Nanoparticle stability in biologically relevant media: influence of polymer architecture

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Electronic Supplementary Information

Content

Characterization

Gel permeation chromatography (GPC). Polymer molecular weight analysis was carried out by GPC performed on a Waters 1515 Isocratic HPLC equipped with two Styragel ® columns (HR4 and HR3, 300 mm ×7.8 mm) connected in series, a differential refractive index detector (Waters 2414) and a UV−visible detector (Waters 2489). HPLC grade tetrahydrofuran (THF) was used as the eluent, at a flow rate of 1 mL/min. Samples were filtered through a 0.22 μm PVDF syringe filter (Millipore) before injection. All measurements were carried out at 30 °C. Molecular weights are reported referenced to monodisperse polystyrene standards (Shodex SL-105). *Nuclear magnetic resonance spectroscopy (^{1}H NMR).* ^{1}H NMR spectra were recorded on a Bruker AV 400 MHz spectrometer in $CDCl_3$ referenced to $CHCl_3$ (7.26 ppm). *Fluorescence spectroscopy (FS).* FS was carried out on a Fluorolog-3 system (HORIBA Jobin Yvon Inc., NJ) at an excitation wavelength of 484 nm, with slit widths of 2.5 nm. Emission spectra were collected from 490 to 620 nm. *Dynamic light scattering (DLS).* DLS experiments were conducted on a Malvern Instruments Nano-ZS ZetaSizer equipped with a 4 mW He−Ne laser operating at 633 nm. All measurements were performed at 25 °C at a scattering angle of 173°. Autocorrelation functions of backscattered light were analyzed using DTS 6.12 software. Cumulants method was used to obtain hydrodynamic radius and polydispersity. Measurements were carried out three times with duration of 150 s each. Three measurements of the electrophoretic mobility for each sample were made using folded capillary cells (Malvern, DTS 1060). The ζ-potential was calculated using the Smoluchowski equation taking the average of the triplicates prepared under the same conditions with 100 runs each. *Transmission Electron Microscopy (TEM).* Bright-field TEM imaging was performed on an FEI Tecnai 12 TWIN Transmission Electron Microscope operated at an acceleration voltage of 100 kV. All TEM images were recorded by a SIS Megaview III wide-angle CCD camera. Copper grids were treated under plasma to render carbon films hydrophilic. Sample grids were prepared by placing a carbon-coated copper grid (Electron Microscopy, Hatfield, PA) onto a droplet of nanoparticle suspension (50 µL). After 5 min, the grid was washed under 8 drops of Nanopure water and placed onto a drop of a 2 wt% aqueous uranyl acetate solution for 20 s. Grids were then blotted with filter paper and allowed to dry at room temperature prior to imaging.

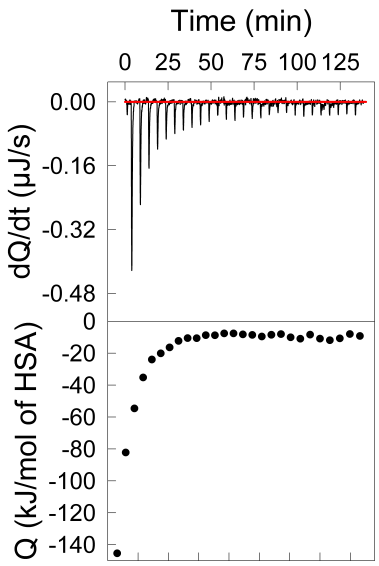

Figure S1. Heat of dilution obtained by titration of 10 µM human serum albumin (HSA) to phosphate buffer saline (PBS) buffer (pH 7.4).

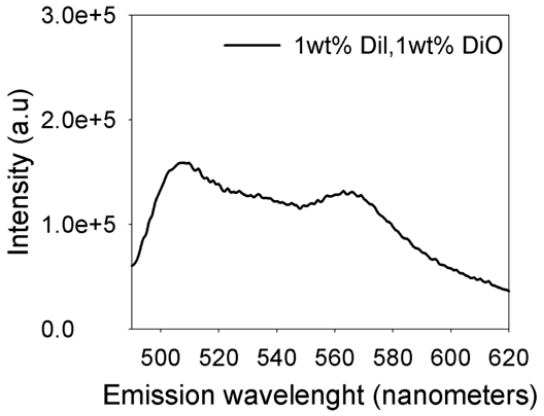

Figure S2. FRET ratio, $I_{565}/(I_{565}+I_{501})$ (normalized to time 0) for the mixture of 1wt% DiO and 1wt% DiI molecules in 90% of FBS.

Polymer synthesis

i. Synthesis of linear diblock copolymers: L45, L75 and L188

Poly(ethylene glycol)methylether (0.285 g, 0.143 mmol) and D,L-lactide (1.71 g, 11.9 mmol) were dried in a screw-capped Schlenk flask equipped with a magnetic stir bar under vacuum (50 mTorr) for ~1 h at 50 °C and allowed to cool to room temperature under argon. Then, dichloromethane (6 mL, such to achieve a monomer concentration of 0.5-2 M) was added *via* syringe and the reagents were allowed to dissolve completely prior to the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (17.9 μl, 0.12 mmol; 1 mol % with respect to D,L-lactide). The reaction was allowed to proceed at room temperature under argon for ~1 h and quenched with an excess of benzoic acid. The mixture was concentrated to ~30% of the original volume under vacuum and added to an excess of 2-propanol (2x). The product was filtered and dried under vacuum at 50 °C overnight. Yield > 95%. L45 (**Figures S3-S5**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 -5.11 (m, 175H), 3.66-3.62 (m, 181H), 3.38 (s, 3H), 1.60 – 1.54 (m, 500H). L75: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.22 – 5.11 (m, 175H), 3.64 (s, 300H), 3.37 (s, 3H), 1.60 – 1.53 (m, 500H). L188: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26 – 5.12 (m, 175H), 3.64 (s, 754H), 3.37 (s, 3H), 1.60 – 1.52 (m, 500H). Gel permeation chromatograms are included in **Figure S10**.

