Probing Side Chain Dynamics of Branched Macromolecules by Pyrene Excimer Fluorescence

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INTRODUCTION

In this study, four different pyrene-labeled polymers were prepared by radical copolymerization of n-butyl methacrylate (n-BMA) and 1-pyrenemethyl methacrylate, 1-pyrenemethoxyethyl methacrylate, 1-pyrenemethoxyethoxyethyl methacrylate, and 1-pyrenemethoxydiethoxyethyl methacrylate to yield $PyEG_0$-PBMA, $PyEG_1$-PBMA, $PyEG_2$-PBMA, and $PyEG_3$-PBMA, respectively. The number of atoms in the side chain of the pyrene-labeled copolymers increased from 3 in $PyEG_0$-PBMA to 12 in $PyEG_3$-PBMA. The increase in side chain length was accomplished by increasing the spacer length between pyrene and the main chain of the polymers using oligo(ethylene glycol) linkers of different lengths. Steady-state fluorescence was used to monitor the efficiency of excimer formation while time-resolved fluorescence was applied to investigate and describe the kinetics of diffusive encounters between the exited and the ground-state pyrenes as a function of spacer length.

The efficiency of excimer formation and the internal dynamics of the pyrene labeled polymers were characterized by applying the fluorescence blob model (FBM) analysis to the fluorescence decays.[1–3] FBM analysis yielded the average number of monomers in a *blob*, N_{blob}, and the rate constant of excimer formation inside a *blob* containing one excited pyrene and one ground-state pyrene, k_{blob}. The product $k_{blob} \times N_{blob}$ provided a measure of the frequency of pyrene-pyrene encounters inside a *blob* as a function of spacer length. By increasing the side chain length from 3 to 12 linker atoms in the $PyEG_X$-PBMA samples, both the side chain mobility and reach increased, thus allowing more efficient excimer formation as demonstrated from the analysis of the fluorescence spectra. In turn, FBM analysis of the fluorescence decays provided a means to represent how the volume probed by an excited pyrene and the kinetics of pyrene excimer formation were affected by the length of the spacer connecting pyrene to the main chain.

The strong effect that the side chain length had on pyrene excimer formation in the $PyEG_X$-PBMA samples suggests that pyrene excimer fluorescence could be an excellent technique to probe the conformation of highly branched macromolecules such as dendrimers or polymeric bottle brushes in solution.

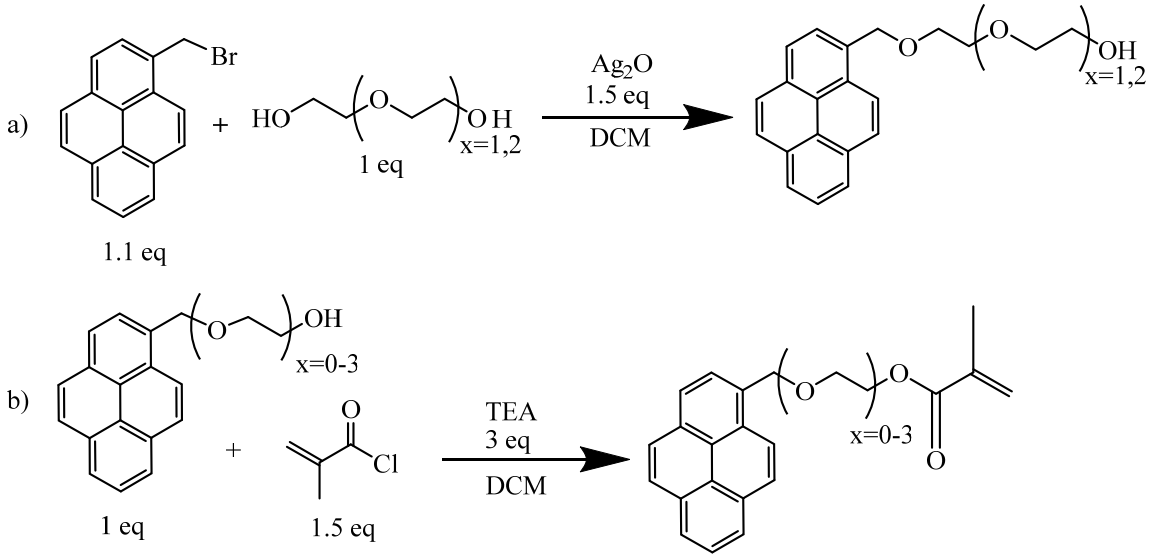
EXPERIMENTALS

Materials: 1-pyrenemethanol, silver(I) oxide (Ag_2O), diethylene glycol (DEG), and triethylene glycol (TEG) were purchased from Sigma-Aldrich. Distilled in glass tetrahydrofuran (THF) was supplied by Caledon Laboratories. PBMA standards were purchased from Polymer Source or Polymer Standards Service (PSS). All chemicals were used as received.

