

Self-assembly and Dis-assembly of Stimuli Responsive Tadpole-like Single Chain Nanoparticles (SCNPs) Using a Switchable Hydrophilic/Hydrophobic Boronic Acid Cross-linker

Junliang Zhang,¹ Joji Tanaka,¹ Pratik Gurnani,¹ Paul Wilson,¹ Matthias Hartlieb,¹ Sébastien Perrier*^{1,2,3}

¹ Department of Chemistry, The University of Warwick, Coventry CV4 7AL, U.K.

² Warwick Medical School, The University of Warwick, Coventry CV4 7AL, U.K.

³ Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, 381 Royal Parade,
Parkville, Victoria 3052, Australia

*Corresponding author: Email: s.perrier@warwick.ac.uk; Tel: +44 2476 528 085

EXPERIMENTAL PART

MATERIALS AND METHODS

Materials.

1, 4-Dioxane was obtained from Fisher Scientific and used as received. Anhydrous Tetrahydrofuran (THF, $\geq 99.9\%$), Dichloromethane (DCM, $\geq 99.5\%$), Isopropylidene glycerol (98%), triethylamine (99%), benzene-1,4-diboronic acid (DBA, $\geq 95.0\%$) were obtained from Sigma Aldrich and used as

received. 4-Acryloylmorpholine (NAM, Sigma-Aldrich, 97%) was filtered through a basic aluminium oxide (activated, basic, BrockmannI, standard grade, B150 mesh, 58Å) column before use to remove the radical inhibitor. 2, 2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, Wako) was used without further purification. dimethyl sulfoxide- d_6 (DMSO- d_6 , 99.9% D atom) obtained from Sigma Aldrich were used for ^1H NMR analysis. 2-(((butylthio)-carbonothioyl)thio)propanoic acid (called (propanoic acid)yl butyl trithiocarbonate (PABTC) in this paper) was prepared according to a previously reported procedure.¹ Glycerol acrylate (GLA) was synthesized by adapting to the published procedure.² Carbon coated copper (300 mesh) TEM grids were obtained from EM Resolutions (Saffron Walden, U.K.) and used as received. Mica discs for AFM were purchased from Agar Scientific Ltd, U.K. and freshly cleaved before use.

Methods.

Nuclear Magnetic Resonance (NMR) spectroscopy (^1H NMR). Spectra were recorded on a Bruker Avance III AV 300 spectrometer (300 MHz) or an HD 400 spectrometer (400 MHz) at 27 °C in deuterated DMSO (DMSO- d_6). Chemical shift values (δ) are reported in ppm. The residual proton signal of the solvent ($\delta_{\text{H}} = 2.51$ ppm) was used as internal reference.

RAFT polymerization. A typical synthesis of the first block is the following: CTA, monomer, solvent (1, 4-dioxane and deionized water) and azoinitiator were charged into a flask having a magnetic stirring bar. The flask was sealed with a rubber septum and degassed with nitrogen for ca. 15 minutes. The solution was then allowed to stir at 70 °C in a thermo-stated oil bath for the desired time. A sample was taken for ^1H NMR (to determine monomer conversion) and SEC analysis (to determine $M_{\text{n,SEC}}$ and D). After reaction, the mixture is cooled down in cold water to room temperature and open to air.

Typical synthesis of the following block. Monomer, initiator and solvent is added to the previous polymerization medium and well mixed. The mixture is then degassed by bubbling nitrogen through the solution for ca. 15 minutes, and the polymerization mixture was allowed to polymerize at 70 °C for the desired time with stirring. A sample was withdrawn from the polymerization medium using a

degassed syringe for ^1H NMR and SEC analysis. After reaction, the mixture is cooled down in cold water to room temperature and open to air.

Determination of monomer conversions. The conversions of the monomers were determined by comparing the integration of the vinyl protons ($\delta \sim 6.50\text{--}5.50$ ppm) to the integration of the three methyl protons belonging to the Z group of the PABTC chain transfer agent ($-\text{CH}_2-\text{CH}_3$) before and after polymerization.

Calculation of $M_{n,th}$. The theoretical number average molar mass ($M_{n,th}$) is calculated using equation (S1).

$$M_{n,th} = \frac{[\text{M}]_0 p M_M}{[\text{CTA}]_0} + M_{\text{CTA}} \quad (\text{S1})$$

where $[\text{M}]_0$ and $[\text{CTA}]_0$ are the initial concentrations (in mol L^{-1}) of monomer and chain transfer agent respectively; p is the monomer conversion as determined by ^1H NMR, M_M and M_{CTA} are the molar masses (g mol^{-1}) of the monomer and chain transfer agent respectively.

Integration in the ^1H NMR spectroscopy of the purified polymers of AB_1 , AB_2 , $\text{AB}_1^{\text{SCNP}}$ and $\text{AB}_2^{\text{SCNP}}$ at different pH values. Due to the high DP of the polymers, the integration of the three methyl protons belonging to the Z group of the PABTC chain transfer agent ($-\text{CH}_2-\text{CH}_3$) will not be accurate. Therefore, the integration of the diol of AB_1 and AB_2 after precipitation was used as internal reference respectively to integrate the peaks between $\delta = 1.90$ and 1.30 ppm for AB_1 and the peaks between $\delta = 2.00$ and 1.28 ppm for AB_2 and this integration was used as internal reference respectively for $\text{AB}_1^{\text{SCNP}}$ and $\text{AB}_2^{\text{SCNP}}$ at different pH values. Considering the targeted DPs and the quantitative conversion of the monomers, the integration of the diol peaks was assumed to be 40 for AB_1 and 160 for AB_2 .

Method of the calculation of hydrolysis of $\text{AB}_1^{\text{SCNP}}$ at pH 2.36. The percentage of hydrolysis was calculated according to equation:

$$n(\text{percentage of hydrolysis}) = n(\text{hydrolyzed diol}) / n(\text{reacted diol before hydrolysis}) \times 100,$$

where $n(\text{hydrolyzed diol})$ is the amount of hydrolyzed diol, $n(\text{reacted diol before hydrolysis})$ is the total amount of reacted diol after cross-linking reaction. The total amount of reacted diol after cross-linking

reaction: $n_{(\text{reacted diol before hydrolysis})} = \int (\text{peak a and a}')_{\text{linear}} - \int (\text{peak a and a}')_{\text{SCNP at pH10.02}}$, the amount of hydrolyzed diol: $n_{(\text{hydrolyzed diol})} = \int (\text{peak a and a}')_{\text{SCNP at pH2.36}} - \int (\text{peak a and a}')_{\text{SCNP at pH10.02}}$.

Size Exclusion Chromatography (SEC). Number-average molar masses ($M_{n,SEC}$) and dispersity values (\mathcal{D}) were determined using size exclusion chromatography with DMF as an eluent. The DMF Agilent 390-LC MDS instrument equipped with differential refractive index (DRI), viscometry (VS), dual angle light scatter (LS) and dual wavelength UV detectors. The system was equipped with 2 x PLgel Mixed D columns (300 x 7.5 mm) and a PLgel 5 μm guard column. The eluent is DMF with 5 mmol NH_4BF_4 additive. Samples were run at 1 mL/min at 50 °C. Poly(methyl methacrylate) standards (Agilent EasyVials) were used for calibration. Analyte samples were filtered through a nylon membrane with 0.22 μm pore size before injection. Respectively, experimental molar mass ($M_{n,SEC}$) and dispersity (\mathcal{D}) values of synthesized polymers were determined by conventional calibration using Agilent GPC/SEC software.

Differential Scanning Calorimetry (DSC). The experiments were performed to determine the thermal behavior of the synthesized polymers on a Mettler Toledo DSC1. In all tests, a scan rate of 10 K/min was used for three heating and cooling cycles. The glass transition temperature (T_g) value is the maxima of the first derivative of (dH/dT) the second heating run.

Transmission Electron Microscopy (TEM). Samples were prepared by placing a carbon coated copper grid onto a 20 μL droplet of aqueous nanoparticles in a petri dish and allowed to air-dry overnight. The grid was then stained with an aqueous solution of uranyl acetate (0.2 wt%) and allowed to air-dry overnight. TEM images were acquired using a JEOL 2100 transmission electron microscope operating at a 200 kV accelerating voltage. Images were captured using Digital Micrograph® and analysed with ImageJ. Size distributions were produced by measuring at least 100 particles in ImageJ.

Atomic Force Microscopy (AFM). AFM images were acquired in AC mode on a Cypher S system (Asylum Research). The probes used were the AC160TS from Olympus probes with a nominal resonant frequency of 300 kHz and a spring constant of approximately 40 N m^{-1} on a Multimode AFM (Asylum

Research). Images were acquired at a pixel resolution of 512 and a scan rate of 1 Hz. The data were analysed by the Asylum Research software.

Dynamic Light Scattering (DLS). Hydrodynamic diameters (D_h) and size distributions were determined by DLS on a MALVERN Zetasizer Nano ZS operating at 20 °C with a 633 nm laser module. Measurements were made at a detection angle of 173° (back scattering). Measurements were repeated three times with automatic attenuation selection and measurement position. The results were analysed using Malvern DTS 6.20 software, using the multiple narrow modes setting. PDI values were calculated using equation S2.

$$\text{PDI} = \frac{\sigma^2}{d^2} \quad (\text{S2})$$

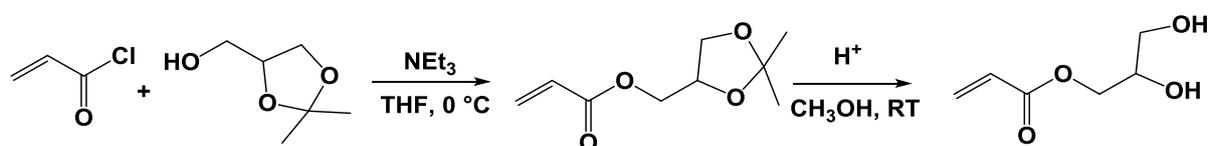
where σ is standard deviation, and d is the diameter.

