

Materials and methods for the chemical synthesis of PFO.

All reagents and solvents were purchased from Sigma-Aldrich, Alfa-Aesar or Fisher Scientific and used without further purification unless stated otherwise. Tetrahydrofuran and toluene were obtained from a solvent purification system (SPS 400, innovative technologies) using alumina as the drying agent.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX400 NMR at 400.13 and 100.61 MHz in CDCl_3 . Proton NMR chemical shifts are reported as δ values in ppm relative to the signal of residual protons in the deuterated solvent (CDCl_3 , $\delta = 7.26$) Data are presented as follows: chemical shift, integration, multiplicity (s = singlet, b.s. = broad signal with no resolved multiplicity, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constant(s) (J) are in Hz. Multiplets / broad signals are reported over the range (in ppm) they appeared. Carbon NMR data were collected relative to the corresponding solvent signals CDCl_3 (77.16). The molecular weights of the polymers were determined by size exclusion chromatography using a Viscotek TDA 302 analyser including refractive index (RI) and dual laser light scattering ($\lambda = 670 \text{ nm}$, 90° and 7°) detectors in THF with a flow rate of 0.5 ml/min at 35°C .

Monomer **M**

The solution of 2,7-dibromo-9,9-dioctylfluorene (8.24 g, 15.0 mmol) in 210 mL of dry THF was cooled to -78°C , and a 2.38 M solution of BuLi in hexane (6 mL, 14.3 mmol) was added dropwise at this temperature over a period of 37 min. The reaction mixture was cooled to -98°C , before the 11 mL (47.7 mmol) of triisopropyl borate was added, and allowed to reach room temperature. After stirring overnight, it was quenched by pouring onto an ice-water-HCl mixture and extracted with ether. Combined extracts were washed with water, dried with MgSO_4 , and the solvent was evaporated. The crude boronic acid (6.98 g) was purified by column on silica, eluting first with toluene then with ether, and converted to the ester by refluxing in 140 mL of dry toluene with 2,2-dimethyl-1,3-propanediol. The final boronic ester was re-crystallised from acetone 3 times affording 6.54 g (85.5%) of the final boronic ester. M.p. $89\text{--}90^\circ\text{C}$. Calculated for $\text{C}_{34}\text{H}_{50}\text{BBrO}_2$: C 70.23 %, H 8.67 %. Found: 70.35 %, H 8.72 %. MS (EI, base peak): 580.3157 (Calc.: 580.3087). ^1H NMR (CDCl_3 , δ , 400 MHz) 7.79 (1H, d, $^3\text{J} = 7.6$ Hz), 7.73 (1H, s), 7.64 (1H, d, $^3\text{J} = 7.6$ Hz), 7.56 (1H, d, $^3\text{J} = 7.8$ Hz), 7.48-7.40 (2H, m), 3.81 (4H, s), 2.10-1.80 (4H, m), 1.28-0.95 (26H, m), 0.82 (6H, t, $^3\text{J} = 7.1$ Hz), 0.70-0.50 (4H, m). ^{13}C NMR (CDCl_3 , δ , 400 MHz) 153.73, 149.51, 142.58, 140.33, 132.97, 129.97, 128.19, 126.35, 121.47, 121.40, 119.04, 72.52, 55.51, 40.34, 31.93, 30.09, 29.34, 29.32, 23.80, 22.74, 22.17, 14.21.

Polymer **P1**

The solution of the monomer **M** (1.40 g, 2.41 mmol) and tetrakis(triphenylphosphine)palladium (0) (12.0 mg, 10.4 μ mol) in 21 ml of toluene was stirred under argon at room temperature for 10 min. An aqueous solution of tetraethylammonium hydroxide (7 ml, 20%) was added, and the reaction mixture was heated at 115 °C (bath temperature) for 2 hours. The reaction was allowed to reach room temperature before 4-bromobenzoic acid (240 mg, 1.19 mmol) and another portion of the catalyst (7 mg, 6 μ mol) were added, and the reaction mixture was heated at 115 °C for another hour. The end-capping was repeated with hydroxyphenylboronic acid (360 mg, 2.37 mmol) and another portion of the catalyst (7 mg, 6 μ mol). The mixture was allowed to reach room temperature and poured onto 900 mL of methanol. The precipitate was filtered, washed with methanol and subjected to a Soxhlet extraction with methanol (12 hours) and acetone (12 hours) to remove low molecular weight fractions. The final extraction with dichloromethane afforded 879 mg (93.9%) of the target polymer **P1**. $M_n=18000$ Da, $M_w=26000$, PDI=1.4. $^1\text{H NMR}$ (CDCl_3 , δ , 400 MHz) 7.85 (1H, d, $^3J = 7.9$ Hz), 7.71 (1H, d, $^3J = 7.6$ Hz), 7.68 (1H, s), 2.50-1.70 (2H, b.s.), 1.35-1.00 (10H, m), 0.95-0.65 (5H, m).

Polymer **TES-PFO**.

