Probing the Conformations of Pyrene Labeled PEGMA Polymeric Bottle Brushes in Solution by Pyrene Excimer Formation

Janine Lydia Thoma, Jean Duhamel*

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

Introduction

Polymer architecture is a key component in the design of a polymer because it dictates its physical properties and consequently, its desired applications. Polymers with complex architectures can be separated into four categories: star, hyper-branched, brush, and network/gel polymers. Developments in the synthesis of brush polymers were spearheaded by the growing number of their applications, for example, in the biomedical industry as drug carriers and in the synthesis of new super soft materials.[1,2] Brush polymers, also called polymeric bottle brushes (PBBs), offer high functionality with respect to backbone and macromonomer chemical composition which allows for their customization depending on the specific property requirements. What gives a PBB its unique properties are its high grafting density and high degree of polymerization.

The monomer unit of a PBB is a macromolecular monomer, or macromonomer, and has a molar mass usually between 1,000 and 10,000 gmol^{-1}.[3] PBBs can be synthesized using three different approaches: grafting through, grafting to, and grafting from.[4] Each technique has advantages and disadvantages but a grafting through approach is the only approach which guarantees 100% side chain incorporation. However, a high degree of polymerization is not guaranteed depending on the size of the macromonomer. Grafting through involves the synthesis of a macromonomer with a functionalized end group which can then be polymerized through the radical chain polymerization of choice.[4]

Contrary to studies of PBBs by microscopy where the macromolecule is adsorbed onto a 2-D surface, the characterization of PBBs in solution is of interest because it allows scientists to probe the dynamics of their densely packed side chains while being in their native conformation. A PBB will adopt a different conformation, either a polymer coil or an extended rod, depending on whether the side chain of the macromonomer is short or long, respectively. The conformation of PBBs in solution is important to consider when designing a polymer because it will affect the accessibility of the side chains. A technique that allows researchers to probe the dynamics of molecules in solution is fluorescence, and more specifically pyrene excimer formation (PEF). When excited by a photon of light, pyrene can either emit as a monomer and decay with a characteristic monomer lifetime τ_M or encounter another ground-state pyrene molecule and form an excimer, which will emit with a different lifetime τ_E. The ability of a pyrene molecule to form an excimer can be applied to characterize a PBB in solution since PEF depends on the local concentration of ground-state pyrene. After labeling a PBB with pyrene to generate a pyrene-labeled PBB (Py-PBB), the efficiency of PEF can be monitored by fluorescence as a function of side chain length to predict possible changes in the conformation of the Py-PBBs in solution from a polymer coil to an extended rod.

Experimental Procedure

The synthesis of a Py-PBB begins by preparing the pyrene labeled macromonomer by first mono-labeling a monodisperse oligo(ethylene glycol) (OEG) using tosyl chloride, silver oxide, and potassium iodide (Scheme 1). The tosyl group is then replaced with a pyrenyl group by using 1-pyrenemethanol and sodium hydride. Finally, the methacrylation of the pyrene labeled OEG (Py-OEG) is conducted using methacrylic anhydride and 4-dimethylaminopyridine (DMAP). The chemical composition of the pyrene labeled monomers was verified using ^{1}H-NMR. Both time-resolved fluorescence (TRF) and steady-state fluorescence (SSF) measurements were conducted to determine the monomer lifetime τ_M and to ensure that the macromonomers were mono-labeled. These monodisperse macromonomers were then polymerized using a grafting through technique and conventional radical chain polymerization to obtain PBBs using azobisisobutyronitrile (AIBN) as the initiator in tetrahydrofuran (THF).
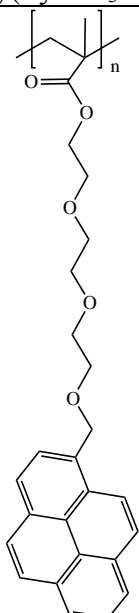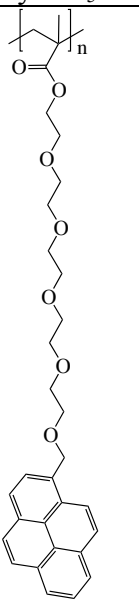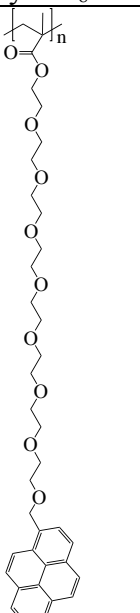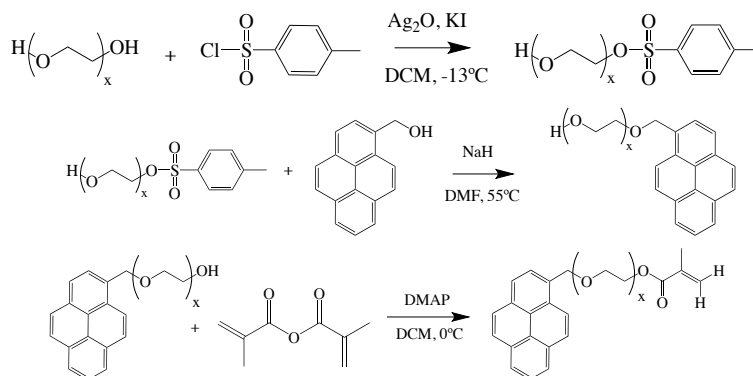

