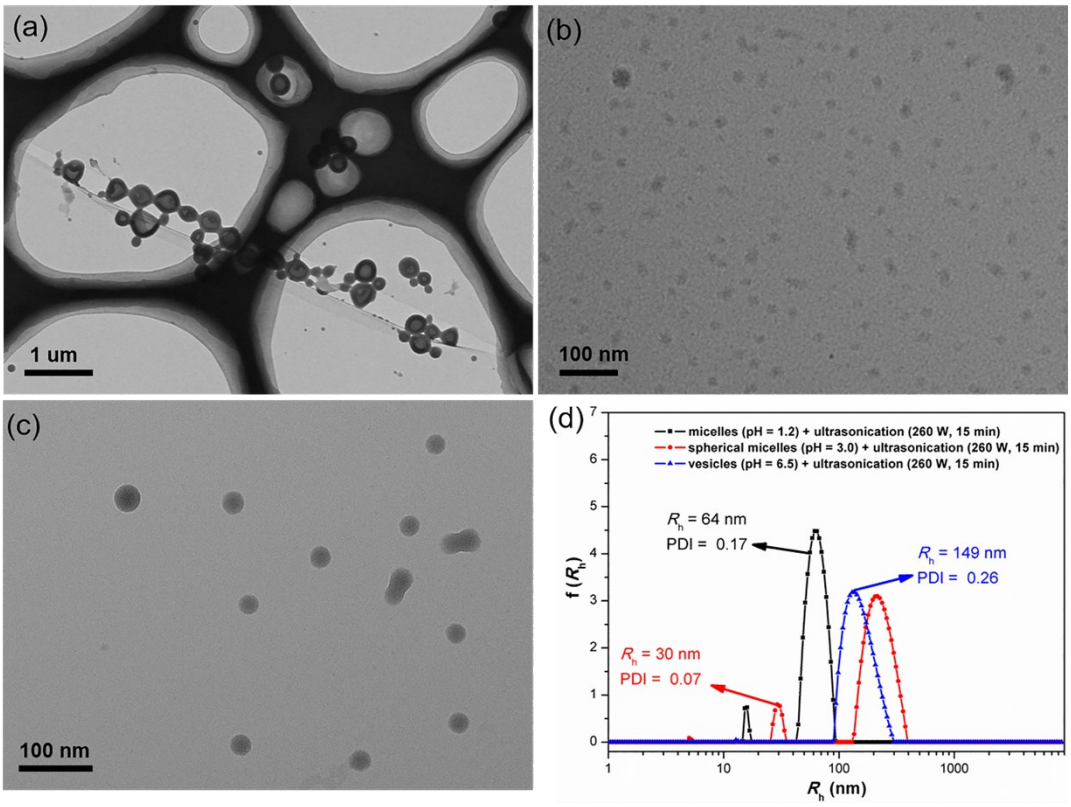


Figure S8. TEM images of (a) vesicles formed at pH of 6.5 and then treated by a high power ultrasound (260 W, 20-25 kHz) for 15 min, (b) the particles formed at pH of 3.0 and then treated by the ultrasound for 15 min, and (c) the particles formed at pH of 1.2 and then treated by the ultrasound for 15 min. (d) DLS curves of the ultrasound treated vesicles (R_h = 149 nm), the ultrasound treated particles formed by the precursor polymer at pH of 3.0 (R_h = 30 nm), and the ultrasound treated micelles formed at pH of 1.2 (R_h = 64 nm).

