

Supporting Information

Surface-Cross-linked Protein-Like Single-Chain Nanoparticle Globules Unexpectedly Stabilized with a Low Cross-linking Degree

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EXPERIMENTAL SECTION

Materials and Instrumentations

Styrene (99%, contains 10-15 ppm 4-tert-butylcatechol as stabilizer) was purchased from Aladdin Reagents. 4-Acryloyloxybenzophenone (ABP, 98%) was purchased from Macklin Reagents. The initiator was 2,2'-azobis (2-methylpropionitrile) (AIBN, 98%) from Energy Chemical. The chain transfer agent (CTA, 98%) utilized was cyanomethyl dodecyl trithiocarbonate, purchased from Sigma-Aldrich. Deuterated solvent CDCl_3 (99.8 atom % D), were purchased from Energy-Chemical. Deuterated solvent C_7D_8 (99.6 atom % D) and $\text{C}_3\text{D}_6\text{O}$ (99.9 atom % D) were purchased from Aladdin Reagents. HPLC-grade THF were purchased from J&K Scientific. Organic solvents including 1,4-dioxane, methanol, tetrahydrofuran (THF) were purchased from Tianjin Tiantai Chemicals. Acetone, toluene and chloroform were purchased from Chron Chemicals.

Styrene was filtered by an alkaline aluminum oxide column to remove the inhibitor before use. 1,4-dioxane was first pre-dried overnight with sodium wire. Then the pre-dried solvent was refluxed with sodium wire and benzophenone under an inert atmosphere until the blue color of the benzophenone carbonyl radical anion is continuously maintained. 2, 2'-azobis (2-methylpropionitrile) (AIBN) was recrystallized from methanol. Other reagents were used directly without purification.

The reversible addition-fragmentation chain transfer (RAFT) copolymerization reactions¹ were carried out in an oil bath (IKA Plate, RCT digital) held by a thermostat at the setting temperature under a N_2 atmosphere. Flow chemistry was performed in a custom-made photochemistry equipment through Teflon tubes (inner diameter = 4.35 mm) with a High-Pressure Pump (Sanotac China). Batch reaction was performed in a three-neck round-bottom flask with a full jacket. Ultra-violet (UV) irradiation was performed using Philips UVA (365 nm, 10 W) lamps.

Characterizations

Nuclear magnetic resonance (NMR) spectra data were recorded on Bruker AVANCEIII600 MHz, AVANCEIII 500 nuclear magnetic resonance instruments using CDCl_3 , C_7D_8 and $\text{C}_3\text{D}_6\text{O}$ as the solvent. Chemical shifts were given in ppm using residue solvent signal as the reference at 25 °C.

The apparent molecular weights and molar mass dispersity of the obtained precursors and SCNPs were measured by a gel permeation chromatography system (GPC, polystyrene calibration) at 35 °C. HPLC-grade THF was used as the eluent with a flow rate of 1.0 mL/min. The GPC system consisted of an Agilent 1260 Infinity II instrument, a HPLC column (PL gel 5 μm Mixed-C, 300 \times 7.5 mm), and a refractive index detector. All samples were tested at a concentration of 1.0 mg/mL.

Dynamic light scattering (DLS) measurements were carried out by using a Malvern Zetasizer Nano-ZS setup equipped with a 10 \times 10 mm quartz cuvette. All samples were tested at a concentration of 1.0 mg/mL in chloroform solution.

UV-vis spectrum of the UVA lamps was measured on an Ocean Optics DH-mini.

Transmission electron microscopy (TEM) measurement was performed on a Hitachi

HT7700 TEM with an accelerating voltage of 120 kV. Samples were dissolved in acetone and diluted to 0.01 mg/mL. Then dripped 6 μL aliquot onto the copper grid and absorbed excess solution with filter paper.

Atomic force microscopy (AFM) images were recorded under a commercial instrument (MultiMode 8, Bruker, Germany) in tapping mode at room temperature in air. The image was confirmed by measurements in $1.0 \times 1.0 \mu\text{m}^2$ sites.

