Electronic Supplementary Information

Modular functionalization and hydrogel formation *via* red-shifted and self-reporting [2+2] cycloadditions

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Materials

All chemicals were used without further purification: 1,6-Dibromopyrene (Synthonix, 98.0 %), copper iodide (Merck, for synthesis), sodium ascorbate (Sigma Aldrich, >99,0 %), *N*,*N*'-dimethyl-ethylenediamine (DMEDA, Alfa Aesar, 95.0 %), sodium azide (Abcr, >99.5 %), sodium hydrosulfide hydrate (Sigma Aldrich), potassium carbonate (Abcr, 99.0 %), iodomethane (Abcr, 99.5 %), palladium(II) acetate (Sigma Aldrich, 97.0 %), triphenylphosphine (Roth, >99.5 %), pentafluorostyrene (Abcr, 98 %), methyl trifluoromethanesulfonate (MeOTf, Sigma Aldrich, >98 %), 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU, TCI, >98.0 %), polyethylene glycol methyl ether thiol (*m*PEG_{44}-SH, M_n = 2,000 g·mol^{-1}), 4arm-polyethylene glycol thiol (sPEG-SH, pentaerythritol core, 20 kDa, JenKem USA, >96 %).

Instrumentation

NMR spectroscopy

NMR measurements were conducted on 400 MHz (9.3 T) Bruker Ascent 400 spectrometer. Coupling constants *J* and chemical shifts δ are displayed in Hz and in ppm, respectively. The signals of deuterated solvents were used as internal standards.

Liquid chromatography mass spectrometry (LC-MS)

LC-MS measurements were performed on an UltiMate 3000 UHPLC System (Dionex, Sunnyvale, CA, USA) consisting of a pump (LPG 3400SZ), autosampler (WPS 3000TSL) and a temperature-controlled column compartment (TCC 3000). Separation was performed on a C18 HPLC column (Phenomenex Luna 5 µm, 100 Å, 250 × 2.0 mm) operating at 40 °C with water/acetonitrile as eluents. The flow was split in a 9:1 ratio, where 90 % of the eluent was directed through a DAD UV-detector (VWD 3400, Dionex) and 10 % was infused into the electrospray source. Spectra were recorded on an LTQ Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a HESI II probe. The instrument was calibrated in the m/z range 74-1822 using premixed calibration solutions (Thermo Scientific).

UV/Vis and fluorescence spectroscopy

UV/Vis and fluorescence measurements were carried out on an QE Pro from Ocean Insight equipped with the light source DH-2000-BAL and the temperature-controlled cuvette holder qpod 2e^{TM} from Quantum Northwest for fiber optic spectroscopy, which enables to successively record UV/Vis spectra in 180° and fluorescence spectra in 90° (Figure S10). Time-resolved measurements were acquired using self-written MatLab scripts.

Tunable Opotek Laser

Laser experiments were conducted using a tunable Coherent Opolette 355 laser from Opotek operated at 410-480 nm with a full width half maximum of 7 ns and a repetition rate of 20 Hz. The emitted pulse, which has a flat-top spatial profile, was expanded to 6 mm diameter using focusing lenses and directed upwards using a prism. The beam was then centered on a glass laser vial which is positioned in a 6 mm diameter slot in a temperature-controlled sample holder. The energy transmitted through the sample holder was measured using a Coherent Energy Max PC power meter.

Ocean Insight LED

Samples were irradiated with a fiber-coupled LED from Ocean Insight (470 nm: 465 ± 10 nm, 33.0 $mW \cdot cm^{-2}$).

pH measurements

pH measurements were carried out on a 907 Titrando from Metrohm.

Rheology

The hydrogels prepared from sPEG-qStyPy (7.0 *wt*%, dissolved in 1.0 mM phosphate buffer, pH 7.4) were characterized *via* rheology using an Anton Paar Modular Compact Rheometer 302 with a plate-plate measuring system. The gap between the 25 mm measuring plate was set to 0.100 mm and a home-built solvent trap, made of a Teflon-ring and silicon oil, was put around the measuring system to prevent evaporation of water. The measuring system was heated by a Peltier element and features a glass plate on the bottom to allow *in-situ* irradiation (Figure S9). The storage modulus *G*' and loss modulus *G*'' were measured in oscillation mode. Amplitude sweeps were acquired with a frequency of $f = 1$ Hz as a function of the strain ($\gamma = 0.1 - 1000$ %). All remaining measurements were carried out with a strain amplitude of $\gamma = 1$ % (in the linear viscoelastic region).