Self-assembly behaviour study of $\text{AB}_1^{\text{SCNP}}$ and $\text{AB}_2^{\text{SCNP}}$ depending on the pH changes by DLS measurements, TEM, AFM, $^1\text{H NMR}$, and SEC analysis. A 1% weight solution of $\text{AB}_1^{\text{SCNP}}$ and $\text{AB}_2^{\text{SCNP}}$ were prepared separately by dissolving the respective SCNPs in deionized water. The initial pH values of the resulting solutions were found to be 10.02 for $\text{AB}_1^{\text{SCNP}}$ and 10.20 for $\text{AB}_2^{\text{SCNP}}$ without adjusting. The pH of the resulting solutions were then adjusted to the certain values as displayed in **Tables S1** and **S2** using 1 M HCl solution. The hydrodynamic diameters (D_h) and size distributions of each pH value were measured by DLS. The solution of $\text{AB}_1^{\text{SCNP}}$ at pH 2.36 was freeze dried to remove the solvent and the obtained material was used for $^1\text{H NMR}$ and SEC analysis. Part of the solution of $\text{AB}_2^{\text{SCNP}}$ at pH 7.60 (when the self-assembly occurred) was taken for SEC, TEM, AFM, and sugar responsive analysis. The solution of $\text{AB}_2^{\text{SCNP}}$ at pH 2.50 was freeze dried to remove the solvent and the obtained material was used for $^1\text{H NMR}$ and SEC analysis.

Sugar responsive study of $\text{AB}_1^{\text{SCNP}}$, $\text{AB}_2^{\text{SCNP}}$, and $\text{AB}_2^{\text{SCNP}}$ self-assembly at pH 7.60. Glucose (10 eq. of $n(\text{diol})$) was added to the solution of $\text{AB}_1^{\text{SCNP}}$ and $\text{AB}_2^{\text{SCNP}}$ (1% weight in H_2O) at $\text{pH} \approx 10$ and the solution of $\text{AB}_2^{\text{SCNP}}$ self-assembly at pH 7.60. The hydrodynamic diameters (D_h) and size distributions of the resulting solutions were measured by DLS. The solutions were then freeze dried to remove the solvent and the obtained materials were used for SEC analysis.

Synthesis of glycerol acrylate (GLA)

Scheme S1. Synthetic route of GLA



First step: Isopropylidene glycerol (19.95 g, 151 mmol, 1 eq), NEt₃ (22.97 g, 227 mmol, 1.5 eq), 0.25 g of hydroquinone (inhibitor), and 200 mL of dried THF were added to a 2 L round bottom flask. Acryloyl chloride (16.4 g, 182 mmol, 1.2 eq) was dissolved in 35 mL of dry THF and added drop wise to the above mixture with stirring in an ice bath over one hour. The mixture was then stirred for 24 hours and filtered. The solvent was removed to obtain a pale yellow solid which was dissolved in 150 mL of DCM and then 100 mL of water was added. The organic phase was extracted with DCM (2 × 100 mL). The organic layer was combined and washed once with 100 mL of brine. The organic phase was dried with magnesium sulphate, filtered, and the solvent was removed to obtain a yellow oil. Half amount of this yellow oil was distilled at 50 °C under vacuum (0.35 mba) to obtain 7.2 g intermediate (solketal acrylate monomer, **SA**). ¹H NMR (**Figure S1**, 400 MHz, DMSO-*d*₆, ppm): δ = 6.38 (*dd*, 1H, *J*₁ = 16.0 Hz, *J*₂ = 4.0 Hz), 6.23 (*dd*, 1H, *J*₁ = 16.0 Hz, *J*₂ = 8.0 Hz), 5.99 (*dd*, 1H, *J*₁ = 8.0 Hz, *J*₂ = 4.0 Hz), 4.33-4.27 (*m*, 1H), 4.23 (*dd*, 1H, *J*₁ = 12.0 Hz, *J*₂ = 4.0 Hz), 4.11 (*dd*, 1H, *J*₁ = 8.0 Hz, *J*₂ = 4.0 Hz), 4.05 (*dd*, 1H, *J*₁ = 8.0 Hz, *J*₂ = 4.0 Hz), 3.72 (*dd*, 1H, *J*₁ = 8.0 Hz, *J*₂ = 4.0 Hz), 1.33 (*s*, 3H), 1.28 (*s*, 3H).

Second step: Solketal acrylate monomer (5 g, 27 mmol) was dissolved in 130 mL of methanol in a 250 mL round bottom flask. Amberlyst resin (2.7 g) was added to the above solution and the resultant mixture was stirred for 24 hours at room temperature. The reaction mixture was then filtered to remove the amberlyst resin and the solvent was removed under reduced pressure to obtain a light brown oil which was then purified by flash column chromatography using chloroform and methanol mixture as the eluent to obtain 2.08 g of product (GLA, colorless liquid). ¹H NMR (**Figure S2**, 300 MHz, DMSO-*d*₆, ppm): δ = 6.38 (*dd*, 1H, *J*₁ = 18.0 Hz, *J*₂ = 3.0 Hz), 6.23 (*dd*, 1H, *J*₁ = 18.0 Hz, *J*₂ = 12.0 Hz), 5.97

(*dd*, 1H, $J_1 = 12.0$ Hz, $J_2 = 3.0$ Hz), 4.94 (*d*, 1H, $J = 6.0$ Hz), 4.68 (*t*, 1H, $J = 6.0$ Hz), 4.17 (*dd*, 1H, $J_1 = 12.0$ Hz, $J_2 = 3.0$ Hz), 4.03 (*dd*, 1H, $J_1 = 12.0$ Hz, $J_2 = 6.0$ Hz), 3.73-3.64 (*m*, 1H), 3.39-3.36 (*m*, 2H).

Synthesis of linear copolymer AB₁

Synthesis of first block **A**: PABTC (13.5 mg, 0.057 mmol, 1.0 eq.), NAM (800 mg, 5.7 mmol, 100 eq.), VA-044 (0.09 mg, 2.8E-04 mmol, 0.0049 eq., 45.8 μ L, 2 mg/mL in H₂O), 1, 4-dioxane (0.353 mL) and H₂O (0.777 mL) were introduced into a flask equipped with a magnetic stirrer and sealed with a rubber septum. The flask was degassed by bubbling nitrogen through the solution for 15 minutes, and placed into a preheated oil bath at 70 °C. After 2 h, the reaction was stopped by cooling the mixture down using a cold water bath. Subsequently, a sample was taken from the reaction mixture for ¹H NMR and SEC analysis. ¹H-NMR (300 MHz, DMSO-*d*₆, ppm): $\delta = 5.11$ (*s*, *broad*, *weak*, CH-S), 3.86-2.89 (*m*, broad, CH₂ polymer), 2.80-0.96 (*m*, CH and CH₂ backbone, CH₃ R-group, CH₂ Z-group), 0.89 (*t*, 3H, $J = 6.0$ Hz, CH₃ Z-group).

Chain extension of first block **A** to obtain **AB₁**: The reaction mixture from the last step was used directly for the chain extension. NAM (640 mg, 4.5 mmol, 80 eq.), GLA (166 mg, 1.14 mmol, 20 eq.), VA-044 (0.30 mg, 9.3E-04 mmol, 0.0016 eq., 150.6 μ L, 2 mg/mL in H₂O), and H₂O (1.025 mL) were introduced into the previous polymerization medium and sealed with a rubber septum. The flask was degassed by bubbling nitrogen through the solution for 15 minutes, and placed into a preheated oil bath at 70 °C. After 2 h, the reaction was stopped by cooling the mixture down using a cold water bath. Subsequently, a sample was taken from the reaction mixture for ¹H NMR and SEC analysis. ¹H-NMR (300 MHz, DMSO-*d*₆, ppm): $\delta = 4.81$ (*s*, OH), 4.64 (*s*, OH), 4.04 (*s*, $-(C=O)-O-\underline{CH_2}-(CHOH)-CH_2OH$), 3.92 (*s*, $-(C=O)-O-CH_2-(\underline{CHOH})-CH_2OH$), 3.83-2.86 (*m*, broad, CH₂ polymer, $-(C=O)-O-CH_2-(CHOH)-\underline{CH_2}OH$), 2.80-0.96 (*m*, CH and CH₂ backbone, CH₃ R-group, CH₂ Z-group), 0.90 (*t*, 3H, $J = 6.0$ Hz, CH₃ Z-group).

Synthesis of linear copolymer AB₂

Synthesis of first block **A**: The first block was synthesized using exactly the same procedure with the synthesis of **AB₁**.

Chain extension of first block **A** to obtain **AB₂**: The reaction mixture from the last step was used directly for the chain extension. NAM (160.9 mg, 1.14 mmol, 20 eq.), GLA (657.6 mg, 4.5 mmol, 80 eq.), VA-044 (0.30 mg, 9.3E-04 mmol, 0.0016 eq., 150.6 μ L, 2 mg/mL in H₂O), and H₂O (1.025 mL) were introduced into the previous polymerization medium and sealed with a rubber septum. The flask was degassed by bubbling nitrogen through the solution for 15 minutes, and placed into a preheated oil bath at 70 °C. After 2 h, the reaction was stopped by cooling the mixture down using a cold water bath. Subsequently, a sample was taken from the reaction mixture for ¹H NMR and SEC analysis. ¹H-NMR (300 MHz, DMSO-*d*₆, ppm): δ = 4.82 (*s*, OH), 4.62 (*s*, OH), 4.02 (*s*, $-(C=O)-O-\underline{CH}_2-(CHOH)-CH_2OH$), 3.91 (*s*, $-(C=O)-O-CH_2-(\underline{C}HOH)-CH_2OH$), 3.79-3.07 (*m*, broad, CH₂ polymer, $-(C=O)-O-CH_2-(CHOH)-\underline{C}H_2OH$), 2.83-0.95 (*m*, CH and CH₂ backbone, CH₃ R-group, CH₂ Z-group), 0.89 (*t*, 3H, *J* = 6.0 Hz, CH₃ Z-group).

General procedure for the intramolecular chain folding to obtain the single chain nanoparticles. The solution of cross-linker benzene-1,4-diboronic acid (DBA) in H₂O (pH \approx 10, adjusted using NaOH) was added dropwise into a premade aqueous solution (pH = 10) of the linear polymer precursor at room temperature. After addition of the solution of DBA, the reaction mixture was freeze dried to remove water to obtain the SCNPs. See the next paragraph for the detailed procedure.

Single chain nanoparticles (SCNP) synthesis. The copolymer precursor was dissolved in deionized water (1 mg/mL for **AB₁** and 0.5 mg/mL for **AB₂**) and the pH of the solution was adjusted to pH \approx 10 using 1 M NaOH aqueous solution. DBA (0.5 eq. of n(diols), 0.5 mg/mL) was dissolved in pH \approx 10 NaOH aqueous solution. The DBA solution was added drop wise to the solution of respective linear precursor at room temperature in 15 minutes for the synthesis of **AB₁^{SCNP}** and 30 minutes for the synthesis of **AB₂^{SCNP}** in order to avoid the intermolecular cross-linking considering the relative large amount of diol in **AB₂**. After addition of the solution of DBA, the reaction mixture was freeze dried to remove water to afford the products as white solids.

