Fluorescence of Polymers: A Probe of Polymer Assemblies

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SUMMARY: In this paper we discuss some general aspects of the use of fluorescence spectroscopy in the characterization of polymers and processes involving polymers. In particular we focus on the use of probes to detect formation of hydrophobic domains or to detect environmental changes because of solvation or pH changes, fluorescence quenching to determine the accessibility of the probe to solution phase small molecules, the use of fluorescence depolarization to characterize segmental motions and the use of energy transfer to characterize morphologies on the length scale of 1-5 nm. We also will compare direct energy transfer (DET) with down-chain electronic energy transfer (EET).

Introduction

The use of fluorescence spectroscopy to characterize polymers has become well established, especially in the last twenty years[1]. Among the advantages of fluorescence spectroscopy are high sensitivity, detection of events at the molecular level and the potential for monitoring events on the nanosecond time scale. Fluorescence can also be used to monitor slow processes like diffusion on a time scale of days. In some cases the polymer of interest inherently contains chromophores (e. g. poly(N-vinylcarbazole) or tryptophan in proteins) but in many cases the fluorophore is not intrinsic to the polymer and has to be added either by covalent bonding to the polymer or allowing it to associate with a polymer via hydrophobic or electrostatic attraction. We will discuss four examples of the application of fluorescence to the study of polymers:

1) Environmentally sensitive small molecules (such as pyrene) that can detect the formation of hydrophobic domains.

2) Fluorescence quenching of small molecule probes or fluorophores covalently bonded to a polymer, in order to assess the accessibility of the probe to solvent or other diffusing species.

3) Fluorescence depolarization to characterize the rotational mobility of a fluorophore.

4) Non-radiative energy transfer between pairs of fluorophores covalently bonded to the same polymer or different polymers. In the former case information about intrapolymer

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dynamics can be obtained. In the latter case polymer diffusion or exchange can be characterized. The detailed time dependence of fluorescence decay for an energy donor surrounded by energy acceptors can be analyzed in terms of restricted or fractal geometries.[2] Similarly, energy transfer among identical chromophores can be analyzed in terms of an ensemble of polymer configurations, but in general analysis of this kind of data requires computer simulations because analytical expressions are not available.

Environmentally Sensitive Fluorescence Probes

(a) Detection of a Polymer Micelle CMC

There are a large number of fluorophores that "report" their local environmental polarity. The most intensely studied example is pyrene. It is very common to use pyrene to monitor changes in the conformation of a polymer, as in the case of the chain collapse of poly(methacrylic acid) at low pH, or in the formation of hydrophobic domains during polymer self-organization or aggregation. Winnik et al. have used the change in fluorescence intensity and/or spectrum to deduce the apparent CMC of polymer micelles formed from polystyrene-*block*-poly(ethylene oxide) (PS-b-PEO).[3] The concept of the method is very simple. A constant amount of pyrene is placed into contact with a solution containing varying concentrations of a polymer which is believed to exhibit a CMC. It is assumed that when the CMC is exceeded that the polymer will form larger hydrophobic domains that can solubilize the probes. It is implicit in this method that polymers that are not part of a large aggregate will not be as efficient for solubilization. What we wish to demonstrate in the following is that this method is more sensitive to the partition coefficient of the probe in the domain than the CMC. This point has also been made in a recent review by Winnik and Yekta.[4]

