Polymer Chain Dynamics in Solution Probed with a Fluorescence Blob Model

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ABSTRACT
This Account describes a new fluorescence tool that characterizes the chain dynamics of polymers in solution. This new tool, coined the fluorescence blob model (FBM), is employed to analyze the fluorescence decays of polymers randomly labeled with pyrene. The FBM yields the number of monomers making up a *blob* (N_{blob}) where a blob is the volume probed by an excited pyrene. By establishing a relationship between N_{blob} and the lifetime of pyrene used as an internal clock, the FBM provides previously unavailable information about polymer chain dynamics and opens new venues of research, which are described in this Account.

Introduction

The ability to properly characterize the random encounters occurring between two far flung units of a same polymer chain in solution has major implications for the study of any phenomenon involving the dynamics of a polymer chain in solution. Such phenomena are the focus of interest of extremely active research areas with scopes as varied as the folding of proteins,[1] the conformational rearrangement of associative polymers in solution under shear,[2] or the collapse of a polymer coil via a temperature-induced coil-to-globule transition (CGT).[3] The significance of characterizing the large scale motions of a polymer chain in solution is illustrated in Figure 1. If two reactive units A and B are present on a polymer chain undergoing a CGT, a reaction between units A and B will occur only if the chain is flexible enough to enable the A and B units to encounter before the coil has collapsed into a gelled sphere where motions are considerably slowed down. Since proteins are believed to undergo a CGT as they fold from an expanded coil conformation to a dense tertiary structure,[4] such considerations bear some rather momentous implications to predict which amino acids of a folding polypeptide will end up as neighbors in a secondary structure of the final tertiary structure of the protein. This example illustrates the importance of being able to
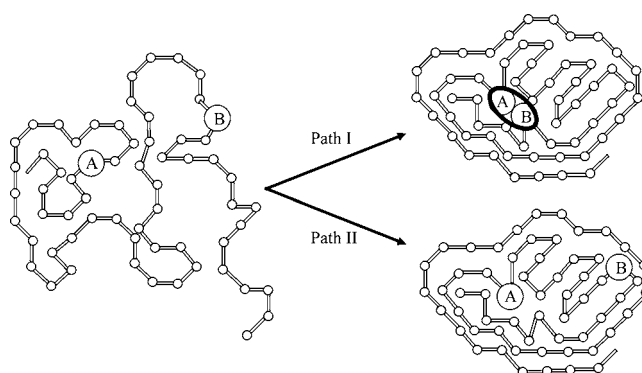
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FIGURE 1. Expanded polymer coil on the left undergoing a CGT and bearing two reactive units A and B. In path I, the chain is flexible enough for units A and B to encounter and react before the CGT ends. In path II, the chain is too stiff for units A and B to encounter before the CGT is complete.
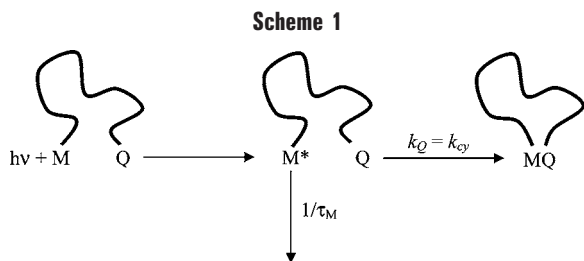
characterize the large scale motions of polymer chains experimentally.

Traditionally, fluorescence has been the technique of choice to study long-range polymer chain dynamics.[5] The most straightforward approach consists in covalently attaching a chromophore and its quencher at the ends of a monodisperse polymer chain[6] and determining the rate constant (k_Q) for the quenching of the excited chromophore from the analysis of the fluorescence decay of the quenched chromophore.[7] Since a quenching event indicates that the two ends of the monodisperse polymer have encountered, k_Q represents also the cyclization rate constant (k_{cy}) of the polymer (Scheme 1).

These elegant fluorescence experiments, introduced some 30 years ago,[6,8] enabled the first quantitative and direct determination of a parameter given by k_{cy} that would reflect the large scale motions of a polymer chain. These experiments demonstrated that k_{cy} decreases with increasing chain length (N) as it becomes more difficult for the two polymer ends to encounter.[7] In the case of polystyrene (PS) in cyclohexane at 34.5 °C, a θ-solvent for PS, k_{cy} decreased as $N^{-1.62}$.[9] In other words, a 4-fold increase in PS chain length results in a 10-fold decrease of k_{cy}. Relationships of the $k_{cy} = f(N)$ type are fundamental in establishing how solvent quality (good versus θ-solvent) or chemical structure affect polymer chain dynamics.

Although the $k_{cy} = f(N)$ trends describe the large scale motions of polymers in solution, it must be recognized that the primary purpose of these experiments was the study of end-to-end cyclization.[7] Inevitably the approach carries some inherent drawbacks when it is diverted to study polymer chain dynamics in solution. The first and foremost drawback is that it probes only the two labeled monomers at the ends of the chain. In the case of a polymer chain made of 100 monomers, 98% of the chain remains invisible, and the proportion of the chain being probed decreases further for larger N values. A second important drawback is that the drastic dependence of k_{cy} with N prevents the study of the chain dynamics of polymers that cannot be synthesized in a monodisperse

Scheme 1

form. Due to the $k_{cy} = f(N)$ dependency, the labeling of the two ends of a polydisperse polymer results in a distribution of k_{cy} values that cannot be easily extracted from the analysis of the fluorescence decays. A third drawback has to do with the study of extremely long or very rigid polymers. Both excessive chain length and rigidity of a polymer limit the number of cyclization events taking place and therefore prevent their detection.

