Electronic Supplementary Information

Influence of the block copolypeptide surfactant structure on the size of polypeptide nanoparticles obtained by mini emulsion polymerisation

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1.0 Methods

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance 400 MHz (^{1}H) as stated in the recorded peaks. All chemical shifts are reported in parts per million (ppm) and analysed relative to the residual nuclei of the reported deuterated solvent.

Dynamic light scattering (DLS) analyses were carried out using a Malvern Zetasizer Nano ZSP instrument (Malvern Instruments, Malvern UK) with a detection angle of 173° and a 3mW He-Ne laser operating at a wavelength of 633nm. 1000µL of solution was used to determine size and distribution of the particles obtained, the measurements were carried out at 25°C and a 10 fold dilution from the stock emulsion in disposable cuvettes.

Nanoparticle tracking analysis (NTA) measurements were performed with a Nanosight NS300 (Malvern, UK). All suspensions were diluted in ultrapure water for measurement following equilibration. Samples were loaded into a laser module sample chamber which allowed for temperature control. Real time video analysis of the nanoparticles was recorded via an in-built sCMOS camera with computer controlled motorized focus. Automatic data analysis was performed on recorded data using the NTA 2.3 software.

Gel permeation chromatography (GPC) was used to determine the molecular weight dispersities (ĐM) and weight average molecular weights (Mw) of the polymers analysed. GPC was carried out using a PSS SECurity GPC system equipped with a PFG 7 µm 8 x 50 mm pre-column, a PSS 100 Å, 7 µm 8 x 300 mm and a PSS 1000 Å, 7µm 8 × 300 mm column in series and a differential refractive index (RI) detector at a flow rate of 1.0 mL min-1 in 1,1,1,3,3,3-hexafluoro-2-propanol (HFiP). The system was calibrated against Agilent Easi-Vial linear poly (methyl methacrylate) (PMMA) standards and analysed by PSS winGPCUniChrom. All GPC samples were prepared using a concentration of 2 mg·mL^{-1}, and were filtered through a 0.2 µm millipore filter prior to injection.

Asymmetrical flow field flow fractionation (AF4) was carried out using an AF2000 system (Postnova Analytics GmbH, Landsberg am Lech, Germany) equipped with an analytical channel containing a regenerated cellulose membrane (10 kDa) and a 350 µm spacer. Detectors connected were; a 280nm Ultraviolet (UV-vis) detector (PN3211), a multi angle light scattering (MALS) detector at 532nm (PN3621), refractive index detector (PN3150) and an on-line dynamic light scattering (DLS) detector

using a Zetasizer flow cell (PN3704). Measurements were performed with a gradient cross flow and a channel flow rate of 0.3 mL/min.

2.0. Synthesis.

NCA Z-L-Lysine, L-Phenylalanine and L-Leucine were all synthesised by previously reported methods.[1, 2], [3] Briefly, ε-carbobenzyloxy-L-lysine (10 g 35.7 mmol), α-pinene (14.5 mL, 91.7 mmol) and THF (150 mL) were added to a three-neck round bottom flask, fitted with a N_2 flow, dropping funnel and condenser and heated to reflux. Triphosgene (8.3 g, 27.9 mmol) was dissolved in THF (100 mL) and added to the refluxing solution dropwise. Once the solution became clear, it was concentrated *in vacuo* to 2/3rds of the volume and precipitated into hexane. The solid collected was dried under vacuum to afford a white fluffy solid (86%).

NCA Z-L-Lysine: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.38 – 7.30 (m, 5H), 7.11 (s, 1H), 5.10 (s, 2H), 4.98 (s, 1H), 4.25 (s, 1H), 3.19 (m, 2H), 1.94 – 1.53 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$, δ): 170.07, 157.07, 152.63, 136.45, 128.72, 128.37, 128.17, 57.57, 40.20, 30.90, 29.25, 21.40.

NCA L-Phenylalanine: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.37-7.29 (m 3H), 7.18 (m, 2H), 6.38, (s, 1H), 4.45 (dd, J = 4.14, 8.05 Hz, 1H), 3.27 (dd, J = 4.04, 14.1 Hz, 1H), 3.00 (dd, J = 8.05, 14.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 168.89, 152.09, 134.02, 129.34, 128.12, 58.98, 37.91.

NCA L-Leucine: ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.68 (s, 1H), 4.35 (d, J = 8.7 Hz, 1H), 1.82 (m, 2H), 1.70 (m, 1H), 0.99 (m, 6H).^{13}C NMR (100 MHz, $CDCl_3$, δ): 169.97, 152.84, 56.27, 40.95, 25.21, 22.84, 21.64.

3.0. Surfactant characterisation

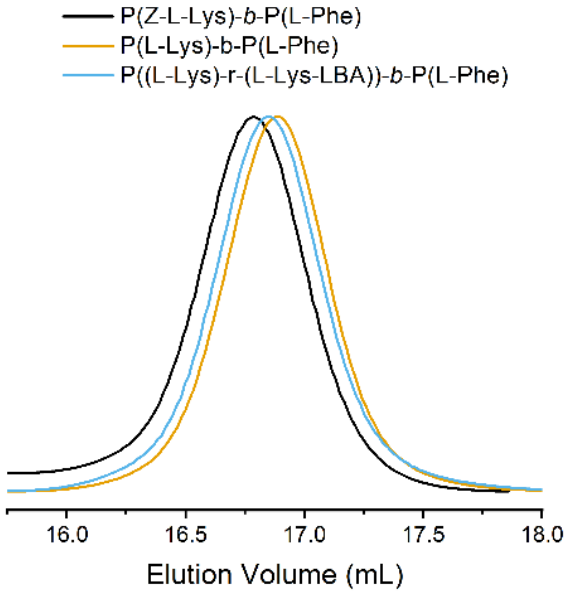

Figure S1: SEC in HFiP of the three stages of surfactant synthesis.

