A Library of Quinoline-labeled Water-soluble Copolymers with pH-Tunable Fluorescence Response in the Acidic pH Region

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Synthesis of bromobenzimidazole:In a round bottom flask of 1000ml, where a magnet and an ice bath were placed, was added 150g KOH and 400ml MeOH. Alongside in a flask of 250ml with a magnet 25g p-bromonitrobenzene and 100ml THF were added. After the solids were completely dissolved in both flasks, the 1000ml flask was placed in a renewed ice bath and the solution of the 250ml flask was added through filter paper to contain any impurities. A condenser was adapted and degassing cycles and Ar/vacuum took place. The system remained under Ar atmosphere for ~15min. Then 17ml of benzylcyanide were slowly added and the reaction was left in an ice bath for ~3hours with continuous renewal of ice. The content of the flask was

precipitated in H_2O and then filtered under vacuum. The precipitate was dried at 75°C for ~2hours and then recrystallized in MeOH. Filtration on porous filter without vacuum followed recrystallization and the pure material was dried at 50°C under vacuum for ~12hours.

Synthesis of 2-amino-5-bromo-benzophenone: In a round bottom of 500ml flask where a condenser, magnetic stirring and oil bath were placed, was added 6g bromobenzimidazole and ~150ml of acetic acid and the mixture heated at 110°C. Addition of 4.5g Fe and 10ml H_2O every 10min was followed. The temperature was removed upon the third addition. After the solution was cooled to ambient temperature, 500ml H_2O was added followed by filtration over a fluted filter to contain iron and washed with ethyl acetate. The filtrate was extracted with EtOAc (3x 40ml) and ultra-pure water (3x 40ml). The organic phase was stirred with magnesium sulfate ($MgSO_4$), then filtered and rotary concentrated in vacuum (rotary evaporation) at 55°C. The purified solid was dried in vacuum for 12hours.

Synthesis of 6-bromo-2-phenyl-4-phenylquinoline: In a round bottom flask of 100ml, where a condenser, magnetic stirring and oil bath were placed, degassing cycles and Ar/vacuum were performed. The flask was cooled under Ar and then 1.75g (6.34mmol) 2-amino- -bromobenzophenone and 20ml of acetic acid (AcOH) were added and the mixture was heated at 110°C. When the 2-amino-5-bromobenzophenone dissolved 2g (9.52mmol) of acetophenone and 1ml of sulfuric acid 95% (H_2SO_4) was added to the reaction. The flask was degassed and filled with argon and heated to reflux for 5days. Then the solution was cooled and precipitated into a mixture of ice/water/ammonia in the ratio of 1/1/0.2. The mixture was filtered and dried under vacuum at 60°C for 12hours. The obtained solid was dispersed in 25ml of absolute ethanol for further purification. The pure solid was filtered and dried under vacuum for 12hours.

The 6-bromo-2-cyanophenyl-4-phenylquinoline and 6-bromo-2-perfluorophenyl-4-phenylquinoline were synthesized according to previous methodology.

Synthesis of the vinyl monomer 2,4-diphenyl-6-(4-vinylphenyl)quinoline (SDPQ): In a round bottom flask of 100ml, where a condenser, magnetic stirring and oil bath were placed, degassing cycles and Ar/vacuum were performed. The flask was cooled under Ar and then 2g (4.44mmol) BrDPQ, 0.86g (5.78mmol) 4-vinylphenylboronic acid, 1.84g (13.32mmol) in 2M aqueous K_2CO_3 solution, 0.15g (0.13mmol) $Pd(PPh_3)_4$ and 50mL of toluene were added and the flask was degassed and it was filled again with Ar. The flask was covered with aluminum foil and the reaction mixture was heated to reflux for 4days. The mixture was filtered through a fluted filter, followed by extraction of the organic layer with EtOAc (3x 40ml) and ultra-pure water (3x 40ml). The organic phase was stirred with magnesium sulfate ($MgSO_4$), then filtered and rotary concentrated in vacuum (rotary evaporation). The solid was dried under vacuum at 40°C for 12hours and then was dispersed in methanol for further purification. The purified yellow solid was filtered and dried under vacuum for 12hours.

The 6-(4-vinylphenyl)-2-cyanophenyl-4-phenylquinoline (SDPQCN) and the 6-(4-vinylphenyl)-2-pentafluorophenyl-quinoline (5FSPQ) were synthesized according to previous methodology.

Synthesis of the watersoluble P(AMPSA-SDPQ) copolymers: In a round bottom flask of 250ml, where a condenser, magnetic stirring and oil bath were placed, degassing cycles and Ar/vacuum were performed. The flask was cooled under Ar and then 10g (0.05mol) AMPSA, 0.040g ($1*10^{-4}$ mol) SDPQ, 0.082g ($5*10^{-4}$ mol) AIBN and finally 100ml DMF were added and the flask was degassed and it was filled with Ar again. The reaction mixture was heated at 110^{o}C for 24hours.

