A general strategy for degradable single-chain nanoparticles via cross-linker mediated chain collapse of radical copolymers

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Electronic Supplementary Information

Contents:

1. Materials and Methods

All reagents and solvents were purchased from Sigma Aldrich and used as received. 2-Methylene-1,3-dioxepane (MDO) was prepared as previously reported.[1] Dry THF was prepared using a Glass Contour - Solvent Purification System. Deionized (DI) water was prepared using an AquaMAX-Basic 321 water purification system. ^{1}H NMR spectra were recorded on a Bruker 400 Ultra Shield spectrometer. Dynamic light scattering (DLS) was conducted on a Malvern Zetasizer Nanoseries at 25 °C. Size exclusion chromatography (SEC) was conducted on a Viscotek TDAmax consisting of a GPCmax integrated solvent and sample delivery module, a TDA 302 Triple Detector Array, and OmniSEC software. 2 x PLgel 5 μm Mixed-C (200-2,000,000) columns were applied in sequence for separation. THF was used as the eluent at 1.0 mL/ min with column and detector temperature at 30 °C, molecular weight values were determined against polystyrene standards.

2. Experimental

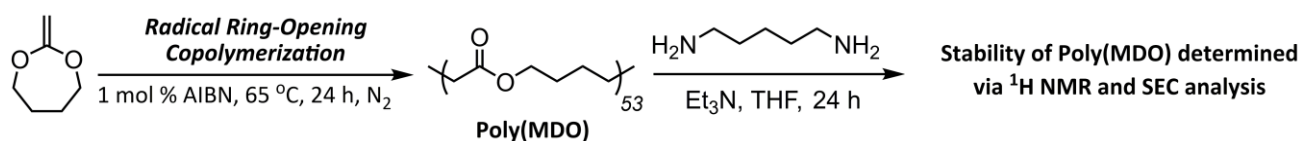### 2.1 Synthesis of Poly(MDO)[2] and Treatment with Diamine

Figure S1. Synthesis of **Poly(MDO)** and subsequent control experiment to determine stability in the presence of primary amine.

Poly(MDO) was prepared and treated with 1,5-diaminopentane and Et$_3$N (under identical conditions to (**DSCNP6**) confirming that main-chain ester hydrolysis or aminolysis does not occur in the presence of primary amines (Figure S1). A 15 mL Schlenk tube was fitted with a magnetic stirrer bar, rinsed with Et$_3$N and dried under high vacuum. 2-Methylene-1,3-dioxepane (10.0 g, 87.61 mmol, 100 eq) and azobisisobutyronitrile (143.8 mg, 0.87 mmol, 1 eq) were added to the Schenk Tube. The reaction mixture was degassed via five freeze-pump-thaw cycles and backfilled with N$_2$ before heating to 65 °C and stirring at 500 rpm for 24 h. After this time the reaction was quenched by rapid cooling, and the polymer isolated as a white waxy solid (8.60 g, 86 % yield) by three precipitations from CHCl$_3$ into hexane. **Poly(MDO)** was analysed via ^{1}H NMR (Fig. S2a) and SEC (Fig. S3). Subsequently, a 250 mL round bottom flask was fitted with a magnetic stirrer bar, thoroughly rinsed with acetone and dried at 100 °C overnight. **Poly(MDO)** (100 mg), 1,5-diaminopentane (16.75 mg), Et$_3$N (40 μL) and dry THF (100 mL) were transferred into the round bottom flask and the solution obtained was stirred at room temperature for 24 h. After evaporation under reduced pressure at 28 °C, the resulting polymer dried under high vacuum at room temperature. Sample obtained was analysed via ^{1}H NMR (Fig. S2b) and SEC (Fig. S3).
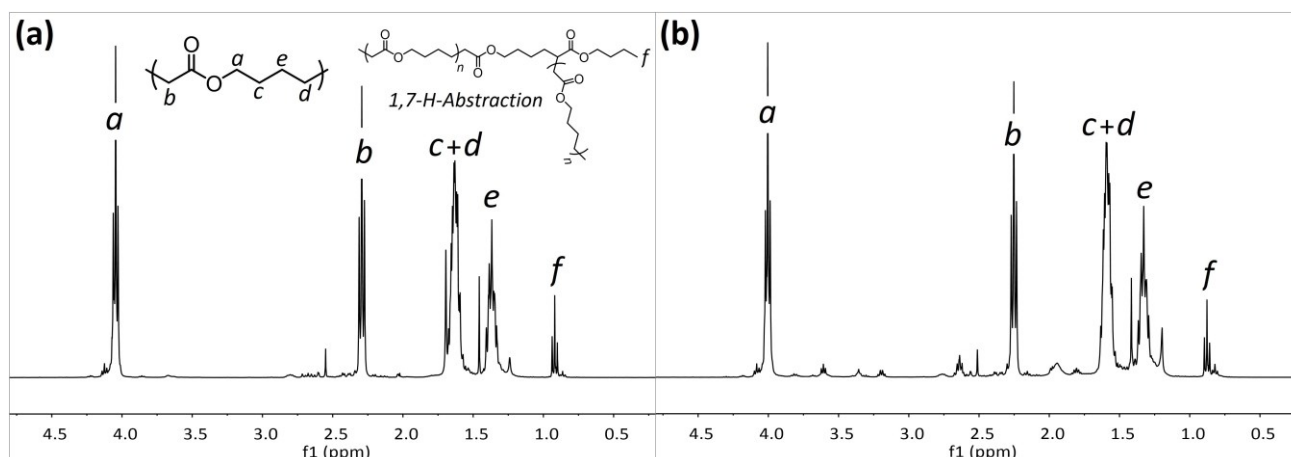
Figure S2. ^1H NMR (CDCl$_3$) spectra of (a) **Poly(MDO)** and (b) **Poly(MDO)** treated with 1,5-diaminopentane and Et$_3$N in THF.
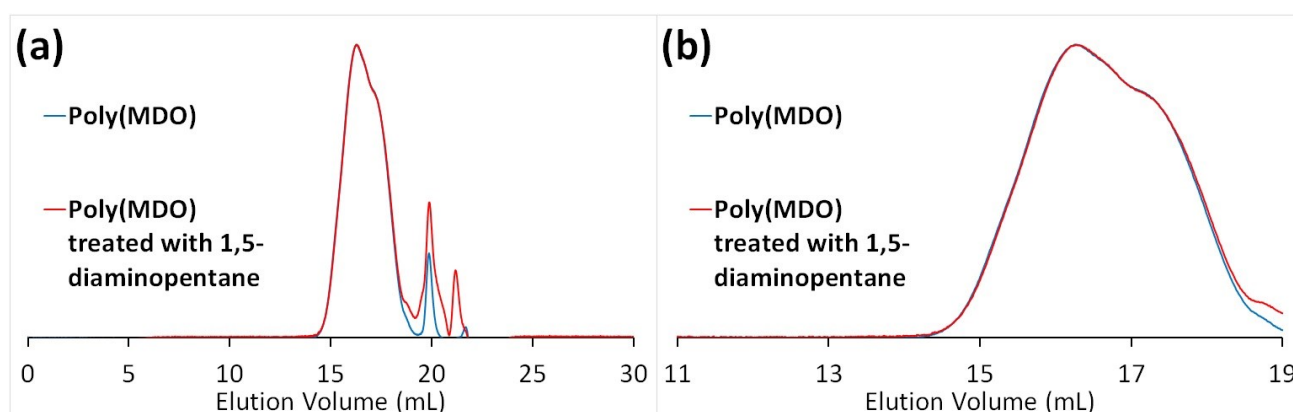

Figure S3. (a) SEC traces of **Poly(MDO)** (blue line) and Poly(MDO) after treatment with 1,5-diaminopentane and Et$_3$N in THF (red line). Peak at 21.0 – 23.0 min = system peak indicating the transition from entropy-dominated size-based separation to enthalpy-dominated interaction chromatography. (b) Expansion of polymer traces to clearly observe no degradation.