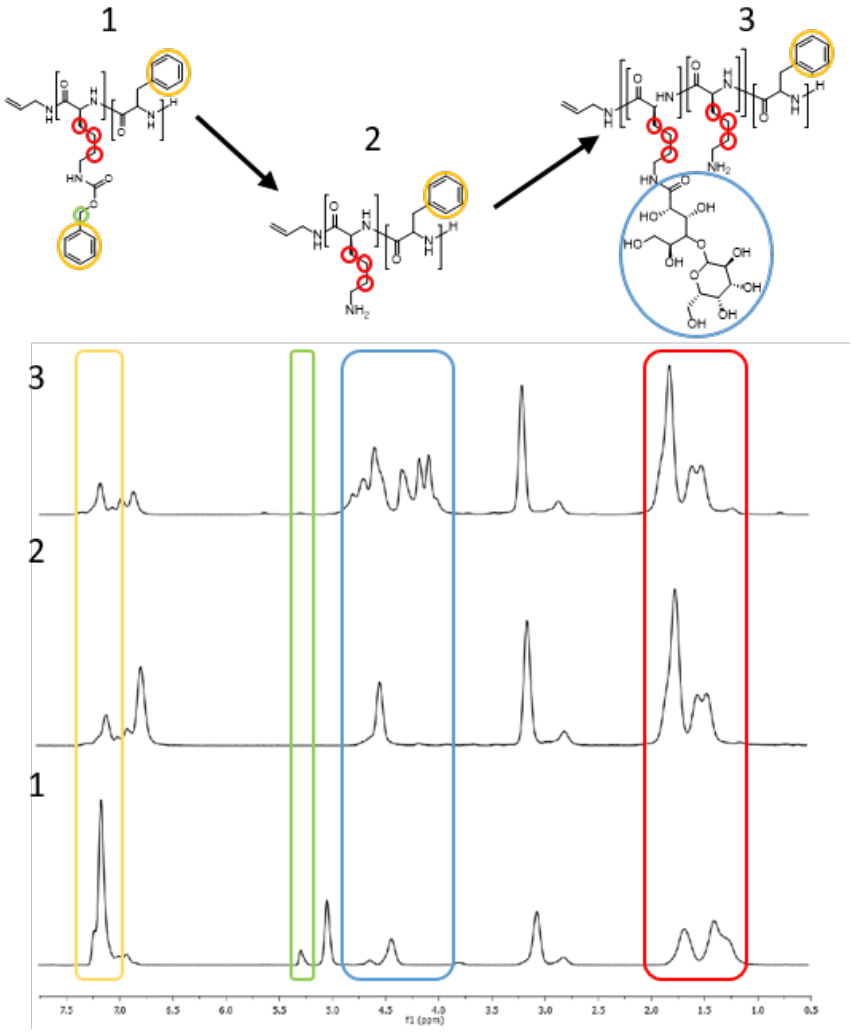

Figure S2: ^{1}H NMR spectra of the three stages of the surfactant synthesis in d-TFA a) protected block polypeptide P(Z-L-Lys)$_{65}$-b-P(L-Phe)$_{14}$ b) deprotected block polypeptide P(L-Lys)$_{65}$-b-P(L-Leu)$_{14}$ c) glycosylated P((L-Lys)-r-(L-Lys-LBA))$_{65}$-b-P(L-Leu)$_{14}$.

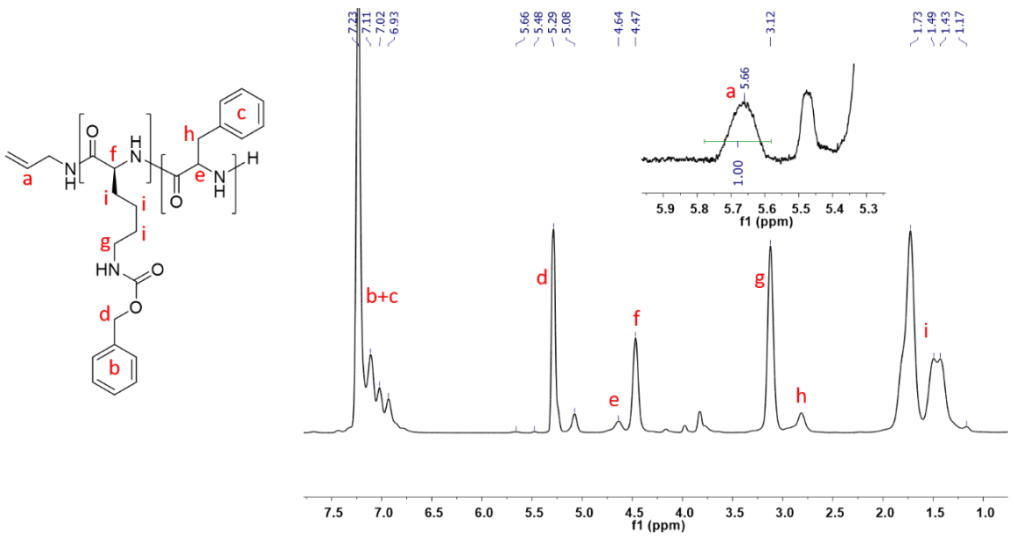

Figure S3: ^{1}H NMR of protected aromatic surfactant (P(Z-L-Lys)-b-P(L-Phe)). Degree of polymerisation is calculated based on the ratio of peaks a:d:h which gave a ratio of 1:65:14.

