Direct monitoring of copolymeric micelles self assembly by a luminescent molecular rotor

Gianfranco Vaccaro, Alberto Bianchi, Michele Mauri, Simone Bonetti, Francesco Meinardi, Alessandro Sanguineti, Roberto Simonutti and Luca Beverina**

Supporting Information

1. **Definition of molecular rotor and detailed description of their use as probes of local versus macroscopic viscosity.**

The term molecular rotor is generally used to describe fluorescent molecules exhibiting an intramolecular rotation in their fluorescent excited state. Typically a molecular rotor is composed by three subunits: an electron-donor (D) part, an electron-acceptor (A) part and a spacing unit rich in conjugated double bonds. This unit links the electronic density of the other two subunits, enhancing the electronic transport from D to A while minimizing the direct overlap between the two fractions and ultimately allowing the three parts to rotate. With such a structure the molecule responds to a photoexcitation with an intramolecular charge-transfer from D to A. When photoexcited, molecular rotors live in a twisted intramolecular charge transfer (TICT) state[1]. If intramolecular rotations are permitted, the rotor relaxes to the ground-state through a not-radiative mechanism. The TICT state formation depends on the local viscosity. As schematically shown in Fig. 1, the three units forming the molecule are able to rotate independently around a single C-N bond. An increase in the viscosity of the local environment can inhibit this type of motion, inducing an adiabatic transition from a not-emissive TICT state to an emissive local excited state (LE). As stated in the Förster-Hoffmann model, by tuning and controlling the microviscosity it is possible to reduce the probability of not-radiative recombination even by several orders of magnitude.

As a consequence, the viscosity of the dispersing medium modulates the emissive properties of molecular rotors. In particular, the luminescence lifetime and quantum yield (QY) of a molecular rotor increase on increasing of system viscosity.

In this context, it worth noting that viscosity probed by fluorescent molecules (e. g. pyrene) and molecular rotors is not quantitatively the same as the macroscopic/bulk viscosity. For this reason it is usually referred to as “micro-viscosity” or “local viscosity” (η). Notwithstanding, the role of viscosity probe of these molecule types is well accredited in literature; for example, the molecular rotors can be used for applications in fluid mechanics, polymer chemistry (sensor of polymerization), cell physiology (sensor of aggregation and conformation changes in protein molecules), etc.

The relation linking the fluorescent lifetime (τ_f) and η is known as Förster-Hoffmann equation[1]:

$$\frac{\Pi_f}{\Pi_0} = z\eta^{\in}$$

Eq. S1

where z and α are parameters associated to the interaction between the environment and the molecular rotor, whereas τ_0 is the radiative lifetime (viscosity-independent rotor constant).

2. Absorption and steady state fluorescence of the molecular rotor AzeNaph1 in MMA solution and PMMA sample.

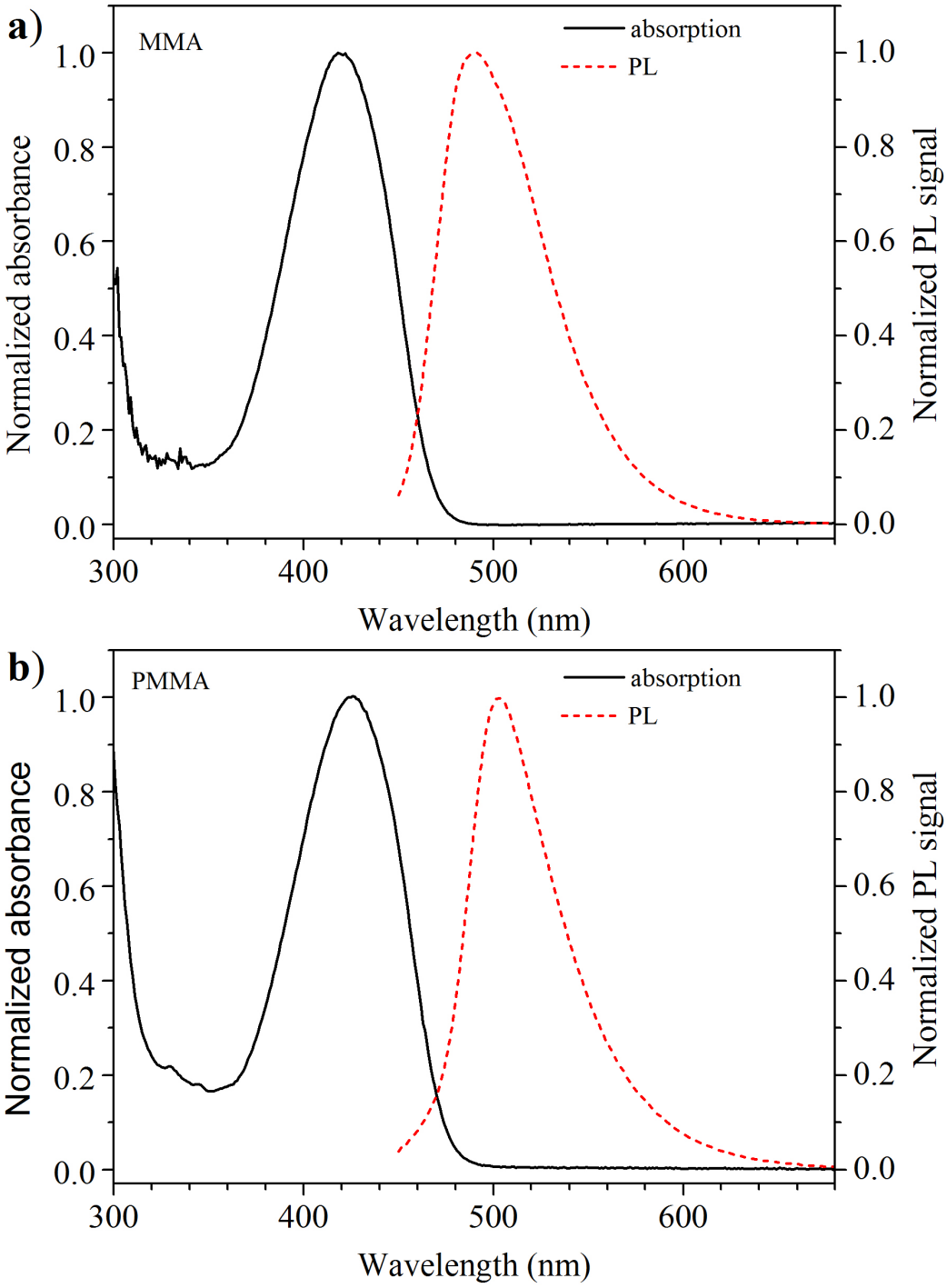


Figure S1. *Absorption and steady state fluorescence (excited at 420 nm) of AzeNaph1 in a 10^{-5} [M] MMA solution a) and in a 10^{-3} [M] PMMA sample b).*

3. Time decay profile as a function of the polymerization time for a solution of AzeNaph1 in styrene containing a radical polymerization initiator.

