

Supporting Information

Enhanced Fluorescence Properties of poly(Phenylene Ethynylene) Conjugated Polyelectrolytes Designed to Avoid Aggregation

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Content

I.	General methods.....	S2
II.	Synthesis.....	S3-S8
III.	NMR spectra.....	S9-S13
IV.	Photophysical measurements.....	S14-S15
V.	Fluorescence Correlation Spectra (FCS).....	S16-S17
VI.	Quenching Experiments.....	S18
VII.	Dynamic Light Scattering (DLS).....	S19

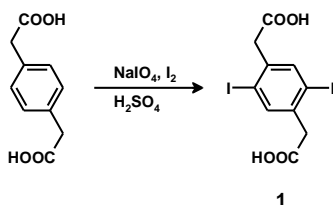
I. General methods

Unless otherwise indicated, all starting materials were purchased from commercial sources (Aldrich, Acros, Strem Chemicals) and used without further purification. All solvents were applied in commercial p.a. quality. For all palladium-catalyzed reactions, the solvents were carefully degassed with argon. ^1H and ^{13}C NMR spectra were recorded on Inova2 (500 MHz) or Varian Gemini-300 spectrometer (300 MHz). The chemical shifts (δ) are reported in parts per million (ppm) using the residual solvent signals as internal standards. The coupling constants are reported in Hertz (Hz). Splitting patterns are designated as s (singlet), bs (broad singlet), d (doublet), t (triplet), and m (multiplet).

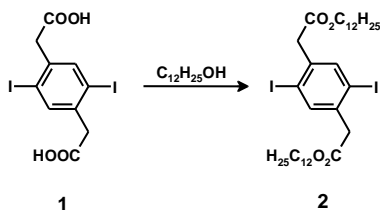
Gel permeation chromatography (GPC) analysis was carried out on a system comprised of a Shimadzu LC-6D pump, Agilent mixed-D column and a Shimadzu SPD-20A photodiode array (PDA) detector, with THF as eluent at 1 mL/min flow rate. The system was calibrated against linear polystyrene standards in THF.

II. Synthesis (Schemes, Procedures, NMR spectra)

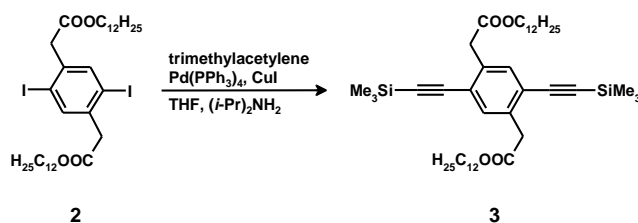
II.I Monomer synthesis



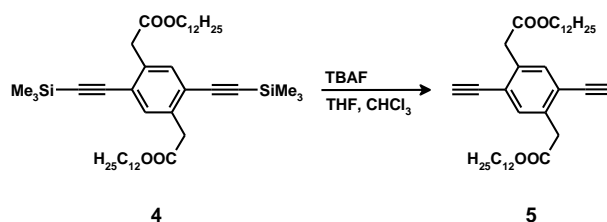
Iodine (2.850 g, 11.23 mmol) and KIO_4 (0.878 g, 3.82 mmol) were slowly added to 95% H_2SO_4 (150 ml). After stirring for 3 h at 25 – 30 °C, a dark brown iodinating solution was obtained. 1,4-Phenylenediacetic acid (1,010 g, 5.20 mmol) was added in one portion to the brown iodinating solution and the resulting solution was stirred overnight at 25 – 30 °C. The yellow reaction mixture was cooled down to room temperature and poured on ice. The precipitate was collected by filtration, thoroughly washed with cold water until the filtrates were neutral and dried. The raw product was stirred in ethanol for 2 h at room temperature to remove side products. The product **1** was filtered and obtained as a white solid (1.622 g, 3.64 mmol, 70 %). ^1H NMR (300 MHz, DMSO-d_6): δ 3.69 (2, 4H), 7.82 (s, 2H), 12.54 (s; 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 44.32, 101.40, 139.17, 140.52, 171.19.



A mixture of **1** (9.0 g, 20 mmol), dodecyl alcohol (80.0 g, 465 mmol) and 85% phosphoric acid (0.5 mL) was heated at 150 °C in a flask equipped with a Dean-Stark trap. After reaction overnight, the solvent was removed under vacuum. The residue was recrystallized in isopropanol to give **2** as an off-white solid (13.4 g, 17.122 mmol, 85%). ¹H NMR (300 MHz, CDCl₃) δ 0.88 (t, 6H, *J* = 6.6 Hz), 1.26 (m, 36H), 1.63 (m, 4H), 3.71 (s, 4H), 4.12 (t, 4H, *J* = 6.6 Hz), 7.73 (s, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 14.36, 22.93, 26.12, 28.75, 29.43, 29.59, 29.81, 29.88, 32.14, 45.31, 65.64, 100.81, 138.85, 140.84, 170.09. MS (ESI) *m/z* ([M + Na]⁺) 805.2160.

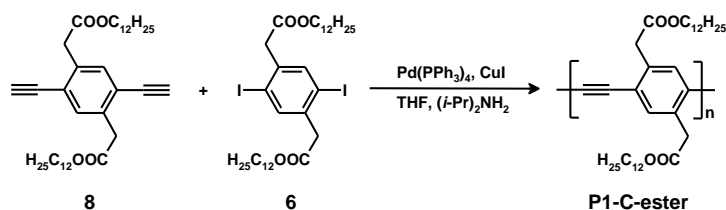


Compound **2** (1.0 g, 1.28 mmol) was dissolved in a mixture of THF (45 mL) and isopropylamine (15 mL), and combined with Pd(PPh₃)₂Cl₂ (14 mg, 0.2 mmol) and CuI (7.5 mg, 0.4 mmol). After degassing the solution, trimethylsilylacetylene (0.359 g, 3.65 mmol) was added, and reacted overnight at room temperature. The solvent was removed and the residue was extracted with dichloromethane/water. The organic layer was washed with saturated ammonium chloride, water and brine, and then dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the crude product was purified by silica chromatography (hexane/dichloromethane, 2/1) to give compound **3** as light yellow oil (0.813 g, 88%). ¹H NMR (300 MHz, CDCl₃) δ 0.23 (s, 9H), 0.88 (t, 6H, *J* = 6.9 Hz), 1.26 (m, 36H), 1.59 (m, 4H), 3.75 (s, 4H), 4.10 (t, 4H, *J* = 6.9 Hz), 7.37 (s, 2H). ¹³C NMR (75 MHz, CDCl₃) δ, 0.08, 14.34, 22.91, 26.07, 28.78, 29.45, 29.58, 29.74, 29.80, 29.86, 29.88, 32.14, 39.60, 65.32, 101.05, 102.68, 123.92, 133.63, 135.59, 170.88. MS (ESI) *m/z* ([M + Na]⁺) 745.5018.

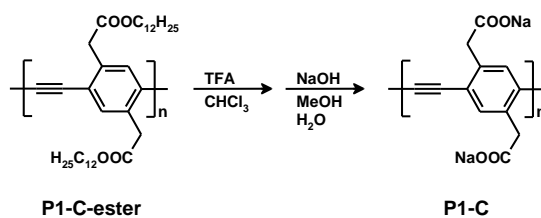


To the solution of compound **4** (0.800 g, 1.11 mmol) in degassed chloroform (50 mL), tetra-*n*-butylammonium fluoride (TBAF, 2.77 mL, 2.77 mmol, 1 M in THF) was added. After reaction overnight, the reaction mixture was passed through a silica column and gave **5** as white solid (0.430 g, 67%). ^1H NMR (300 MHz, CDCl_3) δ 0.88 (t, 6H), 1.26 (m, 36H), 1.61 (m, 4H), 3.33 (s, 2H), 3.78 (s, 4H), 4.10 (t, 4H, $J = 6.6$ Hz), 7.44 (s, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 14.31, 22.89, 25.97, 29.56, 29.66, 29.84, 32.12, 32.98, 39.15, 52.34, 63.15, 81.06, 83.56, 123.34, 134.17, 135.67, 171.16. MS (ESI) m/z ($[\text{M} + \text{Na}]^+$) 601.4227.

