

**A Pyrenyl Derivative with a Four Atom-Linker
that Can Probe the Local Polarity of Pyrene-Labeled Macromolecules**

Shiva Farhangi, Jean Duhamel*

Institute for Polymer Research, Waterloo Institute for Nanotechnology, Department of
Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, ON N2L 3G1,
Canada

* To Whom correspondence should be addressed

ABSTRACT

The fluorescent probe 1-pyrenemethoxyethanol (PyMeEGOH) was designed to replace commercially available 1-pyrenebutanol (PyButOH) as an alternative fluorescent label to probe the internal dynamics and interior polarity of macromolecules by steady-state and time-resolved fluorescence. While excimer formation and sensitivity to solvent polarity are two well recognized properties of pyrene, much less known is that these properties are often mutually exclusive when a 1-pyrenebutyl derivative is used to prepare pyrene-labeled macromolecules (PyLMs). As the sensitivity of pyrene to solvent polarity is a result of its symmetry, attaching a butyl group to pyrene breaks the symmetry of pyrene so that the 1-pyrenebutyl derivatives are much less sensitive to the polarity of their environment compared to unmodified pyrene. This report demonstrates that replacement of a methylene group in the β -position of PyButOH by an oxygen atom such as in PyMeEGOH restores the sensitivity of this pyrene derivative to the polarity of its local environment to the same level as that of molecular pyrene without impeding pyrene excimer formation upon incorporation into PyLMs.

INTRODUCTION

As stated by F. M. Winnik in 1993¹ and supported by more recent reviews,²⁻⁴ “pyrene is by far the most commonly used dye to study macromolecules” due to its ability to form excimer readily upon encounter between an excited and a ground-state pyrene and its sensitivity to the polarity of its local environment. Interactions of pyrene-labeled macromolecules (PyLMs) with themselves and other macromolecules,⁵⁻⁸ surfactants or surfactant micelles,⁹⁻¹² or latex particles¹³⁻¹⁵ lead to changes in the polarity of the local environment of the pyrene probe and its ability to form an excimer. Both effects can be quantified from the ratio of the fluorescence intensity of the excimer over that of the monomer, namely the I_E/I_M ratio, to characterize the efficiency of pyrene excimer formation^{1-4,16} or the ratio of the fluorescence intensity of the first band in the fluorescence spectrum of the pyrene monomer over that of the third band, namely the I_1/I_3 ratio, used to describe the medium polarity.¹⁷⁻¹⁹ While these two features have been well advertised in the scientific literature,^{1-4,16-19} much less mentioned is the fact that the ability of pyrene to probe the polarity of its local environment is strongly reduced upon labeling with one of the many commonly used 1-pyrenebutyl derivatives. Since the variation in the I_1/I_3 ratio with the polarity of the local environment is due to the fact that the band corresponding to the I_1 intensity in the fluorescence spectrum of pyrene is symmetry forbidden, the loss in symmetry induced by the chemical modification of pyrene to generate the 1-pyrenebutyl derivatives is associated with a substantial sensitivity loss of the I_1/I_3 ratio to polarity. This report demonstrates that 1-pyrenemethoxyethanol (PyMeEGOH) which is the chemical equivalent of 1-pyrenebutanol (PyButOH) bare the oxygen atom in the β -position of the spacer can be used as conveniently as PyButOH to label a macromolecule and study its internal dynamics by pyrene excimer fluorescence, but contrary to PyButOH, can probe the local environment of a macromolecule as reliably as molecular pyrene.

This work confirms the insight of earlier reports which suggested but never conclusively demonstrated that introduction of a heteroatom in the β -position of an alkyl substituent of pyrene re-symmetrizes its electronic wavefunction and restores the sensitivity of pyrene to solvent polarity.^{20,21}

EXPERIMENTAL

Materials: 2-Iodoethanol, 1-pyrenemethanol, triethylamine, methacryloyl chloride, butyl methacrylate, and ethylene glycol anhydrous were purchased from Sigma-Aldrich (Reagent Grade). Distilled in glass tetrahydrofuran (THF), toluene, cyclohexane, 2-propanol, and acetonitrile were provided by Caledon Laboratories. HPLC grade ethanol and ethyl acetate were supplied by Fisher Scientific. HPLC grade acetone, 2-butanone, butylalcohol, benzyl alcohol, cyclopentanone, 1,2-dichloroethane, DMF, DMSO, methanol, and o-xylene were obtained from Sigma-Aldrich. HPLC grade hexane and dichloromethane (DCM) were purchased from OmniSolv. All chemicals were used as received.

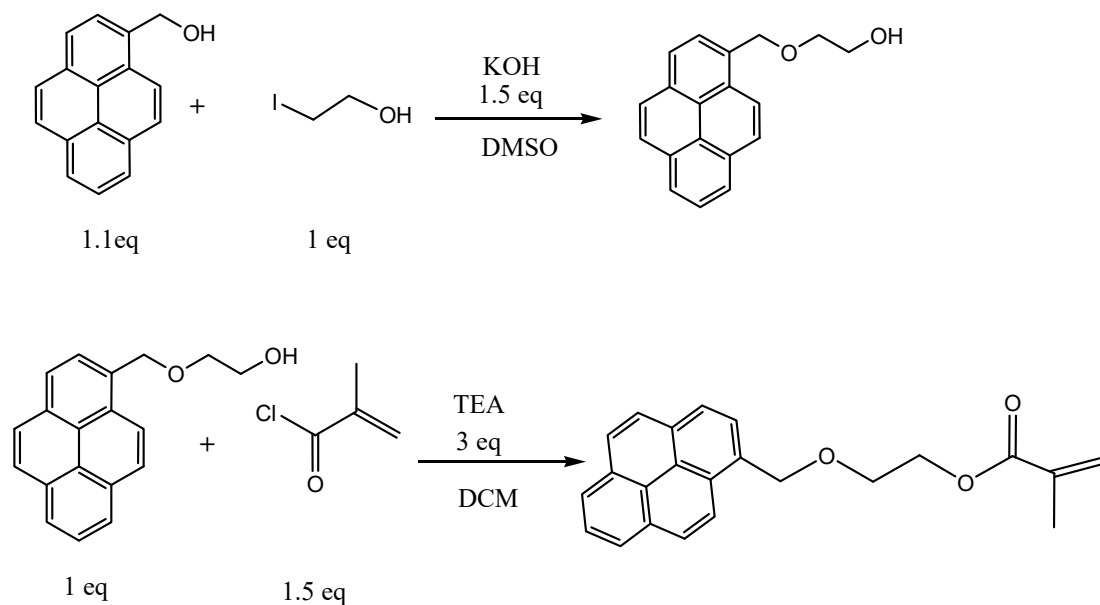
Synthesis of 2-(1-pyrenemethoxy)ethanol (PyMeEGOH): 1-Pyrenemethanol (1.50 g, 6.46 mmol) and ground potassium hydroxide (KOH) (0.50 g, 8.9 mmol) were dissolved in 25 mL of dimethyl sulfoxide (DMSO) and transferred to a 100 mL round bottom flask under N₂ flow. The solution was purged with N₂ for 30 minutes. 2-Iodoethanol (1.00 g, 5.82 mmol) was added drop wise to the reaction mixture. The solution was stirred under N₂ at room temperature for 24 hrs. After the reaction was complete, the solution was washed with 0.5 M HCl, a saturated sodium chloride aqueous solution, and water in that sequence. The solvent was removed with a rotary evaporator. The yellow remaining crude product was dissolved in a minimum amount of DCM before being purified by silica gel column using a 55:45 ethyl acetate:hexane mixture. The solid PyMeEGOH

was then re-crystallized using a 1:10 ethyl acetate:hexane mixture and a pale-yellow solid was obtained in a 38% yield (mp = 65-68 °C).