Static light scattering (SLS) measurements were carried out by using an ALV/CGS-3 light scattering spectrometer, made in Germany, equipped with an ALV/LSE-7004 multiple- τ digital correlator. The light source was a JDS-Uniphase solid-state He-Ne laser with an output power of ca. 22 mW at the operating wavelength of 632.8 nm. The light scattering (LS) cell was held in a thermostat refractive index matching vat filled with purified and dust-free toluene. All samples need to be kept absolutely dust-free and filtered with a 220 nm filter membrane.

The refractive index (RI) increment (dn/dc) was measured by a refractometer (Anton Paar) at 632.8 nm. All sample concentrations need to be configured as accurately as possible, as the effect of concentration on refractive index is very sensitive.

Small angle X-ray scattering (SAXS) experiments were conducted at the France/Xenocs Xeuss 3.0 system with an X-ray source (Ga $K\alpha$, $\lambda = 1.341 \text{ \AA}$) at 25 °C. Glass capillaries of 1.5 mm diameter were used as sample holders. All the sample were exposed by 3600 s relative to buffer blanks at 5 mg/mL in toluene. Scattering data were recorded on an Eiger2R 1M detector at a distance of 800 mm between detector and samples. The scattering vector, q , is defined as $q = (4\pi/\lambda) \sin \theta$, where 2θ represents the scattering angle, respectively.

Additional Characterization Data

Table S1. The number of H atoms from each functional groups on both the precursor chain PS-ABP-5.7 and the obtained SCNPs.

	Styrene	ABP	Backbone
Precursor (before crosslink)	5x	9y	3(x+y)
SCNPs (after crosslink)	5x	9y	3(x+y)- δ

Here x is the number of styrene monomers, and y that of ABP group, and δ the reacted H atom on backbone $-\text{CH}_2-\text{CH}-$. As shown in Figure R3, the area for the phenyl H atoms of styrene are set as a reference and normalized to 100, therefore the change in the area of backbone H on $-\text{CH}_2-\text{CH}-$ group, $(i - i')/100 = \delta/5x$, can be utilized to calculate the value of $X\% = \delta/(x+y) = 5x(i - i')/100/(x+y) = 0.05*0.943*(i - i') = (i - i') * 4.7\%$.

Table S2. The degree of cross-linking (X%) of different SCNPs resulted from PS-ABP-5.7 upon different exposure time.

Exposure Time (min)	Degree of cross-linking (X%)
8	1.2%
15	1.7%
30	2.5%
60	3.1%
90	4.4%
120	5.3%

Table S3. Overview of the precursor PS-ABP-24.4 and its corresponding cross-linking results at different exposure times in acetone/toluene.

Exposure Time (min)	M_n	M_w	PDI	M_n	M_w	PDI
0	29654	34954	1.18			
		Acetone			Toluene	
8	23807	30096	1.26	27019	32615	1.21
15	20183	26322	1.30	31655	42536	1.34
30	15375	23150	1.51	35013	63496	1.81
60	8316	12836	1.54	39411	80073	2.03
90	9213	16986	1.84	40031	92939	2.32

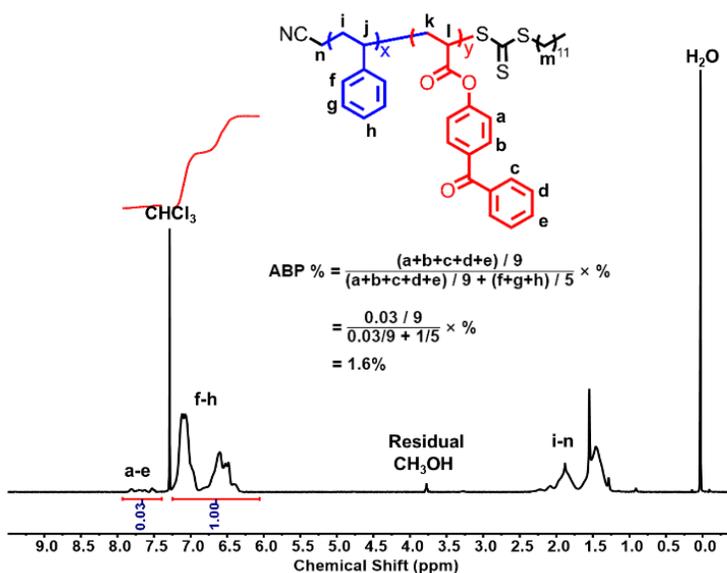


Figure S1. ^1H NMR spectrum of PS-ABP-1.6.

