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

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The detailed analysis of the time resolved luminescence of a specifically designed fluorescent molecular rotor enables the direct monitoring of the self-assembly of a poly(*N*,*N*-dimethylacrylamide)-*block*-polystyrene (PDMA-*b*-PS) copolymer into core–corona nanoparticles. Comparison with bulk PS indicates hard confinement of the micelle core, due to the solvation of the hydrophilic PDMA corona.

Amphiphilic *block* copolymers dispersed in water can self-assemble into a large variety of aggregate structures. Depending on the hydrophilic *vs.* hydrophobic monomers molar ratio, overall chain length and polydispersity, the polymers arrange as spherical micelles, vesicles, polymersomes.[1] The applications of such nanostructures range from micro/nanoelectronics and photonics to bioimaging and drug delivery.[2]

In the case of amphiphilic copolymers, the addition of excess water to a solution of the copolymer in a non-selective solvent induces the assembly of nanostructures. Established experimental methods are generally effective, but still lack a clear rationalization.[3] *In situ*, fast monitoring of the micelle formation cannot be easily accessed through common macromolecular characterization tools. Solution Nuclear Magnetic Resonance (NMR) is quite sensitive to molecular aggregation, mostly evidenced through broadening of resonance line-widths. In a core–corona micelle, however, if the core is below the glass transition (T_g) temperature, its NMR signals completely disappear as a consequence of their extreme broadening, making it thus impossible to quantify for example the molar fraction of organized *vs.* disorganized polymeric chains.[4] Conversely, Dynamic Light Scattering (DLS) provides the micelle hydrodynamic volume, but gives no information about disorganized polymers chains and self-organization dynamics. Transmission Electron Microscopy (TEM) and cryo-TEM images are extremely detailed but require removal or freezing of the solvent, sometimes with extreme alterations.[5] Finally, solution calorimetric techniques are strongly affected by the use of high thermal capacity solvents (like water), intrinsically slow and insensitive to disorganized chains.[6] The direct observation of self-assembly dynamics in terms of organized *vs.* disorganized chains at any given time and under experimental conditions is of crucial importance, particularly for the use of copolymer micelles in drug delivery. In fact, only if the core-forming block is below T_g the hydrophobic payload will be trapped by frozen polymer chains, eventually allowing selective release.[7]

Time Resolved Fluorescence Spectroscopy (TRFS) of suitable fluorescent probes gives a direct and fast access to the polymer aggregation states under various experimental conditions. For instance, the decay of excimers in pyrene labelled polymers is a phenomenon connected to the aggregation state,[8] but the need for covalent functionalization of the polymer limits its scope.[9] Molecular rotors, a class of fluorophores whose emission intensity and lifetime strongly depends on local viscosity, can be directly used – with no need for chemical functionalization – to probe the aggregation state in a variety of experimental situations.[10] Rotors are for example frequently used to study bulk polymerization dynamics[11] as well as aggregation to amyloid fibres in biomedical assays.[12]

We report here for the first time the use of TRFS of a specifically designed molecular rotor as a simple, fast and versatile method for the direct monitoring of the self-assembly of *block* copolymers in solution into core–corona micelles suitable for application in drug delivery. We show in particular that the fluorescence lifetime of our rotor is a sensitive gauge of the molar fraction of disorganized *vs.* self-assembled copolymeric chains as a function of the experimental conditions. We also show that the core of the core–corona micelles is below its T_g temperature, irrespective of its nanometric diameter.