Synthesis

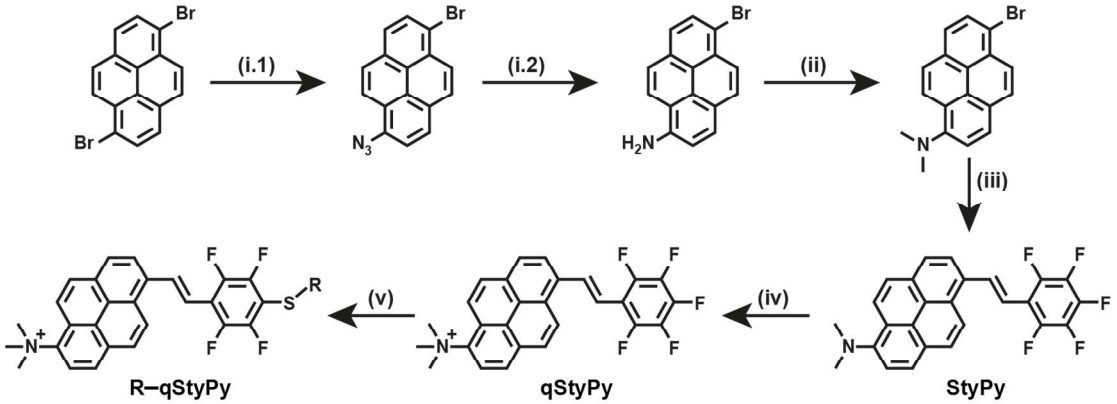
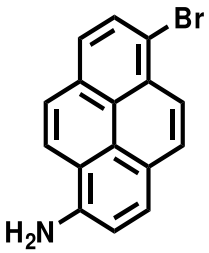


Figure S1: Synthesis and functionalization of qStyPy. Reaction conditions: (i) 1.) NaN_3, CuI, *N*,*N*'-dimethyl-ethylenediamine, sodium ascorbate, N_2; 2.) NaSH · x H_2O, 38 %; (ii) MeI, K_2CO_3, N_2, 100 %; (iii) pentafluorostyrene, $Pd(OAc)_2$, NEt_3, K_2CO_3, N_2, 62 %, (iv) MeOTf, 92 %, (v) R-SH, DBU, > 90 %.

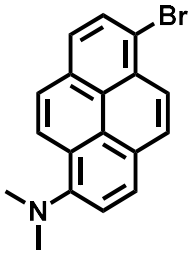
Synthesis of 1-amino-6-bromopyrene



1,6-Dibromo-pyrene (1.00 g, 2.80 mmol, 1.00 eq.), CuI (56.1 mg, 0.295 mmol, 10.5 mol-%) and sodium ascorbate (33.4 mg, 0.169 mmol, 6.0 mol-%) were dispersed in THF (70 mL) and nitrogen-purged (15 min). *N*,*N*'-dimethyl-ethylenediamine (DMEDA, 38.5 mg, 0.437 mmol, 15.6 mol %) dissolved in THF (10 mL) was added to the reaction mixture. Subsequently, NaN_3 (195 mg, 3.00 mmol, 1.07 eq.) dissolved in water (7 mL) was added dropwise and

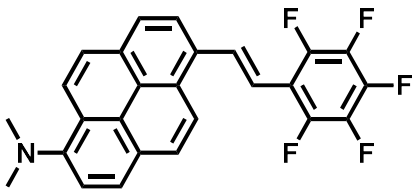
the reaction mixture was refluxed for 15 hours. THF was removed *in vacuo* and the residue was washed twice with water (2 x 30 mL). The aqueous phase was removed *via* centrifugation and subsequently, by lyophilization yielding 928 mg raw product. The raw product was dissolved in THF (95 mL) and mixed with NaSH · H_2O (2.97 g, 40.1 mmol, 13.8 eq.) dissolved in water (9.5 mL). The red solution was refluxed for 3 hours. Subsequently, it was cooled to RT and mixed with chloroform (40 mL) and water (40 mL) and the aqueous phase was extracted twice with chloroform (2 x 40 mL). The combined organic phases were dried *in vacuo* and the residue was purified by flash column chromatography (SiO_2) using pure DCM as the eluent. Yield: 312 mg (37.6 %). ^{1}H-NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.16 (dd, *J* = 8.7, 5.5 Hz, 2H), 8.02 (dd, *J* = 8.7, 6.2 Hz, 2H), 7.95 (q, *J* = 9.2 Hz, 2H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 4.56 (s, 2H) ppm. ^{13}C-NMR ($CDCl_3$, 101 MHz, 25 °C) δ = 141.78, 131.29, 130.75, 130.29, 129.37, 126.88, 125.81, 125.55, 124.12, 122.38, 120.55, 118.89, 116.84, 114.67 ppm.

