

## Supporting Information

# pH sensitive water-in-water emulsions based on the pullulan and poly(*N,N*-dimethylacrylamide) aqueous two-phase system

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## Polymer Synthesis

### RAFT-polymerisation of DMA<sub>24k</sub>

In a dry and nitrogen purged 50 mL Schlenk tube, destabilised DMA (1.0 g, 10 mmol, 250 eq.), EMP (9.0 mg, 0.04 mmol, 1.0 eq.) and AIBN (1.3 mg, 0.008 mmol, 0.2 eq.) were dissolved in DMF (3 mL). The solution was degassed by three freeze-pump-thaw cycles and placed in a pre-heated oil bath (65 °C). Subsequently, the reaction mixture was stirred for 24 h, stopped by cooling down with liquid nitrogen and exposure to air. Afterwards, the polymer was dialysed against deionised water (Spectra/Por 3500 Da) for three days, freeze-dried and a yellow solid (992 mg,  $M_n = 23900 \text{ g}\cdot\text{mol}^{-1}$ ,  $\bar{D} = 1.11$  measured in NMP against PS standards) was obtained.

### RAFT-polymerisation of DMA<sub>80k</sub>

In a dry and nitrogen purged 50 mL Schlenk tube, EMP (9.4 mg, 0.042 mmol, 1.0 eq.), and AIBN (1.4 mg, 0.0085 mmol, 0.2 eq.) were dissolved in DMF (300  $\mu\text{L}$ ) and DMA (5.0 g, 10 mmol, 1200 eq.) added. The solution was degassed by three freeze-pump-thaw cycles and placed in a pre-heated oil bath (65 °C). Subsequently, the reaction mixture was stirred for 19 h, stopped by cooling with liquid nitrogen and exposure to air. Afterwards, the polymer was dialysed against deionised water (Spectra/Por 3500 Da) for three days, freeze-dried and a white solid (4.25 g,  $M_n = 80000 \text{ g}\cdot\text{mol}^{-1}$ ,  $\bar{D} = 1.06$  measured in NMP against PS standards) was obtained.

### RAFT-polymerisation of DMAEMA

In a dry and nitrogen purged 50 mL Schlenk tube, destabilised DMAEMA (2.0 g, 12.7 mmol, 130 eq.), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (27.0 mg, 0.097 mmol, 1.0 eq.) and AIBN (3.1 mg, 0.019 mmol, 0.2 eq.) were dissolved in DMF (5 mL). The solution was degassed by three freeze-pump-thaw cycles and placed in a pre-heated oil bath (65 °C). Subsequently, the reaction mixture was stirred for 24 h, stopped by cooling with liquid nitrogen and exposure to air. Afterwards, the polymer was

dialysed against deionised water (Spectra/Por 3500 Da) for three days, freeze-dried and a yellow solid (1.4g,  $M_n = 13900 \text{ g}\cdot\text{mol}^{-1}$ ,  $\mathcal{D} = 1.2$  measured in DMF against PEG standards) was obtained.

### **Synthesis of PDMAEMA-*b*-POEGMA via RAFT-polymerisation**

In a dry and nitrogen purged 50 mL Schlenk tube, destabilised OEGMA (1.5 g, 3.0 mmol, 84 eq.), PDMAEMA (500 mg, 0.035 mmol, 1.0 eq.) and AIBN (1.1 mg, 0.007 mmol, 0.2 eq.) were dissolved in DMF (5 mL). The solution was degassed by three freeze-pump-thaw cycles and placed in a pre-heated oil bath (65 °C). Subsequently, the reaction mixture was stirred for 24 h, stopped by cooling with liquid nitrogen and exposure to air. Afterwards, the polymer was dialysed against deionised water (Spectra/Por 3500 Da) for three days, freeze-dried and a yellow solid (1.99 g,  $M_n = 108000 \text{ g}\cdot\text{mol}^{-1}$ ,  $\mathcal{D} = 1.37$  measured in DMF against PEG standards) was obtained.

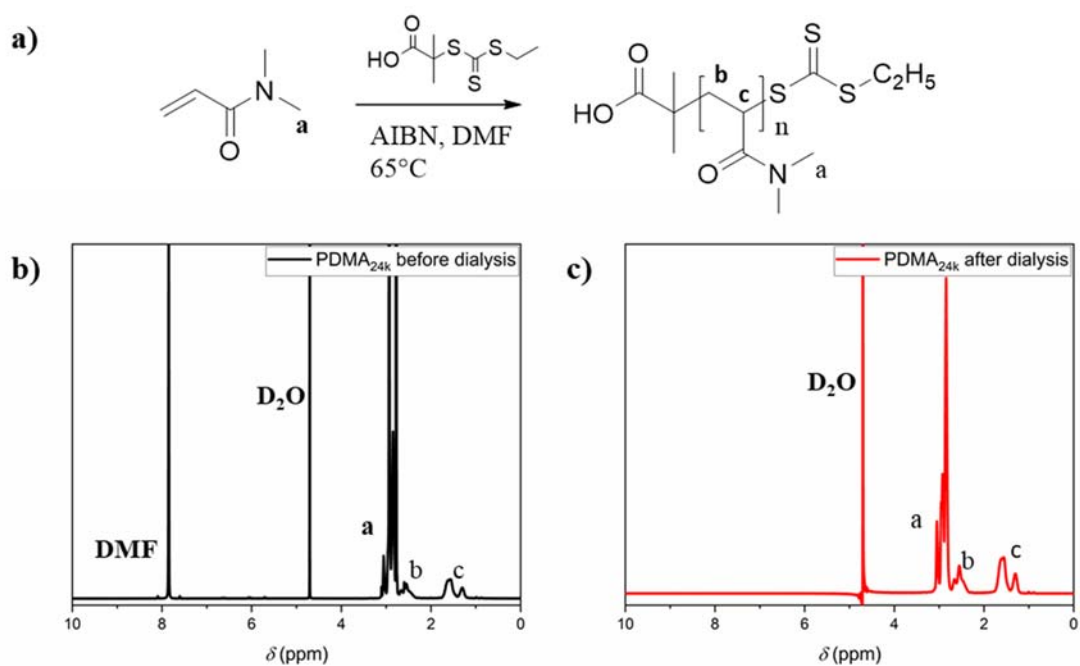
### **Formation of Rhodamine B labelled PDMA<sub>10</sub><sup>6</sup>**