**Scheme 1:** Synthesis of the Py-OEG macromonomer.

Results and Discussion

Three Py-PBBs were synthesized with tri-, penta-, and octa(ethylene glycol) using the protocol outlined in Scheme 1. Their chemical structure and linker length from the polymer backbone to the center of the pyrenyl group (d_{BB-Py}) are shown in Table 1.

Table 1: Chemical structure and d_{BB-Py} (Å) for each Py-PBB.

| Sample	Poly(Py-EG$_3$-MA)	Poly(Py-EG$_5$-MA)	Poly(Py-EG$_8$-MA)
Structure			
Distance (Å)	17.5	24.0	33.8

The ^{1}H-NMR spectrum and the fluorescence data acquired for the pyrene labeled tri(ethylene glycol) (Py-EG$_3$-MA) macromonomer are shown in Figure 1.
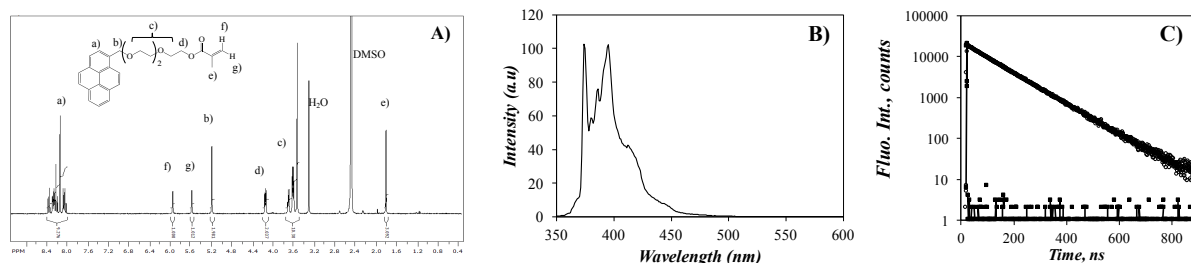
Figure 1: A) ^{1}H-NMR spectrum, B) SSF spectrum, and C) TRF decay for Py-EG$_3$-MA in THF. λ_{ex}= 344 nm, [Py]= 2.5 x 10^{-6} M.

All the peaks from the ^{1}H-NMR spectrum in Figure 1A were assigned confirming the synthesis of Py-EG$_3$-MA. The SSF spectrum (Figure 1B) shows only monomer emission, represented by the series of sharp peaks found between 375 and 410 nm and the lack of a broad structureless emission centered around 480 nm that would indicate PEF. The TRF decay shows mono-exponential behaviour with a lifetime of 270 nm in THF with a pre-exponential contribution of 0.98 thus indicating that a single fluorescence species is present in solution.