After cooling down to room temperature the resulting copolymers were precipitated in EtOAc and filtrated. Then, the crude copolymer was dissolved in ultra-pure water and there it was changed into the sodium salt form through neutralization with an excess of NaOH. Then the copolymer was further purified through dialysis (membrane cut-off: 12kDa), which was immersed in a large excess of water at 22^{o}C for several days by replacing the water twice a day, monitoring the pH and the conductivity until it was similar to the values of the deionized water. The membrane then was removed and the copolymer solution was freeze-dried, collected and stored in a desiccator.

The synthesis of the watersoluble P(AMPSA-SDPQCN) and P(AMPSA-5FSPQ) copolymers were synthesized according to previous methodology using the same reagents' ratios.

Synthesis of the watersoluble P(DMAM-SDPQ) copolymer: In a round bottom flask of 250ml, where a condenser, magnetic stirring and oil bath were placed, degassing cycles and Ar/vacuum were performed. The flask was cooled under Ar and then 10g (0.10mol) DMAM, 0.080g ($2*10^{-4}$ mol) SDPQ, 0.17g ($1*10^{-3}$ mol) AIBN and finally 100mL THF were added and the flask was degassed and it was filled with Ar again. The reaction mixture was refluxed for 24hours. After cooling down to room temperature the resulting copolymers were precipitated in Et_2O and filtrated. Then, the crude copolymer was dissolved in ultra-pure water and further purified through dialysis (membrane cut-off: 12kDa), which was immersed in a large excess of water at 22^{o}C for several days by replacing the water twice a day, monitoring the conductivity, until it was similar to the value of the deionized water. The membrane then was removed and the copolymer solution was freeze-dried, collected and stored in a desiccator.

The synthesis of the watersoluble P(DMAM-SDPQCN) and P(DMAM-5FSPQ) copolymers were synthesized according to previous methodology using the same reagents' ratios.

Synthesis of the watersoluble P(MAPTAC-SDPQ) copolymer: In a round bottom flask of 250ml, where a condenser, magnetic stirring and oil bath were placed, degassing cycles and Ar/vacuum were performed. The flask was cooled under Ar and then 20ml (0.045mol) of 50% wt MAPTAC in H_2O, 0.036g ($9*10^{-5}$ mol) SDPQ, 0.074g ($4.5*10^{-4}$ mol) AIBN and finally 100ml DMF were added and the flask was degassed and it was filled with Ar again. The reaction mixture was heated at 110^{o}C for 24hours. After cooling down to room temperature the resulting copolymers were precipitated in EtOAc and filtrated. Then, the crude copolymer was dissolved in ultra-pure water and further purified through dialysis (membrane cut-off: 12kDa), which was immersed in a large excess of water at 22^{o}C for several days by replacing the water twice a day, monitoring the pH and the conductivity, until it was similar to the values of the deionized water. The membrane then was removed and the copolymer solution was freeze-dried, collected and stored in a desiccator.

^{1}H NMR characterization of monomers and copolymers

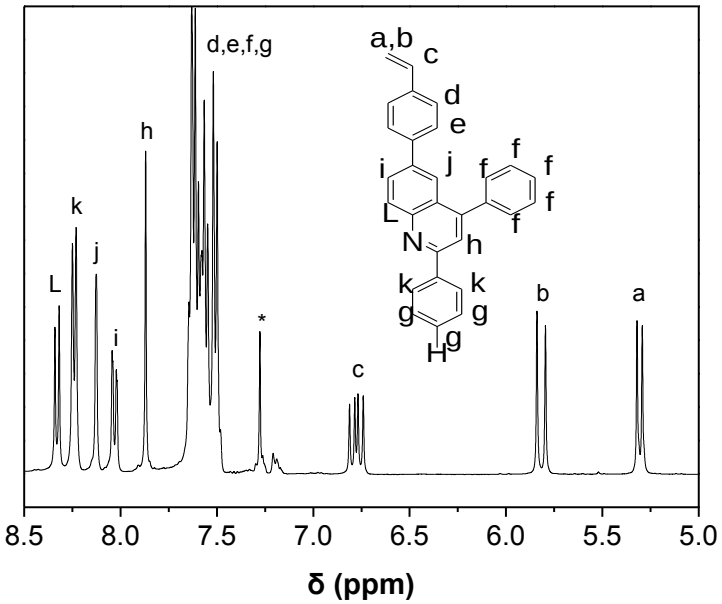

Figure S1. ^{1}H NMR spectrum of SDPQ in $CDCl_3$.