In a dry, argon purged 100 mL round bottom Schlenk flask, PDMA (0.05 g, 0.0001 mmol, 1.0 eq.) was dissolved in dry DMSO (5 mL). Hexylamine (70  $\mu\text{L}$  from a 0.01 mL Hexylamine in 1 mL DMSO stock solution, 0.00026 mmol 2.5 eq.) was added, placed in a pre-heated oil bath (50 °C) and stirred overnight. Afterwards, the reaction mixture was cooled down to ambient temperature, Rhodamine B ITC (0.42 mg, 0.0008 mmol, 7.5 eq.) was added and the solution stirred over night at 50 °C. The mixture was cooled down to ambient temperature and diluted with deionised water. Afterwards, the polymer was dialysed against deionised water (Spectra/Por 3500 Da) for three days, freeze-dried and a purple solid (45.7 mg) was obtained.

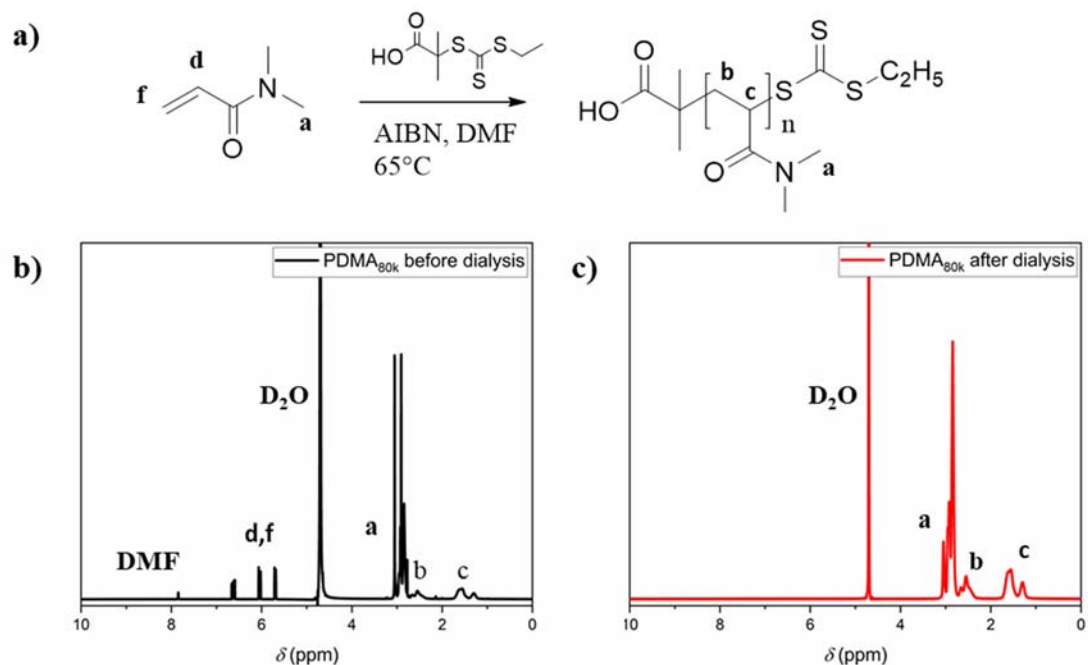
### **Formation of FITC labelled Pullulan**

Based on the literature,<sup>1,2</sup> pullulan (300 mg) was dissolved in DMSO (3 mL) containing pyridine (50  $\mu$ L). Subsequently, FITC (30 mg, 0.077 mmol, 1.0 eq.) and dibutyltin dilaurate (6 mg, 0.0095 mmol, 0.12 eq.) were added to the solution and the mixture was heated up for 2 h at 95 °C. Afterwards, the mixture was cooled down to ambient temperature and precipitated several times in ethanol, followed by dialysis against deionised water (Spectra/Por 3500 Da) for five days. Finally, the product was freeze-dried and an orange solid (302 mg) was obtained.