The three Py-PBBs synthesized were characterized by gel permeation chromatography (GPC). A sample GPC trace from poly(Py-EG$_3$-MA) is shown in Figure 2. The DRI signal corresponding to the polymer overlaps the absorbance signal confirming that the polymer is labeled with pyrene. No absorption is observed in the low molecular weight region where

unattached Py-EG3-MA monomer would elute confirming that all pyrene labels are covalently attached to the PBB.
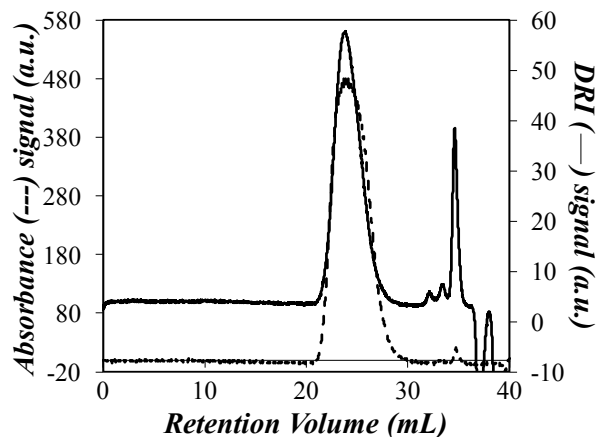
Figure 2: GPC trace of poly(Py-EG$_3$-MA) acquired with (---) UV and (—) DRI detectors.

A PBB can be viewed as an elongated cylinder where each monomer can be represented by a slice whose volume is proportional to R^2, where R is the radius of the cylinder. Ideally each monomer unit contains one pyrenyl group such that the local concentration of pyrene should be proportional to R^{-2}. Since the local concentration $[Py]_{loc}$ is proportional to the average rate constant $<k>$ of PEF, $<k>$ is expected to decrease as the length of the macromonomer linker increases. The fluorescence decays were fitted globally according to the Model Free Analysis (MFA). MFA yielded $<k>$ which was found to decrease with increasing side chain length shown in Figure 3A, as expected.
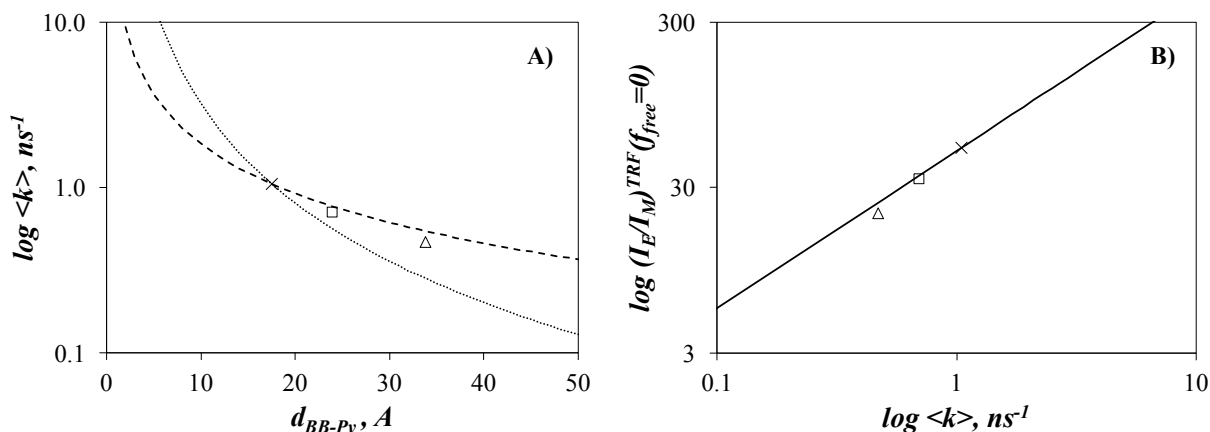
Figure 3: A) Plot of $<k>$ versus (d$_{BB-Py}$) in degassed THF compared to the scaling laws of (---) a coiled side chain with an r_{EE} equal to n and ($\cdots$) an elongated side chain with an r_{EE} equal to n^2, normalized to poly(Py-EG$_3$-MA). B) plot of $(I_E/I_M)^{TRF}(f_{free}=0)$ as a function of $<k>$ in degassed THF. (×) poly(Py-EG$_3$-MA), (□) poly(Py-EG$_5$-MA), and (△) poly(Py-EG$_8$-MA). λ_{ex}= 344 nm, [Py]= 2.5 x 10^{-6} M.

