Supplementary Information

Self-assembly of double-hydrophilic biocompatible block copolymers in concentrated aqueous solution

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1. Polymer synthesis:

Materials

2-(Methacryloyloxy)ethyl phosphorylcholine (MPC > 99 %) was kindly donated by Biocompatibles Ltd, (Farnham, UK). Monohydroxy-capped poly(ethylene oxide) precursors with mean degrees of polymerization of 22, 45 and 114 (as judged by ^{1}H NMR) were purchased from Fluka and used as received. ME-Br initiator was synthesized as previously described[1]. MP-Br initiator and rhodamine methacrylate monomer syntheses are detailed below. Column chromatography grade silica gel 60 (0.063-0.200 nm) was purchased from E. Merck (Darmstadt, Germany). Cu(I) Cl (99.995%), 2,2'-bipyridine (bpy, 99%), 2-bromoisobutyl bromide (BIBB), triethylamine and all other chemicals were purchased from Aldrich. BIBB was vacuum-distilled from calcium hydride and all other chemicals were used as received.

1.1 m-Methylphenyl-2-bromoisobutyryl bromide (MP-Br) ATRP initiator synthesis

3-Methylphenol (13.52 g, 0.125 mol, 1.0 equiv), triethylamine (52.2 mL, 0.375 mol, 3.0 equiv), and 4-(dimethylamino)pyridine (1.53 g, 12.5 mmol, 0.1 equiv) were dissolved in 250 mL of dry dichloromethane in a 1 L two-neck round-bottomed flask under a nitrogen atmosphere. This flask was immersed in an ice bath for 15 min, and then BIBB (34.49 g, 0.15 mol, 1.2 equiv) was added dropwise to the stirred solution using an addition funnel over 1 h. After stirring for 24 h at 20 °C the reaction mixture was filtered to remove the triethylamine hydrobromide by-product. This solution was washed three times with a saturated aqueous solution of sodium hydrogen carbonate (500 mL) and three times with deionized water (500 mL). The purified organic solution was dried using anhydrous $MgSO_4$ and dichloromethane was removed under reduced pressure. The crude brown product was then purified by column chromatography using silica gel as the stationary phase and a 1:15 ethyl acetate/petroleum ether mixed eluent as the mobile phase. The final 3-methylphenyl bromoisobutyrate (MP-Br) initiator was obtained as a slightly yellow liquid (32.1 g, 87%) and was stored in a freezer under nitrogen prior to use.

^{1}H NMR (CD_2Cl_2): δ 7.37 (t, 1H), 7.17 (d, 1H), 7.01 (d, 2H), 2.45 (s, 3H), 2.14 (s, 6H);

MS (EI+): m/z = 256 Da.
Microanalyses: Calculated for $C_{11}H_{13}BrO_2$: C = 51.38; H = 5.10; Br = 31.08. Found: C = 51.61; H = 5.25; Br = 31.09.

1.2. ATRP synthesis of PMPC homopolymers

A typical protocol for the controlled polymerization of 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) *via* methanolic ATRP to yield

$PMPC_{50}$ is as follows. MP-Br initiator (0.1500 g, 0.54 mmol, 1 eq), 2,2'-bipyridine (0.1671 g, 1.1 mmol, 2 eq) and MPC (8.033 g, 26.8 mmol, 50 eq) were weighed into a round-bottomed flask and degassed under nitrogen for 15 min. Methanol was separately degassed under nitrogen and added to the flask *via* syringe to produce a 50 w/w % MPC solution. After purging with nitrogen for a further 5 min, Cu(I)Cl catalyst was added to the stirred solution under a constant flow of nitrogen at 20 ^{o}C. The reaction mixture immediately turned a dark brown and became progressively more viscous. Polymerization was terminated after 105 min by quenching with excess ethanol and water. The PMPC homopolymer was then passed through a silica column (ethanol eluent) to remove residual Cu(II)Cl. In order to remove residual bpy, PMPC was precipitated from ethanol into a ten-fold excess of non-solvent (either THF or ether). The purified PMPC homopolymer was analyzed by ^{1}H NMR (in 3:1 v/v% $CDCl_3$: CD_3OD) and GPC (using a 3:1 v/v % chloroform: methanol mixed eluent) calibrated with near-monodisperse poly(methyl methacrylate) standards. Two other PMPC homopolymers with target DPs of 15 and 30 respectively were also synthesized using this protocol.

1.3. Synthesis of rhodamine 6G 4-(2-(methacryloyloxy)ethyl)piperazine amide fluorescent monomer

Synthesis of rhodamine 6G 4-(2-hydroxyethyl)piperazine amide

In a round-bottomed flask, rhodamine 6G (10.0 g, 0.021 mol) was dissolved in N-(2-hydroxyethyl)piperazine (20.0 g, 0.154 mol). The flask was fitted with a reflux condenser, placed under nitrogen and heated to 90 °C for approximately 24 h. After cooling, the solution was dissolved in the minimum amount of methanol and poured into 500 mL water. After filtering, the aqueous solution was saturated with sodium chloride and extracted with 50 mL aliquots of a 2:1 isopropanol: dichloromethane mixture until only a faint color remained in the aqueous phase. The combined organic phases were dried over anhydrous sodium sulfate, filtered

and dried by evaporation. The rhodamine 6G 4-(2-hydroxyethyl)piperazine amide intermediate was obtained as a dark red powder (7.2 g, 65 % yield) and used without further purification.