Interestingly, all these drawbacks could be eliminated at once if instead of targeting the chain ends, one would attach the labels at internal positions of the chain. Ideally, these labels would be attached randomly along the chain to simplify the labeling chemistry. The random labeling of a chain would enable the study of (1) a polymer chain in its entirety since fluorescent tags would be randomly distributed along the chain, (2) polydisperse chains since the study would focus on the dynamics of the chain interior instead of the chain ends, and (3) long and stiff polymers since the distance spanning two labels could be shortened at will by increasing the labeling level to allow encounters between the chromophore and its quencher.

It was thus unfortunate that despite these obvious advantages, it was not until 1999 that any quantitative information about the long scale motions of a polymer in solution could be retrieved from the fluorescence study of a randomly labeled polymer. This situation was due to the absence of mathematical tools that could handle the kinetics of encounters occurring between labels randomly attached onto a chain. As a matter of fact, the random labeling of a polymer with a chromophore and its quencher generates a distribution of chain lengths spanning the two molecules,[10] which, according to the $k_{cy} = f(N)$ relationship established for end-labeled monodisperse polymers,[7] leads to a distribution of rate constants, which was intractable experimentally.

A solution to this roadblock was proposed in 1999 in the form of the fluorescence blob model (FBM).[11] The FBM is based on the fundamental assumption that upon excitation, a chromophore attached onto a polymer backbone will probe a restricted volume inside the polymer coil while it remains excited. This assumption is based on the observation that a given unit of a polymer chain cannot move freely inside the polymer coil since it must drag along the chain to which it is attached and weave its way inside the polymer coil. Furthermore, an encounter between the chromophore and its quencher can be probed by fluorescence only if the chromophore is still excited at the time of the encounter. If the distance separating a chromophore and its quencher inside the polymer coil is too large, the chromophore will not be able

to encounter the quencher before it emits a photon. It follows from this line of thoughts that during its lifetime, an excited chromophore probes a restricted volume inside the polymer coil, which was referred to as a *blob*. The blob was then used as a unit volume to partition the polymer coil into a cluster of blobs. The random labeling of the polymer coil implied that the quenchers would distribute themselves randomly among the blobs as shown in Figure 2. Without the polymer chain, Figure 2 is analogous to a cluster of jointed surfactant micelles loaded with chromophore and quencher molecules. Using the mathematical treatment derived 30 years ago by Tachyia to describe the kinetics of encounter between an excited chromophore and its quencher in surfactant micelles,[12] the analogy between blobs and surfactant micelles was employed to derive a mathematical equation that describes the kinetics of encounters between a chromophore and its quencher randomly attached onto a polymer. The result of this derivation[11] is shown in eq 1, which describes the time dependence of the concentration of the excited chromophore (M^*).

$$[M^*]_{(t)} = f_{\text{diff}} \exp\left[-\left(A_2 + \frac{1}{\tau_{\text{M}}}\right)t - A_3(1 - \exp(-A_4 t))\right] + f_{\text{free}} \exp(-t/\tau_{\text{M}}) \quad (1)$$

The A_2, A_3, and A_4 parameters used in eq 1 are functions of $\langle n \rangle$, the average number of quenchers per blob, k_{blob}, the quenching rate constant of an excited chromophore located in a blob containing a single quencher, and the product $k_e[\text{blob}]$ where k_e is the rate constant to exchange a quencher between blobs and $[\text{blob}]$ is the concentration of blobs inside the polymer coil. The relationship existing between the A_2, A_3, and A_4 parameters and $\langle n \rangle$, k_{diff}, and $k_e[\text{blob}]$ is given in eq 2.

$$A_2 = \langle n \rangle \frac{k_{\text{blob}} k_e[\text{blob}]}{k_{\text{blob}} + k_e[\text{blob}]}$$

$$A_3 = \langle n \rangle \frac{k_{\text{blob}}^2}{(k_{\text{blob}} + k_e[\text{blob}])^2}$$

$$A_4 = k_{\text{blob}} + k_e[\text{blob}] \quad (2)$$

In eq 1, the lifetime of the chromophore is referred to as τ_{M}. The fractions f_{diff} and f_{free} represent those excited chromophores that are being quenched by diffusional encounters with a quencher and those that are too isolated to encounter a quencher and fluoresce with a decay time equal to τ_{M}, respectively.

