Supporting Information

Giant polymersomes from non-assisted film hydration of phosphate-based block copolymers

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Materials and Instrumentation

Dialysis was performed using a Pre-wetted Spectra/Por® 6 Standard Regenerated Cellulose tubing (45*29 mm, MWCO 1000).

Polymer analysis

^{1}H NMR, ^{31}P NMR and ^{1}H DOSY spectra were measured on a Bruker 500 AMX NMR. All spectra were recorded at room temperature in $CDCl_3$. Gel Permeation chromatography (GPC) measurements were performed in THF with a PSS SecCurity system (Agilent Technologies 1260 Infinity). Sample injection was performed by a 1260-ALS autosampler (Agilent) at 30 °C with a flow rate of 1 ml/min. SDV(PSS) columns with dimensions of 0.8*30 cm, 10 µm particle size, and pore sizes of 10^6, 10^4, and 500 Å were employed. The 1260-RID and UV 1260-VWD detectors (Agilent) were used for detection. The thermal properties of the synthesized polymers have been measured by differential scanning calorimetry (DSC) on a Mettler Toledo instrument DSC823 under nitrogen atmosphere at a heating rate of 10 $K.min^{-1}$. The glass transition temperatures and melting temperature were determined from midpoints in the second heating using the STARe software of Mettler-Toledo. The interfacial tensions of the block copolymers between $CHCl_3$ and H_2O were measured at the spinning drop tensiometer SVT 20N from DataPhysics. A glass capillary was filled with block copolymer mixture in $CHCl_3$ (1.0 mg/mL, ~1.2 g) and a small droplet of H_2O (~0.012 g). Then the capillary was placed horizontally and equilibrated at 20 °C for 10 min under rotation at 8000 rpm to obtain one cylindrical droplet at the axis of rotation. The interfacial tension based on the theory of Vonnegut was measured over 10 min.[1]

Microscopy imaging

Optical and fluorescence microscopy were carried out on an inverted fluorescence microscope Leica DMi8. Images were recorded with a Hamamatsu Orca Flash 4.0 digital Camera C11440 on phase contrast, Cy5 filter (649/666; 1000 ms) and TRITC filter (535/590, 1000 ms) using a Leica HC PL

FLUOTAR L 20x/0.40 Corr PH 1 (506243) air objective and a Leica HC PL FLUOTAR L 40x/0.60 Corr PH 2 (506203) air objective. The vesicles were analysed using ImageJ.

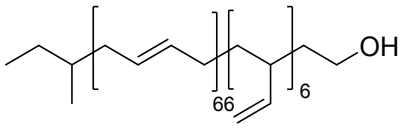
Chemicals

Chemicals and reagents were obtained from Sigma Aldrich, Acros Organics, ThermoFisher, Invitrogen™, polymer Source™ and were used as received unless otherwise stated. Dry tetrahydrofuran and toluene were purchased from Sigma Aldrich or Acros Organics with an AcroSeal®, a seal advertised for dry solvent, stored under inert atmosphere over molecular sieves and was used as such. All reactions involving oxygen/moisture sensitive reagents were performed with anhydrous solvents under a positive pressure of anhydrous argon, using standard Schlenk techniques. Cooling of reaction mixtures to 0 °C was achieved using an ice/water bath. Heating was performed using Drysyn® heating blocks or oil bath.

SYNTHETIC PROCEDURES AND CHARACTERIZATION

Starting Materials

*Polybutadiene (PB-OH) (**1**)*



According to a modified literature procedure,[2] in a flame-dried flask, under inert atmosphere, butadiene (10 g, 0.19 mol) was cryo-transferred to cyclohexane (200 mL) using a isopropanol/dry ice cooling bath. *Sec*-butyllithium (1.4 M, 1.9 mL) was then added while cooling. The polymerisation was continued overnight at room temperature. Hydroxylation was achieved by adding an excess of ethylene oxide (4 mL) by cryo-transfer to the reaction mixture. After 30 min at low temperature, the

mixture was left for 4 h at room temperature. The polymer was terminated using methanol. **^{1}H NMR** (300 MHz, $CDCl_3$) δ_H/ppm 5.60 (dtd, *J* = 17.9, 6.1, 2.9 Hz, 6H, 6* C**H**=CH_2), 5.53–5.29 (m, 134H, 67*C**H**=C**H**), 5.00 (tq, *J* = 12.1, 4.0 Hz, 12H, 6* CH=C**H$_2$**), 3.73–3.61 (m, 2H, C**H$_2$**-O), 2.23–1.91 (m, 274H, C**H$_2$** and C**H**), 1.80–1.00 (m, 17H, C**H$_2$** and C**H**), 0.97–0.76 (m, 6H, 2* C**H$_3$**).

^{1}H NMR

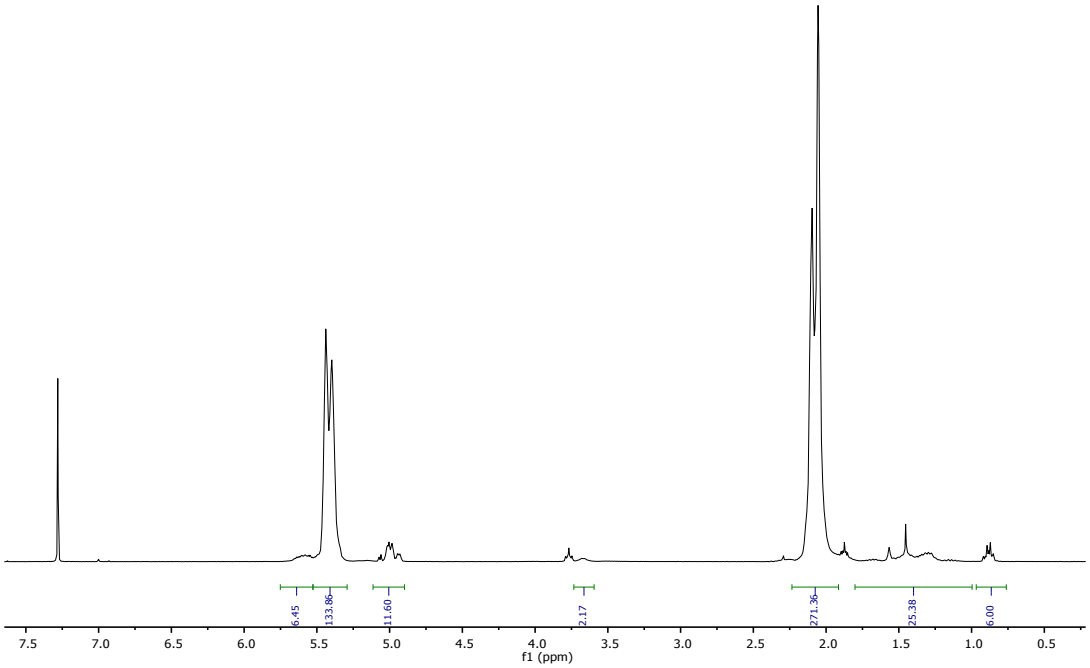
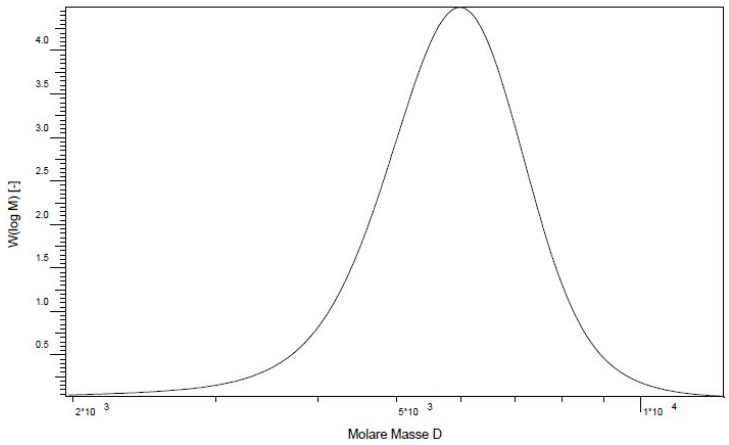


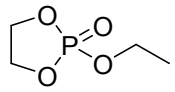
Figure S1. ^{1}H NMR spectra of the PB-OH macroinitiator.

Molar Mass Dispersity (THF, PB calibration)



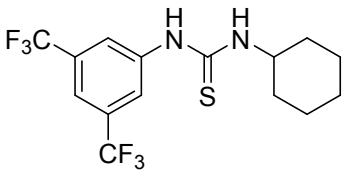
Mn	MW	D	Vp	Mp	Area
5608.27	5937.27	1.06	27.07	5930.26	0.12

*Ethyl Ethylene Phosphate (EEP) (**2**)*



According to a literature procedure,[3] in a flamed dried flask under inert atmosphere, a solution of ethanol (6.32 g, 137 mmol) and triethylamine (13.9 g, 137 mmol) in THF (15 mL) was added dropwise to a stirred solution of 2-chloro-1,3,2-dioxaphospholan 2-oxide (19.6 g, 137 mmol) in THF (80 mL). The reaction was further stirred for 2 h. After completion, the solution was filtrated under inert atmosphere and the filtrate was left overnight at −20 °C for complete precipitation of triethylammonium chloride. Then the solution was concentrated under reduced pressure and distilled at reduced pressure (b.p. 160 °C at $8.0 \cdot 10^{-1}$ mbar) to yield the product as a colourless liquid (12.3 g, 83.0 mmol, 61%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ_H/ppm 4.42-4.24 (m, 4H, O-C**H$_2$**-C**H$_2$**-O), 4.17-4.06 (m, 2H, O-C**H$_2$**-CH_3), 1.28 (t, J = 7.1 Hz, 3H, O-CH_2-C**H$_3$**); **^{31}P NMR** (121 MHz, $CDCl_3$) δ_P/ppm 17.4 (s, 1P).

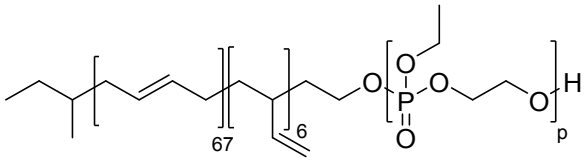
N-[3,5-bis(trifluoromethyl)phenyl]-N'-cyclohexyl-thiourea (TU)



According to a literature procedure,[4] in a flame dried flask, cyclohexylamine (0.91 g, 9.2 mmol) was added dropwise to a stirred solution of 3,5-bis(trifluoromethyl)phenyl isothiocyanate (2.6 g, 9.5 mmol) in THF (10 mL). After stirring for 5 h at room temperature, the reaction mixture was concentrated

under reduced pressure. The crude product was recrystallized from $CHCl_3$ (3.5 mL), filtered, washed with hot $CHCl_3$ and dried under reduced pressure yielding the title product as a white crystalline solid (1.6 g, 4.4 mmol, 48%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ_H/ppm 7.73 (d, *J* = 15.2 Hz, 3H, Ar-**H**), 5.99 (br s, 1H, N**H**), 4.20 (br s, 1H, N**H**), 2.08 (dq, *J* = 12.5, 3.9 Hz, 2H, Cy-**H**), 1.68 (ddt, *J* = 37.5, 13.2, 4.3 Hz, 4H, Cy-**H**), 1.42 (tdd, *J* = 11.4, 8.1, 3.6 Hz, 2H, Cy-**H**), 1.34–1.05 (m, 3H, Cy-**H**).

PB-b-PEEP

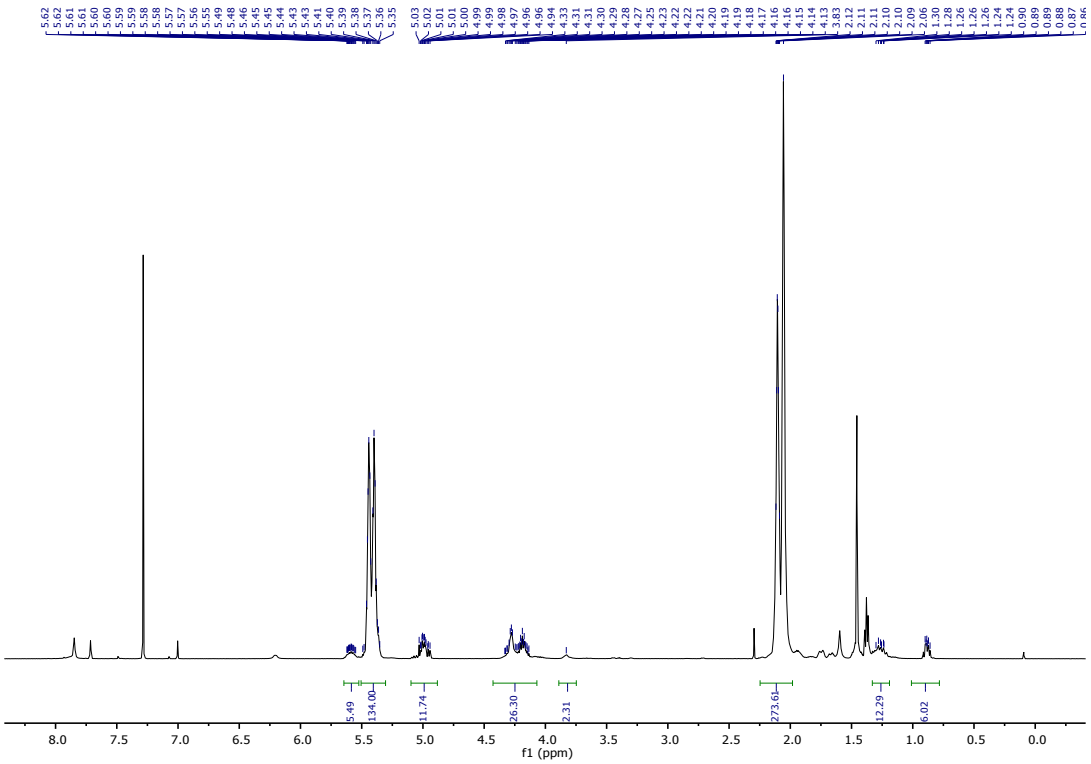


General procedure

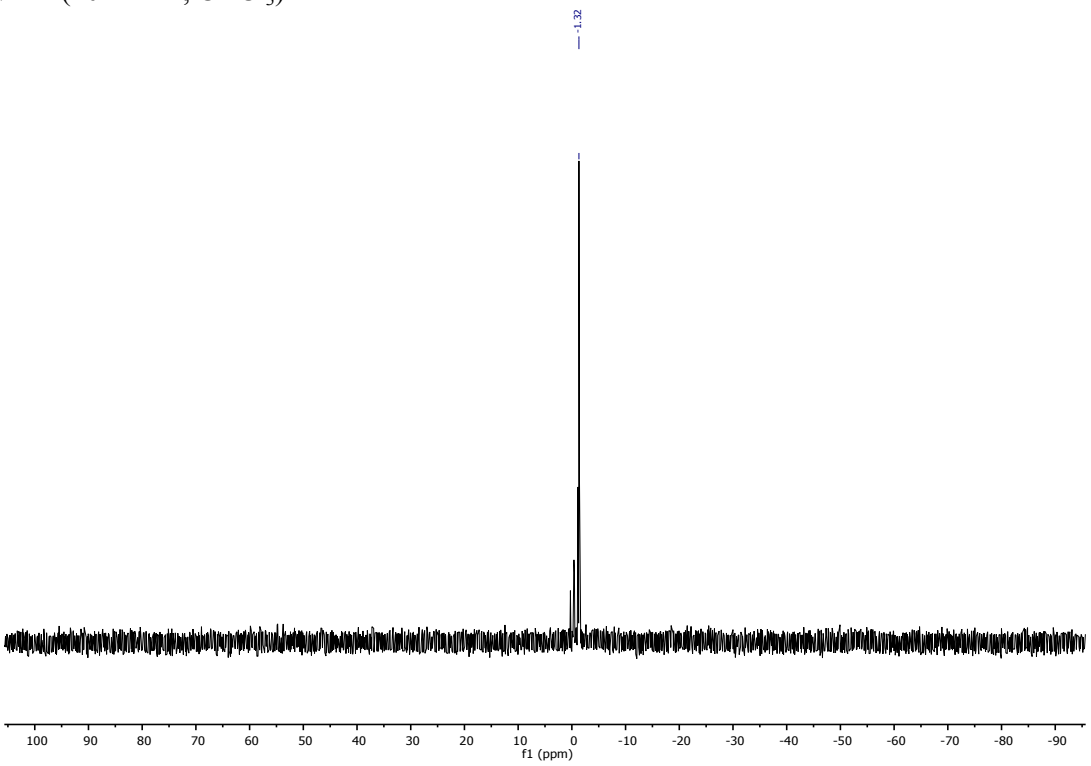
PB-OH and TU were dried under reduced pressure from toluene. EEP and DBU were distilled and stored under inert atmosphere. Based on a modified literature procedure,[3] to a solution of PB_{73}-OH (400 mg, 100 mmol, 1.0 eq) and TU (190 mg, 500 mmol, 5.0 eq) in dry THF (2.0 mL) under inert atmosphere, EEP (100p mmol, p eq) was added. After cooling the solution to 0 °C, DBU (75 µl, 500 mmol, 5.0 eq) was added. The polymerisation was carried out for 30 min and terminated using acetic acid in THF (300 µL, 5.0 mmol, 1.0 M, 50 eq). The colourless solution mixture was dialysed against H_2O in a 1000 MWCO regenerated cellulose dialysis tube for 24 h. The resulting white suspension dissolved in THF and dried under reduced pressure to obtain a pale yellow sticky oil. **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H/ppm 5.59 (dddd, *J* = 14.6, 12.9, 5.9, 3.1 Hz, 6H, 6*C**H**=CH_2), 5.56 – 5.26 (m, 134H, 67*C**H**=C**H**), 5.21–4.89 (m, 12H, 6*CH=C**H$_2$**), 4.49–3.97 (m, p*6H, p*(C**H$_2$**-O)$_3$P=O), 3.83 (s, 2H, C**H$_2$**-OH), 2.24–1.88 (m, 274H, C**H$_2$** and C**H**), 1.98–1.88 (m, 7H, C**H$_2$** and C**H**), 1.75 (dt, *J* = 13.4, 4.1 Hz, 5H, C**H$_2$** and C**H**), 1.67 (dt, *J* = 13.1, 3.9 Hz, 4H, C**H$_2$** and C**H**), 1.38 (t, *J* = 6.9 Hz, p*3H, p*C**H$_3$**-CH_2-O), 1.15 (m, 1H, C**H$_2$** and C**H**), 0.96–0.72 (m, 6H, (C**H$_3$**)$_2$-CH). **^{1}P NMR** (202 MHz, $CDCl_3$) δ_P/ppm −1.3 (s, 1P).