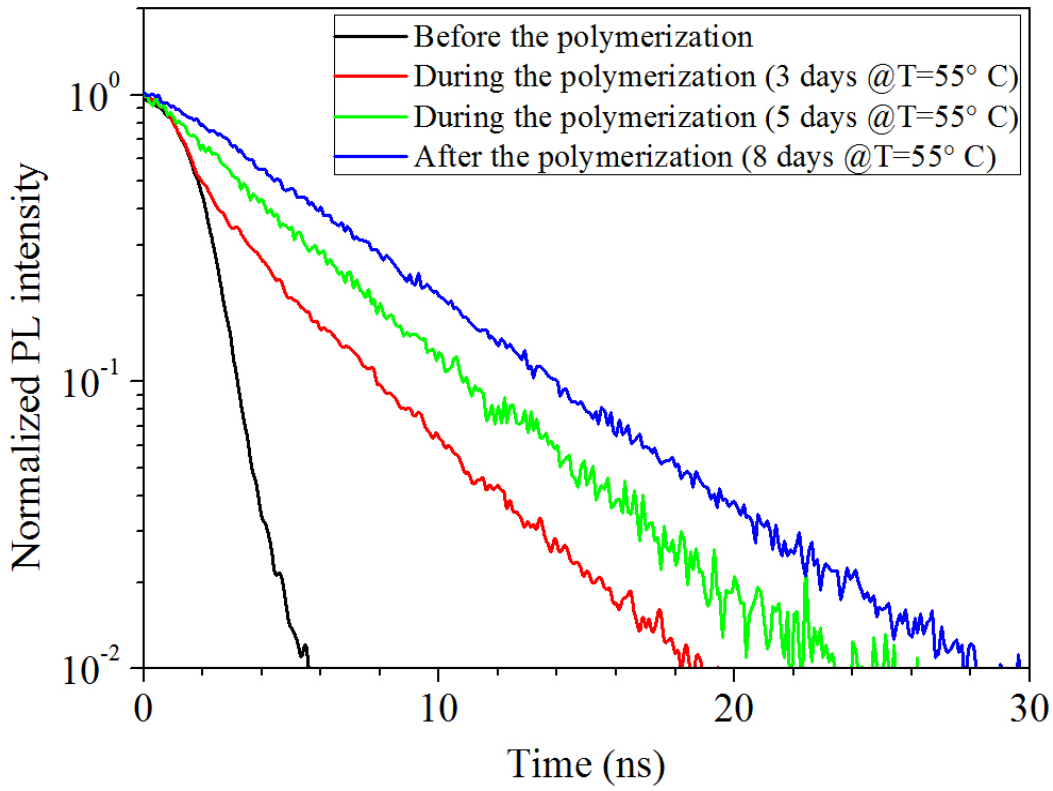


Figure S2. *Time decay profile of the AzeNaph1 acquired before, during and after the polymerization at T~55°C in a styrene solution containing lauryl peroxide as the radical polymerization initiator. The decay lifetime gradually increases on increasing of polymerization degree (before and after the polymerization the decay lifetime is <1 ns and 5.8 ns, respectively). The sample was excited at 405 nm and monitored at 480 nm.*

4. Time decay profiles of AzeNaph1 dispersed in different polymers at room temperature.

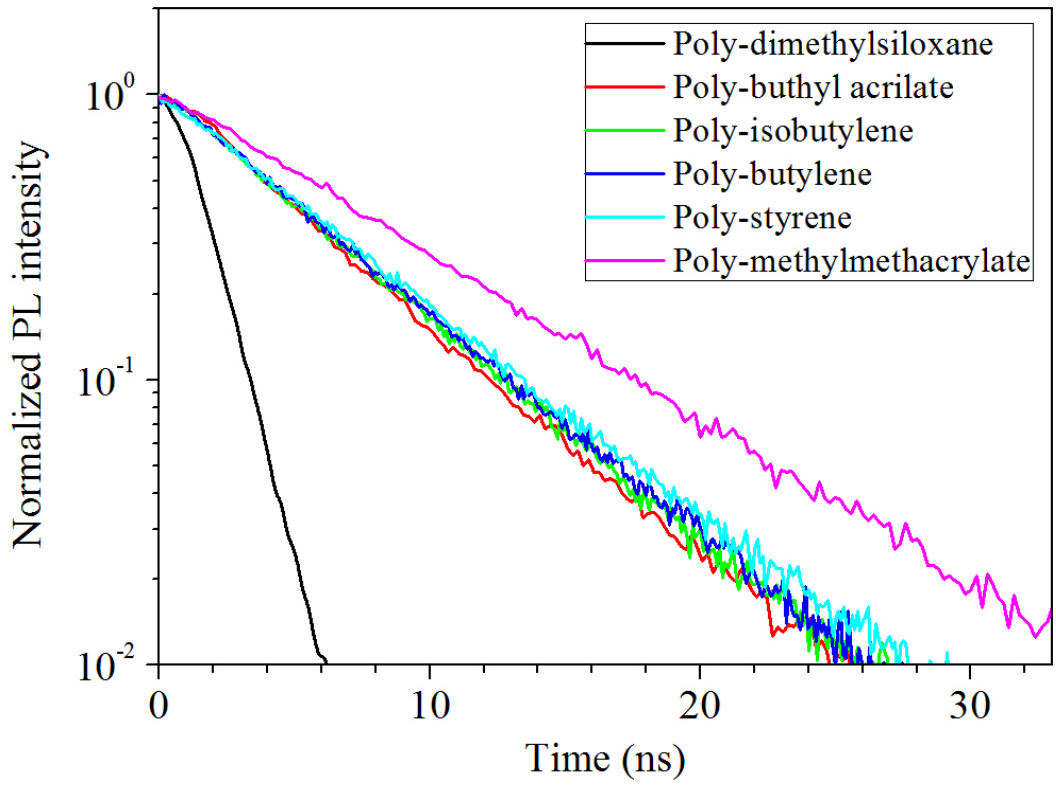
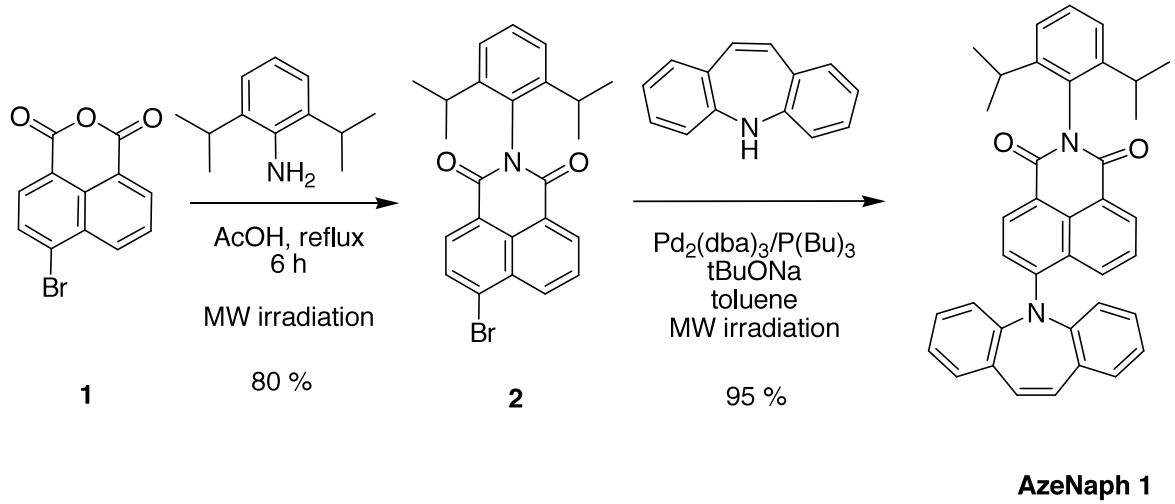


