

Functionalized Triblock Copolymer Vectors for the Treatment of Acute Lymphoblastic Leukemia

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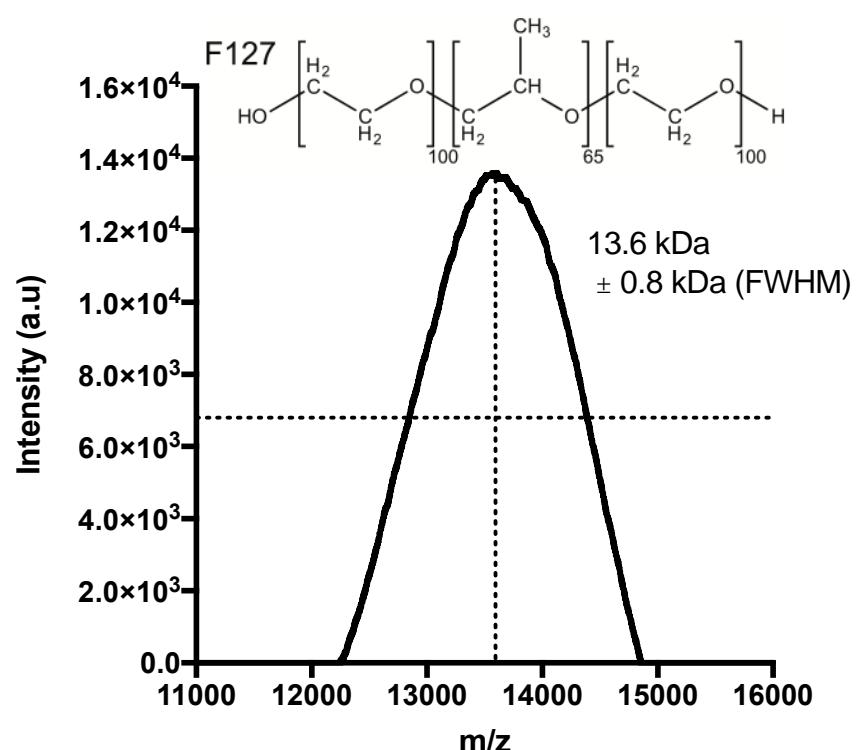
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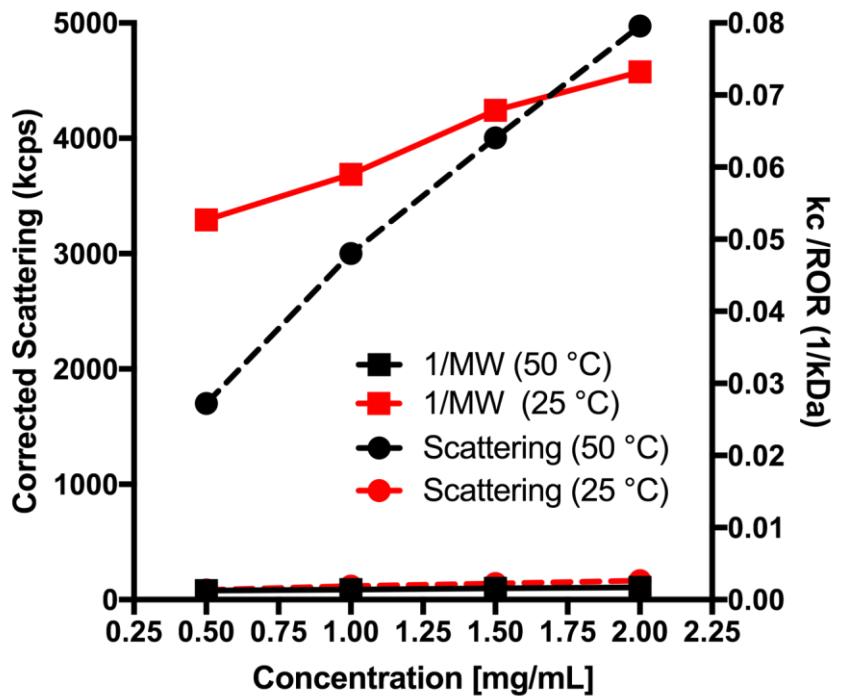
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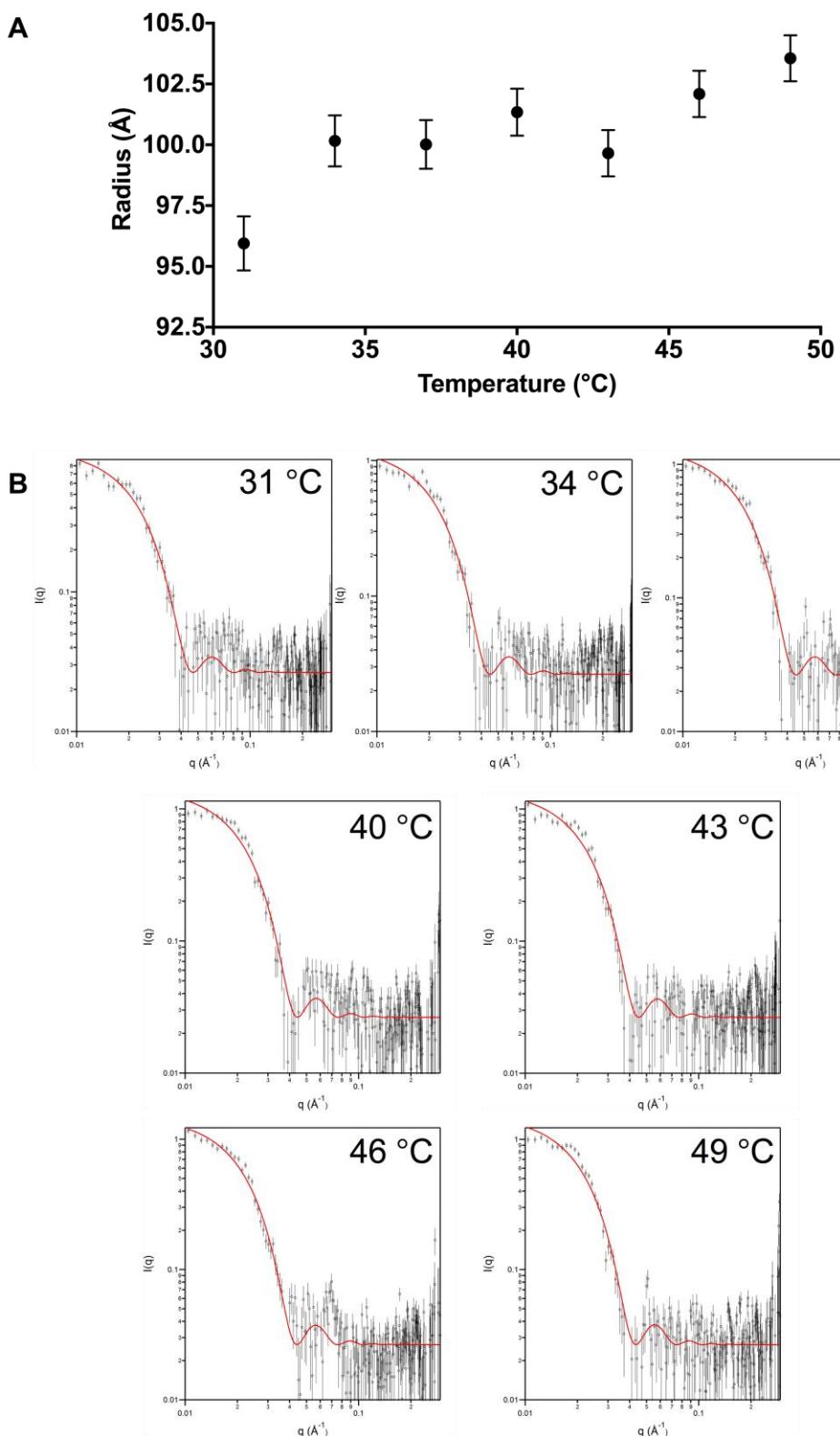
Supplementary Information.



