Supplementary Material (ESI) for Polymer Chemistry

Supplementary information for:

Thermoresponsive giant biohybrid amphiphiles

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Characterisation of the bromine-terminated polymers

P(EGMEA,MEEA)$_{60}$-Br

^{1}H NMR ($CDCl_3$) δ (ppm): 4.17 (br, 4H); 3.62 (br m, 4H); 3.52(br m, 4H); 3.34 (br, 3H); 3.33(br, 3H); 2.34 (br, 2H); 1.90 (br, 1H); 1.65 (br, 2H); 1.49 (br, 1H).

FT-IR ν_{max} (cm^{-1}): 2923, 2879, 2820, 1729, 1449, 1248, 1164, 1107, 1030, 859.

GPC: M_n : 8 348 g mol^{-1}; PDI : 1.07

MALDI-TOF MS: m/z center of peak: 9 209

P(EGMEA,MEEA)$_{79}$-Br

^{1}H NMR ($CDCl_3$) δ (ppm): 4.17 (br, 4H); 3.61 (br m, 4H); 3.51(br m, 4H); 3.34 (br, 3H); 3.32(br, 3H); 2.33 (br, 2H); 1.90 (br, 1H); 1.64 (br, 2H); 1.50 (br, 1H).

FT-IR ν_{max} (cm^{-1}): 2924, 2879, 2819, 1729, 1449, 1247, 1163, 1107, 1030, 858.

GPC: M_n : 10 667 g mol^{-1}; PDI : 1.11

MALDI-TOF MS m/z: center of peak: 12 302

P(EGMEA,MEEA)$_{191}$-Br

^{1}H NMR ($CDCl_3$) δ (ppm): 4.20 (br, 4H); 3.65 (br m, 4H); 3.55(br m, 4H); 3.37 (br, 3H); 3.35 (br, 3H); 2.37 (br, 2H); 1.93 (br, 1H); 1.67 (br, 2H); 1.52 (br, 1H).

FT-IR ν_{max} (cm^{-1}): 2924, 2879, 2819, 1729, 1448, 1247, 1163, 1107, 1029, 857.

GPC: M_n : 25 879 g mol^{-1}; PDI : 1.16

MALDI-TOF MS m/z: center of peak: 29 307

Characterisation of the azide-terminated polymers

P(EGMEA,MEEA)$_{60}$

^{1}H NMR ($CDCl_3$) δ (ppm): 4.13 (br, 4H); 3.58 (br m, 4H); 3.48(br m, 4H); 3.30 (br, 3H); 3.29(br, 3H); 2.31 (br, 2H); 1.86 (br, 1H); 1.61 (br, 2H); 1.46 (br, 1H).

^{13}C NMR ($CDCl_3$) δ (ppm): 174.4, 71.9, 70.3, 70.2, 68.8, 63.4, 63.3, 58.9, 58.7, 41.2, 35.8, 35.0, 34.3.

FT-IR ν_{max} (cm^{-1}): 2922, 2879, 2820, 2111 (azide), 1730, 1449, 1248, 1164, 1108, 1030, 859.

GPC: M_n : 8 271 g mol^{-1}; PDI : 1.08

MALDI-TOF MS: m/z center of the peak: 9 433; details of individual peaks given in Table S1.

P(EGMEA,MEEA)$_{79}$

^{1}H NMR ($CDCl_3$) δ (ppm): 4.10 (br, 4H); 3.55 (br m, 4H); 3.45(br m, 4H); 3.27 (br, 3H); 3.25(br, 3H); 2.27 (br, 2H); 1.83 (br, 1H); 1.58 (br, 2H); 1.42 (br, 1H).

^{13}C NMR ($CDCl_3$) δ (ppm): 174.3, 71.8, 70.2 ,70.1 ,68.8, 63.3, 63.2, 58.8, 58.6, 41.1, 35.7, 34.9, 34.2.

FT-IR ν_{max} (cm^{-1}): 2924, 2879, 2820, 2111 (azide), 1729, 1449, 1248, 1164, 1108, 1030, 859.

GPC: M_n : 10 569 g mol^{-1}; PDI : 1.13

MALDI-TOF MS m/z: center of the peak: 12 185

P(EGMEA,MEEA)$_{191}$

^{1}H NMR ($CDCl_3$) δ (ppm): 4.12 (br, 4H); 3.58 (br m, 4H); 3.47(br m, 4H); 3.29 (br, 6H); 2.30 (br, 2H); 1.85 (br, 1H); 1.60 (br, 2H); 1.44 (br, 1H).