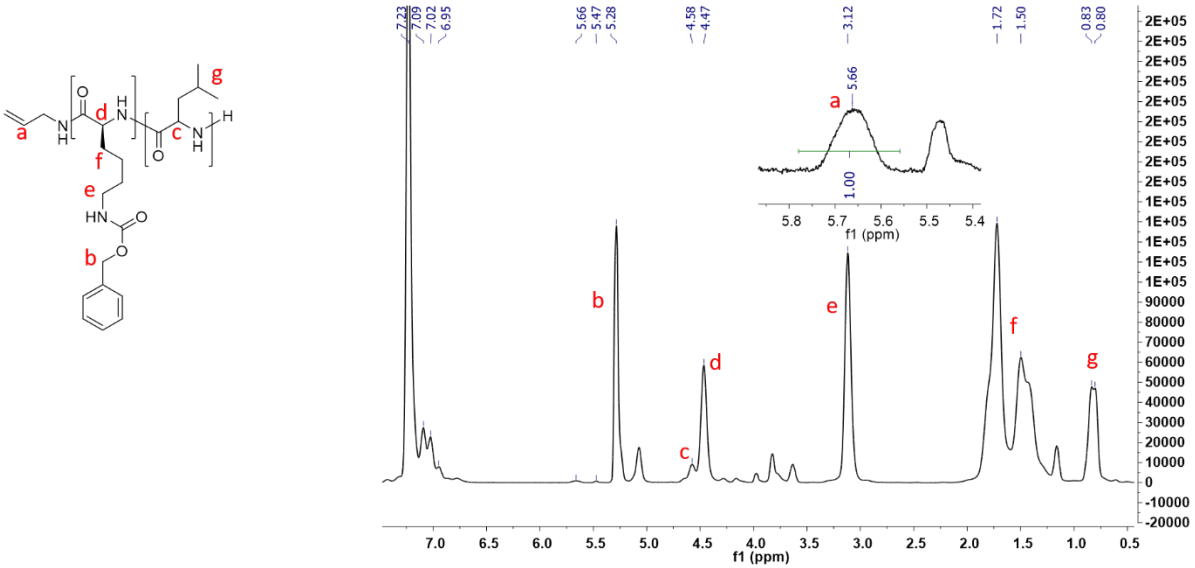

Figure S4: ^{1}H NMR of protected aromatic surfactant (P(Z-L-Lys)-b-P(L-Leu)). Degree of polymerisation is calculated based on the ratio of peaks a:b:g which gave a ratio of 1:56:11.

Table S1: The ^{1}H NMR in d-TFA and HFiP GPC values obtained for the two different surfactant variations at the three stages of synthesis.

	S-Phe			S-Leu		
	M_n^{theo}	**NMR**	**GPC (Mn) (Đ)**	**Theory**	**NMR**	**GPC (Mn) (Đ)**
	L-Lys(50), L-Phe(10)	L-Lys(65), L-Phe(14)		L-Lys(50), L-Leu(10)	L-Lys(56), L-Leu(11)	
Protected	14,577	19,115	16,800 (1.15)	14,2137	15,923	11,900 (1.07)
Deprotected	7,875	10,403	14,500 (1.05)	7,535	8,417	11,300 (1.08)
Glycosylated	12,977	16,865	15,100 (1.06)	12,637	14,199	11,700 (1.08)