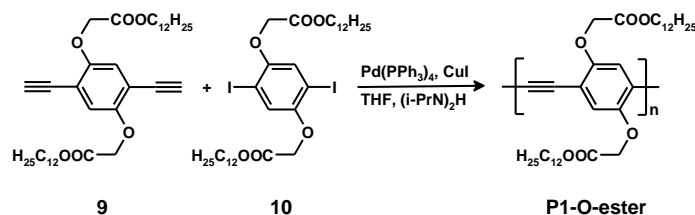
II.II Polymer synthesis



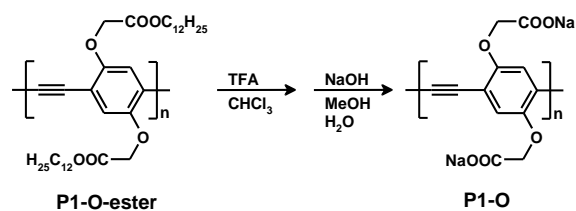
A solution of **8** (0.60 g, 0.1 mmol) and **6** (0.081 g, 0.10 mmol) in 20 mL of THF and 5 mL of diisopropylamine was degassed 1 h before $\text{Pd(PPh}_3)_4$ (0.012 g, 0.01 mmol) and CuI (0.010 g, 0.05 mmol) were added. The mixture was degassed for additional 30 min and then stirred for 1 d at 50 °C. A solution of 0.002 g (0.01 mmol) tert-butyl 4-iodophenylacetate in 2 mL of degassed THF was added. After 6 hours, a solution of tert-butyl 4-ethynylphenylacetate in 2 mL of THF was charged. After reaction at 50 °C for additional 12 hours, the solution was cooled down to room temperature, diluted with 20 mL CHCl_3 and passed through a short alumina oxide column. The yellow solution was concentrated to 2–3 mL and precipitated into methanol. The yellow polymer was collected by centrifugation. After redissolving in CHCl_3 , the polymer **P1-C** was precipitated again 3 times in methanol and obtained as a yellow solid (0.097 g, 87 %). ^1H NMR (500 MHz, CDCl_3): δ 0.87 (m, 6H), 1.24 (m, 36H), 1.60 (m, 4H), 3.87 (m, 4H), 4.10 (m, 4H), 7.51 (m, 2H). GPC (THF): $M_n = 15,600$ g/mol, $M_w = 25,800$ g/mol, PDI = 1.65.



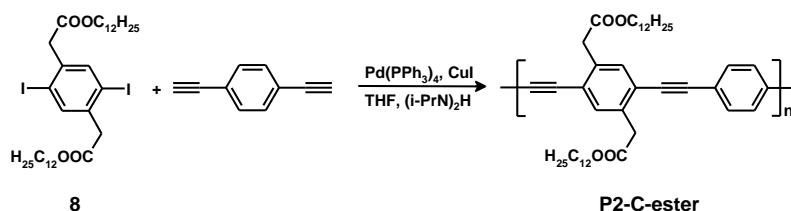
The CPE precursor **P1-C-ester** (0.085 g) was dissolved in 100 mL of chloroform and treated with 50 mL of TFA overnight at room temperature. The solvents were completely removed under vacuum. The residue was dissolved in THF (150 mL) and a solution of sodium hydroxide (10 eq. to ester group) in a mixture of 5 mL methanol and 5 mL of water was added. The mixture was stirred at 50 °C overnight. The solvents THF and methanol were removed under vacuum, and 100 mL water was added to the residue, followed by an additional reaction at 50 °C overnight. The mixture was concentrated to 2-3 mL and the polyelectrolyte **P1-C** was precipitated into a mixture of acetone and methanol (95/5 v/v). The precipitate was collected by centrifugation and dissolved again in 2-3 mL of water. The polymer was additionally precipitated 3 times into a mixture of acetone and methanol. The polymer was dissolved in 30 mL of water (pH = 10), filtered through a membrane filter (0.22 μm pore size) and subjected to dialysis using a dialysis tube (molecular weight cutoff 12,000 – 14,000 Da) against water (pH = 10) over 3 d. CPE **P1-C** was obtained by freeze drying of the aqueous solution as a yellow solid (0.035 g, 88 %). ^1H NMR (500 MHz, D_2O) δ 3.82 (m, 4H), 7.56 (m, 4H).



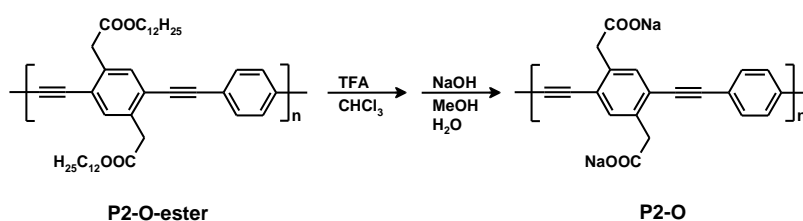
The polymer precursor **P1-O-ester** was synthesized in the same procedure as the polymer precursor **P1-C-ester** using monomer **9** (0.060 g, 0.10 mmol), monomer **10** (0.081 g, 0.10 mmol), $\text{Pd(PPh}_3)_4$ (0.012 g, 0.01 mmol) and CuI (0.010 g, 0.05 mmol). The polymer precursor was obtained as a yellow solid (0.090 g, 86 %). ^1H NMR (500 MHz, CDCl_3): δ 0.87 (m, 6H), 1.25 (m, 36H), 1.66 (m, 4H), 4.21 (m, 4H), 4.67 (m, 4H), 6.91 (m, 2H). GPC (THF): $M_n = 11,900$ g/mol, $M_w = 23,100$ g/mol, PDI = 1.94.



The hydrolysis of polyelectrolyte precursor **P1-O-ester** (0.055 g) was achieved following the same procedure as described above for CPE **P1-C**. The polyelectrolyte **P1-O** was obtained as a yellow solid (0.032 g, 80 %). ^1H NMR (500 MHz, D_2O / MeOD): δ 4.47 (m, 4H), 6.98 (m, 2H).



Polymer **P2-C-ester** was synthesized in the same procedure as polymer **P1-C** using the monomer **8** (0.100 g, 0.13 mmol) and 1,4-diacetylenebenzene (0.016 g, 0.13 mmol), $\text{Pd(PPh}_3)_4$ (0.010 g, 0.01 mmol) and CuI (0.005 g, 0.02 mmol). The polymer **P2-C** was obtained as a yellow solid (0.068 g, 80 %). ^1H NMR (500 MHz, CDCl_3): δ 0.90 (b, 6H), 1.28 (b, 36 H), 1.68 (b, 8H), 3.80 (b, 4H), 4.15 (t, 4H), 7.53 (b, 6H). GPC (THF): $M_n = 19,000$ g/mol, $M_w = 34,200$ g/mol, PDI = 1.80.