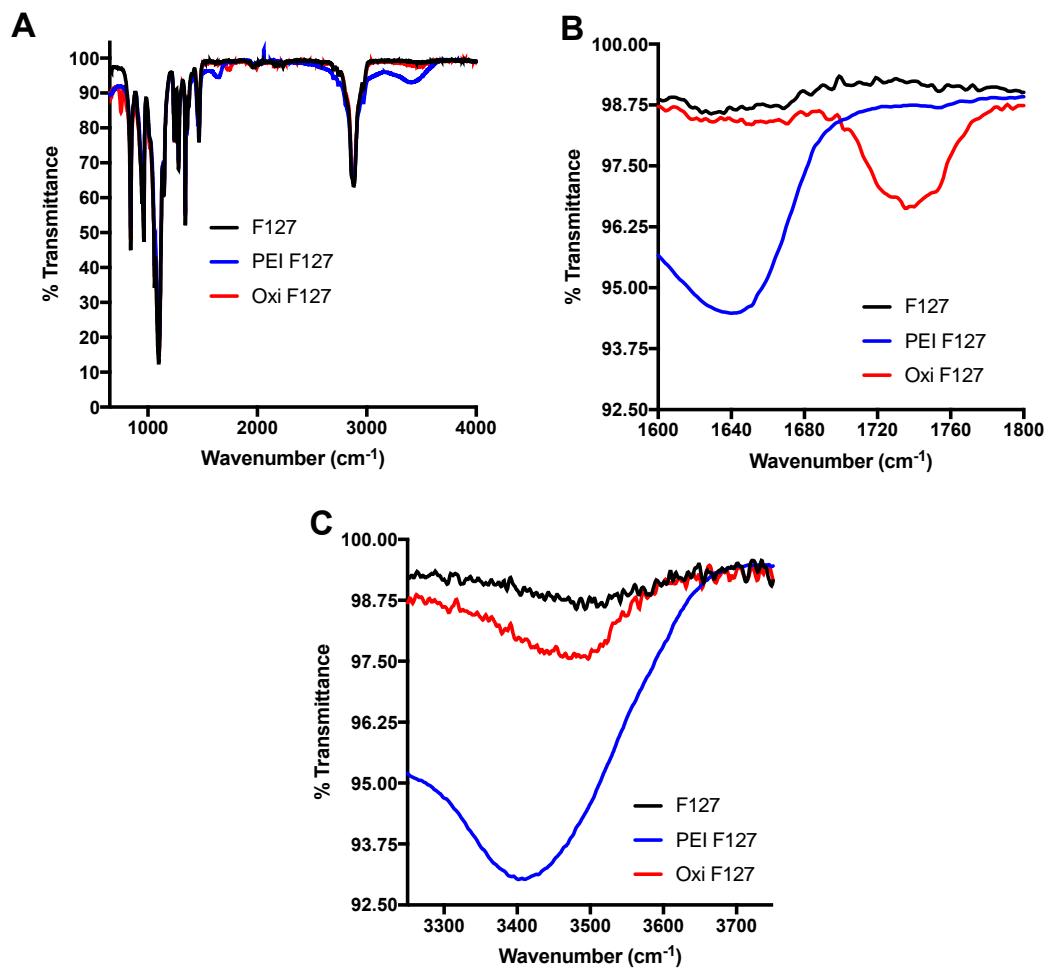
Supplementary Figure 1: MALDI-TOF Mass Spectroscopy from F127, showing a peak intensity of 13.6 kDa.



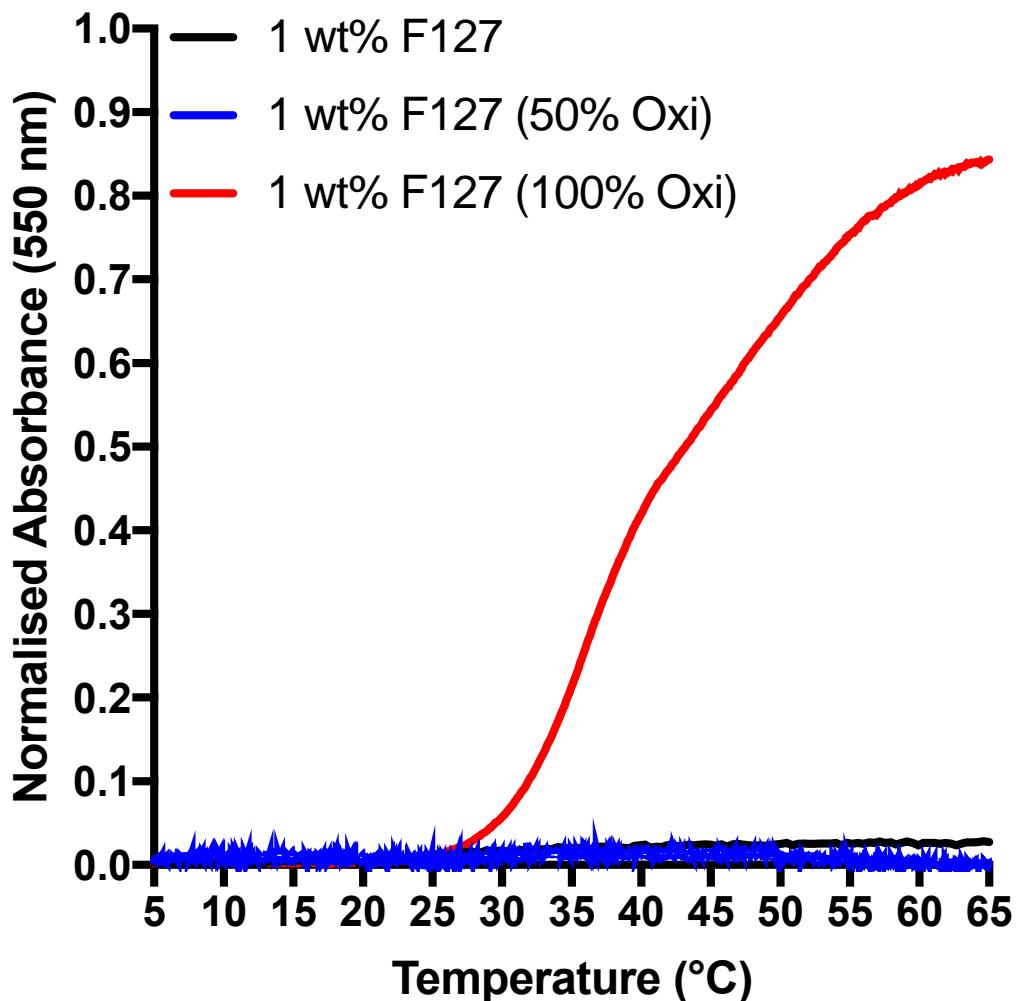
Supplementary Figure 2: DLS studies using previously reported refractive index increment values[42] for F127 at 25 °C and 50 °C allow for the estimation of the molecular weight of the monomer and micelle species as 21.9 ± 0.59 kDa and 891 kDa ± 17.3 kDa respectively.