Choice of an Appropriate Chromophore—Quencher Pair

Special attention must be paid to the choice of a chromophore since it is essential that its lifetime match the time window over which chain dynamics occur. Unfortunately, this requirement reduces the number of potential probes. That and the fact that a macromolecule is viewed through the lens of a bulky chromophore remain two notable inconveniences for any fluorescence study of polymer chain dynamics.
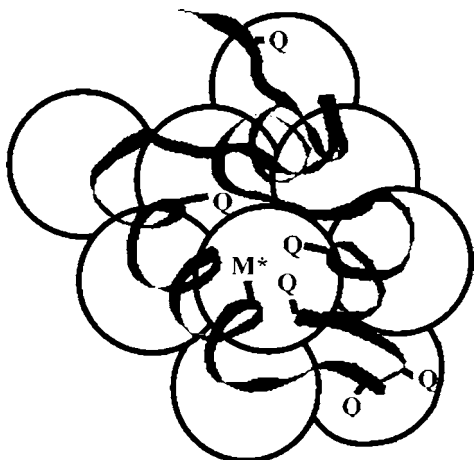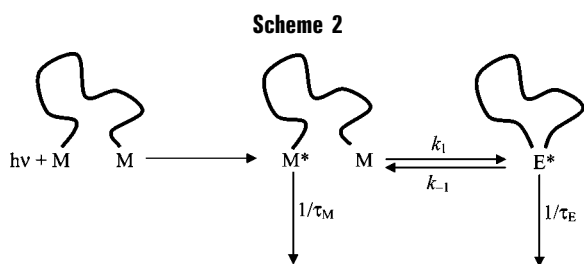
FIGURE 2. Polymer coil onto which a chromophore (M) and several quenchers (Q) are randomly attached. The polymer coil is partitioned into a cluster of blobs.

Scheme 2

By and large, the majority of studies characterizing the long-range motions of polymers by fluorescence quenching experiments use the chromophore pyrene.[7,13,14] The reasons for choosing pyrene reside in its relatively large extinction coefficient, its long lifetime (200–300 ns), which enables the study of the long time scales associated with the large scale motions of polymer chains, and its ability to form an excimer (E) upon diffusion-controlled encounters between an excited pyrene and a ground-state pyrene. The kinetics of excimer formation have been intensively studied and are well-described by the Birks' scheme (Scheme 2).[15]

In Scheme 2, k_1, k_{-1}, and τ_E are the rate constant for excimer formation by diffusion, the dissociation rate constant of the excimer, and the excimer lifetime, respectively. In many reports, k_{-1} is found to be very small, and the dissociation of the excimer is often neglected.[10,11] If k_{-1} is set to equal zero, Scheme 2 is equivalent to Scheme 1. Since the FBM deals with the quenching of a chromophore, the FBM applies to a polymer chain randomly labeled with pyrene, and the time-dependent concentration profile of an excited pyrene monomer is actually well-fit with eq 1.[11,16,17]

Validity of the Fluorescence Blob Model

The first study aimed at establishing the validity of the FBM was conducted with a series of polystyrenes randomly labeled with pyrene having pyrene contents ranging from 0.2 to 7.0 mol %.[11] Three monodisperse polystyrenes ($M_w/M_n < 1.15$) were prepared by anionic polymerization with M_n equal to 6000 (MD6K), 40 000 (MD40K), and
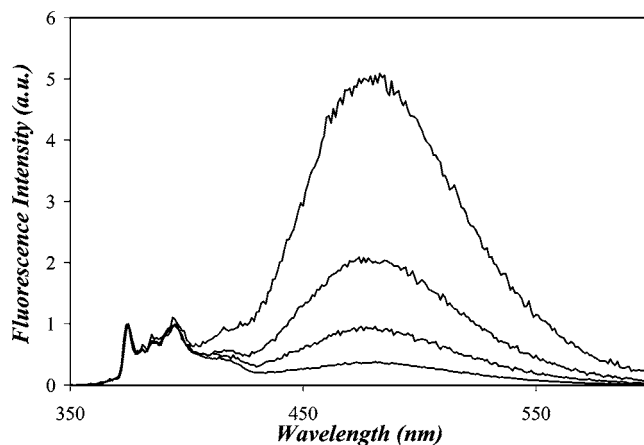

FIGURE 3. Fluorescence spectra in THF of MD110K randomly labeled with pyrene. Pyrene content from bottom to top is 1.3, 2.5, 4.6, and 7.0 mol %. [Py] = 2.5×10^{-6} M.
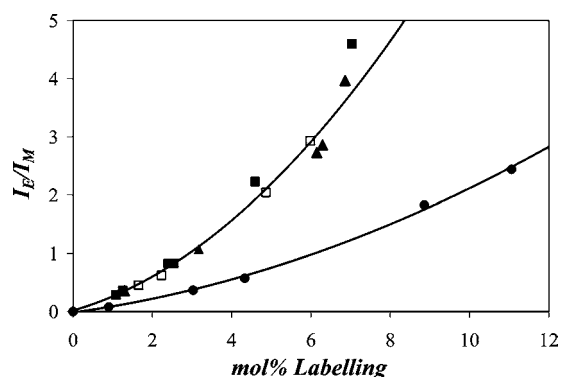

FIGURE 4. I_E/I_M for Py–PS in THF as a function of pyrene content in mole percent: (●) MD6K, (□) MD40K, (■) MD110K, and (▲) PD110K. [Py] = 2.5×10^{-6} M.