The hydrolysis of polymer **P2-C-ester** was achieved following the same procedure as described above for polyelectrolyte **P1-C**. **P2-C** was obtained as a yellow solid (0.027 g, 60 %). ^1H NMR (500 MHz, MeOD): δ 3.77 (b, 4H), 7.53 (b, 6H)



$$\begin{array}{c}
 \text{COOC}_{12}\text{H}_{25} \\
 | \\
 \text{O} \\
 | \\
 \text{[} \text{---} \text{C}_6\text{H}_3 \text{---} \text{C}_6\text{H}_4 \text{---}]_n \\
 | \\
 \text{O} \\
 | \\
 \text{H}_{25}\text{C}_{12}\text{OOC}
 \end{array}
 \xrightarrow[\text{CHCl}_3]{\text{TFA}}
 \xrightarrow[\text{MeOH, H}_2\text{O}]{\text{NaOH}}
 \begin{array}{c}
 \text{COONa} \\
 | \\
 \text{O} \\
 | \\
 \text{[} \text{---} \text{C}_6\text{H}_3 \text{---} \text{C}_6\text{H}_4 \text{---}]_n \\
 | \\
 \text{O} \\
 | \\
 \text{NaOOC}
 \end{array}$$

P2-O-ester **P2-O**

$$\begin{array}{c}
 \text{9} \\
 + \\
 \text{6} \\
 \xrightarrow[\text{THF, (i-PrN)}_2\text{H}]{\text{Pd(PPh}_3)_4, \text{CuI}} \\
 \text{P3-C/O-ester}
 \end{array}$$

$$\text{P3-C/O-ester} \xrightarrow[\text{CHCl}_3]{\text{TFA}} \text{Intermediate} \xrightarrow[\text{MeOH, H}_2\text{O}]{\text{NaOH}} \text{P3-C/O}$$

The hydrolysis of polymer **P3-C/O-ester** (0.075 g) was achieved following the same procedure as described above for the polyelectrolyte **P1-C**. CPE **P3-C/O** was obtained as a yellow solid (0.030 g, 83 %). ^1H NMR (500 MHz, D_2O / MeOD): δ 4.40 (m, 4H), 4.53 (m, 4H), 7.05 (m, 2H), 7.5 (m, 2H).