The *block* copolymer of choice is poly(*N*,*N*-dimethylacrylamide)-*block*-polystyrene (PDMA-*b*-PS) obtained by reversible addition–fragmentation chain transfer (RAFT) polymerization.[13] The fine tuning of reaction conditions (see ESI† for synthesis and characterization of the PDMA-*b*-PS copolymer) enabled the preparation of the target polymer $PDMA_{817}$-*b*-PS_{105}, a molar ratio suitable for the formation of core–corona micelles (Scheme 1a). The fluorescent probe of choice is the donor–acceptor derivative **AzeNaph1** (Scheme 1b) whose

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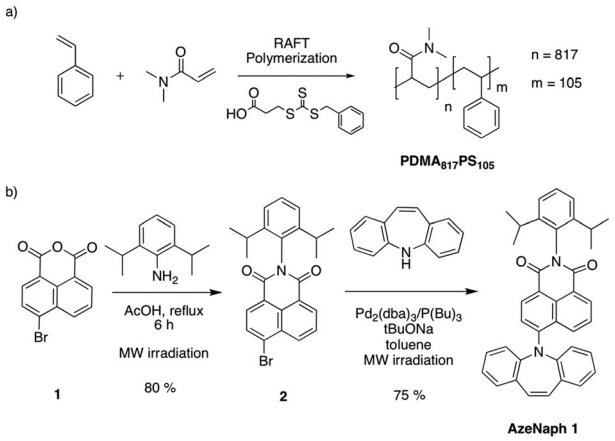
† Electronic supplementary information (ESI) available: Synthesis and NMR characterization of all new materials. Details on DLS and TEM characterization, details on the luminescence behaviour of rotors. See DOI: 10.1039/c3cc44590a



Scheme 1 (a) Synthesis of PDMA-*b*-PS. (b) Synthesis of **AzeNaph1**.

fluorescence lifetime strongly depends on the rotation of its electron donating and rigid dibenzoazepine residue with respect to the electron deficient naphthalene imide residue. Such rotation is progressively hindered upon increase of the local viscosity and is eventually almost stopped in a rigid system. We designed this particular structure in order to provide at the same time negligible water solubility (common rotors like thioflavine and 2-(4-(dimethylamino)benzylidene)malononitrile[12] are at least partially water soluble, see ESI† for details), sensitivity at the high viscosity limit, high Stokes shift (to limit re-absorption) and stability.

Photophysics of **AzeNaph1** is especially suitable for studying nanoparticle formation processes. First of all, its fluorescence lifetime is extremely sensitive to the environment, ranging from hundred ps for liquids and polymers above T_g to 6–8 ns for glassy polymers, with a lifetime characteristic of the employed polymer (see ESI† for details). Just by recording the rotor luminescence lifetime as a function of the polymerization time, it is possible to easily follow the bulk polymerization kinetics of styrene into polystyrene (see Fig. S2, ESI†).

Even more interestingly, the emission decay of **AzeNaph1** behaves as a perfect single exponential in homogeneous samples. The decay is fast in liquid samples, slow in solid ones but always well fitting in a monoexponential law. This is not a common characteristic of standard molecular rotors. For example, both thioflavine and 2-(4-(dimethylamino)benzylidene)malononitrile show a biexponential decay under all experimental conditions (see ESI†). This is the reason why in all previous studies of polymerization kinetics based on the luminescence of a probe, authors mainly focused on the luminescence intensity instead of lifetime.[10,11] As shown in the following, time resolved luminescence is a more appropriate and informative technique, particularly in the case of inhomogeneous samples like core–corona micelles.

During its self-assembly, the *block* copolymer we prepared goes from a random coil configuration to a core–corona structure having a dense, stiff PS core and a swelled PDMA corona. Such behaviour is clearly evidenced by DLS, TEM and NMR characterization. In short, we promoted the self-assembly by the slow addition of deionized water to a copolymer solution in dimethylformamide (DMF). DLS analysis shows that the micelles had a hydrodynamic radius (R_h) of 62 nm (Fig. 1, left). The TEM image (Fig. 1, right) obtained after solvent evaporation shows the PS cores as black spots of 8–10 nm radius whilst the PDMA coronas are disorganized as a consequence