^{1}H NMR (400 MHz, 3:1 $CDCl_3$: CD_3OD) δ 7.52 (m, 2H), 7.37 (m, 1H), 7.16 (m, 1H), 6.73 (s, 2H), 6.55 (s, 2H), 3.46 (t, 1H, J = 5.50 Hz), 3.40 (t, 2H, J = 5.62 Hz), 3.29 (q, 7.27 Hz), 3.16 (br m, 2H + MeOH), 2.94 (br t, 2H, J~5.1 Hz), 2.54 (br t, 2H, J~5.0 Hz), 2.40 (t, 1H, J = 5.38 Hz), 2.24 (t, 2H, J = 5.62 Hz), 1.97 (s, 6H), 1.17 (t, 6H, J = 7.21 Hz) ppm

^{13}C NMR (400 MHz, 3:1 $CDCl_3$: CD_3OD) δ170.42, 160.21, 159.08, 157.63, 138.00, 136.75, 133.94, 133.05, 132.82, 132.27, 130.42, 128.20, 116.52, 96.66, 61.20, 55.50, 52.98, 46.41, 41.25, 20.00, 16.23 ppm.

MS (EI+), m/z = 527 Da

Synthesis of rhodamine 6G 4-(2-(methacryloyloxy)ethyl)piperazine amide

In a round-bottomed flask was placed rhodamine 6G 4-(2-hydroxyethyl)piperazine amide **(**neutral form, 1.0051 g, 1.9 mmol) and methacrylic acid (20.0 mL, 20.3 g, 0.236 mol). To this mixture was added 50 mL chloroform and 12.6 mg BHT. Once a homogeneous solution had formed, methacrylic anhydride was added (2 mL, 2.07 g, 13.4 mmol). After 40 h, 2 mL methanol was added to quench residual methacrylic anhydride and the reaction mixture was stirred for a further 2 h at 20 °C. Chloroform was evaporated at 30 °C under reduced pressure and the residue was poured into 200 mL diethyl ether. After filtration and washing with diethyl ether, the solid residue was partitioned between dichloromethane (100 mL) and water (50 mL). Sodium hydrogen carbonate was added until gas evolution ceased and the aqueous phase was washed with aliquots of dichloromethane (3 x 50 mL). The combined organics were washed with water (five 50 mL portions) and finally with a saturated sodium bromide solution (50 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated. After precipitation into diethyl

ether, the dark red solid product (0.859 g, 76 % yield) was found to be ≥ 95 % pure by ^{1}H NMR.

^{1}H NMR (400 MHz, 3:1 $CDCl_3$: CD_3OD) δ 7.78 (m, 2H), 7.65 (m, 1H), 7.46 (m,1H), 7.00 (s, 2H), 6.85 (s, 2H), 6.09 (s, 1H), 5.63 (s, 1H), 4.23 (t, 2H, J = 5.62 Hz), 3.55 (q, 4H, J = 7.15 Hz), 3.41 (br m, 4H), 2.65 (t, 2H, J = 5.75 Hz), 2.37 (br m, 2H), 2.32 (br m, 2H), 2.21 (s, 6H), 1.93 (br s, 3H), 1.40 (t, 6H, J = 7.21 Hz) ppm.

^{13}C NMR (400 MHz, 3:1 $CDCl_3$: CD_3OD) δ 173.16, 167.20, 156.97, 156.08, 153.43, 136.05, 131.51, 130.28, 129.69, 128.97, 127.51, 126.05, 119.66, 113.49, 93.64, 61.84, 56.32, 53.47, 52.69, 47.53, 41.79, 38.44, 19.80, 18.52, 13.76 ppm.

ES (EI+) m/z = 595 Da

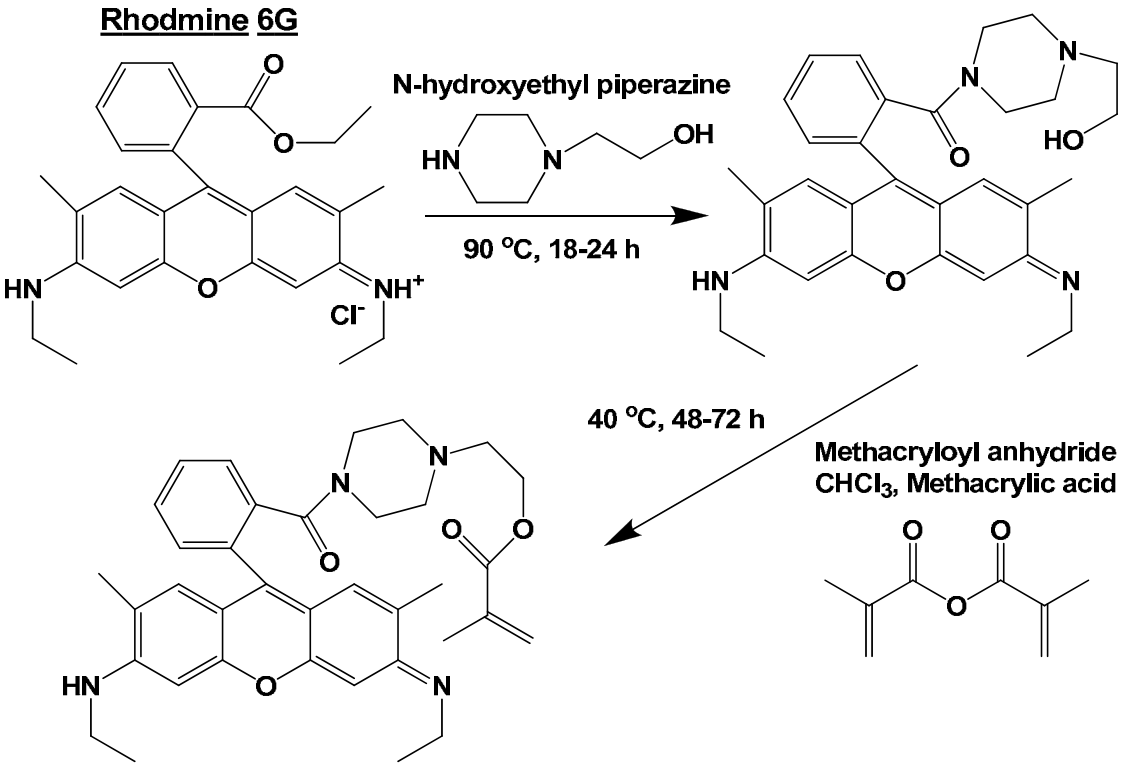

Figure S1. Synthetic route to rhodamine-piperazine ethyl methacrylate (RhMA)