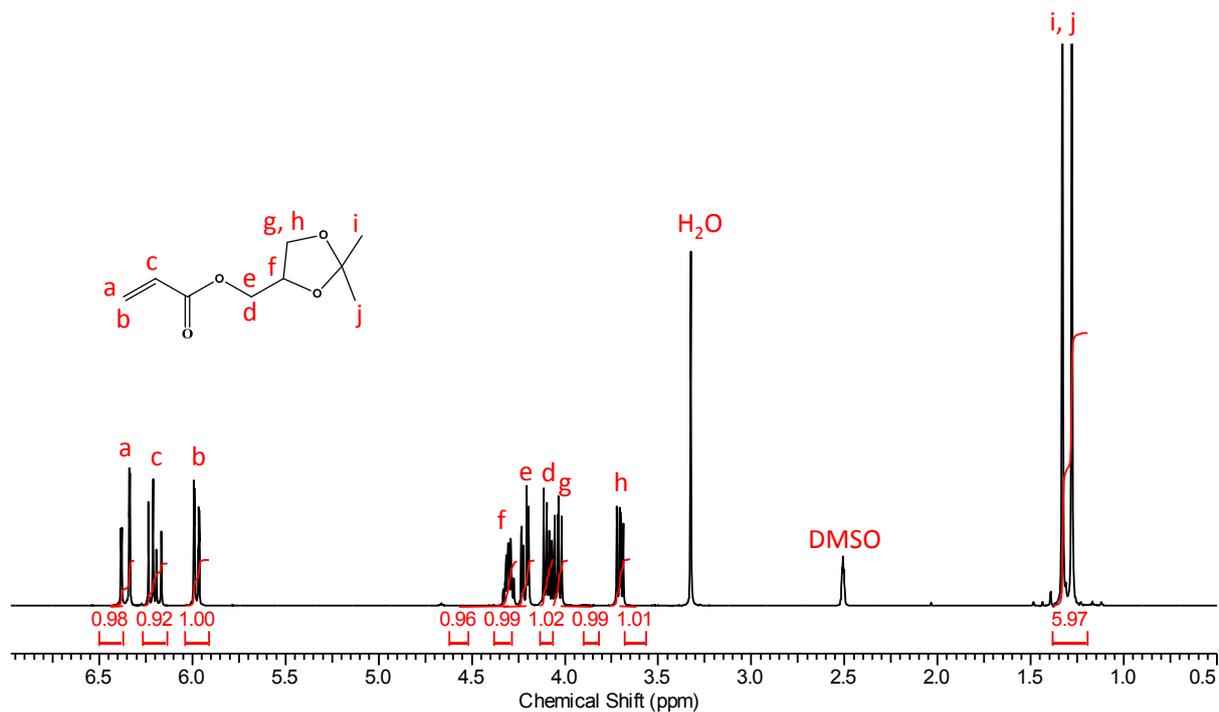


Figure S1. ¹H NMR spectrum (DMSO-*d*₆, 400MHz) of SA.

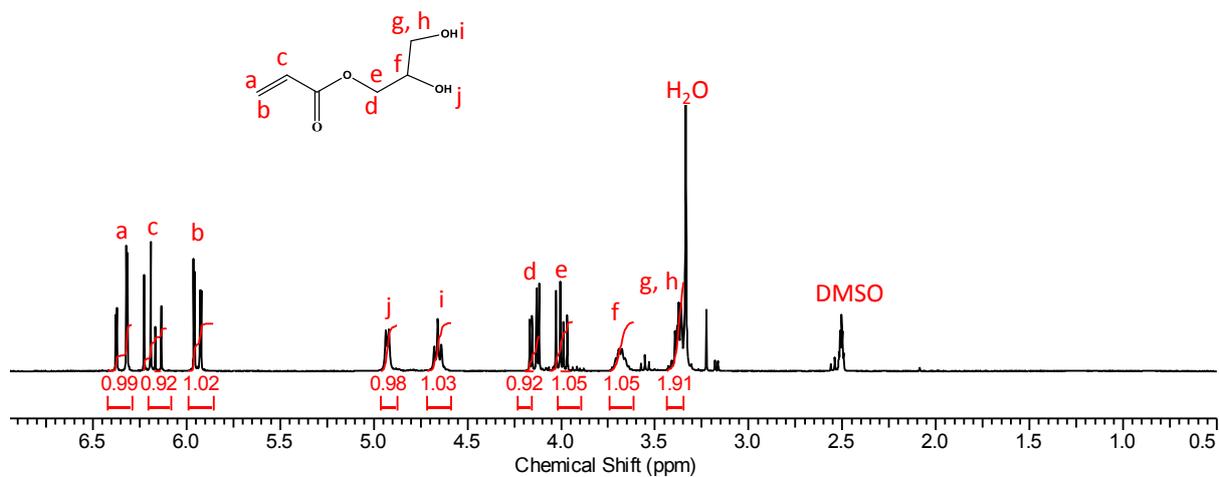


Figure S2. ¹H NMR spectrum (DMSO-*d*₆, 300MHz) of GLA.

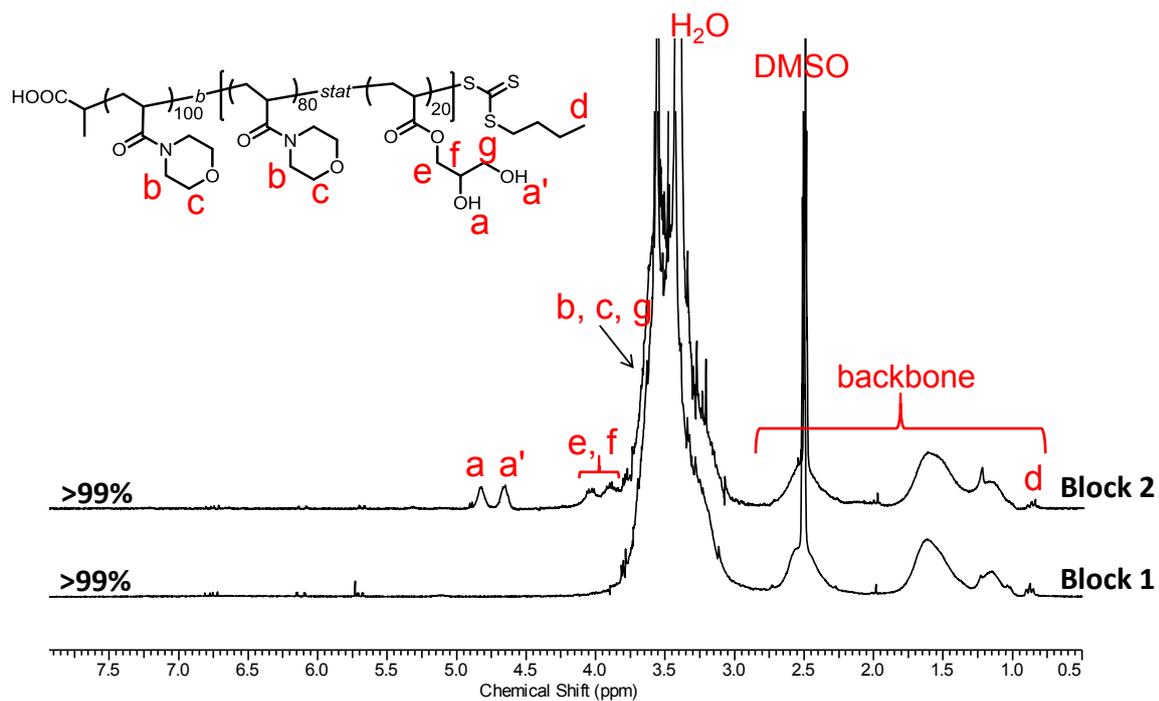


Figure S3. ^1H NMR spectrum ($\text{DMSO-}d_6$, 300MHz) of AB_1 ($\text{PNAM}_{100}\text{-}b\text{-P}(\text{NAM}_{80}\text{-}\text{stat}\text{-GLA}_{20})$) showing the monomer conversion for each block after iterative RAFT polymerization.

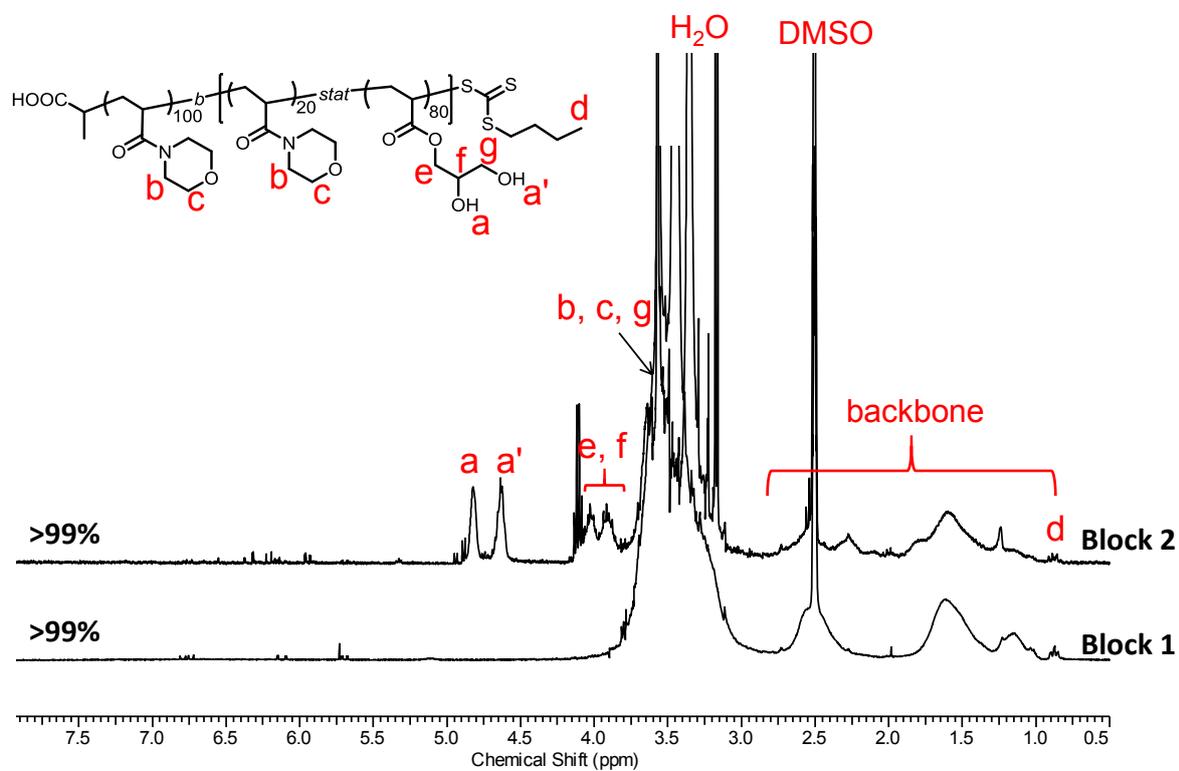


Figure S4. ^1H NMR spectrum ($\text{DMSO-}d_6$, 300MHz) of AB_2 ($\text{PNAM}_{100}\text{-}b\text{-P}(\text{NAM}_{20}\text{-}\text{stat}\text{-GLA}_{80})$) showing the monomer conversion for each block after iterative RAFT polymerization.