Synthesis of 6-bromo-1-(dimethylamino)pyrene



1-Amino-6-bromopyrene (293 mg, 0.991 mmol) and K_2CO_3 (702 mg, 5.08 mmol, 5.1 eq.) were dispersed in DMF (11 mL), nitrogen-purged (15 min) and subsequently mixed with iodomethane (350 µL, 798 mg, 5.62 mmol, 5.7 eq.). The reaction mixture was heated to 120 °C and stirred for one hour. Afterwards, it was cooled to RT, quenched with water (20 mL), and the aqueous phase was extracted with DCM (40 mL). Removing the solvent *in vacuo* yielded the product quantitatively. Yield: 321 mg (99.9 %). ^{1}H-NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 8.47 (d, *J* = 9.3 Hz, 1H), 8.27 (d, *J* = 9.2 Hz, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 8.13 (dd, *J* = 8.3, 1.3 Hz, 1H), 8.06 (dd, *J* = 9.1, 1.3 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.94 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.76 (dd, *J* = 8.2, 0.9 Hz, 1H), 3.06 (s, 6H) ppm. ^{13}C-NMR ($CDCl_3$, 101 MHz, 25 °C) δ = 149.80, 130.93, 130.22, 129.10, 126.81, 126.57, 126.04, 125.52, 124.91, 124.49, 124.08, 123.96, 119.24, 117.18, 45.80 ppm. LC-MS calculated [$C_{18}H_{14}BrN^+$]: 323.0304 g·mol^{-1}, found [$C_{18}H_{14}BrN^+$]: 323.0302 g·mol^{-1}.

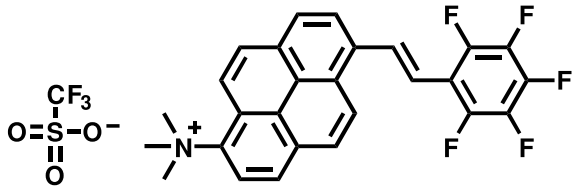
Synthesis of 1-dimethylamino-6-pentafluorstyryl-pyrene (StyPy)



6-Bromo-1-(dimethylamino)pyrene (321 mg, 0.9898 mmol), $Pd(OAc)_2$ (11.6 mg, 0.0517 mmol, 5.2 mol-%) and PPh_3 (61.6 mg, 0.235 mmol, 24 mol-%) were dissolved in DMF (20 mL) and nitrogen-purged (10 min). The solution was mixed with K_2CO_3 (144 mg, 1.04 mmol, 1.1 eq.), NEt_3 (1.5 mL, 10.8 mmol, 10.9 eq.) and pentafluorostyrene (550 µL, 773 mg, 3.98 mmol, 4.0 eq.). The reaction mixture was heated to 110 °C and stirred for 48 h in a sealed Schlenk flask to the exclusion of light and air. Subsequently, it was mixed with water (40 mL) and the aqueous phase was extracted with chloroform (3 x 100 mL). The combined organic phases were removed *in vacuo* and

the raw product was purified *via* flash column chromatography (SiO_2) using DCM as the eluent. Remaining impurities were removed by recrystallization from acetone yielding the product as a yellow powder. Yield: 270 mg (62.3 %). ^{1}H-NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 8.52 (d, J = 16.4 Hz, 2H), 8.27 (t, J = 9.4 Hz, 2H), 8.14 (d, J = 8.2 Hz, 2H), 8.07 (d, J = 9.2 Hz, 2H), 7.77 (d, J = 8.2 Hz, 1H), 7.19 (d, J = 16.5 Hz, 1H), 3.09 (s, 6H) ppm. ^{19}F-NMR ($CDCl_3$, 377 MHz, 25 °C) δ = -142.71 (dd, J = 21.7, 7.7 Hz, 2F), -156.55 (m, 1F), -162.83 (td, J = 21.3, 7.6 Hz, 2F) ppm. ^{13}C-NMR ($CDCl_3$, 101 MHz, 25 °C) δ = 128.39, 126.05, 116.92, 45.93 ppm. ESI-MS: calculated [$C_{26}H_{17}F_5N^+$]: 438.1276 g·mol^{-1}, found [$C_{26}H_{17}F_5N^+$]: 438.1279 g·mol^{-1}.