Control experiment SEC analysis

Poly(MDO)
M_n = 6,090 Da
M_w = 20,135 Da
M_p = 17,725 Da
$Đ_M$ = 3.31

Poly(MDO) treated with 1,5-diaminopentane
M_n = 5,635 Da
M_w = 19,465 Da
M_p = 17,315 Da
$Đ_M$ = 3.45

2.2 Synthesis of Poly(MDO-*c*-NHSMA)

A 15 mL Schlenk tube was fitted with a magnetic stirrer bar, rinsed with Et$_3$N and dried under high vacuum. 2-Methylene-1,3-dioxepane (3.88 g, 34 mmol, 85 eq), methacrylic acid *N*-hydroxysuccinimide ester (1.10 g, 6 mmol, 15 eq), azobisisobutyronitrile (6.6 mg, 40 µmol, 0.1 eq) and 1,4-dioxane (1.66 g, 25 wt %) were added to the Schenk Tube. A small aliquot (*time = 0 h*) was then extracted for monomer conversion determined by ^1H NMR (CDCl$_3$ passed over Na$_2$CO$_3$) spectroscopy. The reaction mixture was degassed via three freeze-pump-thaw cycles and backfilled with N$_2$ before heating to 70 °C and stirring at 500 rpm for 6 h. After this time the reaction was quenched by rapid cooling, and a second aliquot (*time = 6 h*) extracted for monomer conversion analysis. Monomer conversions were determined by comparing the 1,4-dioxane protons present at δ 3.71 ppm with the residual MDO vinyl protons at δ 3.42 ppm and the residual NHSMA vinyl protons at δ 6.37 and 5.84 ppm. Quenching the reaction before complete NHSMA consumption eliminates the formation of homoMDO polymer chains. The polymer was isolated as a white solid (1.50 g, 83 % yield) by three precipitations from THF into hexane.

2.3 Synthesis of Degradable Single-Chain Nanoparticles (DSCNP1-6)

The reagents and quantities for each degradable single-chain nanoparticle (**DSCNP1-6**) preparation are provided in Table S1. Six 250 mL round bottom flasks were fitted with magnetic stirrer bars, thoroughly rinsed with acetone and dried at 100 °C overnight. **Poly(MDO-*c*-NHSMA)** (100 mg) was transferred into each round bottom flask followed by the stated quantity of dry THF, each solution was left to stir at room temperature for 1 h to ensure complete polymer dissolution.

Separately, a stock solution (1 mg/ mL) of diamine cross-linker was prepared by adding 50 mg of 1,5-diaminopentane to 50 mL of dry THF. The stated quantity of stock solution was added to each round bottom flask, and the solutions obtained allowed to stir at room temperature for 30 mins. After this time Et₃N (40 μL) was added to each of the six solutions. Amide bond formation was allowed to proceed for 24 h at room temperature. After this time precipitation of *N*-hydroxysuccinimide was observed in **DSCNP5** and **DSCNP6**, both of these solutions were filtered to remove the precipitated by-product. All six reactions were then separated into two portions (40 mL and 60 mL). The 40 mL portion was reduced under rotary evaporation to approximately 4 mL (10 mg/ mL polymer conc.) before accurate dilution with dry THF to 8 mL (5 mg/ mL polymer conc.) for SEC analysis. The second 60 mL portion was directly analysed by DLS analysis, then extensively evaporated to dryness before ^{1}H NMR analysis (20 mg in 0.4 mL CDCl₃), leaving 40 mg available for degradation study.

Sample ID	Poly(MDO-*c*-NHSMA)	NHSMA Quantity	Target Cross-Linking (%)	1,5-DAP (m_r = 102.18 Da)	Et₃N (m_r = 101.19 Da)	Dry THF	1,5-DAP Solution (1 mg/ mL)
DSCNP1			0	-		100 mL	-
DSCNP2			10	**0.05 eq** 1.68 mg 0.0164 mmol		98.3 mL	1.7 mL
DSCNP3	**100 mg** 48 mol % NHSMA 60 wt % NHSMA	**1 eq** 60 mg 0.328 mmol	25	**0.125 eq** 4.20 mg 0.0410 mmol	**1 eq** 33.19 mg/ 46 μL 0.328 mmol	95.8 mL	4.2 mL
DSCNP4			50	**0.250 eq** 8.38 mg 0.0820 mmol		91.6 mL	8.4 mL
DSCNP5			75	**0.375 eq** 12.57 mg 0.1230 mmol		87.4 mL	12.6 mL
DSCNP6			100	**0.50 eq** 16.76 mg 0.1640 mmol		83.2 mL	16.8 mL

Table S1. Reagents and quantities employed during synthesis of degradable single-chain nanoparticles (**DSCNP1-6**). 1,5-DAP = 1,5-diaminopenate, Et₃N = trimethylamine.

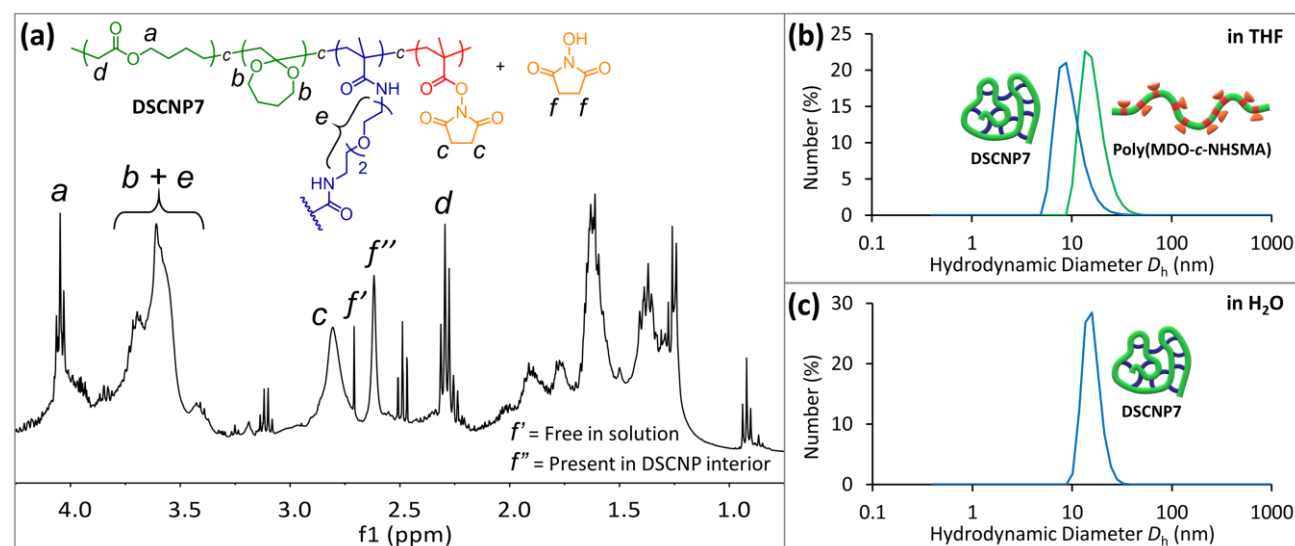## 2.4 Synthesis of Water Soluble Degradable Single-Chain Nanoparticle (DSCNP7)

Figure S4. (a) ^{1}H NMR (CDCl₃) spectra of **DSCNP7**. (b) DLS (number) analysis of **Poly(MDO-*c*-NHSMA)** and **DSCNP7** in THF (3 mg/ mL at 25 °C). (c) DLS analysis of **DSCNP7** in H₂O (3 mg/ mL at 25 °C).