Figure S3. *Time decay profiles of the AzeNaph1dispersed in different polymers at room temperature. The samples were excited at 405 nm and monitored at 480 nm.*

5. Synthetic procedure for the preparation of AzeNaph1.

Molecular rotor AzeNaph1 was prepared according to the protocol shown in Scheme S1. In details, 4-Bromo-1,8-naphthalic anhydride **1** (2.00 g, 7.22 mmol) and 2,6-diisopropylaniline (1.42 g, 8.00 mmol) were suspended in acetic acid (20 ml). The suspension was refluxed in a focused microwave oven at a constant power of 100 W for 6 h in open vessel configuration (ambient pressure). The brown solution was cooled at ambient temperature to give the imide **2** as a yellow solid. The precipitate was filtered, washed on the filter with little acetic acid and dried till constant weight (2.52 g, 5.78 mmol 80 % yield). Analytical data were in agreement with those previously published[2]. The imide 2 (2.00, 4.58 mmol), dibenzoazepine (0.89 g, 4.60 mmol) and tBuONa (0.63 g, 6.54 mmol) were suspended in 30 ml of anhydrous toluene. In a different flask, $Pd_2(dba)_3$ (120 mg) and $P(tBu)_3$ (1.33 ml of a 1 M solution in toluene) were suspended in 10 ml of anhydrous toluene. The purple suspension was stirred at ambient temperature and under nitrogen atmosphere for 10 min and then transferred through a cannula in the flask containing the suspension of the other reagents. The reaction mixture was placed in a focused microwave oven and heated under nitrogen atmosphere at a constant power of 90 W for 5 h. Solvent was removed under reduced pressure to give a yellow residue that was taken up with 150 ml of CH_2Cl_2 and filtered through a plug of silica. The filtrate was evaporated under reduced pressure to give a yellow oil. Sonication in MeOH gave a bright yellow suspension that was filtered to give the molecular rotor AzeNaph 1 as a yellow solid. (2.40 g, 4.37 mmol, 95 % yield). ^{1}H NMR ($CDCl_3$) δ 8.48 (d, 1H, J=7.24), 8.32 (d, 1H, J=8.72), 7.61 (d, 2H, J = 7.92), 7.58 (d, 1H, J =6.95), 7.57 (t, 1H, J=7.97), 7.54 (d, 2H, J=7.71), 7.46 (d, 1H, J=7.02), 7.45 (t, 1H, J=6.69), 7.42 (t, 1H, J=7.75), 7.37 (dd, 1H, J=8.86, 0.98), 7.28 (d, 2H, J=7.74), 7.15 (t, 1H, J=8.86), 7.04 (s, 2H), 6.79 (d, 1H, J=8.72), 2.70 (quin, 2H, J=6.81), 1.12 (dd, 12H, J=6.85, 2.38). ^{13}C NMR ($CDCl_3$) δ165.5, 164.7, 150.8, 146.5, 144.2, 136.3, 133.7, 132.7, 132.6, 132.1, 131.6, 131.2, 131.0, 130.0, 129.9, 129.1, 129.0, 128.5, 124.7, 123.7, 122.6, 114.0, 111.9, 29.9, 24.8.



Scheme S1. *AzeNaph1 synthesis protocol.*

6. ^{1}H and ^{13}C NMR spectra of AzeNaph1 in $CDCl_3$ (Figure S4 and S5).

^{1}H and ^{13}C NMR spectra were recorded using a Bruker AMX-500 spectrometer operating at 500 and 125.70 MHz, respectively. Coupling constants are presented in Hz.