300 MHz ^1H NMR (DMSO- d_6) *PyMeEGOH*: δ 3.56-3.7 (m, 4H, $-\text{CH}_2\text{-CH}_2\text{-OH}$), δ 4.6 (t, 1H, OH), δ 5.2 (s, 2H, $-\text{CH}_2\text{-O}$), δ 7.9-8.4 (m, 9H, Py H's). ^1H NMR spectrum of *PyMeEGOH* is shown in Figure S1 in Supporting Information (SI).

Synthesis of 2-(1-pyrenemethoxy) ethyl methacrylate (PyMeEG-MA): *PyMeEGOH* (1.00 g, 4.31 mmol) was dissolved in the presence of distilled triethylamine (1.30 g, 13 mmol) in 20 mL of dichloromethane (DCM) and transferred to a 50 mL round bottom flask. The solution was cooled down to 5 °C and purged with N_2 for 20 minutes. Methacryloyl chloride (0.68 g, 6.45 mmol) was added drop wise. The solution was stirred under N_2 at room temperature for 24 hrs. When the reaction was complete, the reaction mixture was washed with 0.5 M HCl, saturated sodium carbonate aqueous solution, and water in that sequence. A rotary evaporator was used to remove the solvent. The remaining crude product was yellow and it was dissolved in a minimum amount of DCM before being purified by silica gel column using a 60:40 DCM:hexane mixture. The solid was then re-crystallized in methanol to obtain a pale-yellow solid in 85% yield (mp = 42-45 °C). The overall synthetic procedure is shown in Scheme 1.

300 MHz ^1H NMR (CDCl_3) for *PyMeEG-MA*: δ 1.89 (s, 3H, CH_3 -), δ 3.8-4.4 (m, 4H, $\text{O-CH}_2\text{-CH}_2\text{-O}$), δ 3.39 (t, 2H, Py-CH_2 -), δ 5.5 (s, 1H, $=\text{CH}_2$), δ 6.1 (s, 1H, $=\text{CH}_2$), δ 7.9-8.4 (m, 9H, Py H's). ^1H NMR spectrum of *PyMeEG-MA* is shown in Figure S2.

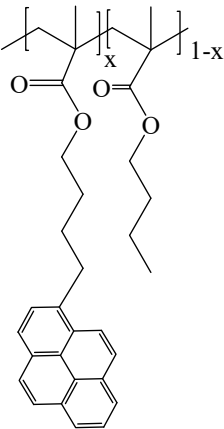
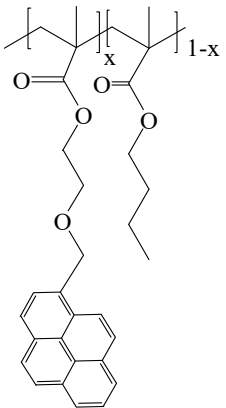


Scheme 1. Synthetic procedure applied to prepare PyMeEG-MA.

Random copolymerization: The pyrene-labeled poly(butyl methacrylate)s (Py-PBMAs) were prepared by radical copolymerization of 1-pyrenebutyl methacrylate (PyBut-MA) or PyMeEG-MA with butyl methacrylate (BMA) to yield PyBut-PBMA or PyMeEG-PBMA, respectively. Their chemical structure is shown in Table 1. The overall number of moles of BMA was kept constant while the ratio of PyBut-MA or PyMeEG-MA to BMA monomer was altered to achieve different molar percentages of pyrene contents ranging between 1 and 10 mol%. The copolymerization was carried out in an oil bath at 65 °C using AIBN as the initiator. The mono methyl ether hydroquinone (MEHQ) stabilizer present in the BMA supplied by Sigma-Aldrich was removed by filtration through a column filled with MEHQ inhibitor-remover beads (Aldrich). The reaction mixture was outgassed for 20 minutes with a gentle flow of nitrogen to remove oxygen, a well-known inhibitor of radical polymerization. The copolymerization was stopped at a conversion of 0.2 or less to avoid any possible compositional drift during the copolymerization. ^1H NMR was used to follow the conversion as a function of reaction time. Once the desired conversion was achieved, the reaction vessel was placed on ice and the polymer was precipitated

in methanol, re-dissolved in THF, and re-precipitated in methanol 5-7 times to remove any unreacted PyMeEG-MA monomer. The final yield was approximately 10% in each copolymerization. A same protocol described in an earlier publication was applied to prepare the PyBut-PBMA samples.²²

Table 1. Chemical structure, pyrene content, absolute M_n , and PDI values of the PyBut_PBMA and PyMeEG-PBMA samples.

Chemical Structure PyBut-PBMA	x mol %	M_n kg/mol	\bar{D}	Chemical Structure PyMeEG-PBMA	x , mol %	M_n kg/mol	\bar{D}
	0.6	163	1.9		0.3	135	2.0
	2.2	296	1.4		1.8	160	1.8
	3.0	197	1.4		2.7	117	2.0
	3.6	264	1.7		3.8	100	2.2
	5.3	275	2.0		4.6	190	1.7
	7.2	416	1.8		5.4	303	1.5

Molecular weight determination: The absolute molecular weight of the polymers was determined using Gel Permeation Chromatography (GPC) with a Viscotek instrument equipped with a 305 Triple Detector Array that combined a refractive index (DRI), viscosity, and UV-Vis absorption detectors. The molecular weight distribution of the samples was obtained using a flow rate of 1 mL/min of THF at 35 °C passing through three 300×8 mm² PolyAnalytik SuperesTM linear mixed bed columns with molecular weight cut off of 400×10³, 4×10⁶, and 20×10⁶. Typical GPC traces collected with a PyMeEG-PBMA sample having a pyrene content of 3.8 mol% (PyMeEG(3.8)-

PBMA) are provided in Figure S3 in SI. GPC analysis was also applied to establish that the sample of pyrene-labeled PBMA was free of any unreacted pyrene-labeled monomer. Pyrene content, absolute number-average molecular weight (M_n), and the polydispersity indices (PDIs) of all samples studied in this report have been listed in Table 1.

Pyrene content determination: The pyrene content of the samples was determined by UV-Vis spectroscopy with a Varian Cary 100 Bio spectrophotometer. The sharp absorption peak at 344 nm of the pyrene derivatives was used to calculate the pyrene content λ_{Py} of the Py-PBMA samples. To this end, the Py-PBMA samples were dissolved in THF to generate a polymer solution of known massic concentration [Poly] in g/L. After diluting the polymer solution to bring the absorbance of the pyrene label at 344 nm below 2.0, the molar concentration of pyrene [Py] in mol/L was determined from the molar absorption coefficient of 1-PyButOH and 1-PyMeEGOH found to equal 42,000 and 45,000 $M^{-1}cm^{-1}$ in THF, respectively. λ_{Py} was obtained by taking the ratio [Py]/[Poly] from the diluted polymer solutions used to acquire the absorption spectra. The molar fraction of pyrene labeled monomer in the polymer expressed as (x) could be determined using Equation 1 where M and M_{Py} represent the molar mass of the unlabeled and pyrene-labeled monomer, respectively.

$$x = \frac{M}{M - M_{Py} + 1 / \lambda_{Py}} \quad (1)$$

In Equation 1, M for BMA equals 142 g/mol and M_{Py} equals 342 or 344 g/mol for PyBut-MA or PyMeEG-MA, respectively.