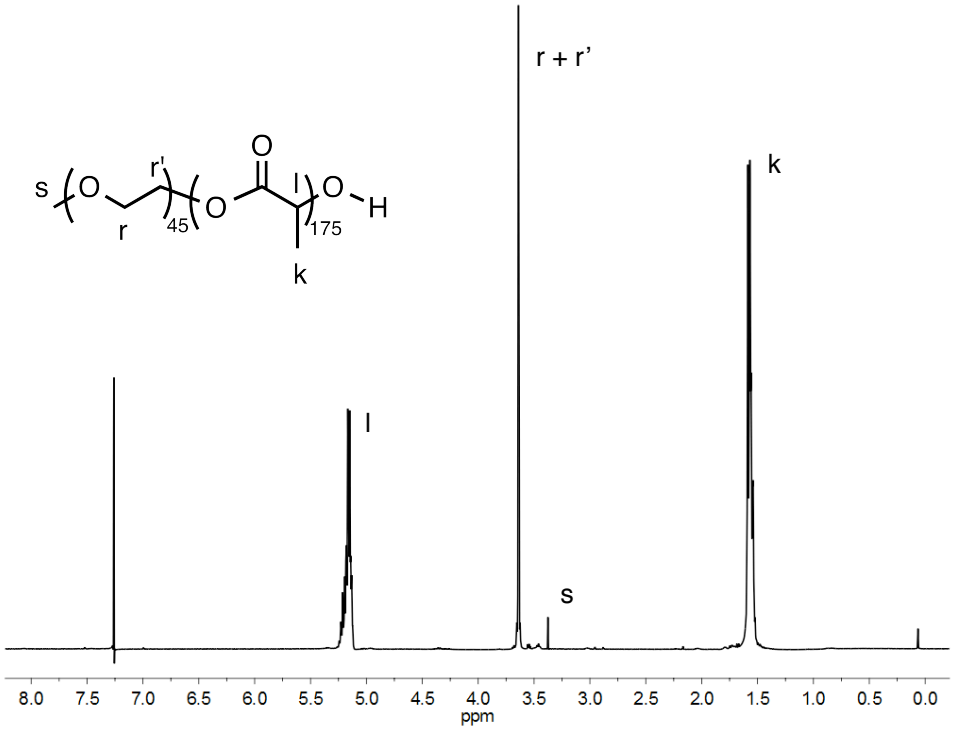

Figure S3. ^{1}HNMR spectrum and peak assignments of PLA_{175}-*b*-PEG_{45} (L45).

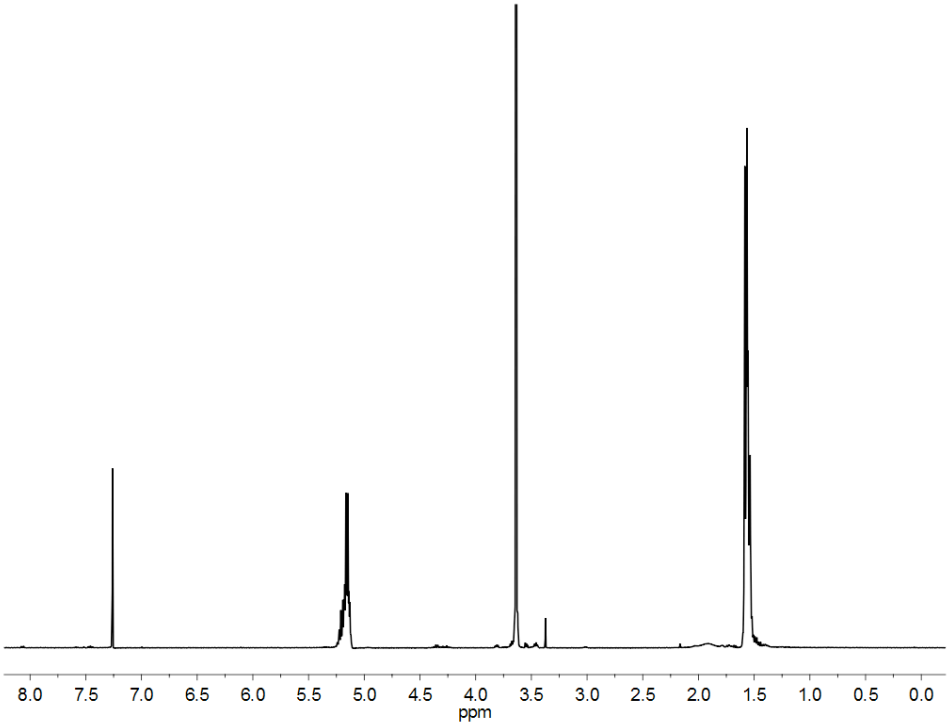

Figure S4. ^{1}HNMR spectrum of PLA_{175}-*b*-PEG_{75} (L75).

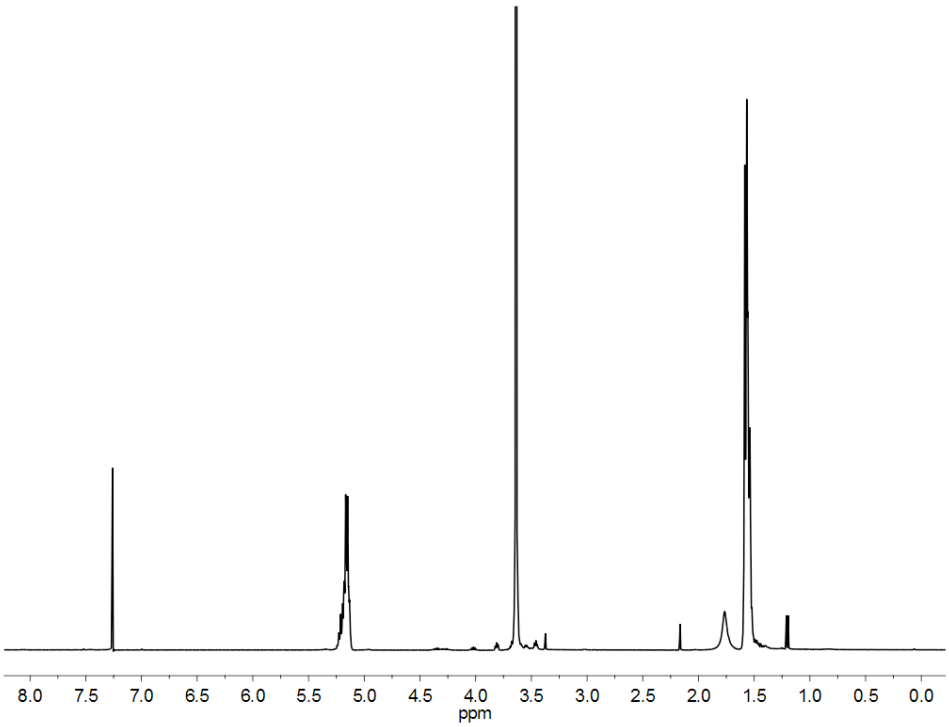

Figure S5. ^{1}HNMR spectrum of PLA_{175}-*b*-PEG_{188} (L188).

ii. Synthesis of the linear-dendritic copolymer D45, ***5***

a) Synthesis of hydroxyl terminated dendron G_2-OH, **3**

5-Amino-1-pentanol was conjugated to 1 through the carboxylic acid group at the focal point. For this, a dry round bottom flask, equipped with a magnetic stir bar and a septum, was charged with the dendron (0.3 g, 0.672 mmol) and 1,1'-carbonyldiimidazole (CDI) (0.131 g, 0.806 mmol) in CHCl3. After 2 h of reaction at room temperature, this solution was added dropwise to a flask containing 5-amino-1-pentanol (0.083 g, 0.806 mmol) previously dissolved in anhydrous chloroform. The reaction was performed at room temperature for 3h. Then, the salts were filtered off and the mixture was washed with brine (50 mL, 3x). 1HNMR spectrum and peak assignments of the conjugate are shown below. Yield: 97%.1H NMR (300 MHz, CDCl3) δ 6.59 – 6.37 (m, 1H), 4.31 (d, 4H), 4.18 (t, 4H), 3.78 – 3.54 (m, 6H), 3.25 (d, 2H), 1.62 – 1.48 (m, 4H), 1.42 (d, 6H), 1.37 (d, 6H), 1.27 (s, 3H), 1.22 – 1.05 (m, 6H).