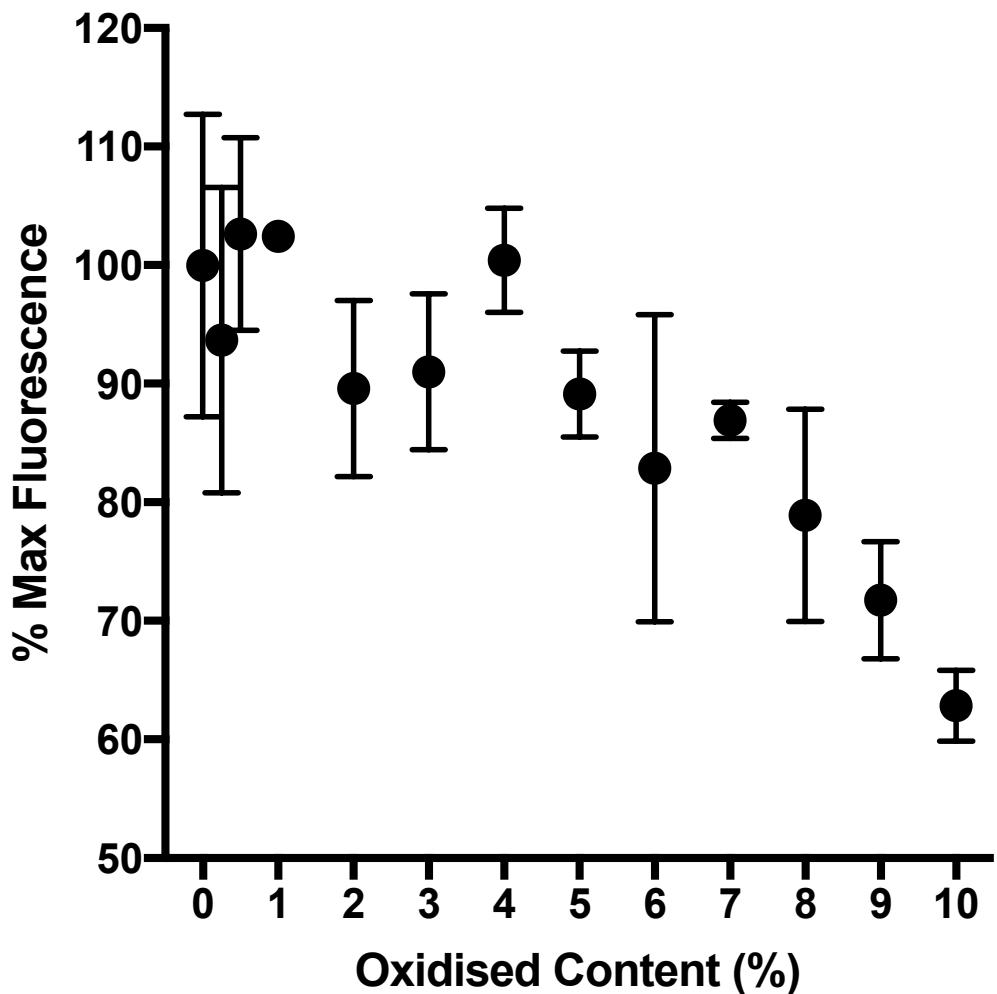
Supplementary Figure 3: SAXS data from 2 wt% F127 at various temperatures. **A.** Temperature (°C) vs. Radius (Å) between temperature 31 °C – 49 °C at 3 °C intervals highlighting increases in radii with temperature. **B.** SAXS profiles from 2 wt% F127 at various temperatures. Top row (left to right) 31°C, 34 °C, 37 °C, 40 °C. Bottom row (left to right) 43 °C, 46 °C, 49 °C.



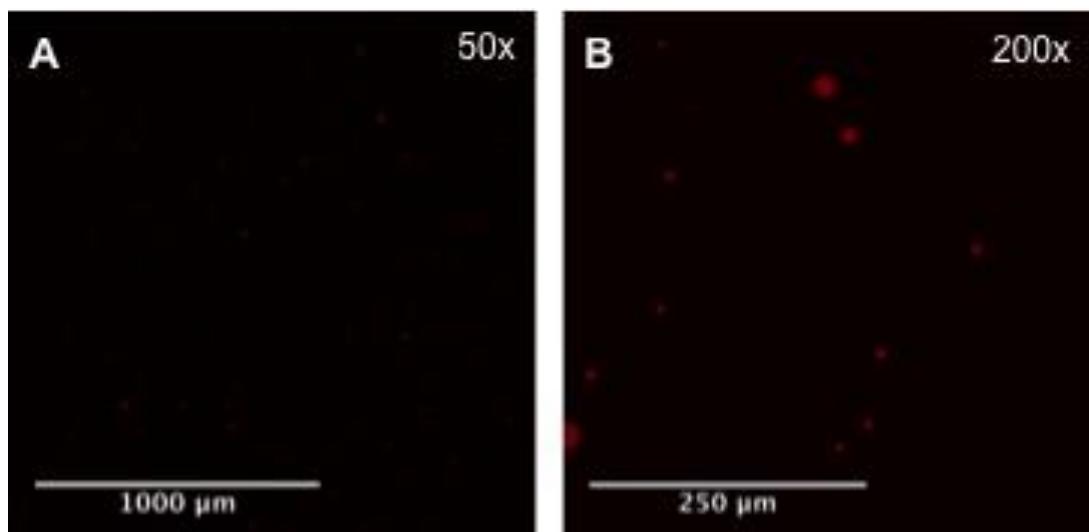
Supplementary Figure 4: FT-IR spectra from F127, oxidised F127 and PEI-F127, highlighting the presence of a carbonyl group (C=O stretch) at 1740 cm^{-1} indicative of successful oxidation and amine/amide groups (N-H stretch) at 3410 cm^{-1} . **A.** Full spectrum, **B.** carbonyl stretch insert and **C.** amine/amide stretch insert.



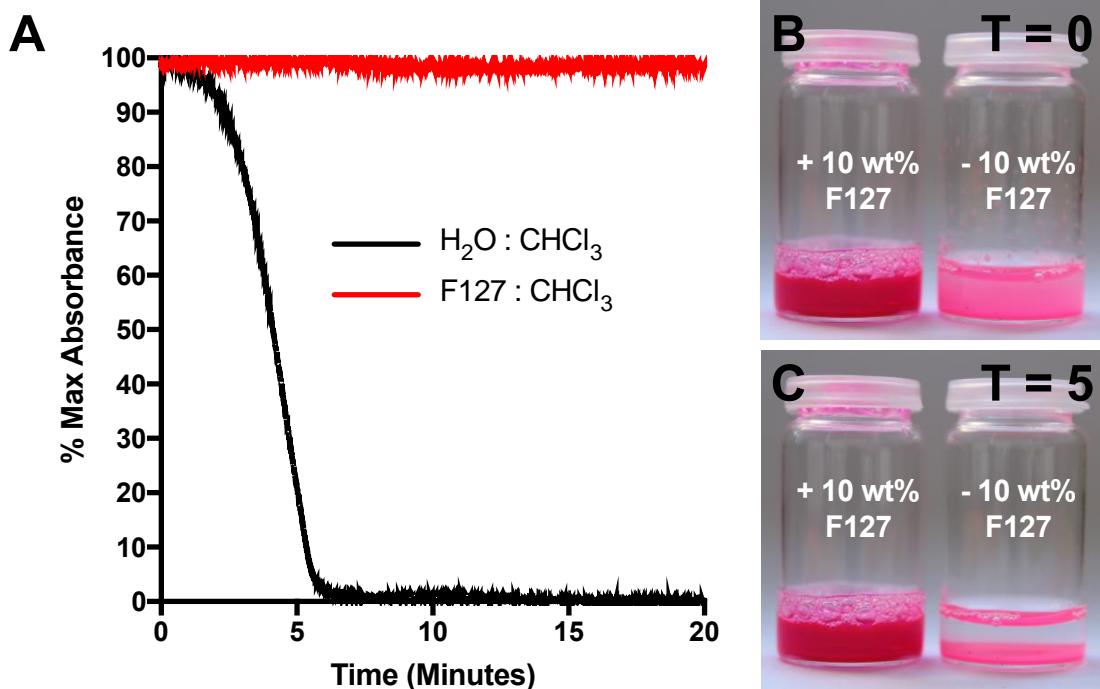
Supplementary Figure 5: Cloud point transitions of Pluronic F127 micelles (100% oxidised), F127 (50% oxidised) and F127 (0% oxidised) at 1 wt% between 5 °C and 65 °C. A ramp rate of 1 °C/min was used and turbidity was monitored by monitoring the absorbance at 550 nm.