Steady-state fluorescence measurements: Steady-state fluorescence spectra were acquired on a Photon Technology International LS-100 equipped with an Ushio UXL-75 Xenon lamp and a PTI 814 photomultiplier detection system. The spectra of all pyrene derivatives were obtained using the right angle geometry. Firstly the solution was diluted down to an optical density of ~ 0.1 corresponding to a pyrene concentration of $\sim 2.5 \times 10^{-6}$ M that is low enough to avoid any possible interpolymeric interaction. The solution was deoxygenated with a gentle flow of N_2 for approximately 30 minutes in order to remove oxygen which is a known quencher of pyrene fluorescence. The degassed solution was sealed and excited at 344 nm. The emission spectrum was acquired from 350 to 600 nm. The fluorescence intensity of the monomer (I_M) and excimer (I_E) were calculated by integrating the fluorescence spectrum from 372 to 378 nm and from 500 to 530 nm, respectively. The I_E/I_M ratio is a qualitative measure of the efficiency of excimer formation for the pyrene-labeled polymer. The ratio I_1/I_3 was obtained by taking the ratio of the peak intensity at about 375 nm over that at about 386 nm. The exact peak position varied slightly depending on the organic solvents used to prepare the Py-PBMA solutions.

Time-resolved fluorescence measurements: The solutions that were prepared to acquire the fluorescence spectra were used to acquire the fluorescence decays. An IBH Ltd. time-resolved fluorometer equipped with an IBH 340 nm NanoLED was used to excite the solutions at 344 nm. The monomer and excimer fluorescence decays were acquired by monitoring the emission at 375 and 510 nm with a cut off filter of, respectively, 370 and 480 nm, to minimize the detection of light scattered by the solution.

Fluorescence Decay Analysis – The Fluorescence Blob Model. The fluorescence decays of the pyrene monomer and excimer were fit globally according to the Fluorescence Blob Model (FBM) based on equations that have been derived earlier and which are provided as supporting

information (see Equation S1-S2 in SI).²³⁻²⁵ More background information on the FBM has been provided in SI. The FBM retrieves the molar fraction f_{diff} , f_{k2} , f_{free} , f_{E0} , and f_{EL} of all the pyrene species in solution. These are the pyrenes that are, diffusing in the solution according to the motion of the structural units of the polymer that they are bounded to (Py_{diff}), close to each other and rearrange rapidly with a rate constant k_2 (Py_{k2}), isolated in pyrene-poor domains of the PyLM where they cannot form excimer and emit with the lifetime of the pyrene derivative τ_M (Py_{free}), present as a properly stacked ground-state dimer that can generate an excimer upon direct excitation ($E0$) that emits with a lifetime τ_{E0} , and found as improperly stacked dimer that leads upon direct excitation to the formation of a long-lived excited dimer (EL) that emits with a lifetime τ_{EL} .

Within the framework of the FBM, the polymer coil is divided into a cluster of identical *blobs*. Diffusive motion of Py_{diff} is described by the three parameters $\langle n \rangle$, k_{blob} , and $k_e \times [blob]$ which are the rate constant of encounter between two structural units bearing a pyrene label which are both inside a same *blob*, the average number of pyrenes per *blob*, and the product of the rate constant of exchange of pyrene moieties between neighboring *blobs* in the polymer coil. The parameters were optimized with the Marquardt Levenberg–algorithm.²⁶ Fits were deemed satisfactory if the χ^2 value was smaller than 1.3 and the residuals and autocorrelation function of the residuals were randomly distributed around zero.

RESULTS AND DISCUSSION

Pyrene derivatives are ubiquitous fluorescent probes used to provide information about the internal dynamics of macromolecules or the polarity of their microenvironment. Consequently, the characterization of a new pyrene derivative such as PyMeEGOH developed for the fluorescent

labeling of macromolecules must include a study of its response to solvent polarity and ability to report on the internal dynamics of macromolecules. To this end, the steady-state fluorescence spectra of PyMeEGOH and PyButOH were acquired in 21 solvents covering a wide range of dielectric constants between 1.9 for hexane and 78.5 for water. They are shown in Figure 1. The fluorescence spectra were normalized to an arbitrary value of 100 at 375 nm which corresponds to the 0-0 transition of pyrene.

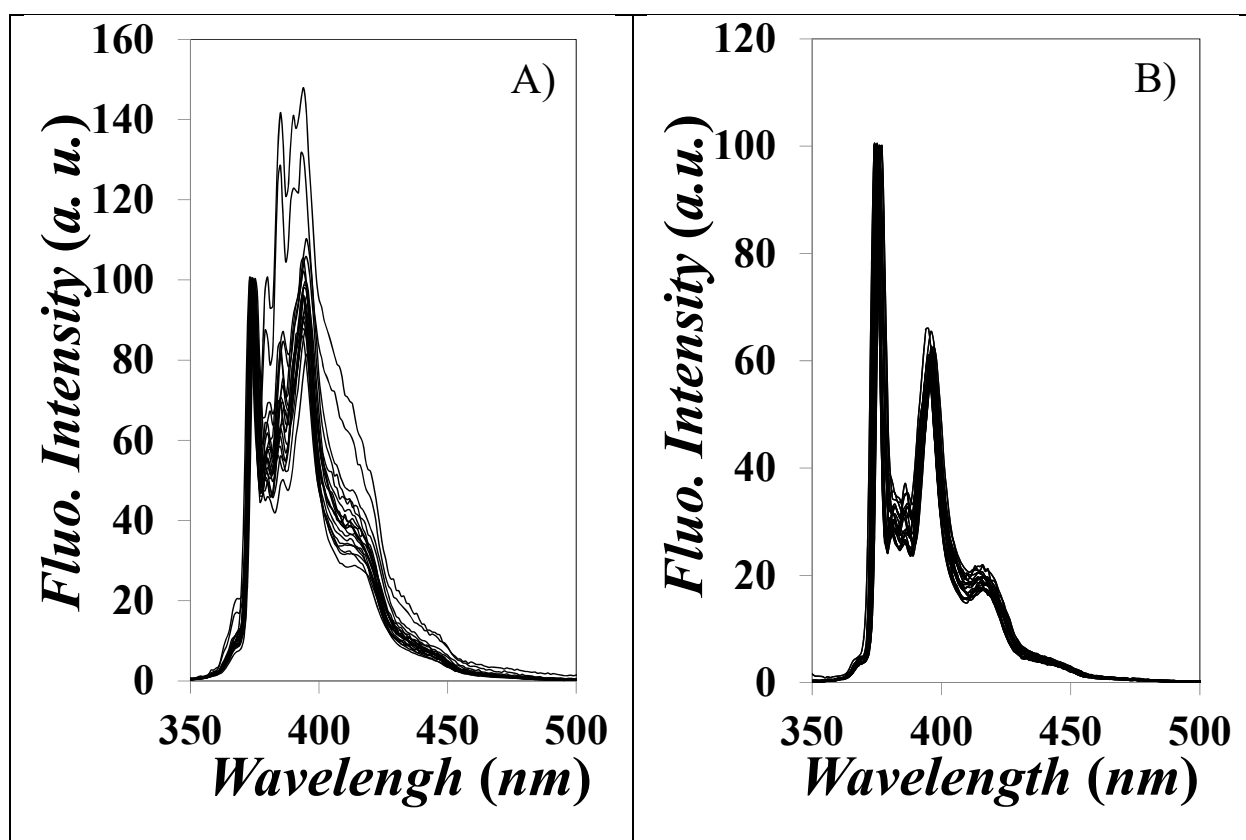


Figure 1. Steady-state fluorescence spectra of A) PyMeEGOH and B) PyButOH in the 21 solvents listed in Table 2. The solvent polarity increases from top (hexane) to bottom (DMSO) in Figure 1A). $[Py] = 2.5 \times 10^{-6}$ M, $\lambda_{ex} = 344$ nm.