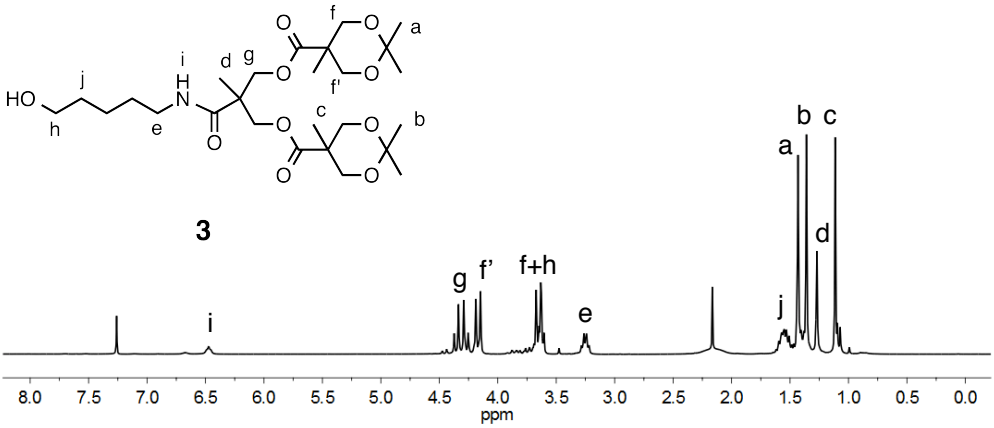

Figure S6. [1]HNMR spectrum and peak assignments of G_2-OH (**3**).

b) Synthesis of PLA_{175}-b-G_2, **4**

D,L-lactide was polymerized from the focal hydroxyl end of 6, according to the protocol outlined for the synthesis of linear amphiphiles. The resulting polymer was purified by precipitation in methanol (2x) and dried under vacuum. Yield: 94%.1H NMR (400 MHz, CDCl3) δ 6.61 (d, 1H), δ 5.24 – 4.96 (m, 175H), 4.37 – 4.23 (m, 4H), 4.20 – 4.10 (d, 4H), 3.70-3.64 (d, 4H) 3.25 – 3.20 (q, 2H), 1.75 - 1.30 (m, 540H), 1.11 (s, 6H).

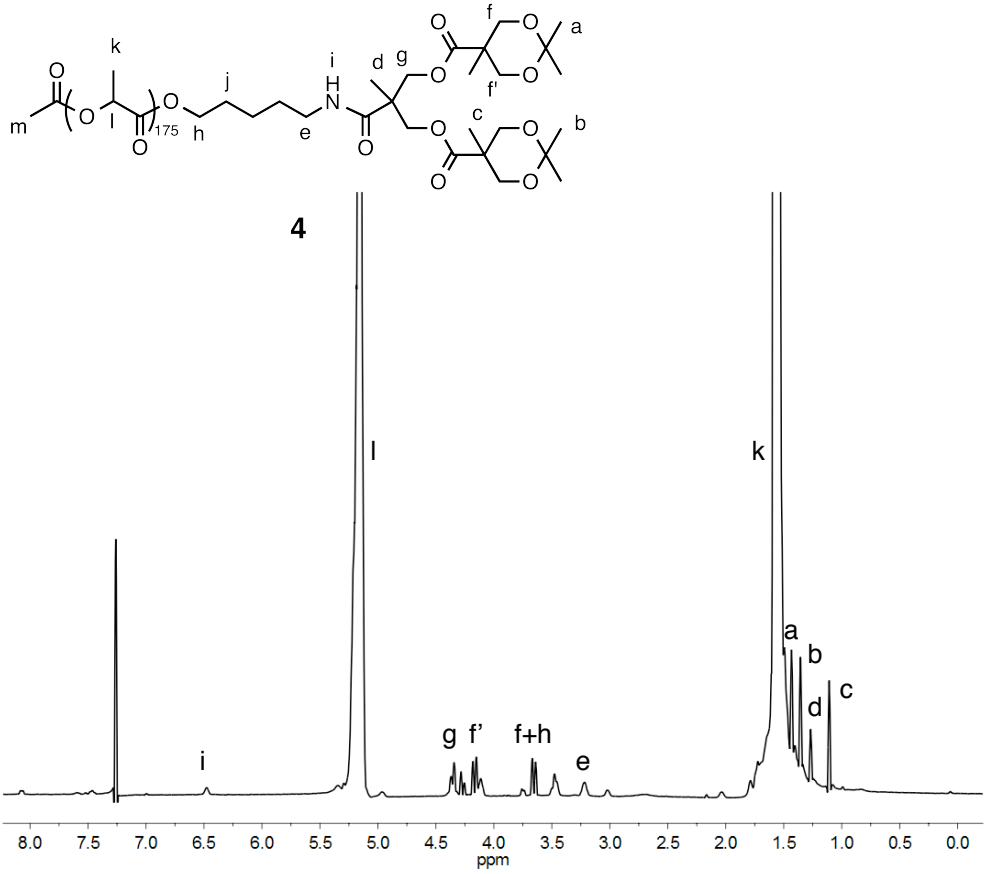

Figure S7. [1]H NMR spectrum and peak assignments of **4**.

c) Poly(ethylene glycol) conjugation

Esterification of the terminal hydroxyl group of PLA was done by dissolving the polymer (0.85g, 0.068 mmol) in 8 mL of anhydrous dichloromethane followed by the addition of triethylamine (9.4 μL, 0.067 mmol) and acetic anhydride (32 μL,0.34 mmol). The reaction was allowed to proceed for 12 h at room temperature under stirring. Deprotection of the acetonide groups of the resulting polymer was done by dissolving the polymer in a solvent mixture consisting of THF and methanol (3:2 v/v). Then, Dowex-H+ resin (0.5 w/wp) was added and the reaction was stirred for 12 h at 50 °C. The resin was filtered off and carefully washed with THF, the filtrate was concentrated under vacuum. The deprotected polymer (0.70 g, 0.056 mmol) was transferred to a round bottom flask, equipped with a stir bar and a septum, and dissolved in anhydrous dichloromethane. 4-nitrophenyl chloroformate (0.226 g, 1.12 mmol) was then added, followed by pyridine (0.09 mL, 1.12 mmol; dropwise). The reaction was allowed to proceed for 24 h at room temperature. Salts were filtered off and the mixture was washed with brine (3x). Purification was done by precipitation into cold methanol (2x) and the white precipitate was collected and dried under high vacuum. Yield: 93%. 1H NMR (400 MHz, CDCl3) δ 8.29 – 8.24 (m, 8H), 7.38 (t, 8H), 5.30 – 5.01 (m, 175H), 4.57 – 4.46 (m, 4H), 4.46 – 4.31 (m, 4H), 4.14 – 4.01 (m, 4H), 3.23 (d, 2H), 2.10 (d, 3H), 1.57 (t, 525H), 1.38 (s, 3H), 1.31 (s, 6H).

