Cyclization dynamics of polymers: 10 Synthesis, fractionation, and fluorescent spectroscopy of pyrene end-capped polystyrenes

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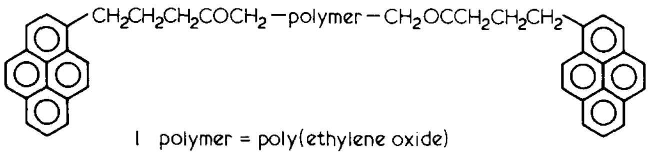
The rate constant for end-to-end cyclization (k_1) for polymer chains is predicted to decrease sensitively with increasing chain length. In this paper the techniques are examined critically for extracting values of k_1 from experiments involving intramolecular pyrene excimer formation in polymers of the form pyrene-polystyrene-pyrene. For significance, results require samples of appropriately narrow molecular weight distribution ($\bar{M}_w/\bar{M}_n \leqslant 1.13$), as well as corrections for polydispersity differences among the samples. Particular attention is focussed both on experimental techniques and on the models used to interpret the kinetics of intramolecular pyrene excimer formation.

Keywords

INTRODUCTION

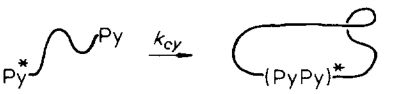
Several years ago Wilemski and Fixman[1] presented a theoretical treatment which related the rate constant, k_{cy}, for end-to-end cyclization of a polymer chain to the slowest internal relaxation time (τ_1) of the chain. They suggested that if a polymer could be synthesized containing a fluorescent group on one end, and an efficient quencher on the other end, it would be possible to carry out fluorescence quenching experiments and determine both k_{cy} and τ_1. Such an experiment could be carried out unambiguously, and if the Wilemski–Fixman [WF] theory withstood experimental scrutiny, there would be a direct means of determining τ_1 at the molecular level, uncontaminated by rotational or translational diffusion of the macromolecule.

Among the fluorescent chromophores that might be suitable for testing the WF theory, pyrene has several features which recommend it. Pyrene is intensely fluorescent with a long fluorescent lifetime ($\approx$ 200 ns). Its excited singlet state survives long enough to probe events which occur on the nanosecond and microsecond time scale. Singlet excited pyrene reacts with pyrene in its ground electronic state to form an electronically excited, tightly bound dimer (an excimer) which is also fluorescent[2]. Whereas pyrene emits blue light ($\lambda_{max} = 380$ nm) the excimer emits green light ($\lambda_{max} = 480$ nm), facilitating its detection. The excimer forming reaction [$Py^* + Py \rightarrow (PyPy)^*$] occurs on every encounter; it is diffusion controlled. At room temperature and below, excimer dissociation back to $Py^* + Py$ is normally negligible. It is presumed that the intramolecular analogue of this reaction would also show diffusion-controlled kinetics[3]. There are no photoproducts produced in the reaction.



1 polymer = poly(ethylene oxide)
2 polymer = polystyrene

Cuniberti and Perico[4] were the first to recognize the feasibility of using intramolecular pyrene excimer formation in polymers to test the ideas developed by Wilemski and Fixman. They prepared samples of poly(ethylene oxide) containing pyrene groups at each end (**1**). When these were excited with u.v. light, they observed green excimer fluorescence in competition with blue fluorescence from locally-excited pyrene groups. They explained their results in terms of diffusion controlled intramolecular end-to-end cyclization of the polymer chains.[4]

 (1)

In our own laboratory we used pyrene excimer formation to study cyclization dynamics in polymers by preparing polystyrene samples end-capped with pyrene groups (**2**). These were studied by steady-state fluorescence and fluorescence decay techniques[5]. While the pyrene excimer technique seemed promising, it became clear, from this work[5] and the publications of Cuniberti and Perico[4], that there are a number of pitfalls in the method. Some of these problems are related to the purity

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of the materials studied, others to the adequacy of interpreting the raw data from spectroscopic measurements. Until these problems could be solved, any experiments would remain ambiguous in their implications. It would be impossible to generate experimental data sufficiently accurate to subject the WF theories to experimental scrutiny.

In this paper several of these difficulties are examined. The number of problems which can be overcome are described and the precautions necessary for carrying out a proper analysis of the data obtained are stressed.

Mita, Horie and their co-workers[6] have also studied cyclization dynamics by a flash photolysis technique, examining triplet–triplet annihilation between anthracene groups attached to the ends of polystyrene chains. There are considerable differences between their results and those reported here. The interested reader is referred to their papers for details.

EXPERIMENTAL

Synthesis of the polymers

Hydroxy-terminated polystyrenes were synthesized at the Centre des Recherches sur les Macromolecules. The polymers were prepared by the initiation of styrene polymerization by naphthyl potassium in tetrahydrofuran, (THF), under argon at −60°C. The polymerization was rapid and exothermic. After the addition was complete, the solution temperature increased to −30°C. This solution was cooled to −60°C and quenched by the addition of 2 ml of ethylene oxide for 50 g styrene and an anticipated polymer molecular weights of 10000. For lower molecular weights the amount of ethylene oxide added was typically three times the theoretical amount required. After 3–4 min vigorous stirring at −60°C, the solution gelled, stirring ceased and the gel was allowed to stand for 1 h and to cool to room temperature.

When the last traces of the rose-pink colour of the benzyl carbanion disappeared, the solution was quenched with 3 ml glacial acetic acid. The gel collapsed immediately. When the solution became homogenous, the polymer was precipitated by the slow addition of the reaction mixture to excess methanol: 5 l of methanol were used for 600 ml THF containing 50 g polymer.

In attempts to prepare polymer of $\bar{M}_n \leqslant 2500$ the lowest weight components failed to precipitate, and, in addition, the polymer contained significant amounts of naphthalene. Polydispersities $[\bar{M}_w/\bar{M}_n]$ were typically 1.2–1.6 with two of the low molecular weight preparations giving $\bar{M}_w/\bar{M}_n$ values slightly above 2.

Polystyrene containing a single-OH group was synthesized using standard vacuum line techniques. *Sec*-butyl lithium in hexane was used to initiate styrene polymerization in THF at −78°C (dry ice–acetone). After completion of the polymerization, the polymer 'living ends' were quenched by ethylene oxide via a breakseal connection. The solution was stirred vigorously at −78°C and allowed to cool to room temperature. When the pink colour of the benzyl carbanion had disappeared, the system was opened to the atmosphere and neutralized with glacial acetic acid. The polymer was purified by reprecipitation from toluene solution with methanol.