110 000 (MD110K), and a polydisperse polystyrene ($M_w/M_n = 1.5$) was prepared by free radical polymerization with M_n equal to 110 000 (PD110K). The chains were randomly chloromethylated and reacted with pyrenemethoxide anion to yield polystyrene samples randomly labeled with pyrene (Py–PS). To prevent intermolecular excimer formation, the fluorescence spectra of dilute Py–PS solutions in tetrahydrofuran (THF) were acquired and are shown in Figure 3 for MD110K. It can be seen in Figure 3 that as the pyrene content of Py–PS increases, more encounters occur between the pyrenyl pendants and more excimer is being produced.

The ratio of the fluorescence intensity of the excimer over that of the monomer, the I_E/I_M ratio, is shown in Figure 4 for all Py–PS samples. The I_E/I_M ratio increases with pyrene content, but whereas all I_E/I_M ratios for the larger Py–PS samples cluster on a master curve, those obtained with the short Py–PS sample depart from the master curve. These trends are informative. The main feature of the FBM is that the focus of the study is switched from the entire polymer chain to a single blob. Consequently, if a phenomenon occurring with a polymer can be described by the FBM, this phenomenon should not be affected by polymer chain length, as long as the polymer is long enough to accommodate a sufficiently large number of blobs. According to this statement, the
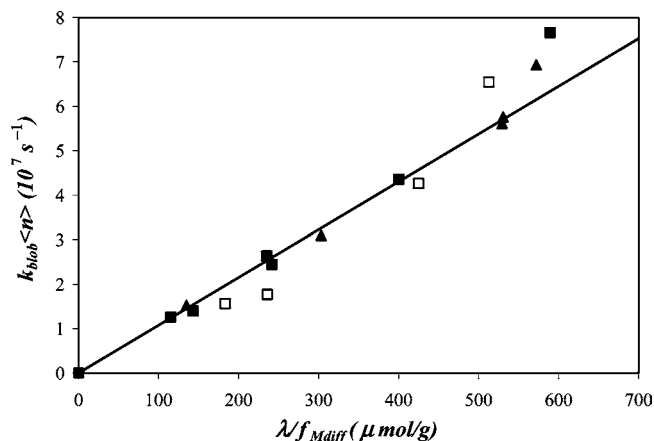
FIGURE 5. Plot of $k_{diff}\langle n \rangle$ versus λ/f_{Mdiff}: (□) MD40K, (■) MD110K, and (▲) PD110K.

trends shown in Figure 4 strongly suggest that the process of excimer formation between pyrenes randomly distributed along a polymer chain should be described by the FBM. Excimer formation appears to be independent of polymer chain length and polydispersity, as long as M_n is sufficiently large. In Figure 4, MD6K in THF appears to be too small.

The fluorescence decays of the pyrene monomer of all Py−PS were acquired and fit with eq 1. The fits were good, and the physical meaning of the parameters $\langle n \rangle$ and k_{blob} was investigated. The first observation made was that all FBM parameters fell on master curves regardless of polymer chain length or polydispersity for the Py−PS having a M_n value larger than 6000, indicating that the FBM does not depend on chain length or polydispersity, as expected. From the analogy existing between blobs and micelles, k_{blob} should be inversely proportional to the volume of a blob (V_{blob}). Since $\langle n \rangle$ represents the average number of pyrenes per blob, the product $k_{blob}\langle n \rangle$ is expected to be proportional to $\langle n \rangle/V_{blob}$, which represents the local concentration of pyrene inside the polymer coil. A plot of $k_{blob}\langle n \rangle$ versus the corrected pyrene content (λ/f_{diff}) of the Py−PS samples is shown in Figure 5. The pyrene content of Py−PS (λ) is replaced by λ/f_{diff} in Figure 5 to account for these pyrene-poor regions, which do not form excimer.[11] Usually f_{diff} is larger than 0.90 for pyrene contents greater than 2 mol %, so that the effect of this correction is small. The linear relationship obtained in Figure 5 confirms that the local concentration of pyrene inside the polymer coil described by the quantity $k_{blob}\langle n \rangle$ increases linearly with pyrene content, as expected from the FBM.

The rate of excimer formation between an excited pyrene and one ground-state pyrene located in the same PS blob, k_{blob}, was found to increase from 1.3×10^7 s^{-1} for a pyrene content of ∼1 mol % to 2.1×10^7 s^{-1} for a pyrene content of ∼6 mol %. Since k_{blob} is inversely proportional to V_{blob}, the increase of k_{blob} with increasing pyrene content suggests that V_{blob} is decreasing. A measure of V_{blob} is offered by the quantity N_{blob}, the number of units

making up a blob, whose expression is given in eq 3.

$$N_{blob} = \frac{\langle n \rangle}{(\lambda/f_{diff})[M_{Py}x + M_u(1 - x)]} \tag{3}$$

In eq 3, M_{Py}, M_u, and x represent the molar mass of a monomer labeled with pyrene, the molar mass of an unlabeled monomer, and the mole fraction of labeled monomers in the polymer, respectively. N_{blob} was found to decrease from 75 units for a pyrene content of ∼1 mol % to 45 units for a pyrene content of ∼6 mol %. The concomitant decrease of N_{blob} and the increase of k_{blob} ($\sim V_{blob}^{-1}$) with increasing pyrene content are internally consistent with the FBM. Since the changes observed for k_{blob} and N_{blob} could be due to increased labeling, which would affect the polymer properties, the trends obtained for k_{blob} and N_{blob} were extrapolated to zero pyrene content. The resulting k_{blob} and N_{blob} values for an ideal polystyrene blob in THF were found to equal 1.0×10^7 s^{-1} and 76, respectively. An N_{blob} value of 76 represents a chain segment larger than MD6K. Therefore, the departure from the master curve of the I_E/I_M ratios obtained with MD6K in Figure 4 is simply due to MD6K being smaller than a single blob.