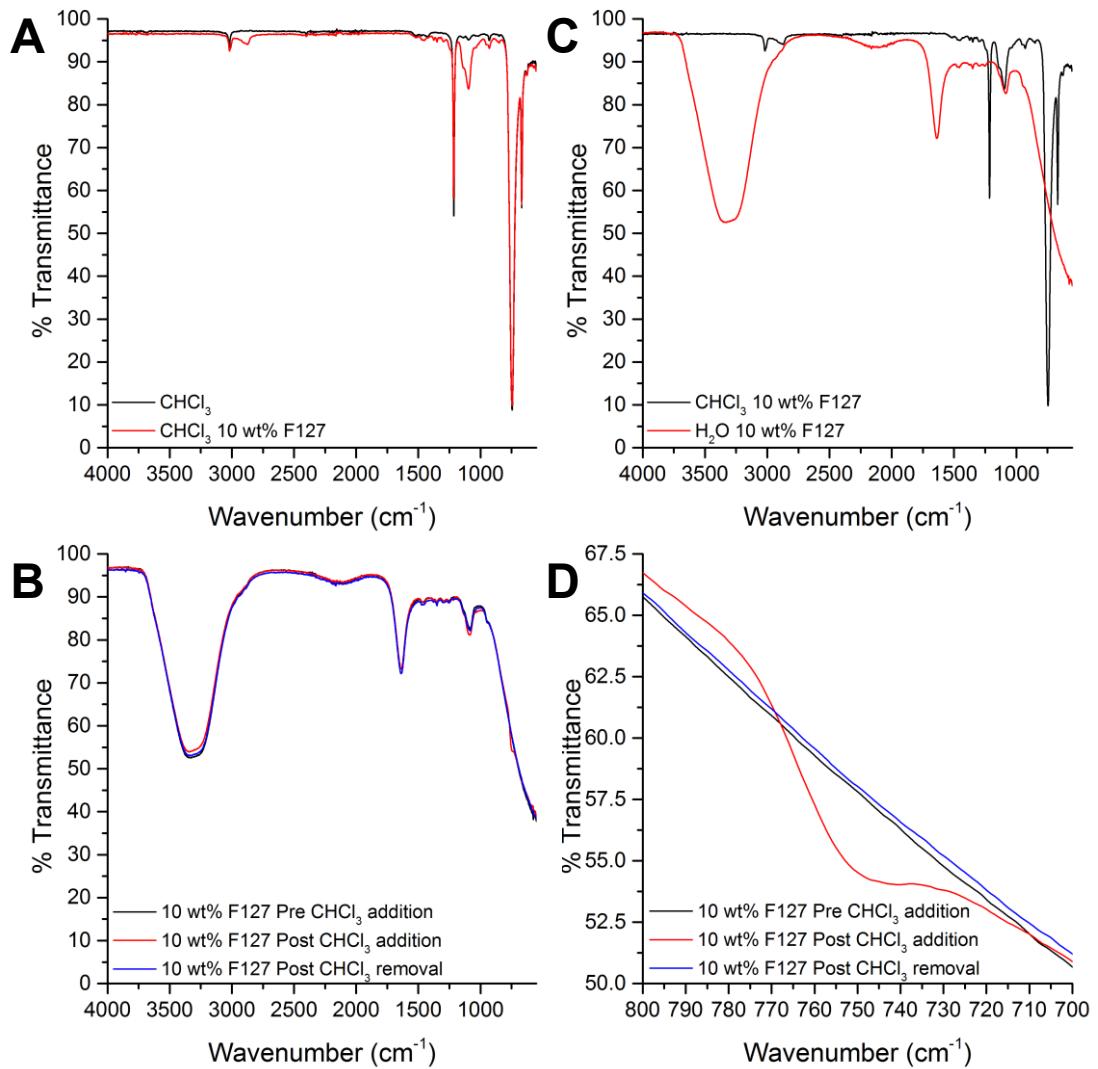
Supplementary Figure 6: Fluorescence intensity of DPH above the CMC/CMT from 5 wt% F127 with increasing oxidised F127 content. Data represent mean \pm SE ($n\geq 3$).



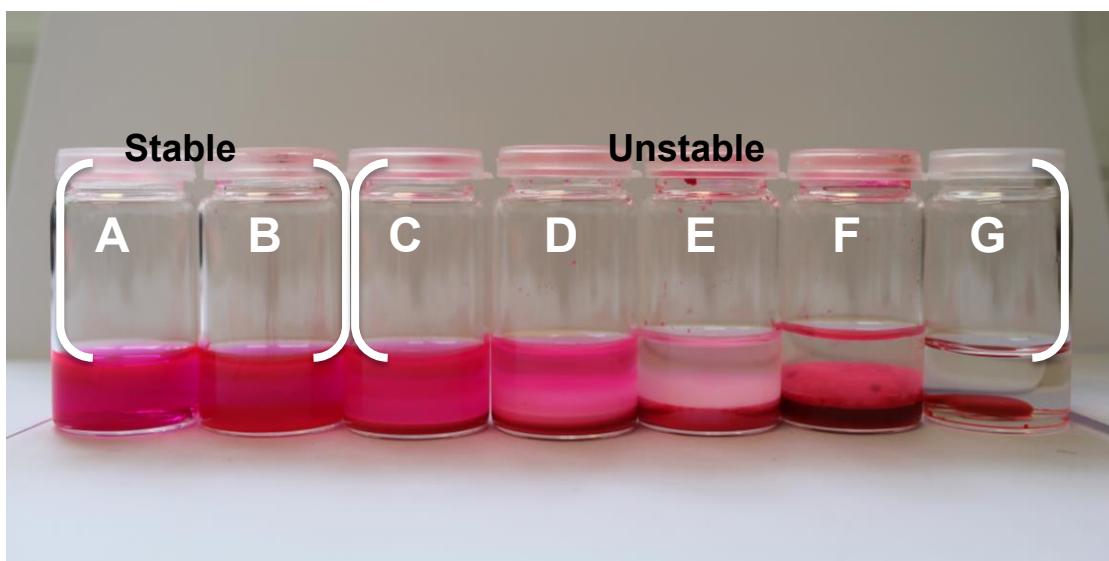
Supplementary Figure 7: Widefield fluorescence microscopy from 10 wt% F127 stabilised H₂O (95%) : CHCl₃ (5%) emulsion showing Nile Red (Log P = 3.834) partitioning into CHCl₃. Performed at 37°C. Magnification equals 50x (**A**) and 200x (**B**). **C.** Structure of the hydrophobic drug analogue Nile Red.



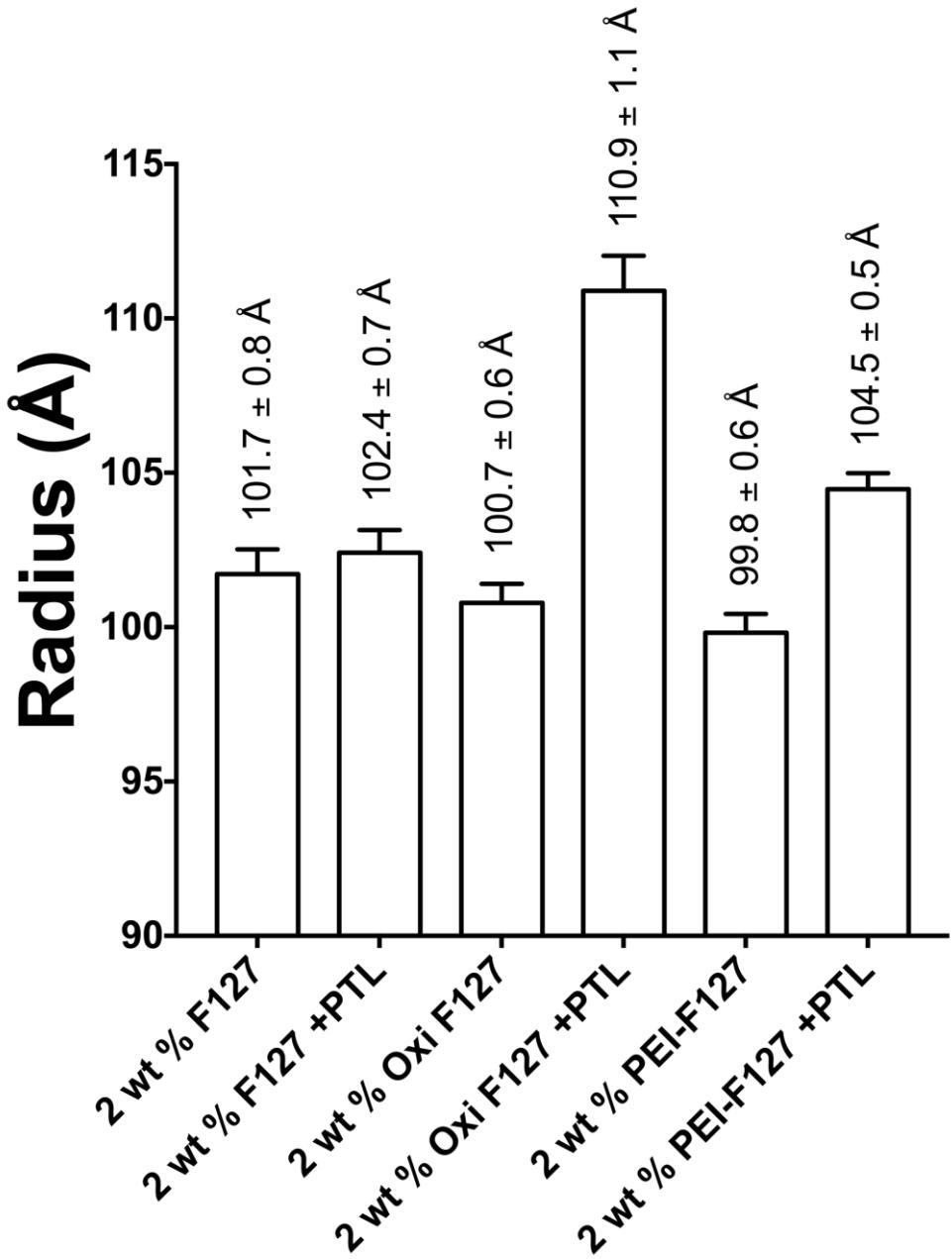
Supplementary Figure 8: **A.** Normalised absorbance at 550 nm and 37 °C over 20 minutes immediately after emulsification of a 10 wt% F127 stabilised H_2O (95%) : CHCl_3 (5%) (black) and H_2O (95%) : CHCl_3 (5%) (red) emulsions containing Nile Red. **B.** Photograph of a 10 wt% F127 stabilised H_2O (95%) : CHCl_3 (5%) emulsion highlighting Nile Red ($\text{Log } P = 3.834$) partitioning into CHCl_3 . **C.** Photograph of an equivalent F127 free unstabilised emulsion emphasising immiscibility of H_2O and CHCl_3 at 0 minutes (left) and 5 minutes (right) after emulsification.