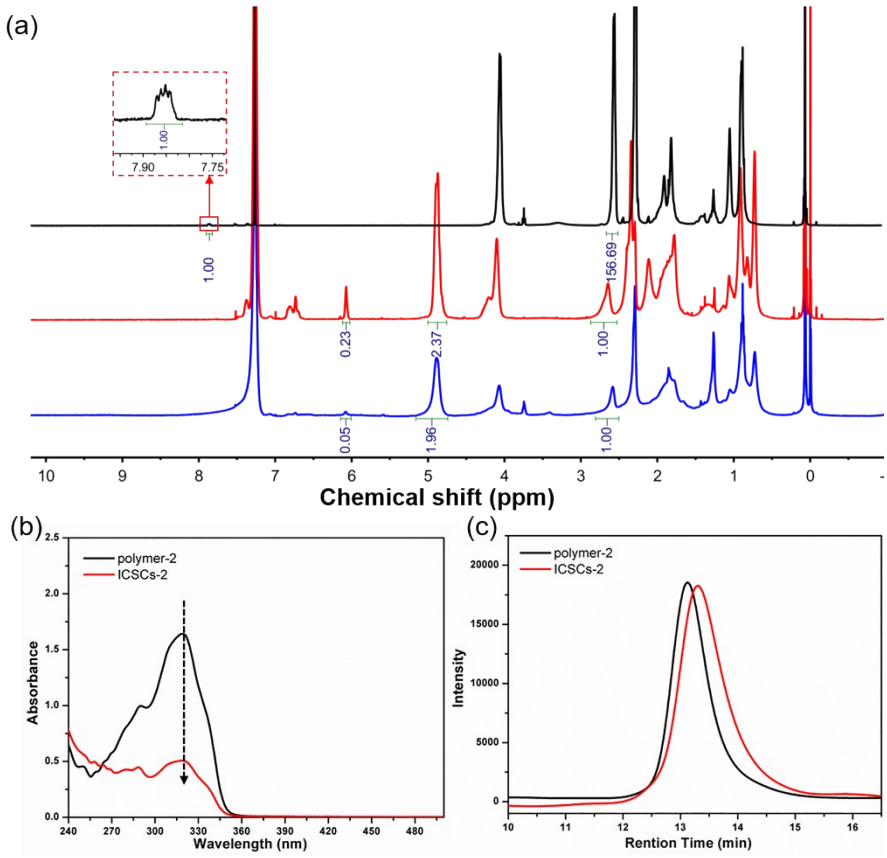


Figure S9. (a) ^{1}H NMR spectra of PDMAEMA$_{157}$ macro-chain transfer agent (black line), PDMAEMA$_{157}$-*b*-P(BzMA$_{372}$-*co*-CMA$_{72}$) (polymer-2) (red line) and ICSC-2 prepared by intrachain crosslinking the P(BzMA$_{372}$-*co*-CMA$_{72}$) block by 365 nm UV irradiation (blue line) till the dimerization degree reaches 69%; and the crosslinking density is 11%. (b) UV-vis spectra of polymer-2 and ICSC-2 at the dimerization degree of 69%. (c) SEC traces of polymer-2 and ICSC-2 measured by using DMF with 1.6 g/L LiBr as eluent at the flow rate of 1.0 mL/min at 50 ℃. The molecular weight and polydispersity index measured by SEC for polymer-2 are 42.9 kg/mol and 1.15, respectively, and those for ICSC-2 are 36.1 kg/mol and 1.18.

Text S4. The Ultrasound-induced ANS Release from the Micelles Formed by Self-assembly of PDMAEMA$_{157}$-*b* P(BzMA$_{372}$-*co*-CMA$_{72}$) (Polymer-2) and ICSC-2. The 2 mm diameter microtip of a commercial 20-25 kHz sonic dismembrator was immersed 1 cm into 2.5 mL of an ANS-loaded micelle suspension contained in a standard quartz cell with a light path of 10 mm. In order to avoid any thermal effects, a pulsed ultrasound was applied (0.5 second pulses applied every 1.5 second), and the quartz cell was placed in an ice bath. The release behavior was characterized by the changes of fluorescence intensity of ANS using an PTI Instruments QM40 Steady-State/Transient Fluorescence Spectrometer. The fluorescence intensity of ANS was recorded every 1 min (pulse time applied in the ANS-loaded micelles). The ultrasound power is 6.5 W and the total pulse time applied in this micelles is 5 min.

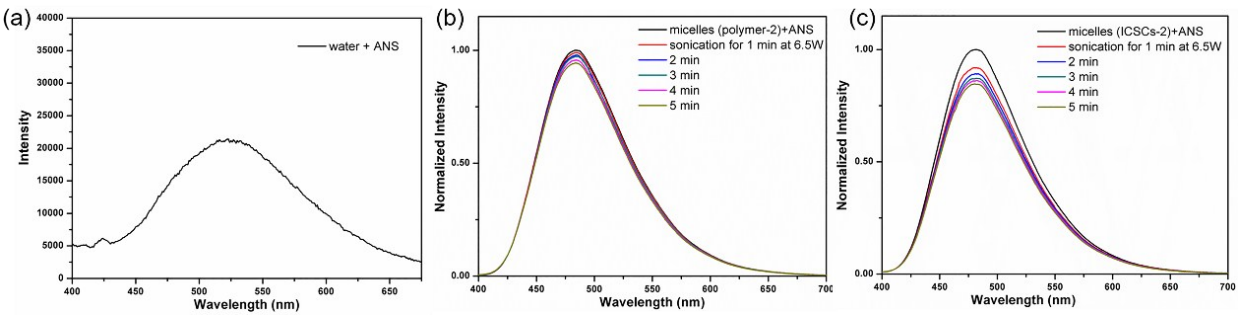


Figure S10. Fluorescence spectra of free ANS in neutral water at the concentration of 67 μM (a), the ANS-loaded micelles formed by polymer-2 (b) and those formed by ICSCs-2 (c) recorded after treated by the ultrasound at 6.5 W for different time.

Text S5. The possible mechanism of ANS release from ICSC-2 micelles. ANS encapsulated in the core of the ICSC-2 micelles can be divided into two parts: one part is located in the interface, and the other part in the central domain of *c*-PBMC. As mentioned before, since *c*-PBMC has an intermediately crosslinked structure, the chain-chain entanglements in the interface between *c*-

PBMCs were largely reduced. Without chain entanglements, the structure stability of the *c*-PBMC/*c*-PBMC interface under the ultrasound treatment should be remarkably weakened. Therefore, the ANS released abruptly from the ICSC-2 micelles at the beginning of the gentle ultrasound treatment should be the ANS molecules located in the interface. We think that the structure at the interface should dissociate, to a certain extent, upon the ultrasound treatment, leading to the abrupt release of the ANS molecules located in the *c*-PBMC/*c*-PBMC interface. Obviously, the structure at the central domain of *c*-PBMC is much stable than that at the interface, and thus the ANS molecules located in the central domain is much less sensitive to the ultrasound treatment and release slowly from the ICSC-2 micelles.

References

1 Y. Zhang and L. Ionov, *Langmuir,* 2015, **31**, 4552-4557.

2 J. Jiang, B. Qi, M. Lepage and Y. Zhao, *Macromolecules,* 2007, **40**, 790-792.