Synthesis of 1-pyrenemethyloxyethyl methacrylate ($PyEG_1$-MA): The synthesis of this pyrene-labeled monomer has been described elsewhere.[2]

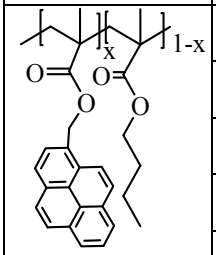
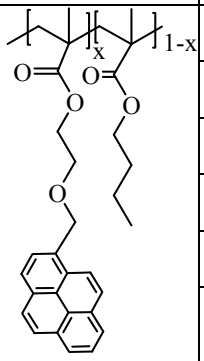
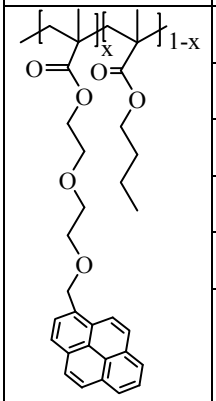
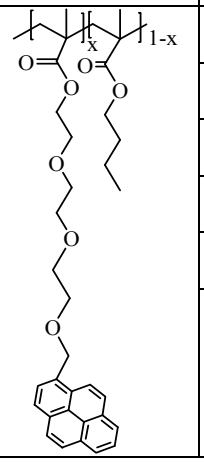
Synthesis of 1-pyrenemethoxyethoxyethanol ($PyEG_2$-OH) and 1-pyrenemethoxydiethoxy ethanol ($PyEG_3$-OH): The same procedure was applied for both compounds. Only the synthesis of $PyEG_2$-OH is described in details. Ag_2O (1.97 g, 8.5mmol) was transferred in a 25 mL round bottom flask. Dichloromethane (DCM) was added and the suspended solution was deoxygenated for 15 minutes. DEG (1.00g, 5.82 mmol) was added and stirred for 45 minutes under nitrogen. 1-(Bromomethyl)pyrene (1.83 g, 6.20 mmol) was pre-dissolved in 5 mL DCM and the solution was added slowly to the reaction mixture. The reaction was stirred for 72 hours under nitrogen at room temperature. The solution was filtered through a Celite® bed. The solvent was removed in a rotary evaporator and the pale-yellow residue was purified by silica gel column chromatography using a 55:45 ethyl acetate-to-hexane mixture. A pale-yellow oil was obtained in a 45% yield.