To a solution of polymer **P1** (120 mg) in 12 ml of dry CHCl_3 the excess of 3-isocyanatopropyltriethoxysilane (0.72 ml) and a catalytic amount of trimethylamine (3 drops) were added. The mixture was stirred at 60 °C for 3 days and cooled down to room temperature. The product was precipitated with methanol filtered and washed excessively with methanol to provide the final compound (112 mg, 93.3 %)

$M_n=17400$ Da, $M_w=27300$, PDI=1.6. $^1\text{H NMR}$ (CDCl_3 , δ , 400 MHz) 7.85 (1H, d, $^3J = 7.7$ Hz), 7.77-7.57 (2H, m), 3.87 (0.06H, q, $^3J = 7.0$ Hz), 3.38-3.27 (0.02H, m), 2.50-1.70 (2H, b.s.), 1.35-1.00 (10H, m), 0.95-0.65 (5H, m).

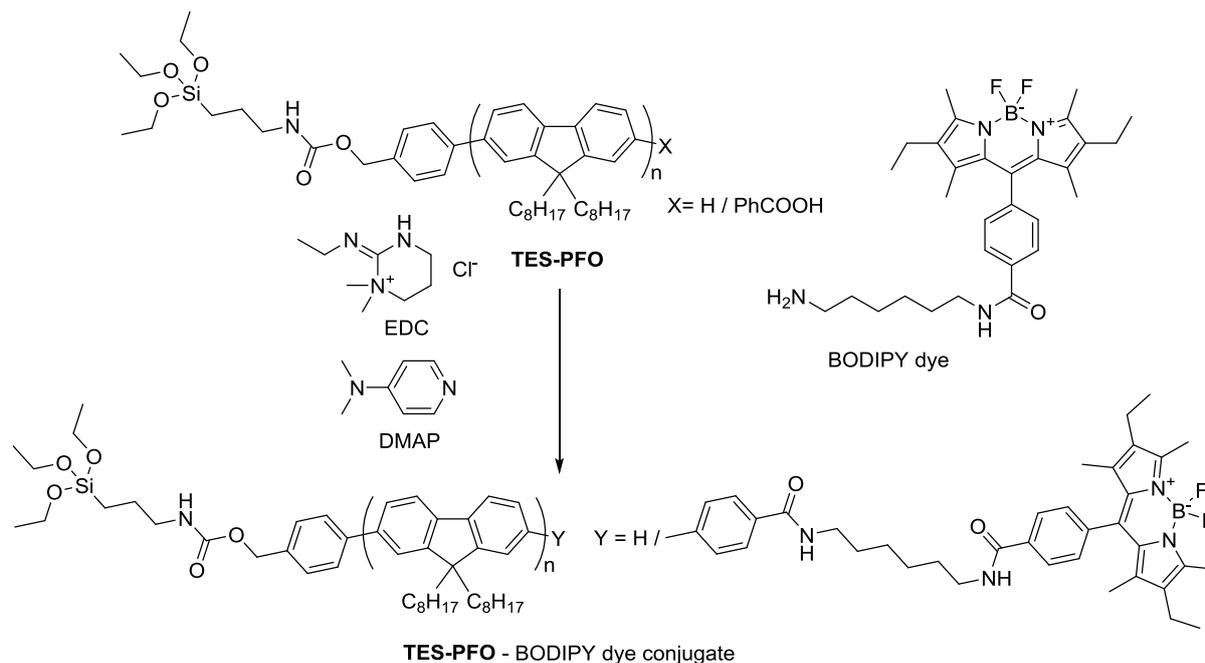
Estimating the ratio of end-capping with **Br-PhCOOH**.

The extent of end-capping with 4-bromobenzoic acid (**Br-PhCOOH**) was estimated by coupling the final polymer **TES-PFO** with BODIPY dye (N-(6-aminohexyl)-4-(2,8-diethyl-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)benzamide) (Scheme S1) and measuring the absorption of the resulting **TES-PFO** – BODIPY dye conjugate at 390 nm and 527 nm. To solution of **TES-PFO** (48.5 mg), BODIPY dye¹ (4.7 mg, 9.0 μ mol) and DMAP (16.7 mg, 137 μ mol) in 10 ml of CH_2Cl_2 was added EDC (18.9 mg, 98.6 μ mol) at once and the reaction mixture was stirred overnight before precipitation with methanol. The product was re-precipitated 3 times and dried in vacuum and the

absorption of its solutions in DCM were analyzed (Figure S8). The ratio of end-capping (ω) was calculated by equation (1) and was found to be 24 %

$$\omega = \frac{\varepsilon_{Pol}^{527} \left[\frac{cm^2}{mg} \right] \cdot M_n \left[\frac{g}{mol} \right]}{\varepsilon_{Dye}^{527} [cm^{-1} \cdot M^{-1}]} \cdot 100 \% \quad (1)$$

where ε_{Pol}^{527} is an observed extinction coefficient for **TES-PFO** – BODIPY dye conjugate at 527 nm (b1 coefficient in the Figure S7), ε_{Dye}^{527} is a molar extinction coefficient of BODIPY dye at 527 nm (b2 coefficient in the Figure S7) and M_n is a number averaged molecular weight of **TES-PFO**



Scheme S1. Preparation of **TES-PFO** – BODIPY dye conjugate

Surface passivation with 1H,1H,2H,2H-Perfluorodecyltriethoxysilane (PFDTES).

