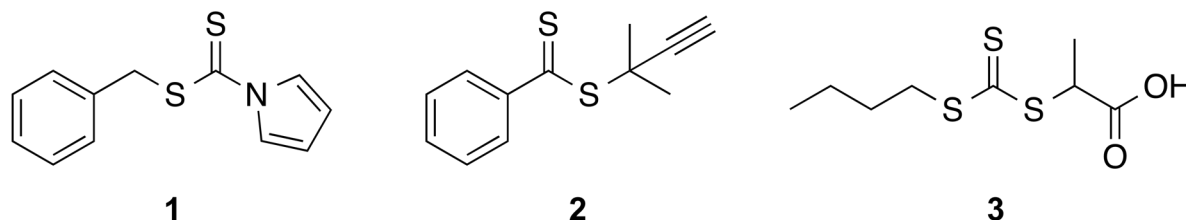


The pH-responsive behaviour of aqueous solutions of poly(acrylic acid) is dependent on molar mass

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Electronic supplementary information

Characterization



Scheme 1. Chain transfer agents used in the synthesis of poly(acrylic acid)

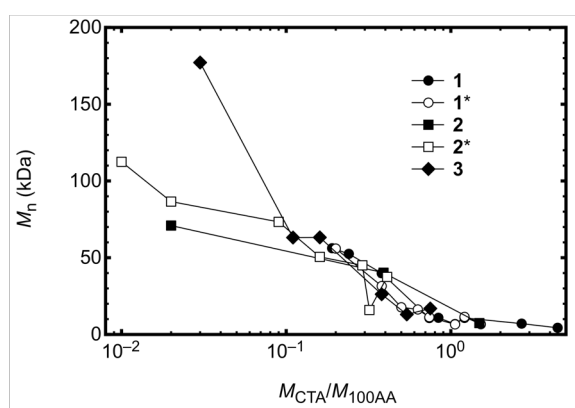


Figure S1. Number average molar mass, M_n , of the ranges of polymers (with and without (*) ACE) at various CTA:AA feed ratios using the chain transfer agent indicated

trimethylsilyldiazomethane then dissolved in THF (solvent filtered to 0.45 μm pore). The eluent was passed through 3 \times PLgel 10 μm mixed-B LS Columns at 1.00 ml/min using a Kinesis 307 Gilson pump. Polymer samples were inserted into the stream via an Anachem 234 auto injector and the refractive index (RI) signal was recorded using an Erma Inc. ERC-7512 RI detector. The system was calibrated using poly(methyl methacrylate) samples.

Polymers prepared in the presence of chain transfer agent (CTA) had much lower molar masses than the polymers synthesized in the absence of CTA, and altering the ratio AA:CTA (AA being acrylic acid) had a dramatic effect on the molar mass of the resulting polymer. Fig. S1 shows that the molar mass dependence of the ratio of AA:CTA was not affected by the incorporation of small amounts of the acenaphthylene (ACE) co-monomer. These low fractions of label facilitate the fluorescence experiments. Ideally the label should be randomly distributed along the polymer chain to ensure that the label exists in a homogeneous environment. At these low concentrations of label, deleterious effects due to different reaction rate coefficients for the polymerization of AA and the addition of ACE can be neglected.

In addition to the study of molar masses on the THF GPC system using an RI (refractive index) detector, the distribution of fluorescence labels was examined via GPC

using a Waters Associates liquid chromatograph equipped with a dual UV-RI system (AD20 Absorbance Detector) / (HP 1047A RI Detector calibrated using the retention time of PAA polymers). For these columns the methylation reaction was not needed (see below). Separation was achieved through two 600mm sulfonated divinylbenzene (DVB) Jordi Gell

A series of poly(acrylic acid) (PAA) samples were synthesized with a range of molecular weights using controlled reversible addition-fragmentation chain transfer (RAFT) chemistry, using three separate chain transfer agents (Scheme 1): 1 (Benzyl-1*H*-pyrrole-1-carbodithioate), 2 (2-cyano-2-propyl benzodithioate) and 3 (2-[[(butylsulfanyl)carbonothioyl]sulfanyl]propanoic acid). A complete list of the poly(acrylic acid) (PAA) samples used in this work are included in Table S1. This includes the chain transfer agent used, as well as the concentration of acenaphthylene (ACE) label. The resultant dispersities are also stated.