Synthesis of 1-trimethylammonium-6-pentafluorstyryl-pyrene triflate (qStyPy)



StyPy (20.6 mg, 47.1 µmol) was dispersed in chloroform (3.0 mL) and mixed with MeOTf (21.4 µL, 31.0 mg, 189 µmol, 4.0 eq.) at RT. The dispersion was heated to 60 °C, so that StyPy fully dissolved. The yellow solution was stirred at 60 °C for 20 h. During the reaction, a pale-yellow precipitate formed. The solution was cooled to 0 °C and the precipitate was collected *via* centrifugation (10,000 RPM, 2 min) and washed with chloroform (3.0 mL). Drying *in vacuo* yielded the quaternized StyPy (qStyPy). Yield: 25.9 mg (91.5 %). ^{1}H-NMR (Acetone-d_6, 400 MHz, 25 °C) δ = 9.04 (d, J = 9.6 Hz, 1H), 8.81 (d, J = 8.9 Hz, 1H), 8.73 (d, J = 9.3 Hz, 1H), 8.66 (d, J = 3.8 Hz, 1H), 8.56 (m, 4H), 8.40 (d, J = 9.3 Hz, 1H), 7.41 (d, J = 16.5 Hz, 1H), 4.41 (s, 9H) ppm. ^{13}C-NMR (Acetone-d_6, 101 MHz, 25 °C) δ = 130.13, 130.05, 128.70, 127.78, 127.29, 126.57, 125.64, 125.29, 124.98, 123.22, 121.67, 119.13, 116.99, 58.45 ppm. ^{19}F-NMR (Acetone-d_6, 377 MHz, 25 °C) δ = -78.84 (s, 3F), -143.60 (m, 2F), -157.83 (m, 1F), -164.78 (d, J = 7.3 Hz, 2F) ppm. LC-MS: calculated [$C_{27}H_{19}F_5N^+$]: 452.1432 g·mol^{-1}, found [$C_{27}H_{19}F_5N^+$]: 452.1433 g·mol^{-1}.

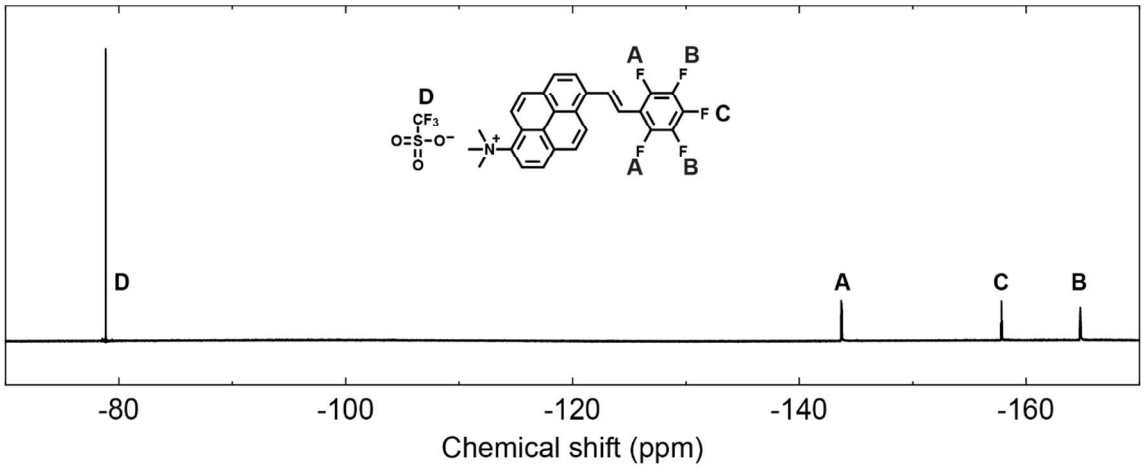
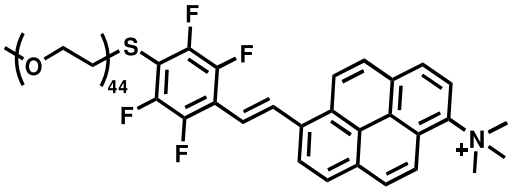


Figure S2: ^{19}F-NMR spectrum of qStyPy in acetone-d_6. The signals are assigned to the fluorine atoms.