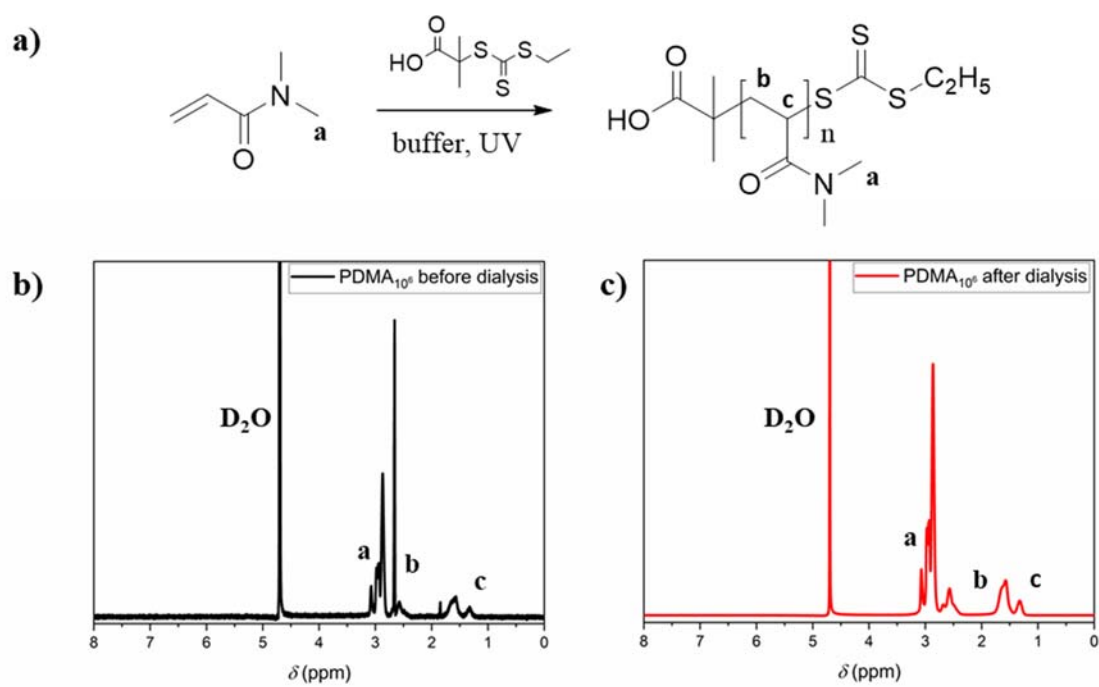
## Polymer analysis



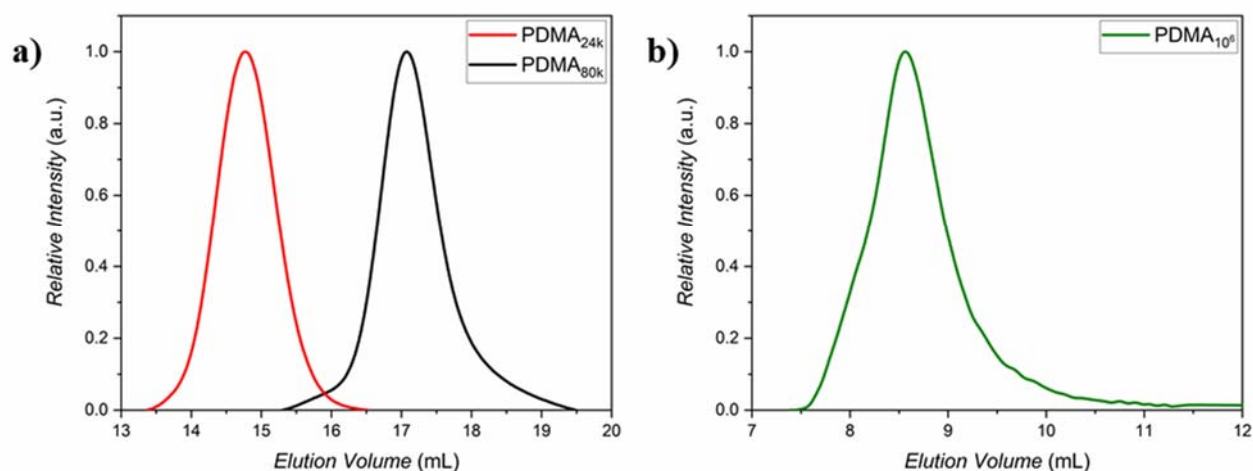
**Figure S1.** (a) Reaction scheme of the RAFT-polymerisation of *N,N*-dimethylacrylamide (DMA), (b and c)  $^1\text{H-NMR}$  of  $\text{PDMA}_{24\text{k}}$  in  $\text{D}_2\text{O}$  (b) before dialysis and (c) after dialysis.



**Figure S2.** (a) Reaction scheme of the RAFT-polymerisation of *N,N*-dimethylacrylamide (DMA), (b and c)  $^1\text{H-NMR}$  of  $\text{PDMA}_{80\text{k}}$  in  $\text{D}_2\text{O}$  (b) before dialysis and (c) after dialysis.



**Figure S3.** (a) Reaction scheme of the photo induced RAFT-polymerisation of *N,N*-dimethylacrylamide (DMA), (b and c)  $^1\text{H-NMR}$  of  $\text{PDMA}_{10}^6$  in  $\text{D}_2\text{O}$  (b) before dialysis and (c) after dialysis.