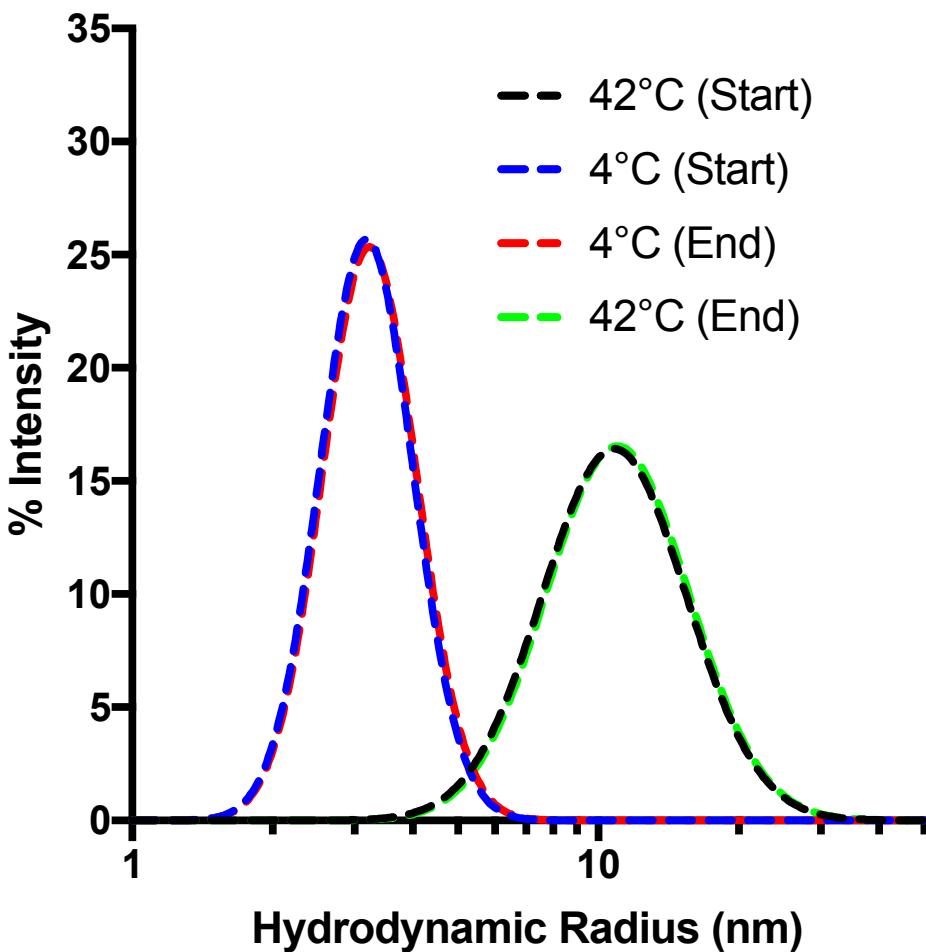
Supplementary Figure 9: FT-IR Spectra emphasizing CHCl₃ removal during rotary evaporation. **A.** CHCl₃ with and without 10 wt% F127. **B.** 10 wt% F127 in CHCl₃ and 10 wt% F127 in H₂O. **C.** 10 wt% F127 in H₂O pre and post (5% (v/v)) CHCl₃ addition and post (5% (v/v)) CHCl₃ removal **D.** Insert of 10 wt% F127 in H₂O pre and post (5% (v/v)) CHCl₃ addition and post (5% (v/v)) CHCl₃ removal.



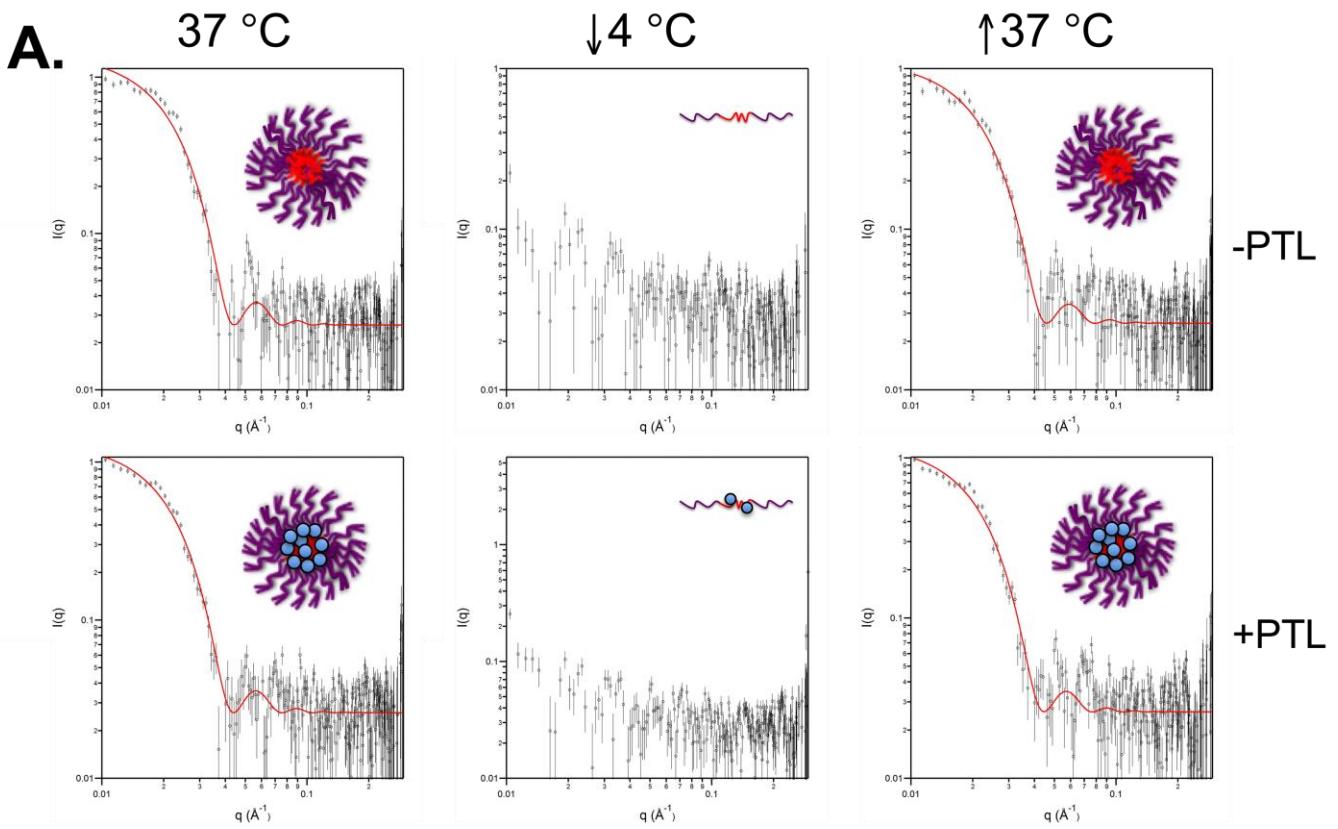
Supplementary Figure 10: Photograph highlighting stability of 1 mg/mL Nile Red in CHCl₃ in 10 wt% F127. 10 wt% F127 supplemented with 1 mg/mL Nile Red in CHCl₃ at room temperature (left to right) to concentrations of (A) 2 % (v/v), (B) 5 % (v/v), (C) 10 % (v/v), (D) 15 % (v/v), (E) 20 % (v/v) and (F) 30 % (v/v) 1 mg/mL Nile Red in CHCl₃ compared to (G) 5 % (v/v) 1 mg/mL Nile Red in CHCl₃ supplemented to H₂O alone.