Synthesis of the pyrene-terminated polymer

The HO-terminated polymer, naphthalene-free, was treated with excess 4-(1-pyrene)-butyryl chloride in toluene–pyridine as described previously[5]. Gel permeation chromatographic analysis showed that the product pyrene-terminated polymer, after synthesis, still contained low molecular weight fluorescent impurities. The previous synthesis procedure was modified as follows: the reaction mixture for 1 g of polymer was diluted with 20 ml of toluene and stirred with 100 mg of activated charcoal and 250 mg. Silica gel for 15 min. It was then filtered and concentrated to 10 ml. The polymer was precipitated by the dropwise addition of this solution to 100 ml of methanol. The polymer synthesized in this way contained no detectable low molecular weight fluorescent or u.v. absorbing impurities.

Fractionation of the polymer

Samples of HO-polystyrene-OH were chromatographed on silica gel columns (40 cm × 1 cm for 2 g polymer) using Machery–Nagel silica gel and eluting with increasing amounts (0.1–5%) methanol in chloroform. The high molecular weight polymers elute from the column before the low molecular weight material. Details have been reported elsewhere[7]. An example is given in *Table 1*. These fractions, after concentrating to 1 ml, were precipitated by dropwise addition to 50 ml of methanol, filtered and dried. Naphthalene eluted from the column in the first fraction.

After the pyrene groups were attached to the chain ends, most polymer samples were further fractionated (tail-clipping) by gel-permeation chromatography [g.p.c.] using a Waters HPLC and three microstyragel columns (10^3, 10^2, 50 nm). These fractions were freeze-dried from benzene. In this form, stored in the dark, these polymer samples are stable for at least three years.

Molecular weight determination

Molecular weights were determined by gel-permeation chromatography using a Waters model 6000 HPLC pump, a Waters model differential refractometer, and microstyragel columns of pore size 10^3, 10^2 and 50 nm. The system was callibrated using polystyrene standards purchased from Pressure Chemical Co.

End group analysis

The number of pyrenes per chain were analysed by u.v. absorption spectroscopy using either a Cary 16 or Cary

Table 1 Fractionation of HO-terminated polystyrene of $\bar{M}_n$ = 8000, $\bar{M}_w/\bar{M}_n$ = 1.58 by silica gel chromatography[a]

Fraction[b]	Weight collected (mg)	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
2	420	>20 000	–
3	370	17 500	1.11
5	180	14 500	1.10
6	130	13 400	1.10
8	160	12 400	1.05
12	150	8200	1.06
13	130	7500	1.08
14	200	5200	1.16
15	290	4700	1.23
16	180	4500	1.19
17	170	4700	1.20
18	100	4100	1.17

[a] Chromoatography of 2.8 g of HO-terminated polystyrene on 280 g of silica gel, packed in chloroform and eluted with 0.01% to 2% methanol/chloroform (vol/vol)

[b] 100 ml fractions

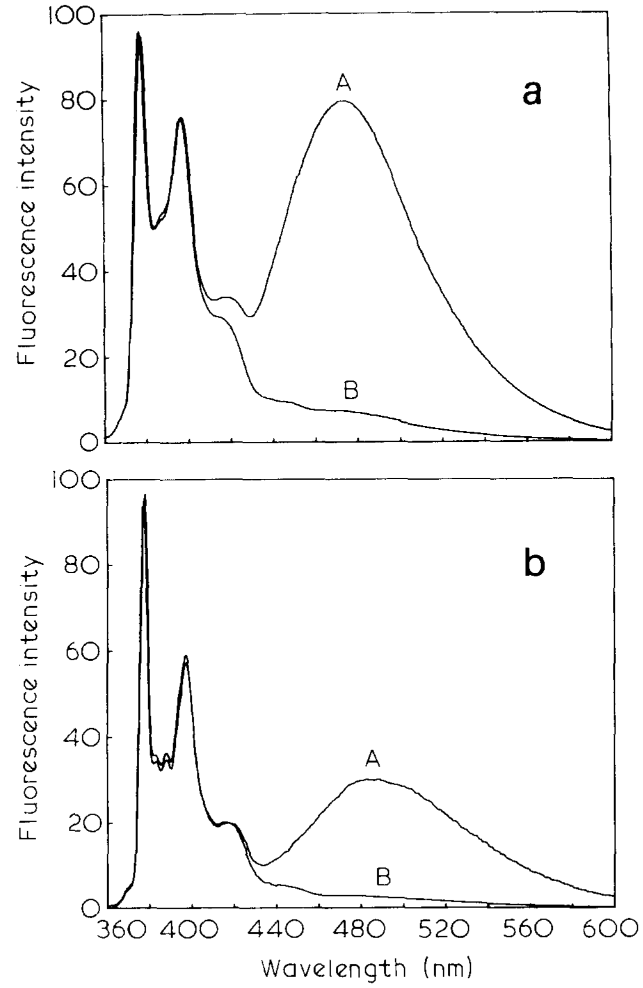


Figure 1 Fluorescence spectra of Py-polystyrene-Py, 2×10^{-6}M (a) In cyclohexane at 34.5°C, A, $\bar{M}_n$ 2900; and B, $\bar{M}_n$ 15600. The curves are normalized at 378 nm. These spectra were obtained with 10 nm emission slits, and are uncorrected for the spectral response of the emission monochrometer and photomultiplier tube. (b) In toluene at 22°C, A, $\bar{M}_n$ 2900; and B, $\bar{M}_n$ 15600. The curves are normalized at 378 nm. These spectra were obtained with 2 nm emission slits, and are fully corrected

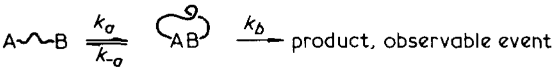
219 spectrometer. For the methyl ester of pyrenebutyric acid in toluene, the extinction coefficient was at 345 nm, $\varepsilon_{345} = 3.83 \times 10^4$M^{-1} cm^{-1}. The absorbances (A) of dilute toluene solutions of polymer samples were then measured, and the concentrations τ of chromophore calculated using $A_{345} = \varepsilon 345$ lc. To conserve valuable polymer samples and to avoid pipetting errors, sub-milligram polymer samples were weighed on a microbalance and diluted to 10.0 ml or 25.0 ml as necessary. Samples used for fluorescence studies showed 1.9–2.1 chromophores per chain. This variation reflects both limitations in the u.v. measurements as well as those in the determination of $\bar{M}_n$ by g.p.c.