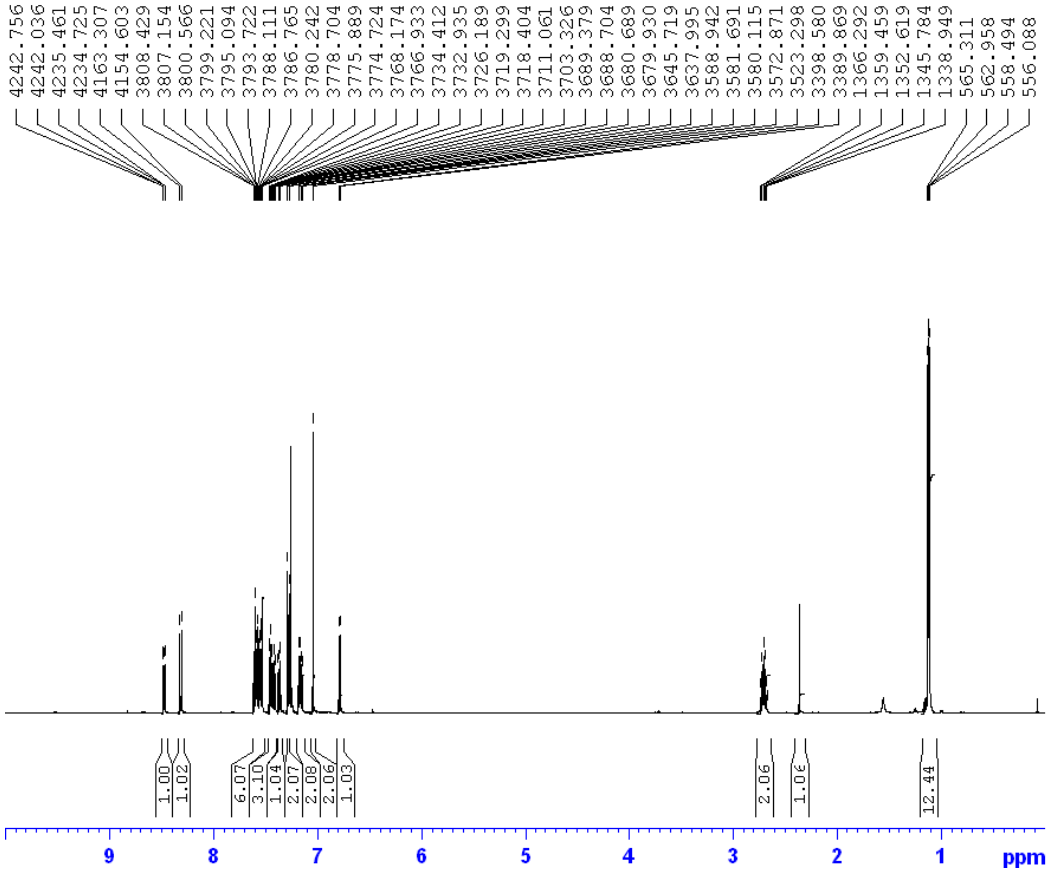


Figure S4. *^{1}H- NMR spectrum of AzeNaph1 in $CDCl_3$.*

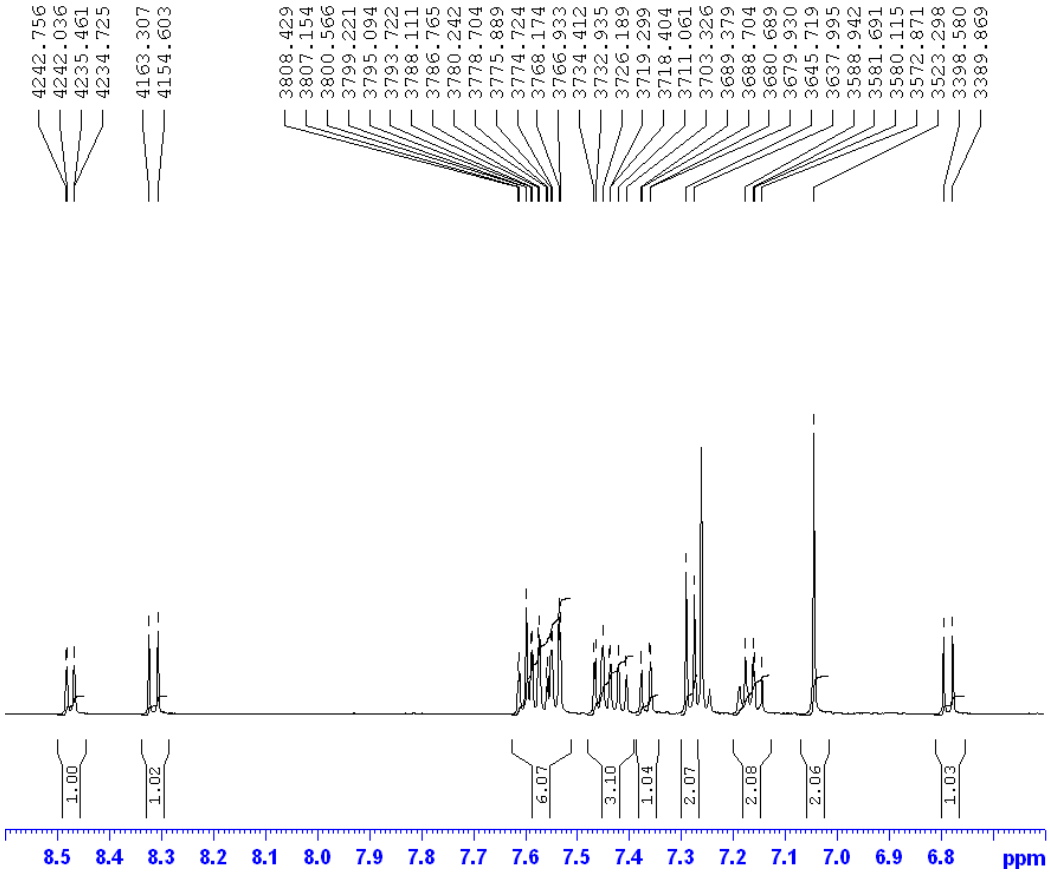


Figure S4a. *^{1}H- NMR spectrum of AzeNaph1 in $CDCl_3$, detail of aromatic spectral region.*

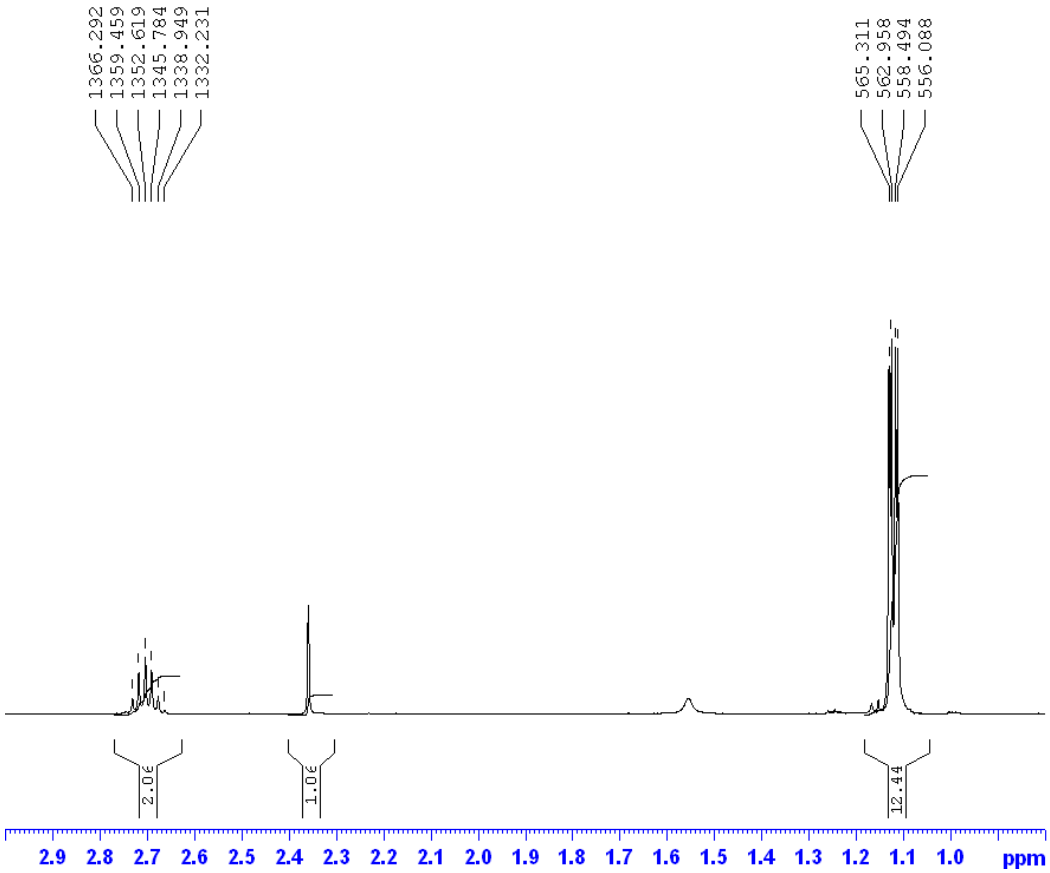


Figure S4b. *^{1}H- NMR spectrum of AzeNaph1 in $CDCl_3$, detail of aliphatic spectral region.*