The hydrophilic cross-linker 1,8-diamino-3,6-dioxaoctane (DOA) was utilized to prepare the water-soluble **DSCNP7** targeted a cross-linking degree of 100 % (conditions detailed below). A reaction duration of 48 h was required to reach a 69 % calculated degree of cross-linking, determined by ^{1}H NMR (Fig. S4a). This increase in cross-linking duration is likely due to the additional bulkiness of DOA. SEC (Table 1 in manuscript) confirmed an apparent decrease in molecular weight for **DCSNP7** (M_p = 22,400 Da). Dynamic light scattering (DLS) analysis in THF (Fig. S4b) further confirmed a decrease in volume when comparing the linear precursor **Poly(MDO-*c*-NHSMA)** (D_h = 16.6 nm) with **DSCNP7** (D_h = 9.8 nm). This difference in D_h (6.8 nm) corresponds to a chain compaction of 41 %. Making a direct comparison in H₂O isn't possible as **Poly(MDO-*c*-NHSMA)** is not water soluble. However, after dispersion in H₂O via solvent evaporation DLS analysis of

DSCNP7 (Fig. S4c) displayed a D_h = 15.8 nm. This nanoparticle solution was very stable with no changes in DLS after one month. The size difference observed for **DCNP7** in THF (D_h = 9.8 nm) and H_2O (D_h = 15.8 nm) could be due to variations in solvation within the nanoparticle interior, or a result of **DSCNP7** aggregation in H_2O. **DSCNP7** successfully underwent chemical degradation into branched oligomers (M_p = 710 Da) via main-chain ester hydrolysis (Table 1 in manuscript).

Detailed conditions for the synthesis of **DSCNP7** are as follows:

Poly(MDO-*c*-NHSMA) (100 mg) was transferred into a 250 mL round bottom flask fitted with a magnetic stirrer bar (previously rinsed with acetone and dried at 100 °C overnight). Dry THF (76.6 mL) was added and the solution left to stir at room temperature for 1 h to ensure complete polymer dissolution. 1,8-Diamino-3,6-dioxaoctane (0.5 eq relative to NHSMA content, 0.1640 mmol, 23.4 mg) in 23.4 mL of dry THF was added, and the solutions obtained allowed to stir at room temperature for 30 mins. After this time Et_3N (40 µL) was added and amide bond formation was allowed to proceed for 48 h at room temperature. After this time, samples were prepared for ^{1}H NMR analysis (20 mg in 0.4 mL CDCl$_3$), SEC analysis (5 mg/ mL in THF), DLS analysis (3 mg/ mL in THF and H_2O) and degradation study.

2.5 Preparation of DSCNP7 Aqueous Solution

DSCNP7 (20 mg, obtained after NMR sample evaporation to dryness) was transferred into a 25 mL round bottom flask fitted with a magnetic stirrer bar. Acetone (5 mL) was added and this solution was stirred for 15 mins, which resulted in complete nanoparticle dissolution. H_2O (10 mL) was then added dropwise over 30 mins while stirring. The obtained solution was left open to the atmosphere and was stirred at room temperature for 24 h to allow acetone evaporation. After this time, the solution obtained was analysed directly via DLS (3 mg/ mL at 25 °C).

2.6 Degradation of Poly(MDO-*c*-NHSMA) and DSCNP1-7

The degradation of **Poly(MDO-*c*-NHSMA)** and **DSCNP1-7** was performed under accelerated alkali conditions. Each sample (40 mg) was transferred into a small vial fitted with a magnetic stirrer bar. THF (2 mL) was added, followed by KOH (80 mg) in H_2O (2 mL). The obtained solutions were stirred at room temperature for 48 h, to ensure complete main-chain ester hydrolysis. After this time the solutions were acidified with conc. $HCl_{(aq)}$ aliquots and subsequently evaporated to dryness. CHCl$_3$ (5 mL) was then added and the solutions stirred for 2 h, after this time KCl precipitated was removed by filtration and the degraded oligomers obtained after evaporation to dryness. Samples were prepared for SEC analysis (5 mg/ mL in THF).

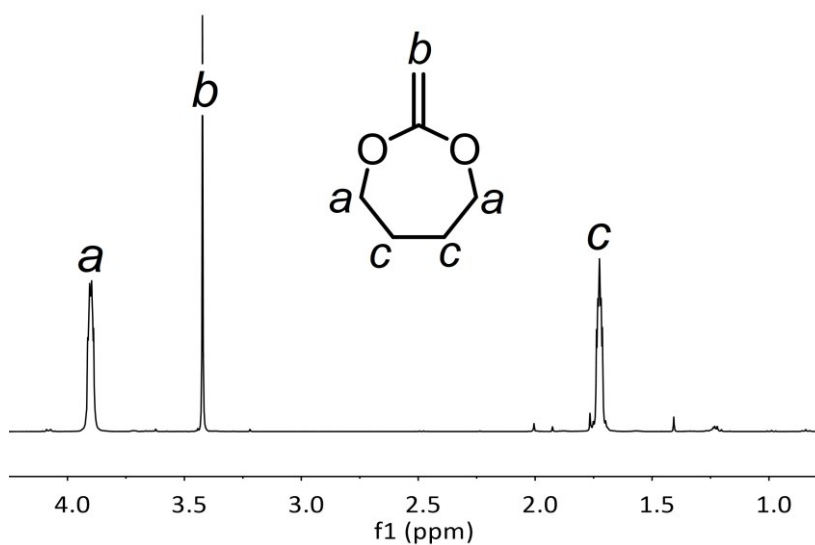# 3. ^{1}H NMR Spectroscopy (MDO, Poly(MDO-*c*-NHMSA) and DSCNP6)

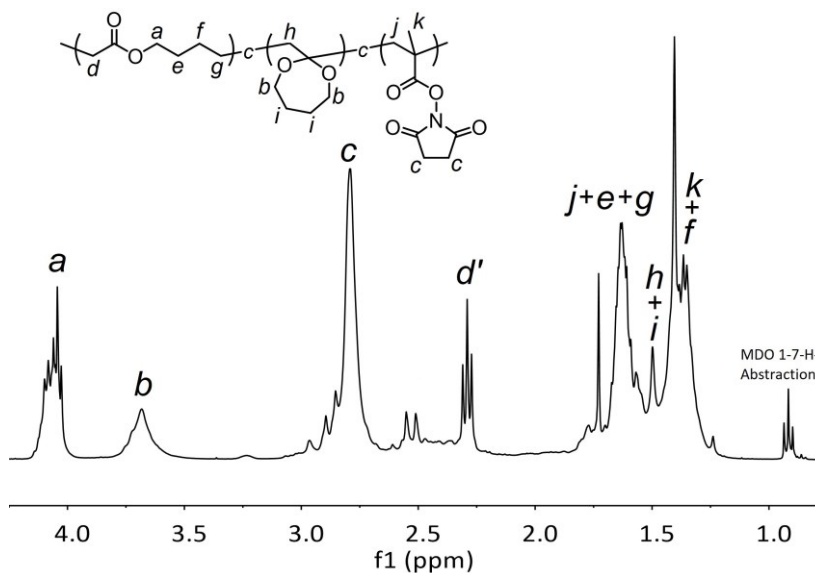**Figure S5.** ^{1}H NMR (CDCl$_3$) spectra of 2-methylene-1,3-dioxepane (**MDO**).