300 MHz ^{1}H NMR (DMSO-d_6) for PyEG$_2$-OH:δ 3.41-3.71 (m, 8H, O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O),δ 4.6 (t, 1H, OH), δ 5.2 (s, 2H, py-CH$_2$-O), δ8.0-8.4 (m, 9H, Py H's).
300 MHz ^{1}H NMR (DMSO-d_6) for PyEG$_3$-OH: δ 3.37-3.72 (m, 12H, O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-OH),δ 4.56 (t, 1H, OH), δ 5.2 (s, 2H, -CH$_2$-O), δ 7.9-8.4 (m, 9H, Py H's).
Synthesis of 1-pyrenemethyl methacrylate (PyEG$_0$-MA), 1-pyrenemethoxyethoxyethyl methacrylate (PyEG$_2$-MA), and 1-pyrenemethoxyethoxydiethyl methacrylate (PyEG$_3$-MA): Only the synthesis of PyEG$_2$MA is described in detail since a similar procedure was applied to prepare PyEG$_0$-MA and PyEG$_3$-MA. PyEG$_2$-OH (1.10 g, 3.43 mmol) and freshly distilled triethylamine (1.04 g, 12 mmol) were dissolved in 30 mL of DCM placed in a 100 mL round bottom flask. The reaction flask was purged with nitrogen for 20 minutes. Afterwards the reaction flask was cooled down using dry ice and methacryloyl chloride (0.62 g, 6.0 mmol) was added drop wise under nitrogen. The reaction mixture was brought to room temperature and the solution was stirred for 24 hrs. After the reaction was complete, the organic phase was washed using an aqueous solution of 0.5 M HCl, saturated sodium carbonate, and saturated sodium chloride, followed by water. The extracted organic phase was rotary evaporated. The remaining crude residues were purified by silica gel column chromatography using a 60:40 ethyl acetate-to-hexane mixture to yield 90% of the desired product. The synthetic procedure and the ^{1}H NMR spectra of PyEG$_0$-MA, PyEG$_2$-MA, and PyEG$_3$-MA are described hereafter.
300 MHz ^{1}H NMR (CDCl$_3$) for PyEG$_0$-MA:δ 1.95 (s, 3H, CH$_3$-),δ 5.5 (s, 1H, =CH$_2$),δ5.9 (s, 2H, Py-CH$_2$-), δ6.4 (s, 1H, =CH$_2$), δ 7.9-8.3 (m, 9H, Py H's).
300 MHz ^{1}H NMR (CDCl$_3$) for PyEG$_2$-MA:δ 1.81 (s, 3H, CH$_3$-), δ 3.73-3.77 (m, 6H, -CH$_2$-O-CH$_2$-CH$_2$-O-),δ4.29-4.32 (m, 2H, COO-CH$_2$-),δ5.2 (s, 2H, Py-CH$_2$-), δ 5.5 (s, 1H, =CH$_2$), δ 6.1 (s, 1H, =CH$_2$), δ 7.9-8.4 (m, 9H, Py H's).
300 MHz ^{1}H NMR (CDCl$_3$) for PyEG$_3$-MA:δ 1.89 (s, 3H, CH$_3$-), δ 3.8-4.4 (m, 8H, CH$_2$-O-CH$_2$-CH$_2$-O-CH2-CH2-O),δ4.21-4.28 (m, 2H, COO-CH$_2$-), δ5.2 (s, 2H, Py-CH$_2$-), δ 5.5 (s, 1H, =CH$_2$), δ 6.1 (s, 1H, =CH$_2$), δ 7.9-8.4 (m, 9H, Py H's).



Scheme 1. Synthetic schemes for the preparation of a) PyEG$_2$-OH and PyEG$_3$-OH and b) PyEG$_x$-MA with $x = 0 – 3$.
Copolymerization of the PyEGX-MA monomers and BMA: The synthesis of the Py-PBMA samples has been described earlier.[3] The chemical structure of the polymers is listed in Table 1.

Table 1. Pyrene content, absolute molecular weight, and PDIs of the PyEG$_X$-PBMA samples.

PyEG$_0$PBMA				**PyEG$_1$PBMA**			
Chemical Structure	Py-content µmol/g (mol %)	M_n kg/mol	PDI	Chemical Structure	Py-content µmol/g (mol %)	M_n kg/mol	PDI
	25 (0.35)	182	1.92		23 (0.32)	164	2.00
	270 (4.0)	204	1.44		123 (1.8)	160	1.80
	352 (5.3)	170	1.39		184 (2.7)	117	2.00
	412 (6.3)	183	1.92		255 (3.8)	100	2.23
	461 (7.1)	164	1.94		304 (4.6)	190	1.73
	525 (8.1)	138	2.20		354 (5.4)	303	1.46
PyEG$_2$PBMA				**PyEG$_3$PBMA**			
Chemical Structure	Py-content µmol/g (mol %)	M_n kg/mol	PDI	Chemical Structure	Py-content µmol/g (mol %)	M_n kg/mol	PDI
	22 (0.32)	179	1.96		23 (0.32)	113	1.56
	123 (1.8)	178	1.56		70 (1.0)	201	1.53
	160 (2.3)	173	1.77		126 (1.9)	117	1.60
	215 (3.2)	191	1.74		142 (2.1)	137	1.48
	258 (3.9)	167	1.56		185 (2.8)	195	1.55
	342 (5.3)	178	1.76		274 (4.2)	157	1.82

Steady-state fluorescence measurements: Steady-state fluorescence measurements were carried on a Photon Technology International (PTI) LS-100 steady-state fluorometer with an Ushio UXL-75Xe Xenon lamp and a PTI 814 photomultiplier detection system. To avoid intermolecular interactions all fluorescence spectra were acquired in dilute solution with a pyrene concentration of 2.5×10^{-6} M. Right angle geometry was used and all samples were deoxygenated by bubbling nitrogen. The solution was excited at 344 nm while the emission spectrum was monitored from 350 to 600 nm.