The basic equation may be written for the total number of moles of probe (m_P^0):

$$[P]_D V_D + [P]_B V_B = m_P^0 \qquad (1)$$

in which $[P]_D$ and $[P]_B$ refer to the concentration of probe in the hydrophobic domain (D) and bulk solution (B) respectively, and V_D and V_B are the corresponding volumes. Normally the volume fraction of the hydrophobic domain is very small such that $V_B \approx V$, the total volume of the solution. The probe may partition between the two phases such that

$$\frac{[P]_D}{[P]_B} = K. \qquad (2)$$

If the system exhibits a CMC behavior then the volume of the domain is given by

$$V_D = V(c - [CMC])/\rho_D \qquad (3)$$

where c is the polymer concentration (g/mL) and ρ_D is the density of the domain, which will be on the order of 1 g/mL. We will consider only the fluorescence intensity in what follows, although in the case of pyrene the relative intensity of the 1,3 vibrational peak in the

fluorescence spectrum or the excitation spectrum can be monitored as well.[3,5] We may write for the total fluorescence

$$F = \phi_{fl}^{D}[P]_D V_D + \phi_{fl}^{B}[P]_B V_B \quad (4)$$

which using eq 1-3 can be rearranged to yield

$$\frac{F}{F_0} - 1 = \frac{\phi_{fl}^{D} - \phi_{fl}^{B}}{\phi_{fl}^{B}} \left\{ \frac{(K/\rho_D)(c - [CMC])}{(K/\rho_D)(c - [CMC]) + 1} \right\} \quad (5)$$

In eq 5 F_0 is the fluorescence intensity of the probe in the bulk solution alone, in the absence of solubilizing polymer. If $\phi_{fl}^{D} = \phi_{fl}^{B}$ no change in fluorescence will be observed, as expected. We have used ionic quenchers (e.g. Tl^+, Cs^+) in the bulk aqueous phase which are excluded from hydrophobic domains to enhance the sensitivity of partitioning experiments of this general type.

The problem with an expression like eq 5 is that it is not very sensitive to the CMC, as illustrated in Figure 1(a). In particular the break in the plot of $(F/F_0) - 1$ vs. c is not related directly to the CMC but is more sensitive to the partition coefficient K.

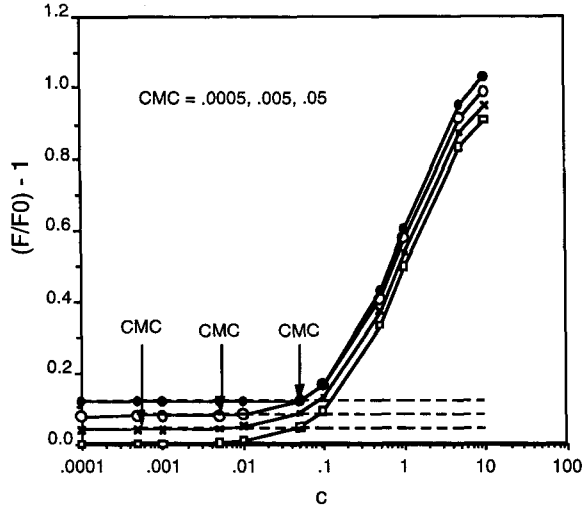


Figure 1 (a) Plot of eq 5 for different CMC values.

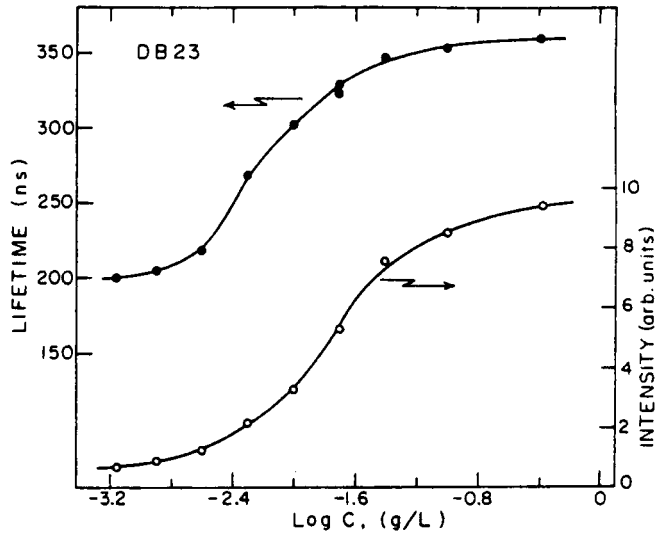


Fig. 1 (b) Plot of experimental data from ref. 3

We prefer to modify the above procedure in the following way: At all polymer concentrations we equilibrate the solution with solid probe, thereby achieving a saturated solution. Therefore $[P]_B$ is a constant, $[P]_B^{sat}$, and using eq 2-4 with this substitution we may write