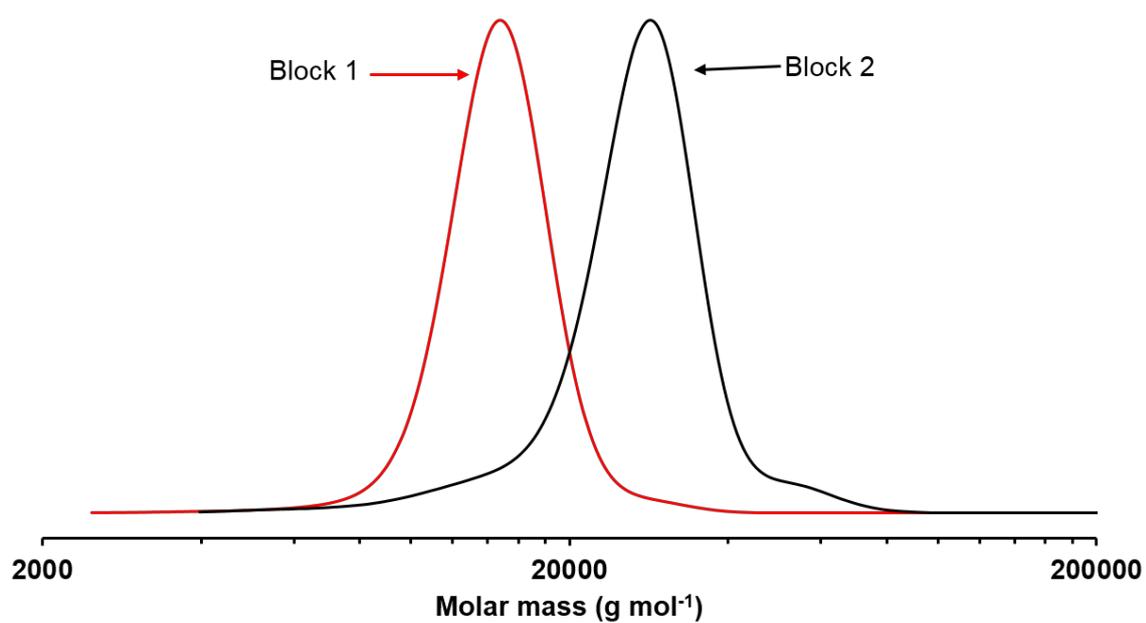


Figure S5. Molecular weight distributions (SEC RI traces in DMF) for successive block extensions of the diblock copolymer AB_1 (PNAM₁₀₀-*b*-P(NAM₈₀-*stat*-GLA₂₀)).

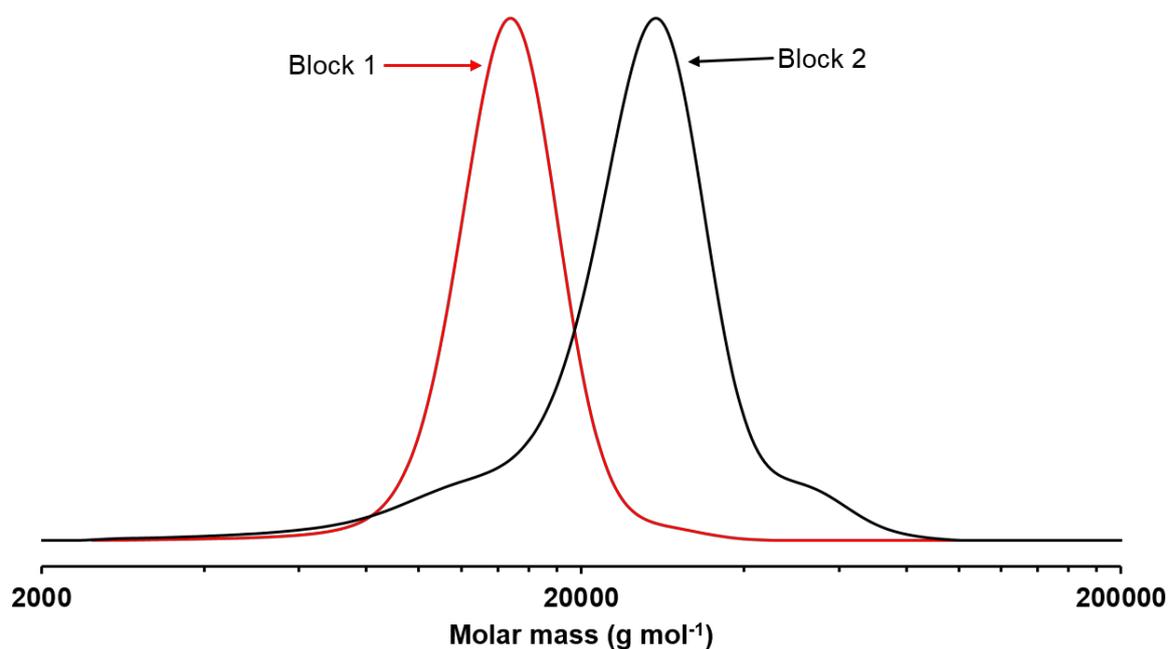


Figure S6. Molecular weight distributions (SEC RI traces in DMF) for successive block extensions of the diblock copolymer AB_2 (PNAM₁₀₀-*b*-P(NAM₂₀-*stat*-GLA₈₀)).

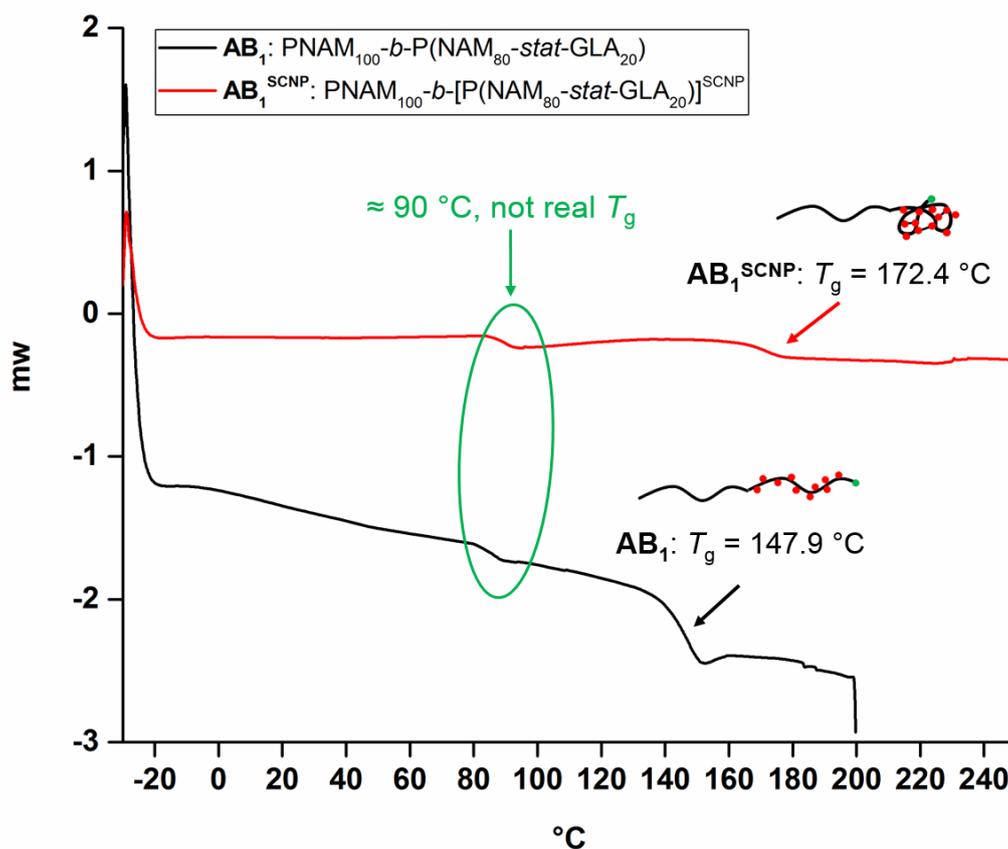


Figure S7. DSC curves of the linear copolymer AB_1 and folded polymer $\text{AB}_1^{\text{SCNP}}$.

During the DSC analysis, the signal around 90 $^{\circ}\text{C}$ displaying the character of a T_g exists in the DSC curves of both AB_1 and $\text{AB}_1^{\text{SCNP}}$. In order to demonstrate this is an artefact due to the analytical instrument rather than a real T_g , the homopolymer PolyNAM₁₀₀ (**A**) and the statistical copolymer Poly(NAM₈₀-stat-GLA₂₀) (**B**₁) were analysed by DSC. Again, a signal around 90 $^{\circ}\text{C}$ was observed for both polymers (see the following figure: **Figure S8**). Since no phase separation should exist in the homopolymer **A** and the statistical copolymer, each copolymer should only display one T_g , 159.2 $^{\circ}\text{C}$ for homopolymer **A** (PolyNAM₁₀₀) and 132.1 $^{\circ}\text{C}$ for the statistical copolymer (Poly(NAM₈₀-stat-GLA₂₀)). Based on these results, we conclude the signal around 90 $^{\circ}\text{C}$ is an instrument artefact.