For substrate passivation, we chose a molecule with a fluorinated carbon tail because its low surface free energy leads to anti-adhesive behaviour with polar and non-polar substances. In order to immobilize PFDTES, glass surfaces were activated as follows. Substrates were sonicated for 30 minutes in 5 M KOH, washed thoroughly with double-distilled water and methanol, and finally sonicated for another 30 minutes in pure methanol. The activated substrates were incubated in a sonicator bath with a 10 mM PFDTES solution in methanol with 1% acetic acid for 1 hour. The residual solvent was discarded and the substrates were cured in a vacuum for one hour. To remove possible impurities, substrates were sonicated in water and methanol for 10 minutes, dried with a nitrogen gas gun, stored under vacuum in a dark place and used within two weeks.

PFO immobilization onto glass substrates.

In order to observe single molecules, we had to surface-immobilize PFO chains at a very low density. With such sparse surface coverage, fluorescence light from individual molecules did not overlap and isolated bright spots corresponding to single molecules can be easily monitored. We chose silane chemistry for its proven record in glass surface functionalization and its notable chemical stability. Prior to PFO attachment, glass surfaces were activated in a similar fashion to PFDTES (see above). Substrates were sonicated for 30 minutes in 1 M KOH, washed thoroughly with double-distilled water and methanol, and finally sonicated for another 30 minutes in pure methanol. The activated substrates were incubated in a sonicator bath with a 10 nM PFO solution in tetrahydrofuran with 1% acetic acid for 2 hours. In case PFO and PFDTES were co-immobilized, this solution also contained 10 mM PFDTES. The residual solvent was discarded and the substrates were cured in a vacuum for one hour. To remove unspecifically adsorbed PFO, the substrates were cleaned by sequentially sonicating for 20 minutes in toluene, acetone and methanol. The clean substrates were dried with a nitrogen gas gun, stored under vacuum in a dark place and used within two weeks.

Single-molecule setup

The basic arrangement of our single-molecule setup has been previously described. We have used specific laser and optics to account for the photoluminescence properties of PFO. Briefly, a PicoQuant 390 nm laser (LDH-D-C-390) beam was reflected by a dichroic mirror (Semrock, Di02-R405-25-D) and focused onto our substrate through a PlanoApo 60x oil objective (Olympus, NA 1.42). For spectroscopic measurements, we used a total internal reflection configuration in order to minimize the contribution of the solvent Raman, whereas intensity measurements were done in epifluorescence mode. In both cases, fluorescence light was collected by an Olympus IX71 inverted microscope, magnified, and filtered (Semrock, FF02-409/LP-25) in order to remove residual laser light. Emission intensity was recorded by an air cooled (-70 C) Andor iXon ultra 897 EMCCD camera with 50 ms integration time. For spectroscopic measurements, a 10:90 beam splitter was placed to direct 90% of emission light onto a Zolix Omni- λ 3007 spectrograph coupled to an Andor Newton 971 EMCCD camera. To improve signal-to-noise ratio, spectral images were binned from the native camera resolution of 1600x400 to 50x200 pixels (50 wavelength values per spectra). Acquisition times were usually set to 1-2 seconds and the spectral window fixed at 415 to 485 nm. With our current optical setup and EMCCD detector size, the entire field of view has dimensions of 50 x 50 μm and each pixel corresponds to 100 nm.

The solvent chamber used in our experiments has been described in our previous work. Briefly, a polytetrafluoroethylene sheet with an etched canal is sandwiched between a microscope slide with

two holes (for solvent inlet/outlet) and the substrate, all held together by two brass plates. We employed HPLC or spectroscopic grade non-degassed solvents.

Data was processed using custom Python scripts and the software package *hmmlearn* was employed for hidden Markov analysis.

SUPPLEMENTARY FIGURES

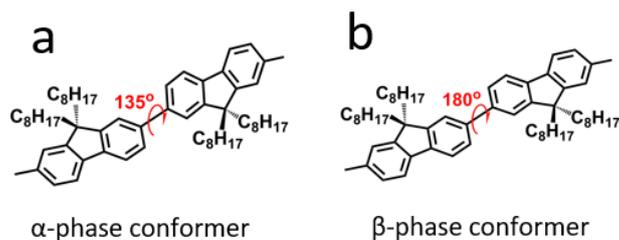


Figure S1. Schematic of the α -conformer (a) and β -conformer (b) and the characteristic twisting angle between consecutive monomers .