The I_1/I_3 ratio for each steady-state fluorescence spectrum was used to characterize the response of the pyrene derivatives to solvent polarity. Rapid visual inspection of Figure 1A indicates that the spectral features of PyMeEGOH, and thus the I_1/I_3 ratios, change substantially with solvent polarity but that these changes are much smaller for PyButOH implying that the former pyrene derivative is a much better probe to monitor the polarity of its environment than the latter. In the fluorescence spectra shown in Figure 1, the intensities I_1 and I_3 correspond to the intensity of the 0-0 and 0-2 transitions of pyrene at about 375 nm and about 386 nm, respectively (see Figure S4).

The I_1/I_3 ratios for PyMeEGOH and PyButOH have been listed in Table 2 for the 21 solvents used. The I_1/I_3 ratios of PyMeEGOH and PyButOH were also compared in Figure 2 to those of molecular pyrene reported by J. K. Thomas¹⁷ and M. A. Winnik.¹⁹ Whereas the I_1/I_3 ratio of PyButOH increases from 2.38 in cyclohexane to 3.86 in DMSO, a 60 % increase, the I_1/I_3 ratio of PyMeEGOH increased from 0.65 in hexane to 1.99 in DMSO, a more than 200% increase representing a range wide enough to easily assess the micropolarity of an environment probed by PyMeEGOH. Most interestingly, the I_1/I_3 ratios obtained for PyMeEGOH were comparable to those of molecular pyrene with a slope in Figure 2 of 1.1 and thus close to unity and a close to zero intercept of 0.25. This result demonstrates that PyMeEGOH is endowed with the same ability to probe the polarity of microenvironments in solution as molecular pyrene with the added benefit that PyMeEGOH can be covalently attached onto macromolecules thanks to its hydroxyl group. This feature was taken advantage of to prepare a series of PBMA samples labeled with different amounts of PyMeEGOH. The ability of the PyMeEG-PBMA series to probe the internal dynamics of PBMA in solution was investigated by comparing its response in terms of pyrene excimer formation to that obtained for a series of more traditional PyBut-PBMA samples which had been

prepared for an earlier study.²² The characteristics of both Py-PBMA samples in terms of M_n , PDI, and molar fraction of pyrene-labeled monomer (x) were provided in Table 1.

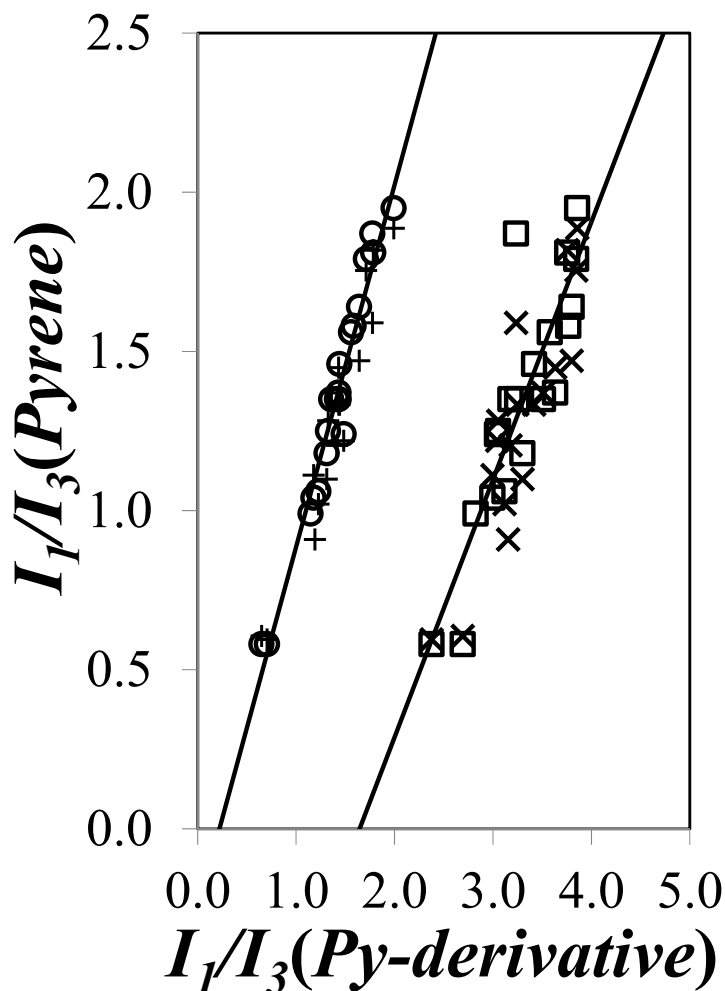


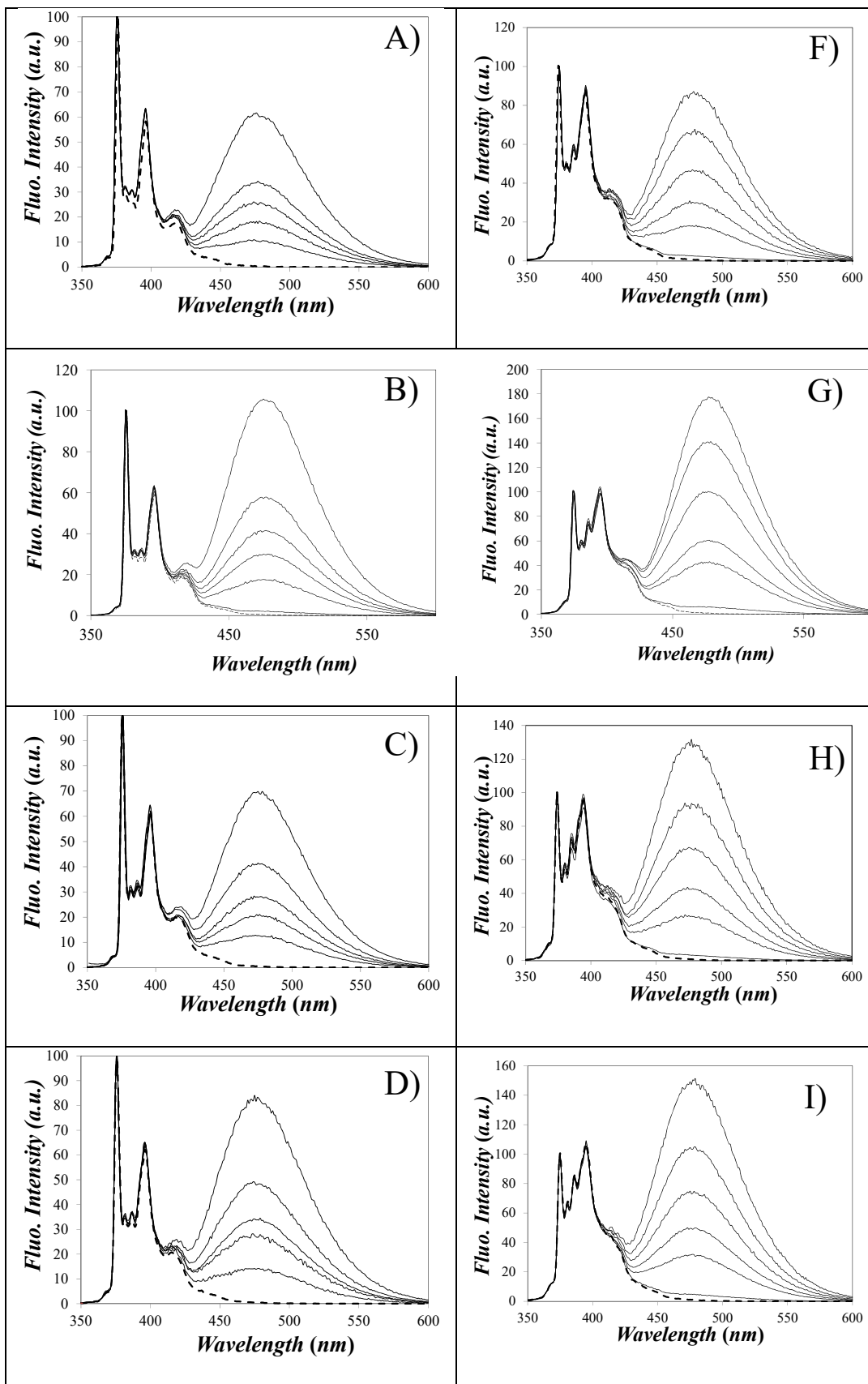
Figure 2. Comparison of the I_1/I_3 ratios of molecular pyrene reported by Thomas (+,×)¹⁷ and Winnik (O,□)¹⁹ as a function of the I_1/I_3 ratio of (+,O) PyMeEGOH and (×,□) PyButOH.