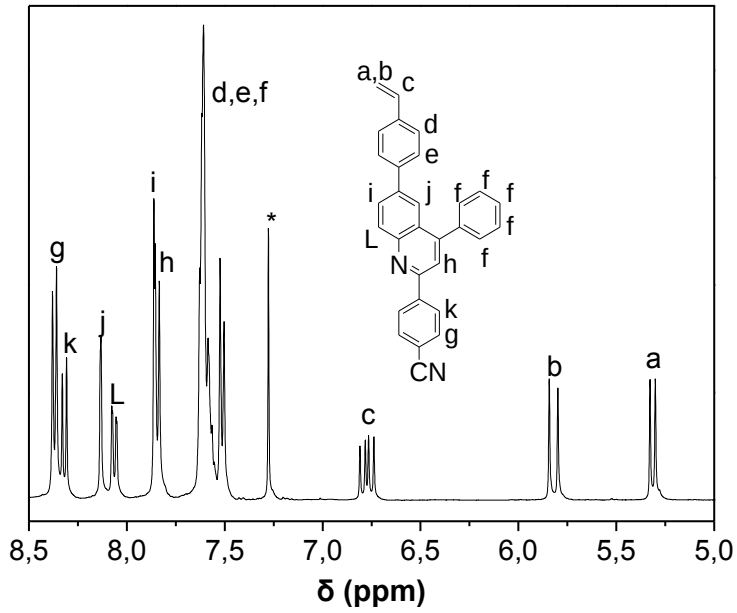

Figure S2. ^{1}H NMR spectrum of SDPQCN in $CDCl_3$.

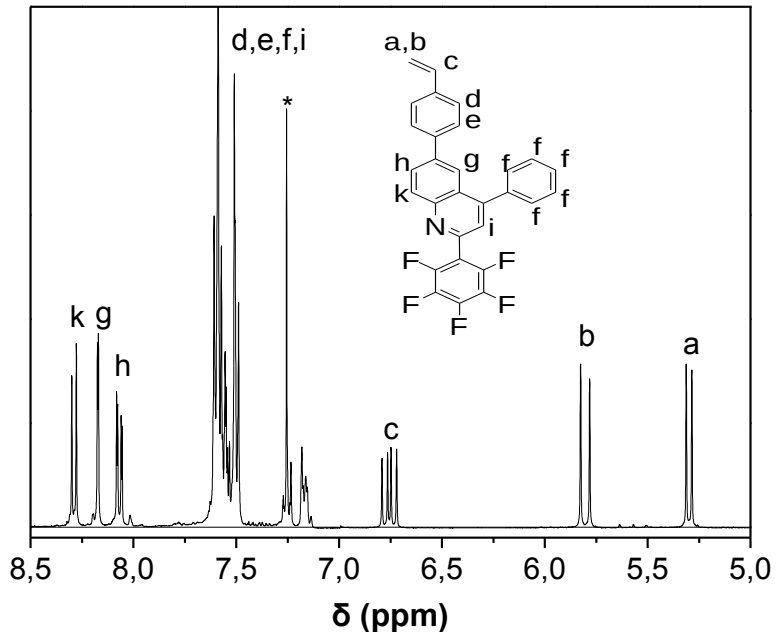

Figure S3. ^{1}H NMR spectrum of 5FSPQ in $CDCl_3$.

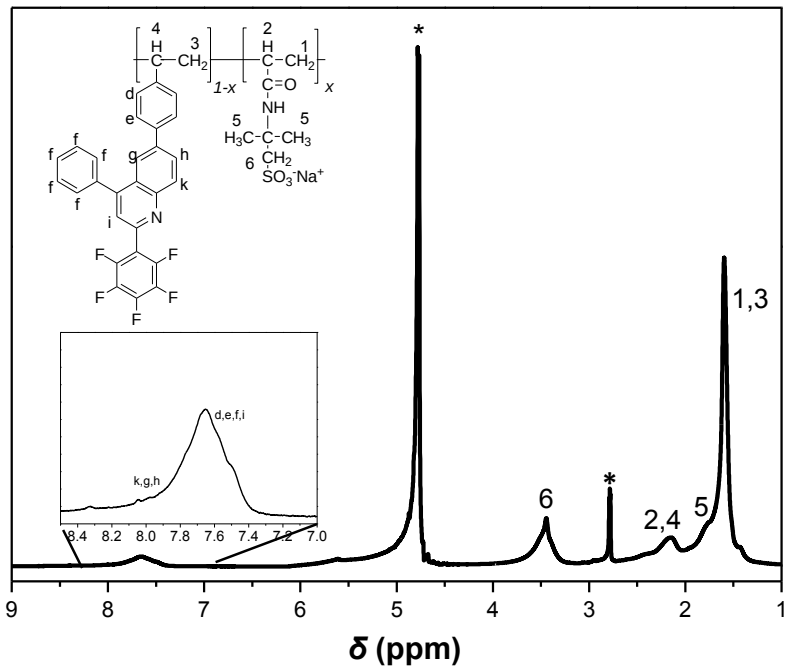

Figure S4. ^{1}H NMR spectrum of the copolymer P(AMPSNa-co-5FSPQ) in D_2O.