^{13}C NMR ($CDCl_3$) δ (ppm): 174.3, 71.8, 70.3, 70.2, 68.8, 63.4, 63.3, 58.9, 58.6, 41.2, 35.9, 35.0, 34.3.

FT-IR ν_{max} (cm^{-1}): 2924, 2879, 2819, 2115 (azide), 1729, 1449, 1247, 1163, 1107, 1029, 857.

GPC: M_n : 26 684 g mol^{-1}; PDI : 1.13

MALDI-TOF MS m/z: center of peak: 29 268

For all polymers, two sets of peaks were observed by ^{1}H NMR corresponding to the two monomers used, and their integration (detailed above) is indicative of the presence of equal amounts of both monomers in the polymers. Most notably, two peaks are seen corresponding to the methyl end group, both integrating to three protons. While it is not possible to differentiate which set of peaks corresponds to which of the two very similar monomers, the relative integration of all peaks clearly confirms a 1 : 1 ratio of the two monomers.

Table S1: Details of the MALDI-TOF MS spectra of P(EGMEA,MEEA)$_{60}$ showing the peaks measured, the predicted values (xEGMEA + yMEEA + end groups + Na) and the difference between measured and predicted values, as well as the polymer composition corresponding to each peak.

Measured	Predicted	Difference	nb EGMEA	nb MEEA	tot n
7827.9	7827.9	0	24	26	50
7869.7	7869.9	0	27	24	51
7913.7	7913.9	0	26	25	51
7956.3	7958.0	2	25	26	51
7999.5	8002.0	2	24	27	51
8043.4	8044.0	1	27	25	52
8086.9	8088.0	1	26	26	52
8130.5	8132.1	2	25	27	52
8174.2	8174.1	0	28	25	53
8218.5	8218.1	0	27	26	53
8260.3	8262.1	2	26	27	53
8304.3	8306.1	2	25	28	53
8347.9	8348.2	0	28	26	54
8390.0	8392.2	2	27	27	54
8434.4	8436.2	2	26	28	54
8477.2	8478.2	1	29	26	55
8521.3	8522.2	1	28	27	55
8566.1	8566.3	0	27	28	55
8608.1	8610.3	2	26	29	55
8651.4	8652.3	1	29	27	56
8695.4	8696.3	1	28	28	56
8740.6	8740.4	0	27	29	56
8781.9	8782.4	0	30	27	57
8827.9	8826.4	-2	29	28	57
8869.5	8870.4	1	28	29	57
8913.4	8914.4	1	27	30	57
8955.8	8956.5	1	30	28	58
8999.4	9000.5	1	29	29	58
9045.9	9044.5	-1	28	30	58
9084.6	9086.5	2	31	28	59
9129.1	9130.5	1	30	29	59
9173.7	9174.6	1	29	30	59
9217.8	9218.6	1	28	31	59
9262.6	9260.6	-2	31	29	60
9305.8	9304.6	-1	30	30	60
9348.5	9348.7	0	29	31	60
9388.4	9390.7	2	32	29	61
9434.2	9434.7	1	31	30	61
9479.5	9478.7	-1	30	31	61
9523.1	9522.8	0	29	32	61
9564.6	9564.8	0	32	30	62
9609.6	9608.8	-1	31	31	62
9652.4	9652.8	0	30	32	62
9693.6	9694.8	1	33	30	63
9738.9	9738.9	0	32	31	63
9782.6	9782.9	0	31	32	63
9824.6	9826.9	2	30	33	63

9869.1	9868.9	0	33	31	64
9913.0	9912.9	0	32	32	64
9955.9	9957.0	1	31	33	64
9996.5	9999.0	2	34	31	65
10043.1	10043.0	0	33	32	65
10085.7	10087.0	1	32	33	65
10129.6	10131.1	2	31	34	65
10171.7	10173.1	1	34	32	66
10215.7	10217.1	1	33	33	66
10259.3	10261.1	2	32	34	66
10302.3	10303.1	1	35	32	67
10346.6	10347.2	1	34	33	67
10391.0	10391.2	0	33	34	67
10434.9	10435.2	0	32	35	67
10476.4	10477.2	1	35	33	68
10521.7	10521.2	0	34	34	68
10566.5	10565.3	-1	33	35	68
10610.2	10607.3	-3	36	33	69
10654.5	10651.3	-3	35	34	69
10694.0	10695.3	1	34	35	69
10741.7	10739.4	-2	33	36	69
10780.7	10781.4	1	36	34	70
	Average:		*30*	*30*	*60*