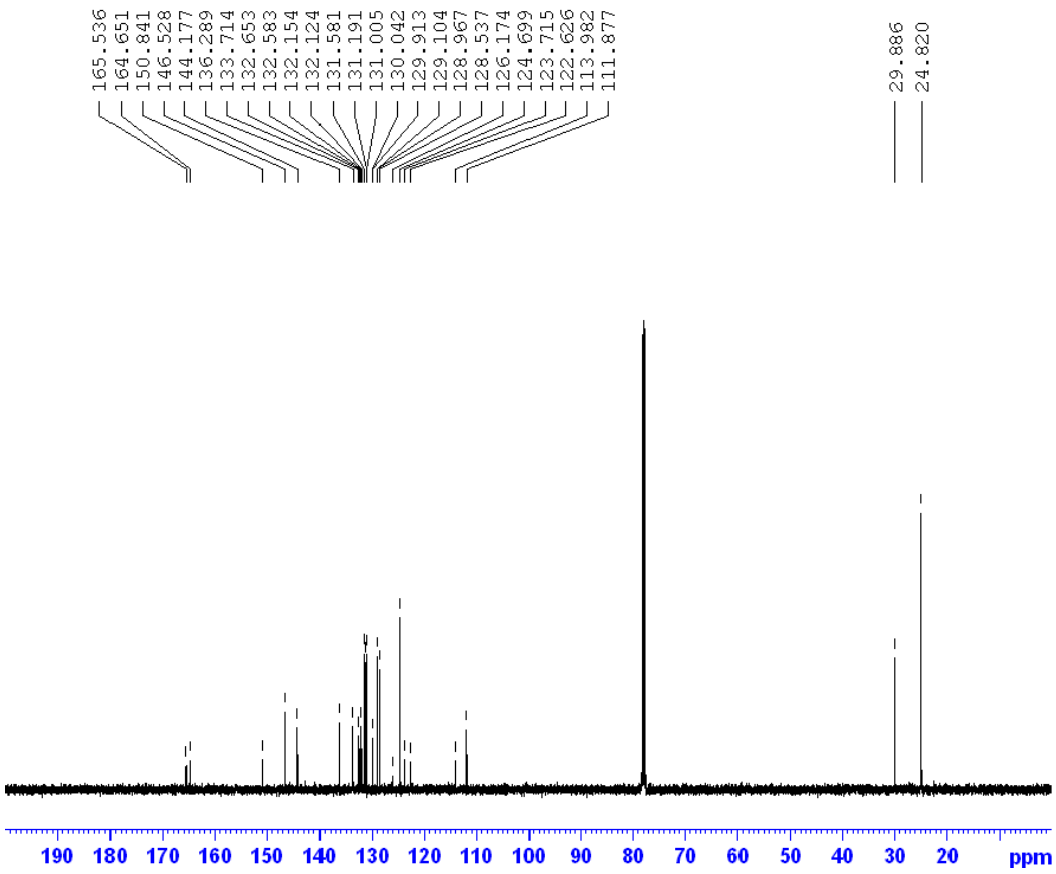
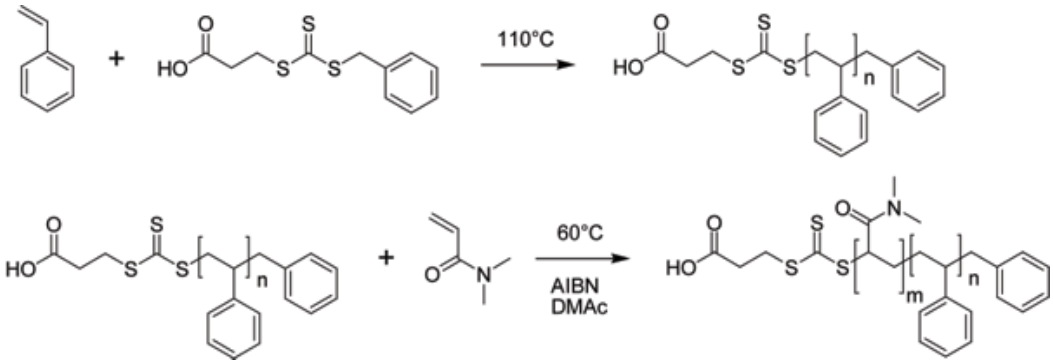


Figure S5. *^{13}C- NMR spectrum of AzeNaph1 in $CDCl_3$.*

7. Details on the synthesis of PDMA-*b*-PS

The block copolymer $PDMA_{817}$-*b*-PS_{105} was prepared according to the three steps procedure shown in Scheme S2.



Scheme S2. *RAFT polymerization of PS_{105}-b-$PDMA_{817}$.*

- **Synthesis of S-3-(benzylthiocarbonothioylthio)propanoic acid**

3-(benzylthiocarbonothioylthio) propanoic acid was synthesized following the reported procedure [3]. 1-mercapto propionic acid (10 g, 0.073 mol) and Na_2CO_3 (10.7 g, 0.08 mol) were stirred in acetone (400 ml), until suspension turned to orange. CS_2 (10.68 g, 0.022 mol) was added and the solution turned bright yellow. After stirring for ten minutes, benzyl bromide (10.26 g, 0.073 mol) was added. Ten minutes later the product was filtered and the solvent was removed under reduced pressure. The residue was added to a saturated solution (700 ml) of brine, extracted with CH_2Cl_2 (2 x 200 ml) and washed with saturated brine solution (3 x 200 ml). After drying the organic extracts over $MgSO_4$ the solvent was removed under reduced pressure to yield (98%) a canary yellow crystalline solid. Tm = 83.6 °C was determined by DSC. FT-IR ATR (cm^{-1}): 669, 838, 1068 (C=S groups), 1204, 1500, 1690 (C=O groups), 2915. ^{1}H NMR ($CDCl_3$): 2.85 (2H, t, S-CH_2-CH_2), 3.62 (2H, t, S-CH_2-CH_2), 4.62 (2H, s, CH_2-Ph), 7.27-7.37 (5H, m, Ph), 9.50-10.05 (1H, COOH). ^{13}C NMR ($CDCl_3$): 31.1 (CH_2-S), 33.0 (S-CH_2-**C**H_2), 41.7 (S-CH_2), 127.9-129.3 (4C, Ph), 134.9 (1C, Ph), 177.8 (C=O), 222.8 (-**C**=S).

- **RAFT polymerization of Polystyrene**

In a 100 ml schlenk tube with septum, 1.5 g (5.51 mmol) RAFT agent 3-benzylsulfanylthiocarbonylsufanylpropionic acid was added and dried under high vacuum for 2 hours. 51.92 ml ($5.51 * 10^{-1}$ mol) of styrene ([M]/[T]=100) were inserted with a cannula and degassed with three or more freeze-pump-thaw cycles. The tube was heated in an oil bath at 110 °C, with magnetic stirring, under argon. After 24 h the mixtures were quenched in liquid nitrogen, and the solid product obtained was dissolved with CH_2Cl_2 and precipitated in methanol. After filtering the sample was dried under high vacuum at 50˚C before use. The polymer was a yellow solid. Mn = 11920 g/mol, Mw = 12638 g/mol and D = 1.06 was measured with GPC. Conversion was determined gravimetrically (100%) and number of monomer units = 105 (n_{PS}) was calculated by comparing the signal area of the ^{1}H NMR peaks of the aromatic group (PS) and methylene group (RAFT agent). T_g = 84˚C was estimated by DSC.