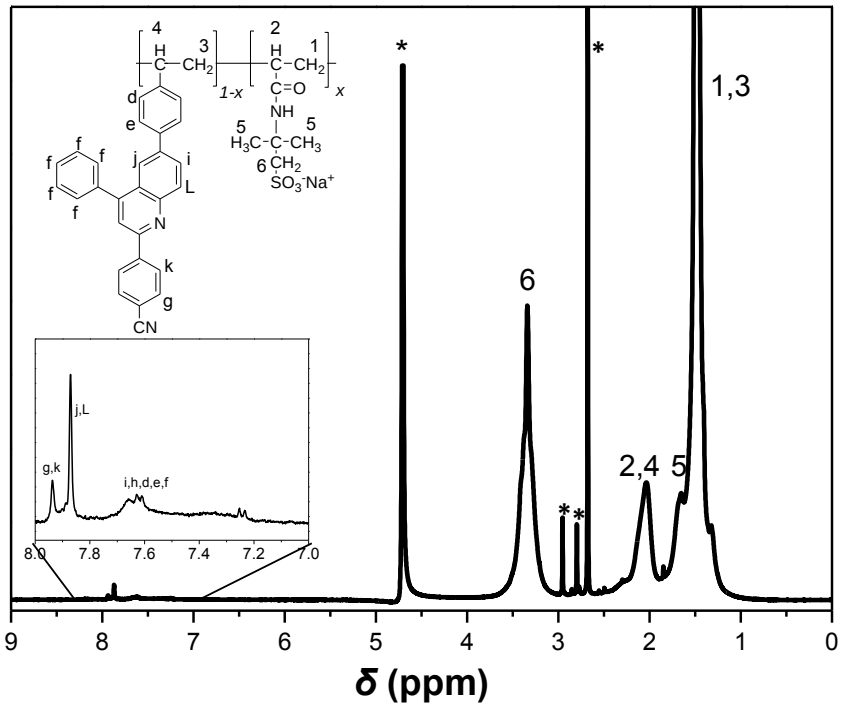

Figure S5. ^{1}H NMR spectrum of the copolymer P(AMPSNa-co-SDPQCN) in D_2O.

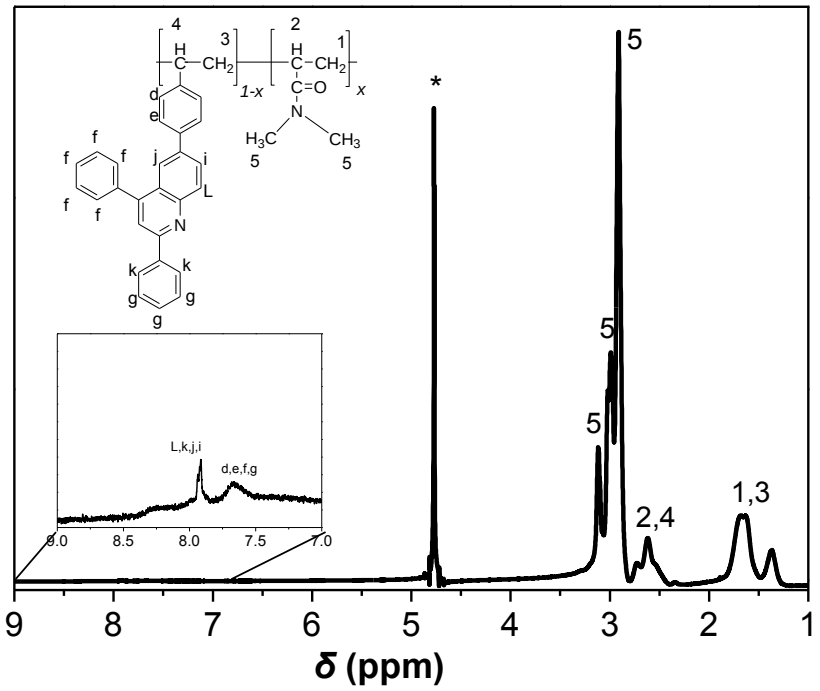

Figure S6. ^{1}H NMR spectrum of the copolymer P(DMAM-co-SDPQ) in D_2O.

Determination of the quinoline content of the copolymers through UV-Vis spectroscopy

In order to determine the SDPQ content of the final products, the UV spectroscopy technique was enabled. Specifically, the SDPQ monomer was selected as reference molecule. Various solutions of known concentration were prepared, using THF as solvent and their absorption was monitored in order to build a calibration curve as it is presented in Figure S7. Then the copolymer P(DMAM-co-SDPQ) was dissolved in the same solvent and its SDPQ content was determined by the Beer-Lambert equation (A=ebC) of the calibration curve. The SDPQ content of the copolymer was found equal to 0.18 mol%.