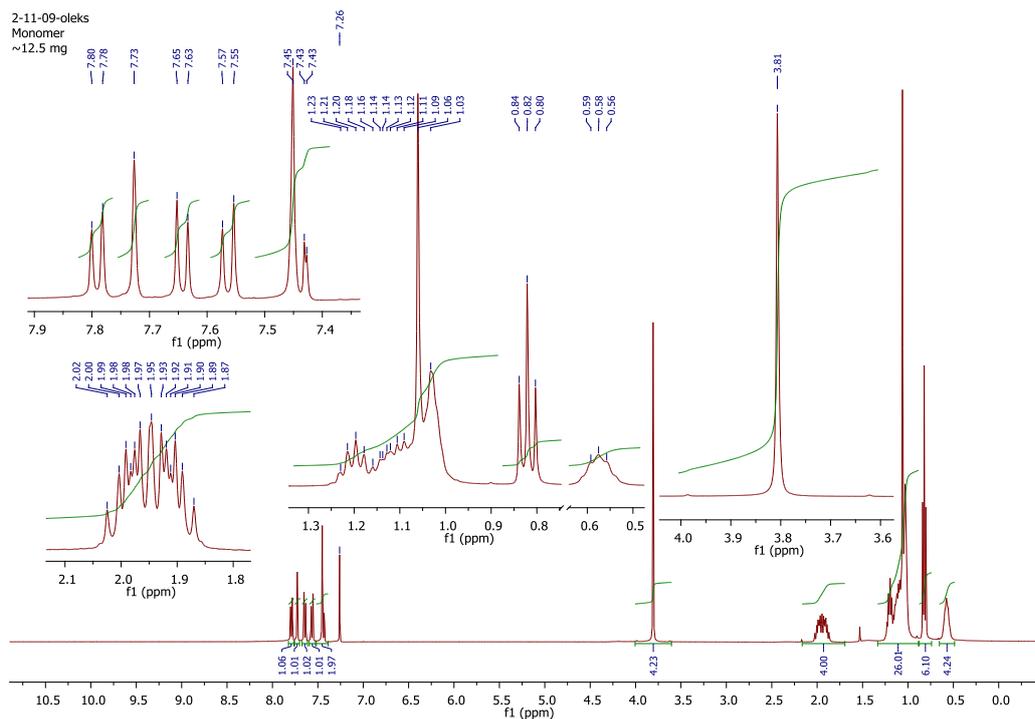


Figure S2. ¹H NMR spectrum of monomer M.

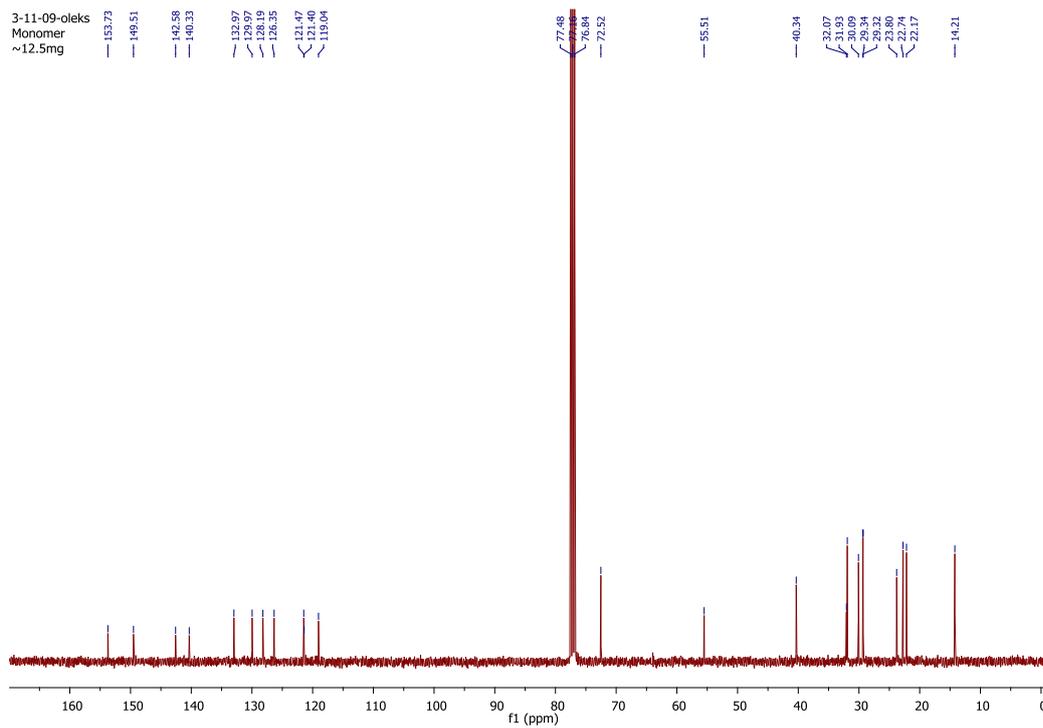


Figure S3. ^{13}C NMR spectrum of monomer **M1**.