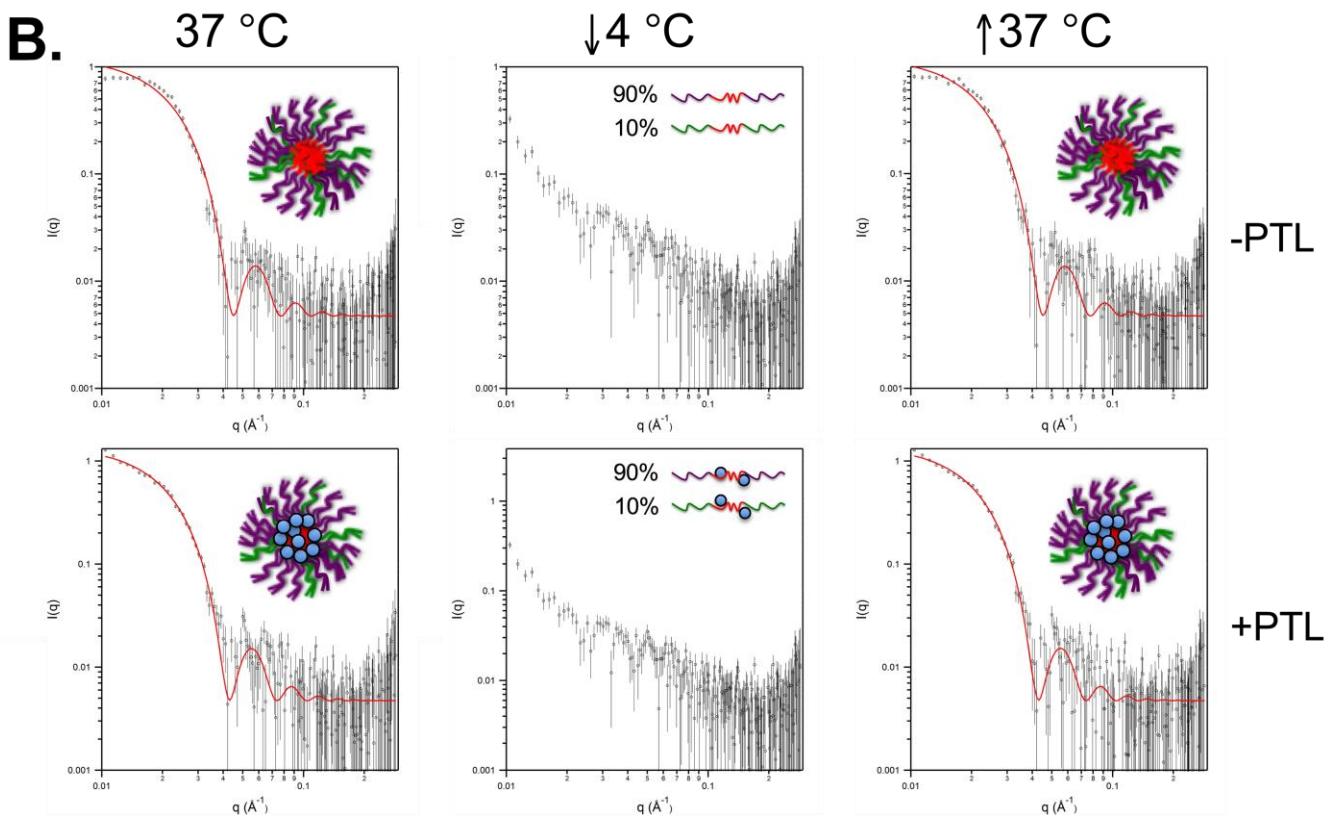
Supplementary Figure 11: Spherical radii derived from SAXS spectra of F127, Oxi-F127 & PEI-F127, highlighting size increases due to differences in packing in the presence of PTL.



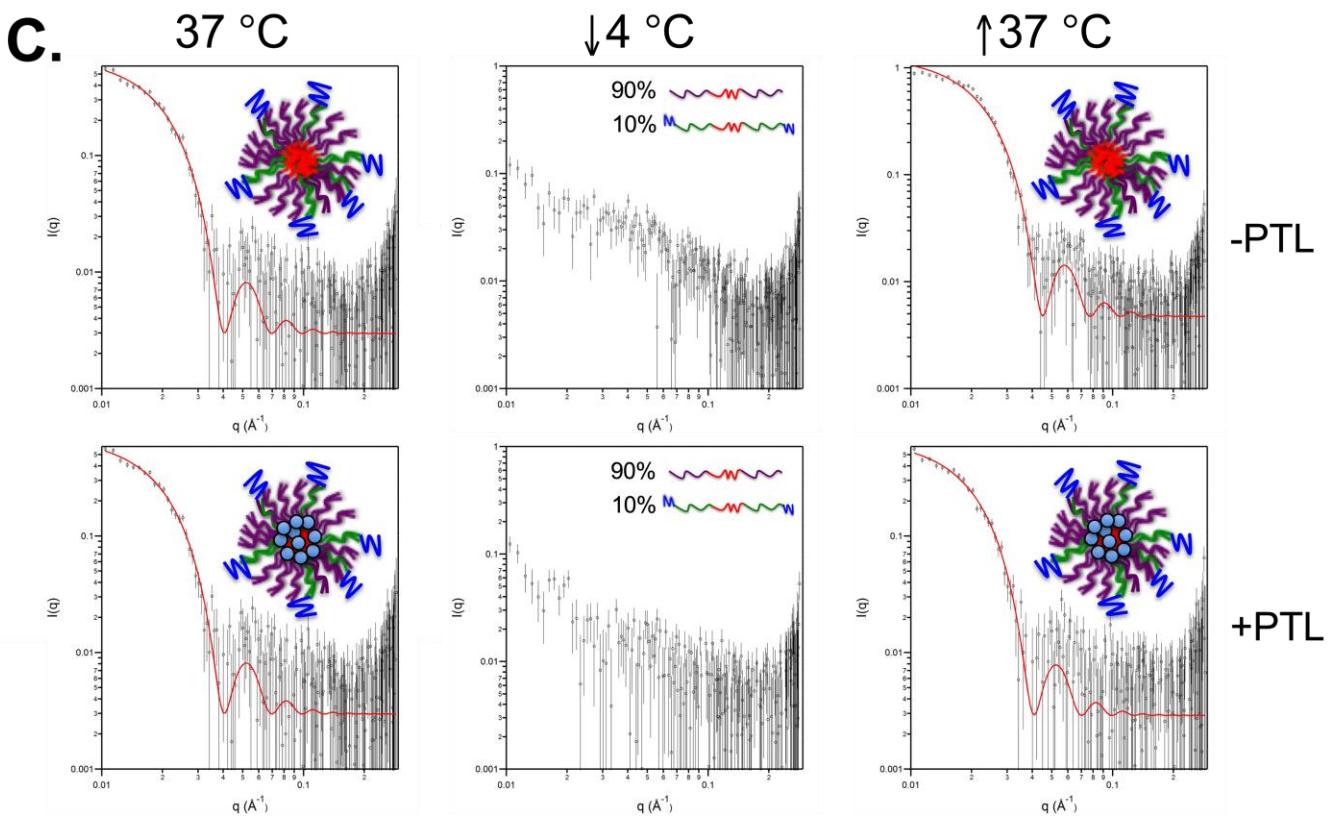
Supplementary Figure 12: DLS spectra from 10 wt% F127 loaded with 0.6 mg/mL PTL and subjected to variations in temperature above (42 °C) and below (4 °C) the CMT, highlighting retention of PTL even in the monomeric phase.