The FBM was validated by a second study of pyrene-labeled poly(N,N-dimethylacrylamide)s (Py−PDMAs) with pyrene contents ranging from 0.2 to 7.3 mol %.[16] The Py−PDMA samples were prepared by free radical copolymerization of N,N-dimethylacrylamide and N-(1-pyrenyl-methyl)acrylamide. Dilute solutions of the Py−PDMA samples were studied in acetone (a θ-solvent) and N,N-dimethylformamide (DMF, a good solvent). The quality of acetone and DMF toward Py−PDMA was established by measuring the second virial coefficient of Py−PDMA in these solvents by static light scattering. The fluorescence decays of the Py−PDMA solutions were acquired and analyzed with the FBM. The fits were good. As with the Py−PS samples, the product $k_{blob}\langle n \rangle$ was found to increase linearly with λ/f_{diff} confirming that the pyrene local concentration inside the polymer coil increases linearly with pyrene content. Within experimental error, the parameters k_{blob} and N_{blob} remained constant with pyrene content and equaled 0.9×10^7 s^{-1} and 60 in acetone and 1.1×10^7 s^{-1} and 26 in DMF, respectively. In acetone, a θ-solvent for Py−PDMA, the polymer is more compact resulting in a larger N_{blob}.

Although the FBM satisfyingly handles the fluorescence decays of linear polymers randomly labeled with pyrene in dilute solution, the inherently fuzzy nature of a blob makes it difficult to describe what a blob exactly represents. To address this concern, poly(L-glutamic acid) was randomly labeled with 1-pyrenemethylamine to yield Py−PGA.[17] PGA in the acid form adopts an α-helical conformation in DMF. Analysis of the fluorescence decays of the pyrene monomer of the Py−PGA samples in DMF yielded an N_{blob} value of 31, suggesting that an excited pyrene can interact with a ground-state pyrene located within 15 amino acids either up or down the α-helix. Molecular mechanics optimizations were conducted by generating

a PGA α-helix *in silico*. Two glutamic acids of the PGA helix were labeled with 1-pyrenemethylamine. The number of amino acids spanning the two pyrenes was increased one amino acid at a time and an optimization was performed for each PGA α-helix labeled with two pyrenes by searching the most favorable conformation of the side chains that would enable the overlap of the chromophores, while holding the α-helix backbone unchanged during the optimizations. The optimizations showed that the two pyrenes could not overlap when they were separated by more than 15 residues, in perfect agreement with the N_{blob} value retrieved experimentally from the FBM analysis of the Py−PGA fluorescence decays.

Since the results obtained with Py−PS,[11] Py−PDMA,[16] and Py−PGA[17] were internally consistent within the FBM framework, they suggest that the FBM is an appropriate tool to study in a quantitative manner the chain dynamics of polymers randomly labeled with pyrene.

The FBM and Polymer Physics

The assumption on which the FBM is based is that a chromophore probes a subvolume of the polymer coil while it remains excited. According to this assumption, shortening the lifetime of the chromophore should yield a smaller V_{blob}. This assumption was tried with the Py−PDMA series. Nitromethane is an efficient quencher of pyrene. The fluorescence decays of the pyrene monomer of Py−PDMA were acquired as increasing amounts of nitromethane were added to the dilute Py−PDMA solutions.[18] In these experiments, nitromethane is an external quencher that diffuses rapidly in solution. It behaves differently from the pyrenyl pendants, which rearrange themselves on a much slower time scale since they are covalently attached to the slow-moving chain. The lifetime of the pyrene label was evaluated as a function of nitromethane concentration from the fluorescence decays of a Py−PDMA sample with a very low pyrene content (0.2 mol %). It decreased with increasing nitromethane concentration.