Time-resolved fluorescence measurements: The fluorescence decays were acquired on a time-resolved fluorometer equipped with an IBH 340 nm NanoLED using the same samples prepared for the steady-state fluorescence experiments. The solutions were irradiated with a pulse of light at 344 nm to excite pyrene. The fluorescence intensity was adjusted to ensure that the number of photons reaching the detector represented less than 2% of the excitation photons. The monomer and excimer fluorescence decays were acquired at 375 and 510 nm, respectively. All acquired decays were fitted according to the FBM analysis. The FBM parameters, pre-exponential factors, decay times, and the corresponding equations used for the analysis of the fluorescence decays can be found elsewhere.[1-3] The quality of the fits was evaluated by the

random distribution of the residuals and the autocorrelation of the residuals and the χ^2 value smaller than 1.30. .

Determination of the Mark-Houwink-Sakurada (MHS) parameters: In this experiment, four narrow molecular weight PBMA standards (M_n in kg.mol^{-1} (PDI) = 2.8 (1.15), 7.0 (1.6), 13 (1.12), 24 (1.25), and 38 (1.15)) were used. The *K* and *a* parameters of the MHS equation were obtained by determining the intrinsic viscosity [η] of the PBMA standards in THF at 25 ºC. A plot of $Ln[\eta]$ as a function of LnM_n yielded a straight line. The slope and intercept of the straight line were calculated to yield *K* and *a* which were found to equal 2.8 (±0.6)×10^{-4} mL/g and 1.09 ± 0.03, respectively. These parameters were used to estimate the hydrodynamic radius of a *blob* (R_{blob}).

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. The molecular weight distribution was characterized by the M_n and PDI values and the pyrene content of the four series of PyEG$_X$-PBMA polymers have been listed in Table 1.

RESULTS AND DISCUSSION

FBM analysis of the fluorescence decays of the PyEG$_X$-PBMA samples yielded the parameters N_{blob} and k_{blob} which were used to determine the product $k_{blob} \times N_{blob}$. Since N_{blob} and k_{blob} did not depend on pyrene content, their values were averaged. As shown in Figure 1, $<N_{blob}>$ increased linearly with increasing spacer length from 40.5 ± 2.3 to 82.7 ± 3.7 indicating that a larger volume was being probed by the excited pyrene inside the polymer coil.

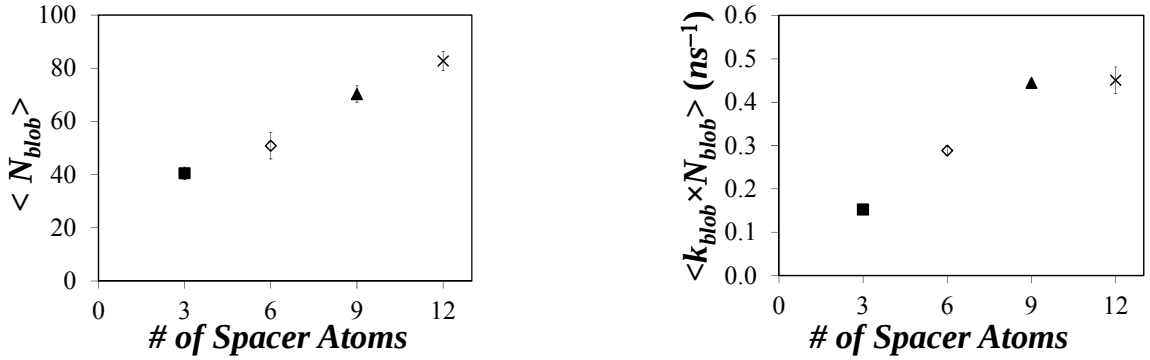


Figure 1. Plot of A) $<N_{blob}>$ and B) $<k_{blob} \times N_{blob}>$ as a function of the number of spacer atoms for (■) PyEG$_0$-PBMA labeled with 4.0, 5.3, 6.3, 7.1, and 8.1mol% pyrene, (◇) PyEG$_1$-PBMA labeled with 1.8, 2.7, 3.8, 4.6 and 5.4 mol% pyrene, (■) PyEG$_2$-PBMA labeled with 1.8, 2.3, 3.2, 3.9, and 5.3 mol% pyrene, and (×) PyEG$_3$-PBMA labeled with 1.0, 1.8, 2.1, 2.7, and 4.3 mol% pyrene in THF. [*Py*] = 2.5 × 10^{-6} M, λ_{ex} = 344 nm.