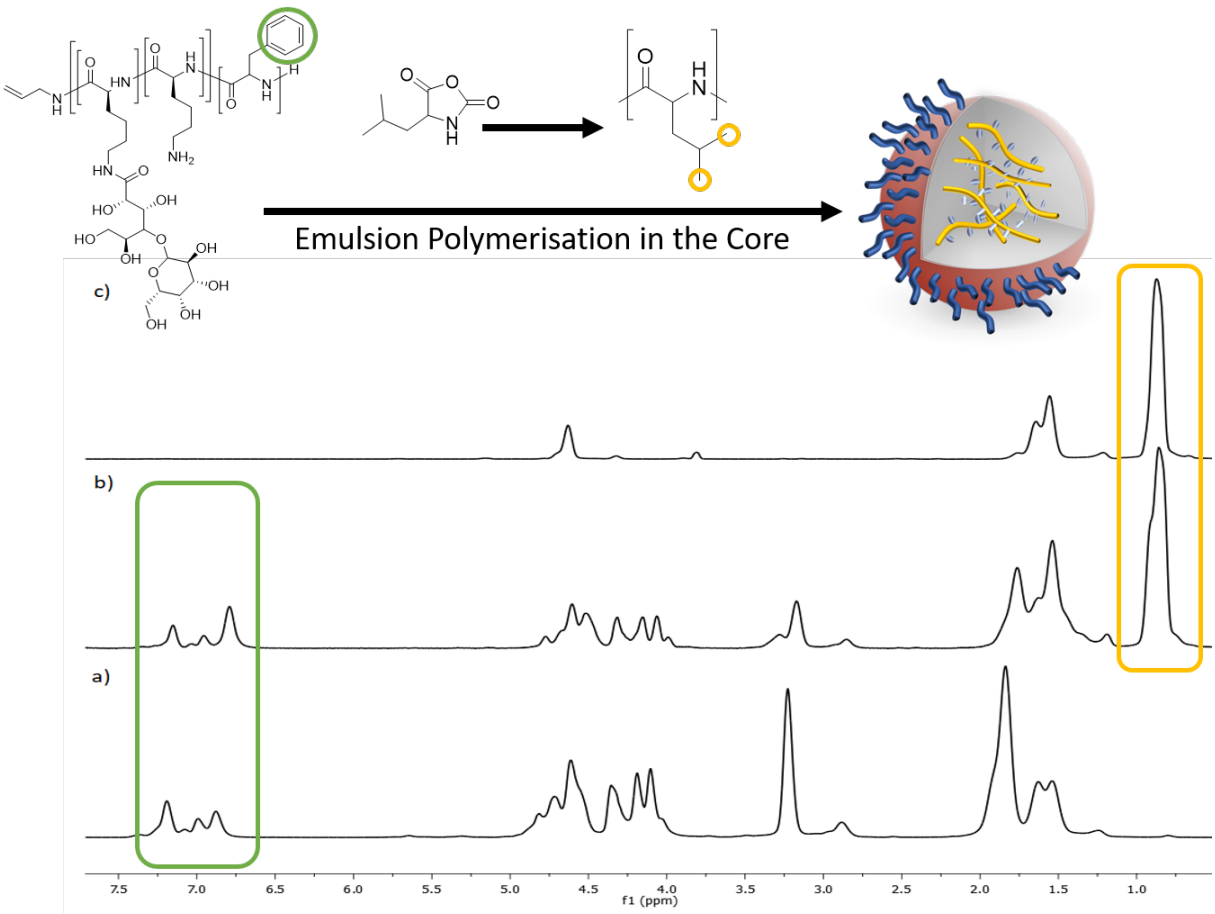

Figure S5: ^{1}H NMR spectra of the surfactant S-Phe (a), the nanoparticle obtained from the miniemulsion polymerisation of the Leu NCA using the S-Phe surfactant, S-Phe/C-Leu (b) and the polyLeu (c). Green dictating the presence of the benzyl moiety in S-Phe (a) and S-Phe/C-Leu (b). Orange dictating the presence of the P(L-Leu) within the nanoparticle.

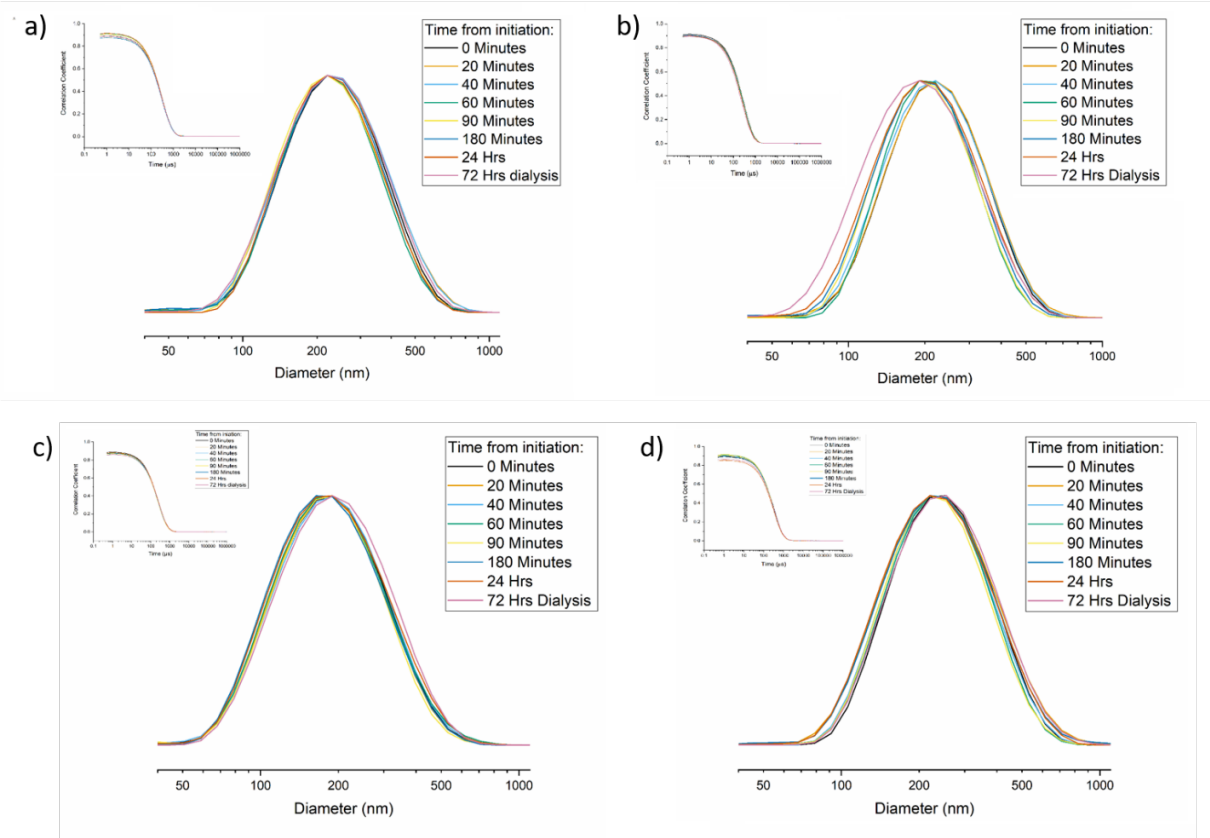

Figure S6: DLS tracking of miniemulsion nanoparticles showing the intensity average traces. a) S-Phe/C-Phe, b) S-Phe/C-Leu, c) S-Leu/C-Phe and d) S-Leu/C-Leu.