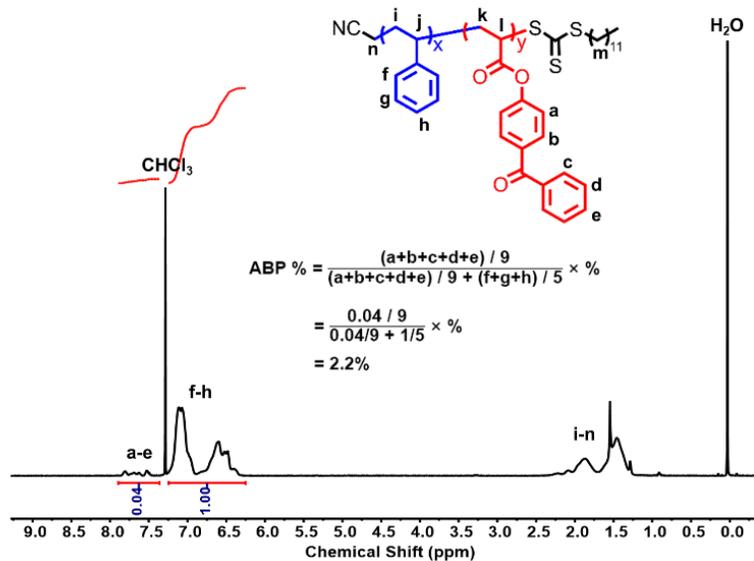


Figure S2. ^1H NMR spectrum of PS-ABP-2.2.

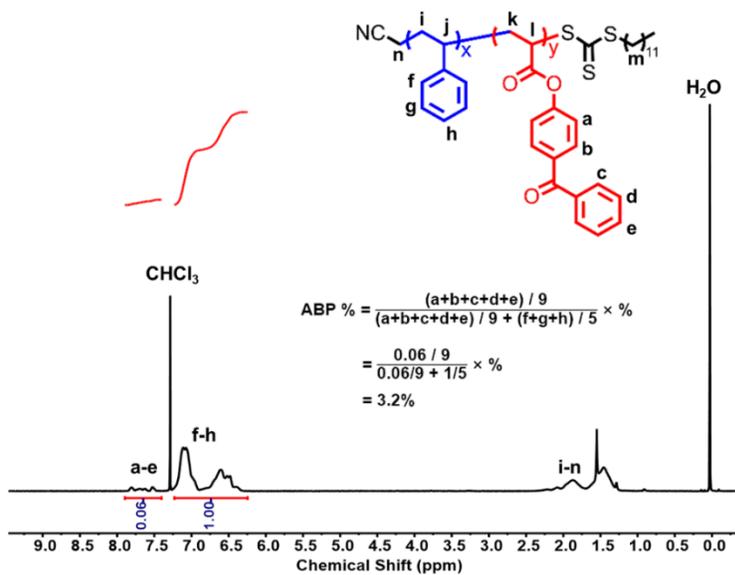


Figure S3. ^1H NMR spectrum of PS-ABP-3.2.