Both N_{blob} and k_{blob} for PyEG$_0$-PBMA were small reflecting the restricted mobility of the pyrene label due to the short 3 atom-long spacer. Interestingly, k_{blob} increased up to PyEG$_2$-PBMA but took a similar value for PyEG$_2$-PBMA and PyEG$_3$-PBMA within experimental error. k_{blob} increased by 2.3 ± 0.3, 2.0 ± 0.2, and 1.2 ± 0.1 folds between PyEG$_0$-PBMA and PyEG$_1$-PBMA, PyEG$_1$-PBMA and PyEG$_2$-PBMA, and PyEG$_2$-PBMA and PyEG$_3$-PBMA, respectively. These results indicated that the mobility of the pyrene label was strongly hindered for PyEG$_0$-PBMA, but that the pyrene label became more mobile and less restrained by the main chain as the side chain connecting pyrene to the PBMA backbone increased in length. The k_{blob} value obtained for PyEG$_2$-PBMA and PyEG$_3$-PBMA reflected mainly the diffusive motion of the side chains.

The N_{blob} value was used to estimate the hydrodynamic radius of a *blob*, R_{blob}. The MHS parameters were determined for the PBMA standards with M_n values ranging between 2.5 and 38 K covering the range of 40 to 83 monomers determined experimentally for the N_{blob} values. The MHS parameters K and a were introduced into Equation 1 to determine the hydrodynamic radius of a *blob*, R_{blob}, which was plotted as a function of the number of spacer atoms in Figure 2.

$$R_{blob} = \left(\frac{3KN_{blob}^{a+1}}{10\pi N_A} \right)^{1/3} \quad (1)$$

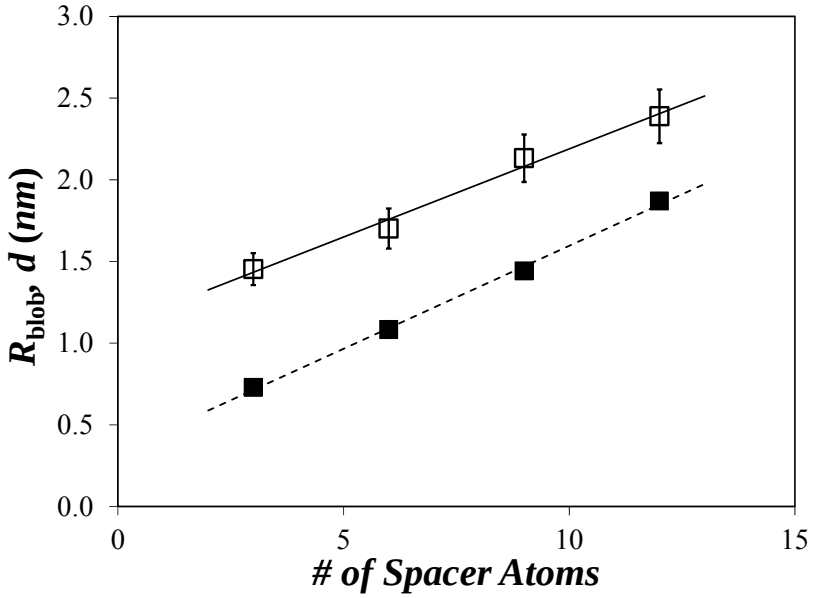


Figure 2. Comparison of R_{blob} (□) and the spacer length d obtained from HyperChem (■) for the PyEG$_X$-PBMA series.

The trends shown in Figure 2 demonstrate that R_{blob} and d, the distance separating pyrene from the PBMA backbone, increase in a similar manner confirming that the increase in the volume probed by the excited pyrene is directly related to the number of spacer atoms. In turn, this study demonstrates that pyrene excimer fluorescence could be applied to probe highly branched macromolecules such as dendrimers and polymeric bottle brushes.

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