III. NMR spectra

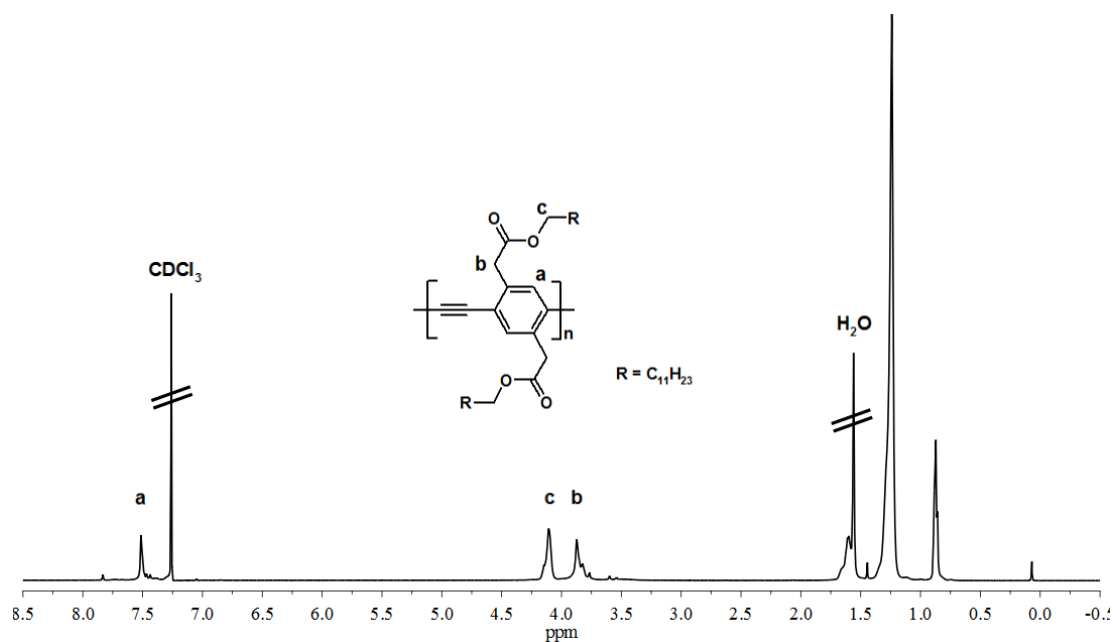


Figure S1: ^1H NMR of polymer P1-C-ester

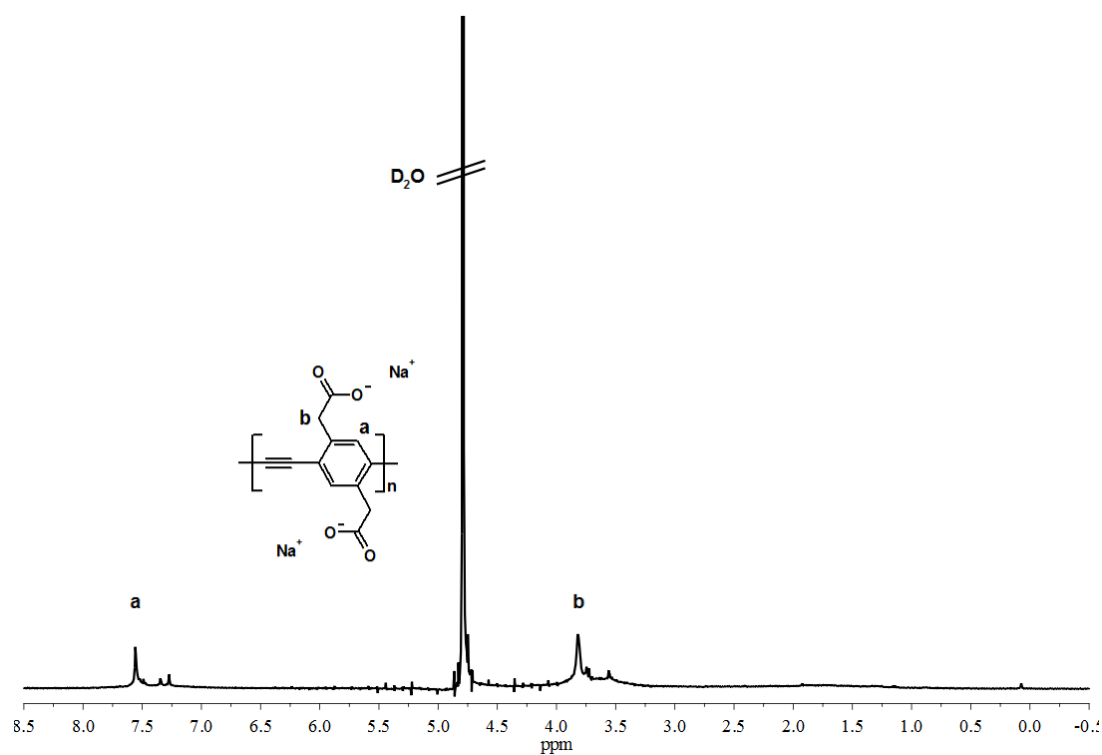


Figure S2: ^1H NMR of CPE P1-C

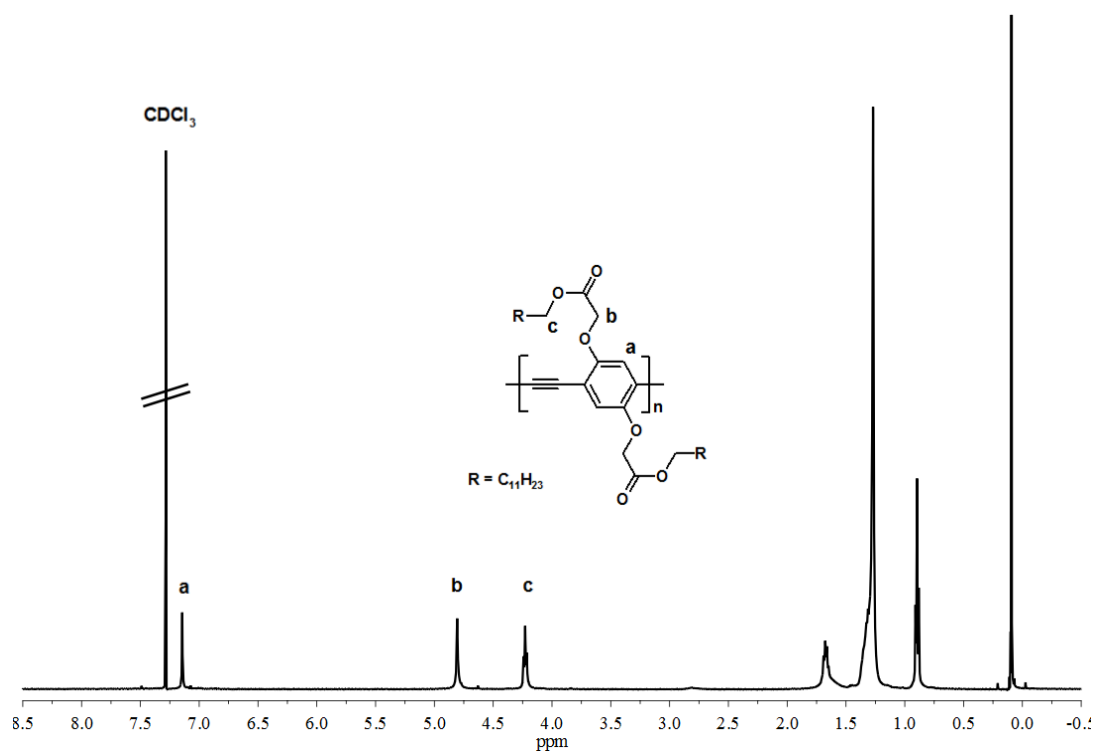


Figure S3: ¹H NMR of polymer P1-O-ester

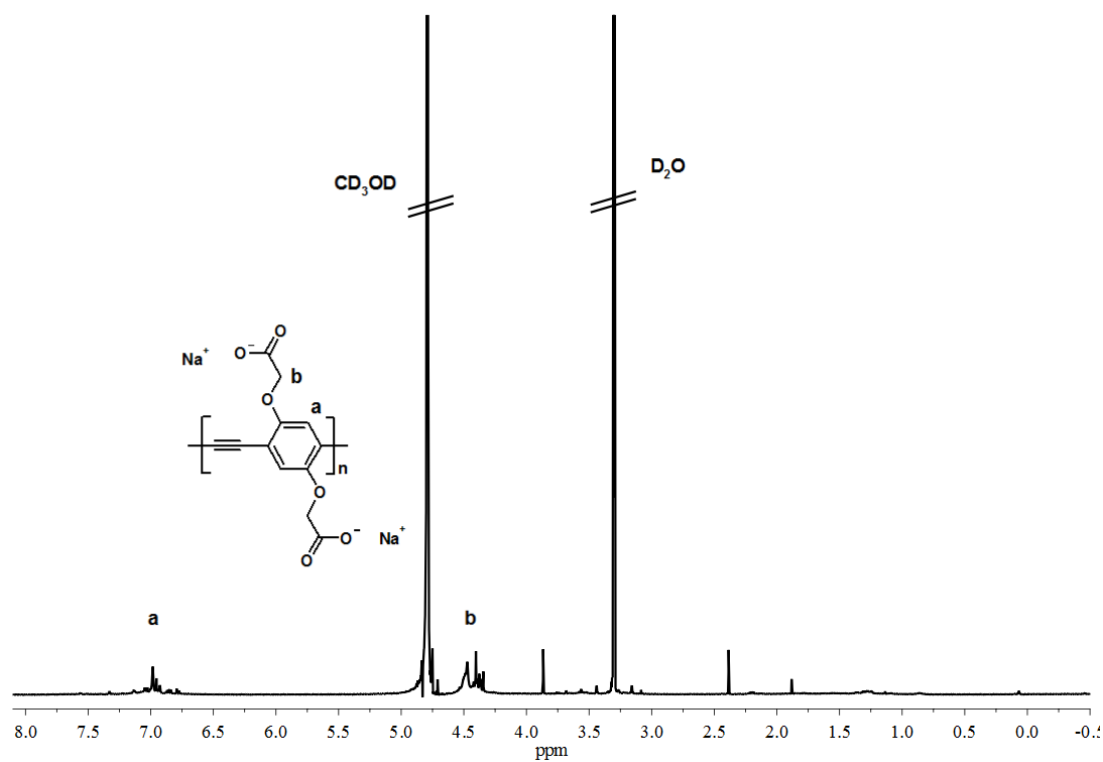


Figure S4: ¹H NMR of CPE P1-O

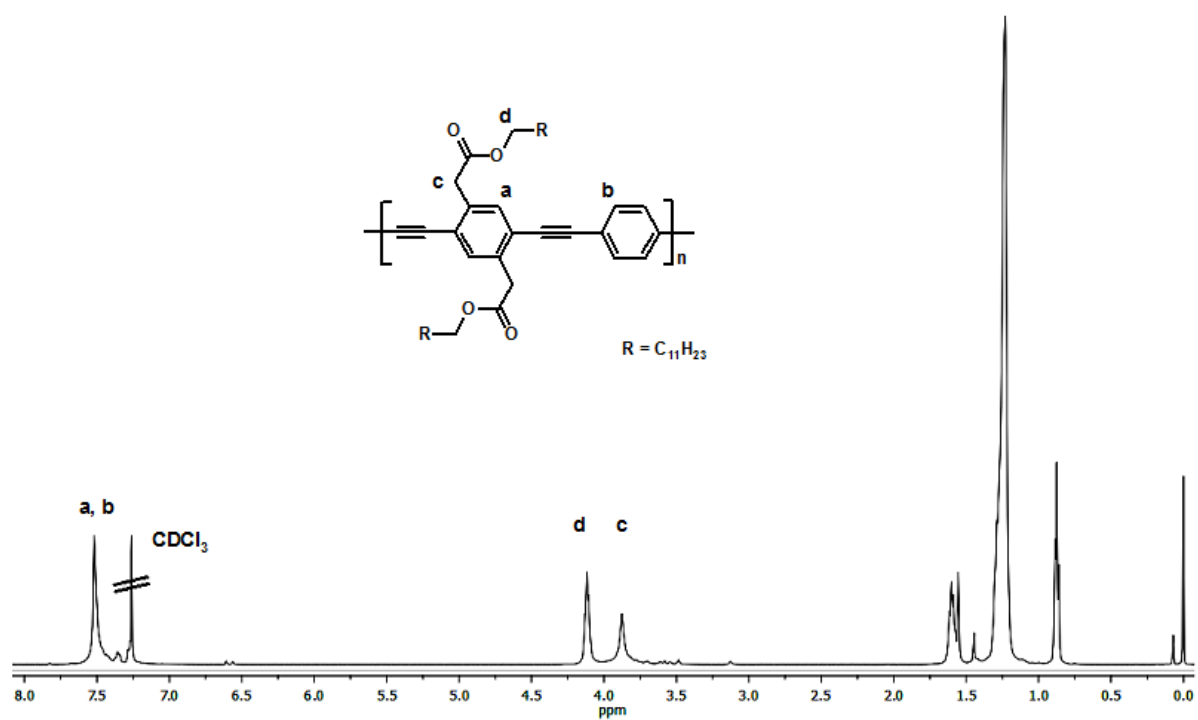


Figure S5: ¹H NMR (500 MHz) of polymer P2-C-ester in CDCl₃.