The activated polymer (0.487 g, 0.037 mmol) was then dissolved in anhydrous dimethylformamide and to this solution were added triethylamine (5 eq) and 2 (1.05 eq). The reaction was carried out at room temperature for 24 h. The resulting solution was concentrated under vacuum before precipitation into cold diethyl ether (2x). The white precipitate (9) was collected and dried under high vacuum. Yield: >86%.1H NMR (400 MHz, CDCl3) δ 5.29 – 5.05 (m, 165H), 4.3-4.1 (b, 12H) 3.65 (d, 730H), 3.38 (s, 12H), 1.55 (m, 524H), 1.25 (s, 3). Gel permeation chromatogram of D45 is included in **Figure S10**.

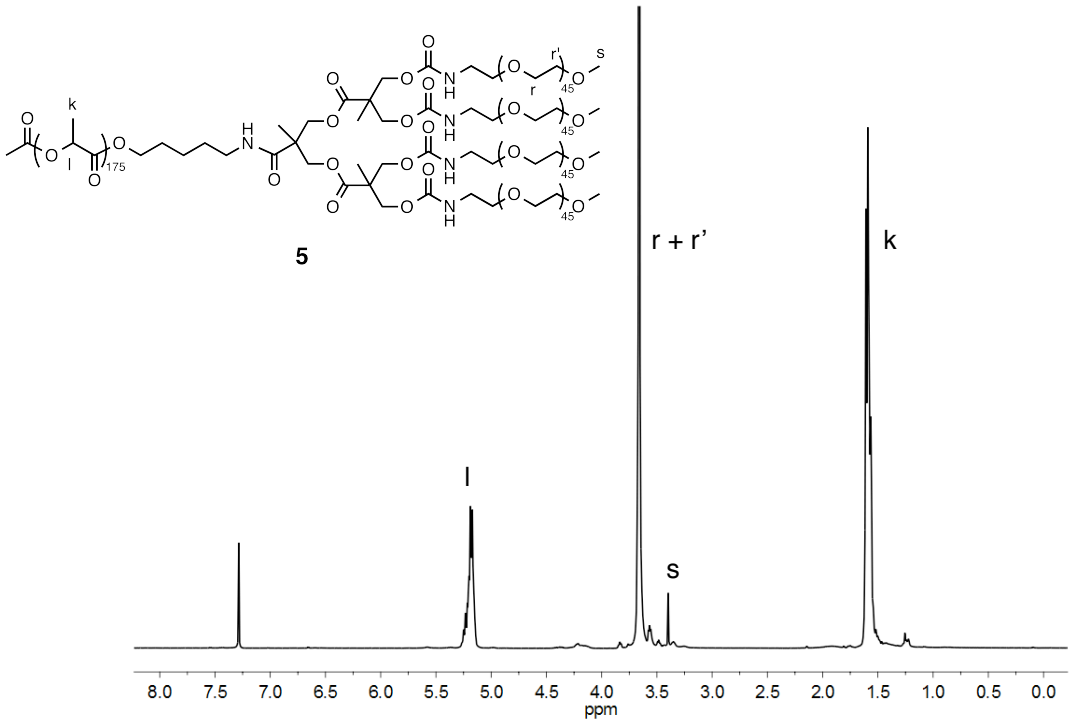

Figure S8. [1]HNMR spectrum and peak assignments of PLA_{175}-*b*-$G_2(PEG_{45})$ or D45 (**5**).

iii. Synthesis of PGMA$_{721}$-g-PEG$_{45}$/PLA$_{28}$ or B45, **6**

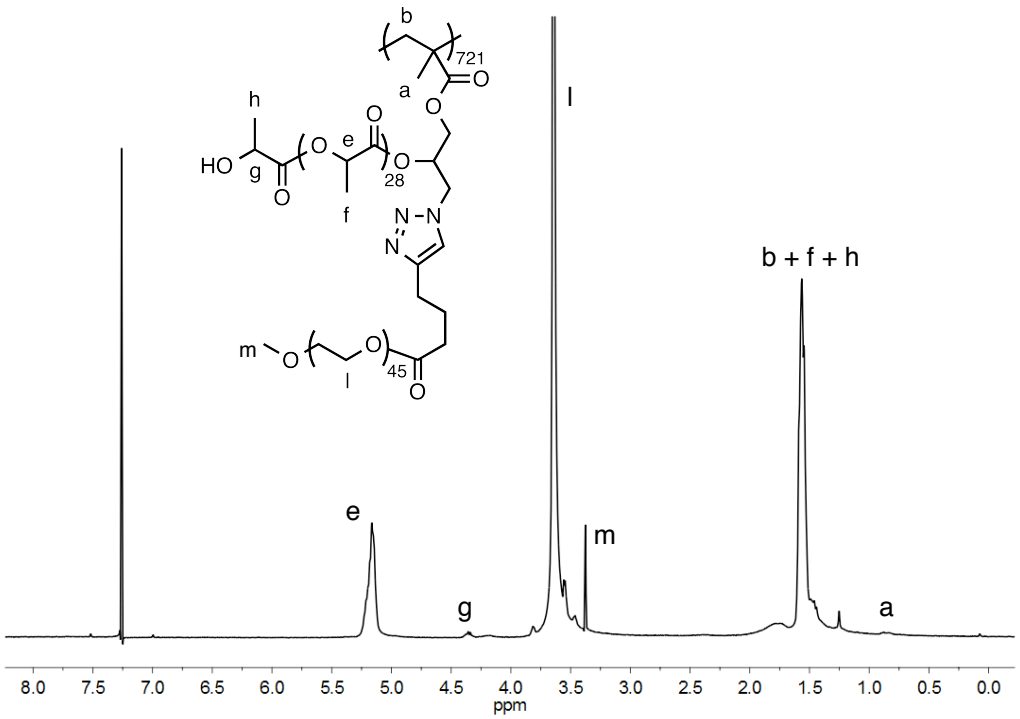

Figure S9. ^{1}HNMR spectrum and peak assignments of PGMA$_{721}$-*g*-PEG$_{45}$/PLA$_{28}$ or B45 (**6**).

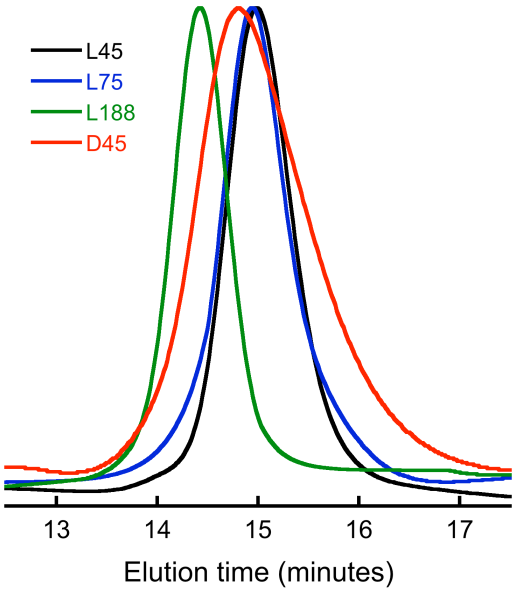

Figure S10. Gel permeation chromatograms of linear and linear-dendritic copolymers (L45, L75, L188 and D45). All distributions are monomodal (with no evidence of macroinitiator), and polydispersity indices ranged from 1.03-1.14 (see Table 1).