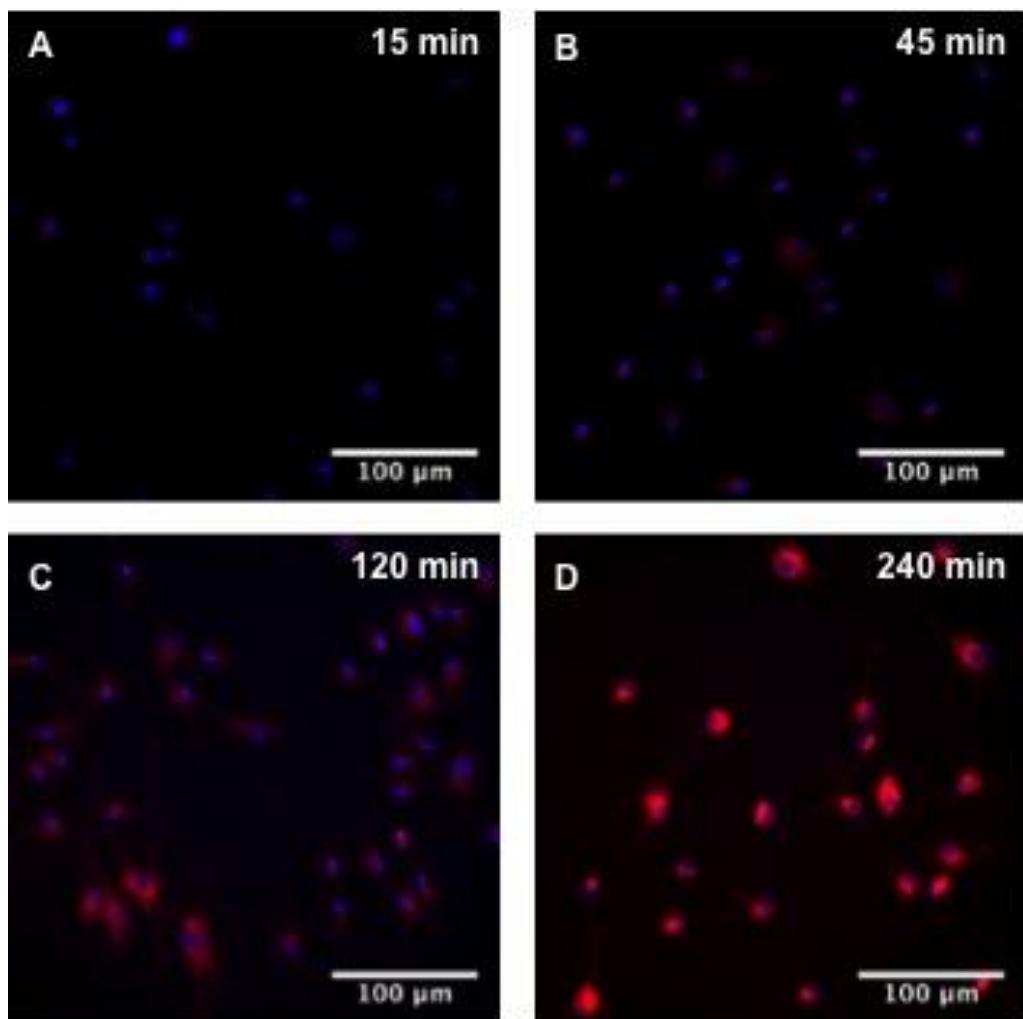
Supplementary Figure 13A: SAXS spectra examining thermal ($37^\circ\text{C} \rightarrow 4^\circ\text{C} \rightarrow 37^\circ\text{C}$) reversibility of Pluronic F127 micelles loaded with and without PTL, highlighting retention of PTL.



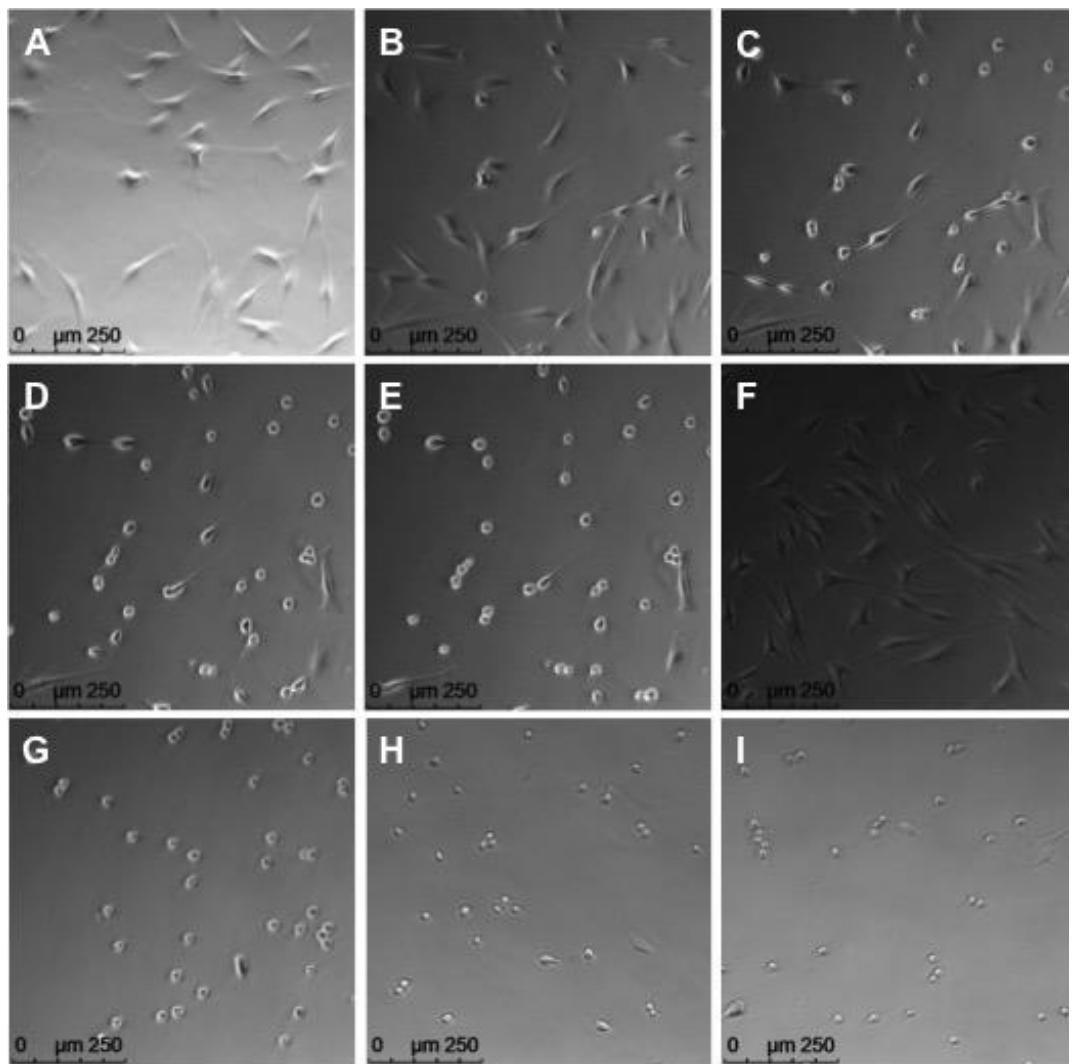
Supplementary Figure 13B: SAXS spectra examining thermal ($37^{\circ}\text{C} \rightarrow 4^{\circ}\text{C} \rightarrow 37^{\circ}\text{C}$) reversibility of Oxi-F127 micelles loaded with and without PTL, highlighting retention of PTL.