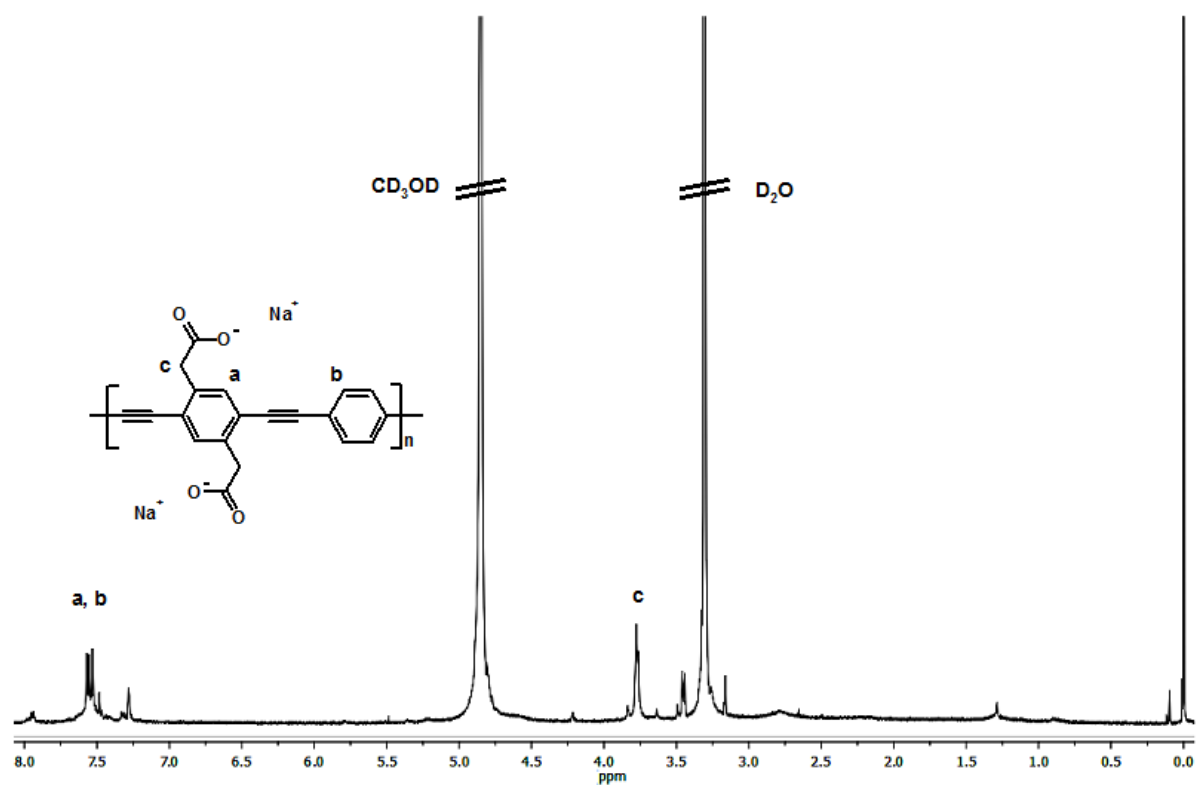


Figure S6: ¹H NMR (500 MHz) of **P2-C** in CD₃OD.

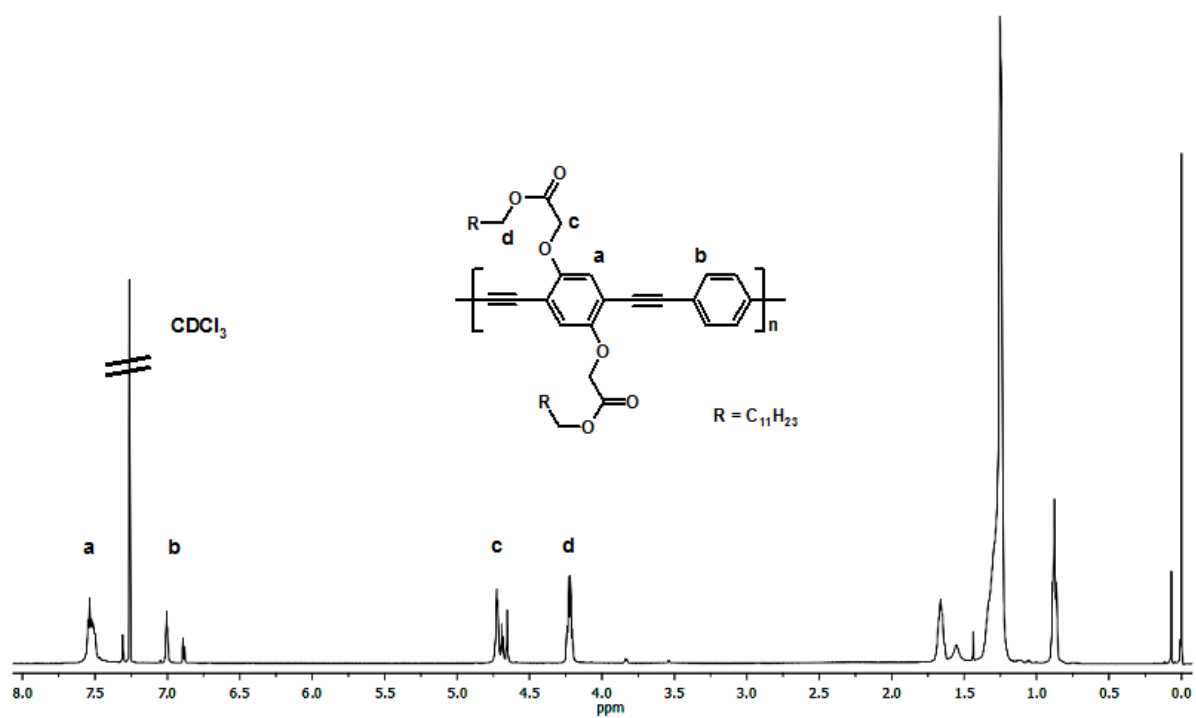


Figure S7: ^1H NMR (500 MHz) of polymer P2-O-ester in CDCl_3 .

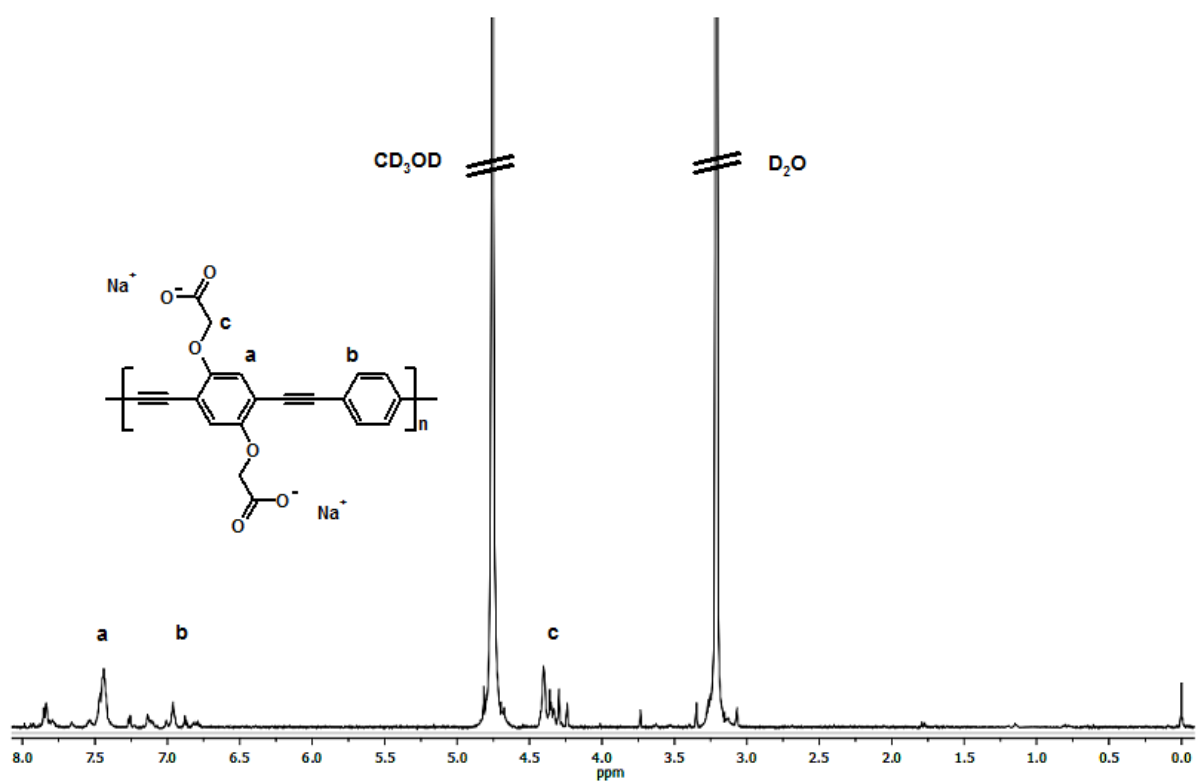


Figure S8: ^1H NMR (500 MHz) of CPE P2-O in CD_3OD .