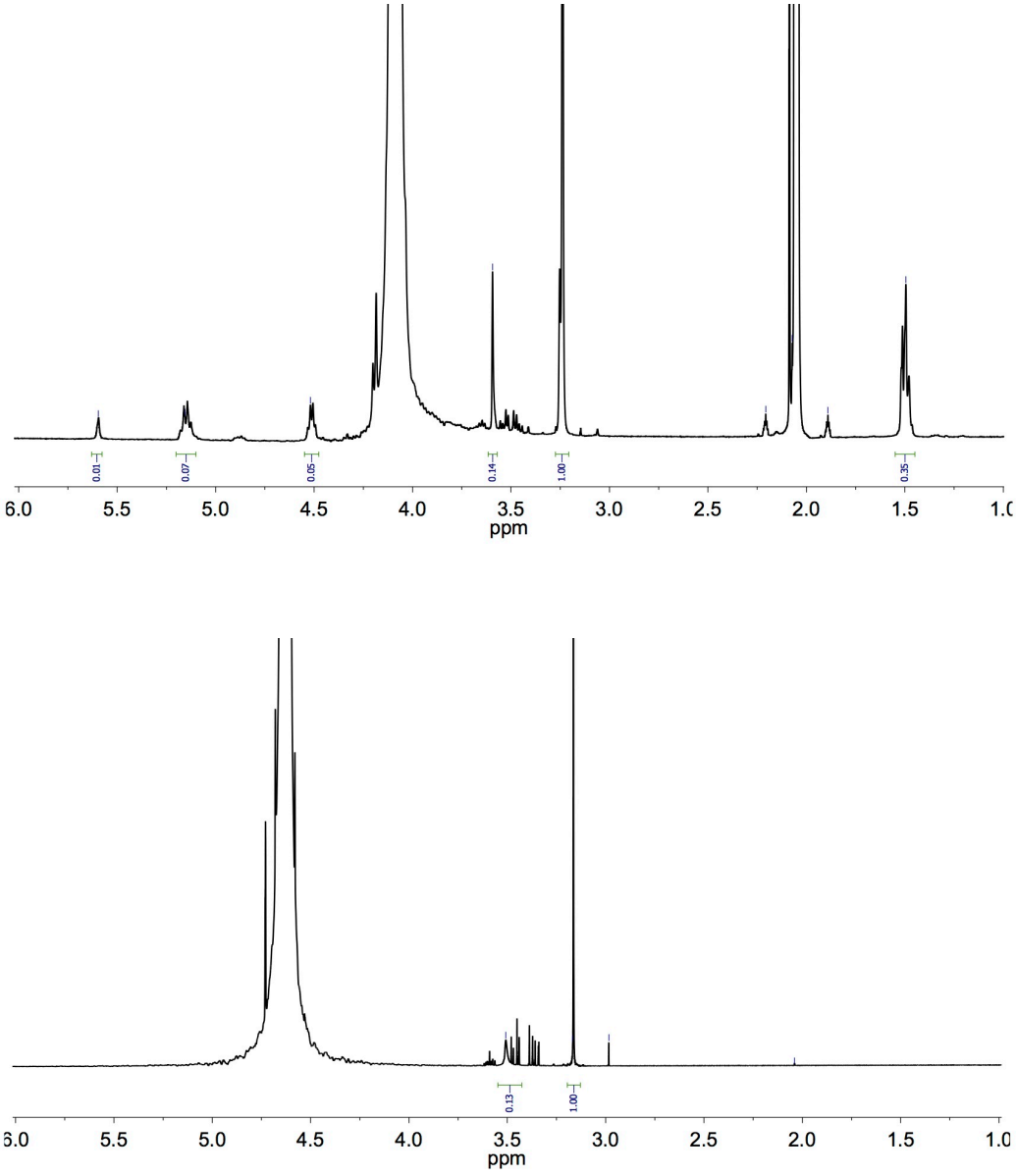

Figure S11. ^{1}HNMR spectra of PLA_{175}-*b*-PEG_{45} (L45) as a unimer in d_6-acetone (top) and in the nanoparticle form in D_2O (bottom). Peak assignments and PEG exposure are given in **Table S1**.

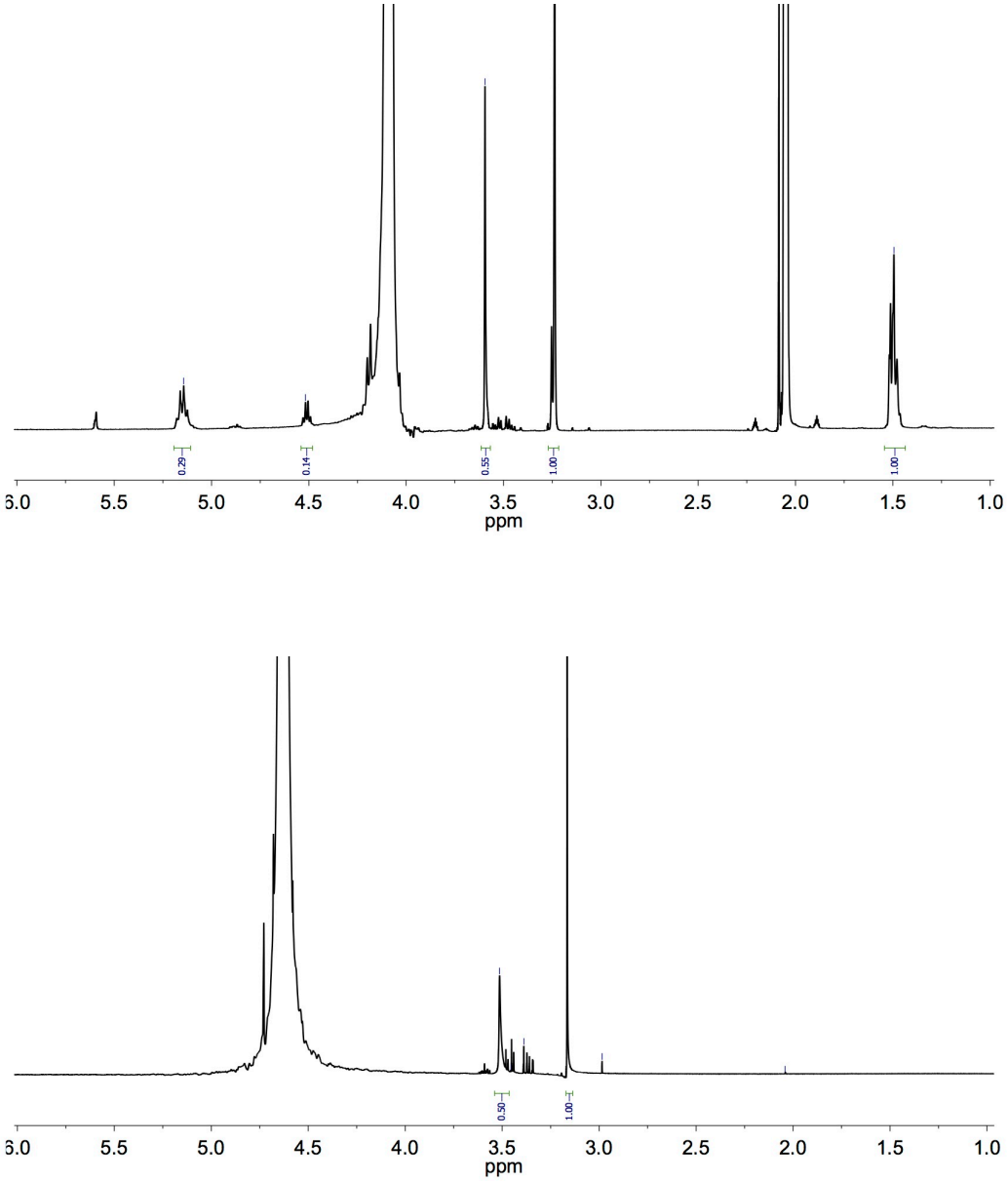

Figure S12. ^{1}HNMR spectra of PLA_{175}-*b*-PEG_{75} (L75) as a unimer in d_6-acetone (top) and in the nanoparticle form in D_2O (bottom). Peak assignments and PEG exposure are given in **Table S1**.