$$\frac{F}{F_{sat}} - 1 = \frac{\phi_{fl}^D}{\phi_{fl}^B} \frac{K}{\rho_D} (c - [CMC]) \qquad (6)$$

where F_{sat} is the fluorescence intensity of the saturated solution. As before, a quencher can be added that is excluded from the hydrophobic domain to increase the dynamic range of the measurement. Eq 6 is a very simple form and the [CMC] is obtained from the ratio of the intercept and slope in the plot of $(F/F_{sat}) - 1$ vs. c. To be of practical use (F/F_{sat}) must exceed unity in the vicinity of the CMC. For example at $c = 2[CMC]$

$$\left(\frac{F}{F_{sat}} - 1\right)_{c = 2[CMC]} = \frac{\phi_{fl}^D}{\phi_{fl}^B} \frac{K}{\rho_D} [CMC] \qquad (7)$$

If we take as a criterion for successful application of this method that the left-hand side of eq 7 is unity, then $(\phi_{fl}^D/\phi_{fl}^B)(K/\rho_D)[CMC] \approx 1$ is required. It is always good practice to establish the apparent CMC for a variety of probes, which will presumably have different values of K and $(\phi_{fl}^D/\phi_{fl}^B)$.

(b) Detection of Acid Diffusion in Films

There are many examples of pH sensitive fluorophores. As part of a collaborative research project[6] we have examined copolymers with a small mole fraction of 4-methyl amino acridine (4MAA). In this particular experiment the film is exposed to an acid with a relatively high vapor pressure, such as trifluoroacetic acid (TFA). As the TFA diffuses into the film the fluorescence increases to a limiting value. These data can be analyzed by diffusion-reaction equations to establish an average diffusion constant of the acid, or even to establish if simple diffusion equations apply. We are using films of this type to establish the diffusive properties of photogenerated acids that are used in certain photolithographic processes.

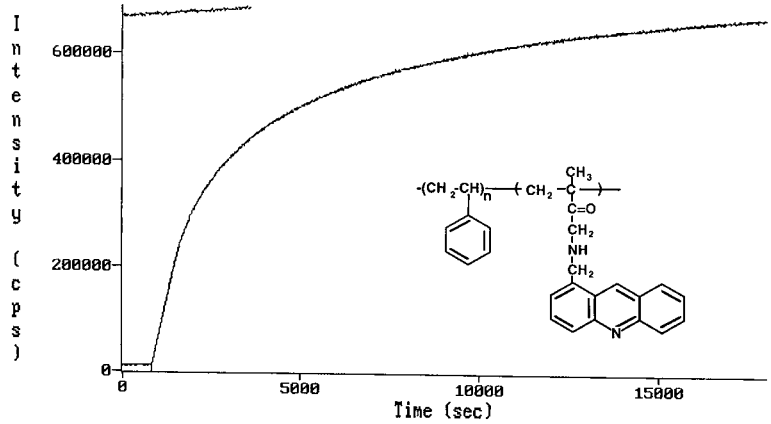


Figure 2 Fluorescence response to TFA diffusing into PS-co-4MAA films

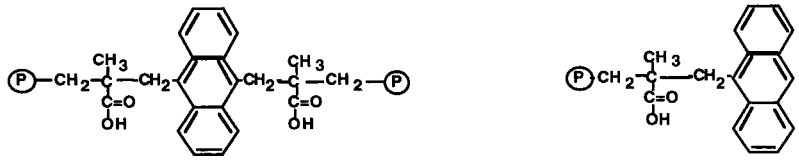
Fluorescence Quenching

An excited state species can be deactivated by collision with a suitable quencher, represented by the following,

$$^1C^* + Q \rightarrow C + Q + \text{heat} \qquad (8)$$

A single state chromophore ($^1C^*$) is denoted in eq 8 because it is this multiplicity that is responsible for fluorescence.[7] The details of the quenching process are often not important so long as it can be established to be short range. In such a case one can ascertain the accessibility of the chromophore to the quencher.