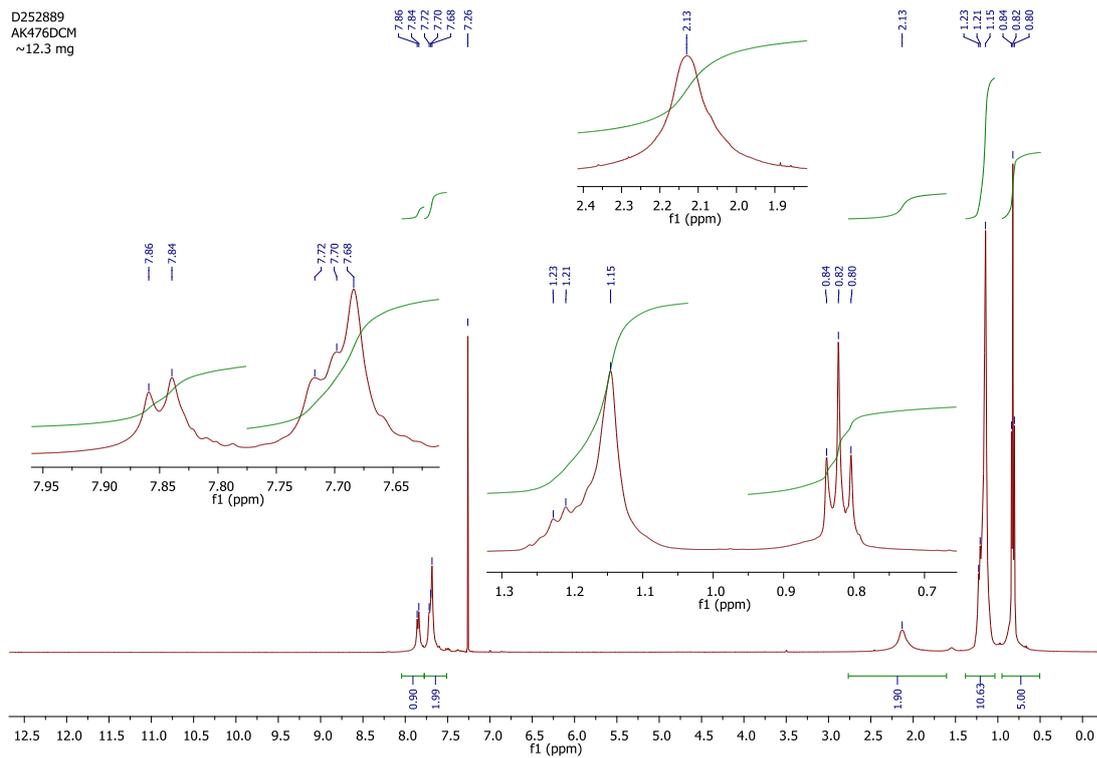


Figure S4. ^1H NMR spectrum of polymer P1.

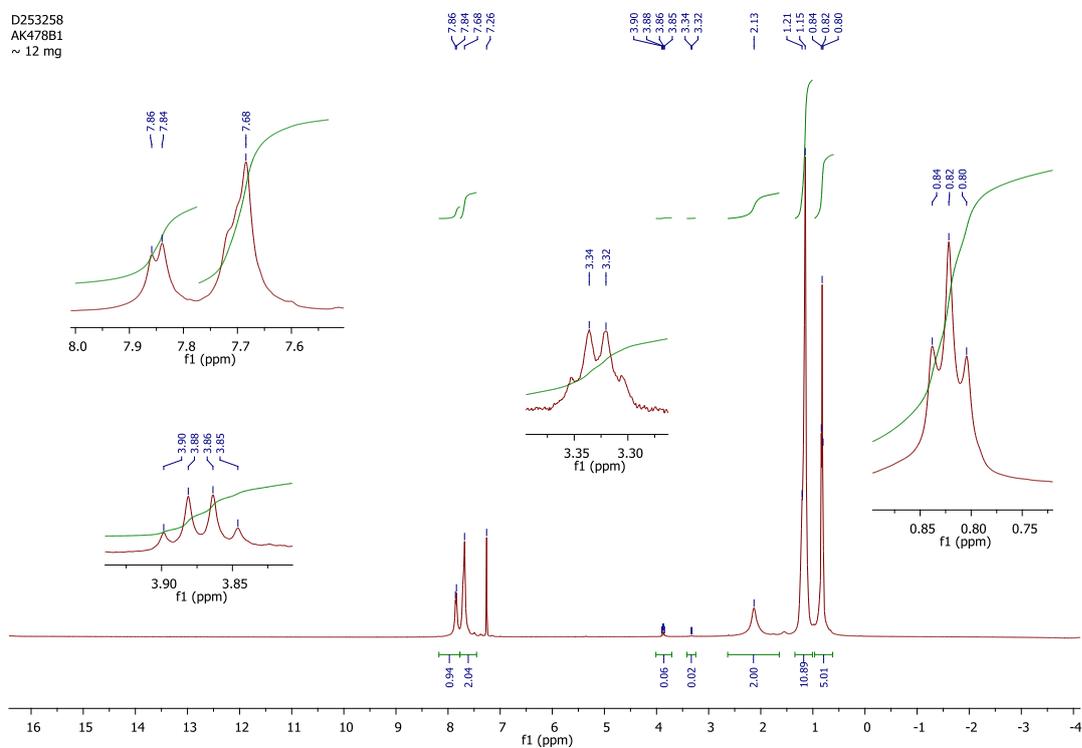


Figure S5. ^1H NMR spectrum of polymer TES-PFO.

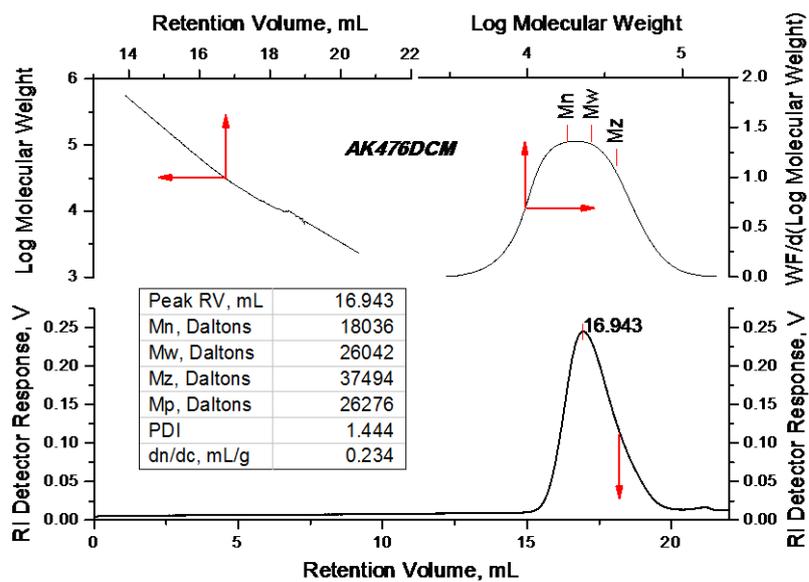


Figure S6. GPC results of for polymer P1.