PB_{73}-b-$PEEP_4$

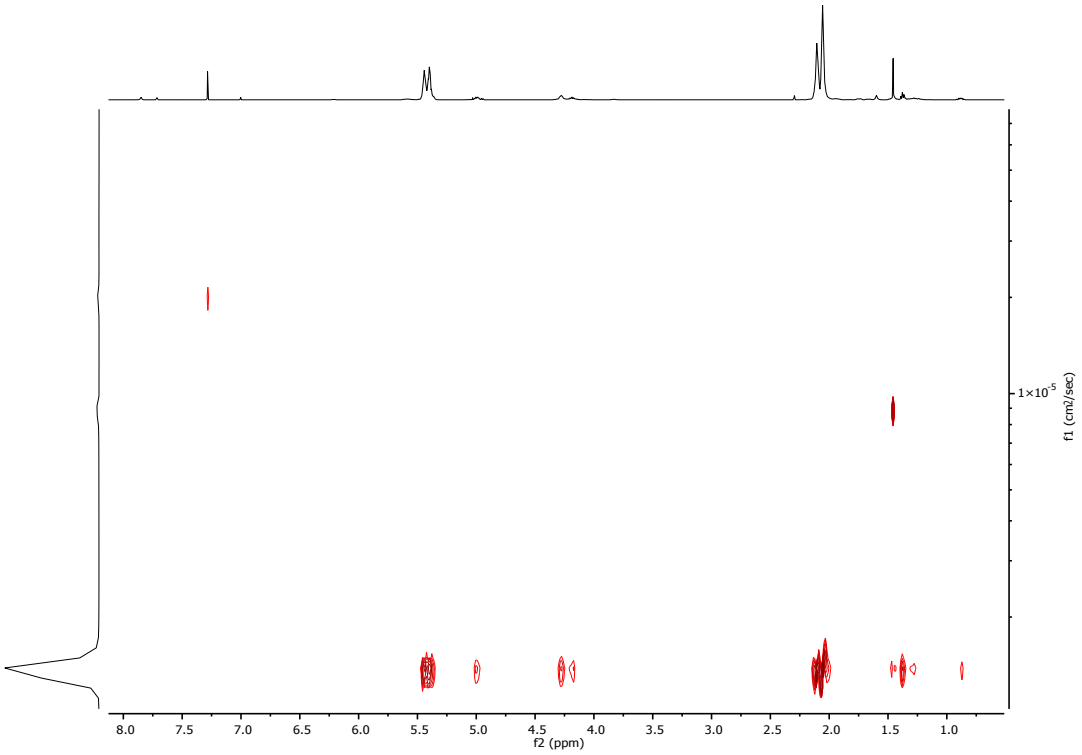
^{1}H NMR (500 MHz, $CDCl_3$)



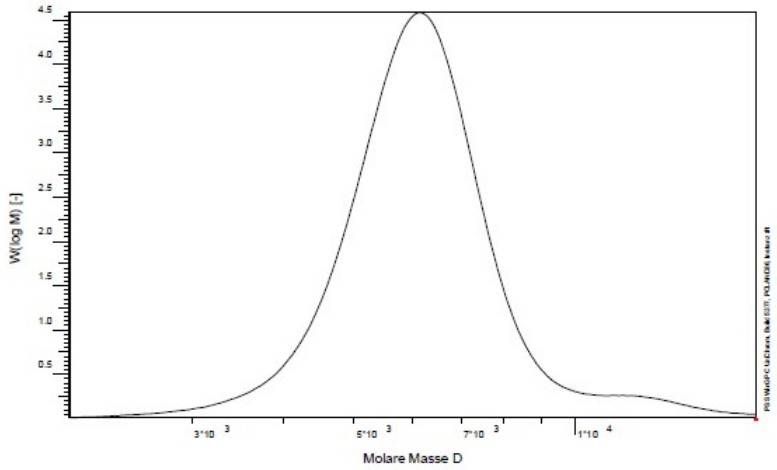
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



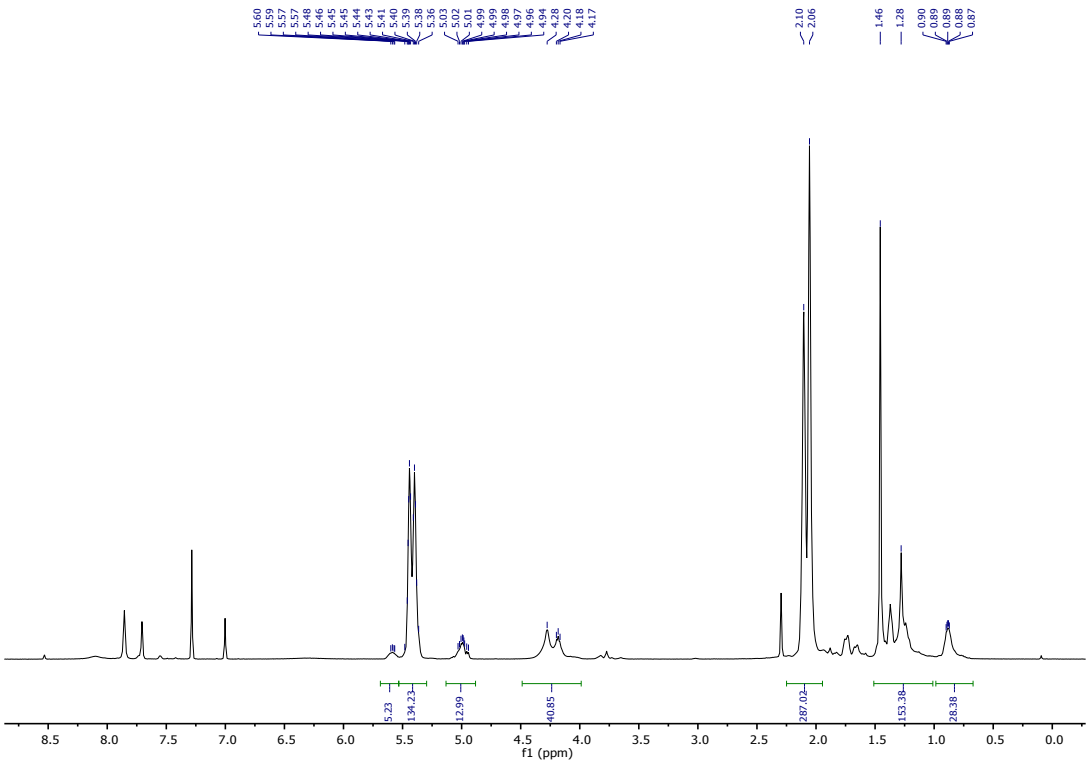
Molar Mass Dispersity (THF, PB calibration)



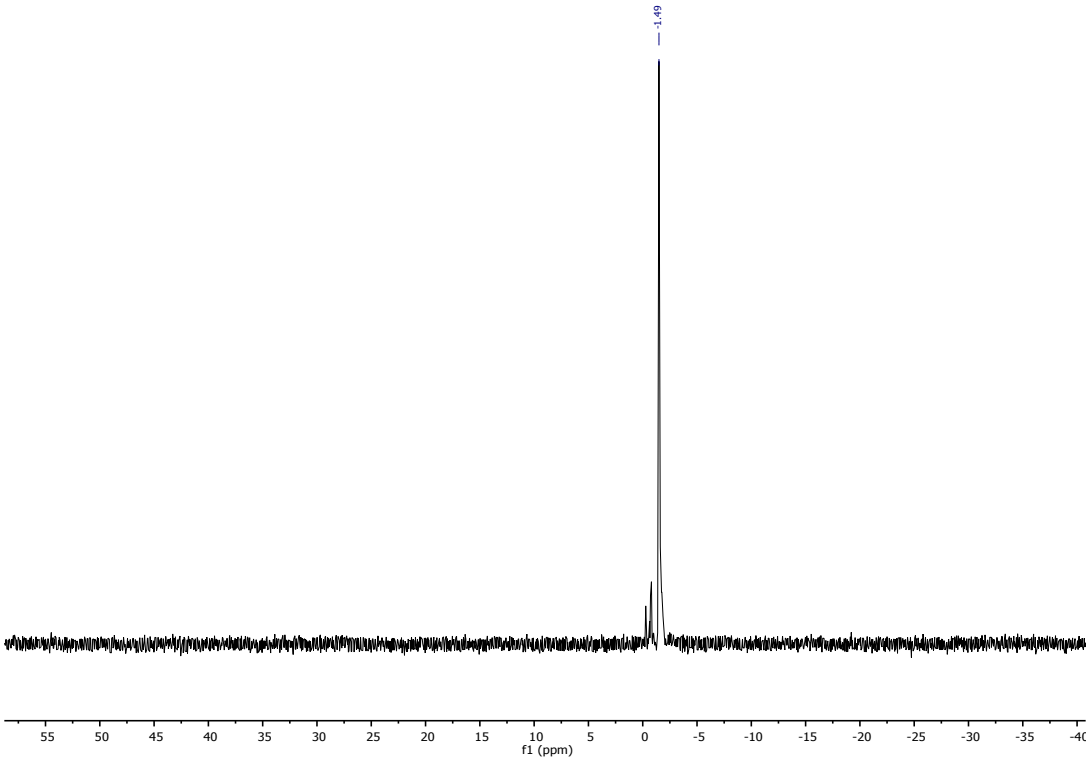
Mn	MW	D	Vp	Mp	Area
5939.55	6371.49	1.07	26.89	6108.08	0.01

PB_{73}-b-$PEEP_7$

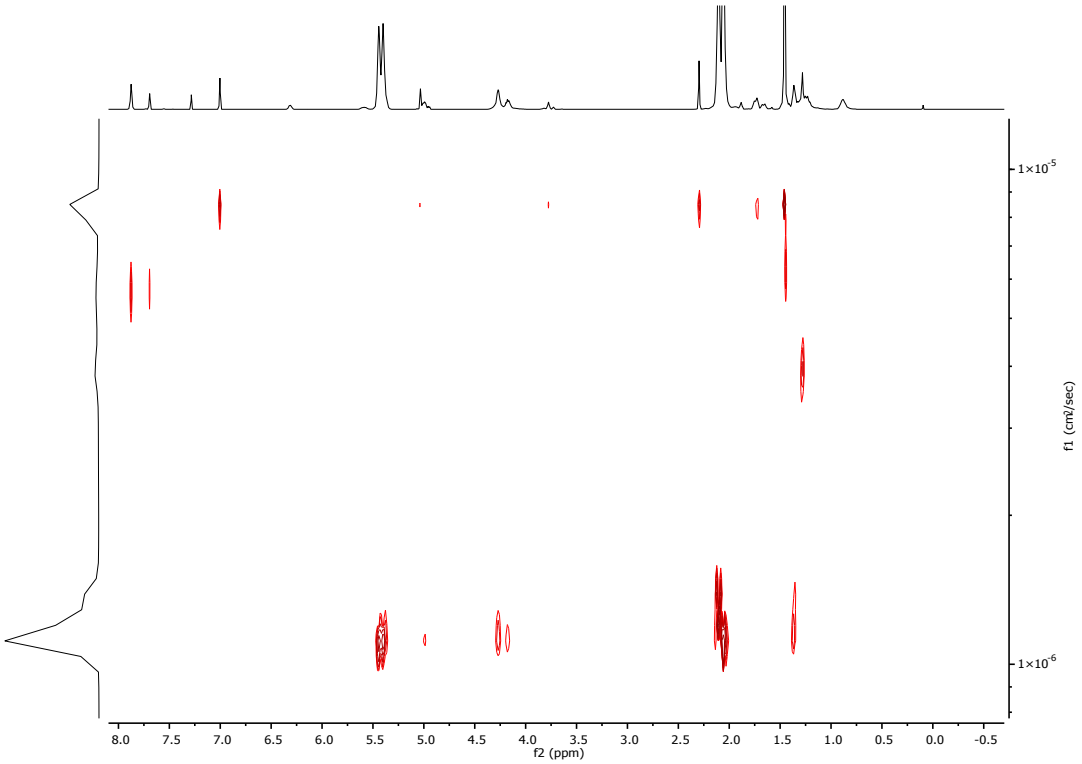
^{1}H NMR (500 MHz, $CDCl_3$)



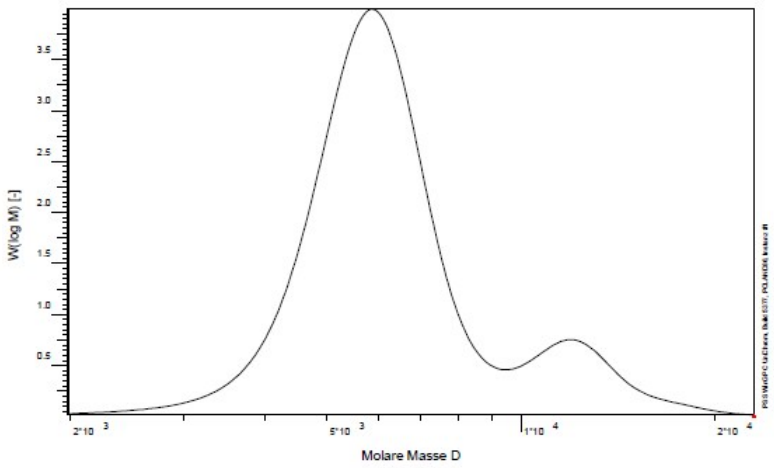
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