Synthesis of *m*PEG$_{44}$-qStyPy



qStyPy (2.89 mg, 4.8 µmol) was transferred into a vial and mixed with a stock solution of *m*PEG$_{44}$-SH (192.2 µL, 50 mg·mL^{-1}, 1.0 eq.) dissolved in acetone. The addition of DBU (1.5 mg, 2.1 µL, 10 µmol, 2.1 eq.) instantly changed the color to deep orange. The solution was stirred for 30 min at 60 °C, cooled to RT and precipitated in cold Et_2O (-6 °C). The precipitate was collected *via* centrifugation, washed with Et_2O (-6 °C) and dissolved in H_2O. Freeze-drying overnight yielded the desired product as an orange powder. Yield: 11.2 mg (90.1 %). ^{19}F-NMR (Acetone-d_6, 377 MHz, 25 °C) δ = -136.49 (ddd, J = 73.9, 23.0, 11.6 Hz), -144.29 (m) ppm. ESI-MS: calculated [$C_{116}H_{200}F_4NO_{44}Na^{2+}$]: 1176.6170 g·mol^{-1}, found [$C_{116}H_{200}F_4NO_{44}Na^{2+}$]: 1176.6167 g·mol^{-1}.

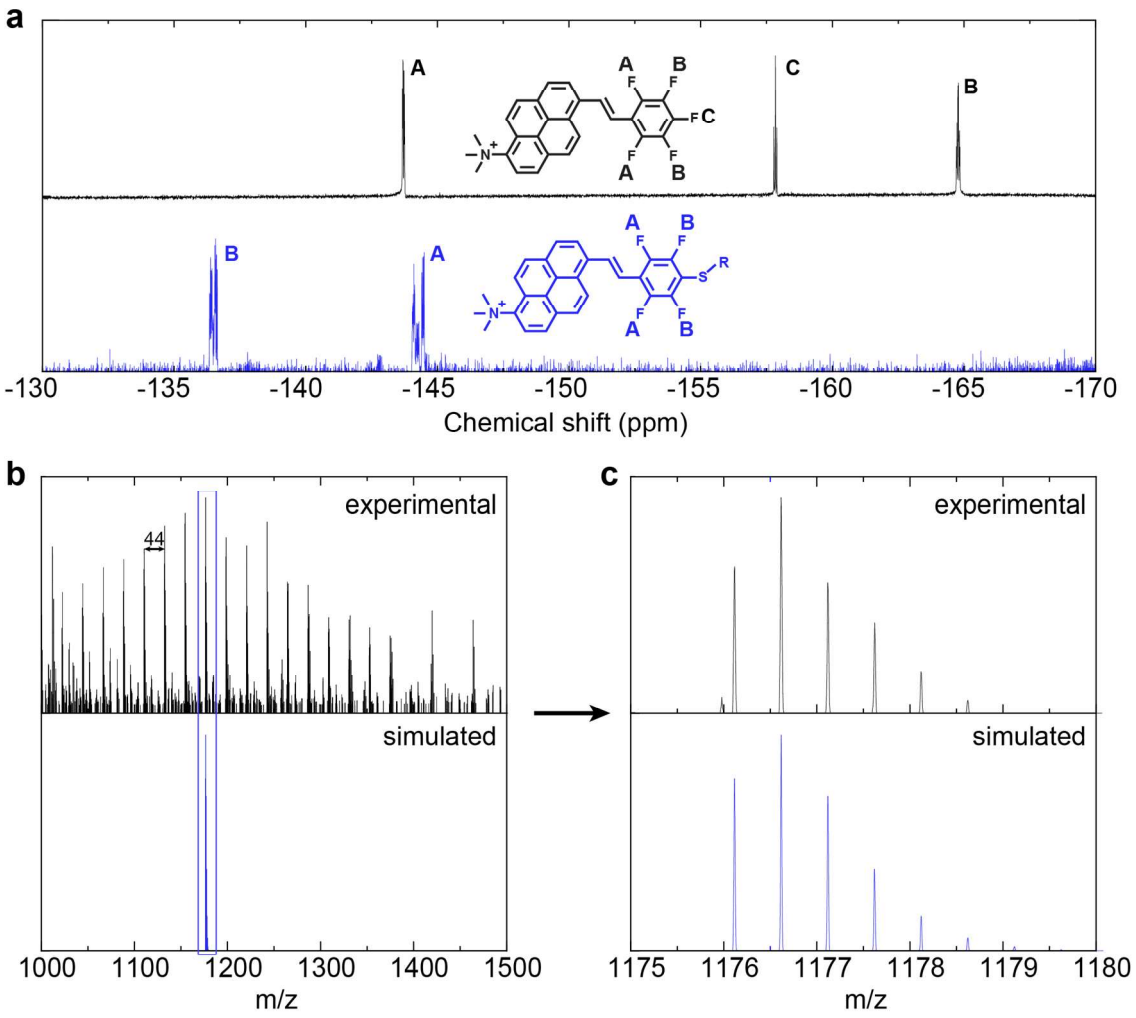


Figure S3: Characterization of *m*PEG$_{44}$-qStyPy. (a) ^{19}F-NMR spectra before (top) and after (bottom) the PFTR in acetone-d_6. The signals are assigned to the fluorine atoms. (b) Experimental (top) and simulated (bottom) mass spectra of *m*PEG$_{44}$-qStyPy. (c) The close-ups of b (highlighted by a blue rectangle) show the isotopic patterns.

