Structure- LCST relationships for end-functionalized water-soluble polymers: an "accelerated" approach to phase behaviour studies

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SUPPORTING INFORMATION





Figure S1. a) Schematic diagram of equipment, and; b) photograph of high throughput (HT) LCST measurement device. Silicone fluid was used as circulating fluid. Hot fluid was circulated in the left side and the cold fluid in the right side. High temperature "Electrolube" black matt paint was used for coating copper base plate. The white internal surface of the box ensures uniform diffuse lighting conditions.

Temperature distribution measurement in a 384 well microplate

Process: In order to confirm that a uniform temperature gradient was achieved, the wells of a clear 384 well polystyrene microplate (24 columns \times 16 rows) were filled with 70 µl distilled water using either a liquid handling robot or a multi-channel micropipette. The microplate was positioned on the black painted copper base plate after equilibrating the two circulators at the desired temperatures for 5 mins (see Figure S1). The whole system was then equilibrated for 10 mins and the temperature in the wells was measured using a wire thermocouple. The results are plotted in Figure S2a–c. The results indicate that a very uniform temperature gradient is obtained across the column axis (Fig. 2a) and the temperature in any given row is very consistent (Fig. 2b).



Figure S2. (a) Temperature distribution data for 384 well microplate in 3D and (b, c) in 2D. Plot (a) was obtained with the circulator temperatures set at 90 °C and 30 °C, respectively. For plot (b) the circulator temperatures were set at 70 °C and 30 °C, respectively. For plot (c) circulator temperatures are specified in the figure.

High throughput LCST determination for pNIPAM

The LCST of pNIPAM was determined using a commercial sample (Scientific Polymer, M_w = 300,000 g/mol). The wells of a clear polystyrene 384 microplate were filled with 70µl 1 wt% pNIPAM solution prepared in distilled water. This plate was then placed on the preheated copper base plate (see Figure S1) and equilibrated for 10 min. Pure deionised water (70µl) was added to the wells of row 9 / column 1 & row 9 / column 24 to allow temperature measurements (although in practice the temperature was not found to differ significantly in the dilute polymer solutions). Images were acquired using a B/W CCD camera (Figure S1). Surface plots were obtained after processing the captured images using ImageJ software. The data shown in Figure S3a was obtained with the circulator temperatures set at 55 °C and 15 °C, respectively, which led to measured temperatures of 38.8 and 21.6 °C in row 9 / column 1 & row 9 / column 24, as indicated in the figure. The LCST phase transition was measured to be 30.6±0.4 °C by defining the transition as the temperature at which a 50% reduction in the maximum "gray value" was observed (see also Fig. S11, below). A close inspection of Fig. S3a shows that each well in column 12 where the LCST phase transition takes place appears to be divided into a white segment and a black segment. Image analysis of a 3×3 well area crossing this transition (Fig. S3b) shows that this is indeed the case and that the LCST transition can be observed *within* a single well. As such, the potential temperature resolution is better than the nominal R/24 °C for a given temperature range (where R = the overall temperature range and 24 is the number columns).



Figure S3. (a) LCST of 1% pNIPAM, $M_w = 300,000$ g/mol; (b) Surface plot showing gray intensity for nine wells covering the phase transition obtained using ImageJ software (an expansion of columns 11–13 from Fig. S3a). The LCST phase transition occurs within the middle column shown here (column 12 from Fig. S3a).

By using circulator temperatures just either side of the LCST phase transition observed in Fig. S3 (40 °C and 32 °C, respectively), it was possible to set up a small overall temperature gradient of just 3.2 °C, as measured from column 1 to column 24. Thus, each column differed by just 0.133 °C (that is, 3.2 / 24). Figure S4 shows the data obtained from this "dynamic expansion" of the phase transition for the sample described in Fig. S3, above. It was still possible to observe a well defined phase transition using image analysis (Fig. S4b), even with such a small temperature gradient and the concomitant "smearing" of the phase transition. Analysis of this data led to the determination of a slightly more precise LCST value of 30.7 ± 0.1 °C under these equilibrium conditions (*c.f.*, 30.6 ± 0.4 °C from measurement in Fig. S3).



Figure S4. (a) Phase transition of pNIPAM over a narrow temperature gradient of 3.2 °C; (b) surface plot obtained using ImageJ software. The LCST was determined to be 30.7 ± 0.1 °C.

Effect of KCl concentration on LCST of pNIPAM

The 16 rows of a clear polystyrene 384 well microplate were filled with 1 wt% pNIPAM solution (Scientific Polymer, $M_w = 300,000$ g/mol) with a different KCl concentration (0–3.75 wt. %) in each row. The circulator temperatures were set as 55 °C and 15 °C, respectively, to give an overall temperature gradient on the plate of 38–21 °C. Figure S5 shows the data that were obtained. For comparison, the LCST for the sample PNIPAM sample at six different KCl concentrations (0, 0.5, 1.0, 2.0, 3.0 and 3.75 wt%) was determined by conventional cloud point measurement (rate of heating 1–1.5 °C/min). These data are superimposed on Fig. S5. A very close agreement was observed between the two methods.