RESULTS AND DISCUSSION

Assessment of the problem

Dilute [10^{-6}M] solutions of Py-polymer-Py show two distinct fluorescence emissions. A structured blue emission, with the first intense band at 380 nm, derives from a locally excited pyrene. This fluorescence intensity is referred to as I_M. A broad, structureless green emission centred at 480 nm originates from the sandwich-shaped pyrene excimer. This fluorescence intensity is referred to as I_E. Examples are shown in *Figure 1*. At these dilutions I_E/I_M values are insensitive to modest changes in concentration. This demonstrates the intramolecular nature of the excimer forming process. A more thorough analysis leads to the conclusion that typically only 0.1% of the excimers at these concentrations may be of bimolecular origin. The I_E/I_M values are related to the rate constants for end-to-end cyclization. The nature of the relation depends upon the detailed mechanism invoked to describe the cyclization process. Consequently, the mechanism of excimer formation must be examined to elucidate the relation connecting I_E/I_M to k_{cy}.

It is very important to recognize that the interpretation given to the results of chemical kinetics experiments depends critically on the assumed mechanism used to calculate rate constants from raw data. The same data, fit to two different mechanisms, can lead to different conclusions. This point must be emphasized for experiments which are intended to use kinetic analyses to test theories.



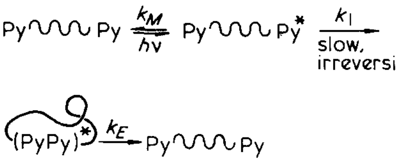
$$\mathrm{A{\sim}B} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} (\mathrm{AB}) \xrightarrow{k_b} \text{product, observable event} \qquad (2)$$

Data from diffusion-controlled reactions are particularly sensitive to misinterpretation. In many 'diffusion-controlled reactions', particularly pyrene excimer formation[2,8], the rates of the diffusion processes [e.g. k_a and k_{-a} in equation (2)] are similar to those of the chemically-activated process [e.g. k_b][9]. Small variations in reaction conditions can affect which step is rate limiting. Partial reversibility of the reaction can become important in some circumstances and not in others. Failure to take proper account of these phenomena can lead to improper conclusions.

To interpret chemical kinetics data properly, it is necessary to establish the reaction mechanism and then to determine the conditions which lead to a change in the rate-limiting step. This is difficult because chemical kinetics alone cannot prove a chemical mechanism. It is useful as a start, however, as it is essential to demonstrate that the reaction rates follow the rate law predicted by the mechanism assumed.

Fluorescence decay experiments are much more sensitive than steady-state I_E and I_M measurements to the details of the process whereby a polymer cyclizes and pyrene groups at both ends come together to form an excimer. Various mechanisms make specific predictions about the form of the decay curves for $I_M(t)$ and $I_E(t)$. These constitute the rate law predicted for each mechanism. For example, in the simple mechanism frequently assumed for intramolecular pyrene excimer formation:

Scheme I



the fluorescence intensity from the locally-excited pyrene $I_M(t)$ is predicted to decay exponentially. $I_E(t)$ is predicted to grow in, from zero intensity at $t = 0$, and subsequently decay. Its time behaviour should be described as a difference of exponentials, with the one of the decay constants being the same as the exponential decay

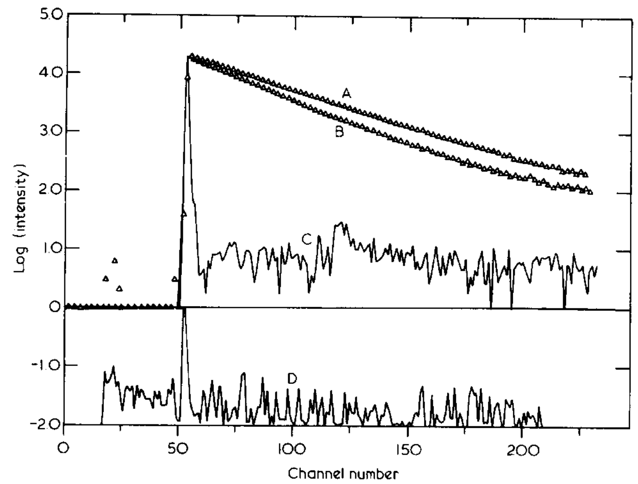


Figure 2 Fluorescence decay curves $I_M(t)$ of Py-polystyrene [M_n 5900] 2×10^{-6}M: A, cyclohexane at 34.5°C, τ, 242.8 ns; B, toluene at 22°C, τ, 192.5 ns; C, the excitation lamp intensity profile; D, log of point-by-point weighted squared deviation from the non-linear least-squares fit

constant of $I_M(t)$. In this mechanism k_1 is the diffusion- or encounter-controlled rate constant for end-to-end cyclization; k_M and k_E describe the sum of radiative and non-radiative first-order decay rates of, respectively, the isolated excited pyrene and the pyrene excimer.

These predictions are quite specific. Early experiments on cyclization dynamics for pyrene-terminated polystyrene samples gave results at odds with these predictions[5]. For example, for a polystyrene of $\bar{M}_w/\bar{M}_n = 1.3$, the fluorescence decay curve shows deviations from exponential behaviour at both short times and long times. This made proper quantitative interpretation of the early experiments impossible.

There are four possible explanations for the non-exponential shape of an $I_M(t)$ decay curve in dilute solution: (1) fluorescent impurities in the sample; (2) incomplete functionalization of the chain ends; (3) effects of broad polydispersity of the polymer samples; and (4) the inadequacy of the mechanism cited in Scheme 1.

(1) Fluorescent impurities are a constant source of concern. It has been found frequently that fractional precipitation of functionalized polymer samples is inadequate for removing low molecular weight impurities. These impurities could be removed by silica gel chromatography or by preparative (5 mg scale) gel permeation chromatography [g.p.c.] on μ-styragel columns. Analysis of the polymers by g.p.c. using u.v. detection was essential for inferring the absence of these impurities.

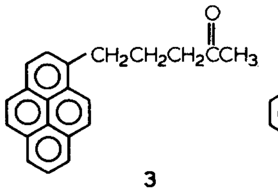
(2) End-group analysis, using u.v. absorption measurements in conjunction with extinction coefficient measurements on low molecular weight compounds is a convenient method for determining the fraction of polymers containing pyrene groups at both ends. As the polymer molecular weights are known only to $\approx 10\%$ accuracy, a similar limit occurs for end-group analyses. Here, the experiments require virtually complete functionalization of both ends of the polymer.