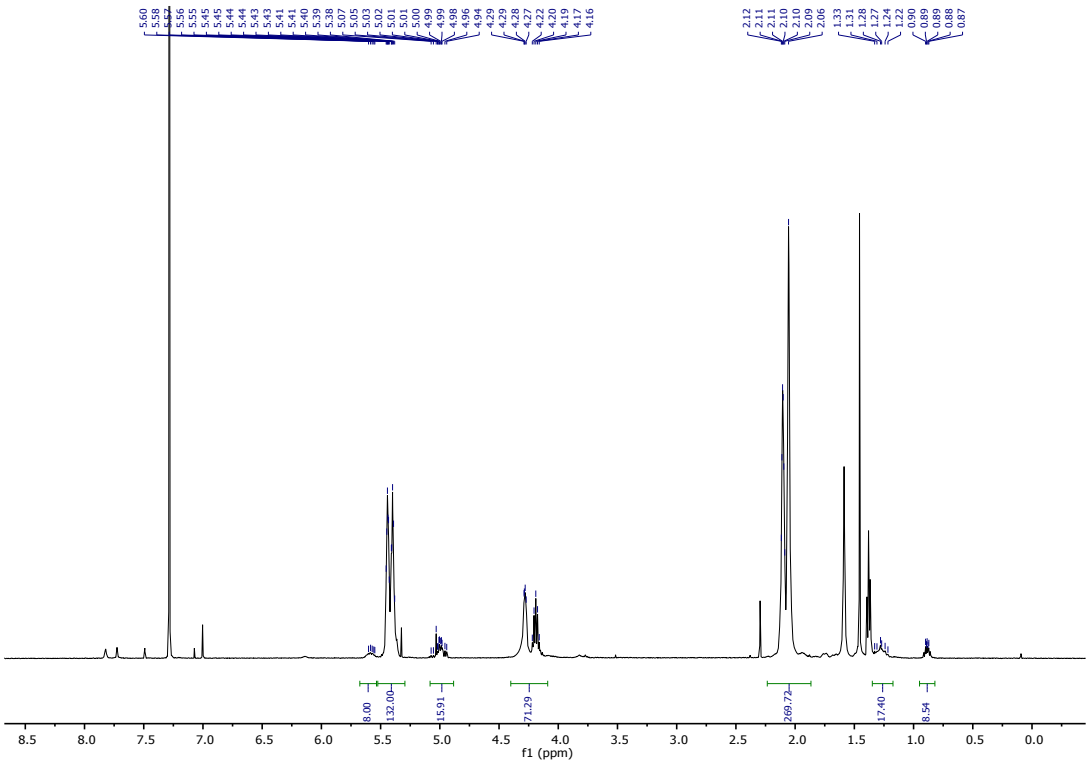
Molar Mass Dispersity (THF, PB calibration)



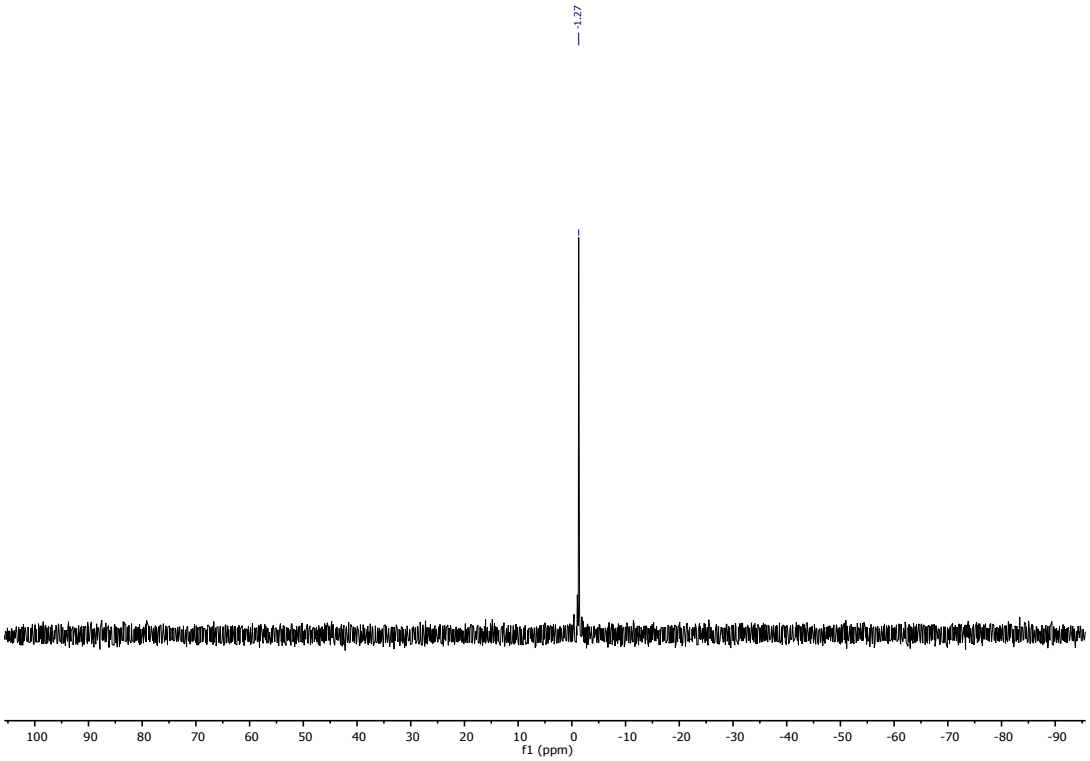
Mn	MW	D	Vp	Mp	Area
5981.06	6767.05	1.13	26.97	5855.30	0.04

PB_{73}-b-$PEEP_{12}$

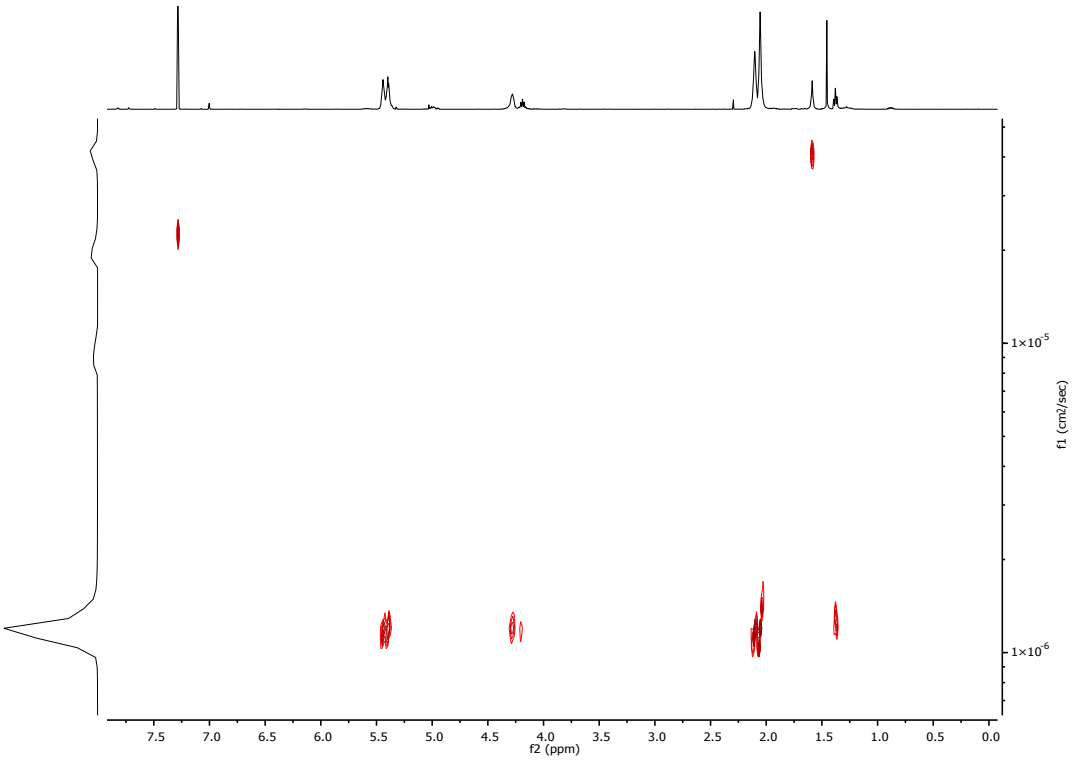
^{1}H NMR (500 MHz, $CDCl_3$)



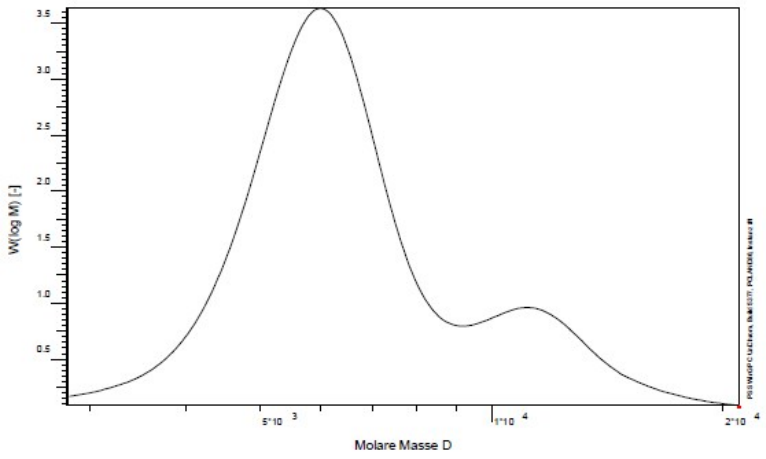
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



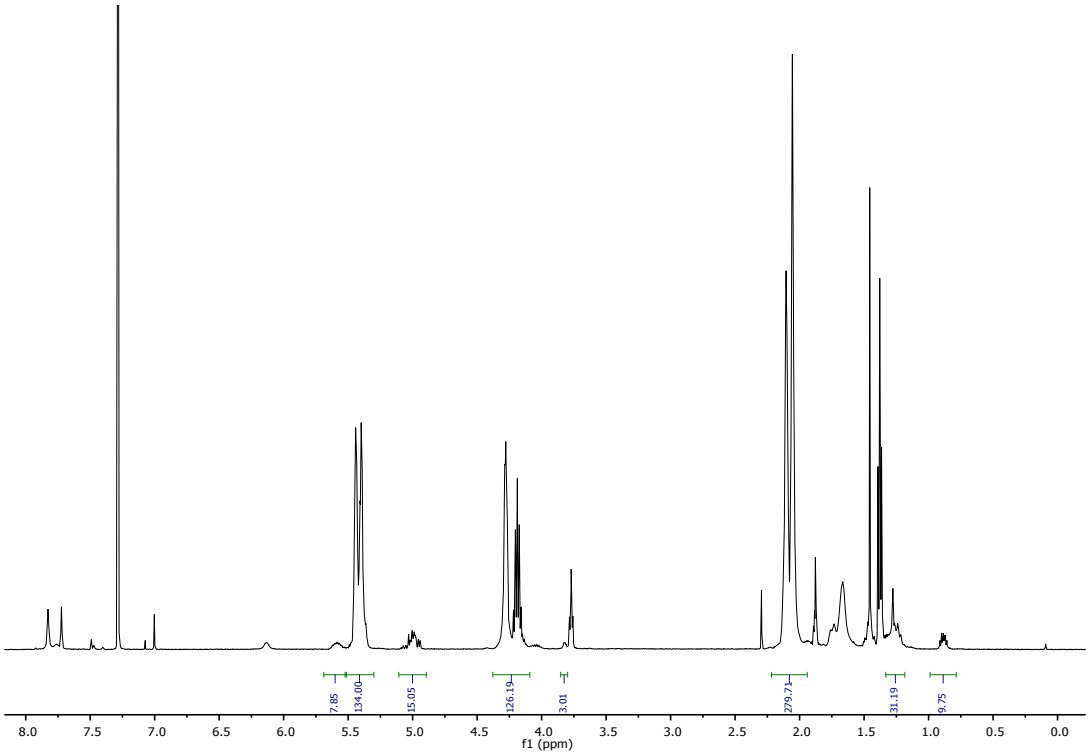
Molar Mass Dispersity (THF, PB calibration)



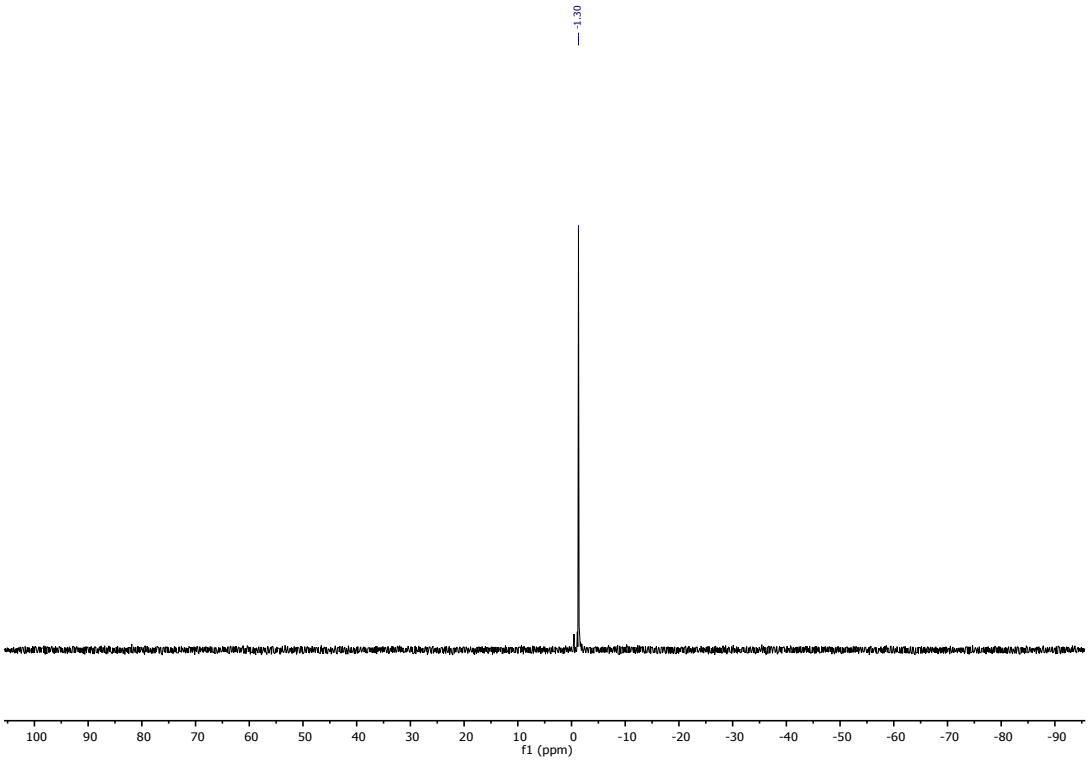
Mn	MW	D	Vp	Mp	Area
6259.61	7140.05	1.14	26.93	5980.04	0.02

PB_{73}-b-$PEEP_{21}$

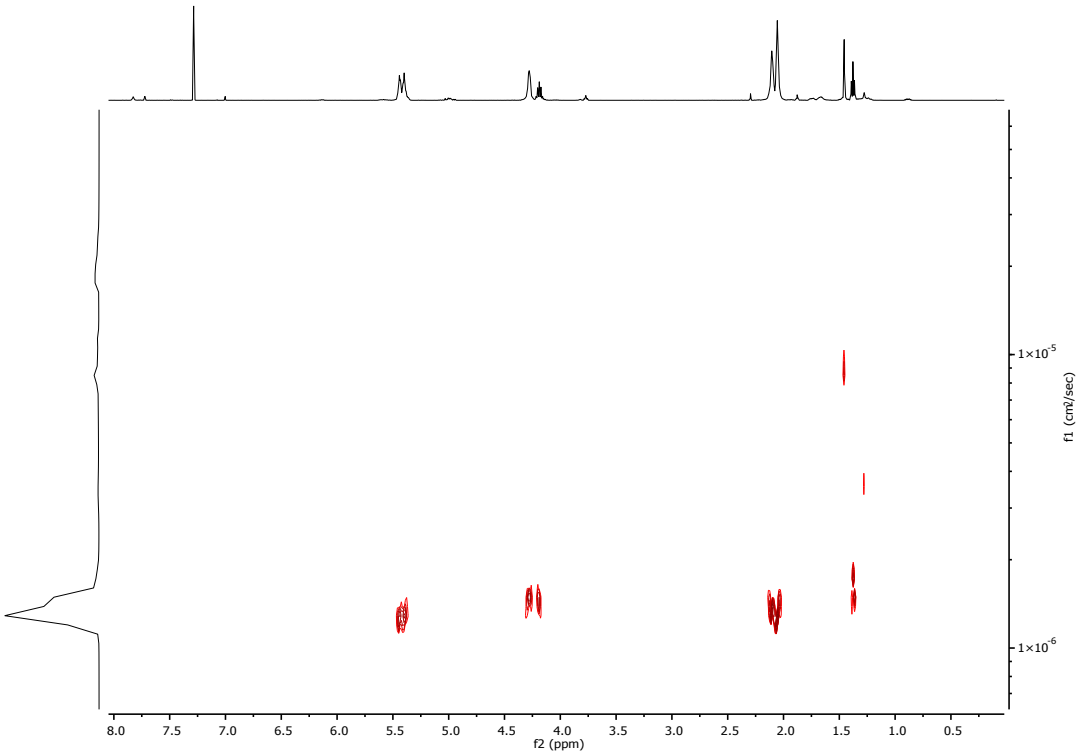
^{1}H NMR (500 MHz, $CDCl_3$)