Gel Permeation Chromatography. Gel permeation chromatography (GPC) was performed on each sample using a tetrahydrofuran (THF)-based system. Samples were prepared for GPC via a methylation reaction with

trimethylsilyldiazomethane then dissolved in THF (solvent filtered to 0.45 μm pore). The eluent was passed through 3 \times PLgel 10 μm mixed-B LS Columns at 1.00 ml/min using a Kinesis 307 Gilson pump. Polymer samples were inserted into the stream via an Anachem 234 auto injector and the refractive index (RI) signal was recorded using an Erma Inc. ERC-7512 RI detector. The system was calibrated using poly(methyl methacrylate) samples.

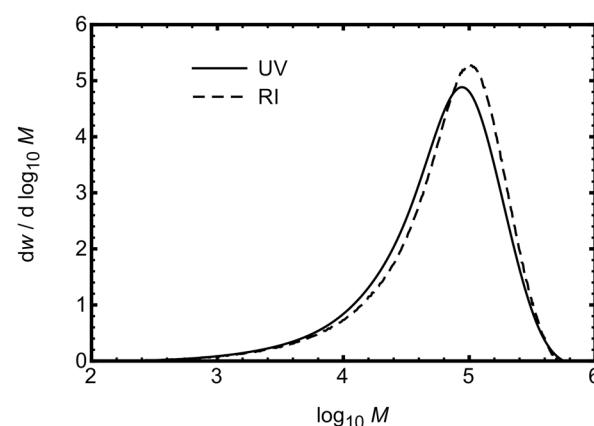


Figure S2. Molar mass distributions for an ACE-labelled polymer, synthesized with no CTA ($M_n = 42.2$ kDa) obtained by both the UV and RI detectors on sulfonated divinylbenzene columns. The distributions are in close agreement

columns and the mobile eluent was 0.1 M tris(hydroxymethyl)aminomethane (TRIS), 0.1 M NaCl, and 0.01 M sodium azide. **Fig. S2** shows size exclusion chromatography-derived molar mass distributions obtained from detection by in-line UV spectroscopy and by differential refractometry. Both distributions can be overlaid (**Fig. S2**) and since the UV detector is sensitive to the presence of residues of ACE, the data indicate that the ACE label was spread evenly throughout the polymer, satisfying the need for a homogeneous distribution of the ACE label. It should be noted that the sulfonated DVB columns used are subject to greater band broadening than that of the THF-based GPC system (using PLGel Mixed B columns), which was used for the data listed in **Table 1**. For this reason, **Fig. S2** exhibits a larger dispersity for these samples than the corresponding data in **Table S1**.

Analysis of poly(acrylic acid), or any charged polymer, by size exclusion chromatography is challenging. The molar masses determined in THF required methylation of the polymer backbone and comparison to a PMMA standard. This methodology was tested by comparison with a GPC on PAA that did not require such methylation. This was achieved using the charged Jordi-Gell DVB-PSS columns. Here, acidic polymers were injected into a set of columns using a TRIS buffer mobile phase. A dual UV-RI detector was used for these tests. The RI detector was calibrated using a set of PAA standards. Using the charged aqueous columns accurate molar masses were determined from the RI detector, and the number average molar mass was within 10% of those determined by the THF system, although large dispersities due to band broadening within the column were observed. The dispersities quoted here are therefore upper limits.