1.4. Rhodamine-labelled P(MPC$_x$-stat-RhMA$_1$) statistical copolymer

The synthesis of rhodamine-labelled PMPC chains was analogous to that of the non-labelled homopolymers, except that 1 eq. of rhodamine 6G 4-(2-(methacryloyloxy)ethyl)piperazine amide was dissolved in the initial solution in addition to the MP-Br, MPC and the 2,2'-bipyridine. DP values of 30 and 50 were targeted for the P(MPC$_x$-*stat*-RhMA$_1$) copolymers after analyses of the purified fluorescent homopolymer by ^{1}H NMR. GPC was conducted using a 3:1 chloroform:methanol mixed eluent calibrated with near-monodisperse poly(methyl methacrylate) standards.

1.5. Transesterification of MP-Br end-groups during MPC homopolymerization in methanol

The *m*-methylphenyl-2-bromoisobutyryl bromide (MP-Br) initiator generates low polydispersity PMPC homopolymers *via* methanolic ATRP. However, partial transesterification of this initiator by methanol leads to the calculation of erroneous (over-estimated) DP values for PMPC homopolymer by ^{1}H NMR, due to the loss of the terminal *m*-cresol group (**see Figure S2**). Such transesterification has been previously reported for certain tertiary amine methacrylates[2,3] and also some ester-based ATRP initiators [4].

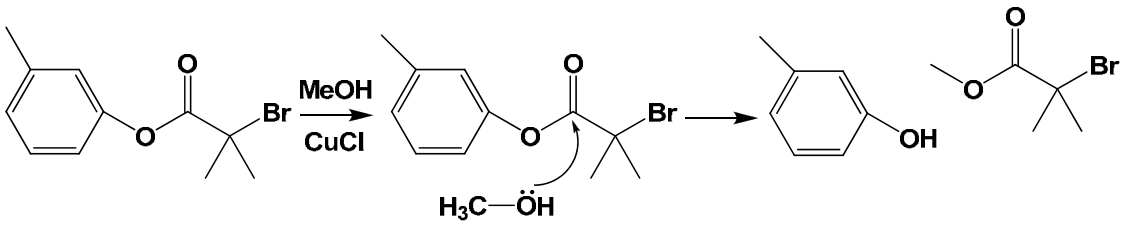

Figure S2. Transesterification of MP-Br in the presence of Cu(I)Cl and methanol.

In order to confirm that transesterification was actually occurring (rather than the problem being simply due to poor initiator efficiency), several control experiments were

conducted. As yet unpublished work in our group has shown that a phenoxy-based ATRP initiator (**see Figure S3**) has enhanced resistance towards transesterification compared to MP-Br when exposed to methanol.[4]

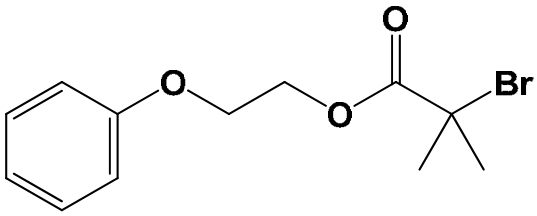

Figure S3 Phenoxy-based ATRP initiator

In order to minimize transesterification of the ATRP initiator end-groups, $PMPC_n$ (where n = 15, 30 and 50) syntheses were conducted using the phenoxy-based initiator in a less nucleophilic solvent (ethanol). These polymerizations were a little less well-controlled, as observed by the slightly higher polydispersities shown in **Figure S4**. However, ^{1}H NMR analysis yielded DP values which were almost identical to those targeted (**Figure S4**). This suggests that transesterification is negligible for MPC polymerizations conducted in ethanol and the initiator end-groups remain essentially intact.

Comparing the GPC traces in **Figure S4** of the PMPC homopolymers synthesized using the two ATRP formulations (i.e. using the phenoxy-based initiator in ethanol or the MP-Br initiator in methanol) indicates that the higher DP values measured by ^{1}H NMR when using the MP-Br initiator are simply due to transesterification, rather than poor initiator efficiency. For example, using MP-Br to target $PMPC_{15}$ suggests an incorrect NMR-derived DP of 27. However, the M_n value (7,700 g mol^{-1}) and the GPC trace for this sample are almost identical to that determined for a targeted $PMPC_{15}$ using the phenoxy-based initiator in ethanol (M_n = 7,400 g mol^{-1}, DP = 16 by NMR).