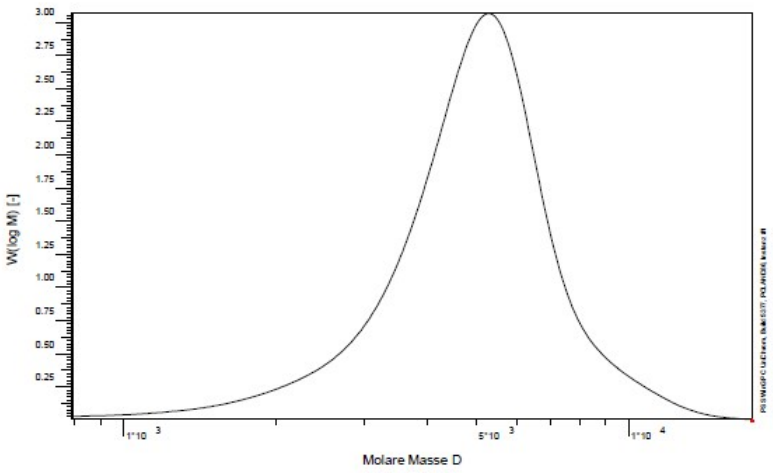
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



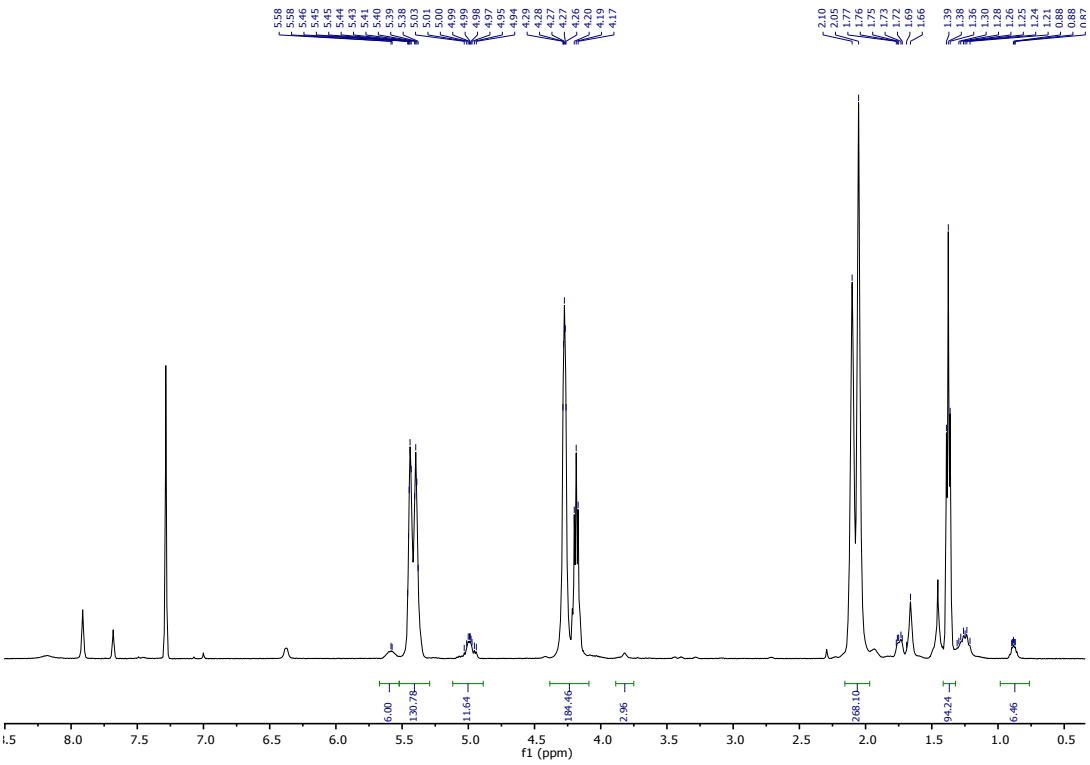
Molar Mass Dispersity (THF, PB calibration)



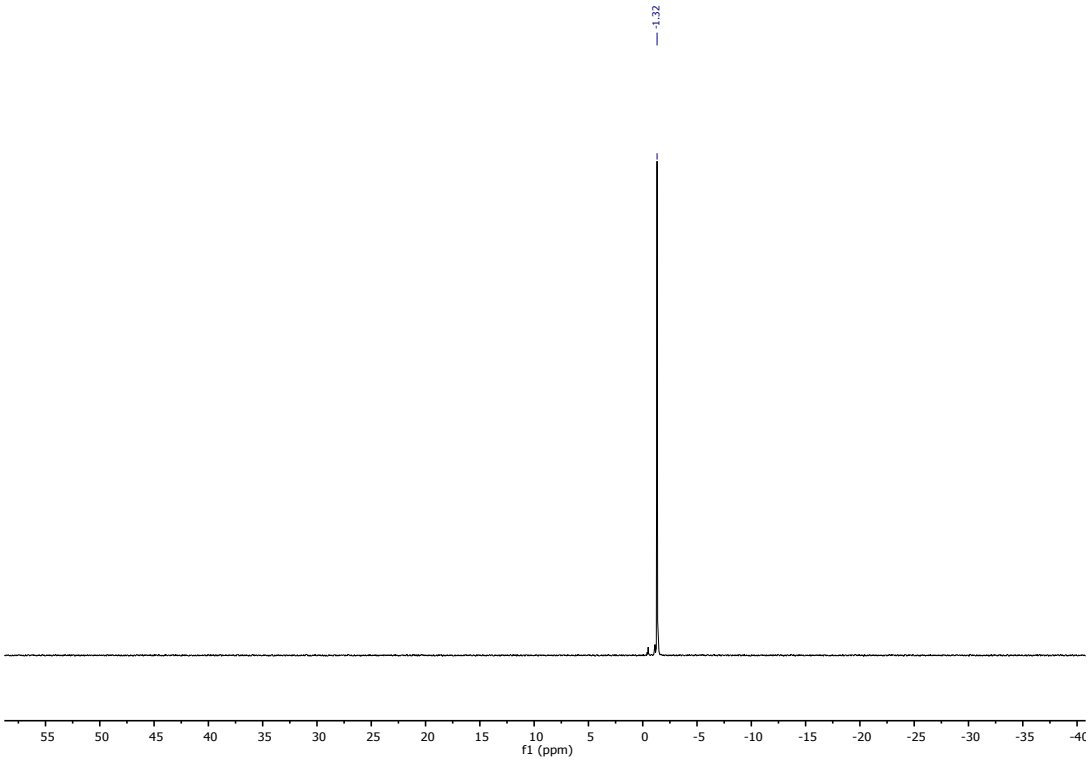
Mn	MW	D	Vp	Mp	Area
4373.84	5183.83	1.19	27.18	5246.41	0.05

PB_{73}-b-$PEEP_{31}$

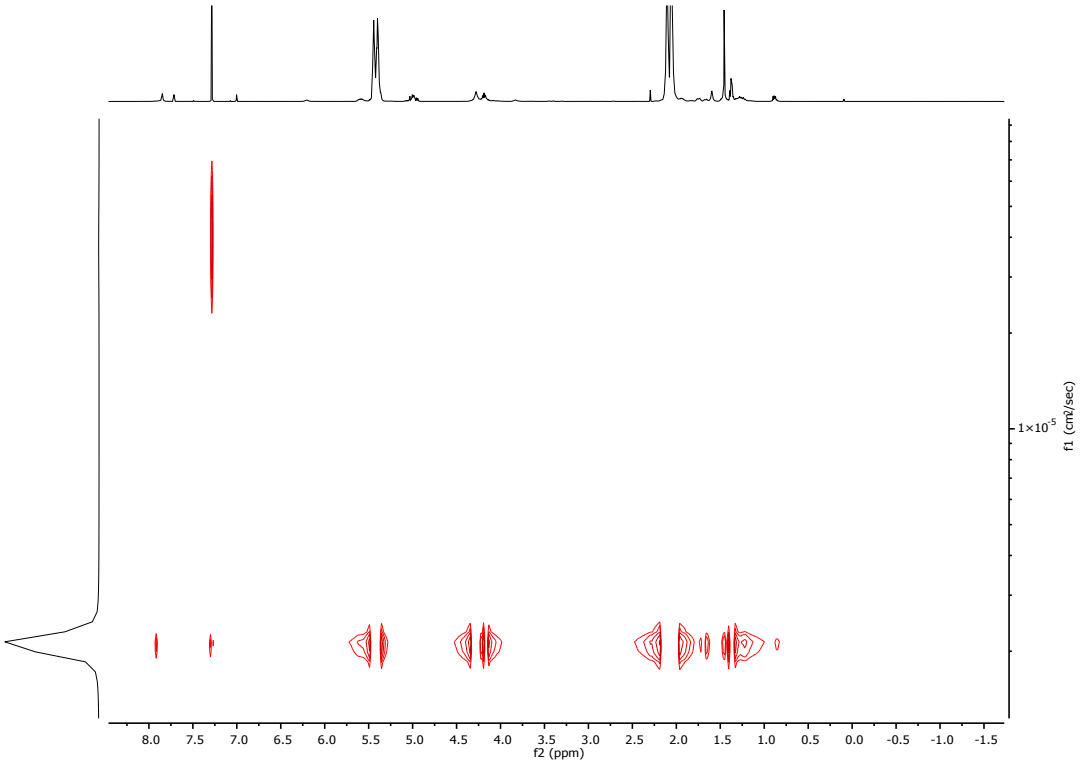
^{1}H NMR (500 MHz, $CDCl_3$)



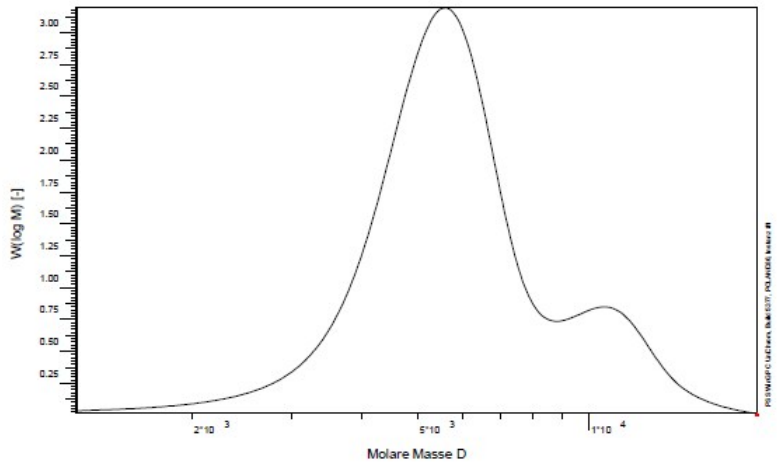
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



Molar Mass Dispersity (THF, PB calibration)



Mn	MW	D	Vp	Mp	Area
5382.44	6322.64	1.17	27.07	5568.65	0.12

PB_{73}-b-PEEP Comparison

^{1}H NMR

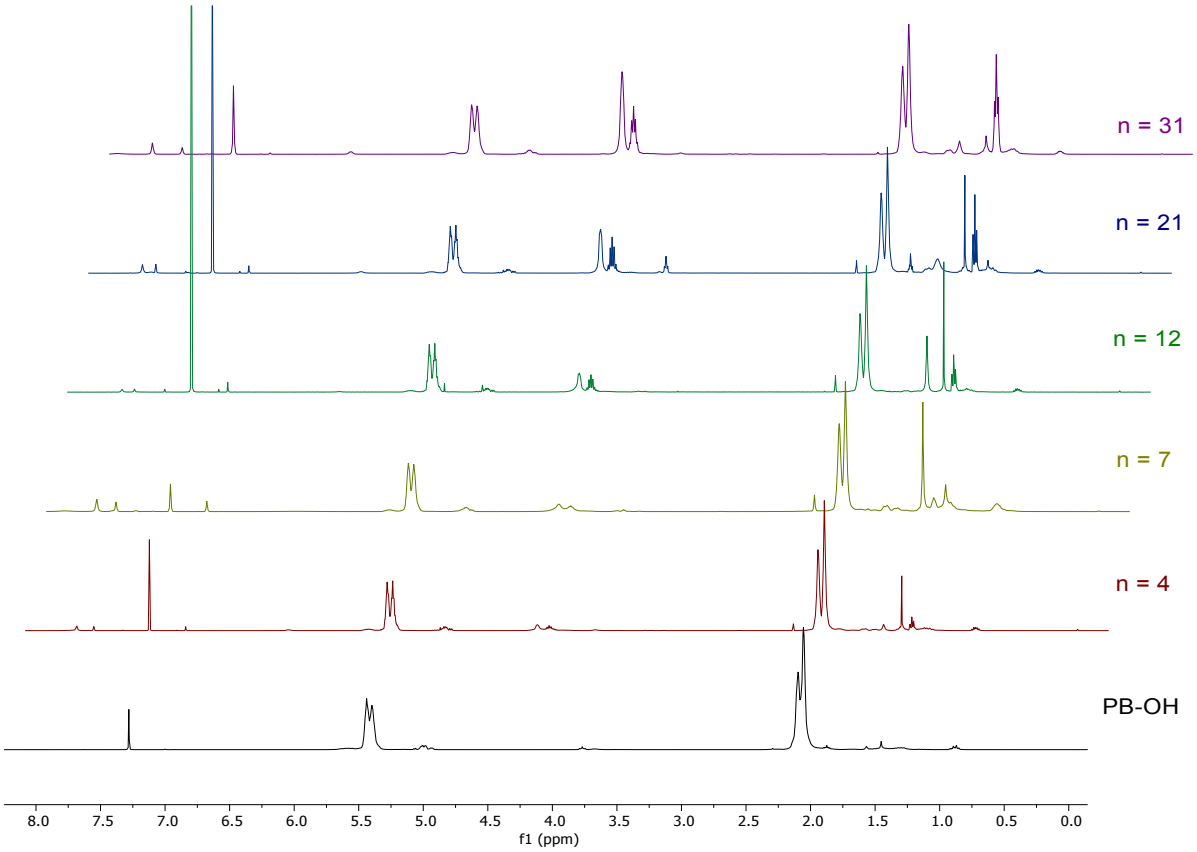


Figure S2. Comparison of the ^{1}H NMR of PB_{73}-b-$PEEP_n$ and PB_{73}-OH

Dispersity (based on elution time)

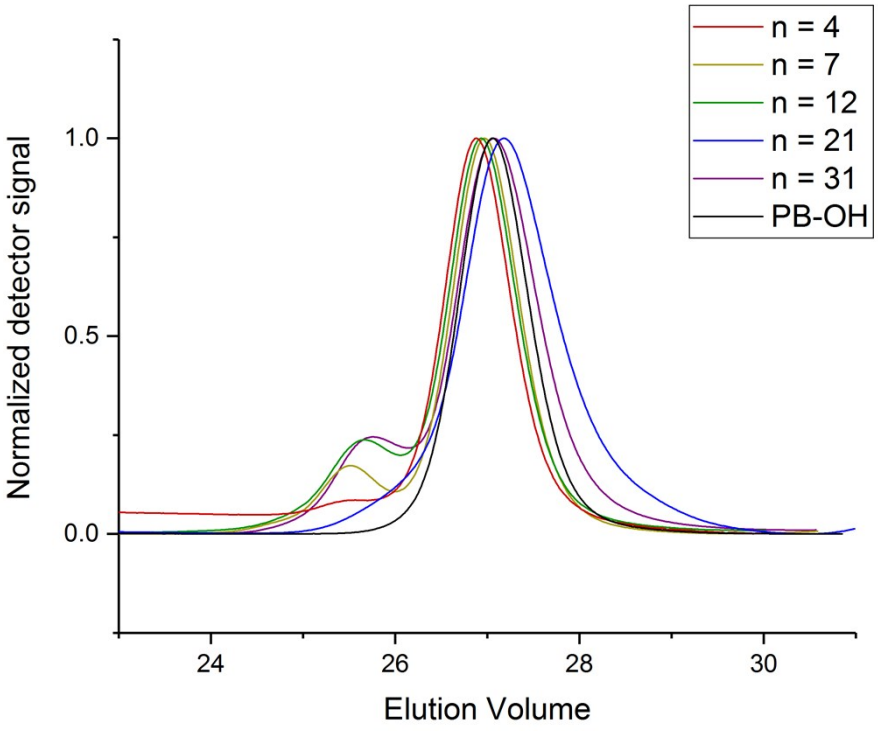


Figure S3. Dispersity of PB_{73}-b-$PEEP_n$ and their macroinitiator PB-OH measured by GPC

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC were run at 10 $K.min^{-1}$ on the neat block copolymers. The cooling and heating curves are labelled with left-handed and right handed arrows respectively and are coloured in black (cooling) and red or blue (heating). When not specified the heating curve (red) correspond to the second heating curve of the neat block copolymers.