N_{blob} was found to decrease linearly with increasing nitromethane concentration and decreasing pyrene lifetime. This result enables one to predict how far within the polymer coil a monomer can move as a function of time, an important piece of information in the context of the discussion generated by Figure 1. The decrease of N_{blob} with increasing nitromethane concentration was accompanied by an increase in k_{blob}. This effect was expected since k_{blob} is inversely proportional to V_{blob}. As N_{blob} decreased with increasing nitromethane concentration, so did V_{blob}. The quenching experiments provided a relationship between k_{blob} and N_{blob} reminiscent of the $k_{cy} = f(N)$ relationship obtained with the cyclization experiments.[7] As shown in Figure 6, k_{blob} scales as $N_{blob}{}^{\gamma}$ where γ equals -1.8 ± 0.3 and -1.4 ± 0.2 in DMF and acetone, respectively. Since k_{blob} is inversely proportional to V_{blob}, these exponents are those expected if V_{blob} were a sphere whose radius R_{blob} would scale as $N_{blob}{}^{\nu}$, with ν equal to 0.6 ± 0.1
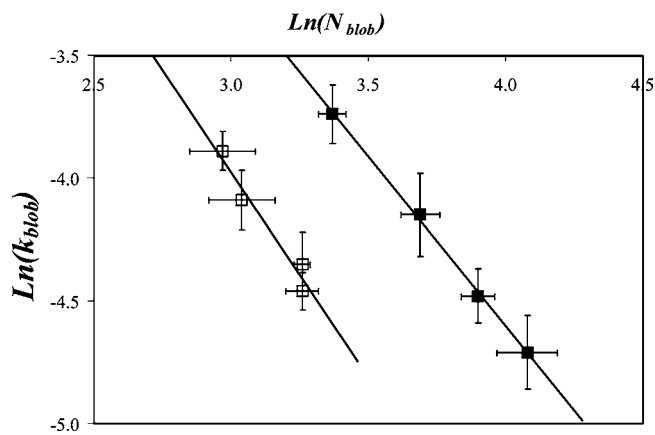

FIGURE 6. Plot of $\ln(k_{blob})$ vs $\ln(N_{blob})$ obtained in acetone (■) and DMF (□). The slopes equal -1.8 ± 0.3 in DMF and -1.4 ± 0.2 in acetone.

in DMF (a good solvent for Py−PDMA) and 0.5 ± 0.1 in acetone (a θ-solvent for Py−PDMA). These values are the Flory exponents for a good and a θ-solvent.[19] Incidentally, these exponents also bracket the γ-value of 1.62 obtained for the $k_{cy} \propto N^{-\gamma}$ relationship for pyrene end-labeled polystyrenes in cyclohexane at 34.5 °C.[7,9]

The Py−PDMA samples were also used to determine the effect of polymer concentration on excimer formation. In these experiments, solutions were prepared in acetone and DMF where small amounts of Py−PDMA (4−10 mg/L) were mixed with large concentrations of naked PDMA (nPDMA) ranging from 15 to 550 g/L.[20] Since acetone, DMF, and PDMA are transparent at 344 nm where pyrene absorbs, these experiments reported on the behavior of isolated Py−PDMA coils in a sea of nPDMA. The fluorescence spectra of the solutions were acquired. The I_E/I_M ratio was determined. As the nPDMA concentration increased, the I_E/I_M ratio decreased. Such a trend is expected because the solution viscosity increases with increasing nPDMA concentration, which hinders the encounters between pyrenyl pendants. However, the decrease of the I_E/I_M ratio was rather modest when compared with the viscosity increase. Increasing the nPDMA concentration in DMF from ∼0 to 330 g/L resulted in a 300-fold increase of the solution viscosity, but the I_E/I_M ratio decreased by only 35%. According to the Birks' scheme,[15] the I_E/I_M ratio is inversely proportional to the solution viscosity. The rather small decrease of the I_E/I_M ratio observed with increasing nPDMA concentration indicates that locally, the chains experience large freedom of motions, as found by others.[21]

The fluorescence decays of the pyrene monomer of the Py−PDMA samples containing increasing nPDMA concentration were acquired and analyzed with the FBM.[20] N_{blob} was calculated. It was found to decrease linearly with increasing nPDMA concentration. Increasing the nPDMA concentration to 500 g/L resulted in a 70% decrease in N_{blob} either in acetone or in DMF from the N_{blob} value obtained under dilute conditions. As for the I_E/I_M ratio, the decrease of N_{blob} with increasing polymer concentration was rather modest when compared with the enormous increase in macroscopic viscosity experienced by
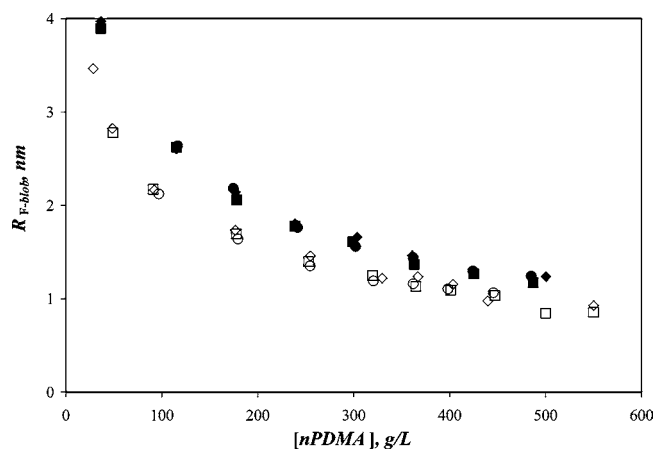
FIGURE 7. Plot of R_{F-blob} as a function of PDMA concentration for P265 ($\diamond$, $\blacklozenge$), P385 ($\bigcirc$, $\bullet$), and P570 ($\square$, $\blacksquare$) in DMF (open symbols) and acetone (filled symbols).

the solution when the polymer concentration increases from ~0 to 500 g/L. As mentioned earlier, this effect is due to the large local mobility of the chain.[21]