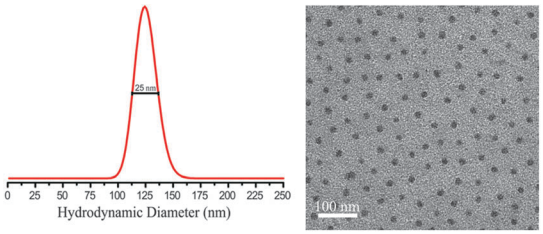


Fig. 1 (left) DLS spectrum of $PDMA_{817}$-*b*-PS_{105} dispersed in water. (right) TEM image of the $PDMA_{817}$-*b*-PS_{105} copolymer.

of solvent evaporation. No contrast reagents were employed, the contrast being connected with the higher density of the closely packed polystyrene cores with respect to the disorganized PDMA surrounding. Finally, the ^{1}H NMR spectrum of the copolymer in $CDCl_3$ (Fig. S8, ESI†) shows all the signals of both blocks, whilst in D_2O, only PDMA signals can be distinguished as those of PS are broadened by their aggregation in the micelle core.[4]

All techniques confirm the formation of core–corona micelles in the presence of excess water. None of them however gave us hints on the self-assembly dynamics and on the minimum amount of water required to complete the process.

We repeated the micelles preparation, in this case starting from a copolymer–**AzeNaph1** (99.9 : 0.1 weight ratio) solution in DMF. We monitored the fluorophore emission lifetime as a function of the water added from 5% v/v to 500% v/v. After each aliquot the sample was equilibrated for 2 minutes (as shown in the ESI,† after 2 min resting time the sample emission behaviour is constant). Fig. 2 shows the **AzeNaph1** photoluminescence (PL) decay profile in pure DMF (blue curve), at 20% v/v (red curve) and 500% v/v (black curve) water content.

The green curve indicates the **AzeNaph1** decay profile in bulk PS. First of all, **AzeNaph1** PL decay at 500% v/v water is superimposable with that in bulk PS. This demonstrates that the local environment experienced by the probe in micelles is indistinguishable from bulk PS, in agreement with DLS, TEM and NMR characterization discussed before. This is also a clear indication that in the presence of excess water all of the rotor molecules are embedded in the nanoparticle core. In the presence of a large excess of water all of the polymers assume a core–corona structure. The rotor, completely insoluble in water, loads in the rigid apolar PS core behaving

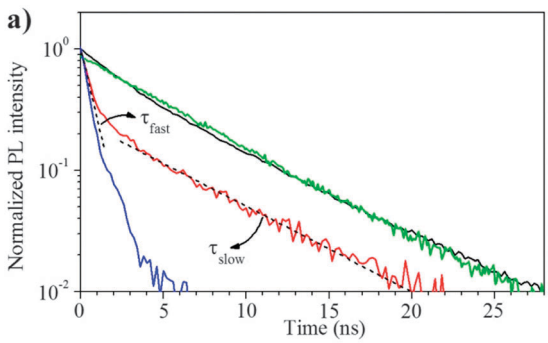


Fig. 2 Time decay profile of **AzeNaph1** in DMF solution containing the $PDMA_{817}$-*b*-PS_{105} copolymer before addition of water (blue curve) and after addition of 20% v/v (red curve) and 500% v/v (black curve) water. The decay profile of **AzeNaph1** dispersed in a polystyrene bulk sample (green curve) is shown as a reference.

accordingly. Conversely, the probe decay in pure DMF is very fast. The red curve shows that at intermediate water content (20%) the probe decay can be fitted in terms of a biexponential decay having a fast component identical to that measured in DMF and a slow one corresponding to that observed in bulk PS. The probe is in this case partially loaded into the rigid cores of micelles and partially dispersed in solution along with the still disorganized copolymer chains. All of the exponential decays obtained for water contents going from 1 to 60% v/v can be fitted in terms of a biexponential decay having the same fast and slow components. We fitted all the normalized data by means of eqn (1),

$$\mathrm{PL}(t) = A_g e^{-\frac{t}{\tau_{slow}}} + (1 - A_g) e^{-\frac{t}{\tau_{fast}}} \quad (1)$$

where PL(t) is the time dependent PL intensity, τ_{fast} = 0.8 ns and τ_{slow} = 5.8 ns are the **AzeNaph1** lifetime in solution and in bulk PS, respectively. The pre-exponential factor A_g corresponds to the fraction of slow decaying probes, which, in the case of complete assembly, is expected to be 1.