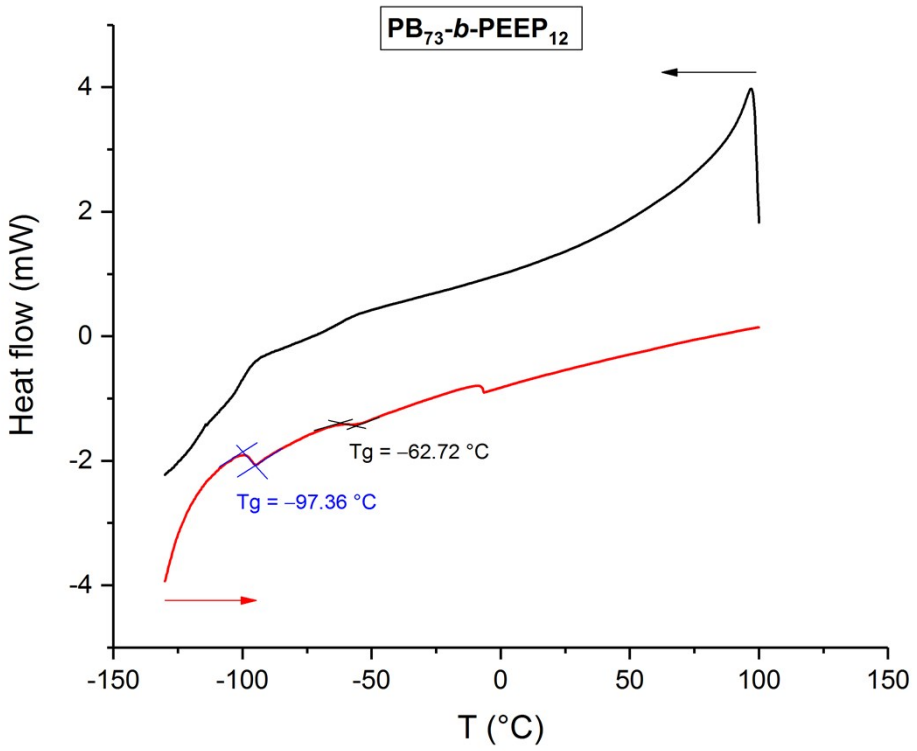


Figure S4. DSC of PB_{73}-b-$PEEP_{12}$.A small drop can be observed in the heating curve at 0 °C corresponding to a normalization of the data by the software and not related to any thermal properties.

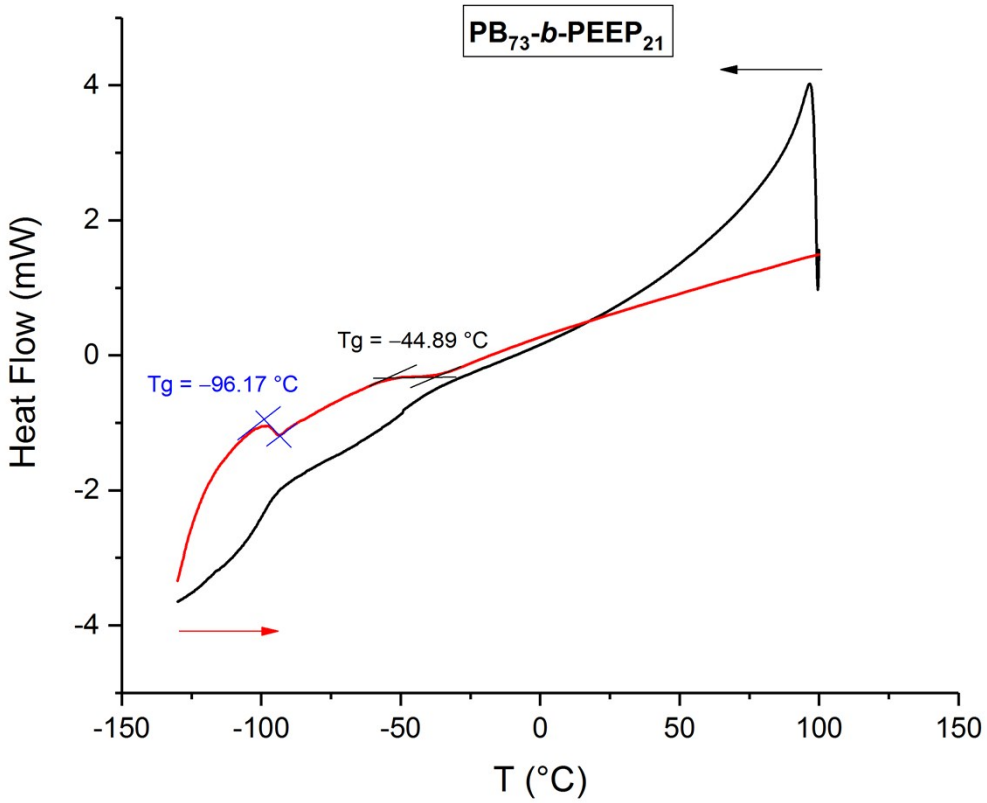


Figure S5. DSC of PB_{73}-b-$PEEP_{21}$.

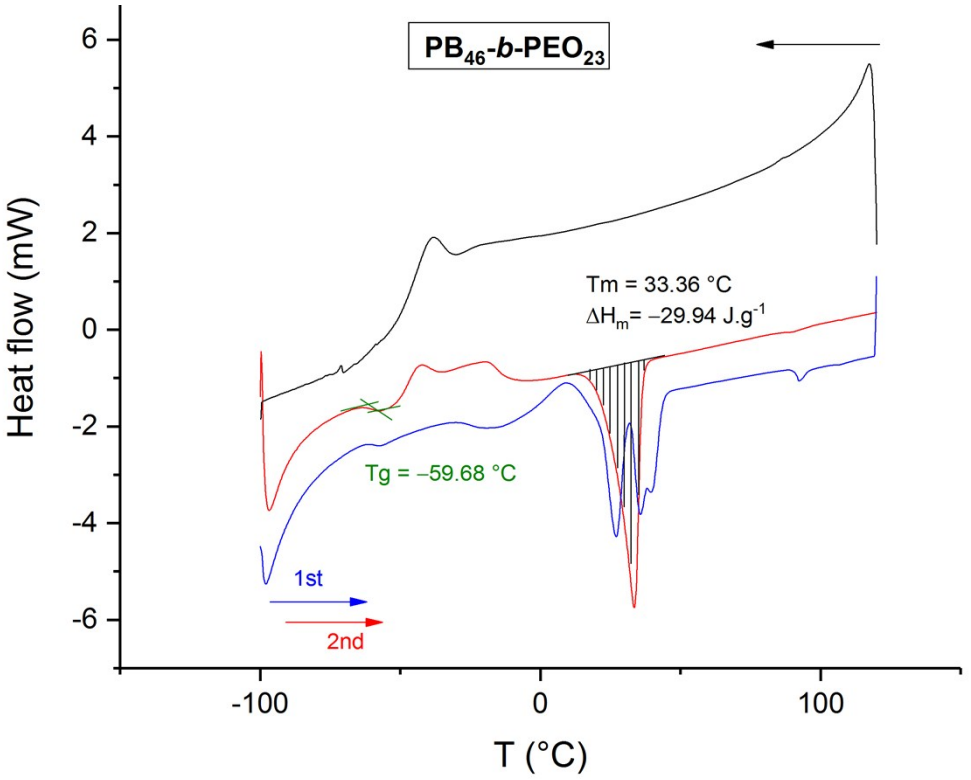


Figure S6. DSC of PB_{46}-b-PEO_{23}. The thermal transition from –50 to 0 °C might correspond to a 2 stages crystalline procedure which explains the lack of clear crystalline temperature T_c in the cooling curve.

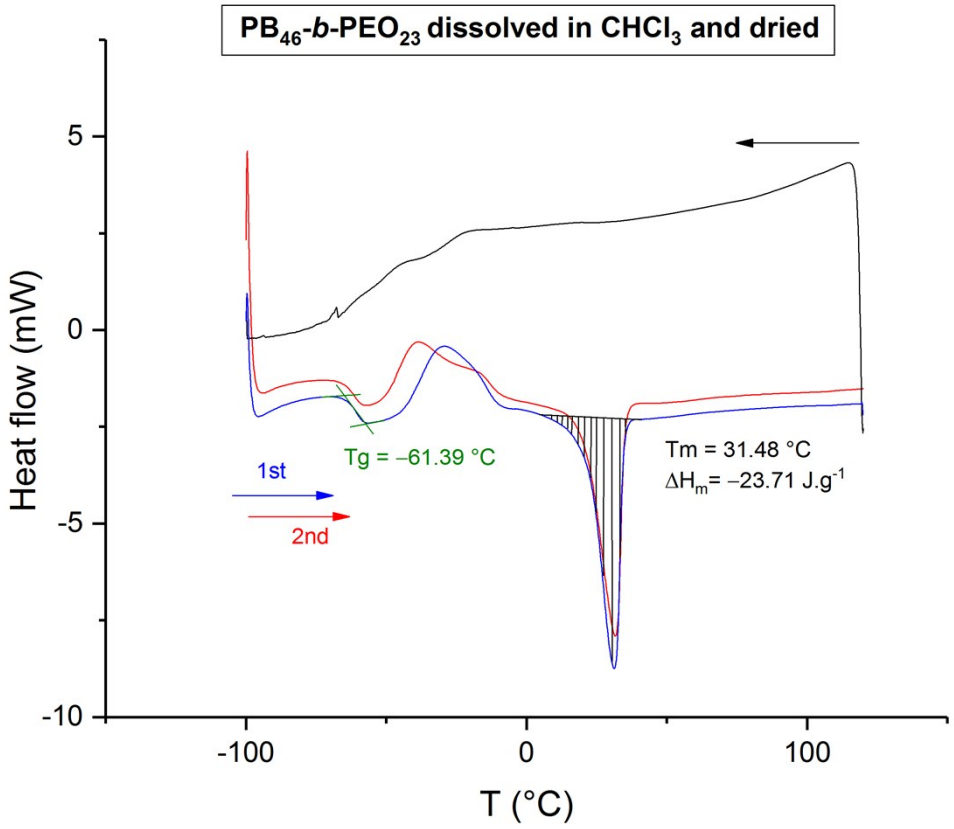


Figure S7. DSC of PB_{46}-b-PEO_{23} dissolved in CHCl3 and dried. In this case PB_{46}-b-PEO_{23} was pre-emptively dissolved in $CHCl_3$ at a concentration of 4.0 mg/mL and then rapidly dried under reduced pressure similarly to the film generation on the Pt-electrodes of the GUV reactor. The first heating curve of the DSC thus corresponds to the neat state of the PB_{46}-b-PEO_{23} found on the Pt-wires. The 1st heating curve of the treated PB_{46}-b-PEO_{23} resemble the 2nd heating curve here and without special treatment rather than the more complex 1st heating curve of PB_{46}-b-PEO_{23} pictured above (**Figure S6**) as obtained from the commercial source.