The 16.3 and 16.9 kDa molar mass distributions. Many of the samples are quite disperse, which will affect the accuracy of the determination of the molar mass of the transition, above which pH-induced conformational behaviour occurs. Samples with the dispersities outlined in **Table S1** of similar molar masses will overlap between each other significantly. The fluorescence lifetime results outlined in the main paper indicate a clear transition between polymers with $M_n = 16$ and 18 kDa. The full molar mass distributions of these polymers are shown in **Fig. S3**. The breadth of these distributions belies the

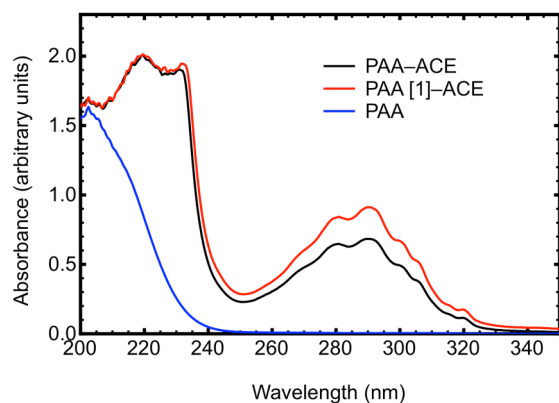


Figure S4. Absorption spectroscopy data of three PAA samples, two of which contain the fluorescent ACE label ($M_n = 42.2$ and 55.9 kDa). The one that does not contain the ACE ($M_n = 58.1$ kDa) shows no discernible absorption at 289 nm. The legend indicates the three polymers tested, where PAA does not contain an ACE label. PAA [1] indicates a polymer ($M_n = 55.9$ kDa) synthesized using chain transfer agent [1]

Absorption spectroscopy. The presence of the fluorescence label ACE was tested using UV/vis absorption spectroscopy. 1 mg mL⁻¹ solutions of polymer were dissolved in ultrapure water and the wavelength examined from 200 to 900 nm using a Specord S-600 spectrophotometer. The presence of the ACE fluorophore was clearly visible from the absorption peak at 289 nm (**Fig. S4**). Samples synthesized with the label showed more absorption at this peak, but the sample synthesized without ACE shows no discernible absorption at all at this wavelength. These experiments do

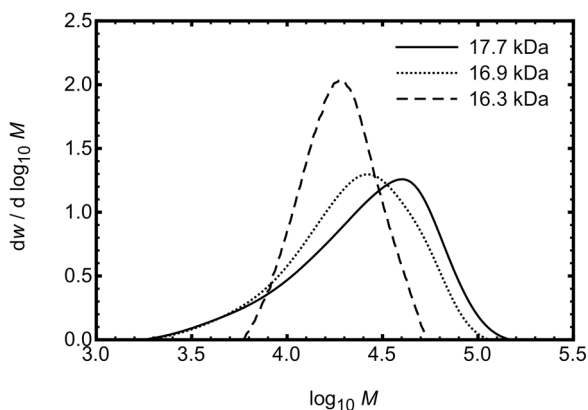


Figure S3. Molar mass distributions of polymers close to the transition discussed in the present work. The dispersity in these samples is considerably greater than that obtained using the THF eluent (**Table S1**)

relatively sharp transition observed in the fluorescence lifetime data. By contrast, it can be seen that in the anisotropy data both the 16.9 and 17.7 kDa PAA behaved as disperse samples, exhibiting a smaller increase in correlation time compared to polymers of greater molar mass.

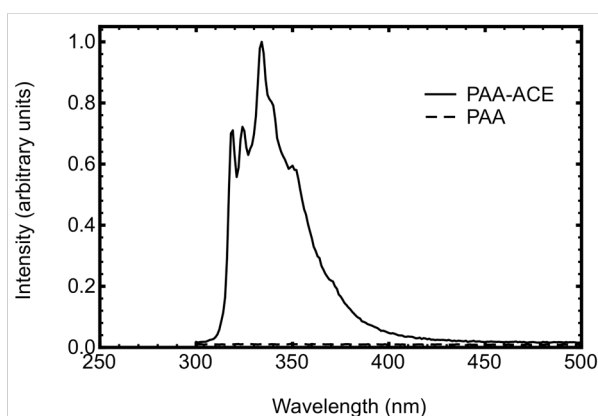


Figure S5. Relative emission spectra of PAA ($M_n = 39.9$ kDa) and P(AA-co-ACE) ($M_n = 55.9$ kDa) polymers, both made in the presence of CTA 1. The excitation wavelength, $\lambda_{ex} = 295$ nm

not demonstrate any polymer conformational behaviour.