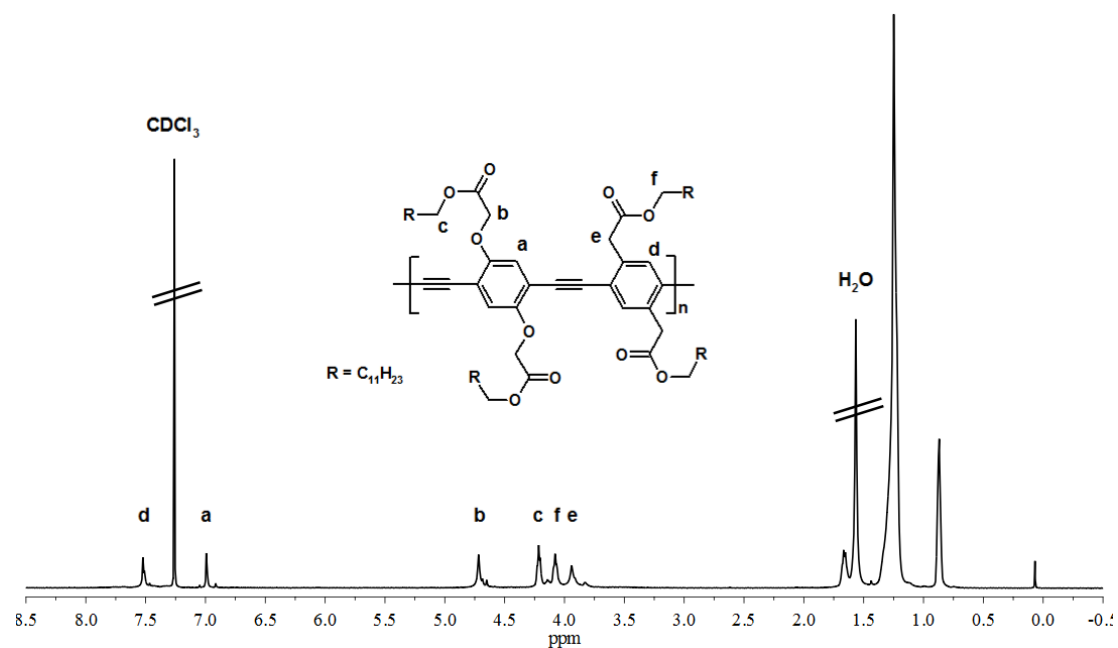


Figure S9: ^1H NMR of polymer P3-C/O-ester

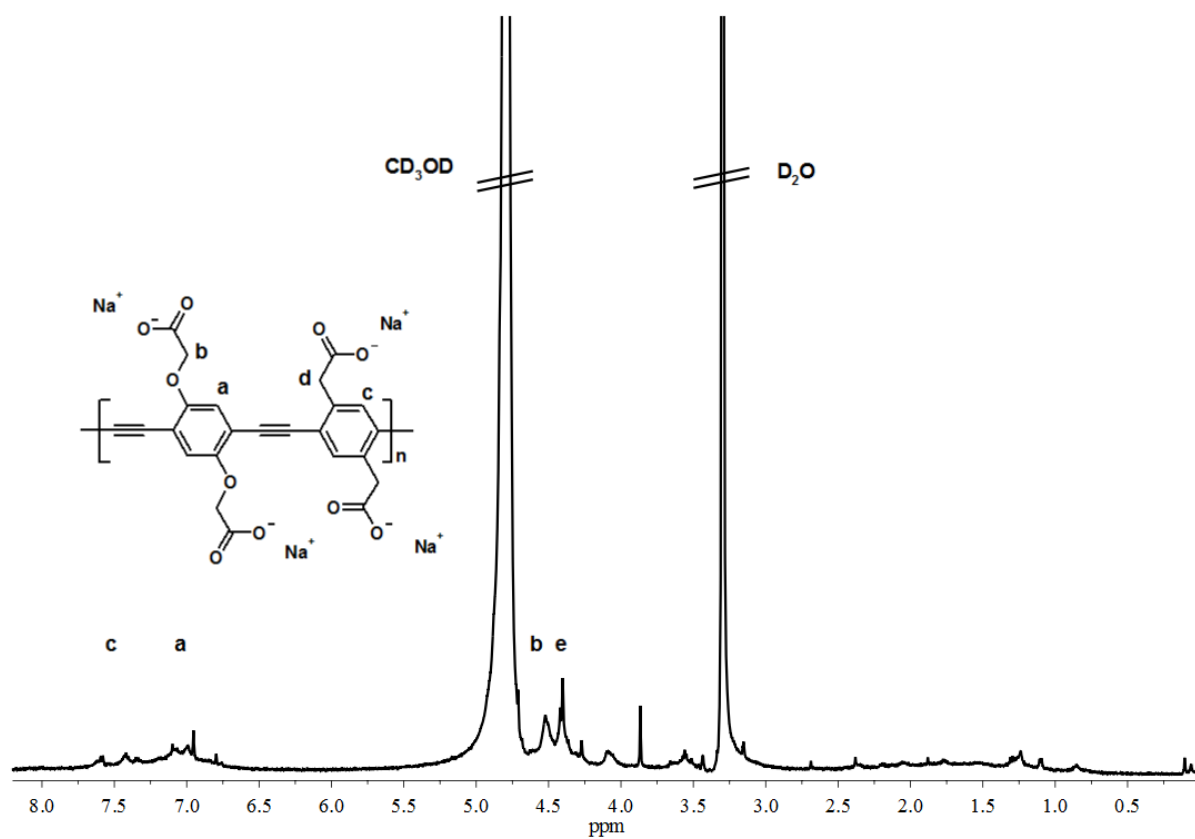


Figure S10: ^1H NMR of CPE P3-C/O

IV. Additional Photophysical Data

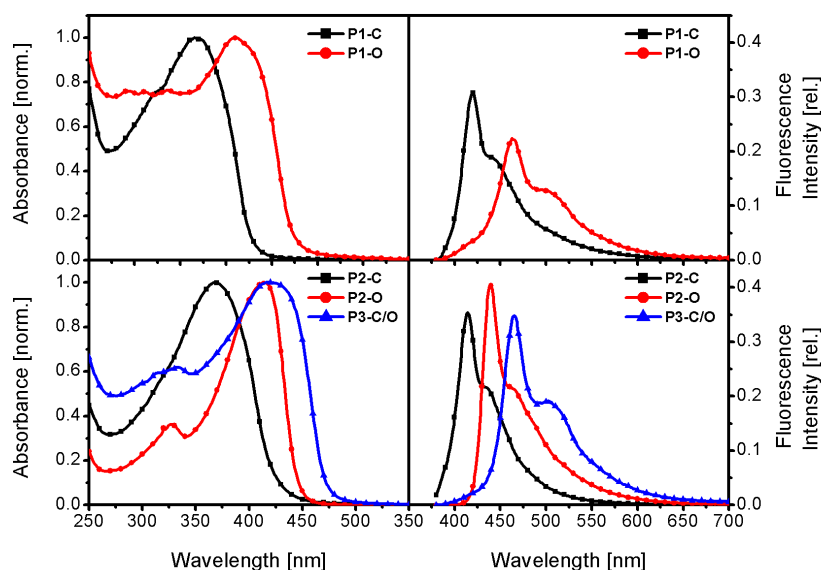


Figure S11: Absorbance and Fluorescence of the homopolymers P1-C & P1-O and the copolymers P2-C, P2-O, P3-C/O in methanol (containing 1 mg/ml NaOH). Fluorescence spectra are area normalized corresponding to the relative quantum yields.