The fluorescence spectra of the PyMeEG-PBMA and PyBut-PBMA samples were acquired in cyclohexane ($\epsilon=2.0$), toluene ($\epsilon=2.4$), THF ($\epsilon=7.5$), DCM ($\epsilon=8.9$), and DMF ($\epsilon=38.3$) and they are shown in Figures 3A-J. The PyMeEG-PBMA samples were found to form excimer as

effectively as the PyBut-PBMA samples, the main difference in the spectra being the strong I_1 peak in Figures 3A-E resulting from the less symmetry forbidden 0-0 transition of the PyButOH derivative.

Table 2: Natural lifetime τ_M , difference $\Delta\tau_M = \tau_M(\text{PyMeEGOH}) - \tau_M(\text{PyButOH})$, and I_1/I_3 ratios of PyButOH and PyMeEGOH in 21 solvents.

Solvent	Dielectric constant (ϵ)	τ_M (ns) PyMeEGOH	τ_M (ns) PyButOH	$\Delta\tau_M$ (ns)	I_1/I_3 PyMeEGOH	I_1/I_3 PyButOH
Water	78.5	195	126	69	1.77	3.23
Dimethyl Sulfoxide	47.2	193	130	63	1.99	3.58
Dimethyl Formamide	38.3	230	163	67	1.78	3.57
Acetonitrile	36.6	273	192	81	1.71	3.84
Methanol	32.7	301	225	76	1.43	3.25
Ethanol	24.6	307	224	83	1.31	3.20
Acetone	19.7	277	195	82	1.64	3.80
Butylalcohol	17.3	295	220	75	1.23	3.12
Cyclopentanone	13.6	193	142	51	1.56	3.57
2-Butanone	13.5	260	187	73	1.58	3.76
2-Propanol	13.4	306	229	77	1.19	3.15
Benzyl Alcohol	11.9	203	145	58	1.48	3.04
1,2-Dichloroethane	10.4	119	80	39	1.44	3.42
Dichloromethane	8.9	155	105	50	1.36	3.36
Tetrahydrofuran	7.5	270	200	70	1.47	3.18
Ethyl Acetate	6.1	266	189	77	1.43	3.63
Chloroform	4.8	121	66	55	1.33	3.05
Toluene	2.4	253	179	74	1.18	2.99
o-Xylene	2.4	218	166	52	1.15	2.82
Cyclohexane	2.0	325	239	86	0.71	2.84
Hexane	1.9	337	257	80	0.65	2.69



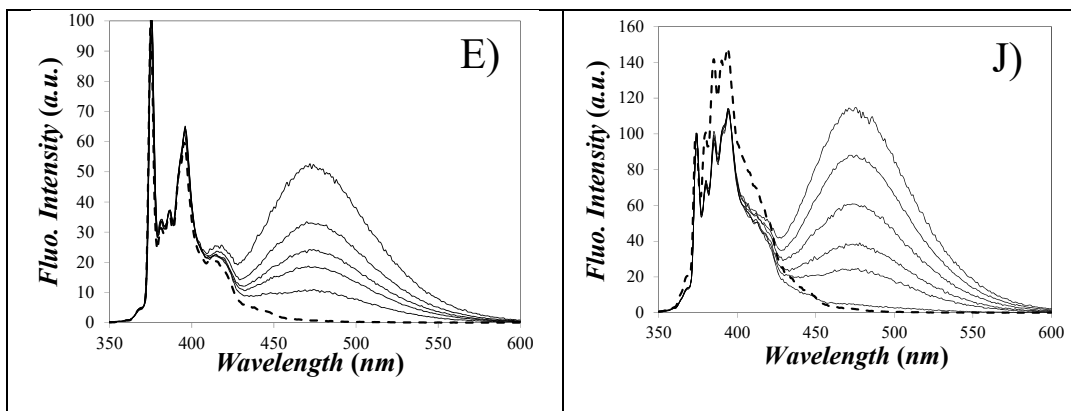


Figure 3. Steady-state fluorescence spectra of PyBut-PBMA labeled with 2.2, 3.0, 3.6, 5.3, and 7.2 mol% pyrene (from bottom to top) in A) DMF, B) DCM, C) THF, D) toluene, and E) cyclohexane and PyMeEG-PBMA labeled with 1.8, 2.7, 3.8, 4.6, and 5.4 mol% pyrene (from bottom to top) in F) DMF, G) DCM, H) THF, I) toluene, and J) cyclohexane. $[Py] = 2.5 \times 10^{-6}$ M, $\lambda_{ex} = 344$ nm. Traces with a dashed line in Figures A-E are for PyButOH and in Figures F-J are for PyMeEGOH.

The nice overlay of the fluorescence spectra in the range of wavelengths where the pyrene monomer emits, namely from 370 to 430 nm, suggests that the I_1/I_3 ratio did not change much with pyrene content. Indeed, the I_1/I_3 ratios averaged over all five Py-PBMA samples were determined to equal 1.03 ± 0.03 , 1.18 ± 0.01 , 1.37 ± 0.06 , 1.34 ± 0.03 , and 1.70 ± 0.04 for PyMeEG-PBMA and 2.71 ± 0.05 , 2.74 ± 0.05 , 3.05 ± 0.11 , 3.18 ± 0.04 , and 3.22 ± 0.07 for PyBut-PBMA in cyclohexane, toluene, THF, DCM, and DMF, respectively, showing very little variation with pyrene content. In all solvents studied, the I_1/I_3 ratios obtained for the PyBut-PBMA samples were in relatively good agreement with those obtained for PyButOH and found to equal 2.84, 2.99, 3.18, 3.36, and 3.57 in cyclohexane, toluene, THF, DCM, and DMF, respectively. Except for cyclohexane in Figure 3J, good agreement was also found for the I_1/I_3 ratios of the PyMeEG-

PBMA samples in toluene, THF, DCM, and DMF and those of PyMeEGOH found to equal 1.18, 1.47, 1.36, and 1.78, respectively.