Fluorescence

Steady state fluorescence measurements can be used to determine the range of wavelengths at which these labels absorb and emit light. In PAA the fluorescent label retains the expected ACE $\lambda_{ex}/\lambda_{em}$ profile of approximately 295/340 nm. Even in the presence of high amounts of CTA the spectral profile of the ACE label remains unchanged. PAA polymers containing no ACE show no luminescence at these wavelengths (**Fig. S5**). No conclusions about the quantity of the effect of ACE can be drawn from these data.

Excited state lifetime measurements are calculated assuming that the fluorescent label, or probe, is dispersed in a homogeneous environment, so that the excited state decay accurately reflects the sensitivity of the species to the solvent and the conformation of the polymer. Assuming this to be the case, the fluorescent intensity $I(t)$ can be modelled using

$$I(t) = A \exp(-t/\tau_1) + B \exp(-t/\tau_2), \quad (\text{S1})$$

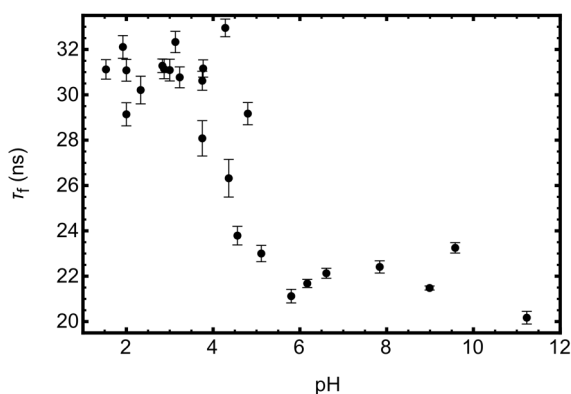


Figure S6. Fluorescence lifetime of P(AA-co-ACE) (no CTA, $M_n = 42.2$ kDa)

requires the important assumption that G lies close to unity. (Here, as in the main article, the subscripts to I refer to parallel and perpendicular orientations of the polarized intensities.)

Fluorescence Lifetime Data

It is known that P(AA-co-ACE) fluoresces with lifetimes of between 20 and 35 ns in aqueous solution, dependent on the pH, and this was confirmed via lifetime analysis of a polymer formed with no CTA, with the fluorescence profile (**Fig. S6**) fitted to a single exponential decay (eqn S2). The full analytical conditions of the data represented in **Fig. 2b** (of the main article) are shown in **Table S2**.

Data fitted to a single fluorescence lifetime give a similar result to those for dual exponential decays, although the quality of fits to the lifetime data is considerably poorer.

Fluorescence Anisotropy Data

The correlation time of P(AA-co-ACE) in aqueous solution varies with pH from 1 to 6 ns, and the results shown in **Fig. 2b** of the article are consistent with this. The CTAs used in these experiments have no effect on τ_c . This was confirmed via time-resolved anisotropy measurements of a polymer formed in the absence of CTA, in which the anisotropic decay was fitted to a single exponential equation. The results of this experiment are shown in **Fig. S7**. The full analytical results of the anisotropic analysis via impulse reconvolution presented in **Fig. 2** (of the article) are shown in **Table S3**.

Data presented in **Fig. 2a** of the article are shown without corresponding residuals. These are shown in **Fig. S8** to demonstrate the quality of the data.

2D DOSY NMR

2D NMR (Diffusion-ordered nuclear magnetic resonance spectroscopy, or DOSY NMR) measurements were used to provide data to test the reliability of the fluorescence results. The pH dependence of the diffusion constant of PAA in

where I_0 is the initial intensity of fluorescence decay and τ the lifetime of two component excited states. τ_f , the final fluorescence lifetime is calculated as

$$\tau_f = \frac{A\tau_1^2 + B\tau_2^2}{A\tau_1 + B\tau_2}. \quad (\text{S2})$$

Excited state lifetime and time-resolved anisotropy measurements were recorded using an Edinburgh Instruments 199 Fluorescence Spectrometer at $\lambda_{ex} = 295$ nm and $\lambda_{em} = 350$ nm. This machine was calibrated with a time region of 0.3980 ns per channel with a variance of 0.0025 and a $G = 1$, which is important because application of the equation for the anisotropy,