The fluorescence decay technique is extremely sensitive to the presence of small amounts of monofunctional polymer chains in a sample. For example, the model compounds containing only one pyrene, the methyl ester **3** and the polymer **4**, show exponential decay curves with lifetimes of ≈ 200 ns in dilute solution, *Figure 2*. As little as $\leqslant 1\%$ of this material, in the presence of a Py-polystyrene-Py sample with a decay time of less than 150 ns, will show up as a long-time tail in a fluorescence decay trace of the mixture. Some polymer samples show pronounced deviations from exponential behaviour at long times under conditions where excimer dissociation is unimportant. By fitting these curves to the sums of two exponential terms and calculating the pre-exponential coefficients of each component, the shapes of these curves can be explained by calculating that the polymer samples contain small percentages of chains bearing only one pyrene.

To substantiate this conclusion, more information is required. The following observations are pertinent. Further reaction of these polymer samples with pyrenebutyryl chloride followed by repurification of the polymer gave samples the $I_M(t)$ curves of which could be fitted to an exponential function with no evidence for a tail at long times. In addition, the excimer fluorescence rise and decay times $I_E(t)$, before and after refunctionalization, were similar: at low concentrations, bimolecular excimer formation can be neglected. Chains with only one pyrene cannot form an excimer. In addition, the excimer fluorescence decay times were similar to that of $I_M(t)$ in the refunctionalized and repurified samples. This result is important. It means that if one can establish that a small degree of incomplete chain functionalization is responsible for the non-exponential $I_M(t)$ decays, the $I_E(t)$ data can be used to obtain quantitative information about cyclization dynamics.

(3) Polydispersity can contribute to non-exponential decays if the fluorescence decay times are a sensitive function of chain length. Under these circumstances, the non-exponential decays for polydisperse samples can be explained by the presence in the sample of short chains with short decay times and long chains with long decay times. From this point of view, it is possible to predict that careful fractionation of the polymer to narrow polydispersities should reduce the magnitude of the deviations from exponential fluorescence decay behaviour. As *Figure 3* indicates, this explanation is substantiated. With polymer samples of $\bar{M}_w/\bar{M}_n \leqslant 1.10$, no deviations from exponential behaviour can be detected over seven lifetimes (99.5%) of the fluorescence decay.

(4) Scheme 1 assumes that pyrene excimer formation is essentially irreversible; that once formed, the pyrene excimer decays radiationlessly or emits a green photon much faster than it can dissociate to the open form of the polymer with a locally excited pyrene. There are indications from studies on pyrene bimolecular excimer formation that excimer dissociation rates are similar in magnitude to those of excimer decay[2,9,10]. It is probable that Scheme 1 should be modified (see later) to include a term (k_{-1}) for excimer dissociation. For this point to be examined, polymer samples are required of high purity and narrow molecular weight distribution, with 100% of the chains substituted at both ends with pyrene groups.



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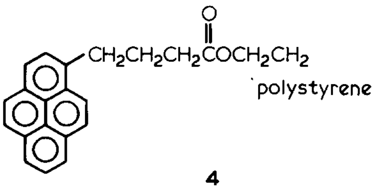

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Fluorescence decay studies

Dilute solutions of molecules containing a single pyrene group show fluorescence decays $I_M(t)$ which can be fitted

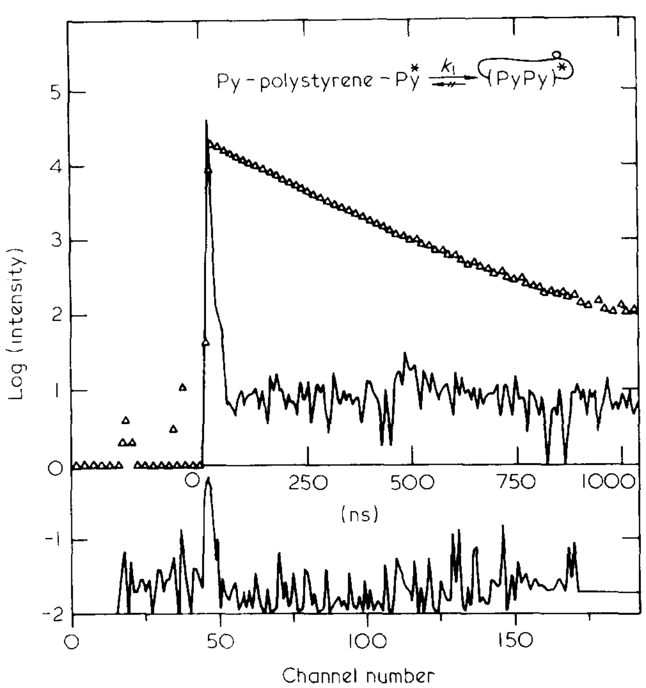
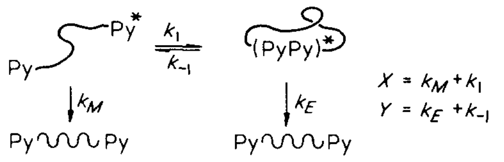


Figure 3 Fluorescence decay curve $I_M(t)$ of Py-polystyrene-Py [M_n 9200], 2×10^{-6}M in toluene at 22°C; M_w/M_n, 1.13; λ^{-1}, 155.8 ns; χ^2, 1.46

to an exponential function over 99.9% (> 10 lifetimes) of the emission decay. Molecules containing two or more pyrenes show fluorescence decay curves which frequently deviate from an exponential form. According to Scheme 2, non-exponential decays should be observed in molecules containing two pyrenes whenever excimer dissociation (described by k_{-1}) is important.

Scheme 2



If k_1 is a constant (this is tantamount to assuming that a single internal relaxation time contributes to the motion of cyclization), Scheme 2 makes specific predictions about the form of the $I_M(t)$ and $I_E(t)$ curves obtained from flash photolysis experiments. It predicts that $I_M(t)$ should decay as a sum of two exponential terms [equation (4)] and $I_E(t)$ should grow in and decay as the difference of two exponential terms [equation (5)]. The decay constants λ_1, λ_2 from both experiments should be identical and the ratio a_3/a_4 should equal 1.0. These constitute testable hypotheses:

$$I_M(t)=a_1 \exp(-\lambda_1 t)+a_2 \exp(-\lambda_2 t) \qquad (4)$$

$$I_E(t)=a_3 \exp(-\lambda_1 t)-a_4 \exp(-\lambda_2 t) \qquad (5)$$

Bimolecular excimer formation between pyrenes unconnected by an intervening chain can be described by a scheme analogous to Scheme 2. Pyrene and excimer fluorescence decay kinetics should similarly be described by equations (4) and (5). Experiments confirm this behaviour, except at very short times. As (bimolecular) k_1 describes a diffusion-controlled process, proximate Py/Py pairs make non-exponential contributions to $I_M(t)$ on the time scale of a few hundred picoseconds, during which time the system relaxes to its steady-state distribution of Py/Py pairs. On the subsequent time scale of nanoseconds and microseconds, k_1 is effectively a constant: many experiments demonstrate that bimolecular excimer kinetics follow equations (4) and (5).