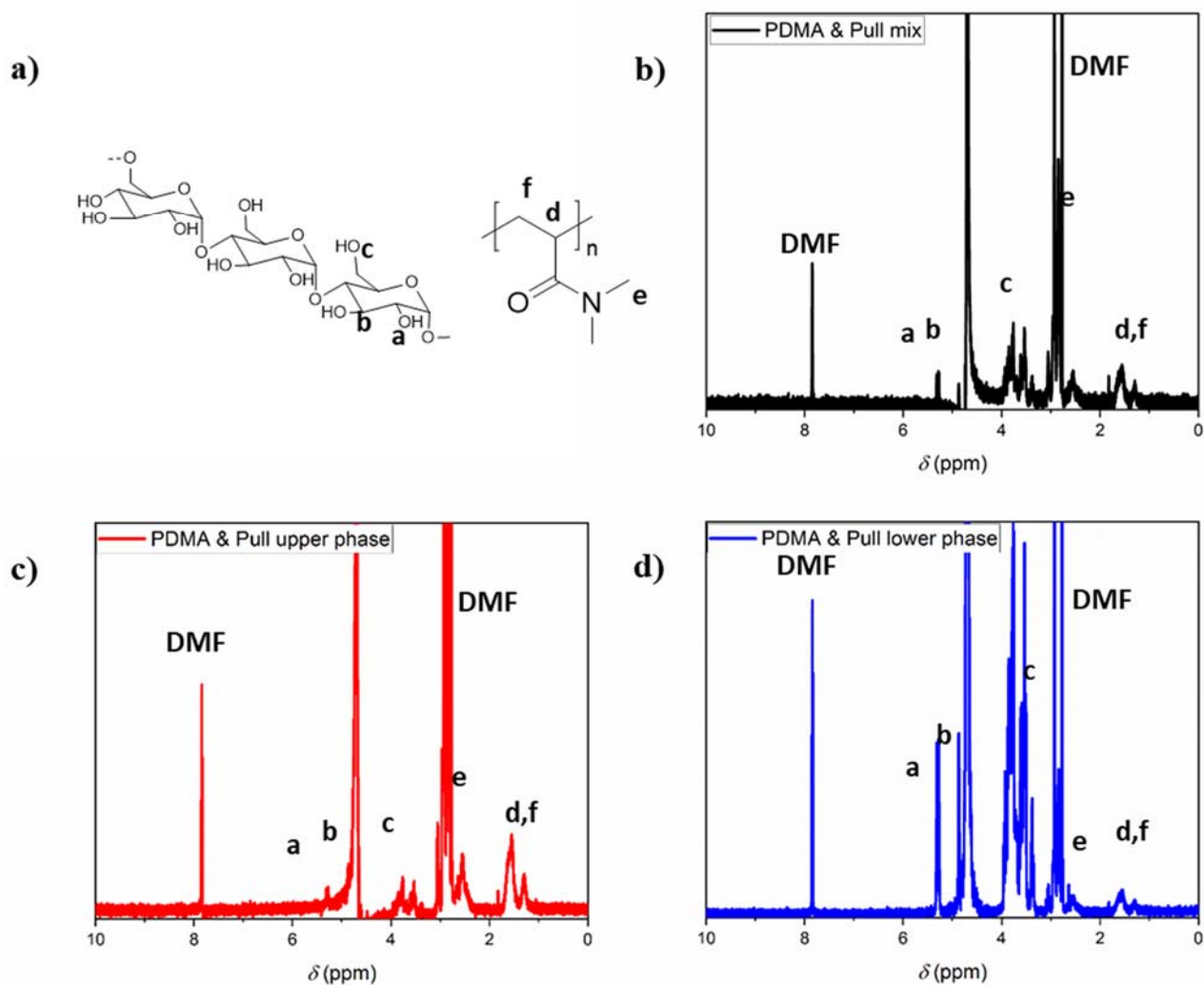
**Figure S4.** (a) Results of SEC measurement of low and medium molar mass PDMA measured in NMP against PS standards and (b) SEC-MALS measurement of UHMW PDMA measured in 0.1 N NaNO<sub>3</sub> buffer.

**Table S1.** Results of SEC measurements.

Polymer	$M_{n, \text{theory}} (\text{g}\cdot\text{mol}^{-1})$	$M_{n, \text{SEC}} (\text{g}\cdot\text{mol}^{-1})$	$D$	$dn/dc (\text{mL g}^{-1})$
PDMA <sub>24k</sub> <sup>a</sup>	24500	23900	1.11	-
PDMA <sub>80k</sub> <sup>a</sup>	118800	80000	1.06	-
PDMA <sub>10<sup>6</sup></sub> <sup>b</sup>	$1.5 \cdot 10^6$	$1.07 \cdot 10^6$	1.40	$0.1728 \pm 0.0039$
PDMAEMA <sup>c</sup>	20400	13900	1.20	-
PDMAEMA- <i>b</i> - POEGMA <sup>c</sup>	55600	108000	1.37	-