- **RAFT polymerization of block copolymer PDMA-b-PS**

RAFT polymerization of block copolymer PDMA-b-PS was performed as shown in literature. [4]

2 g ($1.79 * 10^{-4}$ mol) of PS_{105} were placed in a schlenk tube with septum and dried for 2 hours. 9.22 ml ($8.96 *10^{-2}$ mol) of N,N-dimethylacrylamide and a solution of 2.9 mg AIBN ($1.80 * 10^{-5}$ mol) and 2 ml of N,N-dimethylacetamide (DMAc) were added via cannula in argon atmosphere, resulting in a ratio [T]/[I] = 10. The solution was brought to 50 mL volume with solvent. The solution was degassed with at least three freeze-pump-thaw cycles. The mixture was heated in an oil bath at 80 °C, with magnetic stirring and argon atmosphere. After 24 h the tube was quenched in liquid nitrogen. The solution was placed in a dialysis tube (MWCO of 3500 Da) and dialyzed against Milli-Q water for 3 days to remove the monomer and DMAc. The water solution was dried in rotavapor and then in mechanical pump for one night at 60°C. The product obtained was a slightly yellow solid. Number of monomer units of the second block = 817 (m_{PDMA}) was calculated comparing the signal area of the ^{1}H NMR peaks of the aromatic group (PS) and methyl groups (PDMA).

Molecular weights and molecular weight distributions were determined using a WATERS 1515 system equipped with a HPLC isocratic Pump, WATERS 2414 refractive index detector and four

Styragel columns (HR2, HR3, HR4 and HR5). GPC system was calibrated with Sigma-Aldrich polystyrene standards. M_n = 81 kDa was recorded for $PDMA_{817}$-*b*-PS_{105} in THF at 1.0 mL/min.

8. DSC charatcrization of $PDMA_{817}$-*b*-PS_{105}

Glass transition temperature was determined by differential scanning calorimetry using a Mettler Toledo DSC 821 instrument. $PDMA_{817}$-*b*-PS_{105} in the form of white powder was treated under in vacuum at 110 °C before performing DSC measurements. $PDMA_{817}$-*b*-PS_{105} exhibits a T_g at 120 °C with a heating rate of 20 °C (Fig.S6).

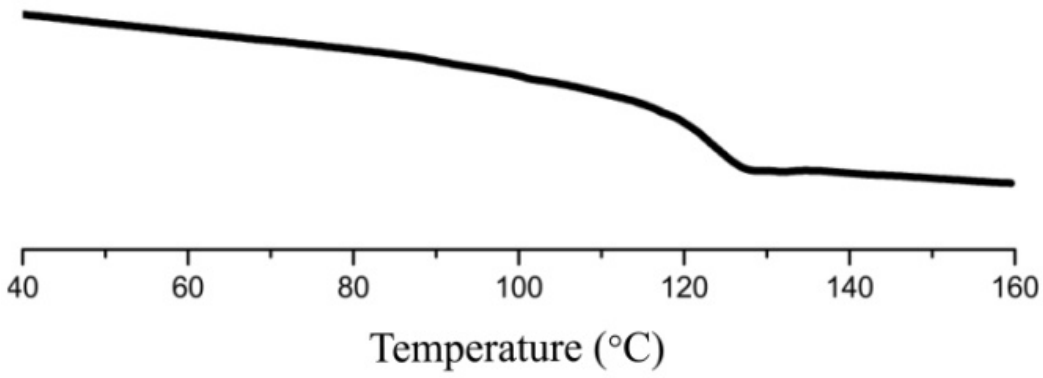


Figure S6. *Third differential calorimetric run of $PDMA_{817}$-b-PS_{105}.*

9. Block-Copolymer $PDMA_{817}$-*b*-PS_{105} correlogram in water.

Dynamic Light Scattering experiments were performed at 25°C on a 90Plus Particle Size Analyzer (Brookhaven Instruments Corporation) with scattering angles of 15° and 90°, equipped with a 35 mW solid state laser and a Brookhaven's TuboCorr correlator with 510 channels.

2 mL of fresh prepared $PDMA_{817}$-b-PS_{105} solution (2% w/w) were pipetted into a 3 mL acrylic square disposable cell. Chromasolv water used as dispersing agent was previously filtered in a 0.2 μm GHP membrane Acrodisc Filter (25 mm Syringe Filter HPLC Certified; PN 4564T; 50/PK). Output is shown in Fig.S7. Particles have a narrow distribution around the mid value (125 nm).

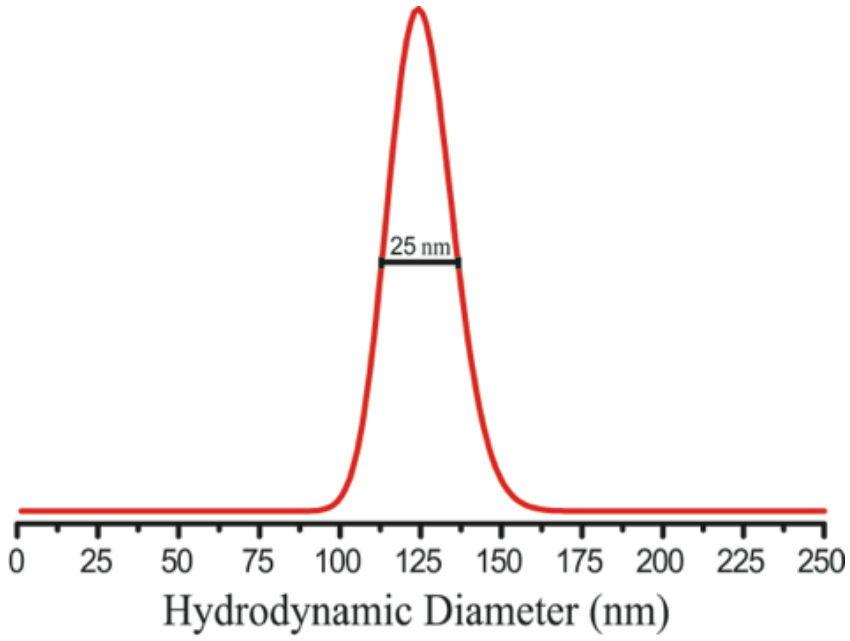


Figure S7. *Particle size distribution of $PDMA_{817}$-b-PS_{105} in water calculated from DLS measurements.*

10. ^{1}H-NMR spectra of copolymer $PDMA_{817}$-*b*-PS_{105} in $CDCl_3$ and D_2O.

^{1}H-NMR spectra were performed on a Bruker AMX-500 instrument operating at 500.13 (^{1}H) MHz. ^{1}H-NMR spectra of PDMA817 PS105 were recorded in non-selective ($CDCl_3$, Sigma Aldrich 99.8 atom % D) and selective solvent (D_2O, Sigma Aldrich 99.9 atom % D), Fig.S7. Sample in D_2O was prepared using a glove bag (Aldrich AtmosBag Z555525-1EA). Both samples were freshly prepared with 30 mg of $PDMA_{817}$-*b*-PS_{105} in 0.75 ml of deuterated solvent and pipetted in a 5 mm thin wall 8" 500 MHz NMR sample tube (Wilmad LabGlass).