One such application of this method that we have pursued is the study of chromophore-tagged polyelectrolytes or polyacids.[8] An on-going example is indicated in Scheme 1:[9]



Scheme 1

These polymers are prepared by anionic polymerization and termination by the appropriate mono- or dibromo anthracene moieties. The objective of these studies is to characterize the efficiency of quenching these differently positioned chromophores by simple monovalent ions. We have concentrated on the high pH regime (ca. 10) where the polyacid is fully deprotonated, so that the polymer acts like a polyanion. By placing the chromophores at these two different locations we hope to understand the differences between the counterion distribution near the center and end of the polyelectrolyte. In all cases the quenching ion is Tl^+ and the counter-ion used to adjust the total ionic strength is K^+ (as KNO_3). Our data may be summarized as follows:

(1) The mid-tagged chain behaves very similarly to the pendent system, displaying strong ionic strength effects and non-linear fluorescence quenching behavior. There is a more significant component of static quenching for the mid-tagged chain than the pendent system. The steady-state quenching data is fit well by an equation similar to one suggested by Morishima et al.[10]

$$I_0/I_q = \{1 + K_{SV}[Tl^+]\}\exp\{n_{max}\, x_{Tl^+}{}^{app}\} \qquad (9)$$

where K_{SV} is the apparent Stern-Volmer constant, which represents quenching by ions outside the "active sphere", n_{max} is the maximum number of ions in the "active sphere" and $x_{Tl^+}{}^{app}$ is the "apparent" mole fraction of Tl^+, which is found to be higher than that computed from the bulk concentration of ions. This may be because of specific binding by Tl^+ to PMA or the inherent limitations of the underlying model. This system displays typical polyelectrolyte effects such as very efficient quenching by an ionic species and a strong sensitivity to ionic strength.

(2) The end-tagged chain shows essentially no polyelectrolyte effect at all, although the quenching curve is well-described by eq 9. In particular the quenching efficiency is relatively low (approximately diffusion limited) and there is essentially no effect of ionic strength. Thus we conclude that the region of high ionic density does not extend beyond the region of the immediate PMA chain. So far as we know experiments that permit this conclusion have not been previously presented.

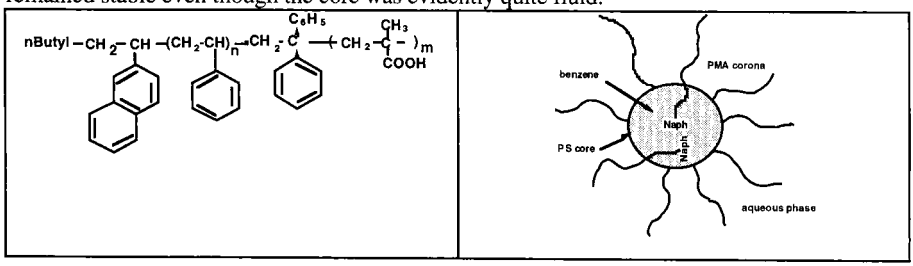
Fluorescence Depolarization

A chromophore with a high degree of symmetry will typically display a simple relationship between the absorption and emission dipole moments, and in the simplest case they are parallel. If the chromophore is able to rotate, then the polarization is lost more quickly. Therefore observation of the time-dependence of depolarization yields information about the dynamics of the probe molecules. Eq 10 is a typical empirical equation used to analyze time-dependent polarization data.