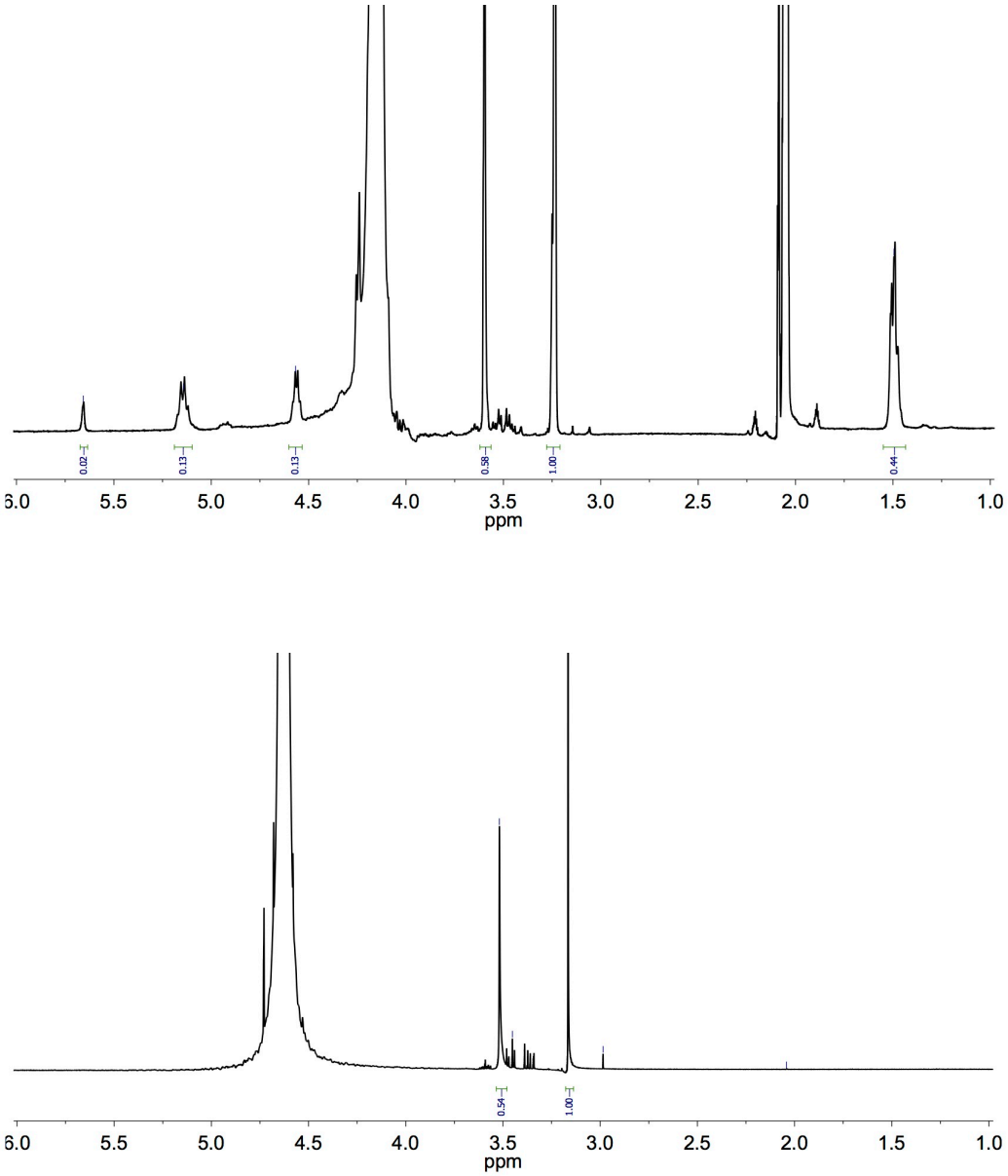

Figure S13. ^{1}HNMR spectra of PLA$_{175}$-*b*-PEG$_{188}$ (L188) as a unimer in d_6-acetone (top) and in the nanoparticle form in D_2O (bottom). Peak assignments and PEG exposure are given in **Table S1**.