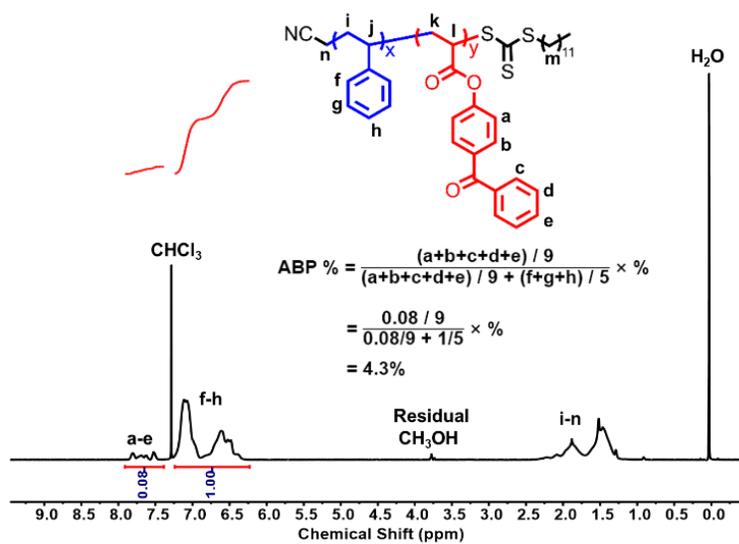


Figure S4. ^1H NMR spectrum of PS-ABP-4.3.

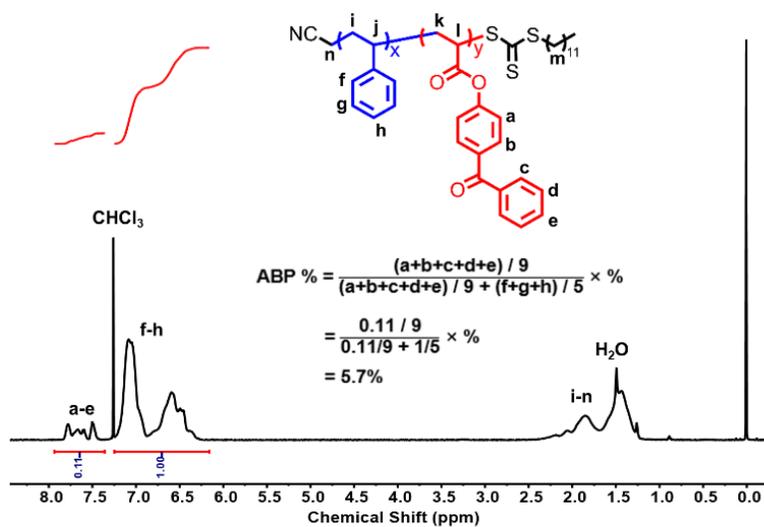


Figure S5. ^1H NMR spectrum of PS-ABP-5.7.

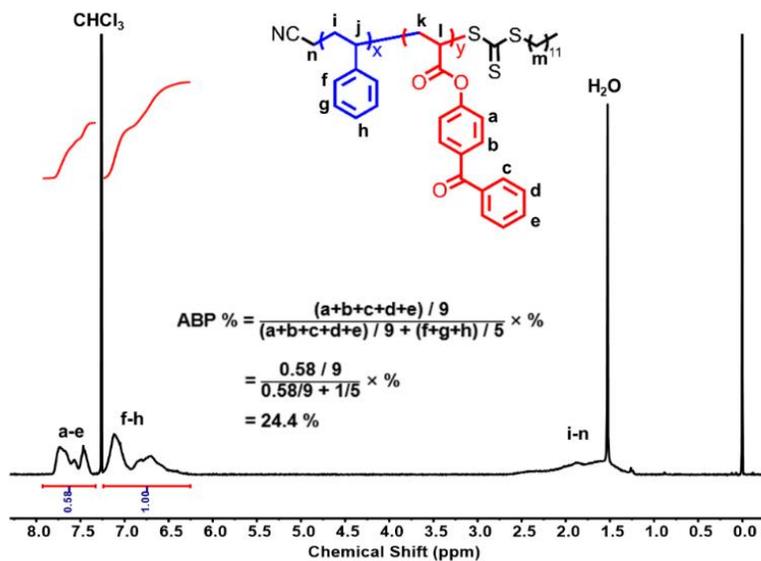


Figure S6. ^1H NMR spectrum of PS-ABP-24.4.