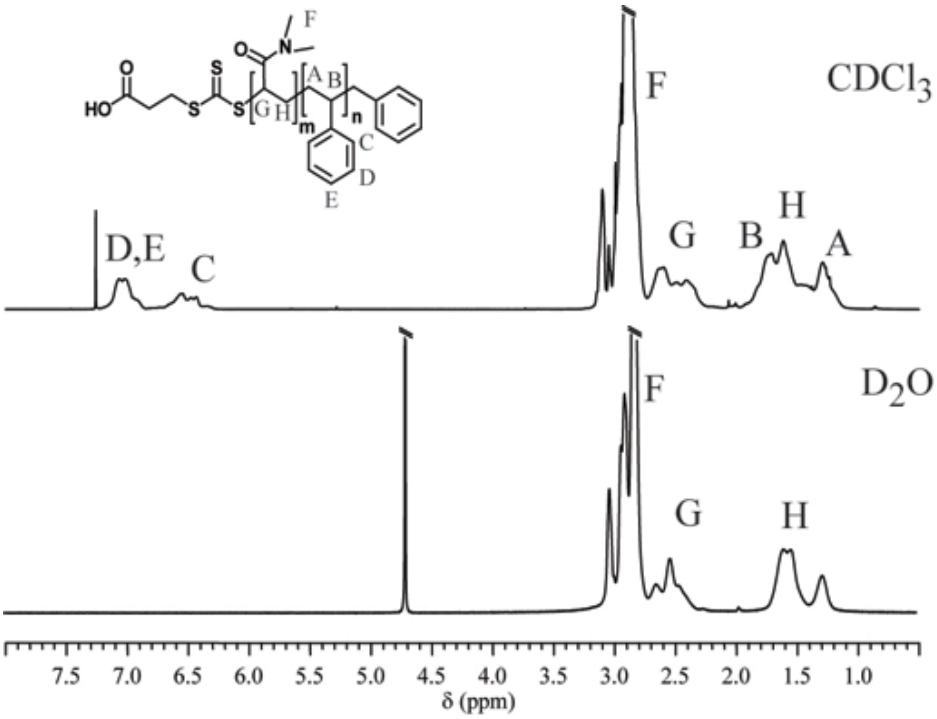


Figure S8. *NMR spectra of $PDMA_{817}$-b-PS_{105} in $CDCl_3$ and D_2O.*

11. Decay profiles of the AzeNaph1 in copolymer DMF solution as a function of the water content.

All sample were excited at 405 nm and monitored at 480 nm. A gradual change from the starting DMF solution containing the copolymer without water and the sample with 500% v/v water is clearly evident. As discussed in the text, we fitted all the decay profiles by the equation 1. We found that in the samples with $H_2O \geq 60\%$ v/v the main component of decay profiles is due to τ_{slow} (A_g~0.9) and only a marginal contribution is attributed to τ_{fast} (see for example the curve with 500% v/v water). It is worth noting that for these samples it is difficult to measure exactly τ_{fast} and, hence, the local viscosity of the environment

explored by the this small fraction of molecular rotors. For this reason, we interpreted the limiting A_g value~0.9 (estimated for sample with $H_2O \geq 60\%$ v/v) as a consequence of the presence of a small fraction of rotors in the micelle corona.

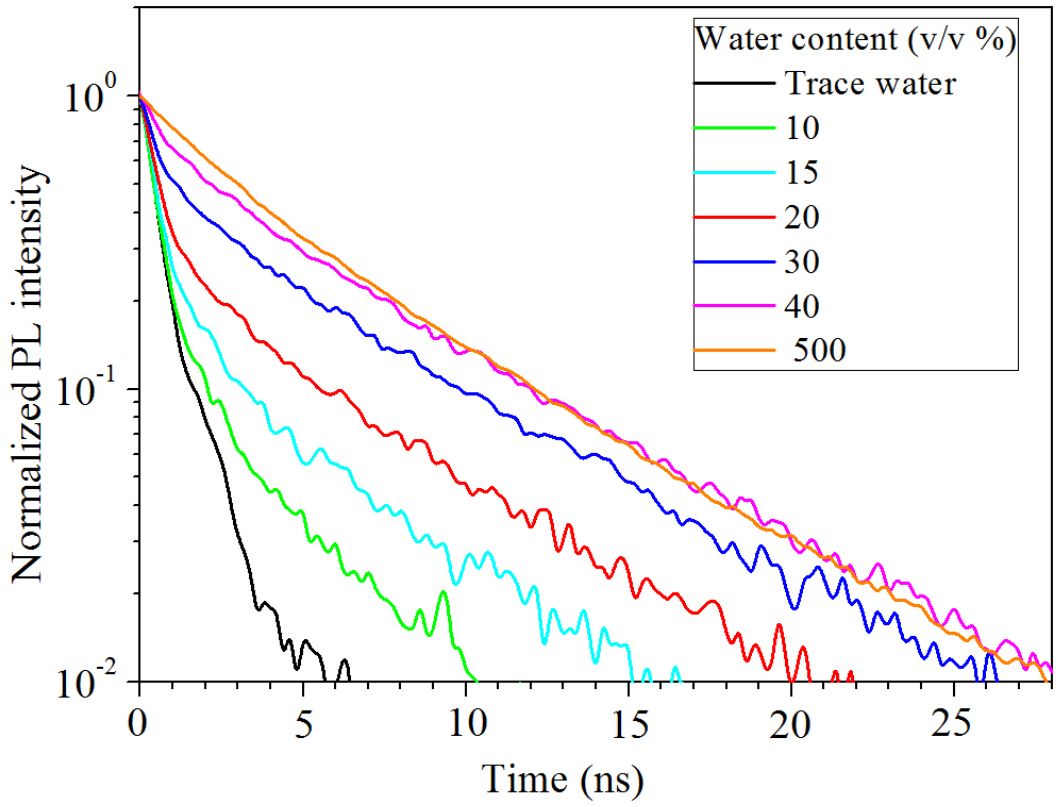


***Figure S9**. Decay profiles of the AzeNaph1 in a DMF solution containing 2% w/w of $PDMA_{817}$-b-PS_{105} at different amount of dropped water.*

12. Relative population with slow lifetime, obtained by fitting the time decay profiles of AzeNaph1 to Eq. 1 (main text), as a function of elapsed time after water addition.