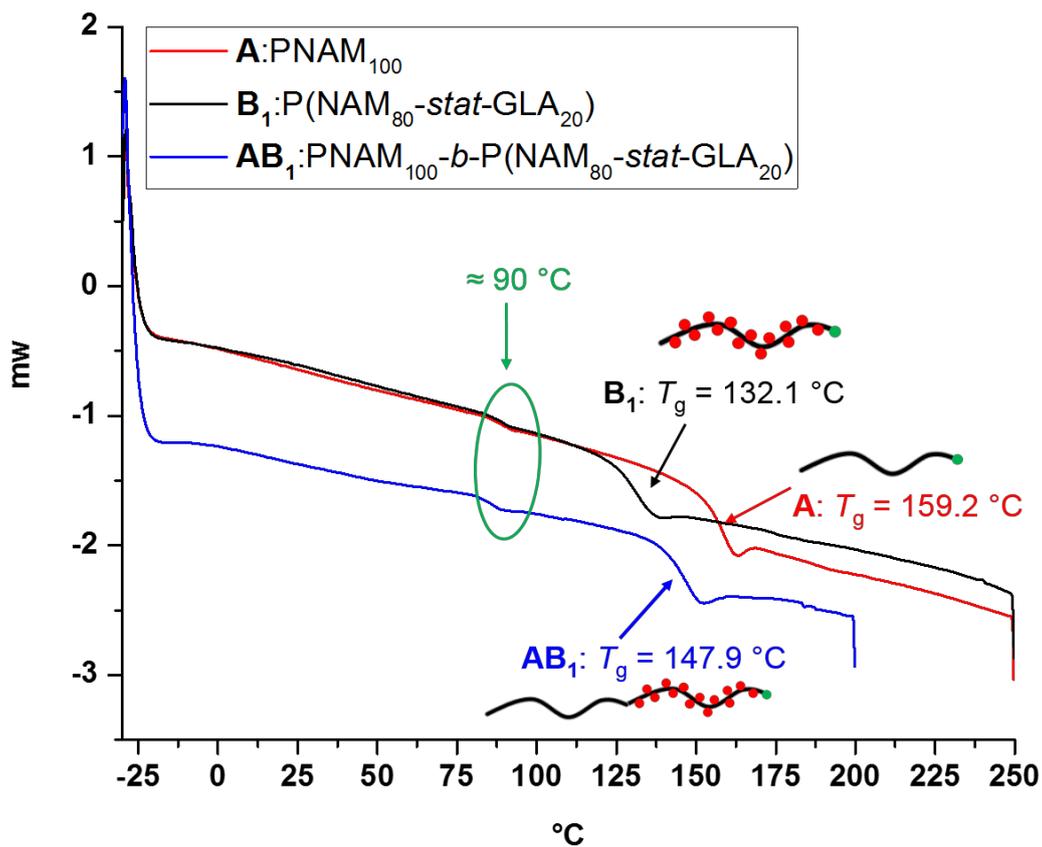


Figure S8. DSC curves of the homopolymer A, statistical copolymer B₁, and AB₁.

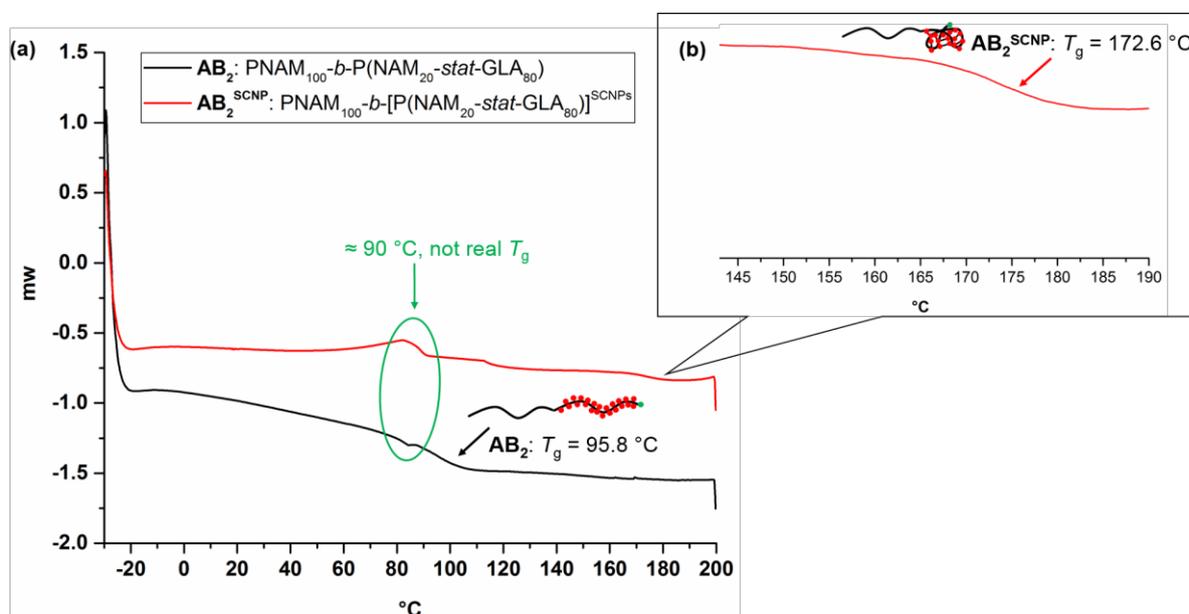


Figure S9. DSC curves of (a) the linear copolymer AB₂ and the folded polymer AB₂^{SCNP}; (b) a zoomed in figure of the folded polymer AB₂^{SCNP}.

Table S1: Hydrodynamic sizes of $\text{AB}_1^{\text{SCNP}}$ at different pH values obtained by DLS in H_2O .

pH	D_h (nm)	PDI
10.02	6.1	0.05
8.45	6.1	0.05
7.10	6.2	0.05
6.28	6.3	0.05
5.66	6.2	0.06
3.28	5.8	0.05
2.36	5.9	0.06

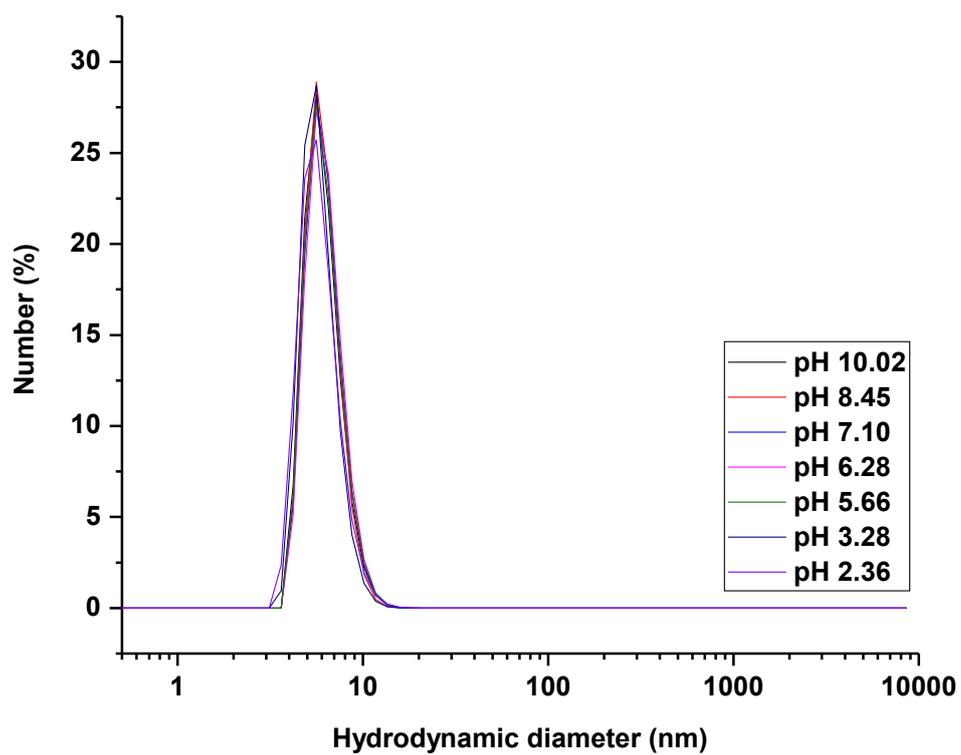


Figure S10. Average hydrodynamic size distributions of $\text{AB}_1^{\text{SCNP}}$ at different pH values obtained by DLS in H_2O .

Table S2: Hydrodynamic sizes of $\text{AB}_2^{\text{SCNP}}$ at different pH values obtained by DLS in H_2O .

pH	D_h (nm)	PDI
10.20	5.0	0.08
8.89	5.0	0.06
8.00	111.2	0.04
7.60	245.5	0.02
6.55	6.3	0.04
4.40	6.8	0.04
3.08	6.6	0.05
2.50	6.5	0.07

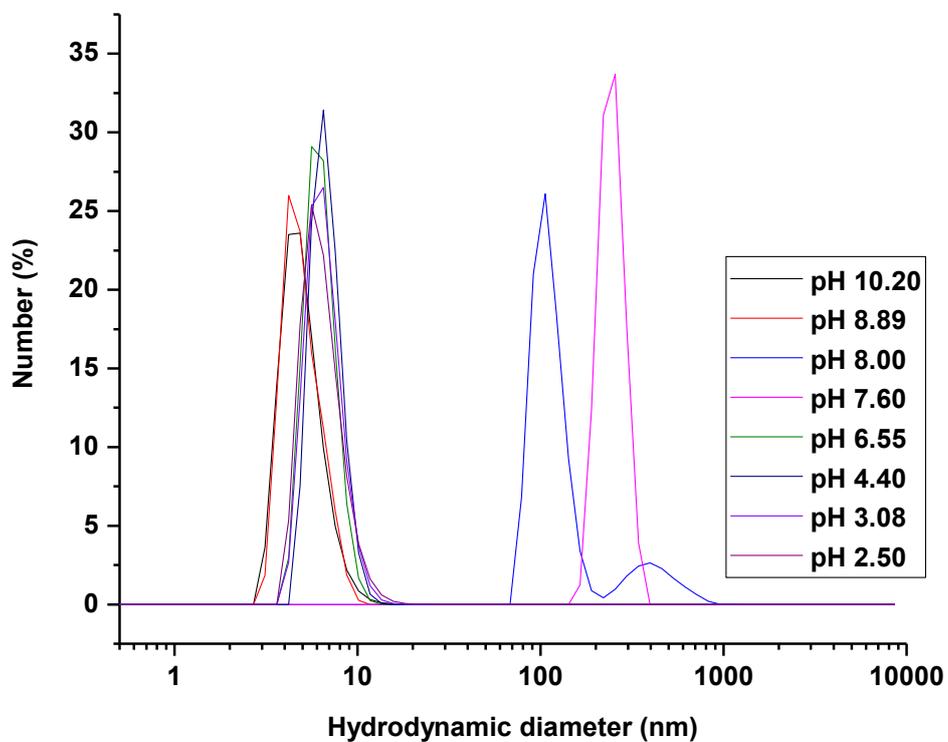


Figure S11. Hydrodynamic size distributions of $\text{AB}_2^{\text{SCNP}}$ at different pH values obtained by DLS in H_2O .