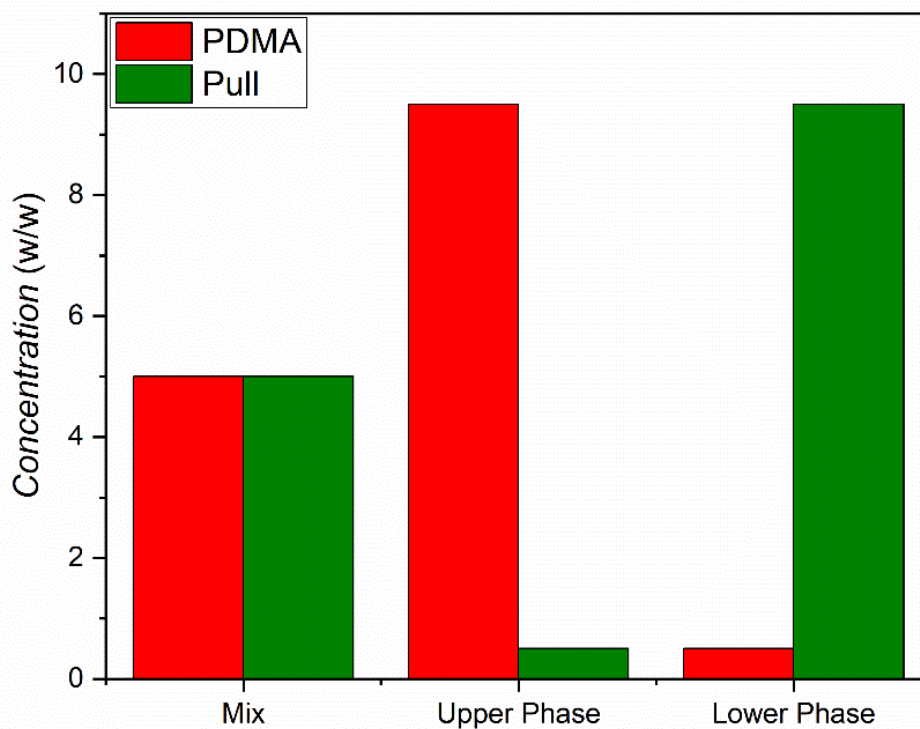
a) low and medium molar mass PDMA measured in NMP against PS standards, b) SEC-MALS measurement of UHMW PDMA measured in 0.1 N NaNO<sub>3</sub> buffer, c) measured in DMF against PEG standards.

## ATPS and water-in-water emulsion



**Figure S5.** (a) Structures of Pull and PDMA and (b-d)  $^1\text{H-NMR}$  in  $\text{D}_2\text{O}$  of the (b) mixture, (c) upper and (d) lower phase after 24 h for the combination  $\text{PDMA}_{10}^6$  & Pull using DMF as internal standard.