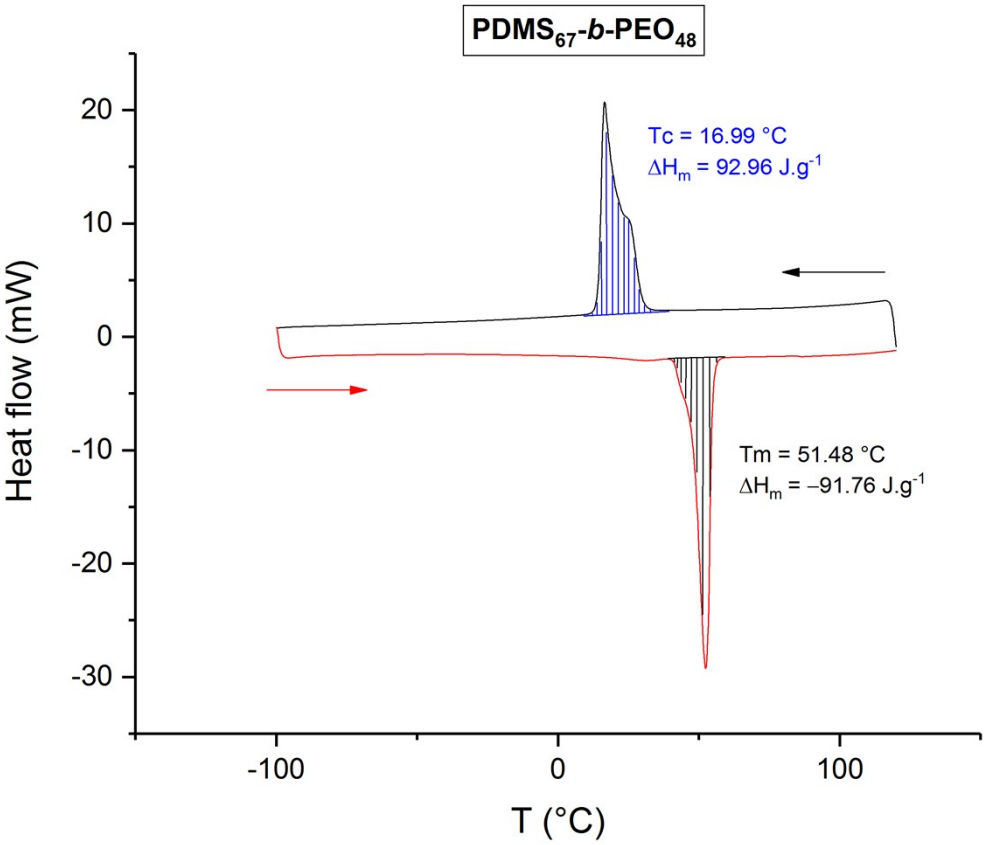


Figure S8. DSC of $PDMS_{67}$-b-PEO_{48}

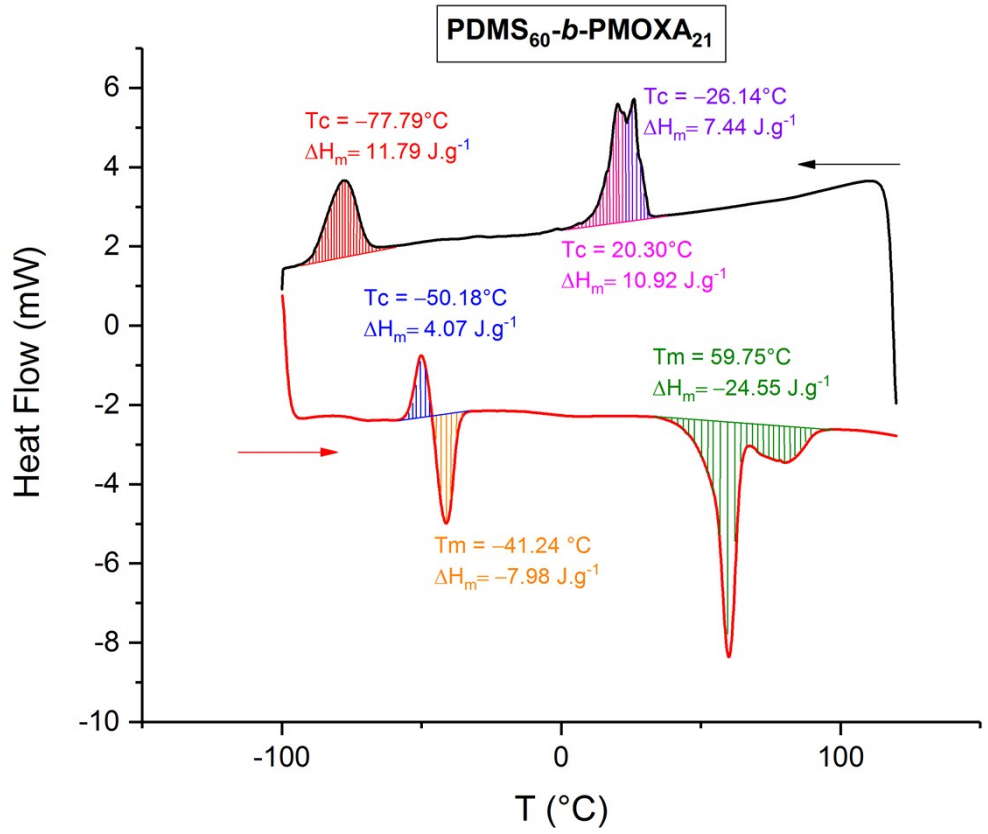


Figure S9. DSC of $PDMS_{60}$-b-$PMOXA_{21}$

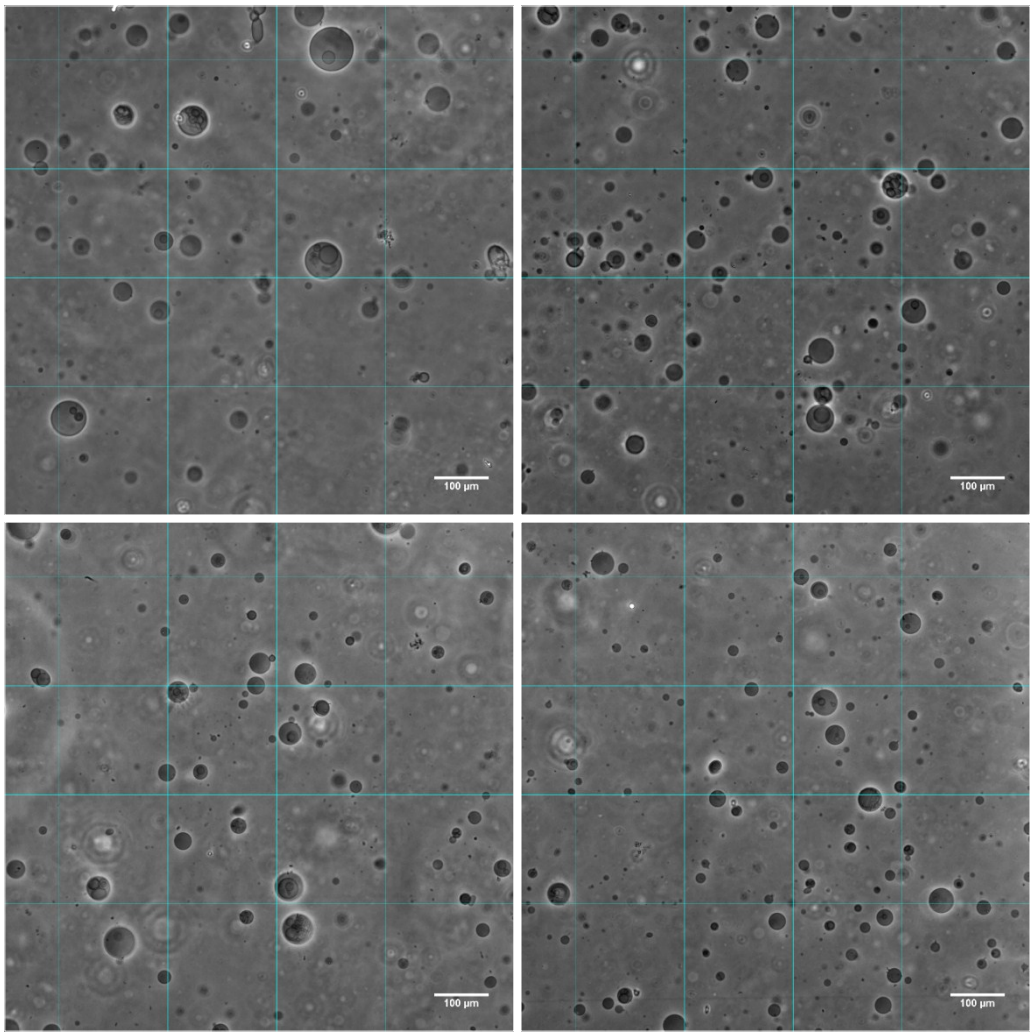
ELECTROFORMATION (EF) AND NON-ASSISTED FILM HYDRATION (NA-FH)

Based on a modified electroformation literature procedure[5], using our homemade device, a solution of block copolymer in $CHCl_3$ (4.0 mg/mL) was loaded on Pt wires and dried under reduced pressure overnight. The cell was fill with a sucrose solution (100 mM in MilliQ H_2O), and connected to a generator. A sinusoidal function was applied (10 V, 10 Hz, 50 min then 10 V, 4 Hz, 15 min) at room temperature (this step was omitted for the non-assisted film hydration). The electrophoresis sucrose solution (50 µL) was then diluted into an equiosmotic glucose solution (100 mM in MilliQ H_2O, 200 µL) for facilitating visualisation of the vesicles at the bottom of the well due to density differences. When using hydrophobic dye like Nile red (NR), the stock solution of block copolymer in $CHCl_3$ (4.0 mg/mL) was doped with 2% of a stock solution of Nile Red (NR) in toluene (0.5 mg/mL) before coating the electrodes and drying. When using hydrophilic dye like Alexa Fluor 647 (AF^{647}), the film hydration was carried out in a AF^{647}-aqueous solution (10µmol/L in 100 mM sucrose in MilliQ H_2O) and diluted (50 µL) into a AF^{647}-free glucose solution (250 µL) for observation. Thus any AF^{647} in the extra-vesicular media is diluted by a factor of 5 and allows a contrast to be observed between encapsulated AF^{647} and free AF^{647}. For the kinetics of non-assisted film hydration, the protocol was slightly modified in order to minimise effects due to volume modifications in the reactor. 10 µL of sucrose solution rather were diluted into 100 µL of glucose instead than 50 µL in 250 µL.

The scaling up film hydration protocol in a round bottom flask was carried out as followed. 1 mL of a PB-*b*-PEEP solution in $CHCl_3$ (4 mg/mL) was added to a 25 mL round bottom flask and concentrated under reduced pressure until all solvent was evaporated. An invisible thin film of the neat polymer was thus obtained. An aqueous sucrose solution (100 mM, 5 mL) was added and the reaction was let to vigorously stir overnight. Similarly to the electroformation and the non-assisted film hydration protocols, the resulting sucrose solution (50 µL) was then diluted into an equiosmotic glucose solution (100 mM in MilliQ H_2O, 200 µL) for facilitating visualisation of the vesicles at the bottom of the well due to density differences.

Yield and size determination

To determine the number of vesicles formed during EF and na-FH, after the vesicles settle (10 min) we took images of the bottom of the well at magnification 20x. The vast majority of vesicles are found at the bottom due to the density difference of the equiosmotic solution of sucrose and glucose and no significant discrepancy was observed between settling times. Locations were randomly chosen to cover the whole area of the well for a better estimation of the yield. The images were then divided into 16 squares of 200 μm length to ease of counting (see below).



The number of vesicles was counted manually on a given surface area and their size were determined using ImageJ's measuring tool. The number of vesicles in a given area was then back calculated into the actual number of vesicles generated in the electrochamber as follows:

$$Yield\ in\ reactor = \frac{Nvesicles\ observed * Vwell}{Vobserved * Vdilution}$$

Where $N_{vesicles\ observed}$ is the average number of vesicles per location of an area of 800*800 = $6.4x10^5$ µm²

V_{well} is the total volume of solution of the well (250µL),

$V_{observed}$ is the volume of the observed area (x*y*z =800 µm*800 µm *2525 µm = 1.6 µL),

And $V_{dilution}$ is the volume of added electroformation solution (50 µL).

The yields of the replicates were averaged and the standard deviation was calculated to determine the error as depicted in Table 2 of the article. For the details of each replicates see the '*Electroformation yield*' and '*electroformation size measurements*' sections.

Fluorescence Determination

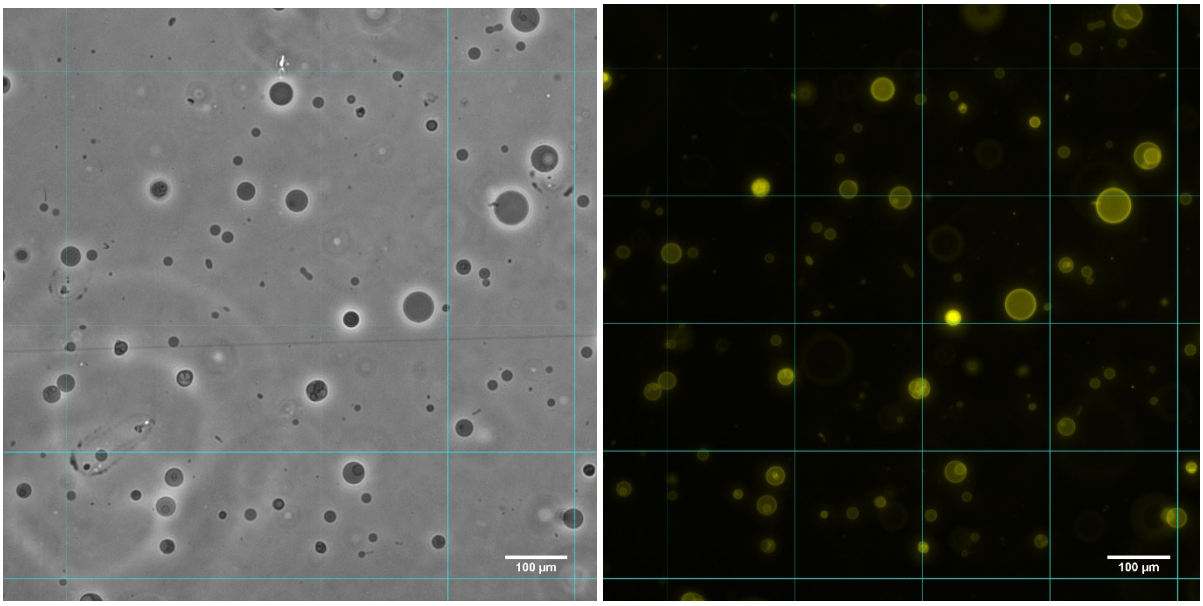
The encapsulation efficiency (ee) was determined by:

$$Encapsulation\ Efficiency = \frac{Nfluorescent\ vesicles}{Ntotal\ vesicles}$$

Where $N_{fluorescent\ vesicles}$ is number of fluorescent vesicles in a location in the relevant channel (Cy5 for AF[647] and TRITC for NR)

And $N_{total\ vesicles}$ is the total number vesicles in that same location.

The encapsulation efficiency at each location were averaged for each replicate. The average of the replicate's encapsulation efficiency and the standard deviation were calculated to determine the global estimated encapsulation efficiency. For the details of each replicates see the '*Electroformation yield*' section.



The degree of fluorescence used for the AF^{647} fluorescence over time was measured using ImageJ's measuring tool with background subtraction as well detailed by Cooper in reference [6]. The initial fluorescence was normalised to 100%.

GUVs yield

Table S1. GUV yield observed for electroformation (EF) and non-assisted film hydration (na-FH) for different block copolymers. The multiple rows per one polymer correspond to the obtained replicated measurements.

Polymer	Product ID	f	Mn	EF Yield (GUV/µL)	na-FH Yield (GUV/µL)
$PB(1,4)_{73}$-b-$PEEP_4$	Synthesised (page S7)	0.13	5000	0.00	0.00
				0.00	0.00
$PB(1,4)_{73}$-b-$PEEP_7$	Synthesised (page S9)	0.21	5000	0.00	0.00
				0.00	0.00
				0.00	0.00
$PB(1,4)_{73}$-b-$PEEP_{12}$	Synthesised (page S11)	0.32	6000	126.84	284.11
				206.89	565.54
				114.13	214.71
				196.45	-
$PB(1,4)_{73}$-b-$PEEP_{21}$	Synthesised (page S13)	0.45	7000	99.77	606.38
				220.54	321.23
				221.87	428.79
$PB(1,4)_{73}$-b-$PEEP_{31}$	Synthesised (page S15)	0.54	9000	0.00	0.00
				0.00	0.00
				-	0.00
$PB(1,2)_{44}$-b-PEO_{14}	P5826-BdEO (polymer	0.20	3000	0.00	-
				0.00	-

	Source)				
$PB(1,2)_{236}$-b-PEO_{100}	Synthesised according to [2]	0.26	17200	0.00	-
				0.00	-
$PB(1,2)_{46}$-b-PEO_{23}	P10351A-BdEO (polymer Source)	0.29	4000	8.51	0.00
				5.00	0.00
				3.76	0.00
				0.00	-
				3.98	-
				0.00	-
$PB(1,2)_{51}$-b-PEO_{28}	Synthesised according to [2]	0.31	4000	0.00	-
				0.00	-
$PB(1,2)_{74}$-b-PEO_{45}	P10946A-BdEO (polymer Source)	0.33	6000	0.00	-
				0.00	-
$PB(1,2)_{85}$-b-PEO_{68}	Synthesised according to [2]	0.39	8000	0.00	-
				0.00	-
$PB(1,2)_{50}$-b-PEO_{41}	P18739-BdEO (polymer Source)	0.40	5000	0.00	-
				0.00	-
				0.00	-
$PB(1,4)_{24}$-*b*-PEO_{14}	P19940-BdEO (polymer Source)	0.32	2000	0.34	-
				1.03	-
				0.00	-
PEO_{46}-B-PB_{23} (50°C)	P10351A-BdEO (polymer Source)	0.29	4000	1.55	-
				1.24	-
				2.32	-
$PDMS_{67}$-*b*-PEO_{48}	P7258-DMSEO (polymer Source)	0.30	7000	0.00	0.00
				0.00	0.00
$PDMS_{60}$-b-$PMOXA_{21}$	Synthesised according to[7, 8]	0.29	6000	4.25	3.09
				1.16	11.34
				6.57	4.02
				-	0.62
$PMOXA_{22}$-*b*-$PDMS_{119}$-*b*-$PMOXA_{22}$	P11170CCC-MOXZDMSMOXZ (polymer Source)	0.31	13000	0.00	0.00
				0.00	0.00

Table S2. GUV yield and encapsulation efficiency (ee) observed for electroformation (EF) and non-assisted film hydration (na-FH) for different block copolymers using Nile Red (NR) as a hydrophobic additive or AF^{647} as a hydrophilic additive. The ee was calculated as a percentage of vesicles which encapsulated the fluorescent dye over the total number of observed vesicles in phase contrast. The multiple rows per one polymer correspond to the obtained replicated measurements.

Additive	***Polymer***	***EF Yield (GUV/µL)***	***EF ee (%)***	***na-FH Yield (GUV/µL)***	***na-FH ee (%)***
NR	$PB(1,4)_{73}$-b-$PEEP_{12}$	124.78	100	681.66	100
		58.78	100	589.05	100
		117.56	100	479.53	100

	$PB(1,4)_{73}$-b-$PEEP_{21}$	130.56	100	1457.78	100
		172.63	100	1382.29	100
		331.03	100	1898.33	100
	$PB(1,2)_{46}$-b-PEO_{23}	0.62	100	0.00	-
		0.00	-	0.00	-
		0.00	-	-	-
AF647	$PB(1,4)_{73}$-b-$PEEP_{12}$	198.77	59	47.95	0.53
		63.42	68	60.02	0.37
		89.72	39	38.98	0.48
	$PB(1,4)_{73}$-b-$PEEP_{21}$	35.75	29	34.65	0.08
		113.85	22	104.57	0.05
		285.40	19	81.47	0.10

Film hydration kinetics

Table S3. Kinetic GUV yield of the non-assisted film hydration (na-FH) of PB_{73}-*b*-$PEEP_{12}$. The kinetic experiments were carried out in triplicate (A, B and C) to determine the average yield and associated standard deviation.