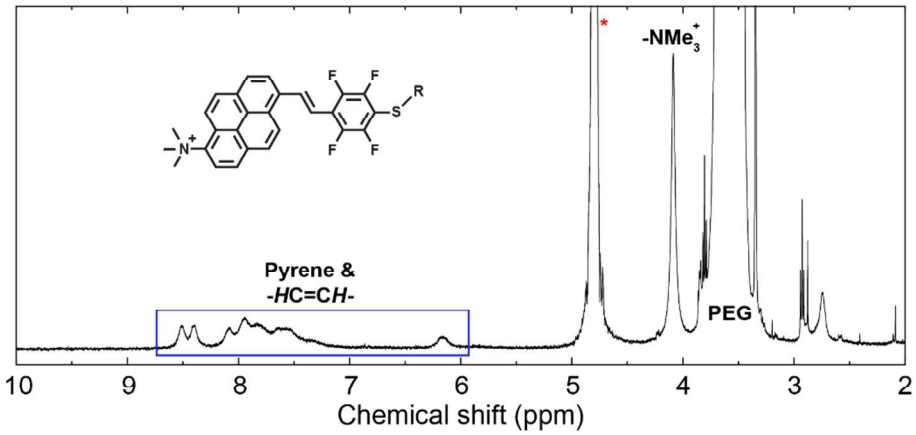
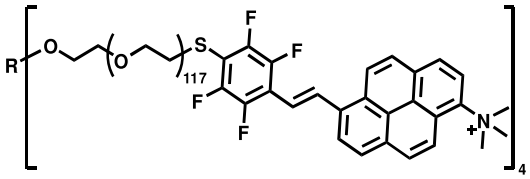


Figure S4: ^{1}H-NMR spectrum of *m*PEG$_{44}$-qStyPy in D$_2$O. The peak broadening of the aromatic signals, highlighted by a blue rectangle, indicate the π–π interactions between the qStyPy-units in water. The solvent residual is highlighted by a red star. Figure S5 in acetone-d_6 shows absence of peak broadening and hence absence of aggregation in good organic solvents.

Synthesis of sPEG-qStyPy



sPEG-SH (19.7 mg, 0.95 µmol) and qStyPy (2.50 mg, 4.2 µmol, 1.10 eq.) were dispersed in acetone (500 µL). Upon addition of DBU (1.3 mg, 1.3 µL, 8.7 µmol, 2.3 eq.), the dispersion instantly became deep orange. The reaction mixture was heated to 60 °C and stirred for 3 h. Subsequently, the solution was cooled to RT and precipitated in cold Et_2O (-6 °C). The precipitate was collected *via* centrifugation, washed with Et_2O (-6 °C) and dissolved in H_2O. Freeze-drying overnight yielded the desired product as an orange powder. Yield: 21.3 mg (97.2 %, d_f > 98 %). ^{1}H-NMR (400 MHz, Acetone-d_6, 25 °C) δ = 9.04 (d, J = 9.6 Hz, 1H), 8.52 (m, 7H), 7.87 (d, J = 8.3 Hz, 1H), 7.46 (dd, J = 46.2, 16.5 Hz, 1H), 4.41 (s, 9H), 3.75 (m, 6H), 3.58 (s, 463H), 3.44 (s, 2H), 3.41 (dd, J = 5.6, 4.1 Hz, 3H), 3.23 (q, J = 6.2 Hz, 2H) ppm. ^{19}F-NMR (Acetone-d_6, 377 MHz, 25 °C) δ = -136.46 (ddd, J = 76.0, 23.1, 11.5 Hz, 2F), -144.22 (ddd, J = 144.4, 23.0, 11.4 Hz, 2F) ppm.