There are fewer detailed studies of intramolecular excimer kinetics. There are some examples in which it can be shown that these equations are obeyed[10,11]. There are more examples, of molecules containing two chromophores connected by a short chain, in which deviations from equations (4) and (5) are documented[12]. While these deviations have not yet been properly explained, it is probable that the origin of the complex kinetic behaviour derives from the contribution of several dynamic modes of chain motion to the cyclization process. The experiments here, with longer chains and very narrow polydispersities, show fluorescence decay behaviour entirely consistent with equations (4) and (5) [see later]. These results are interpreted to mean that a single dynamic relaxation mode of the polymer dominates the cyclization process.

Studies of bimolecular pyrene excimer formation as a function of temperature have indicated that the k_E and k_M steps are only mildly sensitive to temperature changes, whereas excimer dissociation involves a large activation energy barrier[2,8]. By analogy, it is assumed for Scheme 2 that at elevated temperatures k_{-1} will be substantial[18], whereas at low temperatures it will be so small that it can be neglected. In this *low temperature limit*, Scheme 2 reduces to Scheme 1. $I_M(t)$ decays are predicted to be exponential, with a decay constant λ larger than that (λ_{model}) of the model polymer containing only one pyrene:

$$\lambda = k_M + k_1 = \lambda_{\mathrm{model}} + k_1 \qquad (6)$$

Polydisperse polymers introduce complications into the analysis of experimental data. The WF theory preedicts k_1 to be a sensitive function of chain length. Fluorescence decay curves from polydisperse samples will contain a superposition of decay curves from chains of different lengths. For broad polydispersities, $I_M(t)$ could show deviations from exponential behaviour even in the low temperature limit where the contributions of k_{-1} are unimportant. For samples of narrow polydispersities, it is necessary to recognize that measured values of λ_1 and λ_2 and (a_1/a_2) represent averages over a molecular weight distribution, and that these averages differ from those characterizing either $\bar{M}_n$ or $\bar{M}_w$. For this reason angle brackets ($\langle\rangle$) are used around $\langle k_1\rangle$ to emphasize that the value calculated from the data represents an average over a finite molecular weight distribution.

Results in toluene at 22°C. In dilute toluene solution at 22°C, polymer samples **2** fractionated to $\bar{M}_w/M_n \leq 1.1$ show fluorescence decay curves with no detectable deviations from exponential form for 99%–99.5% of the decay. An example is shown in *Figure 3*. The χ^2 values associated with curve fitting are in the range of 1.0–2.0. The autocorrelation function of the residuals show no non-random deviations in the fit beyond the lamp pulse. These samples are thus characterized by single (average) decay rates $\langle\lambda\rangle$. Average cyclization rate constants $\langle k_1\rangle$ for each sample are calculated from the equation:

$$\langle k_1\rangle = \langle\lambda\rangle - \lambda_{\mathrm{model}} \qquad (7)$$

Results in cyclohexane at 34.5°C. In cyclohexane solution at 34.5°C, the θ-temperature, samples of **2** with

Table 2 Decay times and coefficients for decay of the locally-exited state and excimer for the fluorescence of pyrene-terminated polystyrene in cyclohexane at 34.5°C

	Monomer			Excimer		
$\bar{M}_n$	$\langle\lambda_1\rangle^{-1}$ (ns)	$\langle\lambda_2\rangle^{-1}$ (ns)	$\langle a_2\rangle/\langle a_1\rangle$	$\langle\lambda_1\rangle^{-1}$ (ns)	$\langle\lambda_2\rangle^{-1}$ (ns)	$\langle a_3\rangle/\langle a_4\rangle$
2900	83.3	39.3	1.594	69.8	37.0	−0.98
3900	88.6	39.7	0.521	82.4	41.0	−0.97
4500	99.4	44.2	0.362	89.4	42.9	−0.97
6600	120.0	40.3	0.112	115.1	46.8	−0.96
9200	151.9	49.0	0.099	139.6	46.7	−0.95

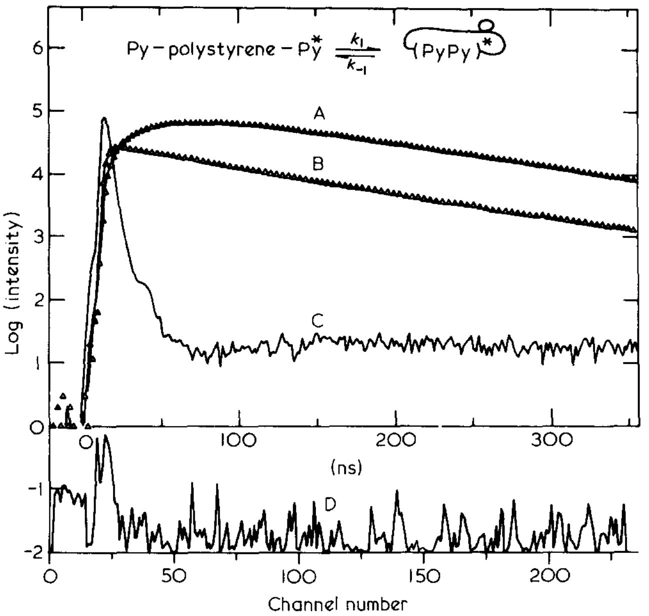


Figure 4 Fluorescence decays of A, $I_E(t)$; B, $I_M(t)$; and C, lamp profile; for a sample of Py-polystyrene-Py [$\bar{M}_n$ 6500], 2×10^{-6} M in cyclohexane at 34.5°C. D, Error profile referring to the fit of the $I_M(t)$ decay. Monomer: λ_2^{-1}, 37.9 ns; λ_1^{-1}, 113.0 ns; a_2/a_1, 0.20; χ^2, 1.13. Excimer: λ_2^{-1}, 37.7 ns; λ_1^{-1}, 108.5 ns; a_2/a_1, −0.93; χ^2, 1.17

$\bar{M}_n > 10^4$ also show exponential fluorescence decays. These decays are characterized by $\langle\lambda\rangle$ values substantially *larger* than for the same sample in toluene at 22°C. λ_{model} is smaller in cyclohexane than in toluene; consequently $\langle k_1\rangle$ values calculated from equation (7) are much larger in cyclohexane than in toluene. Cyclization must occur more rapidly in cyclohexane at 34.5°C than in toluene at 22°C, even though cyclohexane at 34.5°C is the more viscous solvent.