Figure S6. ^{1}H NMR (CDCl$_3$) spectra of **Poly(MDO-*c*-NHSMA)**.
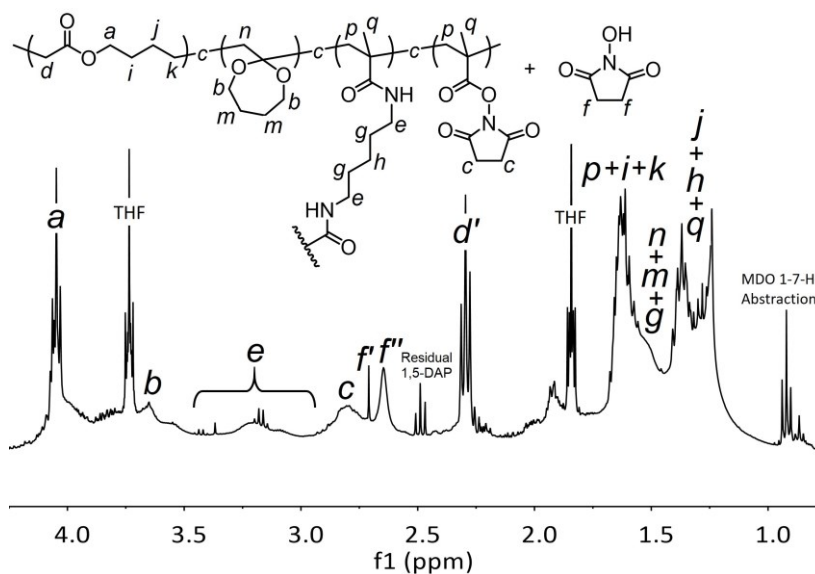

Figure S7. ^{1}H NMR (CDCl$_3$) spectra of **DSCNP6**.

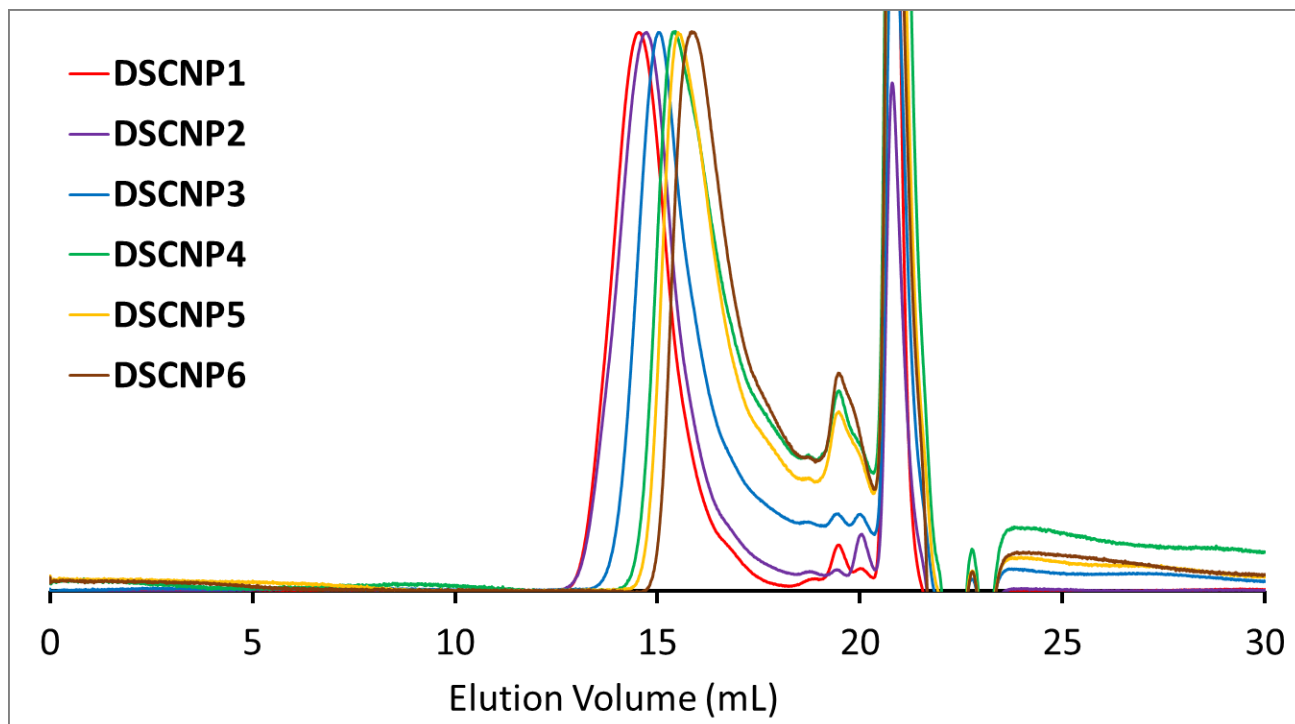## 4. Size Exclusion Chromatography (DSCNP1-6 and DSCNP1-6 After Hydrolysis)