Indeed, Fig. 3 shows that the Ag *vs.* water content plot assumes the form of a titration curve, giving in real time for any specific water content the amount of organized *vs.* disorganized polymer chains. It should be noted that such analysis greatly profits from the peculiar emission properties of **AzeNaph1**. As mentioned previously, our rotor features simple monoexponential decay in all homogeneous samples and a biexponential decay only in the heterogeneous ones. The other commercially available rotors we tested (see ESI†) follow biexponential decay in both homogeneous and heterogeneous samples, thus making data interpretation significantly more complicated.

Our analysis leads to three relevant results. First of all, we clearly show that a 60% water content is enough to induce the complete self-organization of all polymer chains. The large excess of water, prescribed by well-established literature procedures, in our case is unnecessary. Secondly, the self-assembly is a fast process. In all of the cases, the PL signal measured was completely constant for 2 minutes after the addition of water (see Fig. S10 of the ESI†). Finally, our characterization clearly shows that the PS core (whose radius is below 10 nm) is below its T_g. This finding apparently disagrees with the work of Priestley and coworkers,[14] showing that the T_g of PS nanoparticles is size dependent below 100 nm of radius. The case of core–corona nanoparticles is different. The swollen PDMA external corona of the micelles induces a "hydrostatic" pressure on the core surface. This pressure brings the core to the "hard confinement" limit, discussed by the same authors in the case of silica capped PS nanoparticles.[15] In this case the T_g is no longer size dependent. This is a very relevant finding as it ensures that a drug loaded in the micelle core will not be able to escape, through diffusion in a low viscosity environment, prior to selective delivery to the appropriate target.

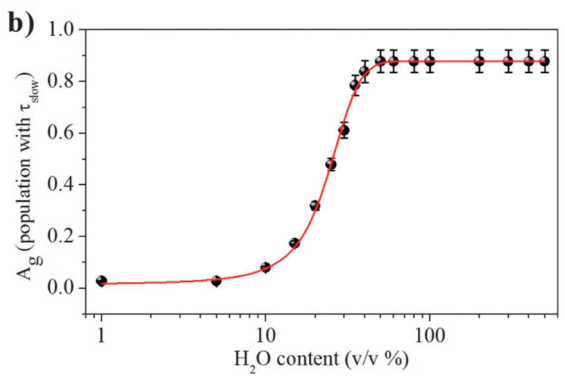


Fig. 3 Relative population with τ_{slow}, obtained by fitting the time decay profiles of **AzeNaph1** to eqn (1), as a function of the H_2O added.

In conclusion, we successfully exploited the time resolved fluorescence decay of a suitable molecular probe to characterize relevant features of the self-assembly of amphiphilic *block* copolymers in water solution: minimum amount of water required to obtain complete self-assembly, minimum equilibration time of the dispersion after changing the composition, and aggregation state of the nanoparticle core. Our analysis is simple, quantitative, fast and non-destructive. These results may support the preparation strategies of self-assembly of amphiphilic *block* copolymers for application in drug delivery, a research field actually particularly active and technologically relevant.