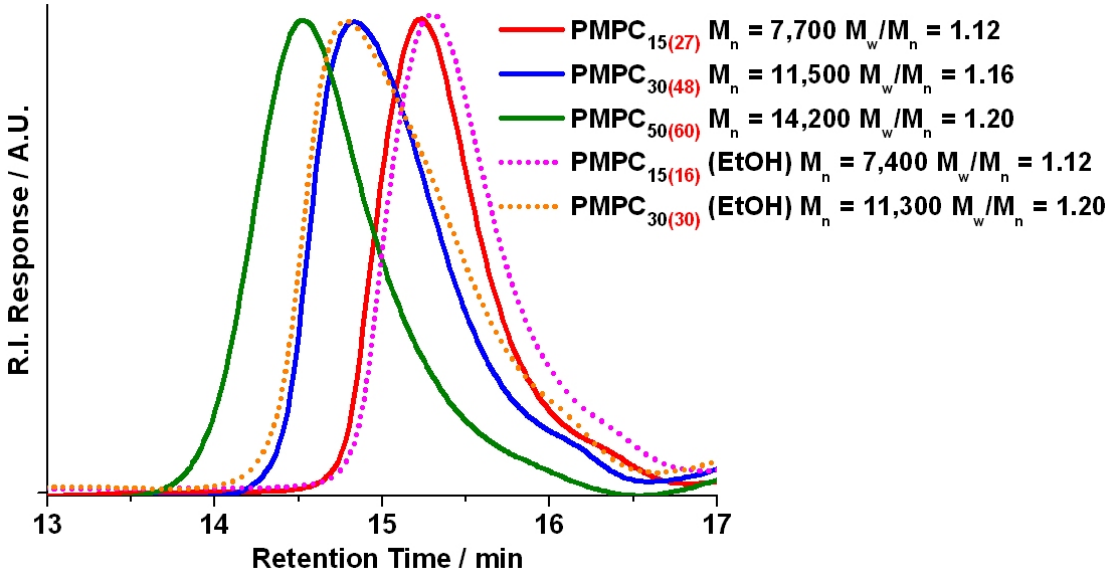

Figure S4. GPC traces, number-average molecular weights (M_n) and polydispersities (M_w/M_n) obtained for the various PMPC homopolymers, illustrating the systematic increase in molecular weight with increasing target DP. The apparent degree of polymerization (as calculated by NMR, but overestimated due to loss of the initiator end-group) is denoted in red parentheses. The eluent is a 3:1 v/v % mixture of Chloroform: methanol with 2 mM LiBr, calibrated with near-monodisperse PMMA standards.

Table S1. Molecular weights and polydispersities of the various PMPC homopolymers and $PMPC_x$-stat-$RhMA_1$ statistical copolymers used in this work.

Degree of Polymerization	M_n (g mol^{-1})	M_n[a] (g mol^{-1})	M_w/M_n[a]
$PMPC_{15}$	4,500	7,700	1.12
$PMPC_{30}$	9,000	11,500	1.16
$PMPC_{50}$	15,000	14,200	1.20
P(MPC_{30}-*stat*-$RhMA_1$)	10,100	11,400	1.17
P(MPC_{50}-*stat*-$RhMA_1$)	15,900	14,000	1.27

[a] Determined by GPC (3:1 chloroform: methanol mixed eluent) calibrated with near-monodisperse PMMA standards

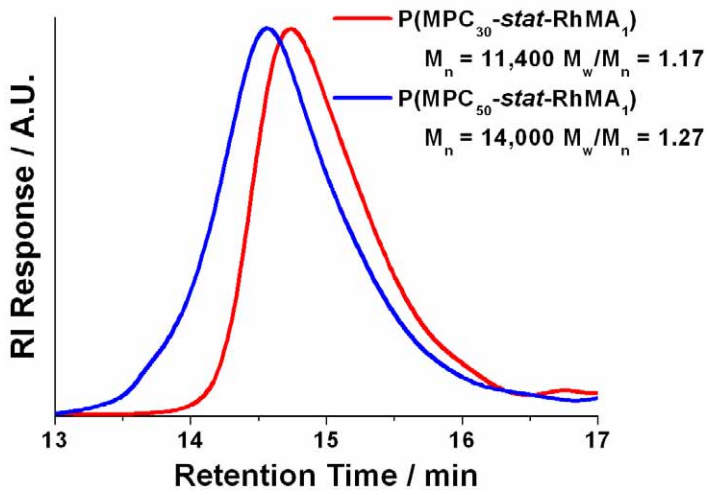

Figure S5. GPC traces, M_n and polydispersities of the rhodamine-containing PMPC statistical copolymers recorded using a 3:1 v/v% chloroform: methanol mixed eluent with 2 mM LiBr calibrated using near-monodisperse PMMA standards.

1.6. ATRP synthesis of PEO-PMPC diblock copolymers

The PEO_m-Br macro-initators were prepared as described previously[5]. A typical protocol for the synthesis of PEO_{45}-$PMPC_{40}$ *via* methanolic ATRP is as follows. PEO_{45}-Br macro-initiator (1.00 g, 0.46 mmol, 1 eq), 2,2'-bipyridine (0.144 g, 0.93 mmol, 2 eq) and MPC (5.553 g, 18.5 mmol, 40 eq) were weighed into a round-bottomed flask. Methanol was separately degassed under nitrogen and added to the flask *via* syringe (9.9 mL) to generate a 40 w/w % MPC solution. After purging with nitrogen for a further 15 min, Cu(I)Cl catalyst was added to the stirred solution under constant nitrogen flow. The reaction mixture immediately became dark brown and was allowed to react for 24 h at room temperature before quenching with excess methanol. The PEO_{45}-$PMPC_{40}$ copolymer was then passed through a silica column using methanol as an eluent to remove the residual ATRP catalyst. This diblock copolymer was then analyzed by ^{1}H NMR and GPC using a 3:1 chloroform: methanol mixed eluent calibrated with near-monodisperse poly(methyl methacrylate) standards. The higher molecular weight PEO_{114}-$PMPC_x$ diblock copolymer syntheses were conducted at 40 ^{o}C in order to fully solubilize the reaction mixture.