Figure S8. SEC analysis (in THF) of **DSCNP1-6**. Peak at 21.0 – 23.0 min = system peak indicating the transition from entropy-dominated size-based separation to enthalpy-dominated interaction chromatography. In order to prevent inter-chain cross-linking in the dry state **DSCNP1-6** samples are not completely evaporated to dryness, therefore additional peaks are present in these traces. Peak at 19.5 – 20 min = residual 1,5-diaminopentane and Et$_3$N. Potential nanoparticle column interactions are observed between 18.0 – 19.5 min, which increase at higher degrees of cross-linking.
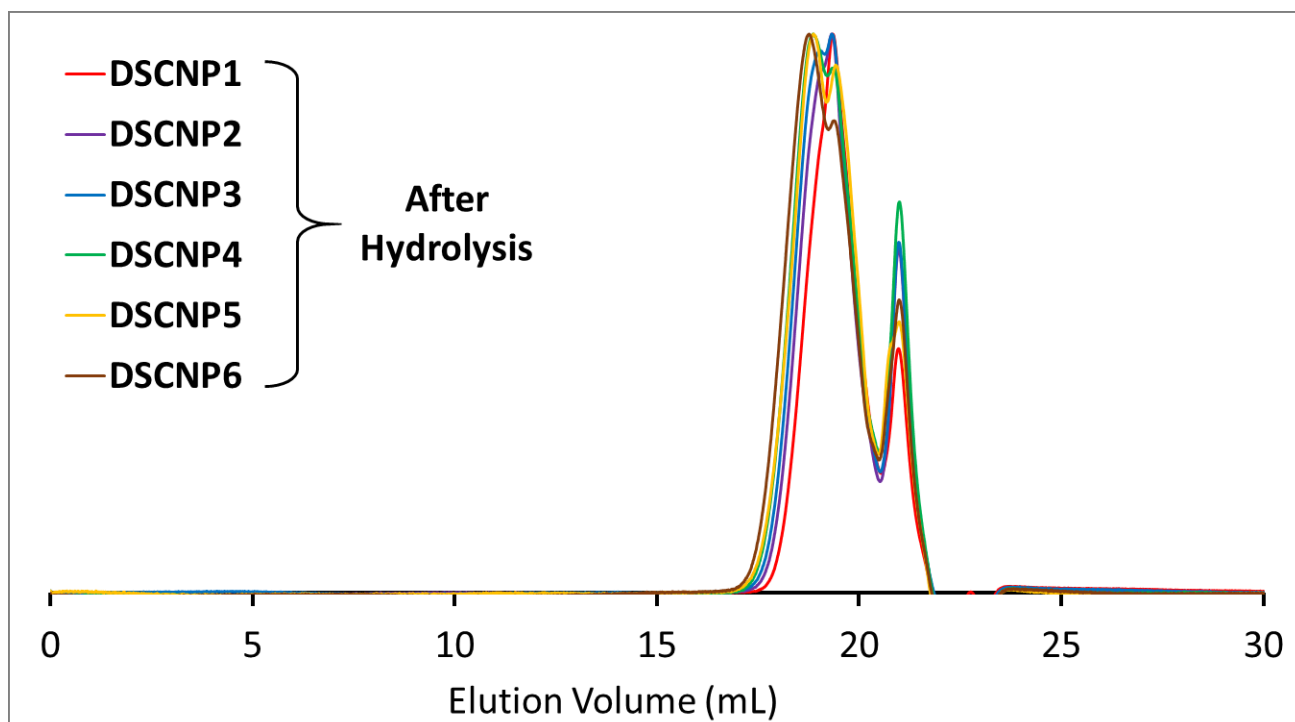

Figure S9. SEC analysis (in THF) of **DSCNP1-6** after main-chain ester hydrolysis. Peak at 21.0 – 23.0 min = system peak indicating the transition from entropy-dominated size-based separation to enthalpy-dominated interaction chromatography.

5. Dynamic Light Scattering Analysis (DSCNP1-6)

| Sample ID | Run | Hydrodynamic Diameter (D_h) | | | | PDI |
		Intensity (nm)	Volume (nm)	Number (nm)	Z-Average (nm)	
DSCNP1 0 % Cross-Linking	1	38.53	22.64	16.85	31.74	0.179
	2	38.66	21.86	15.69	32.20	0.195
	3	39.65	22.15	16.14	31.87	0.176
	4	39.85	21.90	16.06	31.86	0.180
	5	38.59	22.65	16.53	31.69	0.195
	Average	**39.06**	**22.24**	**16.25**	**31.87**	**0.185**
DSCNP2 9 % Cross-Linking	1	36.41	19.46	13.65	29.10	0.179
	2	36.52	19.11	13.30	29.07	0.188
	3	36.42	19.67	14.19	29.13	0.186
	4	36.98	19.03	13.07	29.27	0.185
	5	36.17	19.98	14.49	29.15	0.180
	Average	**36.50**	**19.45**	**13.74**	**29.14**	**0.184**
DSCNP3 23 % Cross-Linking	1	33.15	18.23	12.85	26.82	0.172
	2	33.22	18.55	13.57	27.05	0.184
	3	33.75	18.82	13.79	27.10	0.177
	4	33.20	18.91	13.98	26.94	0.170
	5	33.15	18.43	13.24	26.82	0.170
	Average	**33.29**	**18.59**	**13.49**	**26.95**	**0.175**
DSCNP4 45 % Cross-Linking	1	29.67	16.88	12.26	24.29	0.163
	2	29.70	17.13	12.60	24.27	0.164
	3	29.68	16.80	12.12	24.31	0.162
	4	29.87	16.78	12.22	24.30	0.166
	5	29.70	17.29	12.67	24.40	0.161
	Average	**29.72**	**16.98**	**12.37**	**24.31**	**0.163**
DSCNP5 63 % Cross-Linking	1	26.98	16.06	11.85	22.49	0.150
	2	26.49	16.62	12.42	22.37	0.143
	3	26.59	16.29	12.15	22.37	0.144
	4	27.22	16.23	12.15	22.64	0.161
	5	27.18	15.93	11.50	22.56	0.154
	Average	**26.89**	**16.23**	**12.01**	**22.49**	**0.150**
DSCNP6 80 % Cross-Linking	1	25.88	15.38	11.54	21.45	0.161
	2	26.40	14.86	10.88	21.44	0.169
	3	26.13	15.06	11.05	21.51	0.169
	4	25.94	15.39	11.61	21.67	0.174
	5	26.32	14.82	10.82	21.70	0.175
	Average	**26.13**	**15.10**	**11.18**	**21.55**	**0.170**

Table S2. Dynamic light scattering analysis of **DSCNP1-6** in THF (1 mg/ mL) at 25 °C.
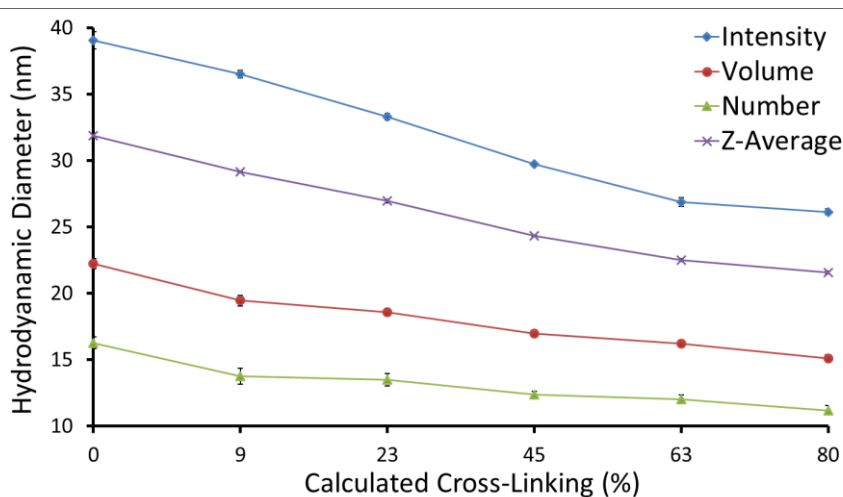