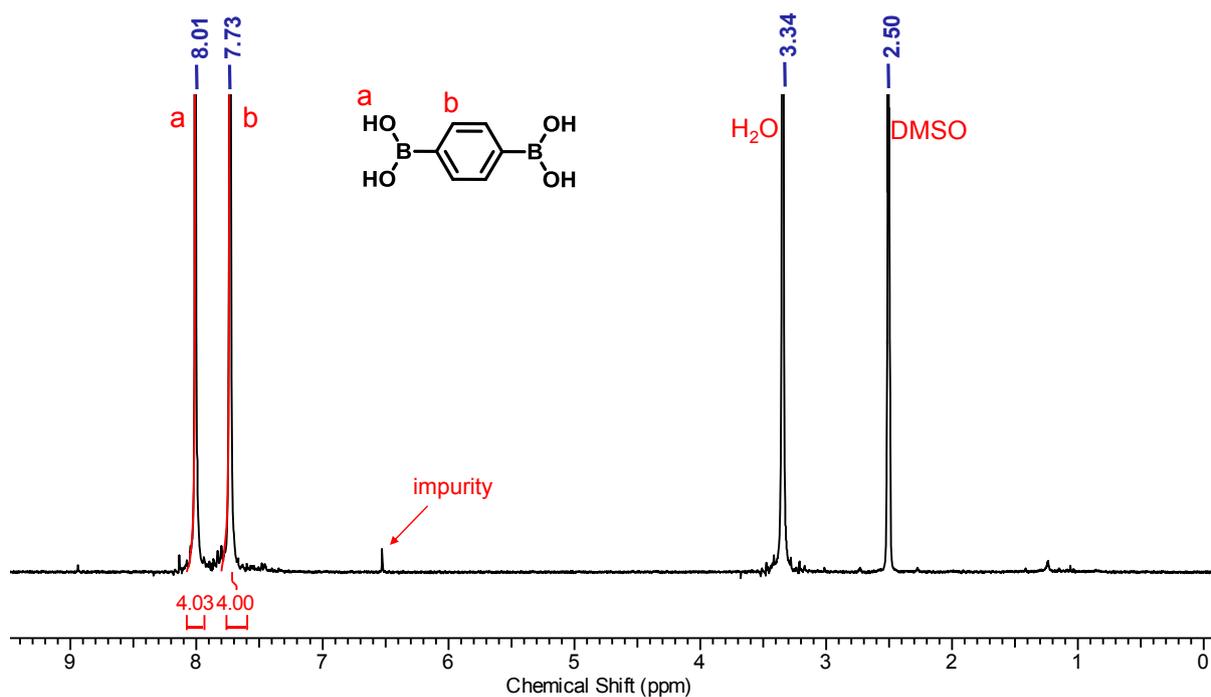


Figure S12. ^1H NMR spectrum (DMSO- d_6 , 300MHz) of free DBA.

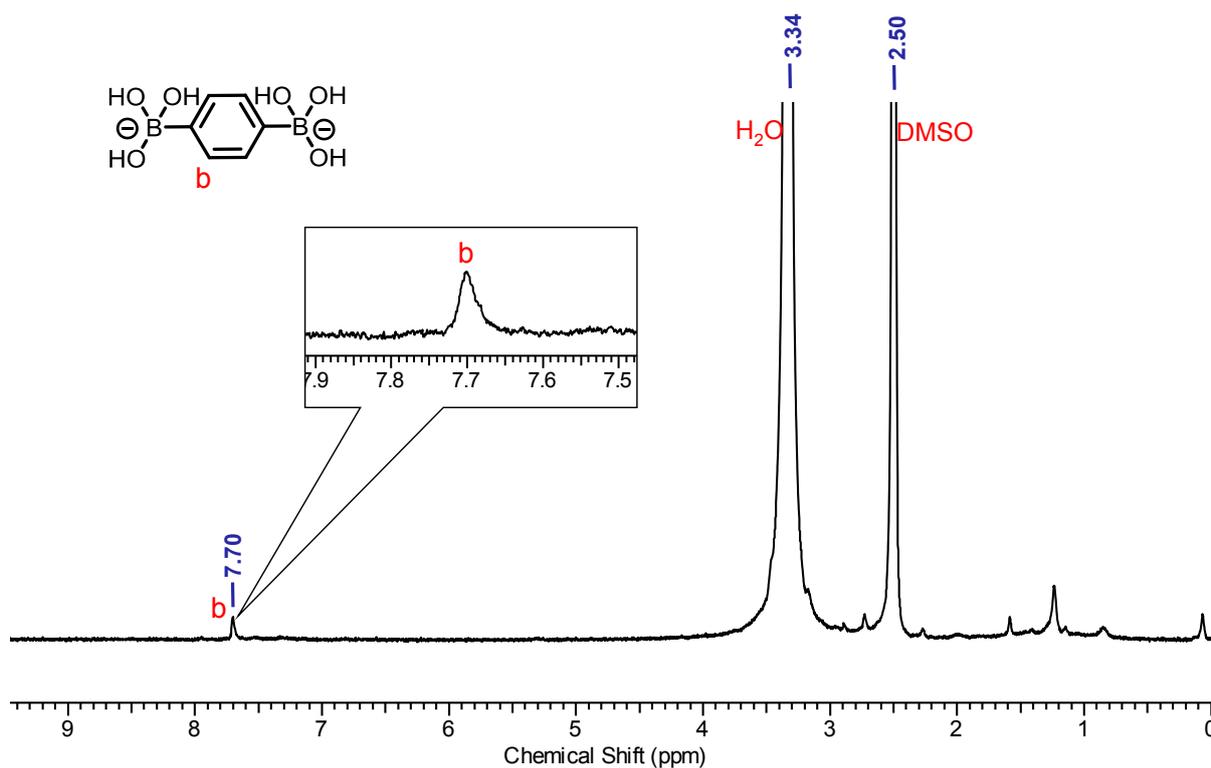


Figure S13. ^1H NMR spectrum (DMSO- d_6 , 300MHz) of free DBA at pH \approx 10.



Figure S14. The picture shows that the negatively charged free DBA ($\text{pH} \approx 10$) is not soluble in $\text{DMSO-}d_6$. The negatively charged free DBA was made by dissolving DBA in the solution of NaOH in H_2O and the pH was adjusted to $\text{pH} \approx 10$ and freeze dried.

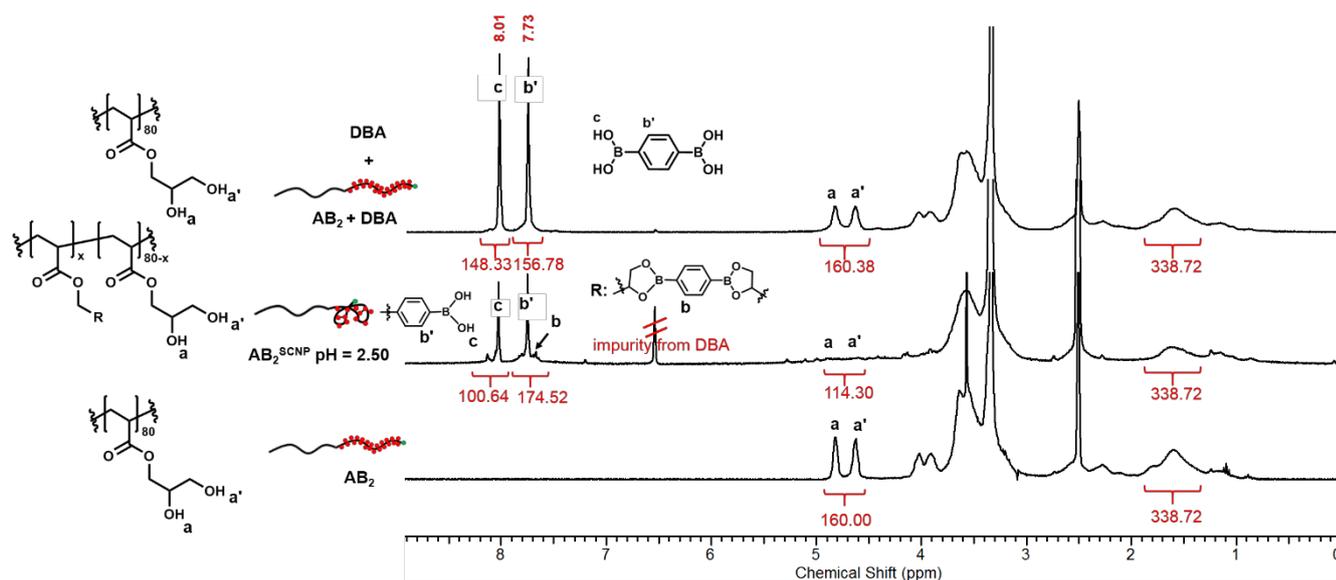


Figure S15. ^1H NMR spectra (300MHz, $\text{DMSO-}d_6$) of linear polymer AB_2 (bottom), folded polymer $\text{AB}_2^{\text{SCNP}}$ at $\text{pH} = 2.50$ (middle), and linear polymer AB_2 mixed with free DBA cross-linker in $\text{DMSO-}d_6$ (top). For comparison with the free DBA, please see **Figure S12**. The integration of the peaks between $\delta = 2.00$ and 1.28 ppm was used as internal reference (see the methods part for how to integrate these peaks). The method for the calculation of the percentage of DBA cross-linker attached to the

polymer backbone is as following: As $\mathbf{AB}_2^{\text{SCNP}}$ at $\text{pH} \approx 10$ was not fully soluble in DMSO, we were not able to obtain the ^1H NMR spectrum at this pH. Therefore, the diol units (peaks a and a') could not be used as reference and the percentage of DBA cross-linker attached to the backbone was calculated according to the integration of peaks c, b', and b. As mentioned in the main text, peak c corresponds to the hydrolyzed DBA units and as shown in the above figure, the integration of peak c equals peak b'. The percentage of hydrolyzed DBA cross-linker: $n(\text{hydrolyzed DBA}) = \frac{\int b'}{\int b+b'} \times 100\% = \frac{\int c}{\int b+b'} \times 100\%$. Therefore, $n(\text{hydrolyzed DBA}) = \frac{100.64}{174.52} \times 100\% = 57.6\%$. If both sides of the DBA were hydrolyzed, then the percentage of attached DBA is 42% ($1 - 57.6\% = 42.4\%$). If only one side of the DBA was hydrolyzed, then the percentage of attached DBA is 84% ($42\% \times 2 = 84\%$). Therefore, the percentage of DBA cross-linker attached to the polymer backbone is between 84% and 42%.