In cyclohexane, the fluorescence spectrum of PyMeEGOH differed greatly from that of the pyrene monomer of the PyMeEG-PBMA samples in Figure 3J. This observation was reflected in the I_1/I_3 ratio of PyMeEGOH and PyMeEG-PBMA that equalled 0.71 and 1.03 ± 0.03 , respectively. This led to the conclusion that the PyMeEG label probed an environment that was much more polar than cyclohexane, possibly that of the ester bonds of PBMA. These interactions between pyrene and the PBMA backbone were further favored by the poor solvent quality of cyclohexane toward PBMA as inferred from intrinsic viscosity measurements. The intrinsic viscosity $[\eta]$ of an unlabeled PBMA sample ($M_n = 337,000$, PDI = 1.48) was found to equal 50.9 ± 0.9 , 50.1 ± 0.9 , and 56.5 ± 1.2 mL.g⁻¹ in toluene, THF, and DCM, respectively, but only 20.4 ± 0.3 mL.g⁻¹ in cyclohexane and 25.9 ± 0.4 mL.g⁻¹ in DMF (see Table S2 in SI). The much smaller $[\eta]$ values obtained for PBMA in cyclohexane and DMF demonstrate that these two solvents are poor solvents for PBMA, whereas toluene, THF, and DCM are good solvents for PBMA. The poor solvent quality of cyclohexane toward PBMA implies that the polymer coil in cyclohexane and DMF has a higher density of ester bonds compared to the other good solvents and that the PyMeEG label in cyclohexane responds strongly to this more polar environment. This conclusion is based on the comparison of the I_1/I_3 ratio of PyMeEGOH equal to 0.71 in cyclohexane and 1.43 in ethyl acetate, the latter solvent being rich in ester bonds resulting in a I_1/I_3 ratio for PyMeEGOH that was twice larger than in cyclohexane. The same effect was not observed in DMF certainly due to the relatively smaller difference in the I_1/I_3 ratio between DMF (1.78) and ethyl acetate (1.43).

The spectra shown in Figure 3 demonstrated that the PyMeEG-PBMA samples formed excimer but the difference in monomer fluorescence induced by polarity changes prevented direct

comparison of the kinetics of pyrene excimer formation based on the I_E/I_M ratio since the intensity of the 0-0 band at ~ 375 nm that is more sensitive to solvent polarity is used for I_M to avoid any overlap with the excimer fluorescence. To this date, a direct comparison of the efficiency of pyrene excimer formation in the Py-PBMA samples can only be achieved by applying the Fluorescence Blob Model (FBM) analysis to the monomer and excimer fluorescence decays.^{3,4,23} To this end, the fluorescence decays of the pyrene monomer and excimer of all PyMeEG-PBMA and PyBut-PBMA samples were acquired in cyclohexane, toluene, THF, DCM, and DMF and they were analyzed globally according to the FBM which has been described in details in SI. For both Py-PBMA series, the monomer and excimer decays were fitted globally with Equations S1 and S2 provided in SI. In a first round of fit, the rate constant k_2 for rearrangement of the pyrene labels was allowed to float. Its value obtained for all constructs of a same polymer series was averaged and the average was fixed in a second round of FBM fitting of the decays. The decay fits were excellent and this analysis yielded N_{blob} , the average number of BMA monomers in a *blob* and k_{blob} , the rate constant describing the diffusive motions inside a *blob* of two monomers bearing a pyrene label. The results of this analysis are discussed hereafter.

The first step in the FBM analysis of the decays consisted in determining the lifetime τ_M of the fluorescent label. To this end, a Py-PBMA sample was prepared with a low (< 0.6 mol%) pyrene content whose monomer decay had a strong ($> 80\%$) contribution of a long-lived pyrene species that was attributed to unquenched pyrene labels that did not form excimer (Py_{free}). This decay time was selected as the natural lifetime τ_M of the pyrene label attached to the polymer. τ_M was found to equal 288, 246, 270, 155, and 228 ns for PyMeEG-PBMA and 224, 180, 195, 110, and 160 ns for PyBut-PBMA in cyclohexane, toluene, THF, DCM, and DMF, respectively. These τ_M values were close to those found for PyMeEGOH and PyButOH in the same solvents (see Table

2), the larger discrepancy in τ_M being observed in cyclohexane for PyMeEG-PBMA. As explained earlier based on the analysis of the I_1/I_3 ratios of PyMeEGOH and PyMeEG-PBMA in cyclohexane, the difference in τ_M value for PyMeEG-PBMA in cyclohexane is due to the sensitivity of this pyrene derivative to the more polar environment generated by the ester bonds of the PBMA backbone (see Figure 3J). It was interesting to note that, τ_M for PyMeEGOH in Table 2 determined for all 21 solvents was on average 67 ± 13 ns longer than for PyButOH. This represents another major advantage for using PyMeEGOH instead of PyButOH as a fluorescent label as its longer lifetime offers a longer temporal window to probe the slow dynamics observed in macromolecules.

Fits of the decays yielded $\langle n \rangle$, the average number of pyrene labels per *blob*, which was used to determine N_{blob} according to Equation S4. Within experimental error, the N_{blob} values obtained for all constructs of a given Py-PBMA series did not depend much on pyrene content as demonstrated in Figure S6. They were averaged and $\langle N_{\text{blob}} \rangle$ was plotted as a function of the inverse of viscosity (η^{-1}) in Figure 4A. The $\langle N_{\text{blob}} \rangle$ values obtained for both Py-PBMA series were comparable although they might appear to be slightly smaller for the PyBut-PBMA constructs despite the relatively large error bars. It must be also pointed out that the different natural lifetimes τ_M used for PyBut-PBMA and PyMeEG-PBMA were not expected to affect much the FBM parameters as can be seen in Supporting Information. In particular the little effect that τ_M had on $\langle N_{\text{blob}} \rangle$ for PyMeEG-PBMA in THF was illustrated in Figure S8B where τ_M was adjusted by adding nitromethane, a well-known quencher of pyrene fluorescence, to the polymer solution.²⁷

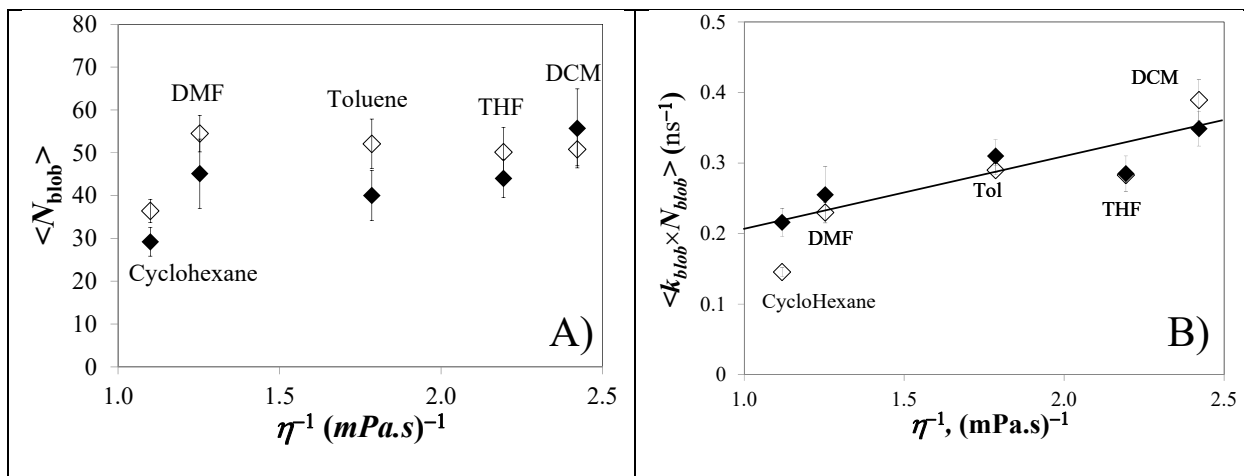


Figure 4. Plot of A) $\langle N_{\text{blob}} \rangle$ and B) $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ as a function of η^{-1} for the Py-PBMA

samples in different solvents. (◆) PyBut-PBMA, (◇) PyMeEG-PBMA.