$$r(t) = \frac{I_{||}(t) - I_{\perp}(t)}{I_{||}(t) + 2I_{\perp}(t)} = (r_0 - r_\infty)\sum_{i=1}^{N} a_i e^{-t/\tau_i} + r_\infty \qquad (10)$$

In this equation r_0 is the value at $t = 0$ and r_∞ is the apparent residual polarization as $t \rightarrow \infty$ (the a_i coefficents sum to unity). $I_{||}(t)$ and $I_{\perp}(t)$ refer to the polarization of the emission with respect to the excitation polarization. In general eq 10 can be used to assess the probe dynamics out to times that are several times the excited state lifetime of the probe. Quite often eq 10 is used only qualitatively, in order to characterize the mobility of a probe species. In principle the lifetimes in eq 10 can be fit to a model for the dynamics of the probe.[11]

An example of the utilization of eq 10 in our own work is to characterize the mobility of a probe in a polymer micelle. The probe was a 2-vinylnaphthalene that was part of a diblock polystyrene-*block*-poly(methacrylic acid) (PS-b-PMA) polymer that was used to prepare the polymer micelles (see Scheme 2). The tagged polymers had to be diluted with untagged polymers so as to avoid depolarization by energy transfer. The time-dependent depolarization polarization was determined as a function of added benzene, which when absorbed by the polystyrene core softened the glass polystyrene and enhanced depolarization was observed. We primarily used r_∞ to characterize this effect, and as can be seen from Figure 3, the behavior of r(t) and r_∞ as a function of added benzene was very systematic. One of the interesting observations was that for relatively large ratios of benzene to styrene (e.g. 9:1) the micelles remained stable even though the core was evidently quite fluid.[12]



Scheme 2

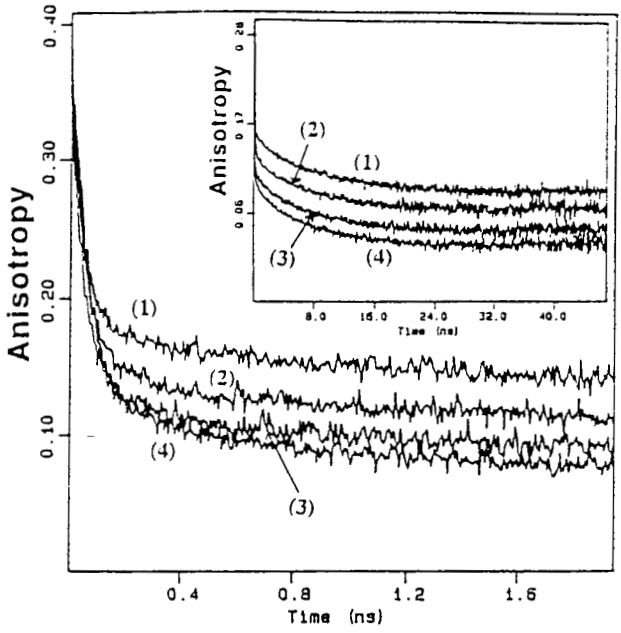


Figure 3 (a) Time-resolved anisotropy for naphthalene labeled PS-b-PMA micelle, as function of added benzene (higher numbers correspond to larger amounts of benzene)