For chains of less than 100 bonds ($\bar{M}_n < 10000$) the fluorescence decay curves of samples in cyclohexane at 34.5°C indicate a more complex behaviour. Deviations from exponential form were noted in the $I_M(t)$ curves, and these deviations became more pronounced for shorter chains. These deviations are explained by noting that the experimental λ values are related to the rate constants in Scheme 2 in a complex way:

$$\lambda_1, \lambda_2 = \tfrac{1}{2}[(Y+X) \mp [(Y-X)^2 + 4k_1k_{-1}]^{1/2} \tag{8}$$

When $k_{-1}=0$, then $4k_1k_{-1} << (Y-X)^2$ and $\lambda_1 = X$ and $\lambda_2 = Y$. Equation (6) is recovered and exponential decays are predicted. If k_{-1} is small but finite, the value of the $4k_1k_{-1}$ term depends linearly on the magnitude of k_1. In the experiments here in cyclohexane, $\langle k_1\rangle$ decreases by three orders of magnitude between polystyrene samples of 50 bonds and those of 2000 bonds. When $\langle k_1\rangle$ is sufficiently large, the $4\langle k_1\rangle k_{-1}$ term is not negligible with respect to $(Y-X)^2$. The $I_M(t)$ decays must be fitted to a sum of two exponentials, equation (4)[13]. An example of a double exponential decay for $I_M(t)$ is shown in *Figure 4*.

Data for $I_M(t)$ and $I_E(t)$ for polystyrene samples of $\bar{M}_n$ 2900–9000 in cyclohexane at 34.5°C are given in *Table 2*. Values are presented for $\langle\lambda_1\rangle$, $\langle\lambda_2\rangle$, $\langle a_2\rangle/\langle a_1\rangle$ and $\langle a_3\rangle/-\langle a_4\rangle$. Several features of the data require comment. First, the $\langle a_4\rangle/-\langle a_3\rangle$ values are very close to −1.0, as required by Scheme 2. For very weak excimers, $|a_4|$ is somewhat smaller than $|a_3|$. Pyrene fluorescence makes a small contribution to the total fluorescence at wavelengths >515 nm. It is very difficult to correct rigorously for this contribution when the excimer emission is very weak. As long as this contribution is small, it has negligible effects on the λ_1 and λ_2 values used to calculate rate constants.

The second noteworthy feature is that $\langle\lambda_1\rangle^{-1}$ for $I_M(t)$ is always $\approx 10\%$ larger than $\langle\lambda_1\rangle^{-1}$ for $I_E(t)$. The larger value of $\langle\lambda_1\rangle^{-1}$ from the $I_M(t)$ measurements might be due to traces (<0.5%) of polymer containing only one pyrene. Samples made up to contain such traces of singly substituted chains have shown somewhat slower decays as the impurity level is increased. Under these conditions, the $\langle\lambda_1\rangle$ values from the excimer decay gives the 'true' long decay constant associated with disubstituted chains. It is, however, possible, that these differences arise in the way the individual components in a polydisperse sample contribute to the average $\langle\lambda\rangle$ values which the computer fits to the fluorescence decay curves. These differences constitute a source of concern which merit further investigation.

The $\langle\lambda_2\rangle$ values from $I_M(t)$ and $I_E(t)$ are of similar magnitude. When $\langle a_2\rangle/\langle a_1\rangle$ is less than 0.15, the $\langle\lambda_2\rangle$ value from the $I_M(t)$ trace is not fitted with great precision, and the precision decreases rapidly as the $\langle a_2\rangle/\langle a_1\rangle$ values become smaller. The short-time component makes so little contribution to the $I_M(t)$ curve that it becomes difficult to measure accurately. When $\langle a_2\rangle/\langle a_1\rangle$ becomes much larger than 2.0, it becomes correspondingly difficult to obtain precise values of $\langle\lambda_1\rangle$ from the $I_M(t)$ traces. It is apparent that the $I_E(t)$ curves give the best values of $\langle\lambda_2\rangle$ for all chain lengths; for short chains, $I_E(t)$ values seem also to give the most reliable values of $\langle\lambda_1\rangle$.

One of the advantages of this more complex behaviour is that from values of λ_1, λ_2, and a_2/a_1, the values of $\langle k_1\rangle$, k_{-1} and k_E can be calculated. The rate constants are related to the measurables via equation (8) and the expression:

$$\frac{a_2}{a_1} = \frac{X-\lambda_1}{\lambda_2 - X} \tag{9}$$

Using these two equations, it is posibble to assess whether k_{-1} and k_E are sensitive to chain length.

The results indicate, *Table 3*, that significant values of k_{-1} and k_E can be obtained from polymer samples of narrow polydispersity, and that the values of these two rate constants are ***independent of chain length.*** This point is very important. From these observations it is concluded that factors affecting the fate of the excimer are local in origin. Its rate of decay, whether by re-opening to give an excited pyrene on one end or by radiative and radiationless decay to ground state, are unaffected by the length or configuration of the intervening chain connecting the pyrene groups. While these conclusions here may probably be made only for cyclohexane solution, there is evidence from studies of toluene solutions at elevated temperatures, where fluorescence decays are no longer exponential, that k_{-1} and k_E are similarly independent of chain length[18].

Two further points need to be emphasized. First, although k_{-1} is significantly smaller than k_E at 34.5°C in cyclohexane, it is not negligible. When $\langle k_1 \rangle$ values are of sufficient magnitude, such a large fraction of the chains cyclize before deactivation that even a small amount of excimer dissociation becomes significant. Apparent $\langle k_1 \rangle$ values calculated from equation (7) [by force-fitting fluorescence decay curves to an exponential form] are too small. Plots of log $\langle k_1 \rangle$ *versus* log N show a significantly smaller slope when apparent $\langle k_1 \rangle$ values (obtained by neglecting the partial reversibility of excimer formation) than when $\langle k_1 \rangle$ values obtained from equations (8) and (9) are used.

The second important point concerns the relation between critical exponents for dynamic and static properties of polymer chains. The ratio $\langle k_1 \rangle / k_{-1}$ is equal to the equilibrium constant for end-to-end chain cyclization $\langle K_1 \rangle$, and provides a measure of the cyclization probability of the chain. As k_{-1} is independent of chain length, $\langle k_1 \rangle$ and $\langle K_1 \rangle$ must depend similarly on the degree of polymerization of the polymer. Translated into theoretical terms, this means that k_{cy} and K_{cy} scale similarly with increasing chain length.