Beside τ_M , viscosity is also expected to slow down the motion of a fluorescent label, which in turn, should reduce V_{blob} and thus N_{blob} .²⁸ Little indication of this effect was observed in Figure 4A where $\langle N_{\text{blob}} \rangle$ did not change much as a function of η^{-1} . Among possible reasons for the apparent lack of response of $\langle N_{\text{blob}} \rangle$ to η^{-1} might be solvent quality toward the polymer.²⁹ It is noticeable that in Figure 4A, the higher viscosity solvents DMF ($\eta=0.802$ mPa.s at 25°C) and cyclohexane ($\eta=0.894$ mPa.s at 25°C) are also the two poorest solvents toward PBMA based on the intrinsic viscosity measurements described earlier. Poorer solvents result in denser polymer coils and thus higher N_{blob} values that would offset the effect imparted by the increased viscosity of DMF and cyclohexane onto N_{blob} . Such effects have been discussed in great details in earlier publications^{Error! Bookmark not defined.-29} and are beyond the scope of this report which focuses on the comparison of the results obtained from the fluorescence response of the PyMeEG-PBMA and PyBut-PBMA series. At this stage, the $\langle N_{\text{blob}} \rangle$ values presented in Figure 4A suggest that this

quantity is similar for PyMeEG-PBMA and PyBut-PBMA and that it does not change much with viscosity for the range of solvent viscosities investigated.

The rate constant of pyrene excimer formation inside a *blob*, k_{blob} , was also retrieved from the FBM analysis of the decays and it was used to determine the product $k_{\text{blob}} \times N_{\text{blob}}$. The product $k_{\text{blob}} \times N_{\text{blob}}$ has been found to represent polymer chain dynamics in solution more faithfully than N_{blob} .^{22,24,25} As for N_{blob} , the products $k_{\text{blob}} \times N_{\text{blob}}$ were insensitive to pyrene content as shown in Figure S6. They were averaged over all constructs for a given Py-PBMA series to yield $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ which was plotted as a function of η^{-1} in Figure 4B. Except in cyclohexane, perfect agreement between the two Py-PBMA series was obtained for $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$. The difference in $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ observed in cyclohexane could not be attributed to different τ_{M} values as the quenching experiment conducted with PyMeEG-PBMA in THF showed in Figure S8B that $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ remained constant within experimental error for τ_{M} values between 100 and 270 ns. We suspect that the difference in $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ values found in cyclohexane between PyMeEG-PBMA and PyBut-PBMA is due to stronger interactions in cyclohexane between the ether bond in the linker of the PyMeEG derivative and the ester bonds of PBMA that hold the pyrene label close to the PBMA backbone. These interactions would hinder the mobility of PyMeEG as reflected by the lower $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ value obtained in Figure 4B in cyclohexane. As a matter of fact, an upcoming study provides evidence that, as the length of the spacer connecting pyrene to the polymeric backbone decreases, the pyrene label probes a smaller V_{blob} .³⁰ The more polar DMF, which like cyclohexane is a poor solvent for PBMA but unlike cyclohexane does not prevent the deployment of the pyrene label into the solution yields the same $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ value for both PyMeEG-PBMA and PyBut-PBMA constructs.

The uptick observed for $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ for a τ_M value of 50 ns in Figure S8B is a direct consequence of the scaling laws that apply to polymers in solution.³¹ Since k_{blob} is the rate constant for diffusive encounters between an excited pyrene and a ground-state pyrene located inside a same *blob*, k_{blob} is a pseudo-unimolecular rate constant equal to the product of the bimolecular rate constant of diffusive encounters k_{diff} times the local pyrene concentration equivalent to one ground-state pyrene inside a *blob* ($k_{\text{blob}} = k_{\text{diff}} \times (1/V_{\text{blob}})$). Using a scaling argument where $V_{\text{blob}} \sim N_{\text{blob}}^{3\nu}$ with ν being Flory's exponent,^{31,32} the product $k_{\text{blob}} \times N_{\text{blob}}$ is found to scale as $N_{\text{blob}}^{1-3\nu}$. Since ν equals 0.5 in a θ -solvent and 0.6 in a good solvent, $1-3\nu$ is negative which implies that $k_{\text{blob}} \times N_{\text{blob}}$ decreases with increasing N_{blob} as is being observed in Figure S8B when τ_M decreases for the PyMeEG-PBMA series in THF. The product $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ obtained for the PyBut-PBMA sample was also given in Figure 5B. Within experimental error, the values of $\langle N_{\text{blob}} \rangle$ and $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ for PyBut-PBMA are similar to those obtained for PyMeEG-PBMA in THF for a lifetime τ_M of 200 ns.

Besides differences in $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ in cyclohexane induced by the poor solvent quality of cyclohexane towards PBMA and interactions between the ether linker of the PyMeEG derivative and the ester bonds of the PBMA backbone, similar $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ values were obtained for the PyMeEG-PBMA and PyBut-PBMA series in all the other solvents that were tried in this study. Furthermore $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ was found to increase with increasing η^{-1} in Figure 4B as would be expected from a diffusion controlled process for pyrene excimer formation. Since $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ has been shown to faithfully represent the internal dynamics of polymers in solution,^{22,24,25} this result demonstrates that the PyMeEGOH derivative used to label PBMA yields the same dynamic information on a polymer in solution as PyButOH does as would be expected since the linker connecting the pyrene derivatives to the PBMA backbone is constituted of a same number of four

atoms. The main difference between these two pyrene derivatives is the ability of PyMeEGOH to probe the polarity of its local environment with a sensitivity that is similar to that of molecular pyrene (see Figures 1A and 2), a feature of this dye whose importance is rooted in the fact that the paper introducing this effect for the first time has been cited more than 1800 times since 1977 according to scifinder!¹⁷ This feature was particularly useful in this study to rationalize why $\langle k_{\text{blob}} \times N_{\text{blob}} \rangle$ took a lower value for PyMeEG-PBMA than for PyBut-PBMA in cyclohexane. As shown in Figures 1B and 3A-E, the fluorescence spectrum of PyButOH responds much more poorly to the polarity of its local environment in comparison to that of PyMeEGOH.

CONCLUSIONS

Macromolecules labeled with a 1-pyrenebutyl derivative are mostly characterized by monitoring changes in pyrene excimer formation since such probes are not much sensitive to the polarity of their environment as illustrated by the present study (see Figures 1B and 3A-E). By comparison, PyMeEGOH differs from PyButOH by the oxygen replacement of the methylene unit in the β -position of the butanol side chain attached in the 1-position of pyrene. Macromolecules labeled with PyMeEGOH report not only on the fluidity of their local environment probed by pyrene excimer formation as efficiently as macromolecules labeled with PyButOH would (see Figure 4), but also on the polarity of the local environment experienced by pyrene in the macromolecule by taking advantage of the excellent fluorescence response to polarity exhibited by PyMeEGOH. Most interestingly, the response to polarity observed for PyMeEGOH was found to be as strong as that of molecular pyrene (Figure 2). Based on these results, PyMeEGOH promises to be a most valuable fluorescent probe to study the internal dynamics and the polarity of the microenvironment generated by macromolecules in solution.

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SUPPORTING INFORMATION AVAILABLE

¹H NMR spectra of PyMeEG-OH and PyMeEG-MA, Traces of gel permeation chromatography, description of the photophysical properties of PyMeEG-OH and PyBut-OH, detailed description of the Fluorescence Blob Model, quenching study of the PyMeEG label by nitromethane, intrinsic viscosity measurements, and parameters retrieved from the FBM analysis of the fluorescence decays.