Time (min)	Replicate A Yield (GUVs/µL)	Replicate B Yield (GUVs/µL)	Replicate C Yield (GUVs/µL)	Average Yield (GUVs/µL)	Std Deviation
5	30.90	-	72.10	51.50	29.13
10	151.41	256.47	305.91	237.93	78.90
30	538.18	432.61	-	485.39	74.65
60	497.50	1076.36	652.00	741.95	299.73
120	898.69	1015.85	652.00	855.51	185.73

Table S4. Kinetic GUV diameter of the non-assisted film hydration (na-FH) of PB_{73}-*b*-$PEEP_{12}$. The kinetic experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

Time (min)	Replicate A Diameter (µm)	Replicate B Diameter (µm)	Replicate C Diameter (µm)	Average diameter (µm)	Std Deviation
5	25.69	-	20.76	23.23	3.49
10	19.70	17.30	18.94	18.64	1.23
30	14.24	20.34	-	17.29	4.31
60	14.81	12.36	10.92	12.70	1.97
120	14.42	15.71	13.35	14.49	1.18

Stability experiments

Table S5. GUV yield evolution over time of PB_{73}-*b*-$PEEP_{21}$ over a period of 33 days. The experiments were carried out in duplicate (A and B) to determine the average yield and associated standard deviation.

Time (days)	Replicate A Yield (GUVs/µL)	Replicate B Yield (GUVs/µL)	Average Yield *PB_{73}-b-$PEEP_{21}$* (%)	Std deviation
0	100.00	100.00	100.00	0.00
4	22.39	19.56	20.97	2.00
6	24.30	6.44	15.37	12.63
33	1.42	9.20	5.31	5.50

Table S6. GUV yield evolution over time of PB_{73}-*b*-$PEEP_{12}$ over a period of 33 days. The experiments were carried out in duplicate (A and B) to determine the average yield and associated standard deviation.

Time (days)	Replicate A Yield (GUVs/µL)	Replicate B Yield (GUVs/µL)	Average Yield PB_{73}-*b*-$PEEP_{12}$ (%)	Std deviation
0	100.00	100.00	100.00	0.00
4	60.28	30.12	45.20	21.33
6	45.64	29.05	37.35	11.73
33	50.00	23.52	36.76	18.73

Table S7. GUV diameter evolution over time of PB_{73}-*b*-$PEEP_{21}$.The experiments were carried out in duplicate (A and B) to determine the average diameter and associated standard deviation.

Time (days)	Replicate A Diameter (µm)	Replicate B Diameter (µm)	Average Diameter PB_{73}-*b*-$PEEP_{21}$ (µm)	Std deviation
0	11.95	10.22	11.09	1.23
4	20.42	14.83	17.62	3.95
6	14.62	7.92	11.27	4.73
33	5.29	7.21	6.25	1.36

Table S8. GUV diameter evolution over time of PB_{73}-*b*-$PEEP_{12}$.The experiments were carried out in duplicate (A and B) to determine the average diameter and associated standard deviation.

Time (days)	Replicate A Diameter (µm)	Replicate B Diameter (µm)	Average Diameter PB_{73}-*b*-$PEEP_{12}$ (µm)	Std deviation
0	17.13	14.67	15.90	1.74
4	12.34	14.39	13.37	1.45
6	14.93	16.37	15.65	1.02
33	18.01	15.12	16.56	2.04

AF^{647} fluorescence encapsulation evolution

Table S9. Fluorescence evolution of AF^{647} encapsulated into PB_{73}-*b*-$PEEP_{12}$ GUVs. The experiments were carried out in triplicate (A, B and C) to determine the average normalized fluorescence and associated standard deviation.

Time (min)	*Normalized Fluorescence (%)*			*Average Normalized Fluorescence (%)*	*Std Deviation*
	Replicate A	Replicate B	Replicate C		
0	100.00	100.00	100.00	100.00	0.00
5	105.62	100.00	98.93	101.51	3.59
10	102.97	105.29	101.21	103.15	2.05
15	111.51	115.94	101.64	109.70	7.32
20	115.88	112.43	115.78	114.69	1.96
25	118.09	115.47	111.50	115.02	3.31
30	133.93	108.86	119.43	120.74	12.59
35	118.76	123.16	118.43	120.12	2.64
40	110.13	126.05	108.95	115.04	9.55
45	110.40	116.25	114.79	113.81	3.05
50	117.90	112.81	115.55	115.42	2.55
55	126.53	115.97	111.81	118.11	7.59
60	115.60	87.11	121.55	108.09	18.40

65	106.92	106.93	116.82	110.22	5.71
70	102.78	96.52	116.50	105.27	10.22
75	94.09	110.81	110.08	104.99	9.45
80	91.99	116.54	112.68	107.07	13.20
85	129.45	117.70	121.65	122.93	5.98
90		97.66	105.86	101.76	5.80
95	99.36	89.80		94.58	6.76
100	93.05	97.77		95.41	3.34

Table S10. Fluorescence evolution of AF^{647} encapsulated into PB_{73}-*b*-$PEEP_{21}$ GUVs. The experiments were carried out in replicate (A, B, C and D) to determine the average normalized fluorescence and associated standard deviation.

Time (min)	*Normalized Fluorescence (%)*				*Average Normalized Fluorescence (%)*	*Standard Deviation*
	Replicate A	**Replicate B**	**Replicate C**	**Replicate D**		
0.00	100.00	100.00	100.00	100.00	100.00	0.00
5.00	85.87	90.50	58.25	100.74	83.84	18.16
10.00	102.37	143.43	90.03	75.20	102.76	29.30
15.00	73.48	72.00	90.25	73.80	77.38	8.61
20.00	74.92	68.58	75.67	64.03	70.80	5.52
25.00	29.99	98.46	87.10	55.66	67.80	31.03
30.00	61.46	56.98	36.84	43.42	49.67	11.49
35.00	74.44	59.20	72.20	43.12	62.24	14.41
40.00	56.01	80.85	66.42	42.79	61.52	16.11
45.00	57.47	114.97	83.93	23.60	69.99	38.84
50.00	60.25		71.06	16.10	49.14	29.12
55.00	31.04		63.91	39.26	44.74	17.11
60.00	57.50			34.04	45.77	16.59
65.00	22.88			32.94	27.91	7.12
70.00	22.72			30.10	26.41	5.22
75.00	23.66			39.62	31.64	11.29
80.00	15.56			33.19	24.37	12.47
85.00	19.32			40.92	30.12	15.27
90.00	16.69			9.20	12.94	5.30
95.00	34.31			-4.59	14.86	27.51

Size measurements and distribution

Table S11. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{12}$ by na-FH. The experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

	Replicate A	**Replicate B**	**Replicate C**	**Average**
Mean diameter (µm)	20.04	17.27	21.58	19.63±2.18

Std dev	17.89	14.71	19.51	-
PDI	0.80	0.73	0.82	0.78

Table S12. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{12}$ by EF. The experiments were carried out in replicates (A, B, C and D) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Replicate D	Average
Mean diameter (µm)	17.13	14.67	11.62	21.16	16.15±4.03
Std dev	15.68	11.04	10.44	20.99	-
PDI	0.84	0.57	0.81	0.98	0.80

Table S13. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{12}$ by na-FH in the presence of Nile Red (NR). The experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	26.70	31.61	23.16	27.16±4.24
Std dev	29.21	28.46	24.15	-
PDI	1.20	0.81	1.09	1.03

Table S14. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{12}$ by EF in the presence of Nile Red (NR). The experiments were carried out in replicate (A, B, C and D) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Replicate D	Average
Mean diameter (µm)	33.08	25.87	27.91	31.71	29.65±3.33
Std dev	24.96	16.72	16.88	23.93	-
PDI	0.57	0.42	0.37	0.57	0.48

Table S15. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{12}$ by na-FH in the presence of AF^{647}. The experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	23.13	22.42	28.87	24.81±3.54
Std dev	18.38	20.32	22.00	-
PDI	0.63	0.82	0.58	0.68

Table S16. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{12}$ by EF in the presence of AF^{647}. The experiments were carried out in replicate (A, B, C and D) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Replicate D	Average
Mean diameter (µm)	25.40	34.82	31.87	29.98	30.52±3.95
Std dev	19.04	22.09	16.20	21.11	-
PDI	0.56	0.40	0.26	0.50	0.41

a) **b)** **c)**

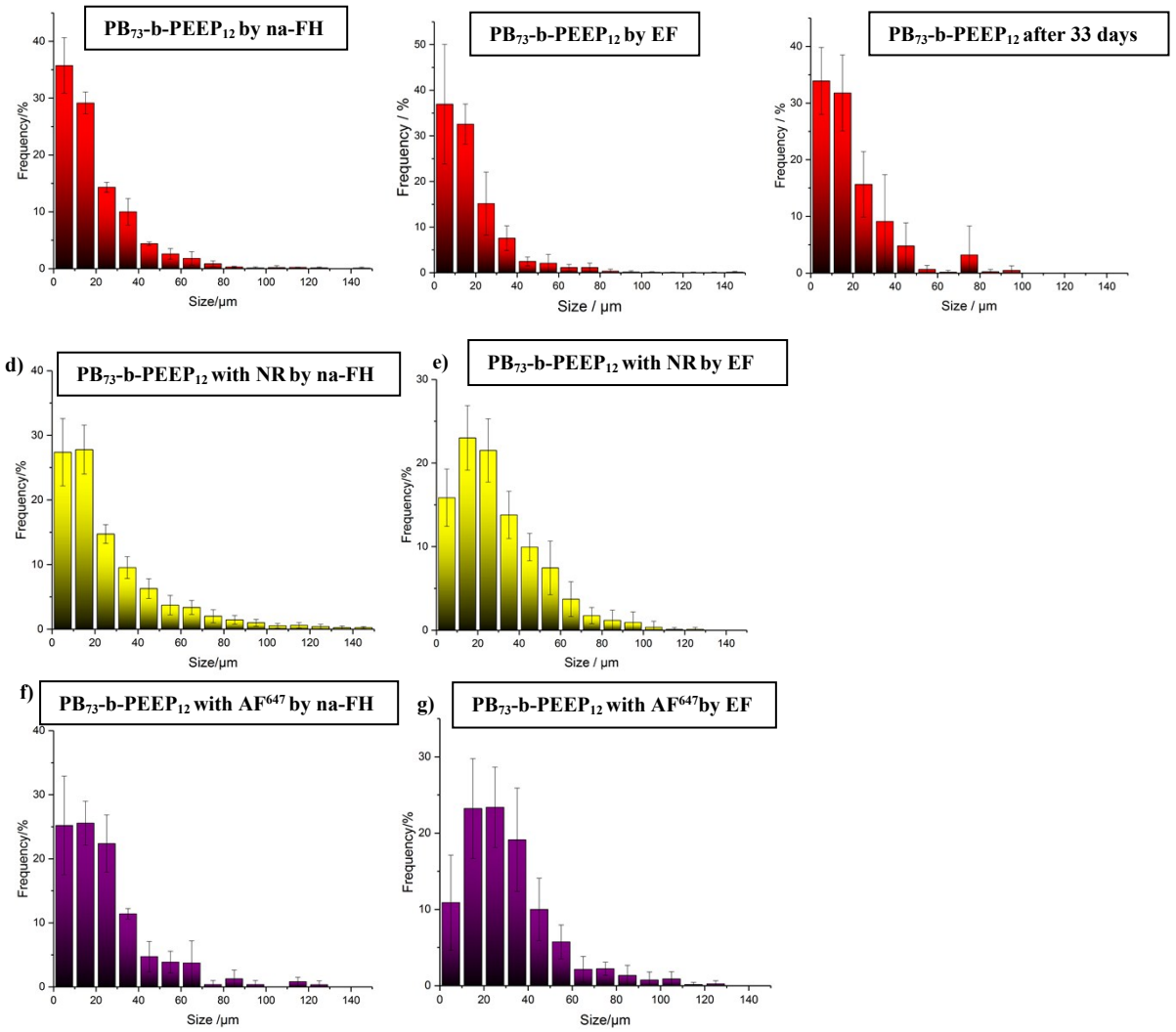


Figure S10. Frequency diagram of the PB_{73}-*b*-$PEEP_{12}$ GUVs diameter a) when freshly formed by na-FH, b) freshly formed by EF, c) after 33 days, d) formed by na-FH in the presence of Nile Red (NR), e) formed by EF in the presence of Nile Red (NR), f) formed by na-FH in the presence of AF^{647}, g) formed by EF in the presence of AF^{647}.

Table S17. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{21}$ by na-FH. The experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	11.62	15.23	15.88	14.24±2.29
Std deviation	9.20	12.18	11.17	-
PDI	0.63	0.64	0.49	0.59

Table S18. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{21}$ by EF. The experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	18.51	14.67	14.17	15.78±2.38
Std dev	14.45	10.51	13.30	-
PDI	0.61	0.51	0.88	0.67

Table S19. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{21}$ by na-FH in the presence of Nile Red (NR). The experiments were carried out in replicate (A, B, C and D) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	14.81	11.99	11.18	12.66±1.91
Std dev	11.82	9.93	7.66	-
PDI	0.64	0.69	0.47	0.60

Table S20. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{21}$ by EF in the presence of Nile Red (NR). The experiments were carried out in replicate (A, B, C and D) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Replicate D	Average
Mean diameter (µm)	19.91	17.97	23.38	17.11	19.59±2.79
Std dev	15.23	11.45	17.42	14.14	-
PDI	0.59	0.41	0.56	0.68	0.56

Table S21. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{21}$ by na-FH in the presence of AF^{647}. The experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	16.73	16.36	18.21	17.10±0.98
Std dev	11.82	11.04	14.58	-
PDI	0.50	0.46	0.64	0.53

Table S22. GUV diameter and polydispersity (PDI) of PB_{73}-*b*-$PEEP_{21}$ by EF in the presence of AF^{647}. The experiments were carried out in triplicate (A, B and C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Replicate D	Average
Mean diameter (µm)	31.62	28.26	22.22	38.58	30.17±8.21
Std dev	18.66	15.39	14.68	20.78	-
PDI	0.35	0.30	0.44	0.29	0.34

a) PB_{73}-b-$PEEP_{21}$ by na-FH b) PB_{73}-b-$PEEP_{21}$ by EF c)

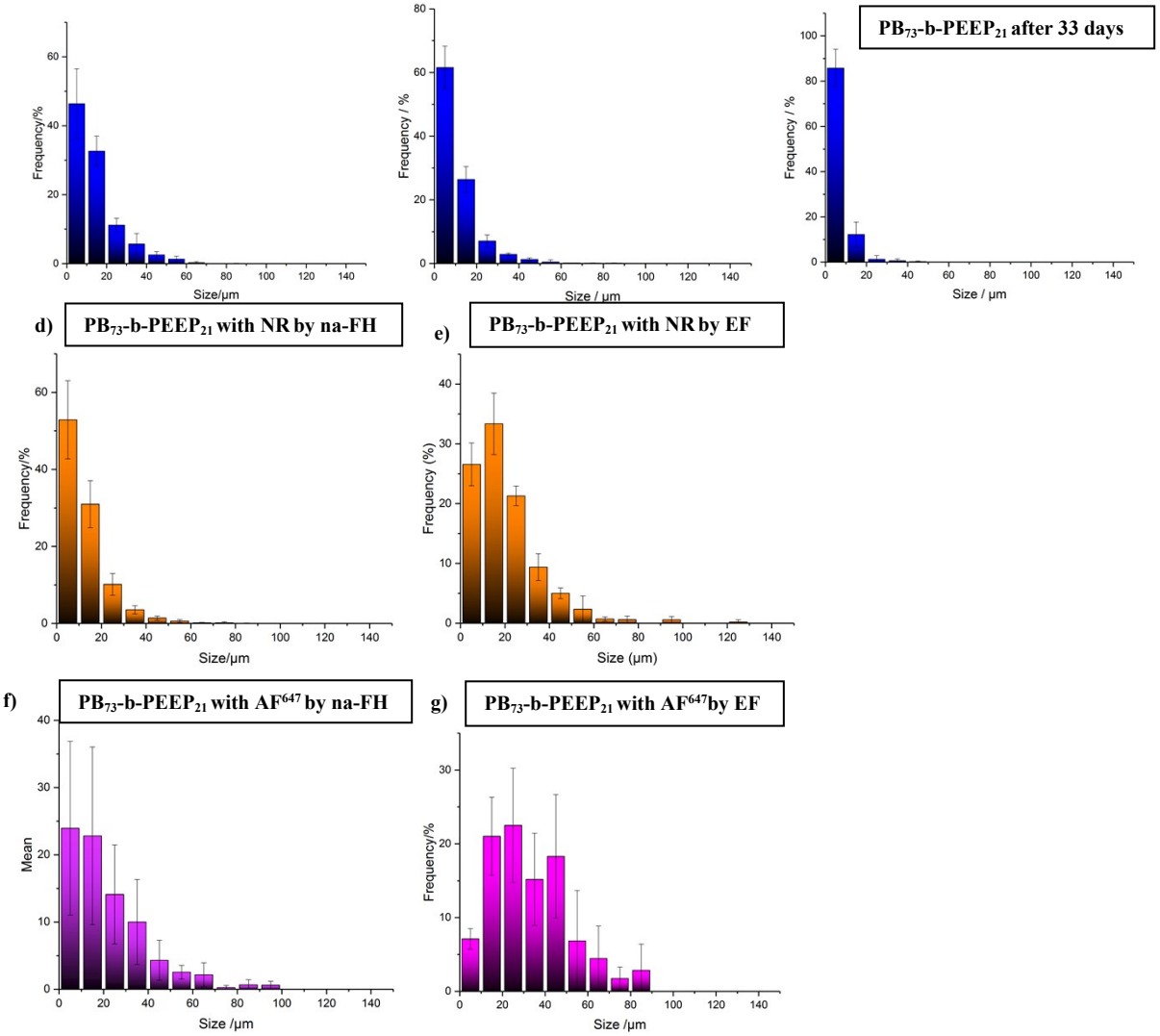


Figure S11. Frequency diagram of the PB_{73}-*b*-$PEEP_{21}$ GUVs diameter a) when freshly formed by na-FH, b) freshly formed by EF, c) after 33 days, d) formed by na-FH in the presence of Nile Red (NR), e) formed by EF in the presence of Nile Red (NR), f) formed by na-FH in the presence of AF^{647}, g) formed by EF in the presence of AF^{647}.