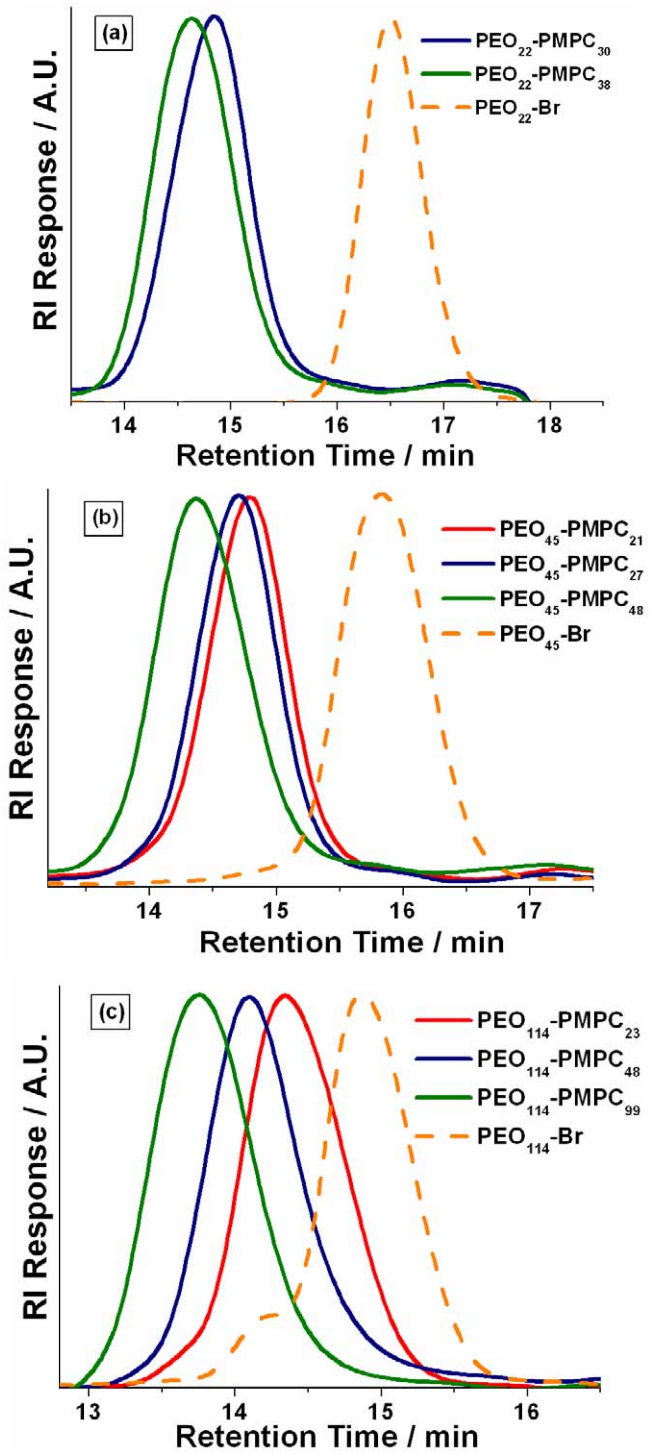

Figure S6. GPC traces obtained for the three series of PEO-PMPC diblock copolymers **(a)** PEO_{23}-$PMPC_x$ **(b)** PEO_{45}-$PMPC_x$ and **(c)** PEO_{113}-$PMPC_x$ used in this work. The GPC protocol involved a 3:1 v/v % chloroform: methanol mixed eluent and PMMA calibration standards.

Table S2. Summary of the PEO mole fractions, molecular weights and polydispersities of the ATRP-synthesized PEO-PMPC diblock copolymers used in this work.

Targeted Composition	Actual Composition[a]	PEO Mol. Fraction	M_n (g mol^{-1})[a]	M_n (g mol^{-1})[b]	M_w/M_n[b]
PEO_{22}-$PMPC_{20}$	PEO_{22}-$PMPC_{30}$	0.10	10,000	13,300	1.11
PEO_{22}-$PMPC_{30}$	PEO_{22}-$PMPC_{38}$	0.08	12,400	15,000	1.14
PEO_{45}-$PMPC_{15}$	PEO_{45}-$PMPC_{21}$	0.24	8,300	14,100	1.10
PEO_{45}-$PMPC_{20}$	PEO_{45}-$PMPC_{27}$	0.20	10,100	15,240	1.10
PEO_{45}-$PMPC_{40}$	PEO_{45}-$PMPC_{48}$	0.12	16,400	19,200	1.12
PEO_{114}-$PMPC_{15}$	PEO_{114}-$PMPC_{23}$	0.42	11,900	19,300	1.12
PEO_{114}-$PMPC_{40}$	PEO_{114}-$PMPC_{48}$	0.26	19,400	24,700	1.13
PEO_{114}-$PMPC_{90}$	PEO_{114}-$PMPC_{99}$	0.15	34,700	29,400	1.16
PEO_{22}-Br[a]	-	-	1,000	2,300	1.11
PEO_{45}-Br[a]	-	-	2,000	4,800	1.13
PEO_{114}-Br[a]	-	-	5,000	12,100	1.10

[a.] Mean degrees of polymerization and molecular weight calculated *via* ^{1}H NMR spectroscopy

[b.] Determined by GPC with a 3:1 v/v % chloroform: methanol eluent system calibrated with near-monodisperse PMMA standards

2. Aqueous phase separation behavior of PEO and PMPC homopolymers

The PEO and PMPC homopolymers were readily dissolved in water at the desired concentration (20-30 w/w%) and degassed under vacuum (5 min) to remove dissolved air. These concentrated aqueous solutions were then mixed in the desired volumes (e.g. 0.50 mL of each solution for the equi-volume mixing experiments) in NMR tubes and the relative heights of each phase were measured at 20 ^{o}C. Phase separation is only reported when a distinct meniscus is generated for the binary mixture.

The partition coefficient of water towards the PEO phase within the aqueous PEO and PMPC homopolymer mixtures, x, was calculated using Eq. 1,

$$x = \frac{pm}{(1-p)n} \qquad \text{(Eq. 1)}$$

Where p is the *post-mixed phase weight fraction* of PEO_{114}, n is the initial *aqueous weight fraction* of PEO_{113} and m is the initial *aqueous weight fraction* of $PMPC_{50}$.