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)}, \quad (\text{S3})$$

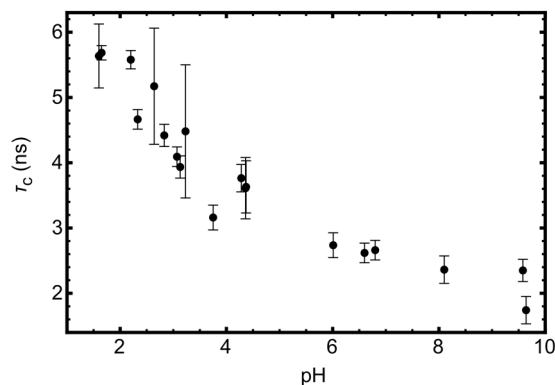


Figure S7. Correlation time of P(AA-co-ACE) (no CTA, $M_n = 42.2$ kDa)

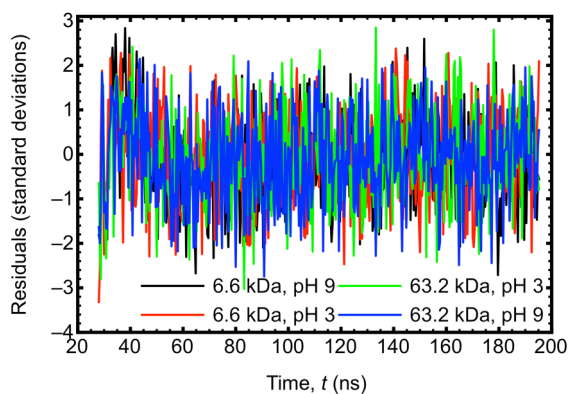


Figure S8. Residuals of data shown in **Fig. 2a** of the article

aqueous solution was measured by comparing the peak diffusion constant of the PAA backbone (1.6 and 2.3 ppm) in D_2O . **Fig. S9** shows three overlaid spectra to demonstrate the shift with hydrodynamic radius of the polymer sample. No change in diffusion position was observed for the smaller molecular weight polymer.

The diffusion coefficients obtained by DOSY NMR are listed in **Table S4**, along with the concentration of PAA used in each experiment.

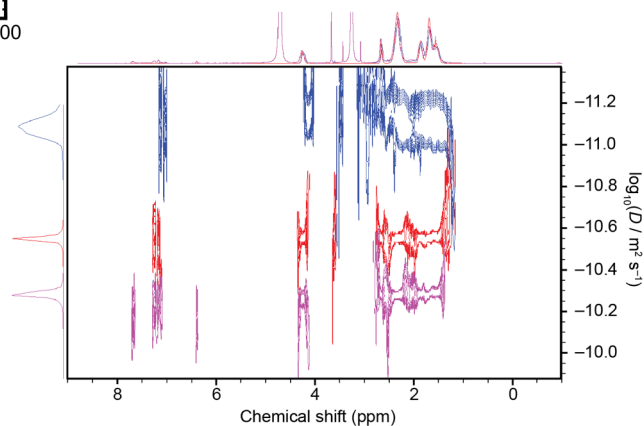


Figure S9. 2D DOSY NMR of PAA polymers: blue, 52.5 kDa, pH 7; red 52.5 kDa, pH 3.3; pink 16.3 kDa, pH 3. 1H NMR of PAA backbones are displayed along the abscissae and the diffusion constant along the ordinates

Table S1. Calculated molar mass (kDa) and dispersity, D , of polymers from varying mole quantities of ACVA : CTA : ACE normalized to 100 moles of AA