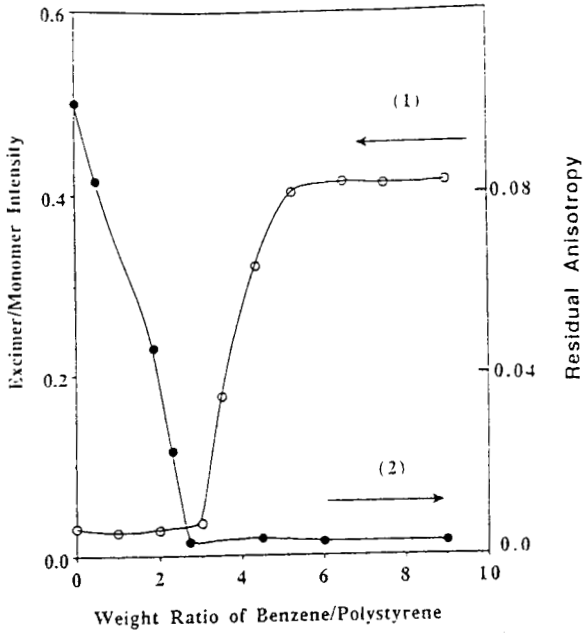
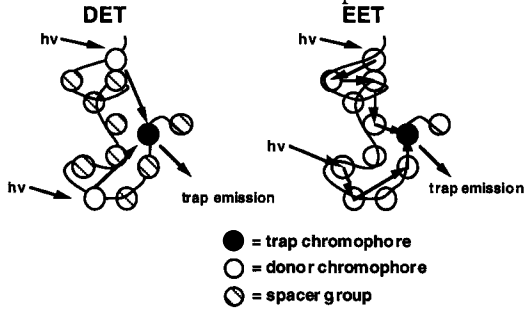


Figure 3 (b) Residual anisotropy (r_∞ in eq 10) as function of ratio of benzene to styrene in PS-b-PMA micelle. Excimer fluorescence for tagged polymer with ca. four chromophores per chain.(from ref. 12)

There has also been an extensive amount of work using tagged polymers analogous to those in Scheme 1 to deduce the rate of polymer segment motion. For example, using end-tagged PMA Ghiggino and Tan. studied the collapse of PMA as the pH was lowered, which greatly increased the residual polarization of the anthracene emission.[13]

Energy Transfer

We distinguish two types of energy transfer, down chain electronic energy transfer (EET) and donor to acceptor direct energy transfer (DET) in which there is an ensemble of acceptors surrounding an isolated donor. These two limits are represented in Scheme 3.



Scheme 3

Except in strictly 1-D systems, analytical solutions for EET are not available. For the case of DET the following general relation was derived by Klafter and Blumen[14]:

$$I(t)_{fl} = e^{-t/\tau_0} \exp\{ - \alpha[A]\Gamma(1-\delta/6)(t/\tau_0)^{\delta/6}\} \qquad (11)$$

In this expression [A] is the concentration of acceptors, $\Gamma(x)$ is the Gamma function and δ is the fractal dimension. In the case of a classical physical dimension $\delta = 2$ or 3 and an exponential decay with $t^{1/3}$ or $t^{1/2}$ results. For a well-defined fractal geometry the density of sites that can accommodate an acceptor falls off like $\rho(r) = \rho_0 r^{\delta-d}$, where d is a physical dimension (2 or 3) and δ is the fractal dimension. Eq 3 with δ equal to some non-integral value often provides a good fit to data for a system with a restricted geometry even if it is not, strictly speaking, a fractal geometry. This can be understood from the distribution of sites. For example, Pekcan et al. have discussed how an energy donor in a cylindrical space yields a decay which is "3-D" at short times (transfer to nearby acceptors) and "1-D" at long times (transfer to distant acceptors, primarily located along the axis of the cylinder).[15] Winnik et al. have used eq 11 and its variants to study a variety of processes:

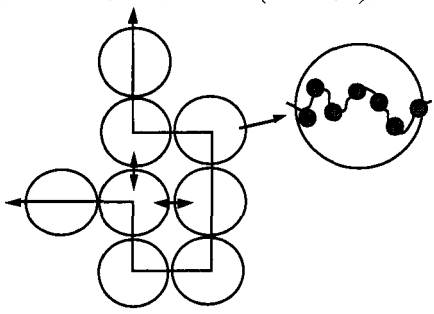
(1) Penetration of latex particles by small molecules[16]

(2) Interpenetration and strongly segregated polymer interfaces[17]