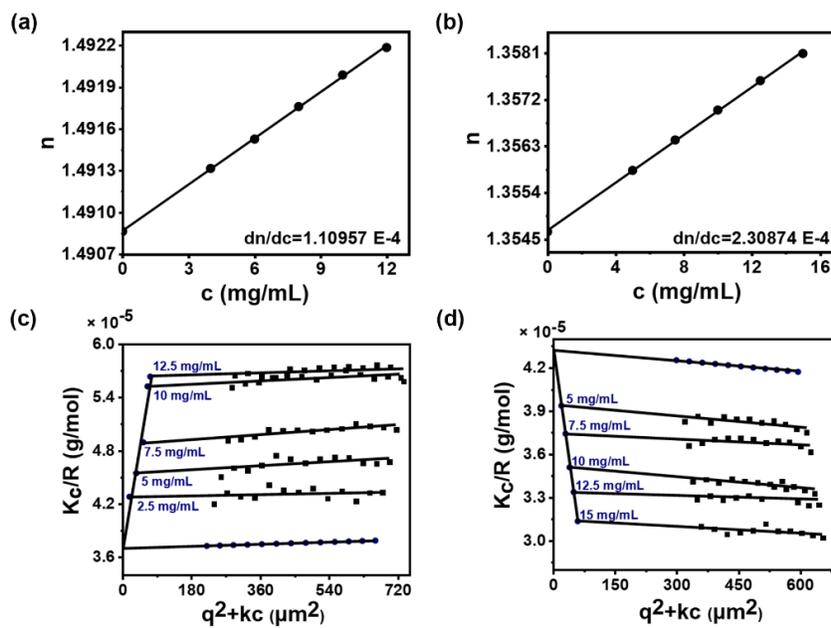


Figure S7. Static light scattering (SLS) analysis of the PS-ABP-5.7 in toluene versus acetone to determine the second osmotic virial coefficient (A_2).² The dn/dc of PS-ABP-5.7 were shown in a) toluene and b) acetone. The Zimm plot PS-ABP-5.7 were shown in c) toluene and d) acetone. where k is a spreading factor to separate superposed data. In toluene, $M_w = 27080$ g/mol, $A_2 = 9.418 \times 10^{-7}$ mol dm^3/g^2 ; In acetone, $M_w = 23070$ g/mol, $A_2 = -4.014 \times 10^{-7}$ mol dm^3/g^2 .

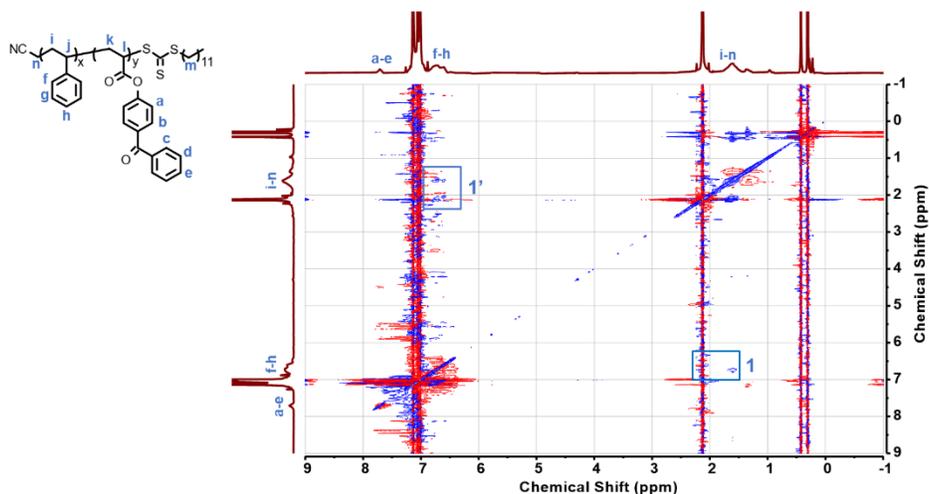


Figure S8. 2D-NOESY spectrum of PS-ABP-5.7 in deuterated toluene.