M_n (kDa)	M_w (kDa)	D	CTA used	ACVA (mol)	CTA (mol)	ACE (mol)
4.4	6.4	1.46	1	1.44	4.45	
6.6	9.5	1.45	1	1.27	1.06	2.49
6.7	10.6	1.58	1	1.33	1.52	1.14
6.8	10.3	1.52	1	1.27	1.06	
7.1	14.3	1.45	1	1.44	2.69	
7.4	15.2	2.04	2	0.44	1.49	
10.7	17.2	1.6	1	0.45	0.74	
10.8	17.2	1.59	1	0.45	1.21	
10.9	22.0	2.02	1	1.44	0.84	
11.3	18.7	1.66	1	0.45	0.74	0.79
11.5	17.0	1.48	1	0.45	1.21	0.91
13.1	18.7	1.43	3	0.03	0.54	0.01
15.9	19.7	1.23	2	0.06	0.32	0.06
16.3	27.4	1.68	1	0.39	0.63	0.3
16.9	20.3	1.2	3	0.4	0.75	0.14
17.7	33.0	1.87	1	0.41	0.5	0.34
26.2	36.0	1.37	3	0.28	0.38	0.03
31.5	53.1	1.69	1	0.45	0.38	0.83
37.5	61.0	1.63	2	0.33	0.41	0.31
39.9	61.4	1.54	1	0.45	0.38	
40.3	64.2	1.59	2	0.19	0.39	
42.2	64.9	1.54		0.88		0.52
45.0	53.4	1.18	2	0.67	0.29	0.64
50.5	90.9	1.79	2	0.3	0.16	0.21
52.5	84.6	1.61	1	0.11	0.24	
55.9	90.9	1.62	1	0.17	0.2	0.49
56.2	94.1	1.68	1	0.22	0.19	
58.1	112.5	1.94		0.87		
63.1	101.0	1.59	3	0.03	0.11	0.02
63.2	76.1	1.2	3	0.1	0.16	0.18
71.0	132.2	1.86	2	0.08	0.02	
73.3	95.3	1.29	2	0.05	0.09	0.04
86.6	157.8	1.82	2	0.01	0.02	0.03
177.2	311.3	1.76	3	0.03	0.03	0.01

ACVA: 4,4'-azobis(4-cyanovaleric acid)

CTA 1: 1H-pyrrole-1-carbodithioate

CTA 2: 2-cyano-2-propyl-benzodithioate

CTA 3: 2-[(butylsufanylcarbonothioyl)sufanyl]propanoic acid

Table S2. Complete analytical results for the data presented in Fig. 1b (of the article). Except where otherwise stated, the uncertainty in all values of τ is less than 0.05 ns.

M_n (kDa)	pH	τ (ns)	χ^2
6.6	2.38	27.5	6.1
6.6	2.5	27.4	1.1
6.6	2.6	25.0	1.7
6.6	2.79	27.8	3.1
6.6	3.61	25.9	2.8
6.6	3.69	23.4	1.3
6.6	5.77	23.6	1.7
6.6	6.39	23.9	4.6
6.6	6.44	24.1	1.4
6.6	8.75	22.9	1.7
6.6	9.1	22.9	2.9
11.3	0.99	25.9	3.2
11.3	2.32	25.7	2.6
11.3	3.27	27.6	2.7
11.3	5.15	26.9	3.5
11.5	1.52	27.3	2.7
11.5	1.87	26.5	2.9
11.5	3.2	24.8	1.6
11.5	3.35	27.5	2.6
11.5	5.26	27.1	4.4
13.1	1.68	26.1	1.4
13.1	1.72	25.7	4
13.1	1.82	25.7	6.7
13.1	2.01	26.0	3.2
13.1	3.67	27.1	2.8
13.1	4.04	26.8	4
13.1	8.08	24.4	3.4
13.1	9.8	26.2	5.2
15.9	1.87	24.9	1.9
15.9	3.64	25.8	1.8
15.9	5.67	26.9	4.4
16.3	2.16	26.4	3.3
16.3	3.48	26.9	3.9
16.3	7.87	22.4	2.9
16.9	1.37	33.1	3.4
16.9	1.42	32.7	2.4
16.9	3.17	36.0	3
17.7	1.93	32.4	3.2
17.7	2.14	32.4	2.7
17.7	2.6	33.1	1.3
17.7	2.9	32.4	3.9
17.7	3.66	32.6	2.8