Since the polymer concentration is the same throughout the solution for nPDMA concentrations larger than the overlap concentration ($C^* = 26$ and 42 g/L for nPDMA in DMF and acetone, respectively), V_{blob} could be determined quantitatively from $N_{blob}M_{DMA}/[nPDMA]$, where M_{DMA} is the molecular weight of *N,N*-dimethylacrylamide. Assuming spherical blobs, the radius of a blob (R_{blob}) was obtained in DMF and acetone as a function of nPDMA concentration. As shown in Figure 7, R_{blob} decreased with increasing nPDMA concentration. For nPDMA concentrations larger than 200 g/L, R_{blob} was found to scale as $N_{blob}{}^{\nu}$ in DMF where ν equalled 0.66, very close to the expected Flory exponent of 0.6 for a polymer in a good solvent.[19] This study is the first, and currently only, example in the literature where R_{blob}, a measure of the distance travelled by an excited pyrene inside the polymer coil, is determined experimentally.

At polymer concentrations larger than C^*, the nPDMA concentration becomes equivalent to the density of the polymer inside the polymer coil (ρ_{coil}). Since the previous study[20] found that N_{blob} decreases with increasing nPDMA concentration above C^*, it can also be said that as ρ_{coil} increases, N_{blob} decreases. On the other hand, the work done by quenching the excited pyrene with nitromethane demonstrated that N_{blob} increases when a monomer is allowed more time to probe its surroundings.[18] Consequently, probing time (t_P) and ρ_{coil} oppose each other in the way they affect N_{blob}. This information is important when dealing with coil-to-globule transitions (CGTs) of a polymer coil because CGTs occur over time so that both t_P and ρ_{coil} increase during a CGT.[22] This concept is illustrated in Figure 8. It was argued earlier,[20] that as a polymer coil undergoes a CGT, N_{blob} can neither increase much with t_P nor decrease much with ρ_{coil} leading to the tantalizing proposal that both effects cancel each other in such a way that N_{blob} remains constant during a CGT! For small N_{blob} values, the above proposal suggests that any unit of the polymer chain will interact with a limited
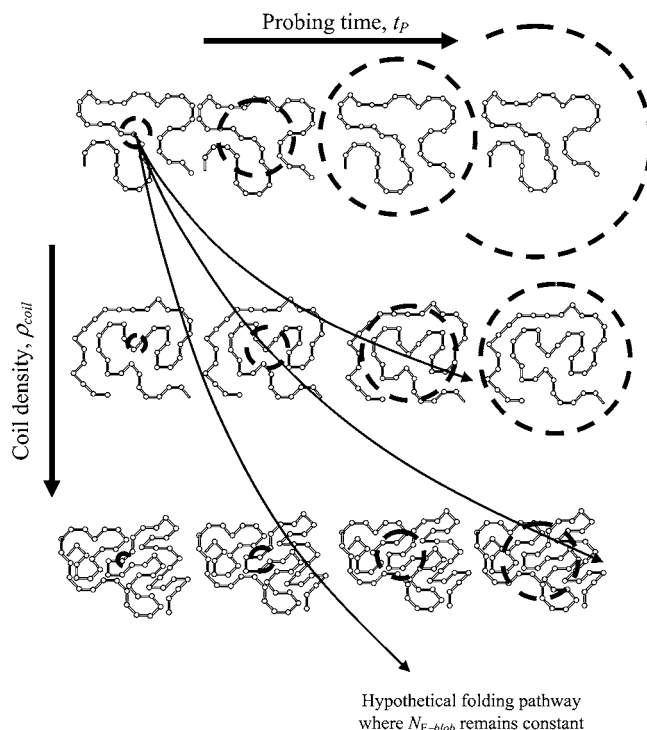

Hypothetical folding pathway
where N_{F-blob} remains constant

FIGURE 8. Illustration of how t_P and ρ_{coil} affect N_{blob} during the CGT of a polymer coil.

number of monomers during a CGT, a conclusion that should drastically limit the number of possible contacts between monomers in the globule state. If proven correct, this proposal should have major implications to answer the question raised in Figure 1 and, incidentally, for the study of protein folding, which is believed to begin with a CGT.[4] As a matter of fact, limiting the number of conformations that a protein can adopt has been a major drive to develop new models to mimic protein folding after Cyrus Levinthal stated his famous paradox.[23]

In a clever development, Prof. Martinho of Lisbon, Portugal, and co-workers applied the FBM to determine the volume of a polymer coil (V_{coil}) in solution. The V_{coil} values that they retrieved for Py—PDMA[24] and Py—PS[25] samples were similar to the hydrodynamic volume of the polymer coil determined by dynamic light scattering. Consequently the FBM was used to determine V_{coil} of Py—PDMA[24] and Py—PS[25] in solution as they underwent a CGT. These studies took advantage of the unmatched sensitivity of fluorescence, which enables experiments to be performed at extremely low polymer concentrations (<10 mg/L), minimizing chain aggregation, which often happens during a CGT where solvent quality toward the polymer is diminished.