Detailed cloud point studies on P(EGMEA,MEEA)$_{79}$

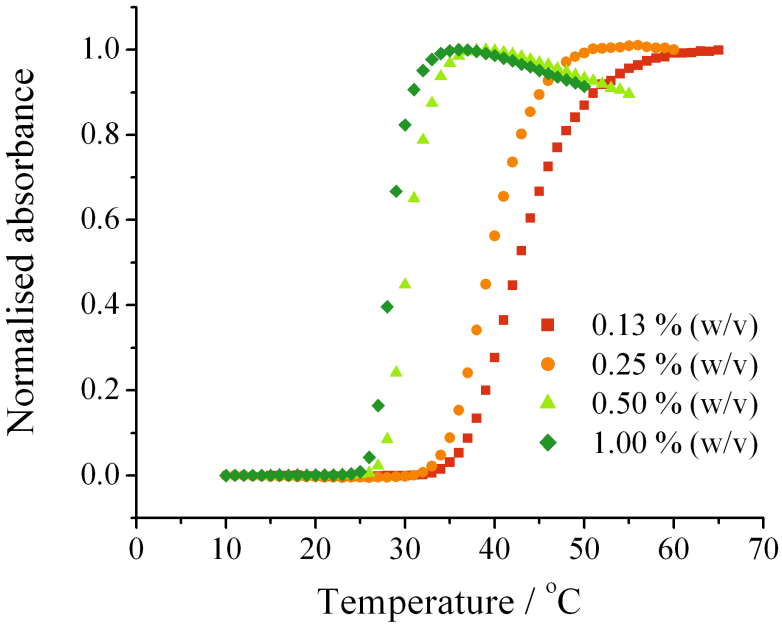

Fig. S1: Cloud point profile of P(EGMEA,MEEA)$_{79}$ at different concentrations in PBS (pH 8.0, 50 mM, 0.1 M NaCl) at a scanning rate of 1 °C min^{-1}.

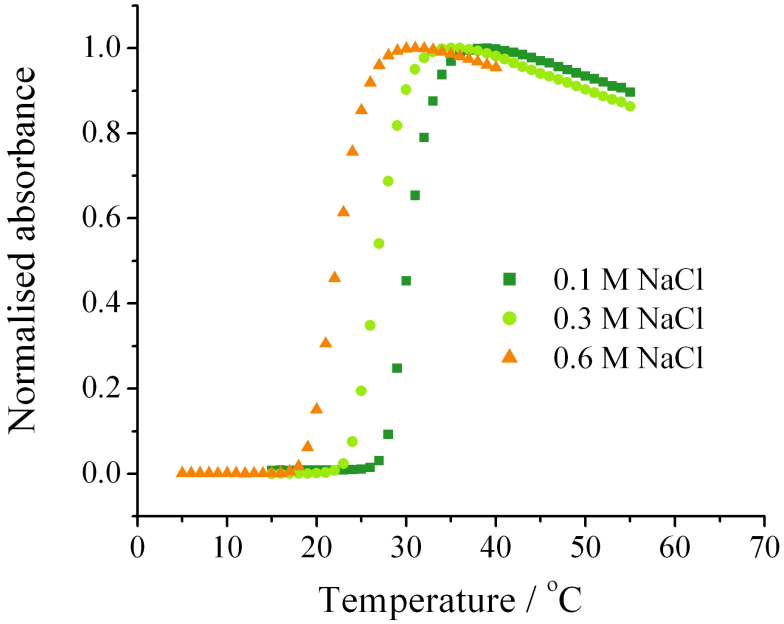

Fig. S2: Cloud point profile of P(EGMEA,MEEA)$_{79}$ with different salt concentrations in PBS (pH 8.0, 50 mM) at a scanning rate of 1 °C min^{-1} and a polymer concentration of 0.5 % (w/v).