17.7	5.13	26.3	3.8
17.7	5.28	28.4	3.1
17.7	6.14	27.8	2.3
26.2	1.73	34.5	2
26.2	3.16	34.2	3.2
26.2	5.05	29.2	3.4
26.2	7.69	25.4	2.7
31.52	1.52	34.7	5.4
31.52	1.75	34.4	1.5
31.52	3.02	34.0	8.1
31.52	3.99	35.5	1.5
37.5	2.58	32.5	2.1
37.5	3.43	32.9	2
37.5	7.66	24.5	2.8
45.1	1.2	33.8	5.7
45.1	3.68	33.8	5.1
45.1	6.22	23.8	3.3
45.1	6.69	25.4	4.6
50.5	1.65	35.6	2
50.5	1.85	34.0	1.6
50.5	2.39	34.6	2.1
50.5	2.99	35.5	2.4
50.5	4.85	30.2	3.7
50.5	8.7	24.6	3.1
56	1.54	36.9	1.9
56	1.83	34.8	2.1
56	1.9	35.4 ± 0.2	3.5
56	2.08	35.6	1.6
56	2.23	34.9	1.8
56	2.3	35.3	6
56	3.42	36.6	2
56	4.7	31.3	3.6
56	5.57	29.0	2.1
56	6.69	25.5	1.3
56	7.29	25.0	2.5
56	9.82	24.5	2.9
63.2	3.36	35.3	2.1
63.2	3.86	34.5	1.8
63.2	5.78	26.5	2
63.2	5.78	26.5	2.5
63.2	6.69	25.5	1.5

Table S3. Full analytical results of data presented in Fig 2 of the article.

M_n (kDa)	pH	τ_c (ns)	Uncertainty (ns)	χ^2
6.6	2.13	1.5	0.11	1.11
6.6	2.38	1.73	0.06	1.01
6.6	2.55	1.86	0.14	0.99
6.6	2.79	1.58	0.05	1.29
6.6	3.61	1.5	0.07	1.1
6.6	3.69	1.36	0.06	1.33
6.6	3.8	1.76	0.1	0.98
6.6	3.82	1.78	0.13	1
6.6	4.24	1.74	0.16	1.21
6.6	5.77	1.92	0.09	0.93
6.6	6.39	1.61	0.06	0.95
6.6	6.44	1.52	0.05	0.97
6.6	7.89	1.64	0.33	1.03
6.6	8.75	1.62	0.07	1.01
6.6	9.1	1.76	0.05	1.31
11.3	1.33	2.14	0.23	0.92
11.3	1.74	2.42	0.28	1.23
11.3	2.13	2.1	0.25	1.11
11.3	3.38	2.15	0.26	1.06
11.3	4.36	1.82	0.22	1.04
11.3	5.79	1.63	0.27	0.96
11.3	7.39	2.06	0.29	1.26
11.3	9.87	1.88	0.17	0.98
13.1	1.72	2.06	0.15	1.11
13.1	1.74	2.3	0.15	1.23
13.1	1.81	2.26	0.16	1.03
13.1	2.01	2.26	0.15	1.04
13.1	3.67	2.08	0.12	1.31
13.1	4.04	2.09	0.15	1.11
13.1	8.08	1.78	0.19	0.98
15.9	1.84	1.57	< 0.005	1.18
15.9	2.05	1.96	< 0.005	1.11
15.9	3.4	1.72	< 0.005	1.04
15.9	3.64	2.19	< 0.005	1.18
15.9	5.67	1.98	< 0.005	1.44
16.3	1.96	2.32	0.34	1.24
16.3	2.16	1.8	0.34	0.98
16.3	3.48	1.79	0.19	1.09
16.3	3.73	2.25	0.22	1.05
16.3	7.87	1.84	0.34	1.09
16.9	1.42	3.1	0.48	0.89
16.9	4.37	2.22	0.21	1.37
16.9	5.07	1.73	0.21	1.06

pH-responsive behaviour of poly(acrylic acid)