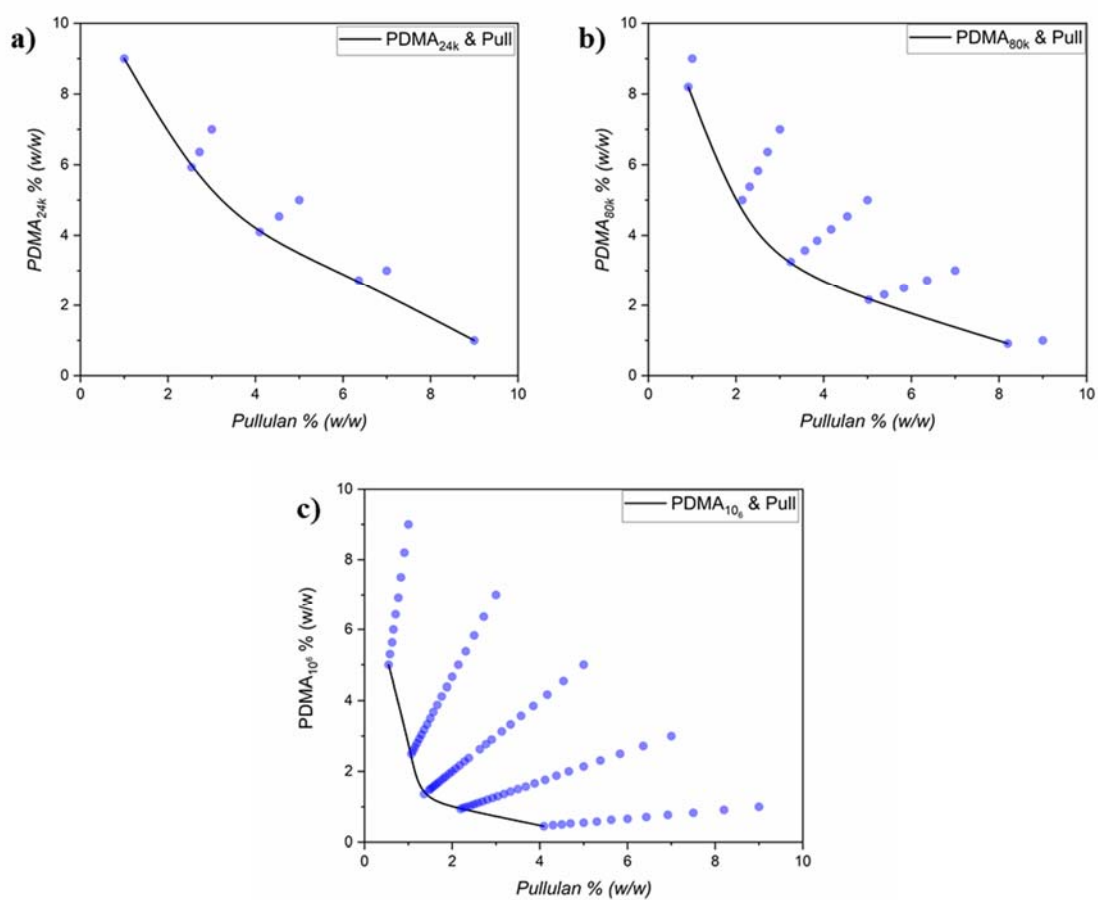




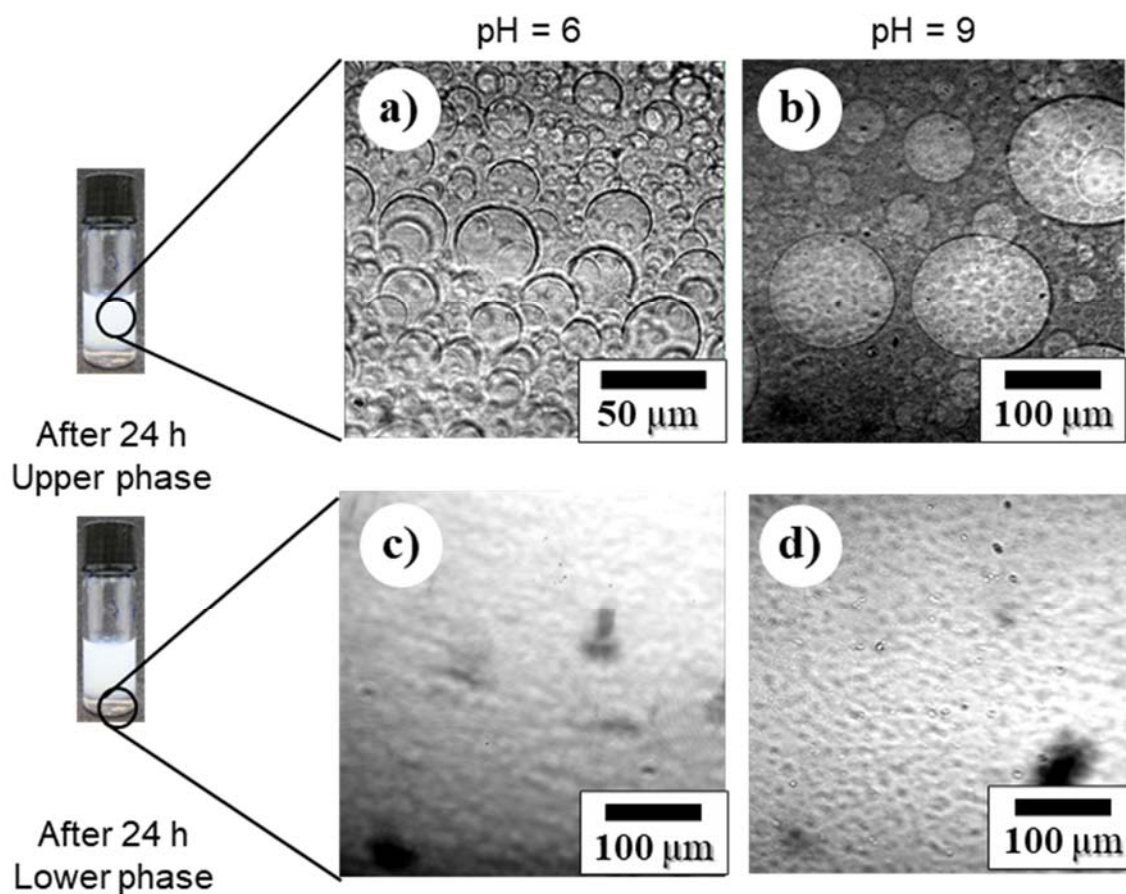
**Figure S6.** Concentration of the polymers directly after mixing and after phase separation (24 h), detected *via*  $^1\text{H-NMR}$  in  $\text{D}_2\text{O}$  of  $\text{PDMA}_{10}^6$  (red) and Pull (green) for the ATPS of UHMW PDMA and Pull using DMF as internal standard.

$$P_{P_x} = \frac{c_{P_x L1}}{c_{P_x L2}}$$

**Equation S1.** For the calculation of the partition coefficient for each polymer in the ATPS with  $P_{P_x}$ -partition coefficient,  $c_{P_x L1}$ -concentration of the polymer in the upper phase (L1) and  $c_{P_x L2}$ -concentration of the polymer in the lower phase (L2).