Table S2: Z-Average sizes for the complete tracking of the nanoparticles during the 24hrs open to air polymerisation and subsequent 72hrs of dialysis purification.

	S-Phe				S-Leu			
	C-Phe		C-Leu		C-Phe		C-Leu	
Time point	Diameter	PDI	Diameter	PDI	Diameter	PDI	Diameter	PDI
0	205.9 ± 6.2	0.18 ± 0.02	196.2 ± 9.2	0.18 ± 0.03	167.9 ± 13.5	0.17 ± 0.02	224.5 ± 14.4	0.17 ± 0.01
20	206.9 ± 4.0	0.20 ± 0.02	197.6 ± 5.6	0.17 ± 0.04	167.6 ± 15.9	0.19 ± 0.03	216.2 ± 15.7	0.18 ± 0.02
40	206.5 ± 1.9	0.19 ± 0.02	194.5 ± 4.5	0.17 ± 0.02	168.0 ± 15.4	0.18 ± 0.02	217.4 ± 11.3	0.16 ± 0.02
60	204.2 ± 2.5	0.19 ± 0.02	189.0 ± 3.0	0.16 ± 0.03	167.3 ± 15.0	0.18 ± 0.03	214.8 ± 10.8	0.16 ± 0.01
90	201.6 ± 1.6	0.18 ± 0.01	181.4 ± 6.3	0.18 ± 0.03	164.5 ± 16.6	0.17 ± 0.02	212.5 ± 9.9	0.16 ± 0.02
180	202.3 ± 2.4	0.18 ± 0.02	180.3 ± 4.2	0.18 ± 0.02	167.2 ± 12.8	0.19 ± 0.01	211.8 ± 13.8	0.19 ± 0.02
24hrs	205.9 ± 6.2	0.18 ± 0.02	178.8 ± 7.9	0.19 ± 0.01	171.9 ± 8.2	0.20 ± 0.02	211.4 ± 12.1	0.19 ± 0.02
Dialysis	203.8 ± 8.8	0.19 ± 0.02	164.4 ± 17.5	0.21 ± 0.02	173.7 ± 6.5	0.18 ± 0.02	224.2 ± 16.0	0.18 ± 0.03

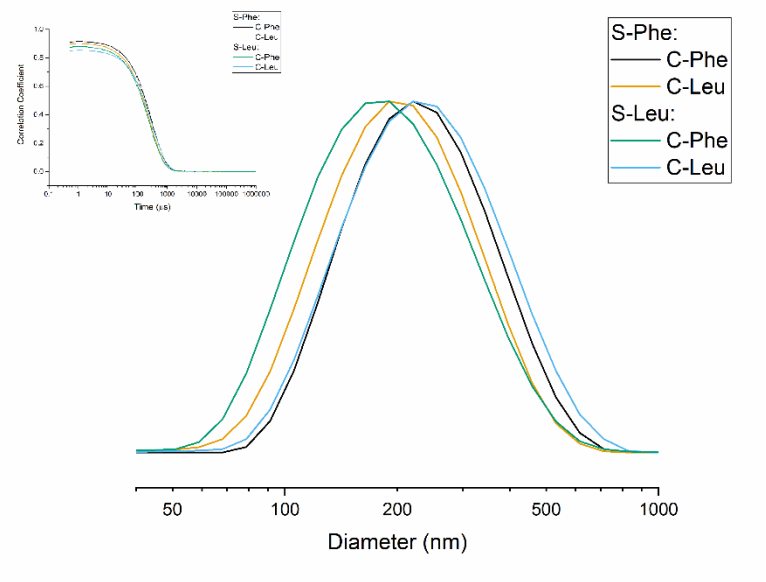

Figure S7: Intensity average DLS traces for the four different compositions of nanoparticles after 24 hrs of open to air polymerisation. Inset: Correlogram of the 4 different samples.

Table S3: Z-average size (nm) as reported by DLS for the 4 variations of miniemulsion nanoparticles after 24hrs open to air polymerisation and their respective p values obtained from a two tail t-test at the 95% confidence level for the comparison of each row and column.

Surfactant	Core		P value
	C-Phe	C-Leu	
S-Phe	205.9 ± 6.2	178.8 ± 7.9	5.5×10^{-8}
S-Leu	171.9 ± 8.2	211.4 ± 12.1	4.6×10^{-7}
P Value	3.3×10^{-9}	4.3×10^{-6}	