Table S23. GUV diameter and polydispersity (PDI) of PB_{46}-*b*-PEO_{23} by EF. The experiments were carried out in replicate (A, B, C, D) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Replicate D	Average
Mean diameter (µm)	37.71	40.55	48.07	22.56	37.22±10.71
Std dev	24.13	25.78	18.99	14.51	-
PDI	0.41	0.40	0.16	0.41	0.35

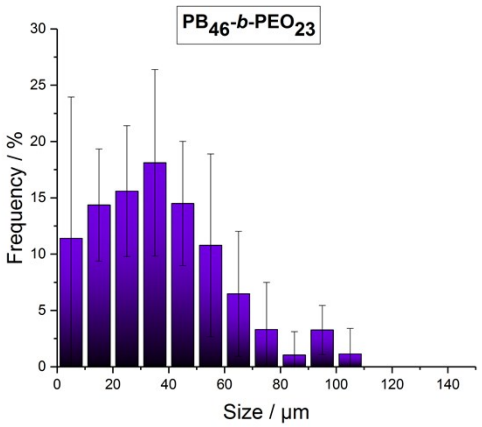


Figure S12. Frequency diagram of the PB_{46}-*b*-PEO_{23} GUVs diameter when freshly formed by EF.

Table S24. GUV diameter and polydispersity (PDI) of $PDMS_{60}$-*b*-$PMOXA_{21}$ by na-FH. The experiments were carried out in triplicate (A, B, C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	18.95	25.49	24.40	22.94±3.50
Std dev	5.35	15.15	10.04	-
PDI	0.08	0.35	0.17	0.20

Table S25. GUV diameter and polydispersity (PDI) of $PDMS_{60}$-*b*-$PMOXA_{21}$ by EF. The experiments were carried out in triplicate (A, B, C) to determine the average diameter and associated standard deviation.

	Replicate A	Replicate B	Replicate C	Average
Mean diameter (µm)	45.77	20.85	13.31	26.64±16.99
Std dev	32.80	9.31	7.04	-
PDI	0.51	0.20	0.28	0.33

POLYMERIC FILM IN BULK BY TEM

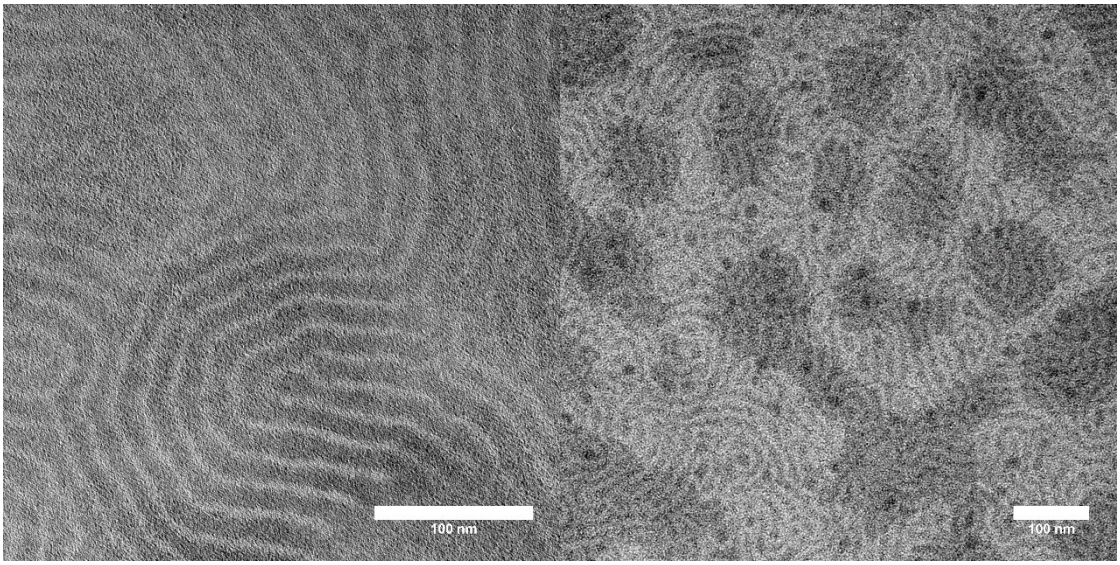


Figure S13. TEM images of drop casted PB_{73}-b-$PEEP_{21}$ B stained with RuO_4. Average lamellar distance: 11.0±1.5 nm (darker stripes) and 4.4±0.7 nm (lighter stripes)

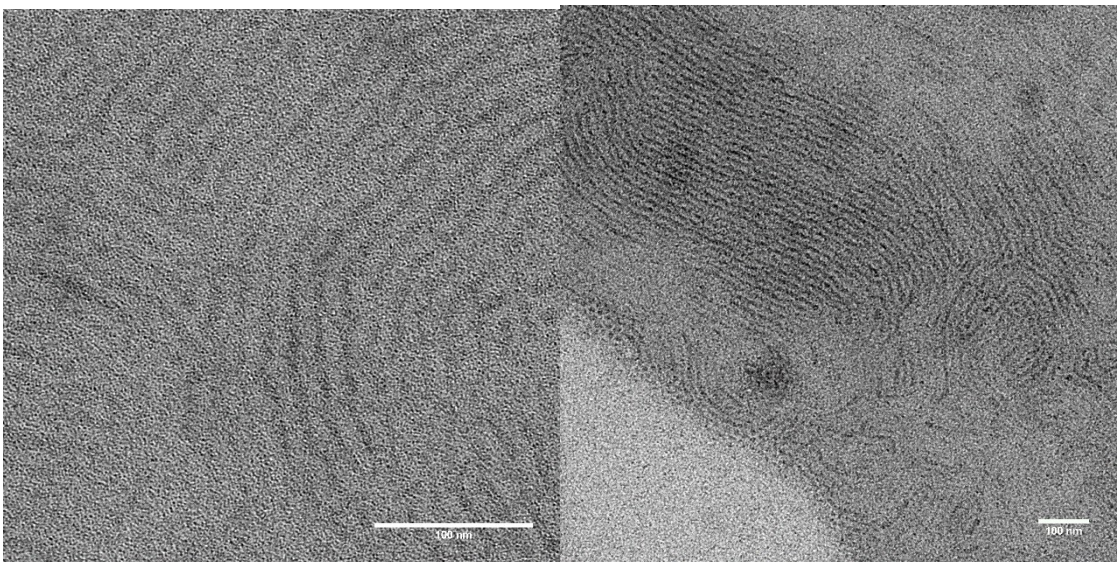


Figure S14. TEM images of drop casted PB_{73}-*b*-$PEEP_{12}$ A stained with RuO_4. Average lamellar distance: 10.4±1.3 nm (lighter stripes) and 3.5±0.4 nm (darker stripes)

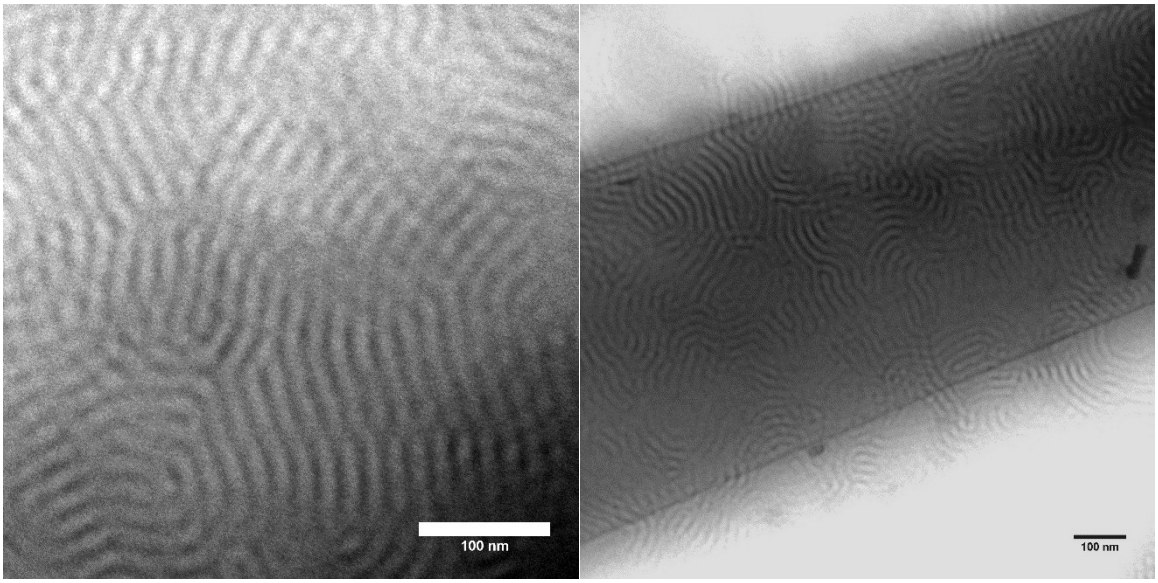


Figure S15. TEM images of drop casted PB_{73}-*b*-$PEEP_{31}$ stained with RuO_4. Average lamellar distance: 8.2±1.1 nm (lighter stripes) and 5.5±0.3 nm (darker stripes)

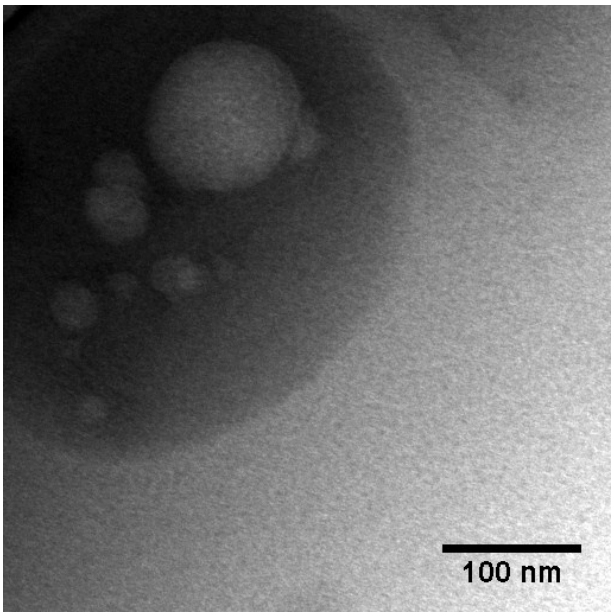


Figure S16. TEM images of drop casted PB_{73}-*b*-$PEEP_4$ stained with RuO_4. No special structure were observed.

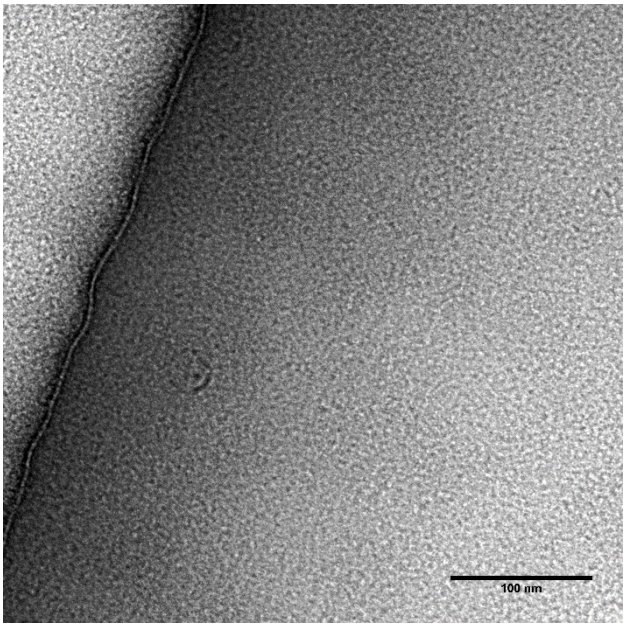


Figure S17. TEM images of drop casted PB_{73}-*b*-$PEEP_7$ stained with RuO_4. No special structure were observed.

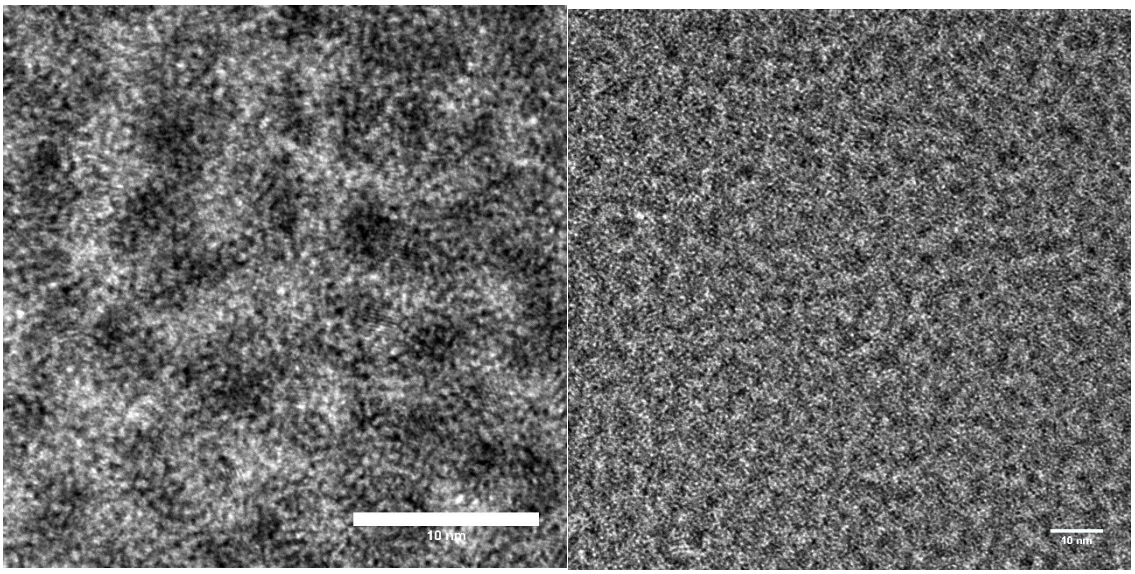


Figure S18. TEM images of drop casted PB_{46}-*b*-PEO_{23} stained with RuO_4. No special structure were observed.

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