Steady-state fluorescence measurements

Steady-state fluorescence measurements provide an additional tool for studying the kinetics of end-to-end cyclization. The ratio of I_E/I_M is very sensitive to chain length and provides a measure of the rate of excimer formation. This application is particularly useful for long chains where cyclization is slow, and $\langle \lambda \rangle$ is approximately equal to λ_{model}. Under these circumstances, equation (7) is no longer useful for obtaining values of $\langle k_1 \rangle$.

According to Scheme 2, I_E/I_M is proportional to k_1 by the relation:

$$\frac{I_E}{I_M} \propto \frac{\phi_E}{\phi_M} = \frac{k_{FE}}{k_{FM}} \left(\frac{k_1}{k_E + k_{-1}} \right) \qquad (10)$$

where ϕ_E and ϕ_M are the absolute quantum yields for excimer and locally-excited pyrene fluorescence, and k_{FE} and k_{FM} are the radiative rate constants, respectively, for excimer and locally excited pyrene emission. As long as the product $(k_{FE}/k_{FM})(k_E + k_{-1})^{-1}$ is independent of chain length, relative values of $\langle I_E/I_M \rangle$ can be used to calculate relative values of $\langle k_1 \rangle$:

Table 3 Rate constants for the cyclization of pyrene-terminated polystyrene[a]. See Scheme 2

		Cyclohexane 34.5°C			Toluene 22°C
$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\times 10^6 s^{-1}$ $\langle k_1 \rangle$	$\times 10^6 s^{-1}$ k_{-1}	$\times 10^7 s^{-1}$ k_E	$\langle k_1 \rangle$ $\times 10^6 s^{-1}$
2900	1.07	17.9	2.12	1.71	6.7
3900	1.08	12.1	2.79	1.73	4.6
4500	1.07	10.2	2.81	1.72	3.5
6600	1.06	5.7	2.55	1.75	2.1
9200	1.13	4.2	(3.95)[b]	1.70	1.2
15 600	1.12	1.1	–	–	0.44
19 700	1.05	0.92	–	–	0.28
25 300	1.22	0.62	–	–	0.29
33 600	1.09	0.26	–	–	0.047
46 100	1.20	0.30	–	–	0.024
99 700	1.18	0.09	–	–	(0.003)[b]

[a] Where both $I_M(t)$ and $I_E(t)$ could both be determined, λ_1 and λ_2 values from the excimer curve were used in conjunction with a_2/a_1 values from $I_M(t)$ to calculate rate constants

[b] Values in parentheses are known with poor precision, typically ±35–50%

$$\frac{\langle I_E/I_M \rangle_1}{\langle I_E/I_M \rangle_2} = \frac{\langle k_1 \rangle_1}{\langle k_1 \rangle_2} \qquad (11)$$

This assumption is normally reasonable, as it has been shown that k_E and k_{-1} are chain-length independent. Only in the case of short chains where cyclization is accompanied by ring strain would these values be expected to depend upon chain length. However, changes in solvent and temperature can affect these rate constants. Equation (11) can not be applied to samples in different sölvents or samples at different temperatures.

Values of $\langle k_1 \rangle$ are calculated using equation (11) in the following manner: the value of $\langle k_1 \rangle_2$ in equation (11) is set to be equal to the value obtained by fluorescence decay measurements on the sample of $\bar{M}_n$6600. Values of $\langle k_1 \rangle_1$ are calculated by comparing I_E/I_M values for other chain lengths to that for the sample of $\bar{M}_n$ 6600. For molecular weights below 25000, values of $\langle k_1 \rangle$ calculated from equation (11) and from fluorescence decay measurements agreed within 5%.

Steady-state emission spectra for two samples of **2** of different chain lengths, in cyclohexane at 34.5°C, are shown in *Figure 1a*. Corresponding spectra of the same two samples in toluene solution at 22°C are shown in *Figure 1b*. These spectra indicate first that I_E/I_M values decrease significantly with increasing chain length. In addition, it is evident from comparison of *Figure 1a* with *1b* that each sample shows substantially more excimer in cyclohexane at the θ-temperature than in toluene at 22°C. This result substantiates the inference made previously that cyclohexane promotes chain cyclization.

When excimer emission is very weak, I_E values must be corrected for residual pyrene fluorescence at wavelengths >500 nm. This is best done by subtracting from the fluorescence spectrum of **2** the corresponding spectrum from the same concentration of a polymer of similar molecular weight containing only one pyrene. At the current limits of excimer detection, negligible bimolecular excimer is formed from dilute solutions [1–2×10^{-6}M] of pyrene-terminated polystyrene of $\bar{M}_n > 10^4$. Consequently, it is sufficient to subtract from the fluorescence spectrum of **2**, the spectrum of a 10^{-6}M solution in the same solvent of a polystyrene sample containing a single

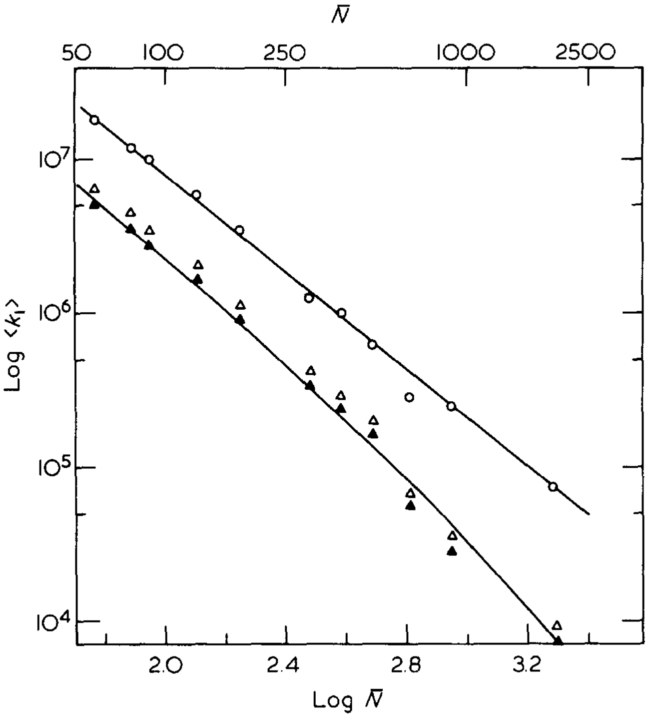


Figure 5 Plot of log $\langle k_1\rangle$ *versus* log $\bar{N}$ (mean chain length) for Py-polystyrene-Py. The average number of bonds between the two pyrenes is indicated at the top of the graph. ○, Cyclohexane at 34.5°C; △, toluene at 22°C; ▲, data from toluene at 22°C renormalized to 34.5°C and to a solvent with the same viscosity as cyclohexane at that temperature

pyrene, after normalizing the two spectra at the (0,0) band of the pyrene fluorescence. It is not sufficient to use a low molecular weight pyrene derivative as the model compound for correcting excimer intensities. Even at 10^{-6}M, molecules such as **3** form non-negligible amounts of excimer. Bimolecular excimer formation is less important for polymer-bound pyrene model compounds because these species have much smaller translational diffusion constants, and excimer formation requires chain interpenetration.