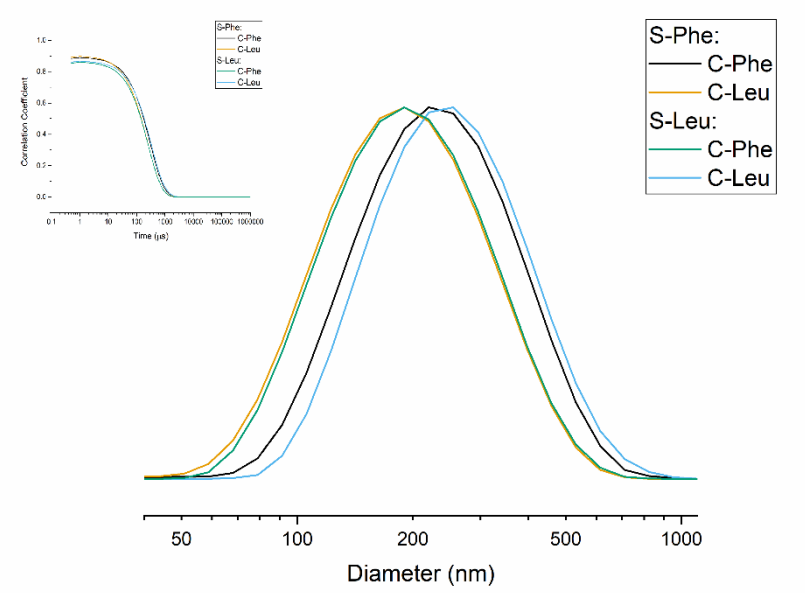

Figure S8: Intensity average DLS traces for the four different compositions of nanoparticles after 72hrs purification by dialysis. Inset: Correlogram of the 4 different samples.

Table S4: Z-average size (nm) as reported by DLS for the 4 variations of miniemulsion nanoparticles after 72hrs purification by dialysis and their respective p values obtained from a two tail t-test at the 95% confidence level for the comparison of each row and column.

Surfactant	Core		P value
	C-Phe	C-Leu	
S-Phe	203.8 ± 8.8	164.4 ± 17.5	1.7×10^{-5}
S-Leu	173.7 ± 6.5	224.2 ± 16.0	1.6×10^{-7}
P Value	3.9×10^{-7}	1.1×10^{-6}	

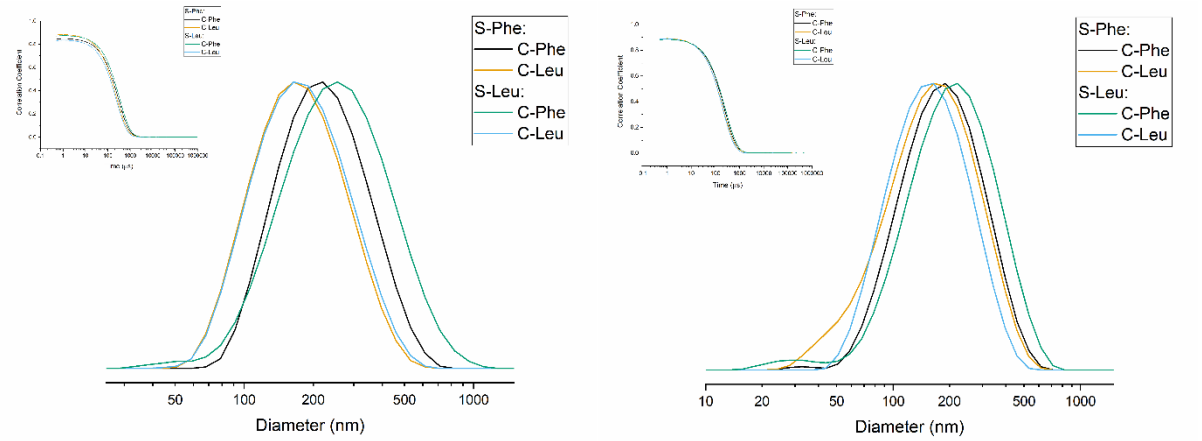

Figure S9: DLS of each variation of nanoparticles after a 10 fold dilution in deionised water (left) and 10mM PBS solution (right).

Table S5: The z-average hydrodynamic diameters as obtained by DLS and the zeta potential when the 10 fold dilution from the stock miniemulsion solution was carried out in 10mM PBS.

Surfactant/Core	H_2O			10mM PBS		
	Size [nm]	PDI	Z-Potential [mV]	Size [nm]	PDI	Z-Potential [mV]
S-Phe/C-Phe	203.8	0.19	41.2	161.3	0.20	13.0
S-Phe/C-Leu	164.4	0.21	51.1	139.2	0.25	16.4
S-Leu/C-Phe	173.7	0.18	48.7	138.6	0.17	16.7
S-Leu/C-Leu	224.2	0.18	49.9	173.1	0.24	13.4

Table S6: Comparison of the diameters of the nanoparticles as reported by the number average from DLS and NTA.

Surfactant/Core	DLS (number average)		NTA	
	Size [nm]	St. dev	Size[nm]	St. dev
S-Phe/C-Phe	134.9	16.0	150.5	10.9
S-Phe/C-Leu	103.3	10.5	134.0	10.7
S-Leu/C-Phe	99.8	9.9	123.5	2.1
S-Leu/C-Leu	153.4	19.1	180.9	7.8