Table S1: Optical properties, fluorescence quenching constants and diffusion times (FCS) in aqueous solution

PPE	H ₂ O (pH = 8)				MeOH ^a		
	Abs. λ_{\max}	PL λ_{\max} ^b	K_{SV} ^c	τ_{Diff} ^d	Abs. λ_{\max}	PL λ_{\max}	K_{SV} ^c
	[nm]	[nm]	[10 ⁶ M ⁻¹]	[μ s]	[nm]	[nm]	[10 ⁶ M ⁻¹]
P1-C	374	422	4.7	76	365	420	3.2
P1-O	411	465	3.6	73	411	463	2.6
P2-C	375	411	0.3	58	368	409	0.1
P2-O	430	512	19.0	223	414	439	1.0
P3-C/O	423	465	4.6	79	419	466	1.6

^a Contains 1 mg/ml NaOH. ^b The excitation wavelength corresponds to the wavelength of the absorption maximum. ^c The Stern-Volmer constants (K_{SV}) are obtained from the steady-state fluorescence emission quenching (Supporting Information). ^d Diffusion time from fluorescence correlation spectroscopy (FCS).

Table S2: Fluorescence lifetime analysis of the polymer precursor P1-C-ester, P1-O-ester, P2-C-ester, P2-O-ester and P3-C/O-ester in CHCl₃.

Polymer	λ_{em}	τ_1 [ns]	A ₁	τ_2 [ns]	A ₂	τ_3 [ns]	A ₃	$\langle\tau\rangle^a$ [ns]	χ^2
P1-C-ester	425	0.06	27.3	0.35	72.5	1.30	0.11	0.27	1.001
P1-O-ester	450	0.41	98.00	0.99	2.0	2.68	0.02	0.42	0.911
P2-C-ester	420	0.30	100.0	0.76	0.0	---	---	0.30	1.030-
P2-O-ester	425	0.33	93.0	0.67	7.0	---	---	0.35	1.010
P3-C/O-ester	450	0.56	79.2	1.53	20.8	---	---	0.76	1.007

^aFluorescence decays are multiexponential, $\langle\tau\rangle$ is the median fluorescence lifetime, $\langle\tau\rangle = \sum(\alpha_i \cdot \tau_i)$

Table S2: Fluorescence lifetime analysis of the polyelectrolytes P1-C, P1-O, P2-C, P2-O and P3-C/O in water (pH = 8) and methanol.

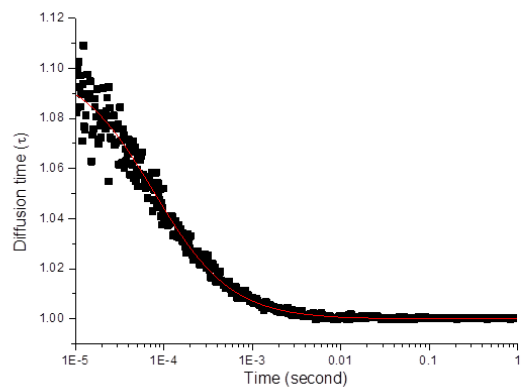
CPE	solvent	λ_{em}	τ_1 [ns]	A ₁ [%]	τ_2 [ns]	A ₂ [%]	τ_3 [ns]	A ₃ [%]	$\langle\tau\rangle^b$ [ns]	χ^2
P1-C	H ₂ O	425	0.40	97.4	1.34	2.60	6.72	0.02	0.43	1.142
	MeOH ^a	425	0.45	98.6	1.32	1.36	6.48	0.00	0.46	1.119
P1-O	H ₂ O	465	0.18	93.8	0.98	6.06	4.45	0.10	0.23	1.129
	MeOH ^a	465	0.54	92.7	1.08	7.08	2.99	0.20	0.58	1.073
P2-C	H ₂ O	420	0.40	42.0	0.63	58.0	---	---	0.56	1.050
	MeOH ^a	420	0.35	32.0	0.63	68.0	---	---	0.44	1.030
P2-O	H ₂ O	420	0.14	99.0	1.46	1.0	4.61	0.00	0.15	1.040
	MeOH ^a	420	0.44	60.0	0.69	40.0	---	---	0.53	1.060
P3-C/O	H ₂ O	465	0.15	96.6	0.77	3.19	3.03	0.16	0.17	1.067
	MeOH ^a	465	0.44	81.7	0.78	18.2	2.54	0.14	0.50	0.956

^acontains 1 mg/ml NaOH

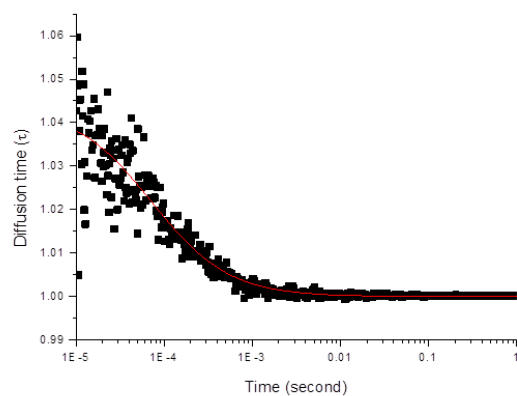
Fluorescence decays are multiexponential, $\langle\tau\rangle$ is the median fluorescence lifetime, $\langle\tau\rangle = \sum(\alpha_i \cdot \tau_i)$

V. Fluorescence correlation spectroscopy (FCS)

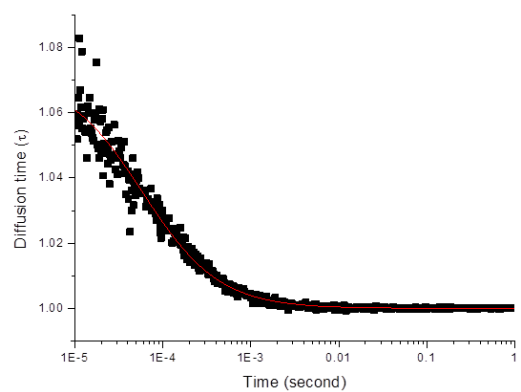
P1-C



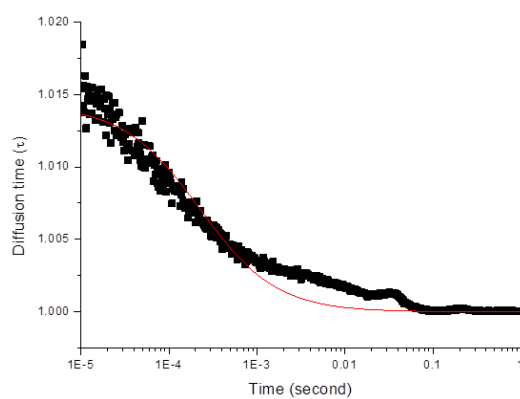
P1-O



P2-C



P2-O



P3-C/O

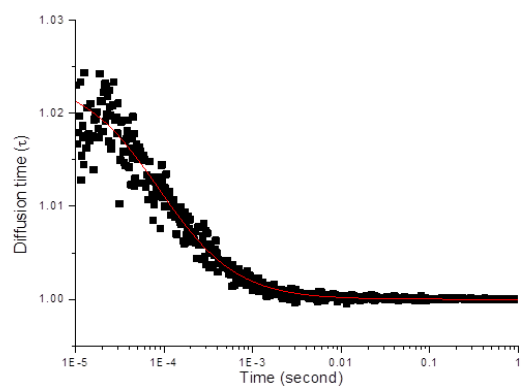


Figure S12: FCS spectra of the polymers P1-C, P1-O, P2-C, P2-O and P3-C/O in water (pH = 8), $c = 0.2 \mu\text{M}$

Table S4: Diffusion time (FCS) of the polyelectrolytes P1-C, P1-O, P2-C, P2-O and P3-C/O in aqueous solution (pH = 8) and the estimated particale size based on a spherical model.

CPE	$\tau_{\text{Diff.}}$ [μs]	Particle size [nm]
P1-C	76	2.05
P1-O	73	1.97
P2-C	58	1.56
P2-O	223	6.02
P3-C/O	79	2.13

VI. Quenching Experiments

For all quenching experiments, stock solutions with a concentration of 1 mM in water (pH = 9.0) were prepared. The concentration of the polymer for each quenching experiment was 0.5 μM . The solutions of methyl viologen (MV^{2+}) were freshly prepared in water (pH 8.0) for quenching experiments.