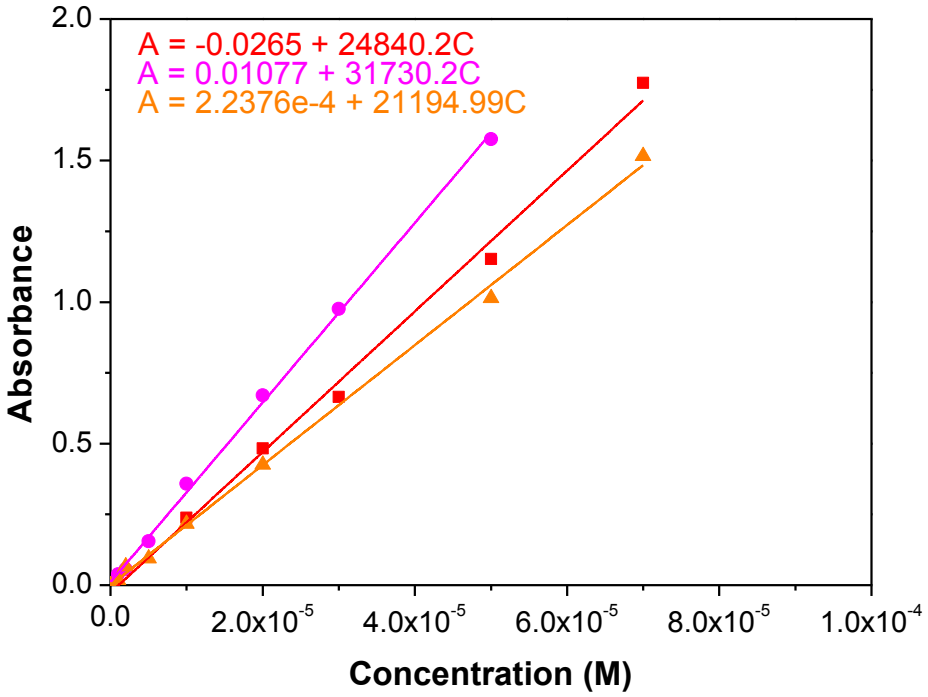

Figure S7. Calibration curves of the SDPQ ((■) red), SDPQCN ((●) magenta), 5FSPQ ((▲) orange) used for the determination of the quinoline content of the P(DMAM-co-Quinoline) copolymers in THF.

The quinoline content (λ_Q) in g/L of the polymers can be obtained by taking the ratio of quinoline concentration in mol/L obtained by UV-Vis absorption measurements after applying Beer-Lambert law with ε_Q and the massic polymer concentration [Poly] in g/L.

$$\lambda_Q = \frac{[Q]}{[Poly]}$$

The molar fraction (x) of quinoline-labeled monomers can be determined from equation:

$$x = \frac{M_o}{{}^{1}/_{\lambda_Q} + M_o - M_Q}$$

where Mo and M_Q are the molar masses of monomers used to prepare a given polymer and the quinoline-labeled monomer, respectively

Knowing the exact SDPQ content of this copolymer, various solutions of known concentration were prepared, using H_2O as solvent and their absorption was monitored in order to build a calibration curve as it is presented in Figure S8. This step was performed in order to avoid solubility issues of the charged copolymers in organic solvents like THF.

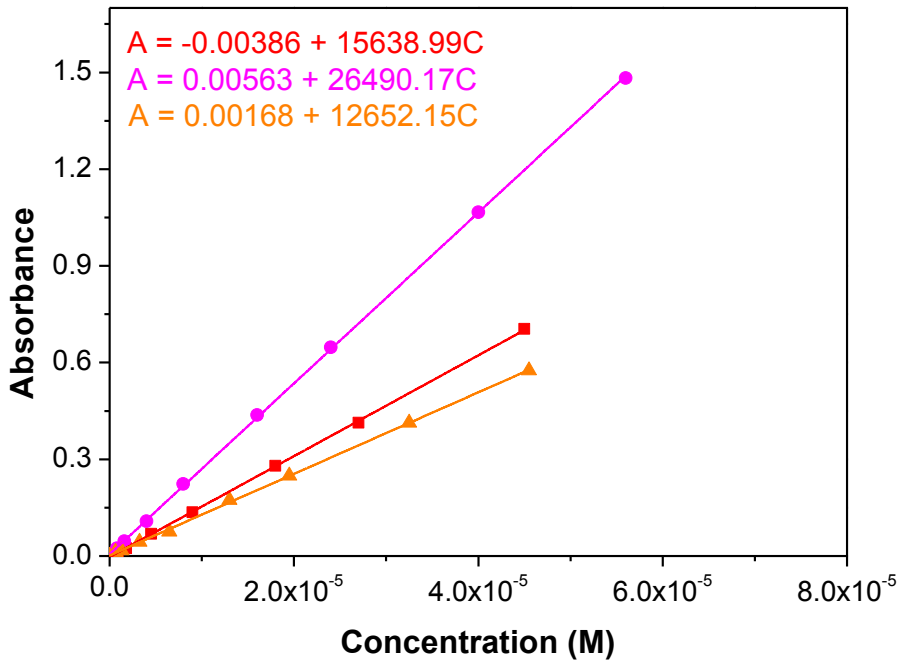

Figure S8. Calibration curves of the P(DMAM-co-SQPQ) ((■) red), P(DMAM-co-SDPQCN) ((●) magenta), P(DMAM-co-5FSPQ) ((▲) orange) copolymers in H_2O, used for the determination of the final quinoline content of the water-soluble quinoline- labeled polyelectrolytes.

The extinction coefficients as determined from Figures S7 and S8 are summarized in Table S1.