300



(a)

(b)

Figure S5. Effect of KCl concentration on LCST for pNIPAM (Scientific Polymer, $M_w = 300,000 \text{ g/mol}$); (a) Agreement between high throughput and more conventional measurements, (b) Surface plot obtained from the image shown in (a).

Synthesis and characterization of end-functionalized pDMAEMA

Synthesis of ATRP initiators: Seven different tertiary bromo-ester ATRP initiators were synthesized by reacting 2-bromo-2-methylpropionic (BMPA) acid with corresponding alcohol in presence of carbodiimidazole (CDI) as a coupling agent. A dichloromethane (DCM) solution of BMPA was added slowly to a dispersion of an equivalent amount of CDI, followed by refluxing for 15 min. An equimolar amount of the corresponding alcohol dissolved in DCM was added was then added this solution and refluxed for 2 h. The solvent was removed by rotary evaporation and the crude product was purified by column chromatography using an appropriate ethyl acetate / hexane mixture. A different purification procedure was used for the initiator based on *N*,*N*-dimethyethanol. This product was purified by washing with water (3 times) followed by drying over Na₂SO₄ and subsequent removal of the DCM by rotary evaporation. Yields were found to be in the range 75–85%. All products were characterized by ¹H and ¹³C NMR spectroscopy, elemental microanalysis, and mass spectrometry.

Synthesis of polymers: Two series of end-functionalized poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA) samples were synthesized by "temporal sequential sampling" aqueous atom transfer radical polymerization (ATRP) methods using CuCl as the catalyst, Bipy as the ligand, and an IPA/water mixture (5% v/v water) as solvent (Scheme S1). All reactions were carried out at ambient temperatures. For the ethyl end-functionalized samples a commercially-available EBIB initiator (Aldrich) was used. In all other cases the initiators were synthesized, as outlined above. Typical conditions used were as follows: monomer:solvent = 1:4 v/v, monomer:initiator:catalyst:ligand = 25:1:1:2 mol. Aliquots were sampled at predetermined reaction times to give a library of molecular weights. The samples were aerated immediately to terminate the polymerization and then purified by passing through a plug of neutral alumina before drying in a Buchi Genevac and vacuum oven at 60 °C overnight. All polymers were characterized by NMR spectroscopy and gel permeation chromatography (GPC).

Scheme S1. Synthesis of end functionalized pDMAEMA using aqueous ATRP (R1 = ethyl; R2 = n-propyl; R3 = n-butyl; R4 = n-octyl; R5 = benzyl; R6 = phenethyl; R7 = N,N'-dimethylaminoethyl



Polymer characterization:

NMR: Polymers were characterized using a Bruker Analytic GmbH 400 MH_z ¹HNMR spectroscopy (in CDCl₃) using TMS as an internal standard.

GPC: Molecular weights and distributions were characterized using a Polymer Laboratories System equipped with a PL-ELS1000 evaporative light scattering detector and a series of PC mix gel column 5 μ m MIXED C and D GPC system. The instrument was calibrated with EasiCal polystyrene standards (from Polymer Laboratories). THF was used as the eluent with 1 % v/v Et₃N additive at 1ml/min flow rate and a column temperature of 40 °C.



Figure S6. Representative ¹H NMR spectrum for polymer R1g (in CDCl₃)



Figure S7. Typical GPC chromatograms (polymers R1a-g).



Figure S8. Comparative GPC chromatograms for polymers R1-7a (DP = 7-9) and R1-7g (DP = 22-23) showing control over MW for the different end functionalities.

Polymer structure	Sample code	DP	<i>M</i> n	GPC results	
(with end group)		(cal) ^a	(cal) [⊳]	<i>M</i> n	PDI
	R1a	9.28	1610	2265	1 20
CH3	R1b	13.27	2237	2745	1.20
H ₃ C-CH ₂ -O ₂ C-PDMAEMA	R1c	16.33	2718	3073	1 17
ĊH ₃	R1d	18.53	3064	3367	1.16
	R1e	20.69	3403	3623	1.14
	R1f	21.80	3578	3662	1.14
	R1g	23.09	3781	3771	1.14
211	R2a	8.10	1438	2062	1.21
	R2b	12.84	2183	2630	1.18
$H_3C - CH_2 - O_2C - PDMAEMA$	R2c	15.77	2644	2864	1.18
	R2d	18.12	3013	3203	1.16
	R2e	20.15	3333	3234	1.17
	R2f	21.24	3504	3287	1.16
	R2g	22.64	3724	3558	1.15
ĊН ₃	R3a	6.96	1273	1956	1.21
$H_3C - (CH_2) - O_2C - PDMAEMA$	R3g	22.19	3667	3797	1.14
ÇH ₃	R4a	8.31	1541	2188	1.19
$H_3C - (CH_2) - O_2C - PDMAEMA$ 7 CH ₃	R4g	22.86	3829	3801	1.14
CH2	R5a	8.71	1582	2157	1.20
$Ph-CH_2-O_2C$ $+$ PDMAEMA CH ₂	R5g	21.71	3626	3808	1.14
ÇH ₃	R6a	7.38	1387	1997	1.24
$Ph-(CH_2)-O_2CPDMAEMA$ CH_3	R6g	22.55	3772	3534	1.16
СН3 СН-	R7a	8.93	1598	1945	1.17
$H_{3}C - N - (CH_{2}) - O_{2}C - PDMAEMA$	R7g	21.56	3583	3610	1.11