Applications of the FBM

The FBM can be viewed as a mathematical tool that handles the kinetics associated with the diffusion-controlled encounters between pyrenes randomly distributed along a polymer chain. As it turns out, the FBM also applies when the ground-state pyrenes are clustered into pyrene aggregates since the pyrene aggregates act as another quencher species randomly distributed inside the
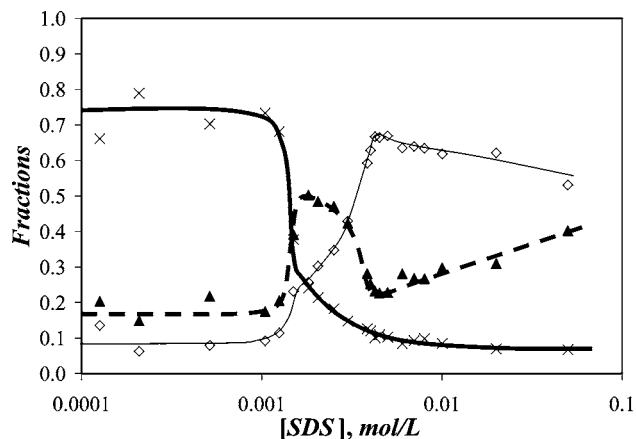
FIGURE 9. Fractions f_{diff} ($\Diamond$), f_{free} ($\blacktriangle$), and f_{agg} ($\times$) for a 1.0 g/L Py−HASE solution as a function of SDS concentration.

polymer coil. Working with pyrene has the additional advantage that a time-resolved fluorescence decay of the pyrene excimer can distinguish between those excimers that are formed quasi-instantaneously by direct excitation of a pyrene aggregate and those that occur over a longer time scale being the result of a diffusional encounter between an excited pyrene and a ground-state pyrene. Global analysis of the fluorescence decays of the pyrene monomer and excimer using the FBM yields the fraction of pyrenes that are aggregated (f_{agg}).[16,17,25−30] This effect can be used to study hydrophobically modified water-soluble polymers (HMWSPs) and hydrophilically modified oil-soluble polymers (HMOSPs).

HMWSPs and HMOSPs are used as rheology modifiers in aqueous solutions[31] and apolar oils,[32] respectively. HMWSPs are constituted of a water-soluble backbone onto which hydrophobic pendants are covalently attached. HMOSPs are made of an oil-soluble apolar backbone into which oil-insoluble polar groups are incorporated. Intermolecular association of the insoluble pendants in solution generates large polymeric aggregates resulting in a substantial viscosity increase of the solution. Since the viscoelastic behavior of these solutions is controlled by the level of association between the insoluble pendants,[2] the determination of f_{agg} is a key element for the characterization of these systems. When the polar groups of a HMOSP are labeled with pyrene or the hydrophobes of a HMWSP are replaced with pyrene, the FBM can be applied to determine f_{agg} experimentally.

The f_{agg} parameter has been determined for a series of Py−PDMA samples in acetone−water mixtures.[16] The f_{agg} parameter was found to increase with the water-content of the mixtures. The f_{agg} parameter was also determined for pyrene-labeled hydrophobically modified alkali-swellable emulsion copolymers (Py−HASE) in basic aqueous solution and THF as a function of pyrene content.[26] The pyrene pendants were not aggregated in THF but showed ∼60% aggregation in aqueous solution. The variation of f_{agg} with the addition of sodium dodecyl sulfate (SDS) to a Py−HASE solution was monitored;[27,28] f_{agg} decreased as the hydrophobic pyrene pendants were incorporated inside SDS micelles (Figure 9).

Maleated ethylene−propylene copolymers (EP) are examples of HMOSPs. Maleated EPs were pyrene-labeled to yield Py−EP, and f_{agg} was determined in hexane, an apolar solvent.[29] Since the distribution of pyrene reflects the distribution of maleic anhydride pendants on the backbone, f_{agg} could be used to determine the inherent level of maleic anhydride clustering on the EPs, which results from the maleation of EP.[32] These experiments illustrated how the clustering of insoluble pendants affects the viscosity of the solution of a modified polymer as the Py−EP sample with clustered maleic anhydrides associated more efficiently in hexane, resulting in a stronger viscosity increase with polymer concentration.[29] The effect on f_{agg} upon adding toluene, which is equally polar as hexane but is aromatic, to a Py−EP solution in hexane was investigated.[30] Addition of toluene resulted in a substantial decrease of f_{agg}. In other words, aromatic compounds are very efficient at reducing the level of association existing between the polar pendants of Py−EP.

The above experiments[16,17,25−30] constituted the first examples in the literature where the behavior of all pendants of a modified polymer were monitored quantitatively whether these pendants were in a dissociated or aggregated state.

Conclusions

The FBM is a new mathematical tool that handles the diffusion-controlled encounters occurring in solution between the fluorescent pendants of a polymer. It has provided some new, previously unattainable insights about the distance travelled by a monomer inside a polymer coil as a function of time and coil density. The validity of the FBM was established by studying a series of pyrene-labeled PS, PDMA, and α-helical PGA. The FBM has been applied to the study of various phenomena such as CGTs or the clustering of the insoluble pendants of modified polymers. It has been shown to be a robust and versatile tool that can answer difficult questions encountered in polymer science.

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