Figure S10. DLS overview of **DSCNP1-6** by intensity, volume, number and Z-Average.
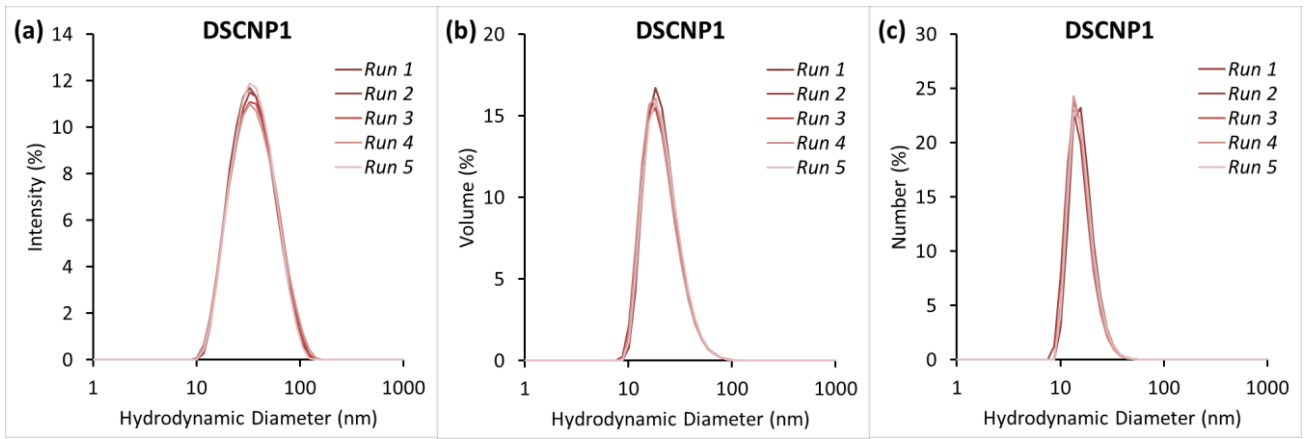
Figure S11. DLS analysis of **DSCNP1** by (a) intensity, (b) volume and (c) number.
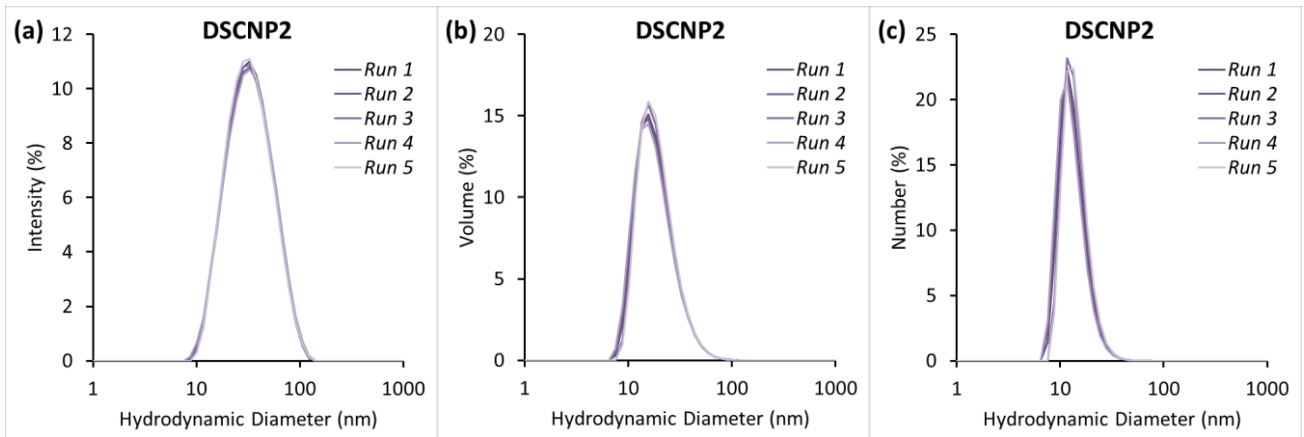

Figure S12. DLS analysis of **DSCNP2** by (a) intensity, (b) volume and (c) number.
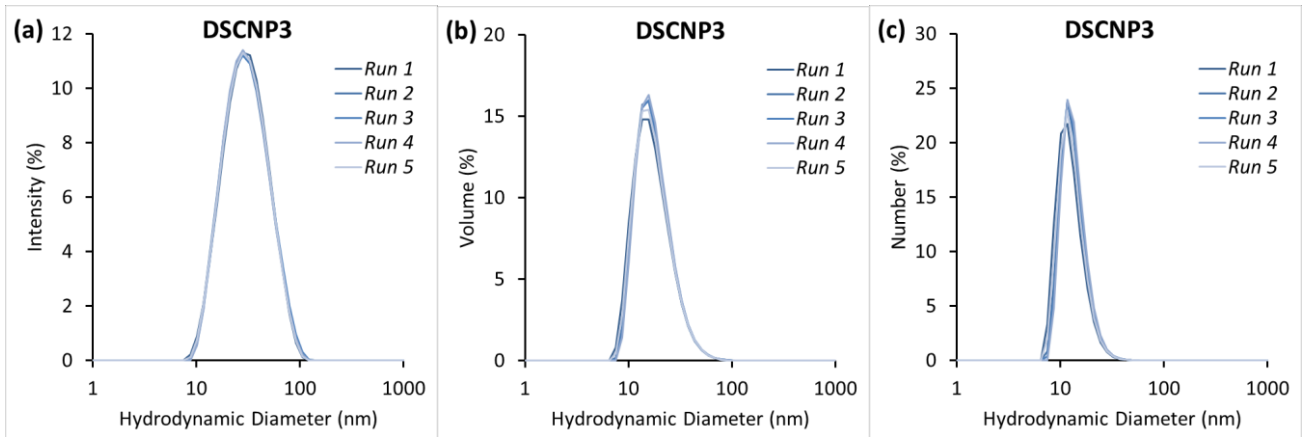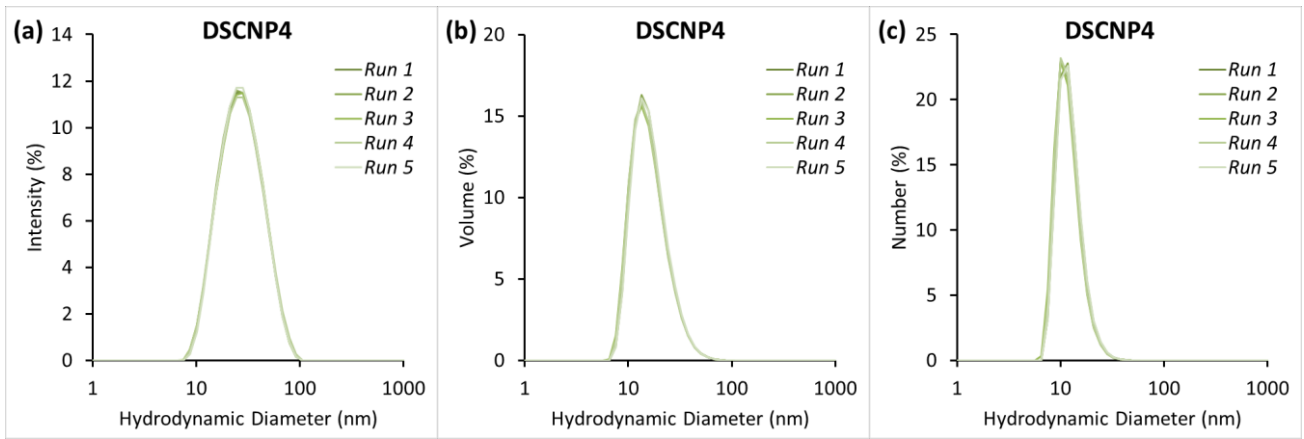

Figure S13. DLS analysis of **DSCNP3** by (a) intensity, (b) volume and (c) number.