The fluorescence intensity ratio of excimer-to-monomer determined by combining the parameters retrieved from the MFA of the TRF decays, namely the $(I_E/I_M)^{TRF}(f_{free}=0)$ ratio, was then compared to $<k>$ as shown in Figure 3B. The solid line is a master curve modeled by Equation 1 using an α value of 0.95 and τ_E value of 50 ns determined from the MFA of the TRF decays acquired for 64 pyrene-labeled macromolecules (PyLMs) in degassed THF.[5] All three Py-PBBs were found to fall close to the master curve demonstrating that they follow the general behaviour expected for PyLMs.

$$\left(\frac{I_E}{I_M}\right)^{TRF}\left(f_{free}=0\right)=\tau_E\langle k\rangle^{\alpha}$$

(1)

If a Py-PBB is viewed as an elongated cylinder, the side chains of the Py-PBB can behave as either a random coil, with an end-to-end distance (r_{EE}) of $n^{0.5}$, where n is the number of atoms in the spacer, or they may stretch out toward the periphery of the Py-PBBs, with an r_{EE} equal to n. $<k>$ for each of the three Py-PBBs can be compared to two scaling laws where $<k>$ scales as either n or n^2 depending on whether the side chain is coiled or elongated, respectively, as shown in Figure 3A. The three Py-PBBs are shown to fall closer to the scaling law associated with side chains which have an r_{EE} equal to $n^{0.5}$ suggesting that the side chains of the PBBs adopt a random coil conformation in THF.

Conclusions

The synthesis of three PBBs was completed and their fluorescence decays analyzed to determine the effect of linker length on polymer conformation. So far a decrease in $<k>$ with increasing side chain has been observed; however there is no evidence of a conformational change which is expected to cause a discontinuity in a plot of $<k>$ versus d_{BB-Py}. This observation is complemented by the results obtained in Figure 3A which indicate that the side chains of the Py-PBBs behave as random coils in THF. The absence of discontinuity might be due to the relative flexibility of the OEG linker and as a result, the conformational change might occur at a higher linker length. The next Py-PBB to be synthesized will contain a linker with twelve ethylene glycol units.

References

1. Qi, Y.; Simakova, A.; Ganson, N. J.; Li, X.; Luginbuhl, K. M.; Ozer, I.; Liu, W.; Hershfield, M. S.; Matyjaszewski, K.; Chilkoti, A. A brush-polymer/exendin-4 conjugate reduces blood glucose levels for up to five days and eliminates poly(ethylene glycol) antigenicity. *Nat. Biomed. Eng.* **2016**, *1*, 0002.
2. Mukumoto, K.; Averick, S. E.; Park, S.; Nese, A.; Mpoukouvalas, A.; Zeng, Y.; Koynov, K.; Leduc, P. R.; Matyjaszewski K. Phototunable Supersoft Elastomers using Coumarin Functionalized Molecular Bottlebrushes for Cell-Surface Interactions Study. *Macromolecules* **2014**, *47*, 7852- 7857.
3. Ito, K. Polymeric Design by Macromonomer Technique. *Progr. Polym. Sci.* **1998**, *23*, 580-620.
4. Verduzco, R.; Li, X.; Pesek, S.L.; Stein, G. E. Structure, Function, Self-Assembly, and Applications of Bottlebrush Copolymers. *Chem. Soc. Rev.* **2015**, *44*, 2405-2420.
5. Farhangi, S.; Casier, R.; Li, L.; Thoma, J. L.; Duhamel, J. Characterization of the Long-Range Internal Dynamics of Pyrene-Labeled Macromolecules by Pyrene Excimer Fluorescence. *Macromolecules* **2016**, *49*, 9597-9604.