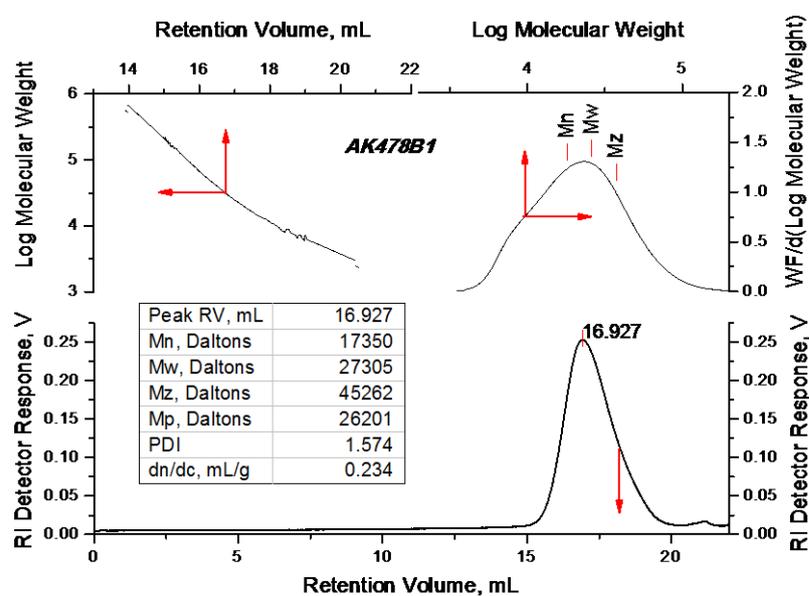


Figure S7. GPC results of for polymer TES-PFO.

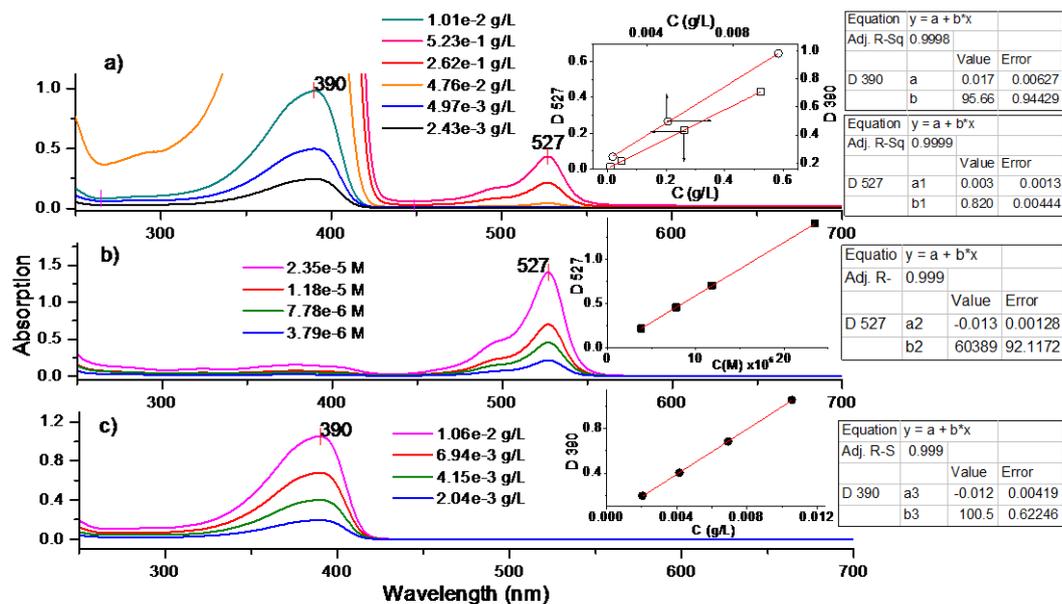


Figure S8. UV absorption spectra of a) TES-PFO – BODYPI dye conjugate, b) BODYPI dye and c) TES-PFO in CH_2Cl_2 solutions at different concentrations.

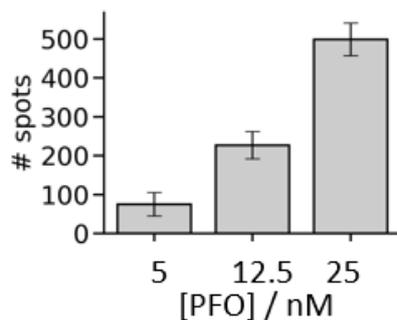


Figure S9. Dependence of the number of spots with the concentration of TES-PFO.