16.9	6.14	1.84	0.21	1.25
16.9	8.25	2.13	0.24	1.11
17.7	2.6	2.5	0.21	0.97
17.7	3.66	2.47	0.14	1.06
17.7	5.28	2.27	0.22	1.2
17.7	7.69	1.69	0.18	1.1
26.2	1.93	4.33	0.6	1.33
26.2	2.14	4.17	0.33	0.95
26.2	2.6	3.53	0.46	1.33
26.2	2.9	3.17	0.33	1.01
26.2	3.66	3.61	0.12	0.98
26.2	5.13	1.98	0.23	0.93
26.2	5.28	2.31	0.3	1.29
26.2	7.69	1.97	0.14	1.1
31.5	1.12	4.25	0.59	1.21
31.5	1.34	3.81	0.31	1
31.5	1.75	3.42	0.06	1.02
31.5	3.02	3.47	0.06	0.97
31.5	4.19	3.08	0.3	1.03
31.5	5.63	2.19	0.09	0.95
31.5	7.66	1.92	0.16	0.99
37.5	1.2	4.1	0.68	1.01
37.5	1.65	3.88	0.59	0.92
37.5	3.68	4.2	0.24	1.11
37.5	6.69	1.54	0.24	1.2
45.1	2.22	4.83	0.18	1.11
45.1	3.43	2.89	0.18	0.95
45.1	3.58	3.36	0.21	0.98
45.1	9.98	2.27	0.23	1.07
50.5	1.83	3.63	0.4	1.04
50.5	1.85	3.39	0.25	1.23
50.5	2.39	3.22	0.24	1.31
50.5	2.99	3.23	0.32	1.26
50.5	4.85	1.96	0.13	1.11
50.5	8.7	2.13	0.16	1.14
56	2	3.81	0.02	1.18
56	2.81	3.31	0.23	0.99
56	2.9	4.48	0.34	1.44
56	3.69	3.95	0.03	1.01
56	5.07	1.95	0.12	1.04
56	6.04	1.46	0.08	1.18
56	7.57	1.85	0.13	1.11
63.2	1.54	3.9	0.23	1.14
63.2	2.15	3.87	0.23	0.98
63.2	3.1	3.85	0.28	1.09
63.2	3.86	3.23	0.42	1.21

63.2	5.57	1.84	0.23	1.09
63.2	5.78	1.88	0.19	1.13
63.2	6.69	2.04	0.19	1.19

Table S4. Diffusion coefficients of polymers as measured by NMR for different concentrations (wt%) of PAA and pH.

M_n (kDa)	pH	wt %	Diffusion coefficient ($\mu\text{m}^2/\text{s}$)
6.6	3.3	6.25	100.0
6.6	4.3	8.75	83.4
6.6	5.8	11.25	98.4
6.8	3	7.5	78.3
6.8	3.8	6.25	76.0
6.8	7.8	7.5	76.7
16.3	2.3	6.25	67.1
16.3	2.7	7.5	69.7
16.3	3.3	6.25	66.1
16.3	3.7	7.5	66.7
16.3	4	6.25	67.5
16.3	5.5	6.25	78.2
16.3	6.3	7.5	74.8
16.3	7.1	6.25	66.1
17.7	2.6	7.5	67.1
17.7	3.4	7.5	66.1
17.7	3.63	12.5	67.0
17.7	4.9	7.5	67.1
17.7	5.2	10	58.7
17.7	5.5	7.5	35.4
17.7	6	12.5	29.9
17.7	7.1	7.5	27.8
26.2	4.3	6.25	41.8
26.2	5.4	6.25	31.1
26.2	5.9	20	21.7
26.2	6.9	6.25	20.9
26.2	7.8	6.25	20.3
26.2	16	6.25	41.7
52.5	2.8	15	30.3
52.5	3.3	22.5	29.6
52.5	4	15	27.5
52.5	4.85	22.5	31.6
52.5	5.9	22.5	8.9
52.5	6.4	22.5	8.1
52.5	7	15	7.9
52.5	7.8	22.5	7.4
55.9	2.34	12.5	24.5
55.9	4.4	8.75	23.2

55.9	5.8	15	11.0
55.9	5.9	12.5	10.2
55.9	6	22.5	10.2
55.9	7.6	8.75	7.9