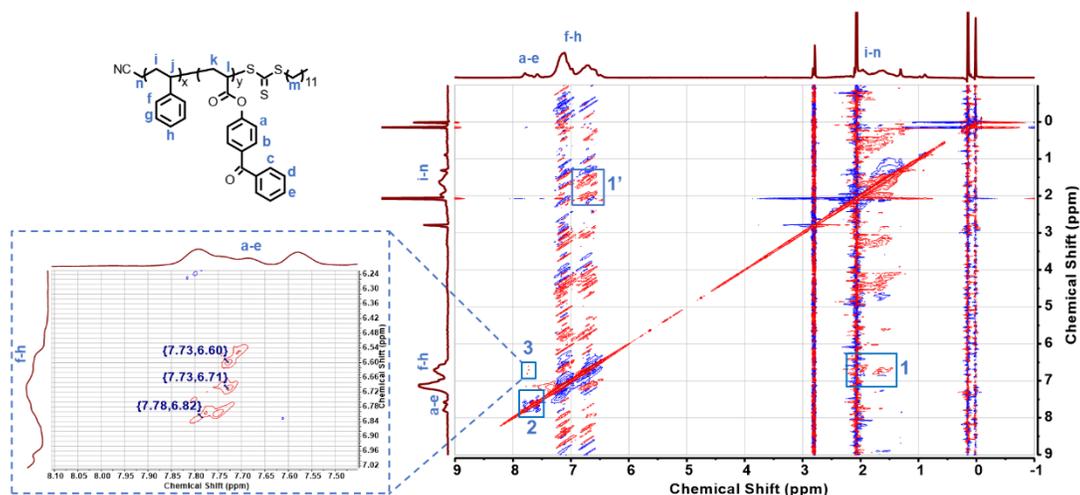


Figure S9. 2D-NOESY spectrum of PS-ABP-5.7 in deuterated acetone.

In deuterated toluene, the 2D-NOESY spectrum of the precursor PS-ABP-5.7 exhibits very weak signal, nearly limited to the correlation signal between the backbone $\text{-CH}_2\text{-}$ with the phenyl groups on the styrene, as labelled by the square 1 and 1' in Figure S8

In deuterated acetone, the 2D-NOESY spectrum of the precursor PA-ABP-5.7 (Figure S9) revealed more diverse range of signals: (i) peaks corresponding to the correlation signal of the backbone $\text{-CH}_2\text{-}$ with the phenyl groups on the styrene (square 1 and 1'); (ii) ABP interacting with itself (square 2); and (iii) weak signals indicating interactions between ABP and the phenyl groups on the styrene (square 3). Although (ii) and (iii) are weak, they do indicate an important point: the precursor chain tends to exhibit a more collapsed state in acetone compared to in toluene.

To enhance the signal, the number of positive contour level is set up at 128. However, the signal-to-noise ratio is still weak, making data analysis challenging. The above 2D-NOESY shows the same results as the 1D selective NOE in the main text.

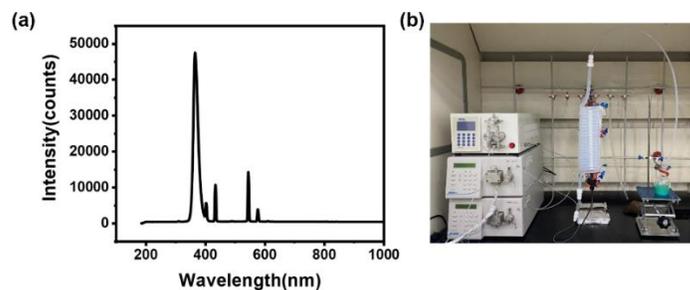


Figure S10. a) UV-vis spectrum of the UVA lamps. b) Custom-made flow chemistry equipment.

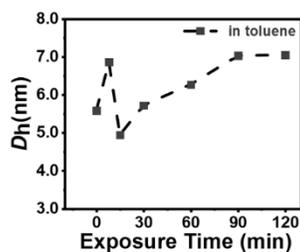


Figure S11. D_h of PS-ABP-5.7 crosslinked in toluene from DLS.