Table S1. Molar Extinction coefficients for the Quinoline monomers and the DMAM-based copolymers

Polymer	Molar Extinction Coefficient ε ($mol^{-1}.L.cm^{-1}$)
SDPQ	24800
SDPQCN	31700
5FSPQ	21200
P(DMAM-co-SDPQ)	15600
P(DMAM-co-SDPQCN)	26500
P(DMAM-co-5FSPQ)	12700

SEC characterization

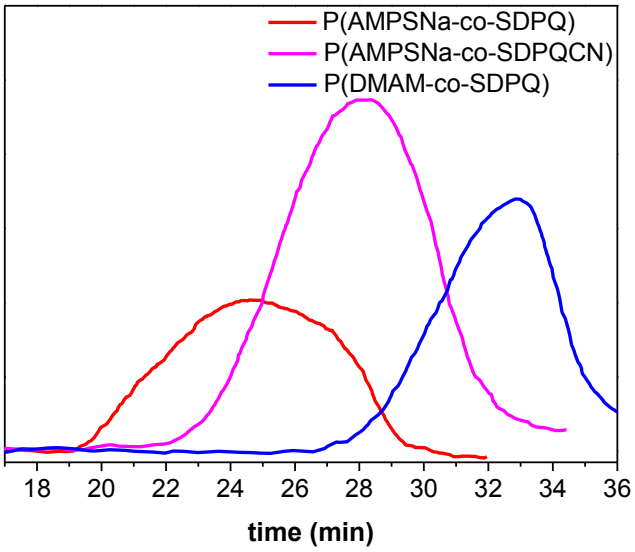

Figure S9: SEC curves of the P(AMPSNa-co-SDPC), P(AMPSNa-co-SDPQCN), P(DMAM-co-SDPQ) copolymers in 0.1M $LiNO_3$ as eluent.

UV-Vis absorption spectroscopy

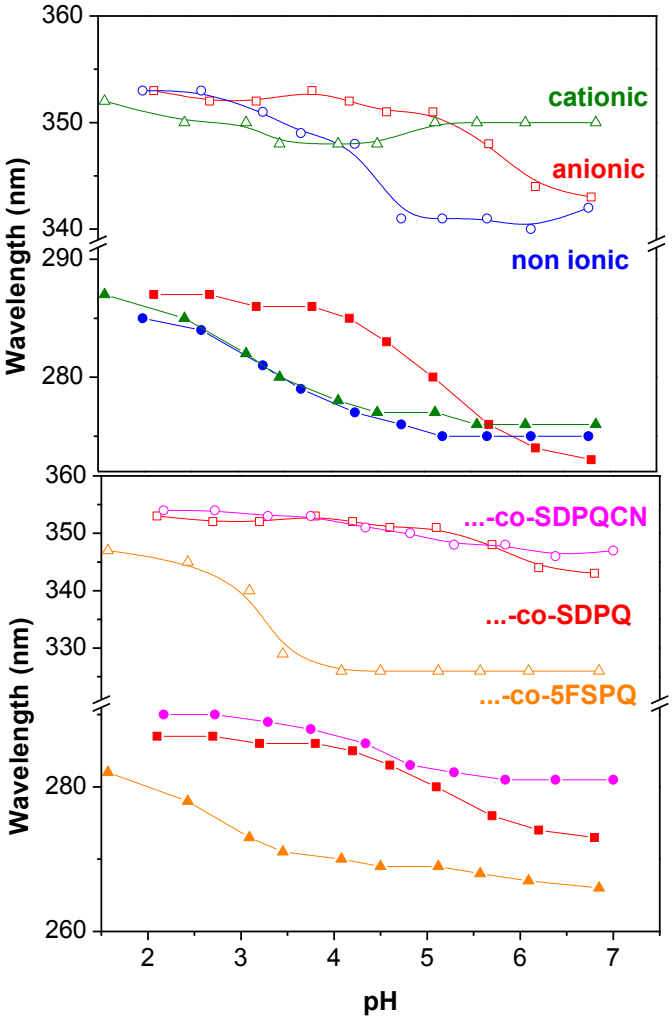

Figure S10. The position of the peaks for the three characteristic wavelengths 280nm (solid), 350nm (dashed) and 390nm (dotted), for the quinoline-labeled copolymers P(AMPSNa-co-SDPQ) (■), P(DMAM-co-SDPQ) (●), P(MAPTAC-co-SDPQ) (▲), P(AMSPNa-co-SDPQCN) (●), P(AMPSNa-co-5FSPQ) (▲). C_Q=2x10^{-5}M.