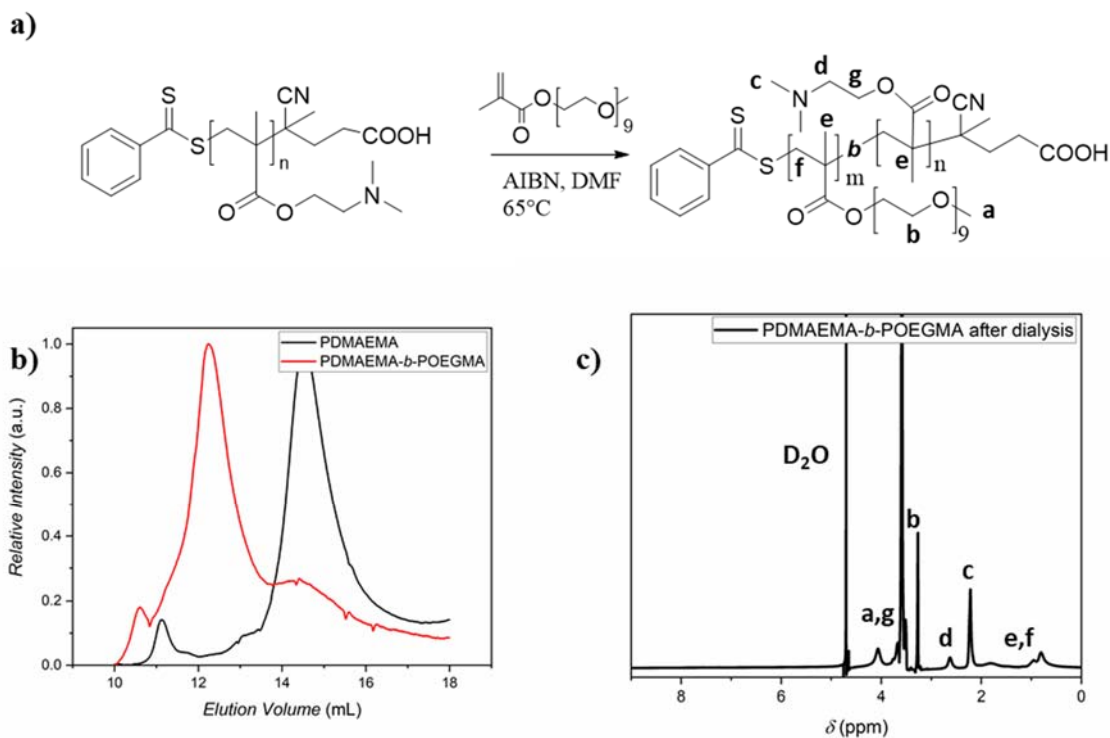
**Figure S7.** Phase diagrams of the ATPS for all polymer combinations, showing the experimental binodals (black curves) and the dilution steps (blue dots) (a) PDMA<sub>24k</sub> & Pull, (b) PDMA<sub>80k</sub> & Pull and (c) PDMA<sub>106</sub> & Pull.



**Figure S8.** (a–d) Bright-field microscopy images of the w/w emulsion of UHMW PDMA<sub>10</sub><sup>6</sup> and Pull (1.5 wt %/1.5 wt %) stabilised with PS nanoparticles (0.1 wt%) after 24 h (a) upper phase at pH = 6, (b) upper phase at pH = 9, (c) lower phase at pH = 6 and (d) lower phase at pH = 9.

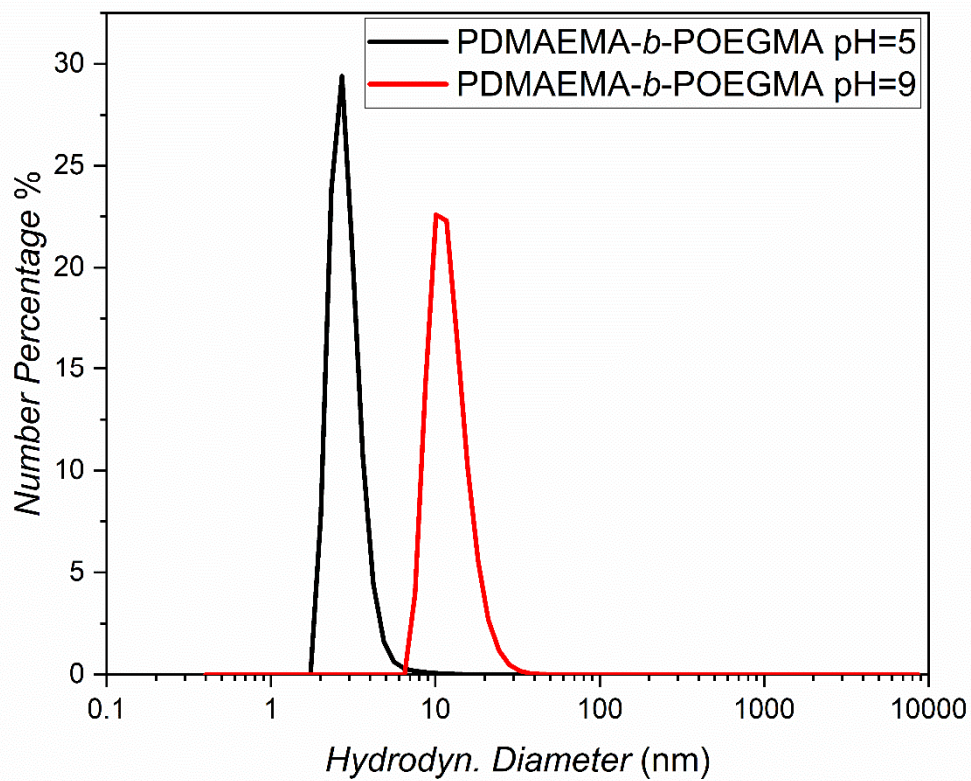
**Table S2.** Average droplet size of 1.5/1.5 wt% ATPS formed by PDMA<sub>10</sub><sup>6</sup> and Pull and 0.1 wt% PS nanoparticles or 1 wt% PDMAEMA-*b*-POEGMA additive before phase separation and after 24 h of phase separation, measured over 30 particles.