Table S1. Molecular weights and molecular weight distributions for end functionalized pDMAEMA

a) Calculated from ¹H NMR and monomer conversion.
b) Calculated DP, including end-functional groups.

High throughput LCST determination for end functionalized pDMAEMA

(i) Effect of end-group functionality for ethyl- and propyl-functionalised pDMAEMA: Rows 2–15 of a clear 384 well polystyrene microplate were filled with 75 μ l aqueous solutions (1wt % of polymer and at pH 9.0-9.5) of end functionalized pDMAEMA (polymers 1a-g and 2a-g, see Table S1) using a multi channel micropipette. The wells of rows 1 and 16 were filled with 75 μ l of pure water as an optical and thermal reference. The LCST phase behavior was investigated as described before using circulator temperatures of 100 °C and 20 °C, respectively, to give an overall temperature range (Figure S9) of 61–39.5 °C.



Figure S9. HT LCST measurement for ethyl- and propyl-functionalized pDMAEMA samples, R1a-g and R2a-g. As might be expect, the effect of the propyl group was over this molecular weight range was more pronounced than the effect of the ethyl group, hence the steeper "gradient" for the LCST variation in samples R2a-g.

(ii) Effect of molecular weight and end-group: In a separate experiment, rows 2–15 of a clear 384 well polystyrene microplate were filled with 75 μ l aqueous solutions (1wt % of polymer and at pH 9.0-9.5) of end functionalized pDMAEMA (polymer 1-7a of DP = 7-9 and R1-7g of DP = 22-23, see Table S1) using a multi channel micropipette. The LCST phase behavior was investigated as described before using circulator temperatures of 90 °C and 20 °C, respectively, to give an overall temperature range (Figure S10) of 55.1–36.4 °C.



Figure S10. HT LCST measurement for end-functionalized pDMAEMA samples, R1-7a (DP = 7–9) and R1-7g (DP = 22-23). In general, the lower molar mass polymers (R1-7a) have *lower* LCST values than the equivalent higher molar mass materials (R1-7g) indicating that the proportionate effect of the hydrophobic end-group is greater in the more oligomeric materials.

Extraction of LCST values by image analysis

LCST values were determined from profiles of the "gray values" in the captured images (*e.g.*, Fig. S10). The LCST was defined as the temperature at which a 50% in Gray Value occurs (* $GV_{1/2}$) between the maximum and minimum values recorded in the centre of each microwell (these positions are denoted by the blue circles in Fig. S11, below). Both the interpolated LCST value and the transition column (*i.e.*, the column in which the transition was observed) are recorded in Table S2.



Figure S11. Representative profile plots of LCST transitions for pDMAEMA solutions (propyl end group, samples R2a and R2g). (a) Sample R2a (DP = 7–9) (row 3 of microplate; Fig. S10), (b) Sample R2g (DP = 22–23) (row 10 of microplate; Fig. S10). $*GV_{1/2} = 50\%$ of change in Gray Value; gray values measured at centres of wells as denoted by blue dots – measurement in the centre of the wells avoids the well edges and edge reflections (the sharp troughs and spikes, respectively, in these plots).

Table S2. LCST values obtained from HT measurements for end functionalized pDMAEMA samples (extracted from data in Fig. S10)

Row	Row no.	Sample	Transition column		
			Column number ^a	Temperature (°C, ±0.2)	
А	1	Water			
В	2	R1a	10	47.8	
С	3	R2a	15	43.7	
D	4	R3a	11	47.0	
E	5	R4a	8	49.4	
F	6	R5a	16	42.9	
G	7	R6a	15	43.7	
Н	8	R7a	5	51.9	
Ι	9	R1g	6	51.0	
J	10	R2g	7	50.2	
K	11	R3g	11	47.0	
L	12	R4g	9	48.6	
М	13	R5g	14	44.5	
N	14	R6g	10	47.8	
0	15	R7g	b	b	
Р	16	Water	_	_	

a) Column in which LCST transition ($^{*}GV_{1/2}$) was observed. b) No LCST transition observed in this temperature range.