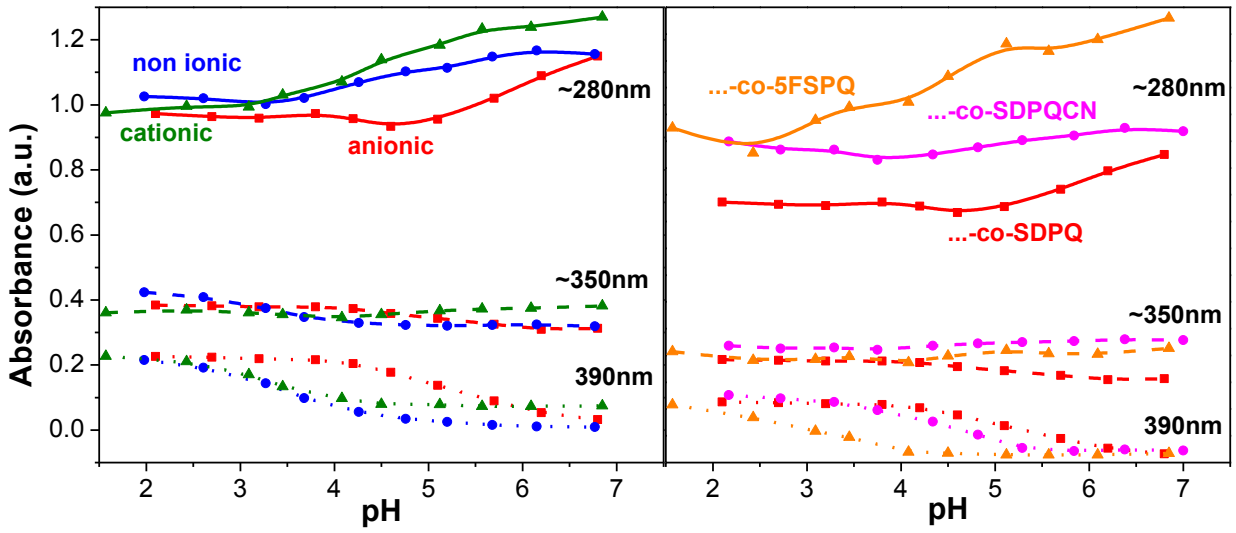

Figure S11. Maximum absorption for the three characteristic wavelengths 280nm (solid), 350nm (dashed) and 390nm (dotted), for the quinoline-labeled copolymers P(AMPSNa-co-SDPQ) (■), P(DMAM-co-SDPQ) (●), P(MAPTAC-co-SDPQ) (▲), P(AMSPNa-co-SDPQCN) (●), P(AMPSNa-co-5FSPQ) (▲). C_Q=2x10^{-5}M.

Photoluminescence Spectroscopy

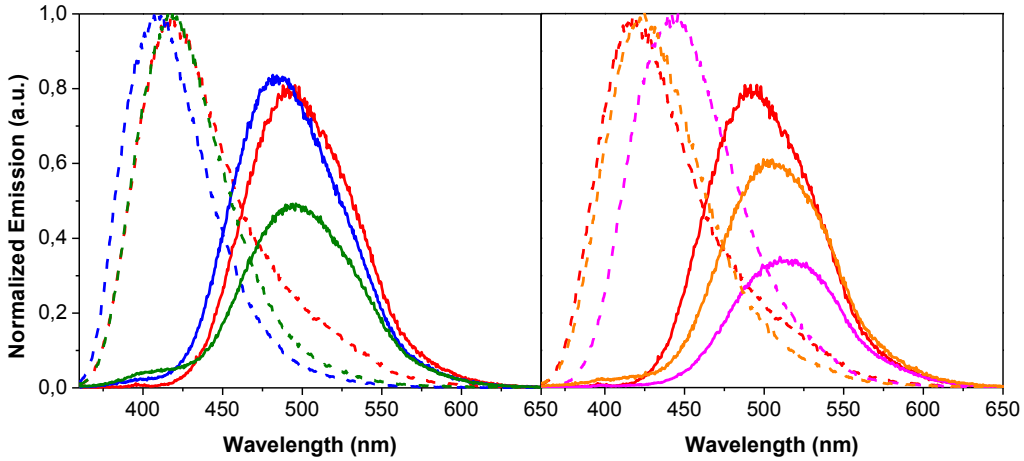

Figure S12. Emission spectra at pH~2 (solid lines) and at pH~7 (dashed lines) for the water-soluble copolymers P(AMPSNa-co-SDPQ) (red), P(DMAM-co-SDPQ) (blue), P(MAPTAC-co-SDPQ) (green), P(AMSPNa-co-SDPQCN) (magenta), P(AMPSNa-co-5FSPQ) (orange), upon excitation at 350nm. C_Q=2x10^{-6}M.

Quantum Yields

The quantum yield of the final water-soluble copolymers was determined using quinine as standard molecule. Quinine, when dissolved in 0.1M H_2SO_4, has Q_R=0.557 when excited at 350nm, a wavelength very close to the second absorbance band of quinoline derivatives. The quantum yield of the copolymers was determined using the equation:

$$Q = Q_R * {}^{I}/_{I_R} * {}^{E_R}/_{E} * {}^{n_1}/_{n_2}$$

where Q = Quantum Yield, I = intergraded intensity, E = Absorbance and n = refraction index, while R stands for the standard (quinine).

The absorption and emission spectra, used for the calculations, are depicted at Figure S13 and Figure S14, respectively.