(3) The core and core-corona boundary layer of polymer micelles.[18]

For EET a much more difficult analysis is required. In particular we have considered chains which contain identical chromophores along the chain and single or multiple traps either located randomly within the chain or at specific locations (e.g. chain ends). If down-chain energy transfer is strictly one-dimensional, then analytical solutions are available.[19] If cross-chain transfer is possible because of chain coiling, then the following elaboration is required[20]:

(1) A model has to be adopted for the energy transfer step between chromophores. We have assumed that transfer occurs only between nearest neighbors of a lattice model treatment of a polymer. Each "bead" of the polymer chain can be taken to be a single chromophore or a group of chromophores that can be treated as a unit (Scheme 4).



Scheme 4

Alternatively the rate of transfer can be assumed to fall off with distance, such as predicted by the Förster equation

$$k_{ET}(r) = \frac{\kappa^2}{\tau_0} (R_0/r)^6 \qquad (12)$$

In eq 12 R_0 is the Förster radius and the value depends strongly on the particular chromophore.[21] The effect of orientation is incorporated into the κ^2 factor which for rapidly rotating chromophores has a value of 2/3. It is common to include this factor in the tabulated R_0 values.

(2) The relation between chromophores can be simulated by a polymer model, either with molecular detail (but usually using only short segments) or based on a lattice with self-avoidance and consideration of the energy of interaction between non-neighboring groups. It is this latter approach we have used. For each conformation a master equation can be written for the probability of excitation residing at site *i* (there are N chromophores/chain):

$$\frac{dp(t)_i}{dt} = \sum_{j \neq i}^{N} \{w_{ji}p(t)_j - (w_{ij} + u_i)p(t)_i\} \qquad (13a)$$

or

$$\frac{d\mathbf{P}(t)}{dt} = \mathbf{W}\mathbf{P}(t) \qquad (13b)$$

In this equation w_{ij} is the energy transfer rate from site *i* to site *j*, which is a function of their separation (and possibly their mutual orientation) and u_i is the rate of transfer from site *i* to a trap. If there is no transfer to a spectroscopically distinct trap, there would not be any effect of energy transfer on the observed photophysics. The trap could be a separate species covalently bound to the host polymer, or it could arise from self-trapping via excimer formation.

For any given model of EET and trapping, the *i*th conformation yields a solution that can be written formally as

$$\mathbf{P}(t)_i = e^{\mathbf{W}_i t}\,\mathbf{P}(0) \qquad (14)$$

$\mathbf{P}(0)$ is the vector of initial excitation probability, usually assumed to have each element equal to 1/N since all N chromophores have equal probability of being excited. $\mathbf{W}_i$ is the matrix of rate constants that corresponds to the *i*th conformation. The observed photophysics is an ensemble average of the survival probability:

$$\langle S(t)\rangle = \frac{\sum_N^1 \langle \mathbf{1}|\, e^{\mathbf{W}_i t}\, |\mathbf{1}\rangle\, e^{U_i/kT}}{\sum e^{U_i/kT}} \qquad (15)$$

where $|\mathbf{1}\rangle$ is a vector of length N with each element of value unity. The energy of the *i*th conformation, U_i, is computed according to the polymer model. The observed fluorescence decay is given by $e^{-t/\tau_0}\langle S(t)\rangle$ where τ_0 is the intrinsic lifetime of the fluorophore.

It is obvious that carrying out this program of calculation requires considerable effort and the fit of the calculated $\langle S(t)\rangle$ to experimental data is not necessarily unique. Additionally the method as discussed above assumes a static model of a polymer in which conformational changes are slow compared to the excited state lifetime.[22] We believe that it is difficult, and perhaps impossible, to use EET to obtain structural information about a polymer system. On the other hand this modeling approach can help elucidate the interplay of polymer properties and polymer photophysics that control down chain energy transfer and the potential of polymers as photon harvesting reagents.[23]

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