Table S3. Summary of the equi-volume aqueous mixing experiments conducted using the various PEO and PMPC homopolymers at 20, 25 and 30 w/w %. The post-mixed relative PEO phase volume (%) is given in parentheses when a two-phase system is observed. Water preferentially segregates into the PEO phase with a partition coefficient, x = 1.38

PEO DP	PMPC DP	20 w/w % Two-phase system?	25 w/w % Two-phase system?	30 w/w % Two-phase system?
PEO_{22}	$PMPC_{15}$	**No**	**No**	**No**
PEO_{22}	$PMPC_{30}$	**No**	**No**	**No**
PEO_{22}	$PMPC_{50}$	**No**	**No**	**Yes (54 %)**
PEO_{45}	$PMPC_{15}$	**No**	**No**	**Yes (57 %)**
PEO_{45}	$PMPC_{30}$	**No**	**Yes (57 %)**	**Yes (56 %)**
PEO_{45}	$PMPC_{50}$	**No**	**Yes (61 %)**	**Yes (56 %)**
PEO_{114}	$PMPC_{15}$	**No**	**Yes (58 %)**	**Yes (57 %)**
PEO_{114}	$PMPC_{30}$	**Yes (62 %)**	**Yes (59 %)**	**Yes (57 %)**
PEO_{114}	$PMPC_{50}$	**Yes (60 %)**	**Yes (60 %)**	**Yes (56 %)**

Table S4. Phase separation behaviour of equal volume mixtures of aqueous PEO_{114} and $PMPC_{50}$ homopolymer solutions of varying initial weight fractions at 20 °C.

PEO_{113} (n)	$PMPC_{50}$ (m)	Two-phase system?	PEO_{114} vol.% after mixing	Partition coefficient of water in PEO (x)
0.12	0.10	No	-	-
0.20	0.10	No	-	-
0.30	0.10	Yes	82.7	1.59
0.40	0.10	Yes	85.2	1.44
0.50	0.10	Yes	86.2	1.25
0.06	0.30	No	-	-
0.12	0.30	Yes	44.9	2.03
0.20	0.30	Yes	46.5	1.30
0.30	0.30	Yes	56.0	1.27
0.06	0.45	No	-	-
0.12	0.45	Yes	35.9	2.10
0.20	0.45	Yes	40.0	1.50
0.30	0.45	Yes	43.9	1.17
			Mean x	1.50 ± 0.50

3. Diblock copolymer phase separation

Each block copolymer was accurately weighed into a glass vial and the corresponding desired weight of water was added. The samples were sealed and left to stand for a week at 20°C, after which they were stirred using a spatula. The samples were then left to stand for a further week prior to SAXS analysis. Briefly, x-ray scattering measurements were conducted at beam lines 2.1 and 6.2 of the synchrotron radiation source (SRS) at the Daresbury Laboratories, Warrington, UK and at BM26 at the European synchrotron radiation facility (ESRF) in Grenoble, France. Both beam lines were configured for SAXS using monochromatic radiation of wavelength λ = 1.54 Å. Static samples had a data collection time of 5 minutes. Background samples of either water of air were

recorded for 30 minutes. The PEO-PMPC diblock copolymer samples were loaded into a multi-faceted holder and sealed with Kapton tape. This consisted of a metal plate, with a 10 x 10 array of 10 mm diameter holes, spaced 10 mm apart (**see Figure S7a**). This enabled the loading of many samples at one time, facilitating rapid sample analysis. The holes also have a slight recess on the 'exit' in order to minimize diffraction due to the sample holder (**see Figure S7b**).

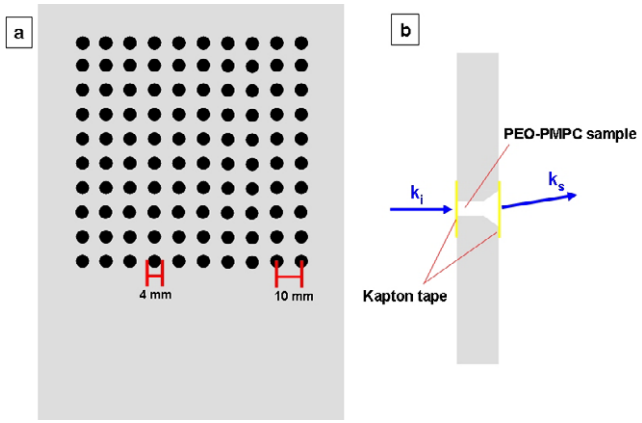

Figure S7. (a) Schematic representation of the sample holder used for SAXS analysis of the PEO-PMPC gels at both the SRS and ESRF facilities. **(b)** Schematic illustration of the geometry of each hole. The sample is sealed using Kapton tape, which is highly transparent to x-rays. The hole has a slight recess for the scattered radiation (k_s) in order to minimize x-ray diffraction and adsorption by the sample holder.

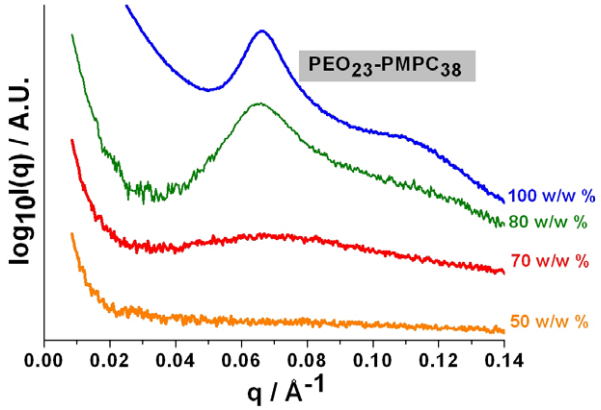

Figure S8. SAXS patterns obtained for PEO_{22}-$PMPC_{38}$ as a function of copolymer concentration in water at 21 ^{o}C.