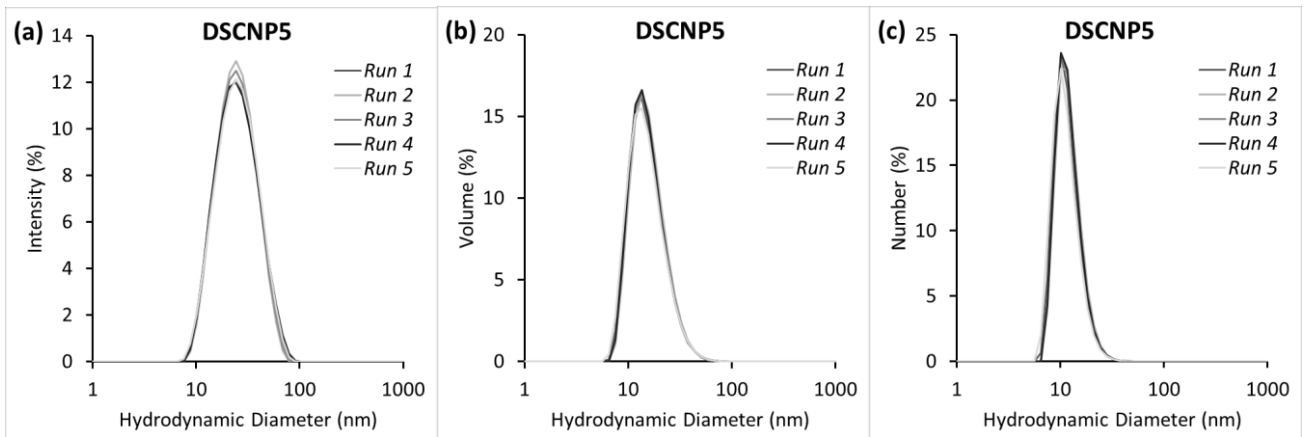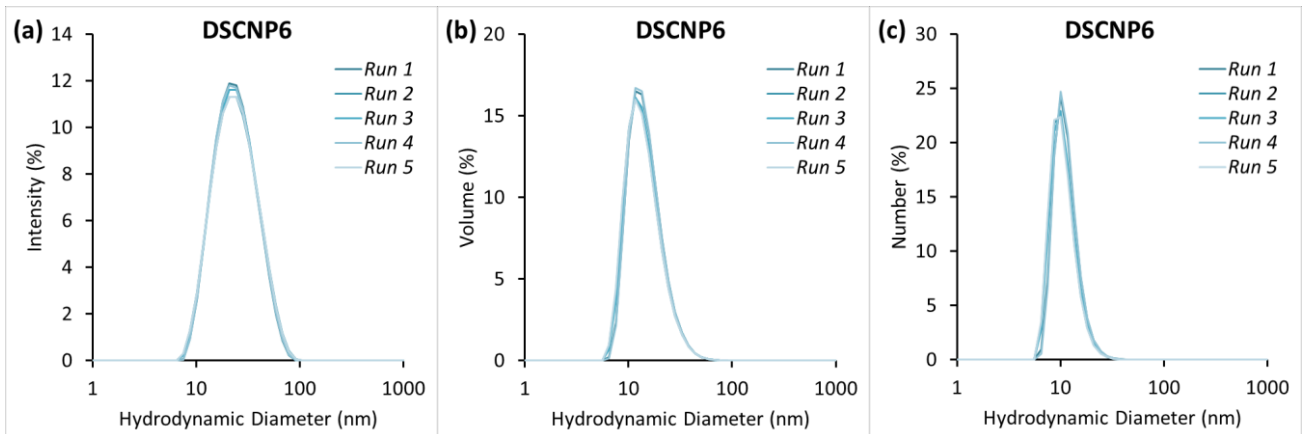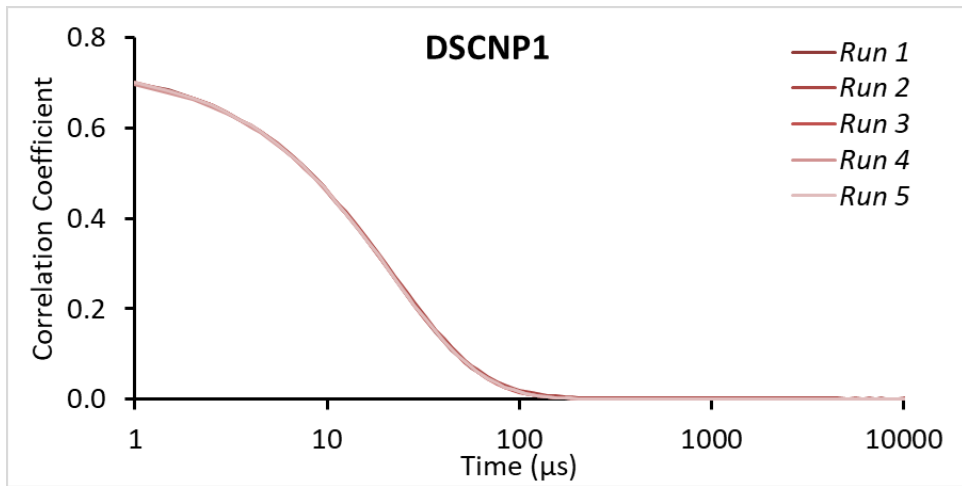**Figure S14.** DLS analysis of **DSCNP4** by (a) intensity, (b) volume and (c) number.

Figure S15. DLS analysis of **DSCNP5** by (a) intensity, (b) volume and (c) number.

Figure S16. DLS analysis of **DSCNP6** by (a) intensity, (b) volume and (c) number.