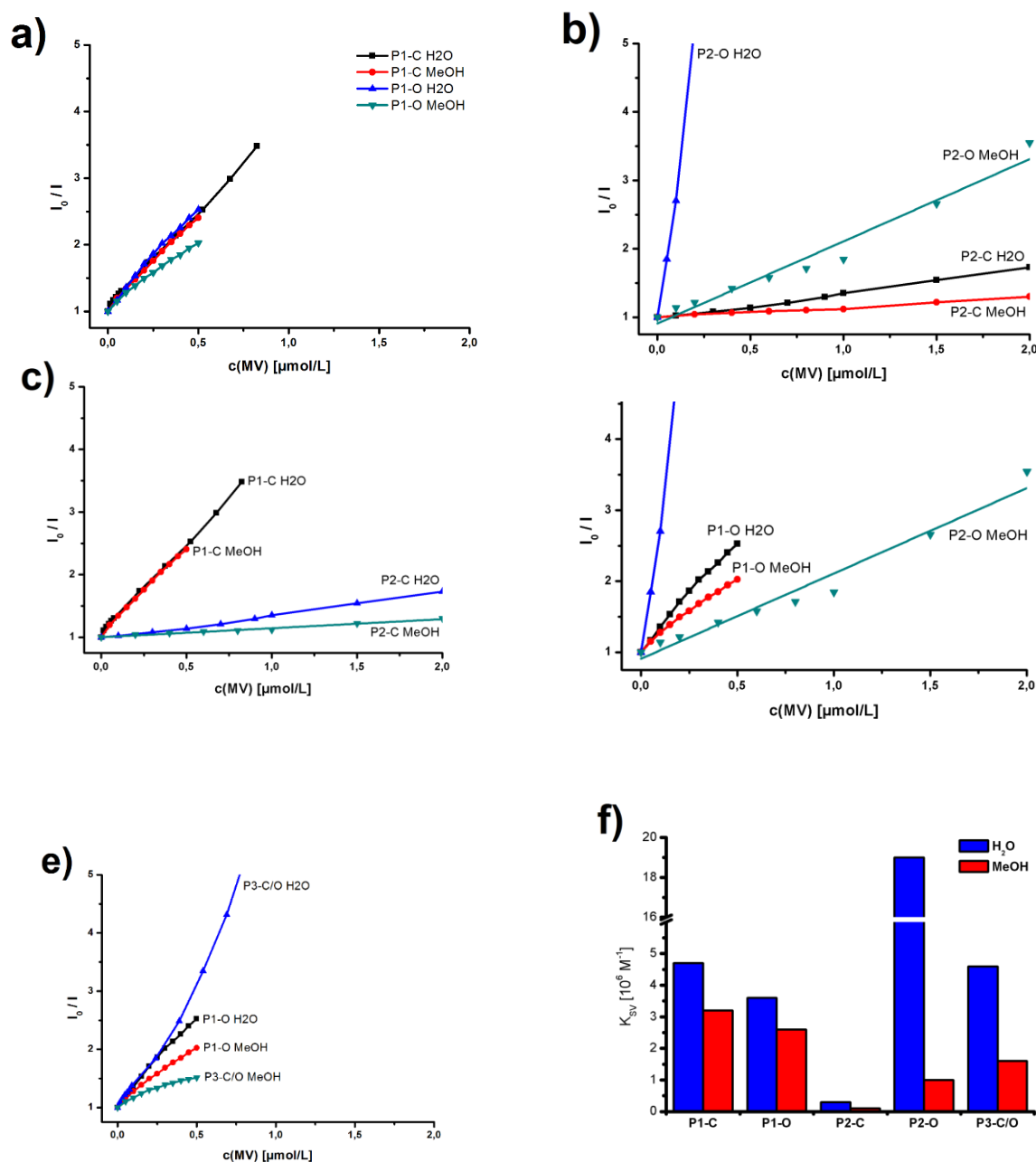


Figure S13: a-e) K_{SV} plots of the CPEs P1-C, P1-O, P2-C, P2-O and P3-C/O in water (pH = 8) and methanol using MV^{2+} as quencher. f) K_{SV} values of all CPEs in water (pH = 8, blue) and methanol (red). The Stern-Volmer constants (K_{SV}) are obtained from the steady-state fluorescence emission quenching. Where plots are curved, the K_{SV} values represent the slope of the linear region at low quencher concentration. The estimated error of the K_{SV} values is $\sim 10\%$.

VII. Dynamic Light Scattering

Dynamic light scattering (DLS) characterization of all the polymers were performed on a Zetasizer Nano (Malvern Instruments, Worcestershire, United Kingdom) at 25°C. Samples (0.1 mM in pH=8 aqueous solution or methonol which contains 1 μ M NaOH) were measured with a quartz cuvette with 1cm O.D.. Three measurement cycles were run for each sample. The data were averaged from 10 light scattering periods of 10 s for each cycle. Average diameter values were calculated using the Malvern Instruments DTS software.

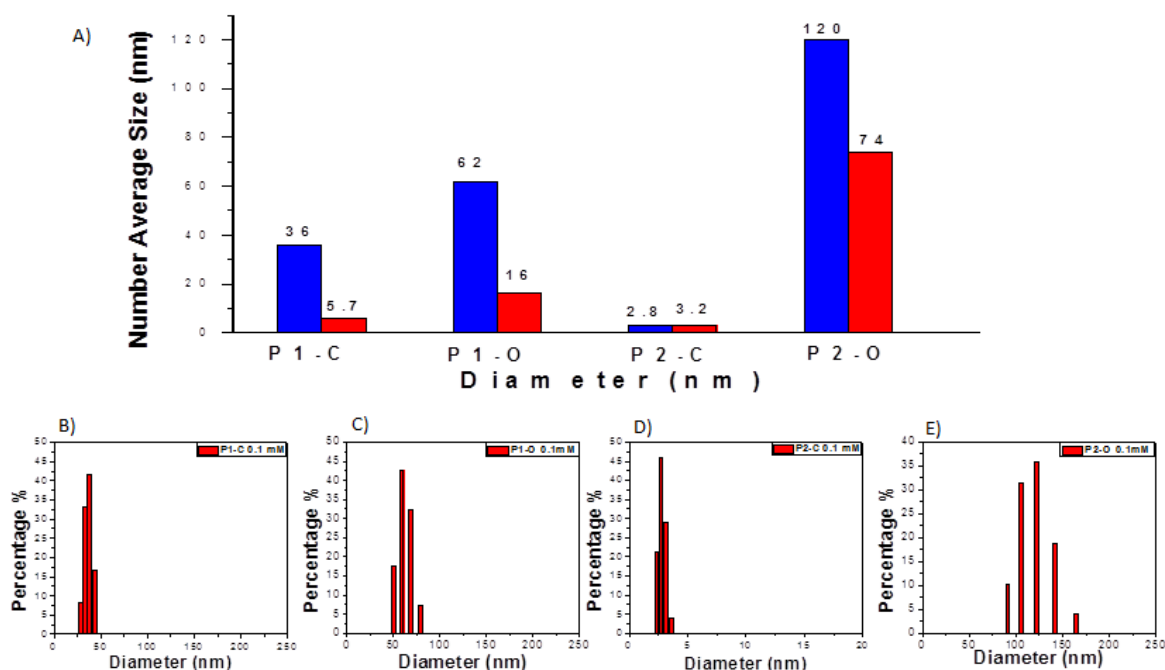


Figure S14: A) polymer size comparison of P1-C, P1-O, P2-C and P2-O at a concentration of 0.1 mM in pH=8 water (blue column) and basic methonol solution (red column). B), C), D), E) size distribution of P1-C, P1-O, P2-C and P2-O in pH=8 water at a concentration of 0.1 mM respectively.