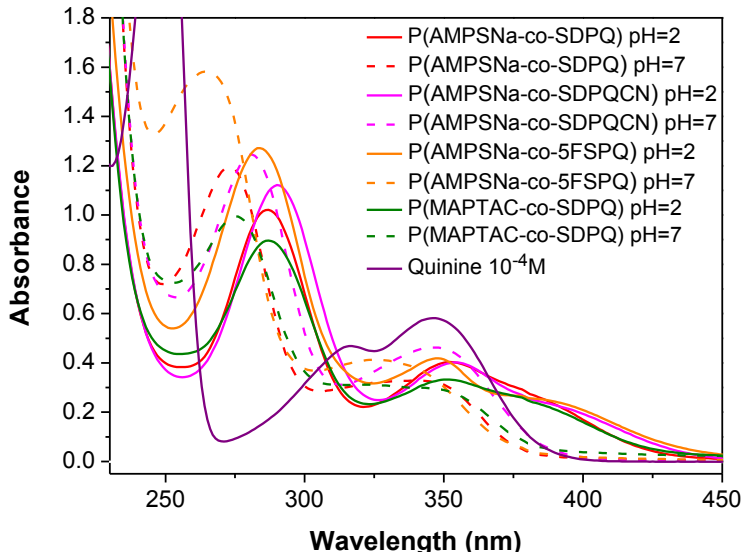

Figure S13. Absorption spectra of the water soluble copolymers at pH~2 and pH~7, as well as the absorption spectrum of quinine, used as standard in order to calculate the quantum yield of the quinoline unit. C_Q=2x10^{-5}M.

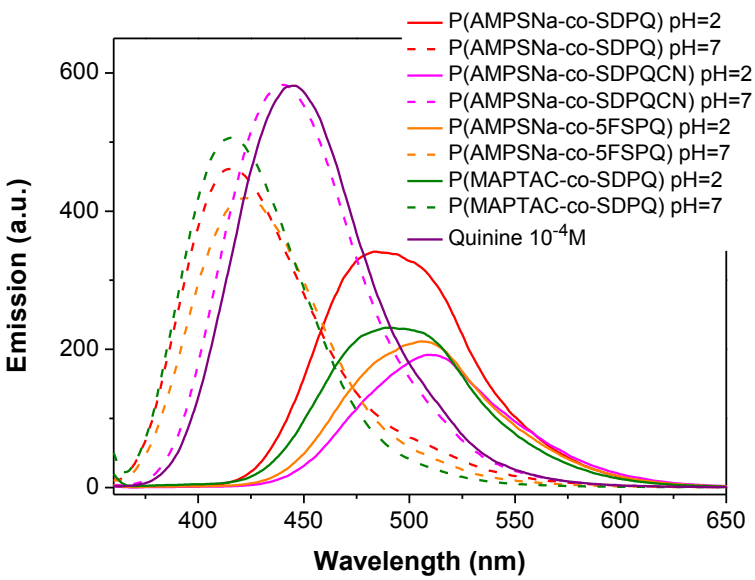

Figure S14. Emission spectra upon excitation at 350nm of the water soluble copolymers at pH~2 and pH~7 as well as the emission spectrum of quinine, used as standard in order to calculate the quantum yield of the quinoline unit. $C_Q=2x10^{-6}M$.

Nile Red probing

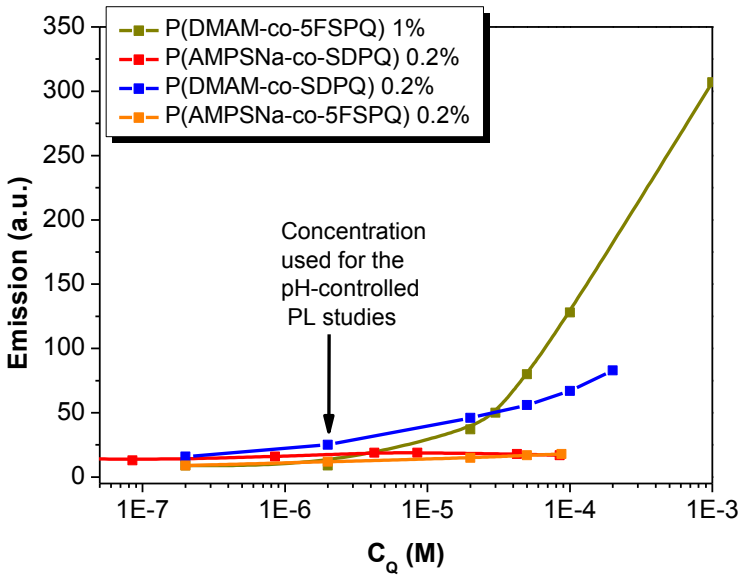

Figure S15: Nile Red probing results. The concentration of Nile Red is 1.6×10^{-6}M and the excitation wavelength is set at 550nm.

Table S2. Molar and mass concentrations of all quinoline- labeled copolymers synthesized in the present study.

Polymer	Polymer Concentration for UV-Vis Study (g/ml)	Quinoline Concentration for UV-Vis Study (M)	
		Based on Feed composition (0.2 mol% Quinoline)	Based on UV-Vis Calibration curves
P(AMPSNa-co-SDPQ)	0.00229	$2\ 10^{-5}$	2.2×10^{-5}
P(AMPSNa-co-SDPQCN)	0.00229	$2\ 10^{-5}$	2×10^{-5}
P(AMPSNa-co-5FSPQ)	0.00229	$2\ 10^{-5}$	3×10^{-5}
P(DMAM-co-SDPQ)	0.000996	$2\ 10^{-5}$	1.8×10^{-5}
P(MAPTAC-co-SDPQ)	0.00221	$2\ 10^{-5}$	1.8×10^{-5}