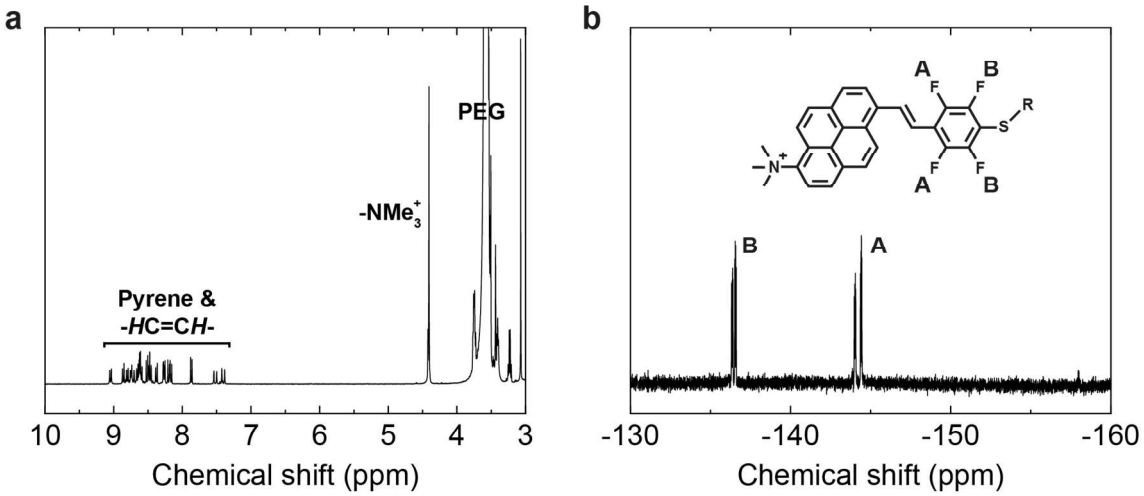


Figure S5: NMR spectra of sPEG-qStyPy in acetone-d_6. (a) ^{1}H-NMR spectrum. The signals are assigned to the functional groups. (b) ^{19}F-NMR spectrum. Signals are assigned to the fluorine atoms.

Overlap concentration of sPEG-OH

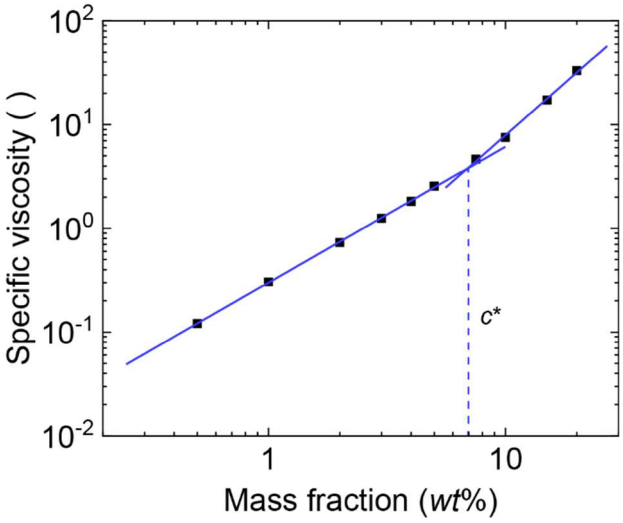


Figure S6: Overlap concentration of sPEG-OH (20 kDa) in water: *c = 6.9 *wt*%.**

Zero-shear viscosity η_0 of sPEG-qStyPy.

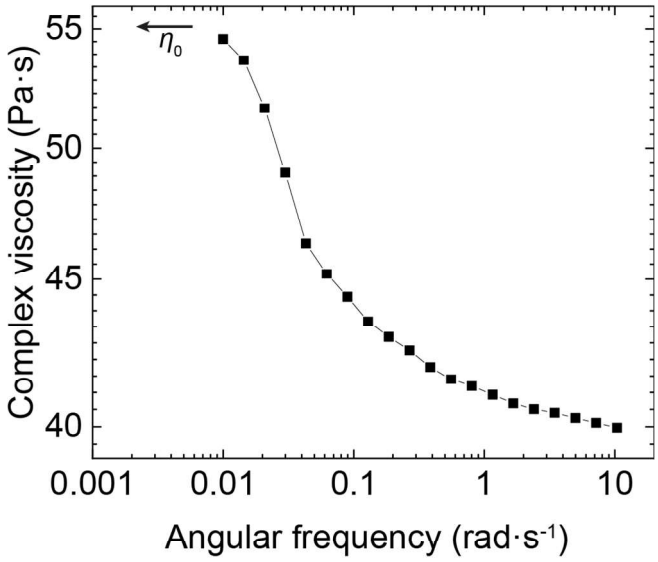


Figure S7: Zero-shear viscosity of sPEG-qStyPy in water (7.0 *wt*%). η_0 is greater than 55 Pa·s.