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Notes and references

1 E. B. Zhulina and O. V. Borisov, *Macromolecules*, 2012, **45**, 4429–4440.
2 H.-C. Kim, S.-M. Park and W. D. Hinsberg, *Chem. Rev.*, 2010, **110**, 146–177.
3 H. Huang, B. Chung, J. Jung, H. W. Park and T. Chang, *Angew. Chem., Int. Ed.*, 2009, **48**, 4594–4597.
4 (*a*) J. S. Hrkach, M. T. Peracchia, A. Domb, N. Lotan and R. Langer, *Biomaterials*, 1997, **18**, 27–30; (*b*) C. R. Heald, S. Stolnik, K. S. Kujawinski, C. De Matteis, M. C. Garnett, L. Illum, S. S. Davis, S. C. Purkiss, R. J. Barlow and P. R. Gellert, *Langmuir*, 2002, **18**, 3669–3675; (*c*) G. M. Wilmes, D. J. Arnold and K. S. Kawchak, *J. Polym. Res.*, 2011, **18**, 1787–1797; (*d*) K. Vijayakrishna, D. Mecerreyes, Y. Gnanou and D. Taton, *Macromolecules*, 2009, **42**, 5167–5174.
5 T. Azzam and A. Eisenberg, *Langmuir*, 2010, **26**, 10513–10523.
6 (*a*) C. J. Lai, W. B. Russel and R. A. Register, *Macromolecules*, 2002, **35**, 841–849; (*b*) J. M. Virgili, A. Hexemer, J. A. Pople, N. P. Balsara and R. A. Segalman, *Macromolecules*, 2009, **42**, 4604–4613.
7 (*a*) K. T. Kim, J. Cornelissen, R. J. M. Nolte and J. C. M. van Hest, *Adv. Mater.*, 2009, **21**, 2787–2791; (*b*) W. Scarano, H. T. T. Duong, H. X. Lu, P. L. De Souza and M. H. Stenzel, *Biomacromolecules*, 2013, **14**, 962–975; (*c*) S. Hocine, D. Cui, M. N. Rager, A. Di Cicco, J. M. Liu, J. Wdzieczak-Bakala, A. Brulet and M. H. Li, *Langmuir*, 2013, **29**, 1356–1369.
8 F. M. Winnik, *Chem. Rev.*, 1993, **93**, 587–614.
9 (*a*) M. Wilhelm, C. L. Zhao, Y. C. Wang, R. L. Xu, M. A. Winnik, J. L. Mura, G. Riess and M. D. Croucher, *Macromolecules*, 1991, **24**, 1033–1040; (*b*) S. C. Lee, Y. K. Chang, J. S. Yoon, C. H. Kim, I. C. Kwon, Y. H. Kim and S. Y. Jeong, *Macromolecules*, 1999, **32**, 1847–1852; (*c*) R. Rutkaite, L. Swanson, Y. Li and S. P. Armes, *Polymer*, 2008, **49**, 1800–1811; (*d*) M. M. Mok and T. P. Lodge, *J. Polym. Sci., Part B: Polym. Phys.*, 2012, **50**, 500–515.
10 (*a*) M. A. Haidekker and E. A. Theodorakis, *J. Biol. Eng.*, 2010, 4; (*b*) K. Y. Law, *Chem. Phys. Lett.*, 1980, **75**, 545–549; (*c*) Y. Shiraishi, T. Inoue and T. Hirai, *Langmuir*, 2010, **26**, 17505–17512.
11 R. O. Loufty, *Pure Appl. Chem.*, 1986, **58**, 1239–1248.
12 N. Amdursky, Y. Erez and D. Huppert, *Acc. Chem. Res.*, 2012, **45**, 1548–1557.
13 (*a*) G. Moad, E. Rizzardo and S. H. Thang, *Polymer*, 2008, **49**, 1079–1131; (*b*) K. H. Wong, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Polymer*, 2007, **48**, 4950–4965.
14 R. D. Priestley, C. J. Ellison, L. J. Broadbelt and J. M. Torkelson, *Science*, 2005, **309**, 456–459.
15 C. Zhang, Y. L. Guo and R. D. Priestley, *Macromolecules*, 2011, **44**, 4001–4006.