REFERENCES

- (1) Winnik, F. M. Photophysics of Preassociated Pyrenes in Aqueous Polymer Solutions and in Other Organized Media. *Chem. Rev.* **1993**, *93*, 587–614.
- (2) Duhamel, J. Internal Dynamics of Dendritic Molecules Probed by Pyrene Excimer Formation. *Polymers* **2012**, *4*, 211–239.
- (3) Duhamel, J. New Insights in the Study of Pyrene Excimer Fluorescence to Characterize Macromolecules and their Supramolecular Assemblies in Solution. *Langmuir* **2012**, *28*, 6527-6538.
- (4) Duhamel, J. Global Analysis of Fluorescence Decays to Probe the Internal Dynamics of Fluorescently Labeled Macromolecules. *Langmuir* **2014**, *30*, 2307-2324.

- (5) Winnik, M. A.; Li, X.-B.; Guillet, J. E. Cyclization Dynamics of Polymers. 13. Effects of Added Polymer on the Conformation and Dynamics of Polystyrene Containing Evenly Spaced Pyrene Groups. *Macromolecules* **1984**, *17*, 699-702.
- (6) Nishikawa, K.; Yekta, A.; Pham, H. H.; Winnik, M. A.; Sau, A. C. Fluorescence Studies of Hydrophobically Modified Hydroxyethylcellulose (HMHEC) and Pyrene-Labeled HMHEC. *Langmuir* **1998**, *14*, 7119-7129.
- (7) Kujawa, P.; Liu, R. C. W.; Winnik, F. M. Do Fluorocarbon, Hydrocarbon, and Polycyclic Aromatic Groups Intermingle? A Study of the Interactions in Water between Fluorocarbon- and Hydrocarbon-Modified Poly(*N*-isopropylacrylamides). *J. Phys. Chem. B* **2002**, *106*, 5578-5585.
- (8) Anghel, D. F.; Alderson, V.; Winnik, F. M.; Mizusaki, M.; Morishima, Y. Fluorescent Dyes as Model 'Hydrophobic Modifiers' of Polyelectrolytes: A Study of Poly(acrylic acid)s Labelled with Pyrenyl and Naphthyl Groups. *Polymer* **1998**, *39*, 3035-3044.
- (9) Hu, Y.-Z.; Zhao, C.-L.; Winnik, M. A.; Sundararajan, P. R. Fluorescence Studies of the Interactions of Sodium Dodecyl Sulfate with Hydrophobically Modified Poly(ethylene oxide). *Langmuir* **1990**, *6*, 880-883.
- (10) Winnik, F. M.; Regismond, S. T. A.; Goddard, E. D. Interactions of an Anionic Surfactant with a Fluorescent-Dye-Labeled Hydrophobically-Modified Cationic Cellulose Ether. *Langmuir* **1997**, *13*, 111-114.
- (11) Anghel, D. F.; Toca-Herrera, J. L.; Winnik, F. M.; Rettig, W.; v. Klitzing, R. Steady-State Fluorescence Investigation of Pyrene-Labeled Poly(acrylic acid)s in Aqueous Solution and in the Presence of Sodium Dodecyl Sulfate. *Langmuir* **2002**, *18*, 5600-5606.

- (12) Relógio, P.; Martinho, J. M. G.; Farinha, J. P. S. Effect of Surfactant on the Intra- and Intermolecular Association of Hydrophobically Modified Poly(*N,N*-dimethylacrylamide). *Macromolecules* **2005**, *38*, 10799-10811.
- (13) Winnik, M. A.; Egan, L. S.; Tencer, M.; Croucher, M. D. Luminescence Studies on Sterically Stabilized Polymer Colloid Particles: Pyrene Excimer Formation. *Polymer* **1987**, *28*, 1553-1560.
- (14) Nakashima, K.; Liu, Y. S.; Zhang, P.; Duhamel, J.; Feng, J.; Winnik, M. A. Picosecond Fluorescence Studies of Energy Transfer on the Surface of Poly(butyl methacrylate) Latex Particles. *Langmuir* **1993**, *9*, 2825-2831.
- (15) Eckert, A. R.; Hsiao, J.-S.; Webber, S. E. Photophysics of Adsorbed and Solution Phase End-Tagged Poly(ethylene oxide). *J. Phys. Chem.* **1994**, *98*, 12025-12031.
- (16) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: New York, 1970; p 301.
- (17) Kalyanasundaram, K.; Thomas, J. K. Environmental Effects on Vibronic Band Intensities in Pyrene Monomer Fluorescence and Their Application in Studies of Micellar Systems. *J. Am. Chem. Soc.* **1977**, *99*, 2039-2044.
- (18) Dong, D. C.; Winnik, M. A. The Py Scale of Solvent Polarities. Solvent Effects on the Vibronic Fine Structure of Pyrene Fluorescence and Empirical Correlations with ET and Y Values. *Photochem. Photobiol.* **1982**, *35*, 17-21.
- (19) Dong, D. C.; Winnik, M. A. The Py Scale of Solvent Polarities. *Can. J. Chem.* **1984**, *62*, 2560–2565.
- (20) Zachariasse, K. A.; Vaz, W. L.; Sotomayor, C.; Kühnle, W. Investigation of Human Erythrocyte Ghost Membranes with Intramolecular Excimer Probes. *Biochim. Biophys. Acta* **1982**, *688*, 323-332.

- (21) Winnik, F. M.; Winnik, M. A.; Ringsdorf, H.; Venzmer, J. Bis(1-pyrenylmethyl) Ether as an Excimer-Forming Probe of Hydrophobically Modified Poly(N-isopropylacrylamides) in Water. *J. Phys. Chem.* **1991**, *95*, 2583-2587.
- (22) Farhangi, S.; Weiss, H.; Duhamel, J. Effect of Side-Chain Length on the Polymer Chain Dynamics of Poly(alkyl methacrylate)s in Solution. *Macromolecules* **2013**, *46*, 9738-9747.
- (23) Duhamel, J. Polymer Chain Dynamics in Solution Probed with a Fluorescence Blob Model. *Acc. Chem. Res.* **2006**, *39*, 953-960.
- (24) Ingratta, M.; Mathew, M.; Duhamel, J. How Switching the Substituent of a Pyrene Derivative from a Methyl to a Butyl Affects the Fluorescence Response of Polystyrene Randomly Labeled with Pyrene. *Can. J. Chem.* **2010**, *88*, 217-227.
- (25) Yip, J.; Duhamel, J.; Qiu, X. P.; Winnik, F. M. Long-Range Polymer Chain Dynamics of Pyrene-Labelled Poly(N-isopropylacrylamide)s Studied by Fluorescence. *Macromolecules* **2011**, *44*, 5363-5372.
- (26) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. *Numerical Recipes. The Art of Scientific Computing (Fortran Version)*; Cambridge University Press: Cambridge, 1992.
- (27) Ingratta, M.; Duhamel, J. Effect of Time on the Rate of Long Range Polymer Segmental Intramolecular Encounters. *J. Phys. Chem. B* **2009**, *113*, 2284-2292.
- (28) Ingratta, M.; Duhamel, J. Effect of Viscosity on Long Range Polymer Chain Dynamics in Solution Studied with a Fluorescence Blob Model. *Macromolecules* **2009**, *42*, 1244-1251.
- (29) Kanagalingam, S.; Spartalis, J.; Cao, T.-C.; Duhamel, J. Scaling Relations Related to the Kinetics of Excimer Formation between Pyrene Groups Attached onto Poly(N,N-dimethylacrylamide)s. *Macromolecules* **2002**, *35*, 8571-8577.

- (30) Farhangi, S.; Duhamel, J. Probing Chain Dynamics of Branched Macromolecules by Pyrene Excimer Fluorescence. *ASAP Macromolecules*, **2015**.
- (31) De Gennes, P. G. *Scaling Concepts in Polymer Physics*. Cornell University Press, Ithaca, 1979.
- (32) Flory, P. J. *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, 1953.

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