Figure. S17 DLS raw correlation data for **DSCNP1**.
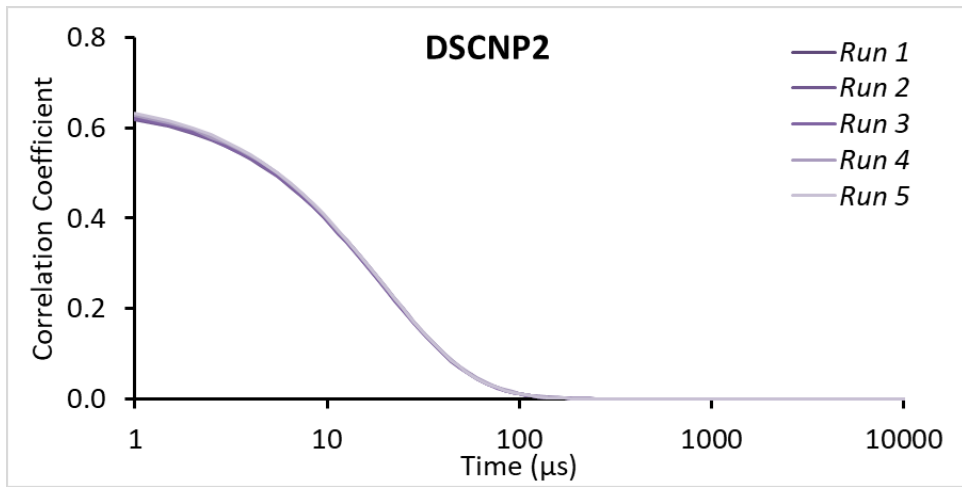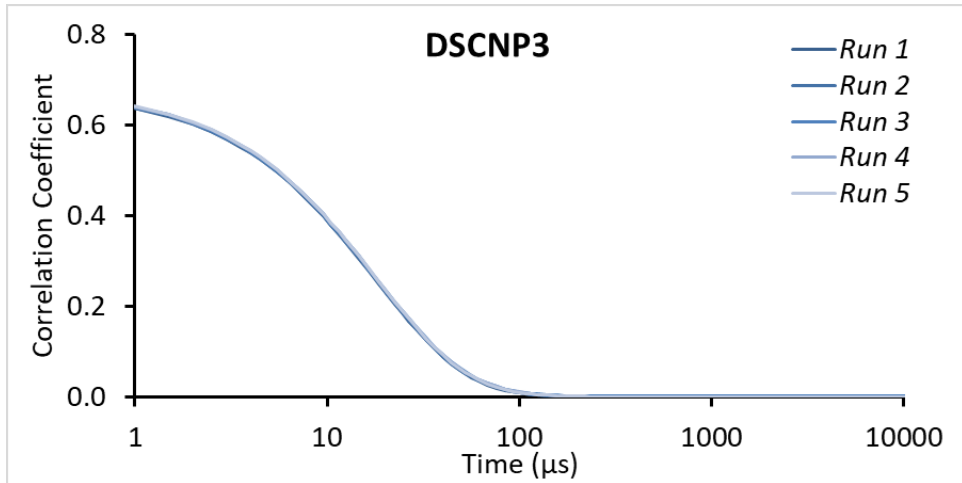

Figure. S18 DLS raw correlation data for **DSCNP2**.
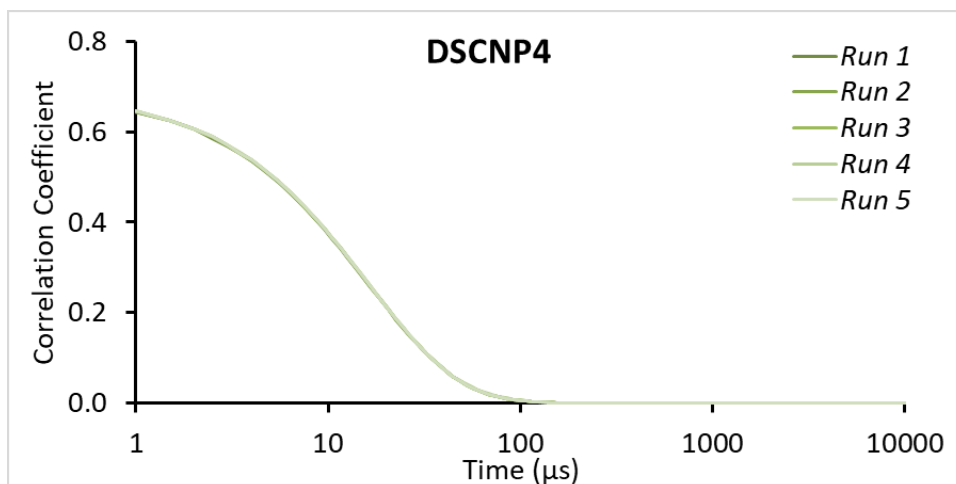

Figure. S19 DLS raw correlation data for **DSCNP3**.

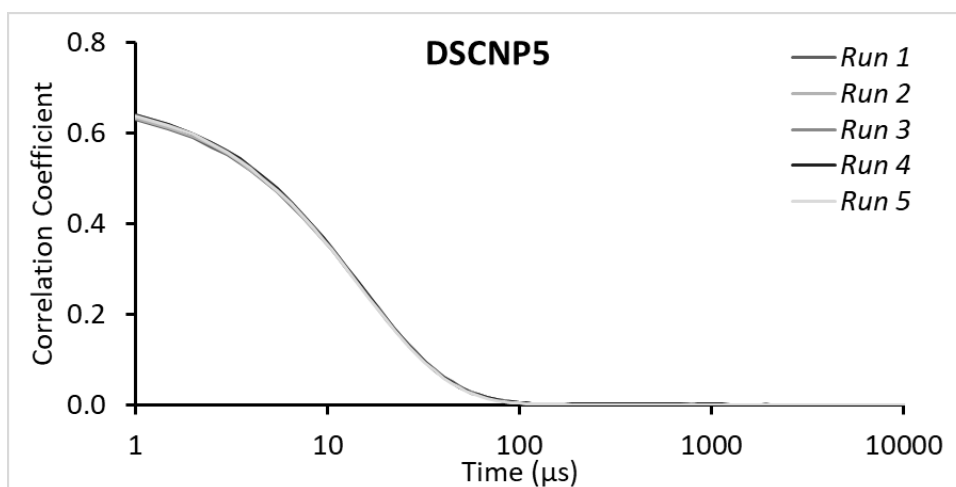**Figure. S20** DLS raw correlation data for **DSCNP4**.

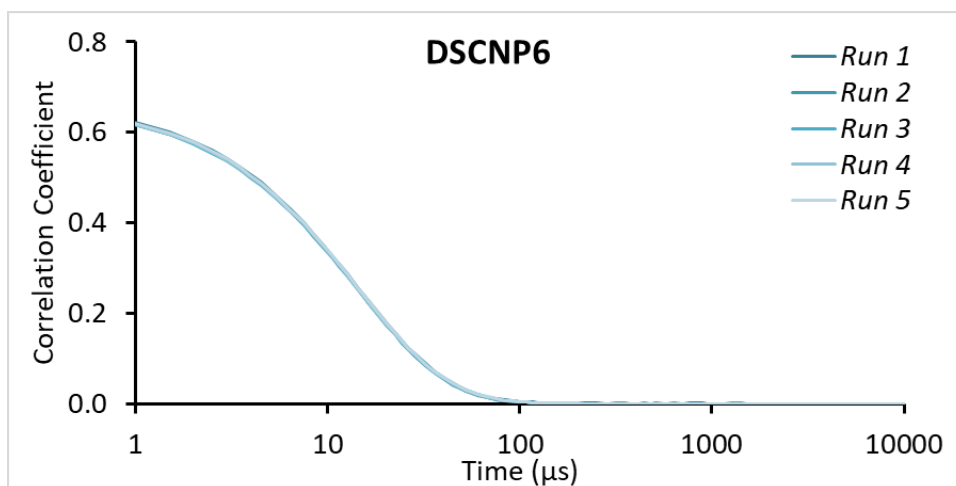**Figure. S21** DLS raw correlation data for **DSCNP5**.

Figure. S22 DLS raw correlation data for **DSCNP6**.

6. References

1   W. J. Bailey, Z. Ni and S. R. Wu, *J. Polym. Sci. Pol. Chem.*, 1982, **20**, 3021-3030.
2   S. Reddy Mothe, J. S. J. Tan, L. R. Chennamaneni, F. Aidil, Y. Su, H. C. Kang, F. C. H. Lim and P. Thoniyot, *Journal of Polymer Science*, 2020, **58**, 1728-1738.