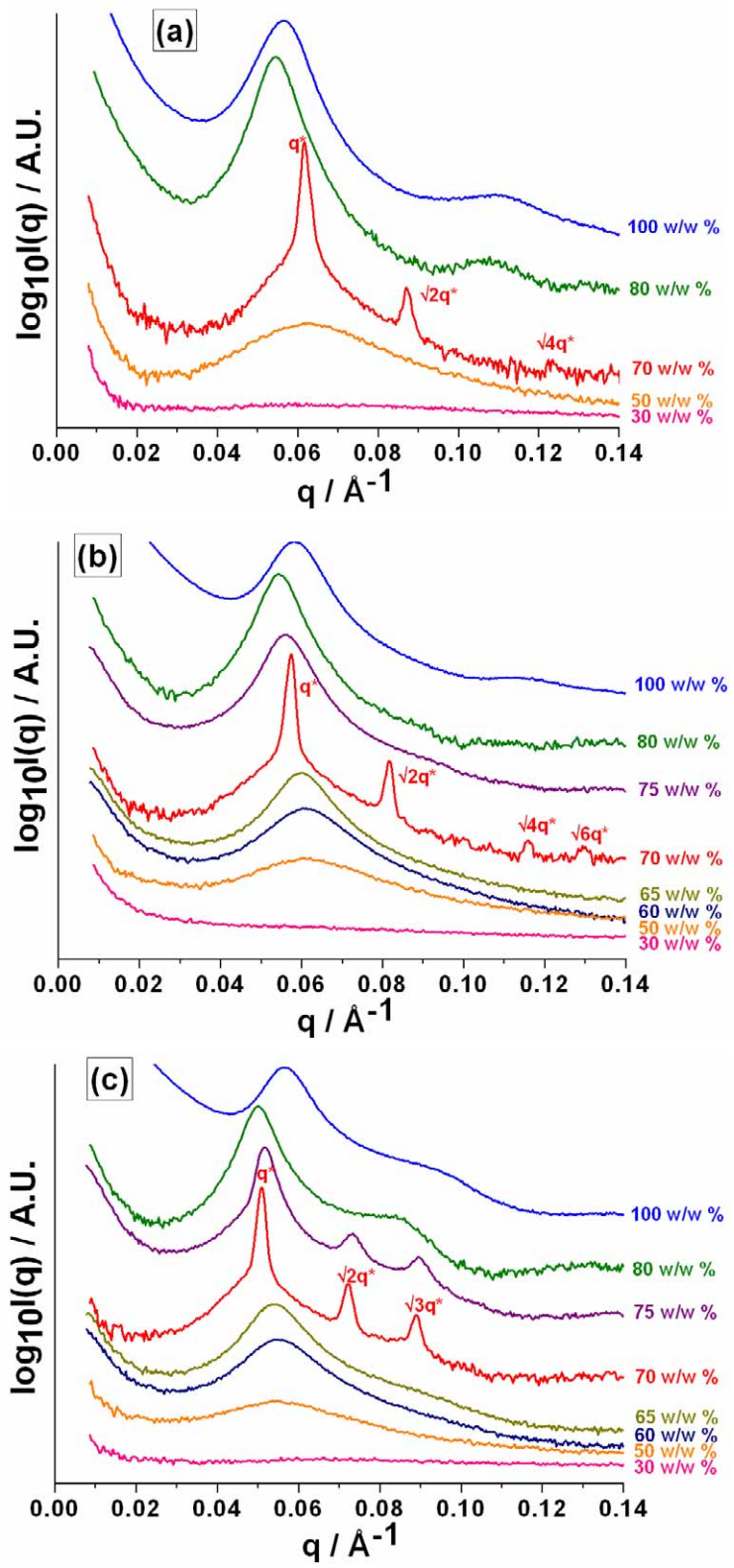

Figure S9. SAXS patterns obtained for **(a)** PEO_{45}-$PMPC_{21}$, **(b)** PEO_{45}-$PMPC_{27}$, and **(c)** PEO_{45}-$PMPC_{48}$ as a function of copolymer concentration in water at 21 ^{o}C