Cyclization dynamics

Here, the chain length dependence of $\langle k_1\rangle$ is examined. In calculating the mean chain length $\bar{N}$ from the polymer molecular weight, it is necessary to take account of the mass of pyrene groups, and the mass and the length of the spacer chains by which they are attached. The end groups comprise 630 mass units of the measured molecular weight and contribute 15 bonds to its length. $\bar{N}$ is calculated, therefore, from the expression:

$$\bar{N}=2[(\bar{M}_n-630)/104]+15 \qquad (12)$$

The end groups make a negligible contribution to $\bar{N}$ when $\bar{M}_n$ is sufficiently large. When $\bar{M}_n$ is small, e.g. <9000–10000, its contribution to the total chain length is significant. One of the experimental advantages of the spacer chain is that it minimizes the possibility that excimer formation between the two chain ends would be inhibited by steric interactions between phenyl groups on the polystyrene chain.

Values of $\langle k_1\rangle$ for pyrene end-capped polystyrene, obtained as described previously, are plotted *versus* mean chain length, $\bar{N}$, in *Figure 5*. At the θ-temperature in cyclohexane, the rectilinear dependence of log $\langle k_1\rangle$ upon log $\bar{N}$, predicted by the Wilemski–Fixman theory, is indeed observed. To compare the magnitude of the slope with that predicted by WF theory, $\langle k_1\rangle$ and $\bar{N}$ must be expressed as identical averages over the molecular weight distributions in each polymer sample. In *Figure 5*, the $\bar{N}$ values, calculated from $\bar{M}_n$, respresent different averages for each sample than do the $\langle k_1\rangle$ values.

As the molecular weight distributions are known for each sample for which $\langle k_1\rangle$ was measured, either $\langle k_1\rangle$ or $\bar{M}$ can be re-normalized to obtain the appropriate comparison*. It is assumed here that for the *i*'th molecular weight component (of mole fraction χ_i) in a polymer sample, $k_{1,i}=AM_i^{-\gamma}$. Consequently the experimental value of $\langle k_1\rangle$ depends upon M according to:

$$\langle k_1\rangle=\sum_i k_{1,i}\chi_i=A\sum_i \chi_i M_i^{-\gamma} \qquad (12)$$

Linear least-squares methods were used to obtain optimum values of A and γ for the various samples shown in *Figure 5*. This treatment gives $\gamma=1.62$. The slope in *Figure 6* is -1.52. The difference in the absolute magnitude of these numbers derives from the fact that the two highest molecular weight samples have broader molecular weight distributions than those of low molecular weight.

In toluene at 22°C, a good solvent for polystyrene, the $\langle k_1\rangle$ values are smaller than for similar chain lengths in cyclohexane at 34.5°C, and the log $\langle k_1\rangle$ *versus* log $\bar{N}$ plot is curved downward for increasing chain length. This behaviour represents an excluded volume effect on the dynamics of cyclization.

The most interesting and delicate aspect of these measurements is their proper interpretation. The WF theory suggests that $k_{cy}^{-1}$is essentially equal to τ_1, the slowest internal relaxation time of the polymer in dilute solution. In principle, this relation applies only to infinitely long chains. Perico and Cuniberti[15] have examined the consequences of adapting the model to accommodate the more realistic situation of finite chains with partially draining hydrodynamic interactions. They conclude that under these circumstances, higher frequency modes must contribute to the magnitude of k_{cy}. Doi's theoretical analysis of the harmonic spring model for chain cyclization leads to the same conclusion[16].

It was surprising, therefore, to find that for polymers of sufficiently narrow molecular weight, that cyclization was accompanied by fluorescence signals that were strictly exponential for 99% of the fluorescence decays. These results require that end-to-end cyclization be dominated by a single (rate-limiting) mode of polymer motion, and that higher frequency motions make relatively unimportant contributions to these measurements. This result is even more surprising, because quasi-elastic light and neutron scattering experiments [where the mgnitude of the inverse scattering vector $|\bar{q}|^{-1}$ is of the order of the polymer dimensions] always show a superposition of dynamic modes of polymer motion[17].

These differences can be explained by first recognizing that the intramolecular fluorescence quenching experiment operates in the internal reference frame of the polymer: only motions which change the distance se-

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* This treatment makes the implicit assumption that $\langle I_M(t)\rangle=\langle A_M\exp(-\lambda_1 t)\rangle$ for a polydisperse sample can be set equal to $\langle A_M\exp(-\langle\lambda_1\rangle t)$. For a critical and incisive discussion of problems associated with this assumption see ref. 14

parating the two ends contribute to k_{cy}. Consequently, translational and rotational diffusion, which contribute to scattering experiments, have no effect on the intramolecular fluorescence quenching experiment. In addition, the normal mode picture [Rouse, Zimm models] of low frequency polymer motion, at least in the limits of the Rouse and Zimm chains, predicts that the motions associated with the even modes [of relaxation times $\tau_0, \tau_2, \tau_4, \ldots$] do not affect the end-to-end displacement. It is presumed that these modes, therefore, do not contribute to these measurements here of cyclization rates.

According to these arguments, these measurements of cyclization dynamics should be sensitive to $\tau_1, \tau_3, \tau_5 \ldots$ Both the Rouse and Zimm models predict that τ_3 and τ_5 should be considerably shorter than τ_1. What is important in a fluorescence decay experiment is not the amplitude of the motion at short times, but rather the area and shape of the $I_M(t)$ *versus* time plot. Higher frequency motions contribute to fluorescence quenching in the short time domain (here, times on the order of nanoseconds), but these contribute negligibly to measurements carried out on a time scale of one to two microseconds.

It is possible, therefore, to rationalize the observations that $I_M(t)$ decays as a single exponential, and suggest that higher-order internal modes make as yet undetectably small contributions to the polymer motion. These experiments offer the opportunity for a direct molecular-level determination of the slowest internal relaxation time of a polymer $[\langle k_1\rangle^{-1} \approx \langle\tau_1\rangle]$, uncontaminated by translational and rotational diffusion, and negligibly affected by higher frequency motions of the chain.

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