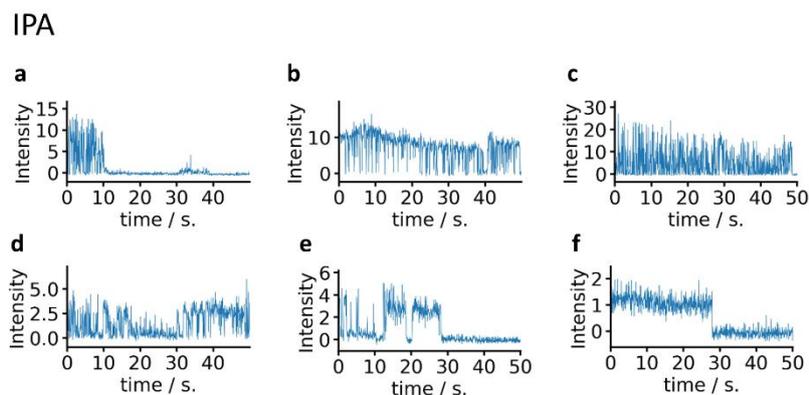


Figure S10. Representative photoluminescence intensity traces for PFO single molecules in isopropanol. Traces a-e show fast blinking dynamics, typical behaviour for PFO in isopropanol. Trace f shows no blinking dynamics in the order of our measurement window (50 ms). Roughly one third of the traces in IPA presented a behaviour like similar to f.

toluene

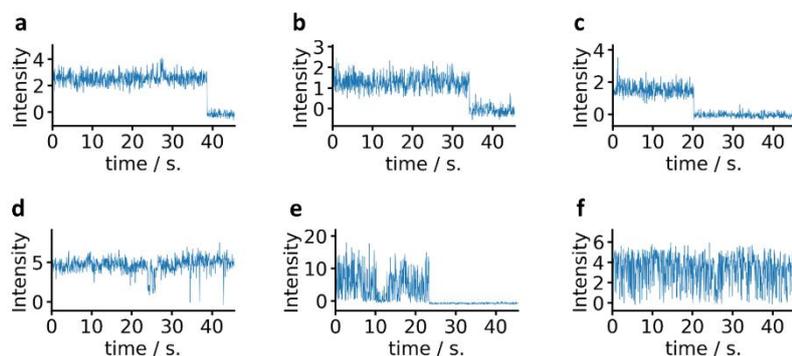


Figure S11. Representative photoluminescence intensity traces for PFO single molecules in toluene. Traces a-d do not show significant blinking dynamics, typical behaviour for PFO in toluene. Traces e-f shows fast blinking dynamics. Roughly one third of the traces in toluene presented a behaviour like the one shown by e-f.

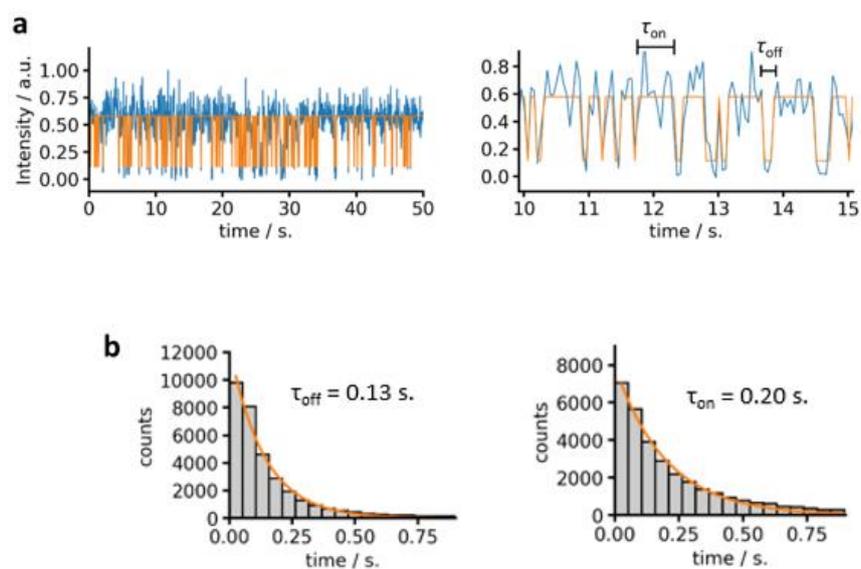


Figure S12. Hidden Markov model (HMM) analysis of blinking events. (a) Example trace of TES-PFO in isopropanol (blue) and idealized fit to HMM (orange). The magnified part on the right displays τ_{on} and τ_{off} dwell times, which are calculated based on the idealized trajectory. (b) Dwell time histograms calculated from hundreds of traces like the one in (a).