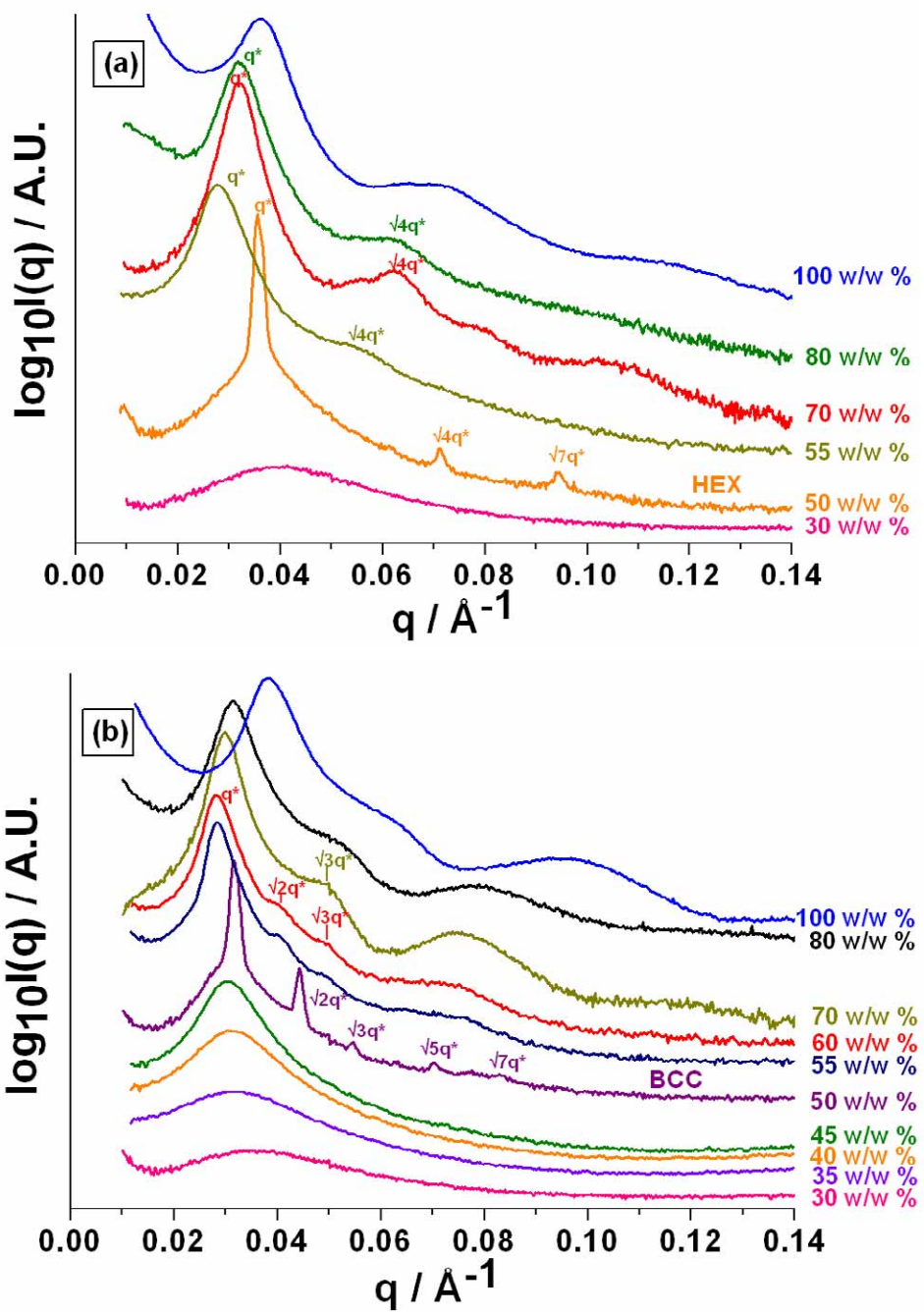

Figure S10. SAXS patterns obtained for **(a)** PEO_{114}-$PMPC_{48}$ and **(b)** PEO_{114}-$PMPC_{99}$, as a function of copolymer concentration in water at 21 ^{o}C

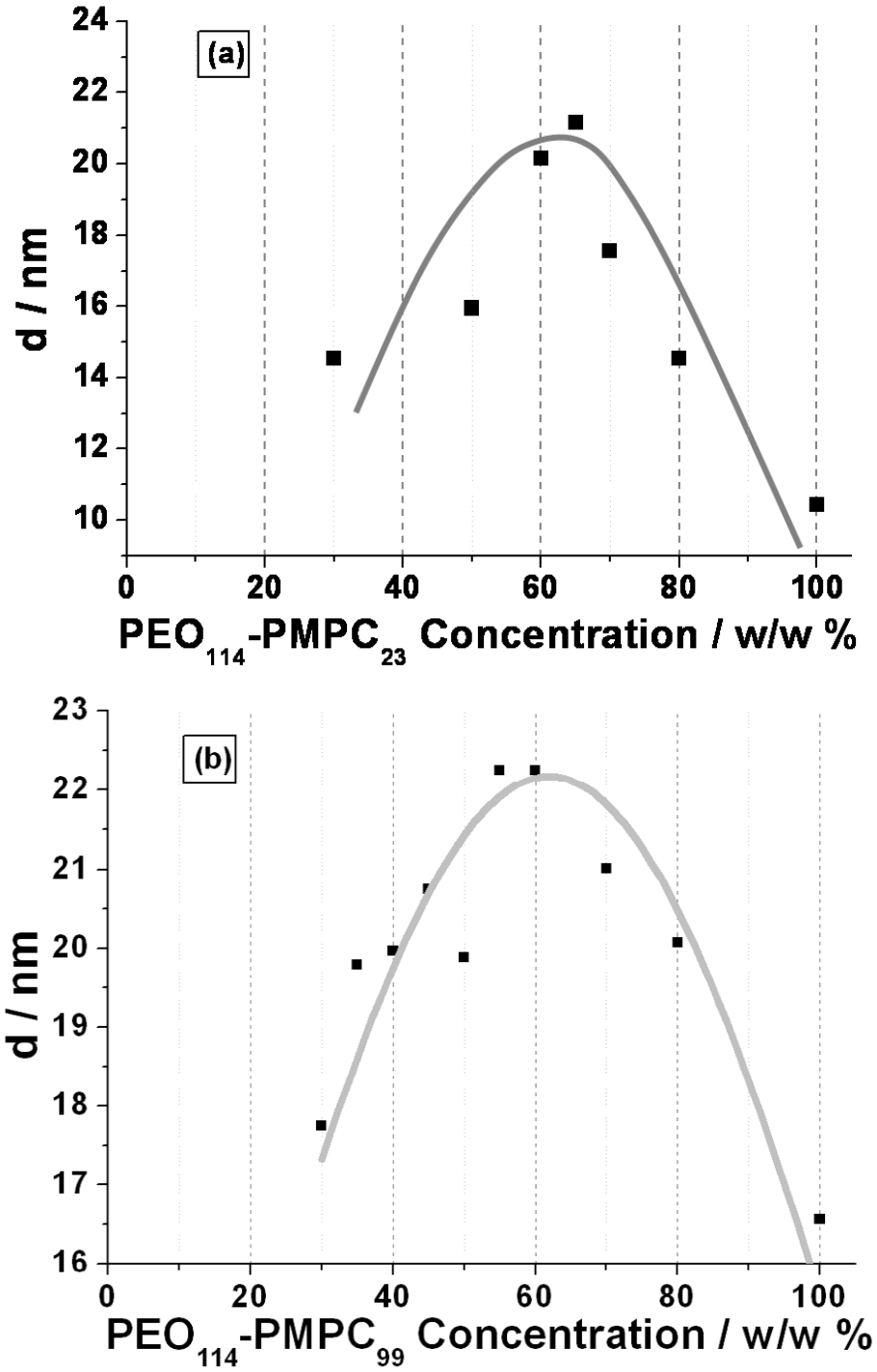

Figure S11. Variation in the SAXS domain size, d, on dilution with water for **(a)** PEO_{114}-$PMPC_{23}$ and **(b)** PEO_{114}-$PMPC_{99}$. The lines are added as guides to the eye.

References

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