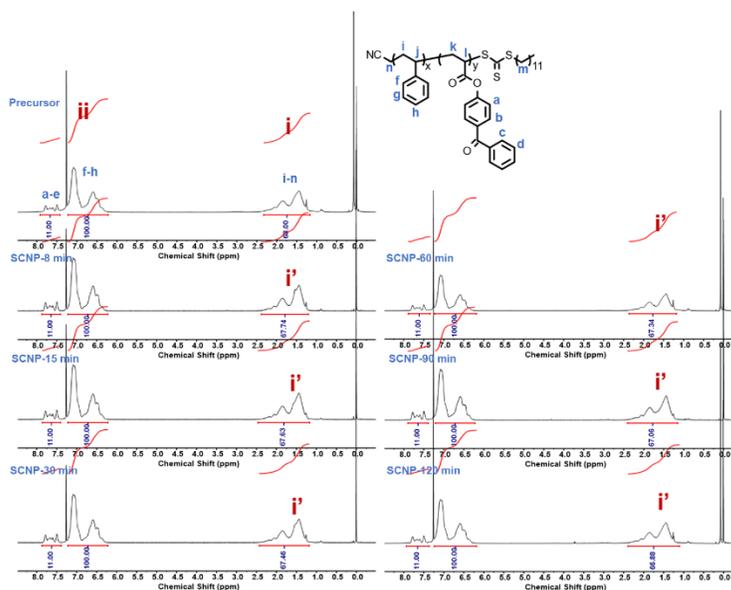


Figure S12. ^1H NMR spectra of precursor and SCNPs resulted at different exposure time.

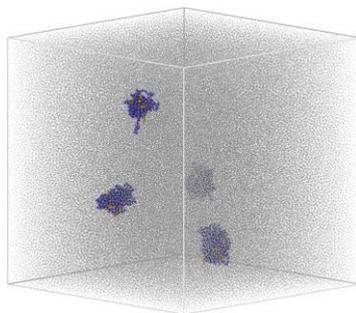


Figure S13. Final snapshot of simulation with 4 particles in a simulation box of $80 \sigma \times 80 \sigma \times 80 \sigma$ the simulation is performed for enough long time of 5×10^7 time steps, with a $dt = 0.005$.

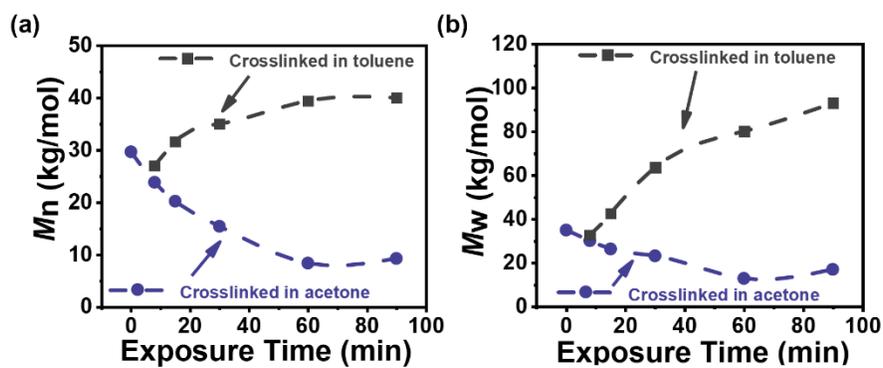


Figure S14. Trends of M_n and M_w of PS-ABP-24.4 in toluene and acetone versus exposure time, respective.

REFERENCE

- (1) Perrier, S. 50th Anniversary Perspective: RAFT Polymerization—A User Guide. *Macromolecules* **2017**, 50 (19), 7433-7447.
- (2) Teraoka, I. Polymer Solutions: An Introduction to Physical Properties, Caption 2.4: static light scattering. **2002**. DOI:10.1002/0471224510