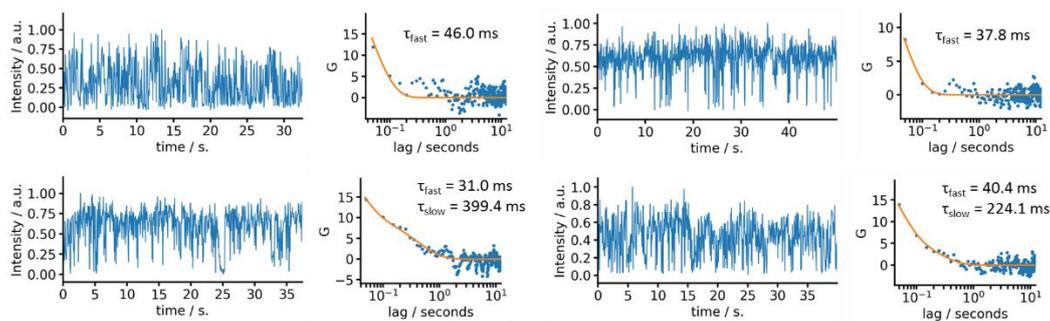
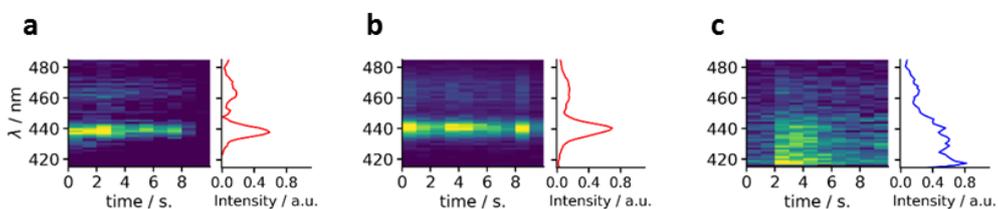


Figure S13. Representative blinking dynamics and corresponding autocorrelation function of the fluctuations obtained in IPA. The autocorrelation function has been fitted to a monoexponential (top traces) or biexponential decay (bottom traces).

IPA



toluene

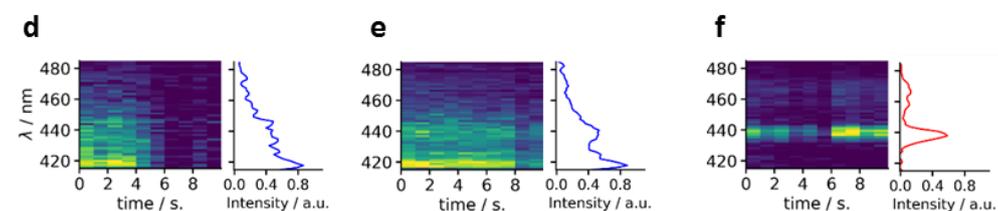


Figure S14. Spectral traces for single molecules of PFO in isopropanol (top) and toluene (bottom). Traces **a, b** and **f** correspond to the beta phase, whereas traces **c-e** show a glassy phase conformation.

IPA → toluene

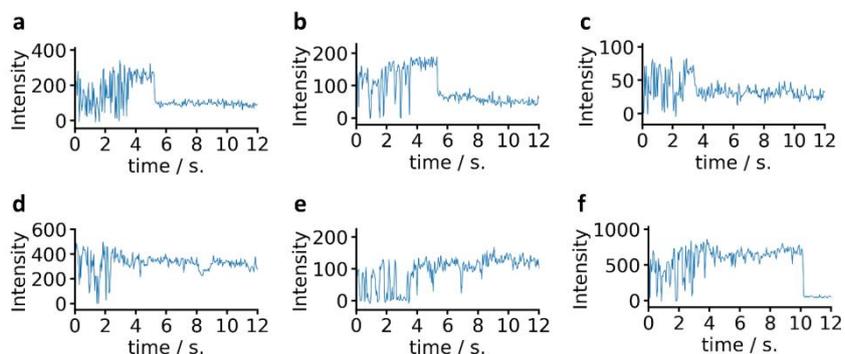


Figure S15. Photoluminescence behaviour for PFO single molecules upon solvent exchange. At around 4 seconds, isopropanol is replaced by toluene. A clear reduction in blinking dynamics is observed for the majority of traces.

toluene → IPA

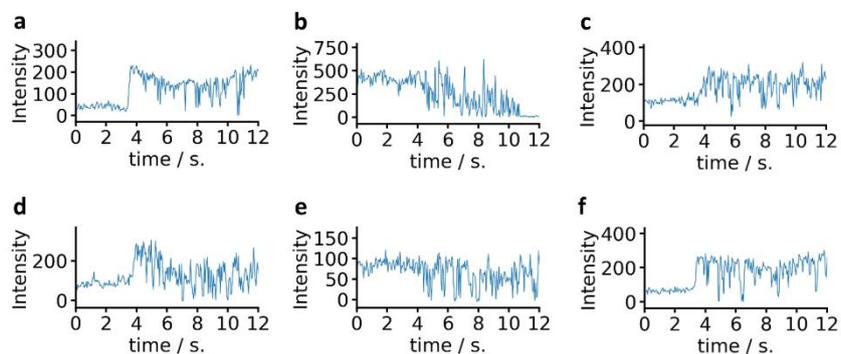


Figure S16. Photoluminescence behaviour for PFO single molecules upon solvent exchange. At around 4 seconds, toluene is replaced by isopropanol. A clear increase in blinking dynamics is observed for the majority of traces.

References

1. I. Yildiz, E. Deniz, B. McCaughan, S. F. Cruickshank, J. F. Callan and F. M. Raymo (2010) Hydrophilic CdSe–ZnS Core–Shell Quantum Dots with Reactive Functional Groups on Their Surface. *Langmuir*, 2010, **26**, 11503-11511.