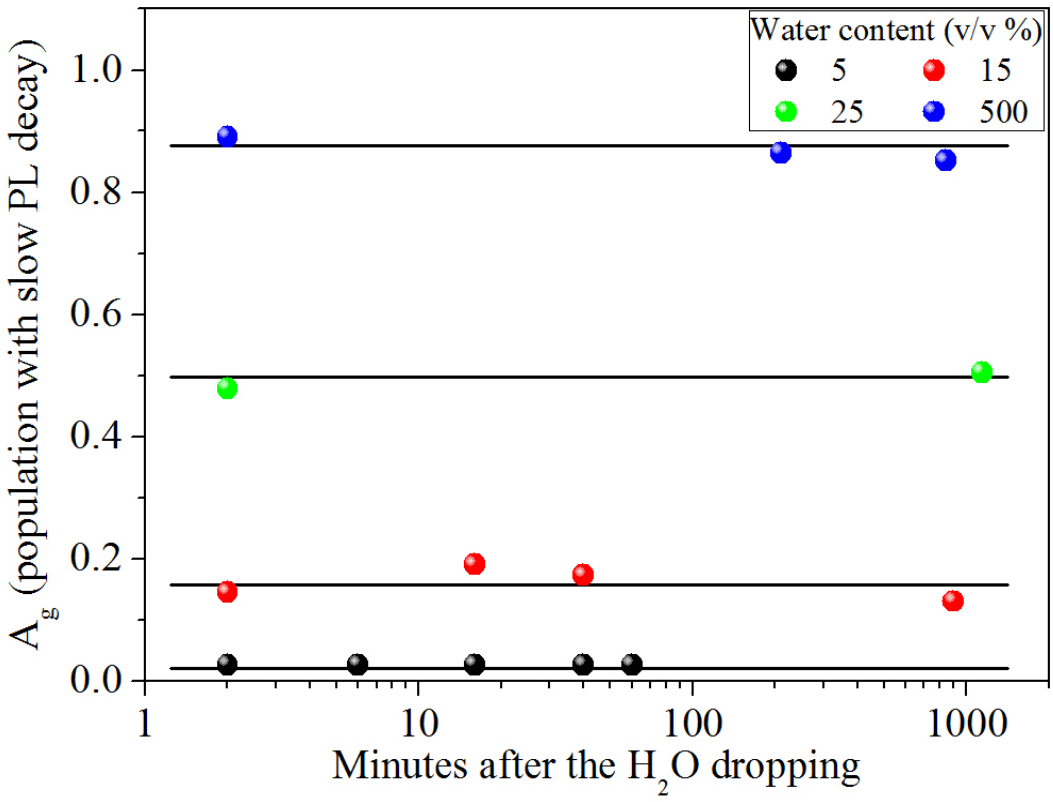
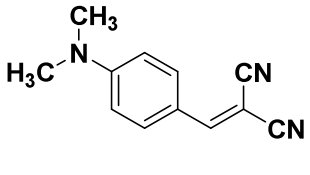


Figure S10. *Relative population with τ_{slow}, obtained by fitting the time decay profiles of AzeNaph1 to Eq. 1, as a function of elapsed time after water dropping.*

13. Thioflavive T and p-N,N-dimethylaminobenzylidenemalononitrile characterization as potential molecular rotors suitable for block-copolymer micelles characterization.

We immediately discarded Thioflavive T as a possible rotor of interest for the present study because of its complete water solubility. Conversely, although in any case at least sparingly soluble in water, p-N,N-dimethylaminobenzylidenemalononitrile (from now on **DiMeCN**) could in principle be used. Figure S11 shows **DiMeCN** emission spectrum in DMF.



DiMeCN

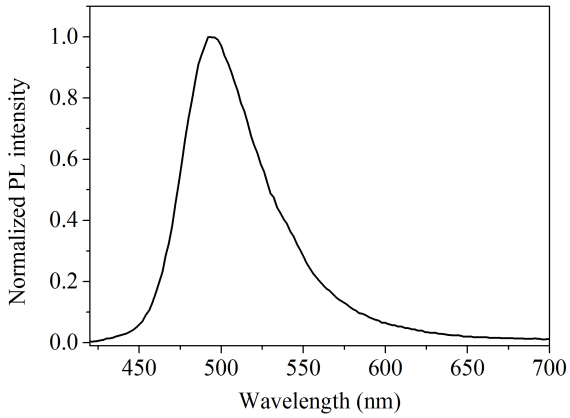


Figure S11. *Emission spectrum of a DiMeCN solution in DMF.*

Figure S12 shows the **DiMeCN** decay profile in a styrene solution (dashed line) compared with the finger response. The two signals are barely distinguishable, thus making hard the precise estimate of the probe luminescence lifetime. Its value is likely below 20 ps.

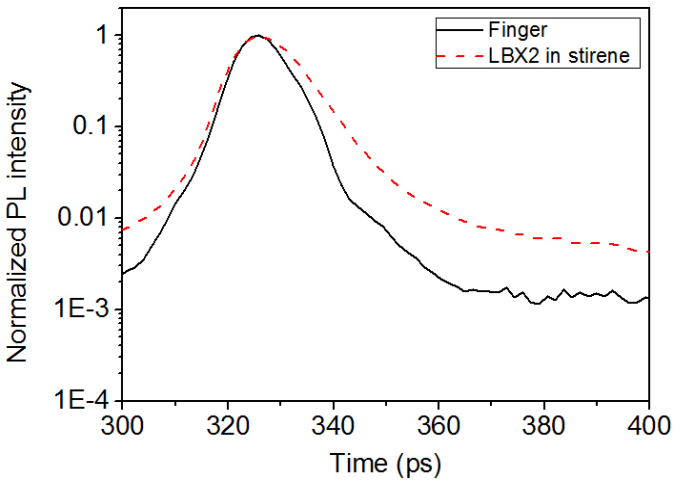


Figure S12. *Time decay profiles of the luminescence of a DiMeCN solution in styrene.*

Figure S13 shows the time decay profile (in this case acquired using a streak camera set-up) of a solid polystirene sample containing DiMeCN. The sample was prepared by bulk polymerization. Indeed, the probe shows a biexponential behaviour even if the sample is completely homogeneous and well below the corresponding glass transition temperature. As our most important finding, the capability to titrate the organized versus disorganized copolymeric chains ratio depending on the experimental conditions, requires a probe possessing a rigorously monoexponential decay in homogeneous samples, we could not use DiMeCN in our study.

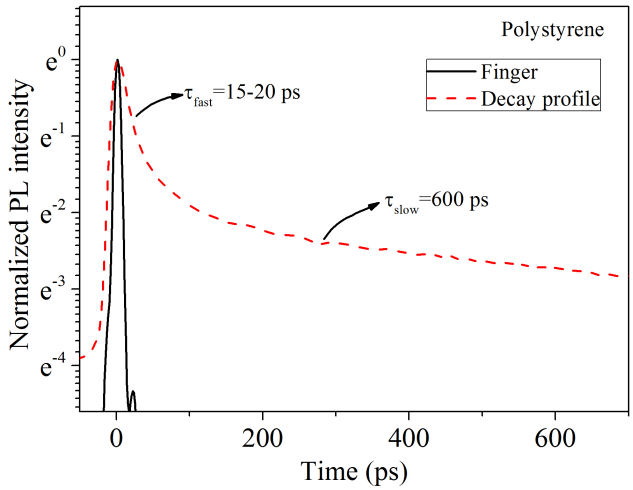


Figure S13. *Time decay profiles of the luminescence of a polystyrene sample containing DiMeCN. Sample prepared by bulk polymerization of the solution used in Figure S12.*

14. SUPPORTING INFORMATION REFERENCES

[1] M. A. Haidekker, E. A. Theodorakis, *Journal of Biological Engineering* **2010**, *4*.
[2] F. Nolde, J. Qu, C. Kohl, N. G. Pschirer, E. Reuther, K. Müllen, *Chemistry – A European Journal* **2005**, *11*, 3959-3967.
[3] J. T. Lai, D. Filla, R. Shea, *Macromolecules* **2002**, *35*, 6754-6756.
[4] a) M. Hernández-Guerrero, T. P. Davis, C. Barner-Kowollik, M. H. Stenzel, *Eur Polym J.* **2005**, *41*, 2264-2277; b) J. Skey, R. K. O'Reilly, *Chemical Communications* **2008**, 4183-4185.