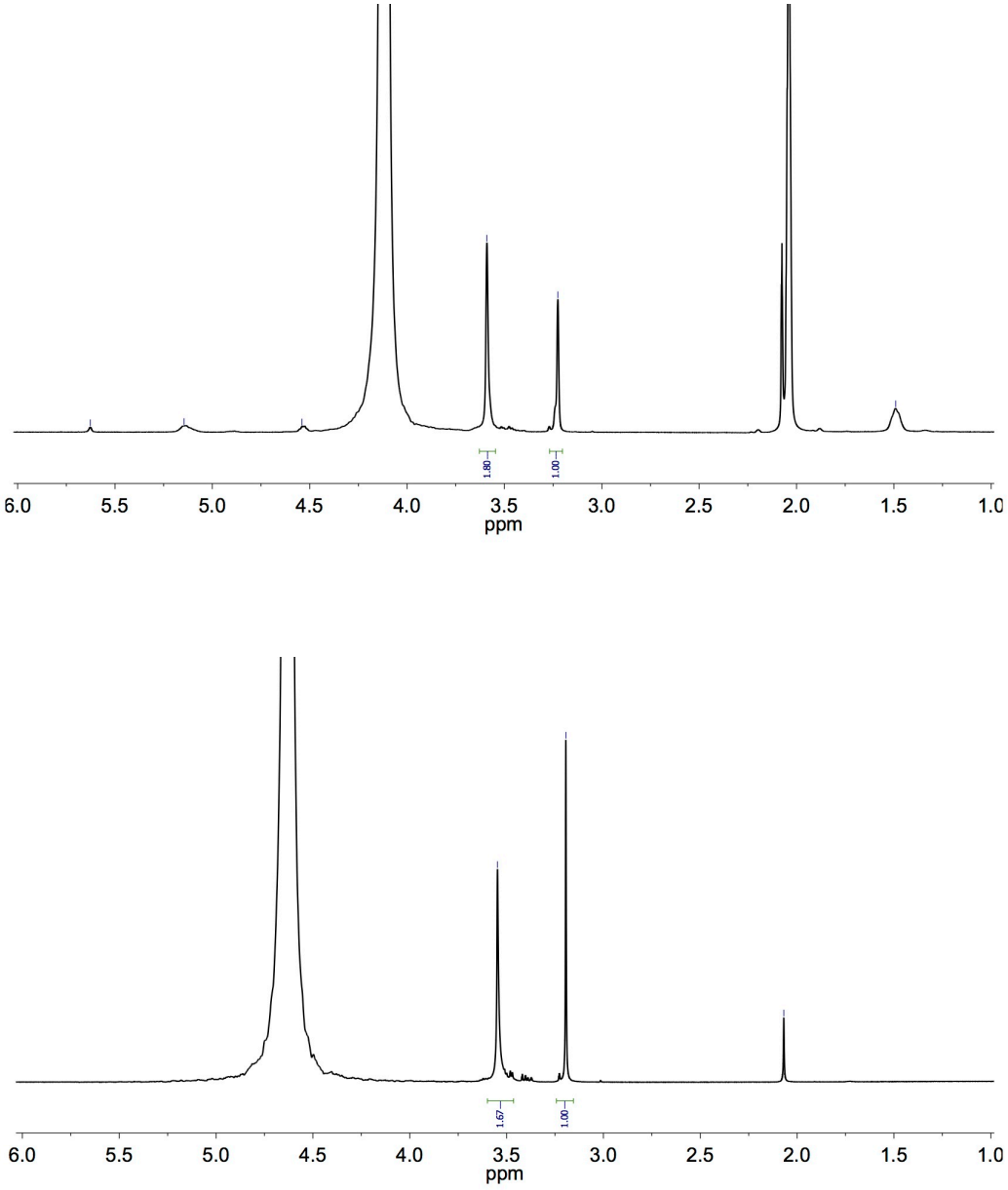

Figure S14. ^{1}HNMR spectra of $PGMA_{721}$-g-PEG_{45}/PLA_{28} (B45) as a unimer in d_6-acetone (top) and in the nanoparticle form in D_2O (bottom). Peak assignments and PEG exposure are given in **Table S1**.

Table S1. Chemical shifts of amphiphiles as unimers (in d_6-acetone) and in nanoparticle form (in D_2O) at room temperature.

	δ in d_6-Acetone (ppm)				D_2O				PEG exposure (%) [a]
	CH (PLA)	CH_3 (PLA)	CH_2CH_2 (PEG)	CH_3 (MeOH)	CH (PLA)	CH_3 (PLA)	CH_2CH_2 (PEG)	CH_3 (MeOH)	
L45	5.16 m	1.49 m	3.59 s	3.24 s	-	-	3.51 s	3.17 s	78.6
L75	5.14 m	1.49 m	3.60 s	3.24 s	-	-	3.51 s	3.16 s	90.9
L188	5.14 m	1.49 m	3.60 s	3.23 s	-	-	3.52 s	3.17 s	93.1
B45	5.15 s	1.49 s	3.59 s	3.23 s	-	-	3.55 s	3.19 s	92.7

[a] PEG exposure was determined from the ratio of the CH_3 signal of methanol in D_2O to that in d_6-acetone

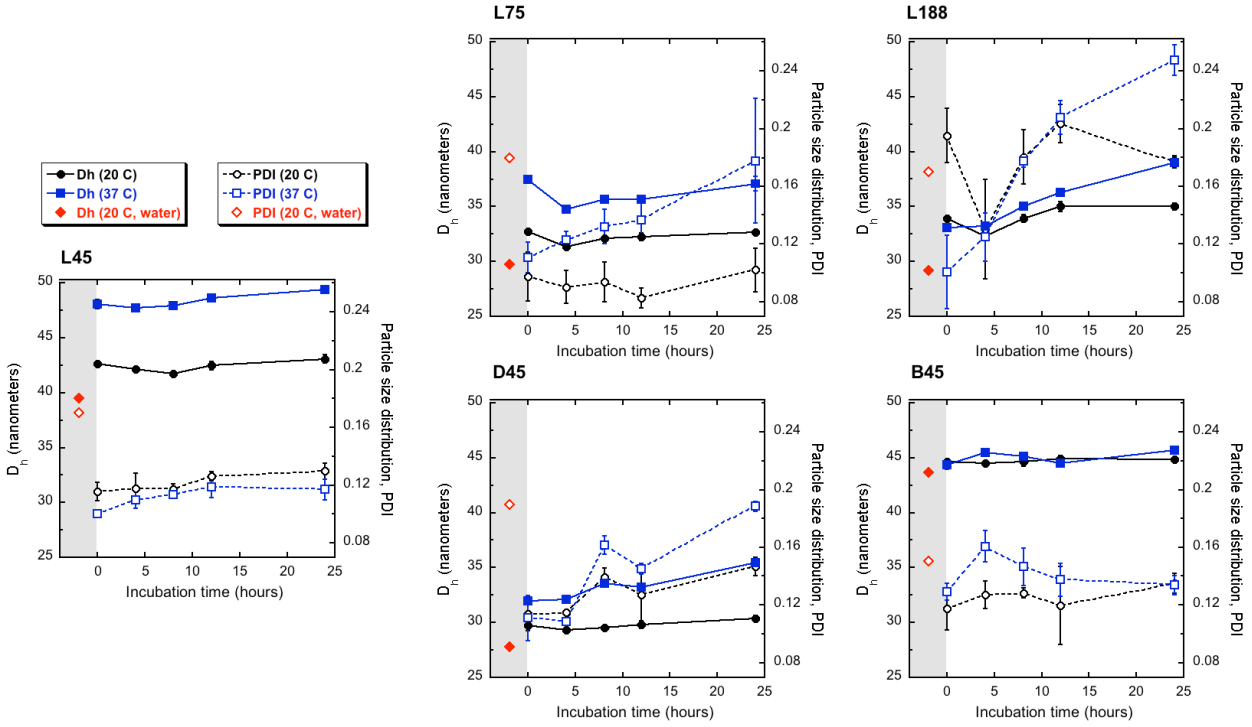

Figure S15. Nanoparticle size (D_h, filled symbols) and particle size distribution (PDI, open symbols) during incubation in 10 mM phosphate buffer saline (PBS). Stability was examined at 20 °C (circles) and 37 °C (squares). Particle size and PDI of samples prior to incubation (*i.e.*, in water at 20 °C) are shown in the shaded regions of each plot. Incubation time of 0 h corresponds to samples examined shortly (~5 min) after addition of NP suspension to a medium containing the buffer. Standard deviations were estimated based on three separate measurements.