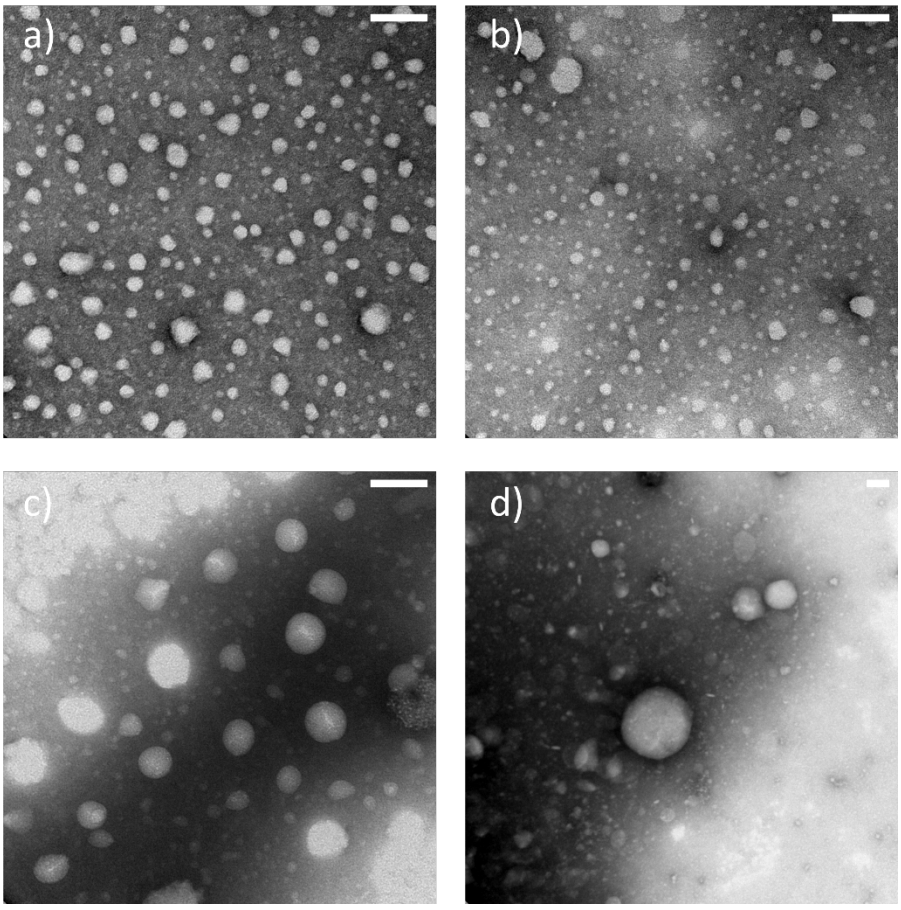

Figure S10: TEM images of nanoparticles S-Phe/C-Phe (a), S-Phe/C-Leu (b), S-Leu/C-Phe (c) and S-Leu/C-Leu (d). Images were taken with a 1% phosphotungstic acid stain, a, b and c have a magnification of 20k and d has a magnification of 8k. Scale bar represents 100nm.

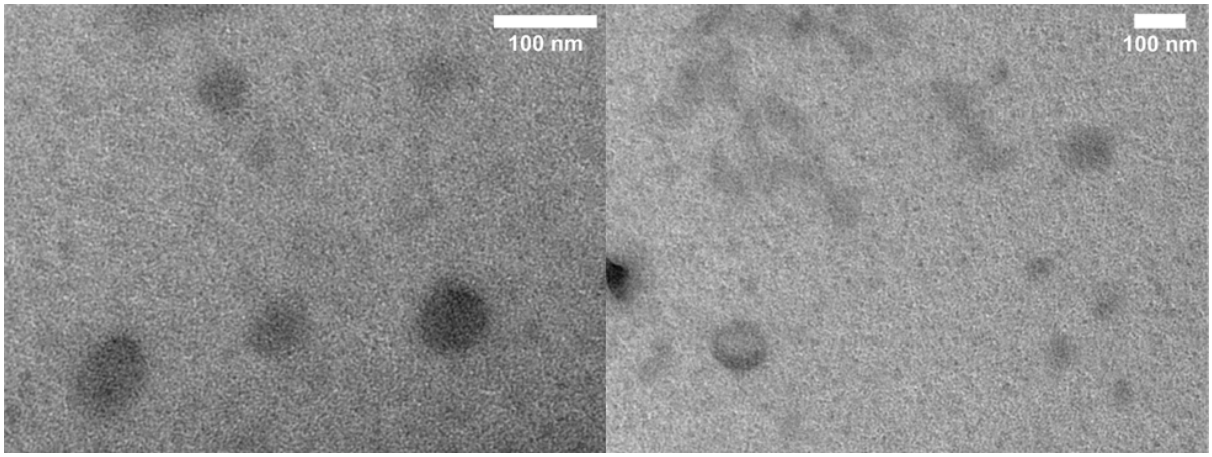

Figure S11: TEM images of nanoparticles S-Phe/C-Phe (left), S-Phe/C-Leu (right). Images were taken without a stain with magnification of 60k and 30k for left and right respectively.