<b>ATPS</b>	<b>Droplet Size Emulsion [<math>\mu\text{m}</math>]</b>	<b>Droplet size after phase separation (24h) [<math>\mu\text{m}</math>]</b>
<b>PDMA &amp; Pull + PS at pH =6</b>	<b>32 <math>\pm</math> 5</b>	<b>49 <math>\pm</math> 22</b>
<b>PDMA &amp; Pull + PS at pH =9</b>	<b>76 <math>\pm</math> 27</b>	<b>118 <math>\pm</math> 53</b>
<b>PDMA &amp; Pull + BCP at pH =9</b>	<b>86 <math>\pm</math> 56</b>	<b>107 <math>\pm</math> 31</b>

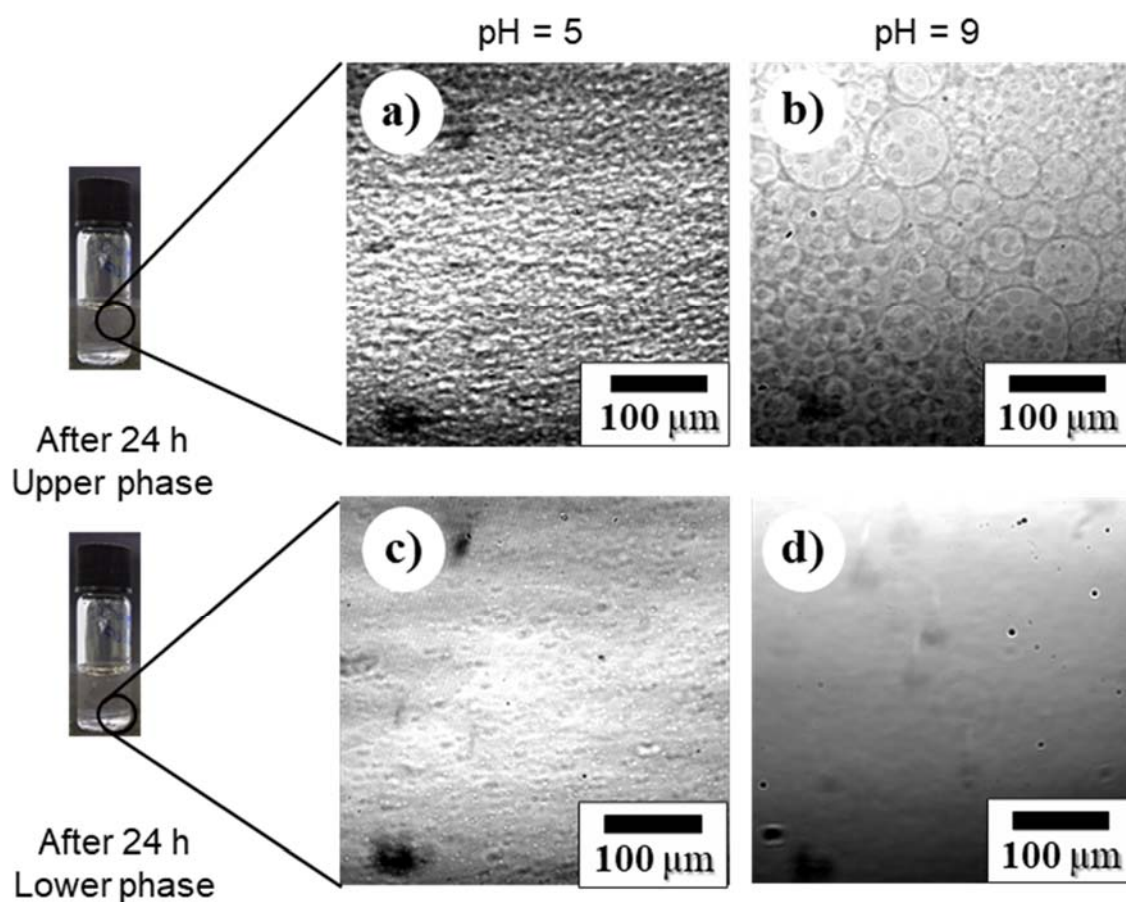


**Figure S9.** (a) Reaction scheme of the synthesis of the block copolymer PDMAEMA-*b*-POEGMA via RAFT polymerisation, (b) SEC measurement of PDMAEMA-*b*-POEGMA measured in DMF against PEG standards and (c)  $^1\text{H-NMR}$  of PDMAEMA-*b*-POEGMA after dialysis.

The formation of the block copolymer PDMAEMA-*b*-POEGMA is indicated by the increase of the molar mass from the first block to the block copolymer as well as the decrease of the elution volume. However, even after dialysis a shoulder is present around the area of the first block in the elugram of the block copolymer sample indicating a residual amount of the first block in the block copolymer.



**Figure S10.** Comparison of number weighted particle size distribution of PDMAEMA-*b*-POEGMA in aqueous solution at pH=5 (1.0 wt%, black curve) and PDMAEMA-*b*-POEGMA in aqueous solution at pH=10 (1.0 wt%, red curve) at ambient temperature.



**Figure S11.** (a–d) Bright-field microscopy images of the w/w emulsion of UHMW PDMA<sub>10</sub><sup>6</sup> and Pull (1.5 wt %/1.5 wt %) stabilised with PDMAEMA-*b*-POEGMA (1 wt%) after 24 h (a) upper phase at pH = 5, (b) upper phase at pH = 9, (c) lower phase at pH = 5 and (d) lower phase at pH = 9.

## References

1. De Belder, A. N.; and Granath, K. Preparation and properties of fluorescein-labelled dextrans. *Carbohydrate Research* **1973**, 375-378.
2. Liu, J.; Yasuhiko T. Photodynamic therapy of fullerene modified with pullulan on hepatoma cells. *Journal of drug targeting* **2010**, 602-610.