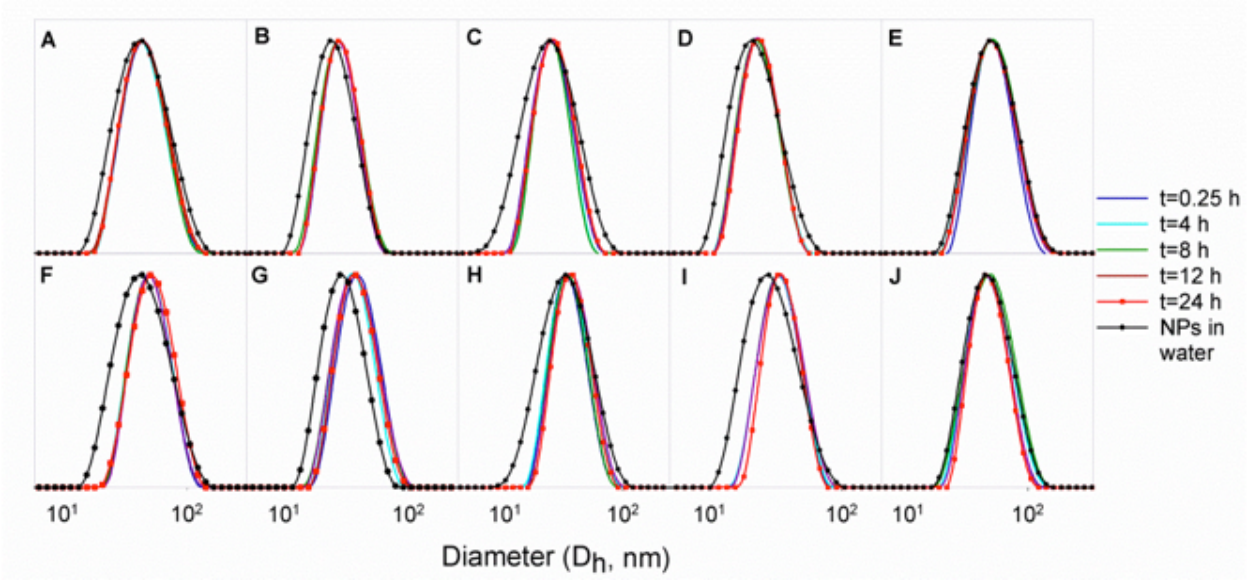

Figure S16. Particle size distributions of nanoparticles incubated in 10 mM PBS, pH=7.4 obtained by DLS. Figures A through E corresponds to self-assemblies of L45, L75, L188, D45 and B45 incubated at 20 °C. Figures F through J are the same nanoparticles incubated at 37 °C. Particle size distributions in water (black circles) are also presented as references for each case.

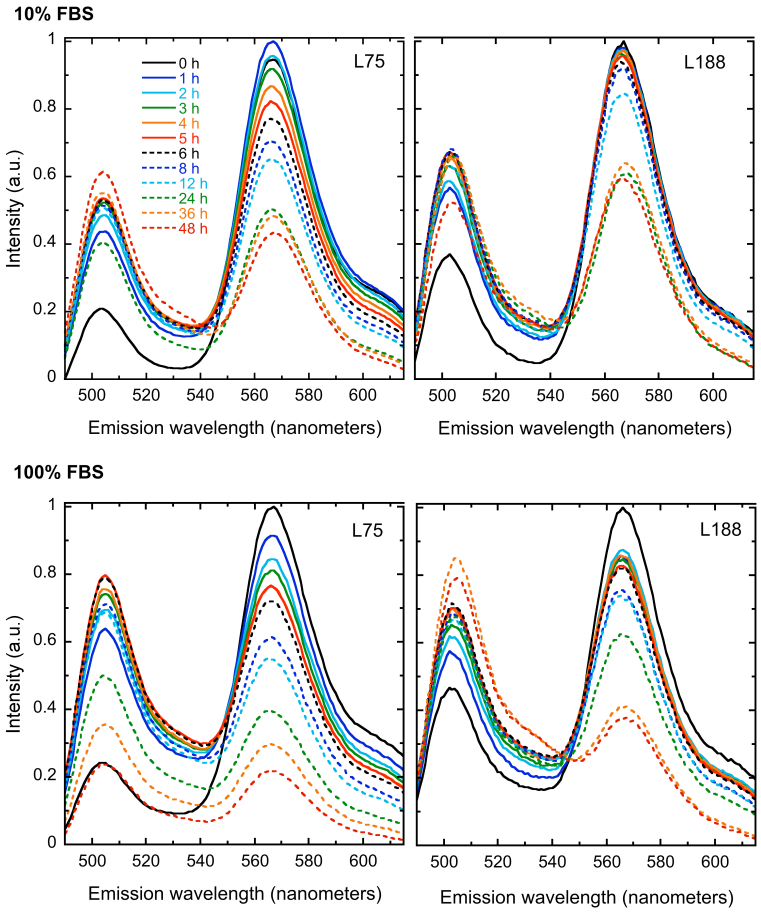

Figure S17. FRET measurements during nanoparticle incubation in fetal bovine serum, showing the shift in primary emission peak from that of the acceptor (DiI, I_{565}) to that of the donor (DiO, I_{501}). Shown are FRET experiments for L75 and L188 in 10% and 100% FBS.

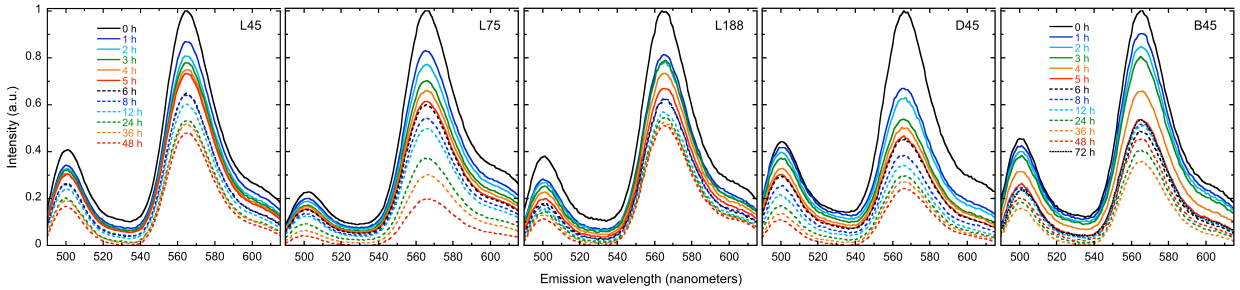

Figure S18. FRET measurements during nanoparticle incubation in PBS 10mM over 48 h (linear series and linear-dendritic sample) and over 72 h (brush amphiphile), showing no shift in primary emission peak.

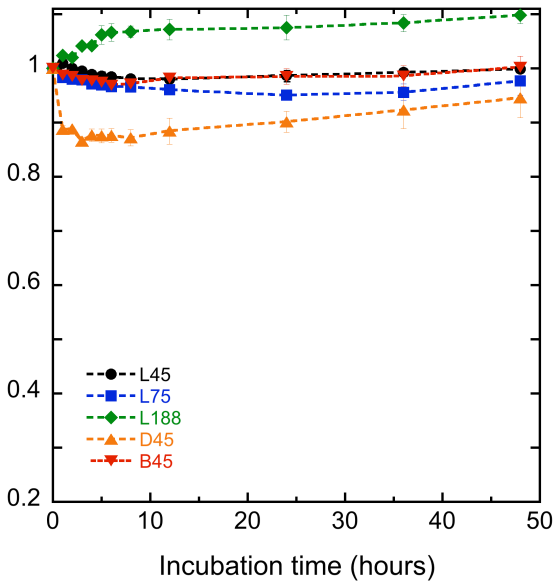

Figure S19. Normalized FRET ratios (FR) during incubation FBS 10 mM.

Bibliography

1. J. L. Santos and M. Herrera-Alonso, *Macromolecules*, 2014, **47**, 137-145.
2. H. Y. Luo, J. L. Santos and M. Herrera-Alonso, *Chem Commun*, 2014, **50**, 536-538.