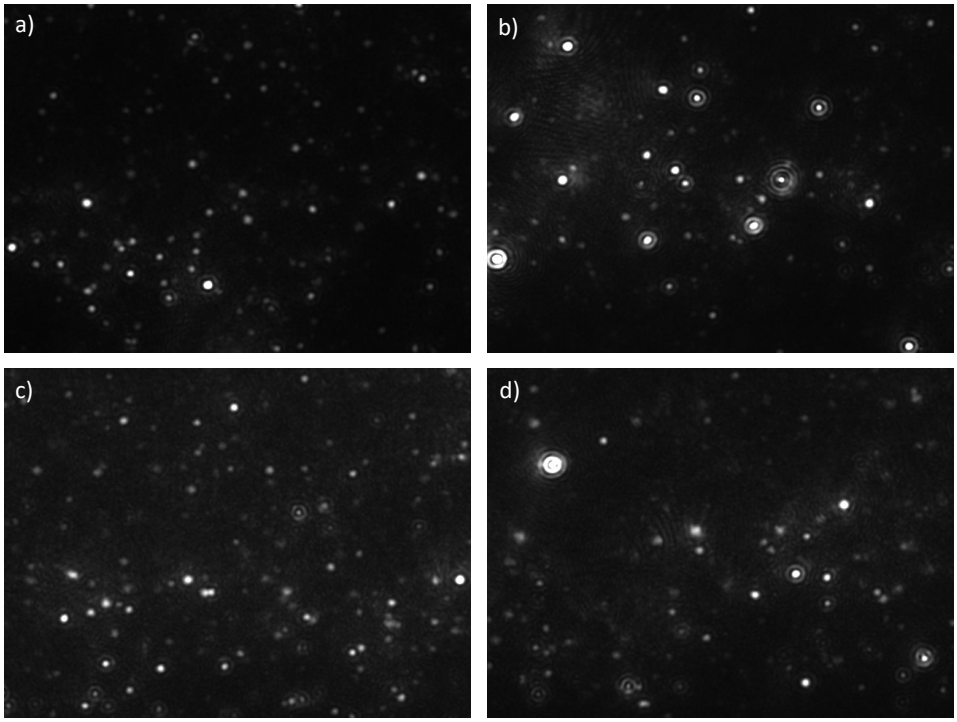

Figure S12: NTA still images of nanoparticles S-Phe/C-Phe (a), S-Phe/C-Leu (b), S-Leu/C-Phe (c) and S-Leu/C-Leu (d)

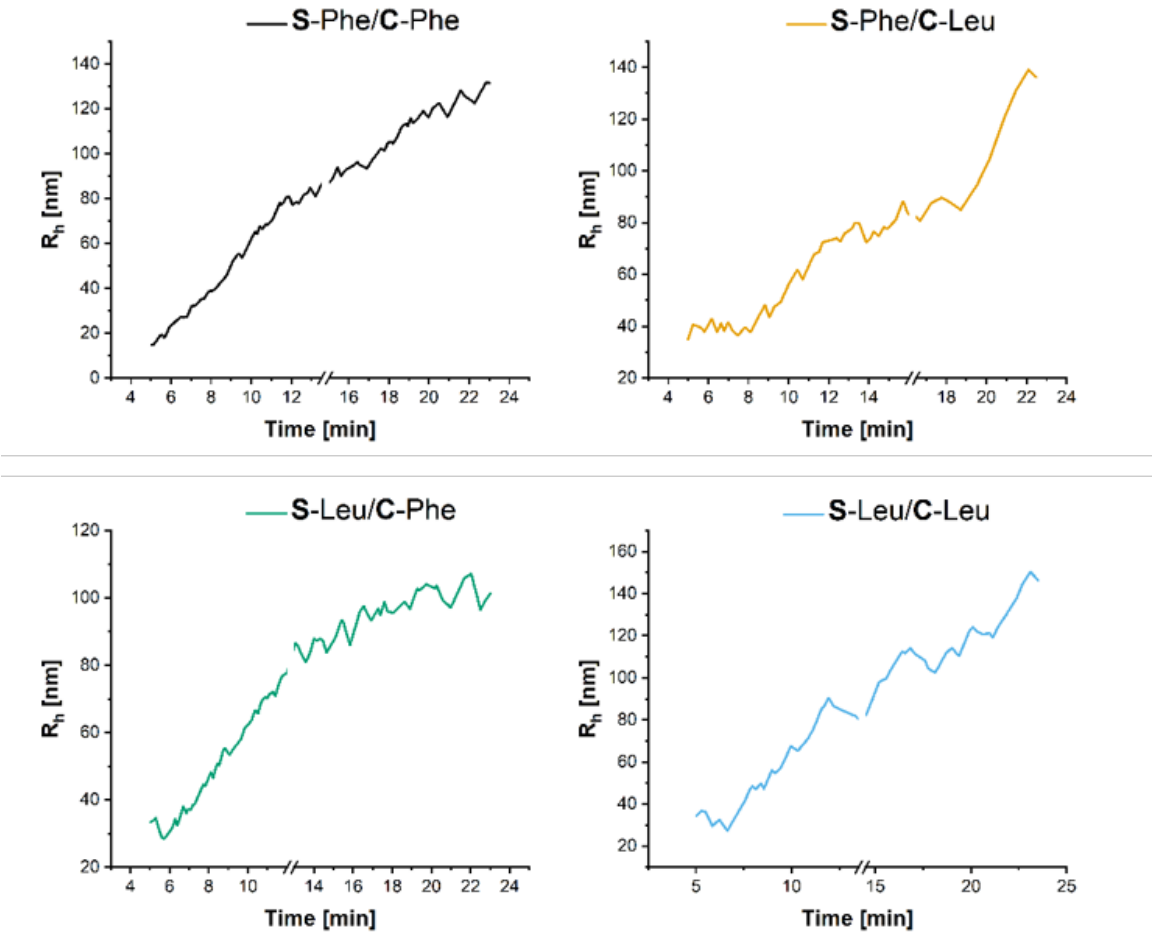

Figure S13: AF4 traces online DLS traces showing the Rh values for the whole sample for each variation tested.

[1] G. J. M. Habraken, M. Peeters, C. H. J. T. Dietz, C. E. Koning and A. Heise, Polymer Chemistry, 2010, 1, 514-524.

[2] J. Sun, X. Chen, C. Deng, H. Yu, Z. Xie and X. Jing, Langmuir, 2007, 23, 8308-8315.

[3] Z.-Y. Tian, Z. Zhang, S. Wang and H. Lu, Nature Communications, 2021, 12, 5810.