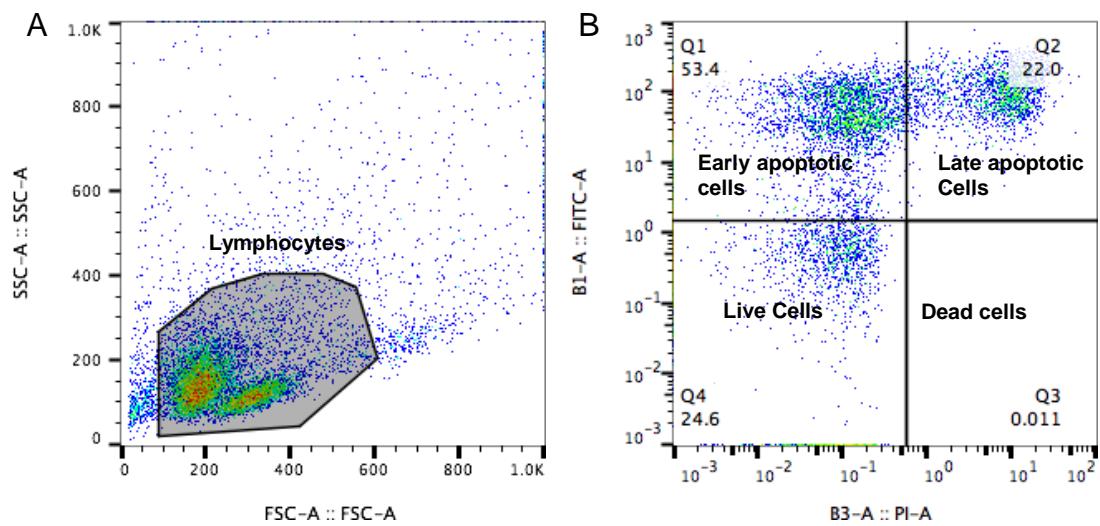
Supplementary Figure 13C: SAXS spectra examining thermal ($37^\circ\text{C} \rightarrow 4^\circ\text{C} \rightarrow 37^\circ\text{C}$) reversibility of PEI-F127 micelles loaded with and without PTL, highlighting retention of PTL.



Supplementary Figure 14: Fluorescence (widefield) imaging of hMSCs incubated with 1.33 wt% RITC-F127 (red) for 15 (A), 45 (B), 120 (C) and 240 (D) minutes and Hoechst 33342 (blue) nucleic acid stain for 30 minutes. Scale bars represent 100 μm .



Supplementary Figure 15: **A-E.** hMSCs treated with 72 μ M PTL, 1.5 wt% F127 after 0, 10, 20, 30 and 45 minutes. **F.** hMSCs treated with 1.5 wt% F127. **G.** hMSCs treated with 72 μ M PTL, 1.5 wt% 10% Oxi F127. **H.** hMSCs treated with 72 μ M PTL, 1.5 wt% 10% PEI F127. **I.** hMSCs treated with 72 μ M PTL, 0.5% MeOH.



Supplementary Figure 16: Flow cytometry gating strategy to assess viability. T-ALL lymphoblasts were initially gated on the basis of forward and side scatter characteristics (A). Viability of lymphoblasts gated in (A) were assessed by Annexin V -FITC and PI staining (B).

Supplementary Table 1. SAXS data parameters, fit parameters and calculated values for 2 wt% F127 in water

Sample	2 wt% Neutral				2 wt% Neutral + PTL			
Temperature (°C)	<i>37</i>		<i>37 return</i>		<i>37</i>		<i>37 return</i>	
Fit parameters								
Scale	0.018		0.018		0.018		0.018	
Radius (Å)	101.67	± 0.80	98.15	± 0.91	102.51	± 0.74	101.06	± 0.78
SLD sphere (Å ⁻²)	9.01E-06	± 3.23E-09	9.04E-06	± 3.56E-09	9.03E-06	± 2.79E-09	9.04E-06	± 2.92E-09
SLD solvent (Å ⁻²)	9.43E-06		9.43E-06		9.43E-06		9.43E-06	
Background (cm ⁻¹)	0.025		0.027		0.027		0.027	
SAXS parameters								
I(0) (cm ⁻¹) [real]	1.37	± 0.03	1.12	± 0.02	1.35	± 0.03	78.81	± 0.83
R _g (Å) [real]	78.69	± 0.63	76.53	± 0.75	79.49	± 0.82	1.24	± 0.02
I(0) (cm ⁻¹) [reciprocal]	1.10	± 0.04	0.95	± 0.06	1.10	± 0.04	1.02	± 0.03
R _g (Å) [reciprocal]	59.71	± 8.18	60.72	± 11.21	63.73	± 13.56	64.89	± 13.40
Calculated parameters								
Volume	4402173	± 104488	3960817	± 110439				
Fraction of PLU (f)	0.20	± 0.04	0.18	± 0.05				
Aggregation number	46	± 2	38	± 2				

Supplementary Table 2. SAXS data parameters, fit parameters and calculated values for 2 wt% negative F127 in water

Sample	2 wt% Negative				2 wt% Negative + PTL			
Temperature (°C)	37		37 return		37		37 return	
Fit parameters								
Scale		0.018		0.018		0.018		0.018
Radius (Å)	100.79	± 0.62	99.93	± 0.63	110.89	± 1.14	110.03	± 1.16
SLD sphere (Å ⁻²)	9.01E-06	± 2.53E-09	9.02E-06	± 2.57E-09	9.17E-06	± 2.44E-09	9.17E-06	± 2.49E-09
SLD solvent (Å ⁻²)		9.43E-06		9.43E-06		9.43E-06		9.43E-06
Background (cm ⁻¹)		0.005		0.005		0.003		0.003
SAXS parameters								
I(0) (cm ⁻¹) [real]	1.05	± 0.03	1.02	± 0.03	0.69	± 0.03	0.67	± 0.02
R _g (Å) [real]	58.49	± 8.16	58.79	± 3.25	87.21	± 1.60	86.95	± 1.62
I(0) (cm ⁻¹) [reciprocal]	1.34	± 0.02	1.29	± 0.03	0.55	± 0.03	0.58	± 0.03
R _g (Å) [reciprocal]	78.44	± 0.62	78.54	± 0.68	68.66	± 11.08	73.91	± 9.90
Calculated parameters								
Volume	4288340	± 79077	4179812	± 78640				
Fraction of PLU (f)	0.20	± 0.04	0.20	± 0.04				
Aggregation number	45	± 2	44	± 2				

Supplementary Table 3. SAXS data parameters, fit parameters and calculated values for 2 wt% positive F127 in water

Sample	2 wt% Positive				2 wt% Positive + PTL			
Temperature (°C)	37		37 return		37		37 return	
Fit parameters								
Scale		0.018		0.018		0.018		0.018
Radius (Å)	99.41	± 0.60	99.41	± 0.61	104.11	± 0.52	104.12	± 0.51
SLD sphere (Å ⁻²)	9.02E-06	± 2.47E-09	9.02E-06	± 2.49E-09	9.02E-06	± 1.92E-09	9.02E-06	± 1.92E-09
SLD solvent (Å ⁻²)		9.43E-06		9.43E-06		9.43E-06		9.43E-06
Background (cm ⁻¹)		0.002		0.002		0.003		0.003
SAXS parameters								
I(0) (cm ⁻¹) [real]	1.28	± 0.02	1.27	± 0.03	1.40	± 0.03	1.46	± 0.01
R _g (Å) [real]	78.21	± 0.59	78.25	± 0.64	81.87	± 0.77	82.74	± 0.22
I(0) (cm ⁻¹) [reciprocal]	0.99	± 0.04	0.97	± 0.61	Insufficient Scattering Data			
R _g (Å) [reciprocal]	60.31	± 8.89	58.40	± 10.74				
Calculated parameters								
Volume (Å ³)	4115470	± 74930	4115470	± 75578				
Fraction of PLU (<i>f</i>)	0.20	± 0.04	0.20	± 0.04				
Aggregation number	43	± 2	43	± 2				