Macroscopic phase separation of sPEG-styrylpyrene (22 kDa)

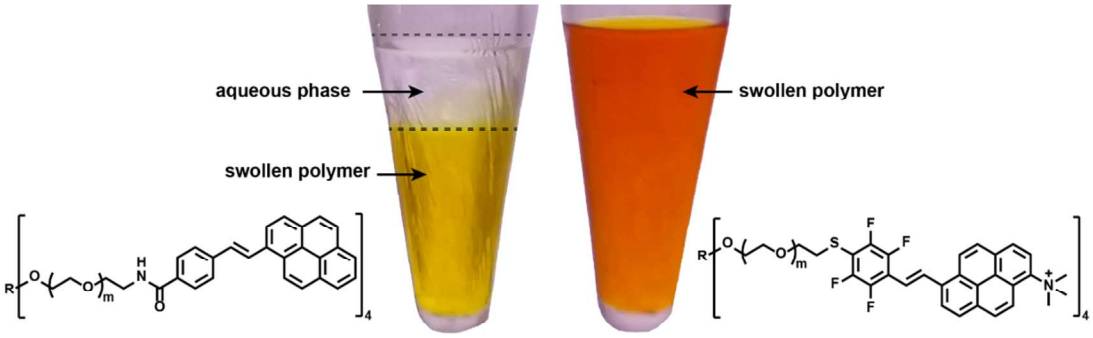


Figure S8: Macroscopic phase separation using the hydrophobic styrylpyrene (left) as opposed to qStyPy (right) at 7.0 *wt*%.

Rheology with *in-situ* irradiation

The samples were irradiated with a fiber-coupled LED from Ocean Insight (470 nm).

Figure S9: Anton Paar Modular Compact Rheometer 302 with fiber-coupled *in-situ* irradiation.

UV-Vis spectroscopy

UV-Vis measurements were carried out on an QE Pro from Ocean Insight equipped with the light source DH-2000-BAL and the temperature-controlled cuvette holder qpod 2e™ from Quantum Northwest. The fiber optic spectroscopy enables to successively acquire UV-Vis spectra in 180° and irradiate samples in 90° (Figure S10). Time-resolved measurements were acquired using self-written MatLab scripts.

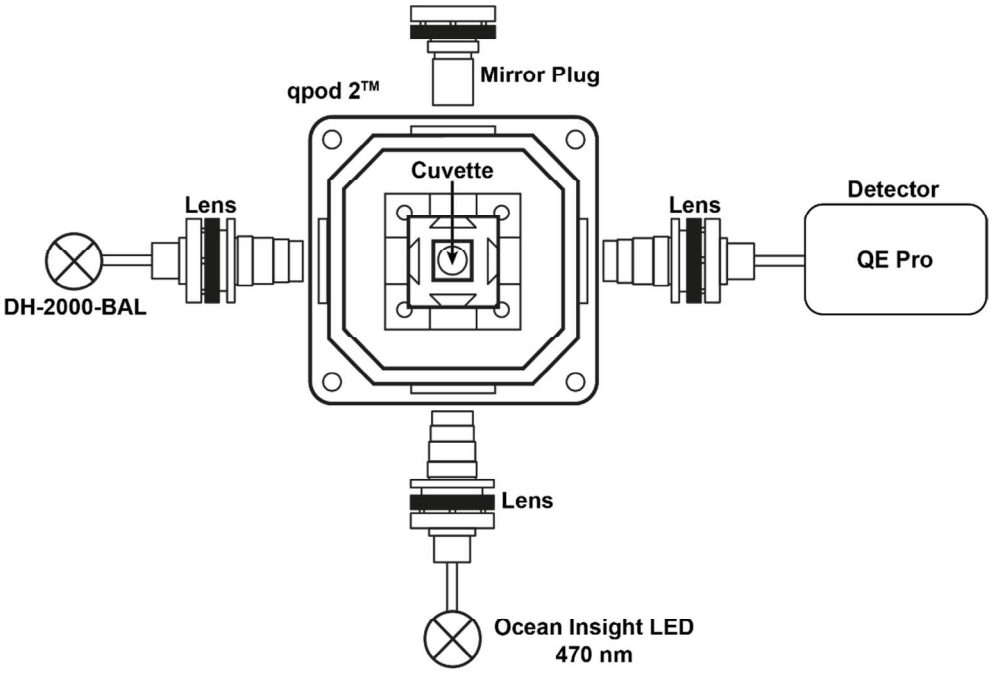


Figure S10: Experimental set-up for successive UV-Vis measurements and sample irradiation.