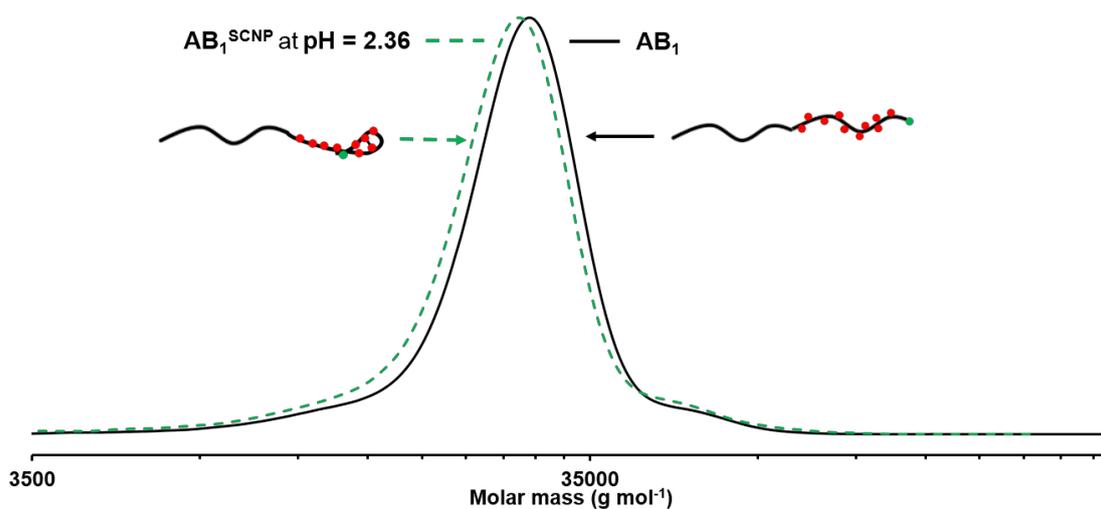


Figure S16. SEC chromatograms (RI traces) obtained in DMF for: \mathbf{AB}_1 ($M_{p,\text{SEC}} = 27200 \text{ g mol}^{-1}$, $M_{n,\text{SEC}} = 23700 \text{ g mol}^{-1}$, $D = 1.14$) and $\mathbf{AB}_1^{\text{SCNP}}$ at $\text{pH} = 2.36$ ($M_{p,\text{SEC}} = 26100 \text{ g mol}^{-1}$, $M_{n,\text{SEC}} = 22000 \text{ g mol}^{-1}$, $D = 1.19$, $\langle G \rangle = 0.96$).

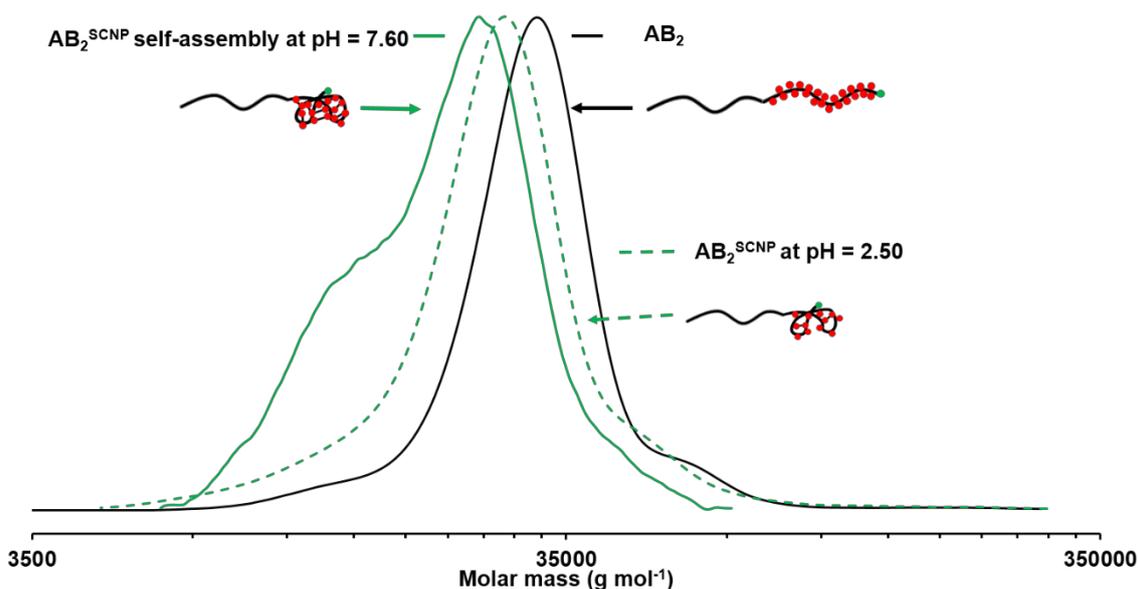


Figure S17. SEC chromatograms (RI traces) obtained in DMF for: AB_2 , $\text{AB}_2^{\text{SCNP}}$ self-assembly at pH = 7.60, and $\text{AB}_2^{\text{SCNP}}$ at pH = 2.50. These samples were run in the same calibration which is different to those in **Figure 1** due to the recalibration of the SEC system when the analysis was carried out.

Table S3. Characterization of the linear copolymers, SCNPs at different conditions by DMF-SEC*.

sample	Composition	$M_{n,\text{th}}^a$ g mol ⁻¹	$M_{p,\text{SEC}}^b$ g mol ⁻¹	$M_{n,\text{SEC}}^b$ g mol ⁻¹	D^b	$\langle G \rangle^c$
AB_1	PNAM ₁₀₀ - <i>b</i> -P(NAM ₈₀ - <i>stat</i> -GLA ₂₀)	28600	28600	25100	1.13	-
$\text{AB}_1^{\text{SCNP}}$ with addition of glucose	PNAM ₁₀₀ - <i>b</i> -[P(NAM ₈₀ - <i>stat</i> -GLA ₂₀)] ^{SCNP}	-	29100	27000	1.08	-
AB_2	PNAM ₁₀₀ - <i>b</i> -P(NAM ₂₀ - <i>stat</i> -GLA ₈₀)	28900	30800	28200	1.16	-
$\text{AB}_2^{\text{SCNP}}$ self-assembly at pH 7.60	PNAM ₁₀₀ - <i>b</i> -[P(NAM ₂₀ - <i>stat</i> -GLA ₈₀)] ^{SCNP}	-	24100	18800	1.17	0.78
$\text{AB}_2^{\text{SCNP}}$ at pH 2.50	PNAM ₁₀₀ - <i>b</i> -[P(NAM ₂₀ - <i>stat</i> -GLA ₈₀)] ^{SCNP}	-	27100	23200	1.16	0.88
$\text{AB}_2^{\text{SCNP}}$ with addition of glucose	PNAM ₁₀₀ - <i>b</i> -[P(NAM ₂₀ - <i>stat</i> -GLA ₈₀)] ^{SCNP}	-	29900	26500	1.14	-
$\text{AB}_2^{\text{SCNP}}$ self-assembly at pH 7.60 with addition of glucose	PNAM ₁₀₀ - <i>b</i> -[P(NAM ₂₀ - <i>stat</i> -GLA ₈₀)] ^{SCNP}	-	29600	28000	1.12	-

* These samples were run in the same calibration which is different to those in **Figure 1** due to the recalibration of the SEC system when the analysis was carried out.

^a $M_{n,\text{th}} = [M]_0 \times p \times M_M / [\text{CTA}]_0 + M_{\text{CTA}}$, p is the monomer conversion determined by ¹H NMR.

^b Determined by SEC in DMF with PMMA used as molecular weight standards, M_p represents the maximum peak value of the size-exclusion chromatogram.

^c Compaction parameter $\langle G \rangle = M_{p,\text{SCNP}} / M_{p,\text{linear}}$, the molecular weight variation caused by the cross-linking reaction (*e.g.* the attached DBA units) was not taken into account.

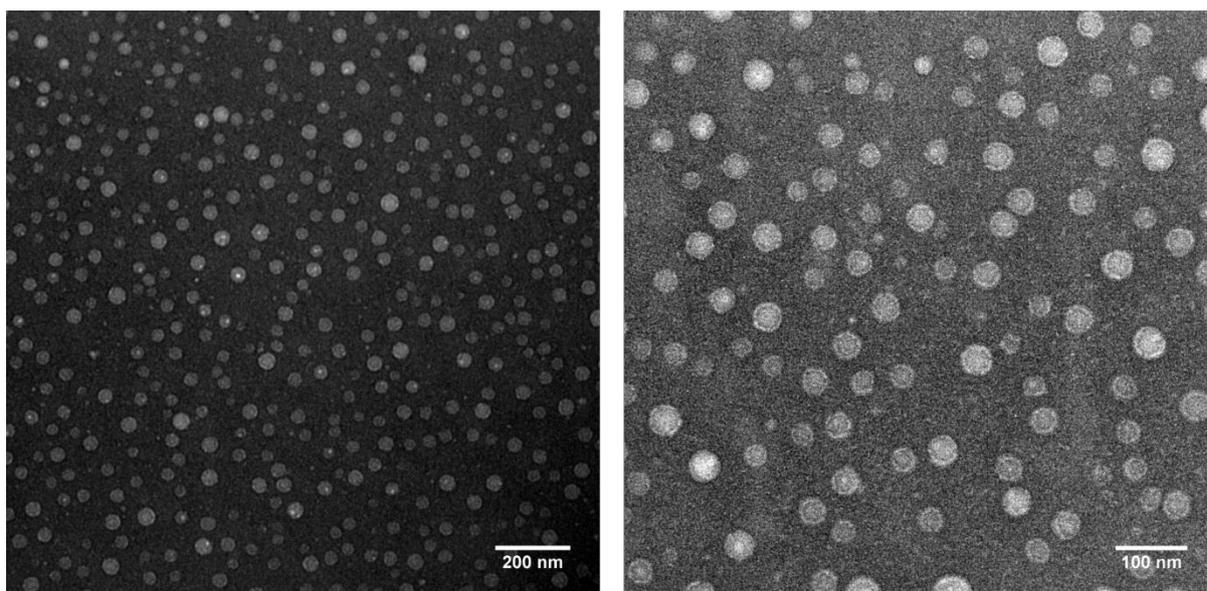


Figure S18. TEM image of micellar structures formed by the self-assembly of $\text{AB}_2^{\text{SCNP}}$ at $\text{pH} = 7.60$ (images taken from different areas of the grid compared to **Figure 5**). Samples were diluted by 10 times of the self-assembly sample using deionized water.