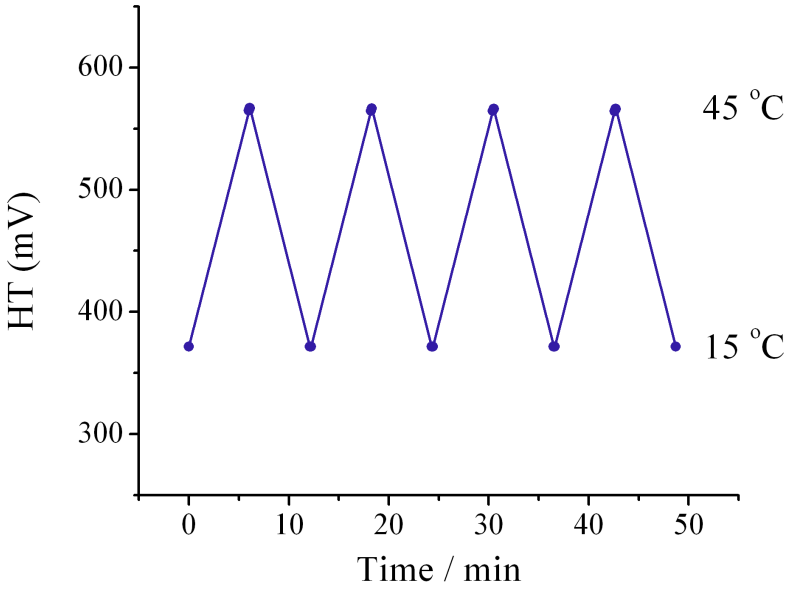

Fig. S3: Reversibility of the thermoresponsive behaviour of P(EGMEA,MEEA)$_{79}$ over five multiple heating and cooling cycles at a scanning rate of 1 °C min^{-1} on 0.5 % (w/v) solutions in PBS (pH 8.0, 50 mM, 0.1 M NaCl).

Mass spectrometry on acetylene-functionalised EGFP

Sequence of the EGFP:

MGSSHHHHHHSSGLVPRGSHMLEKREAEAGRLGAGGPVATMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGK
LTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRI
ELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQS
ALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

M/Z acetylene functionalised EGFP (m/z): 27 561, 27 714

The two peaks observed have a mass difference of 153, corresponding to the addition of one N-propargyl maleimide (135) and one water molecule (18). The first peak is believed to be unfunctionalised EGFP, and the second maleimide-functionalised EGFP.

The first peak is lighter than the expected mass of EGFP (31 082), indicating partial decomposition. Since the protein was later successfully bound to Ni-NTA beads, it is known that the His-tag at the N-terminus is still present, partial decomposition must therefore have mostly taken place at the C-terminus.

FPLC traces of the purified biohybrids

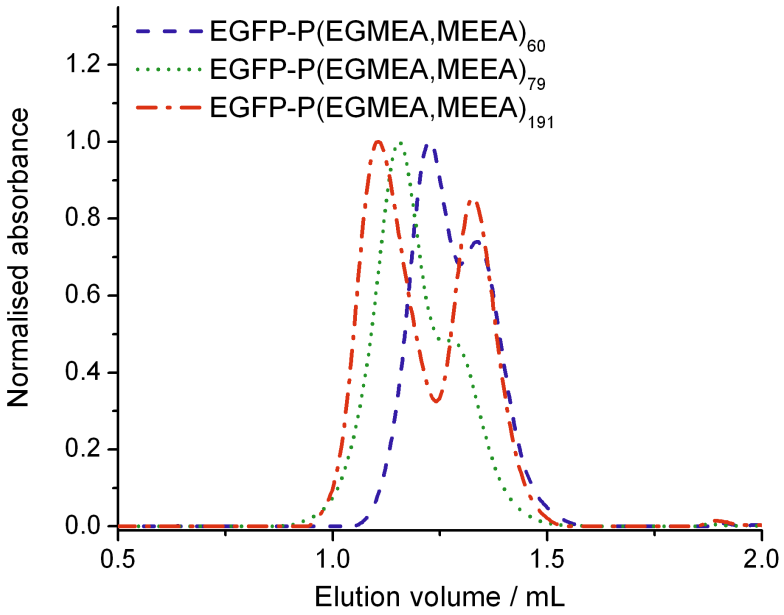

Fig. S4: FPLC trace of the three biohybrids after purification by FPLC ran on a Superdex 75 and monitored at λ = 490 nm.

For EGFP-P(EGMEA,MEEA)$_{191}$, integration of the two peaks indicates that approximately 60% of the proteins present in the final sample are functionalised with thermoresponsive polymers. In the case of the biohybrids with shorter polymer chains, an estimate cannot be given because of the significant peak overlap, but it appears that the proportion of functionalised proteins is higher.