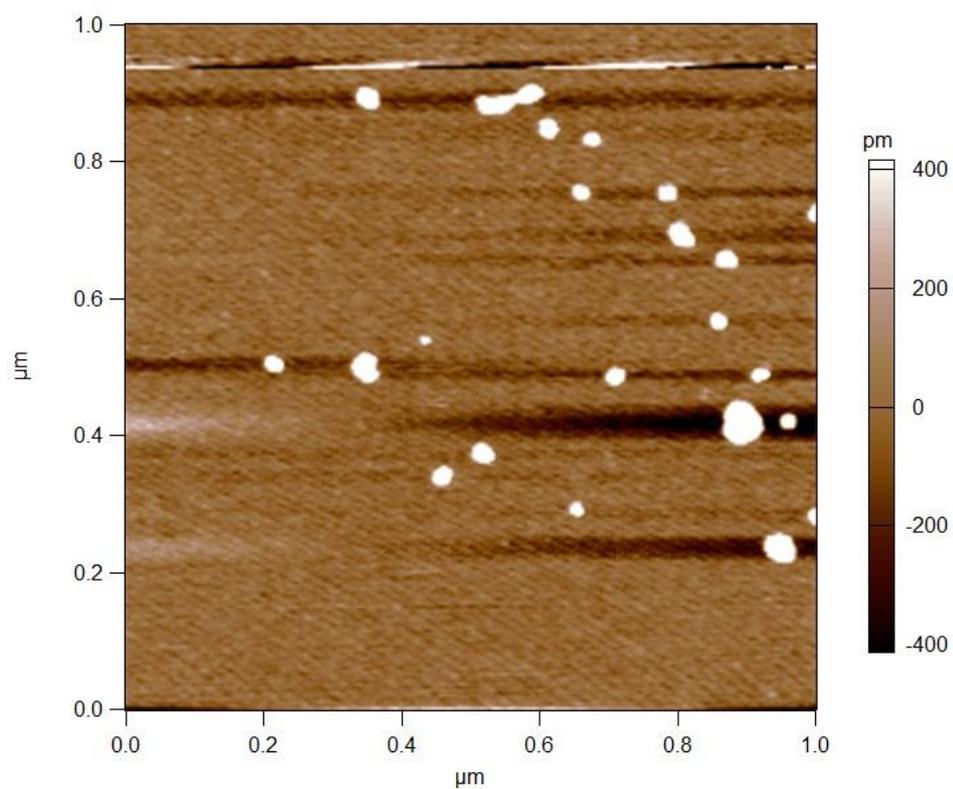


Figure S19. AFM topography image of nanoparticles formed by the self-assembly of $\text{AB}_2^{\text{SCNP}}$. Samples were diluted by 10 times of the self-assembly sample using deionized water and 30 μL of solution was drop-deposited onto freshly cleaved mica disc and then freeze dried to remove the water.

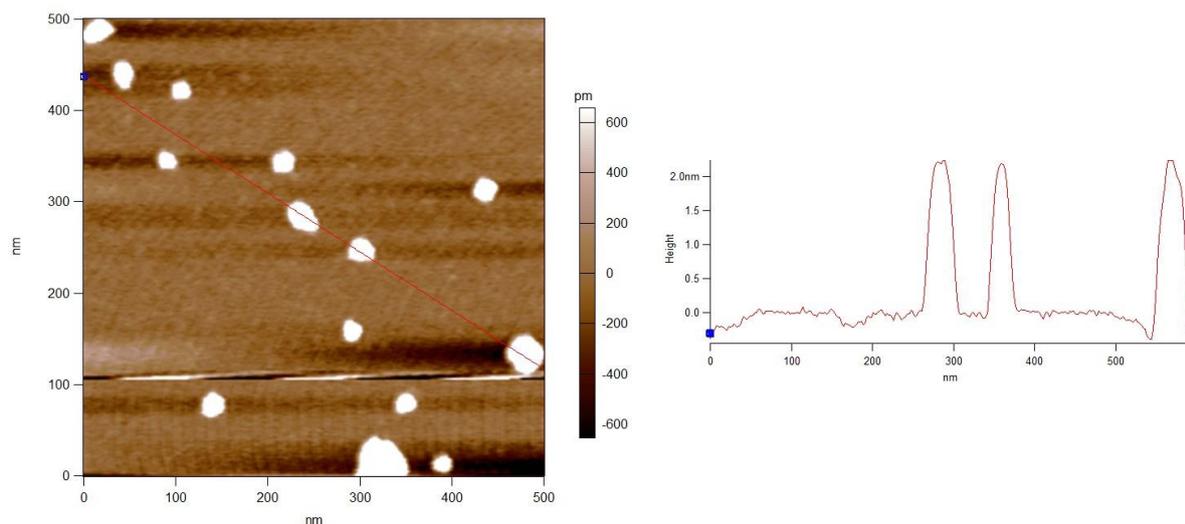


Figure S20. AFM topography image of nanoparticles formed by the self-assembly of $\text{AB}_2^{\text{SCNP}}$. The red line in the topography image shows the analyzed particles. Samples were diluted by 10 times of the self-assembly sample using deionized water and 30 μL of solution was drop-deposited onto freshly cleaved mica disc and then freeze dried to remove the water.

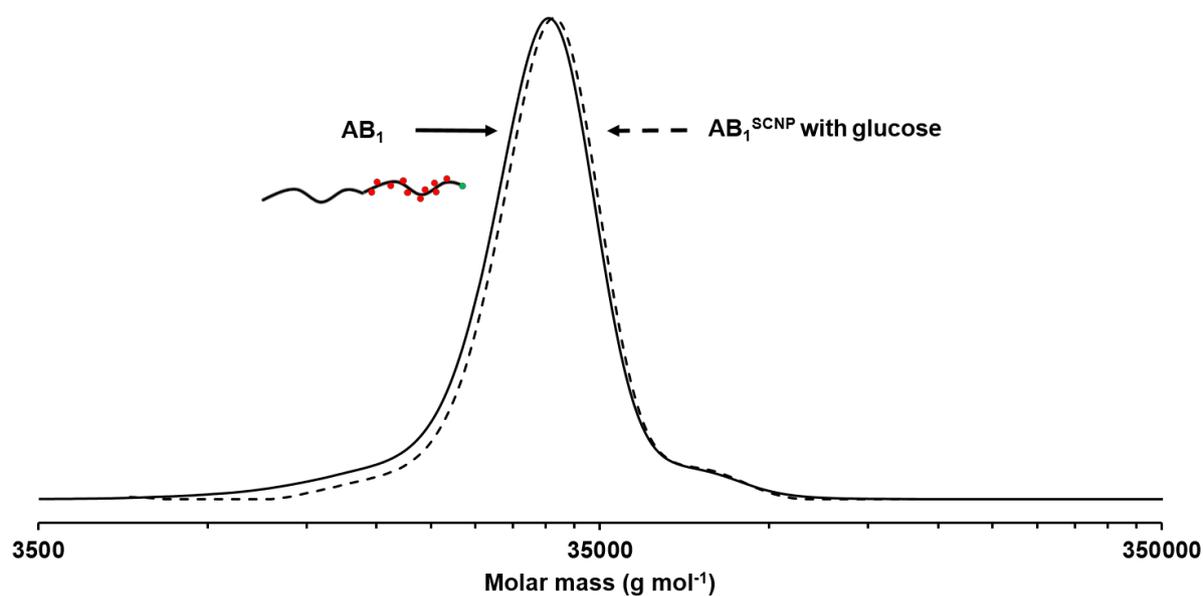


Figure S21. SEC chromatograms (RI traces) obtained in DMF for: AB_1 (solid) and $\text{AB}_1^{\text{SCNP}}$ with addition of glucose at $\text{pH} = 10.02$ (dash). These samples were run in the same calibration which is different to those in **Figure 1** due to the recalibration of the SEC system when the analysis was carried out.

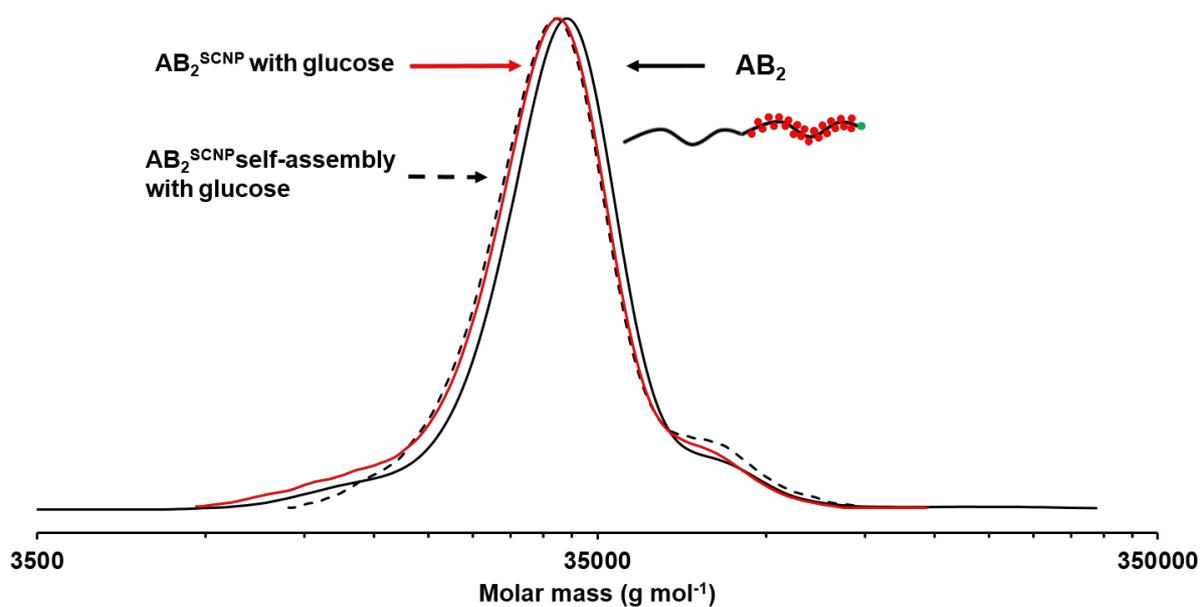


Figure S22. SEC chromatograms (RI traces) obtained in DMF for: **AB₂^{SCNP}** self-assembly with addition of glucose at pH = 7.60 (black dash), **AB₂^{SCNP}** with addition of glucose at pH = 10.20, and linear copolymer **AB₂**. These samples were run in the same calibration which is different to those in **Figure 1** due to the recalibration of the SEC system when the analysis was carried out.

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