Synthesis of Fluorescently Labeled Polymers and Their Use in Single-Molecule Imaging

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ABSTRACT: A series of linear polymers labeled with one perylene diimide per polymer chain were synthesized using living free-radical polymerizations based on nitroxides. Depending on the choice of the initiator, the perylene diimide was either at the end or the middle of the polymer chain. The polymers were synthesized as homopolymers or block copolymers from butyl acrylate, styrene, isoprene, and butadiene. Poly(butadiene) labeled with a perylene diimide dye in the center of the polymer was studied using single-molecule imaging in a poly(methyl methacrylate) host. Most of the dyes remained stationary during the experiments, but approximately 5% of the dyes reoriented. The fraction of dyes reorienting was observed to be independent of pumping intensity. This system is an excellent test bed for studying the local environment of the dye and the heterogeneity of the host polymer.

Single-molecule imaging is a new technique that has been used to probe the dynamics of molecules in local environments in liquids, solids, surfaces, and lipid bilayers.[1−8] This technique should prove a sensitive probe of the local dynamics of polymers in various hosts as it can be used to study the motion of individual polymers rather than the ensemble average. Several researchers have described the behavior of single molecules as dopants in a host polymer, but the host−guest approach relies on the probe fluorophore fitting into a void in the host material.[9−13] Here, we study the motion of *polymer chains*—not the behavior of voids or defects—through the motion of the fluorophores. The fluorophores are covalently inserted into the polymer backbone, and as such, they report on the mobility of the local polymer chain to which the fluorophore is attached.

In this paper we report the synthesis of polymers labeled in the backbone with perylene diimides and demonstrate that individually labeled chains can be observed in a poly(methyl methacrylate) (PMMA) host. We prepared polymers with a single perylene diimide at the end or in the middle of a polymer chain; here, the perylene diimide acts as the reporter of the location and orientation of the localized segment of the labeled chain. This synthesis guarantees only one fluorophore per polymer chain.

We chose perylene diimide as a fluorescent label because of its high quantum yield of fluorescence, relative immunity to photobleaching, and easily accessible fluorescence and absorption maxima.[14−16] Perylene diimides and perylenes have been used in single-molecule spectroscopy by our group and others.[17−19] A drawback of using perylene diimides is the difficulty of reacting the readily available 3,4,9,10-perylenetetracarboxylic anhydride, **1**, with amines.[15,16] The 3,4,9,10-perylenetetracarboxylic anhydride is nearly insoluble in all solvents except concentrated sulfuric acid, and the solubility of the perylene diimides is low unless the amine is bonded to a secondary alkyl or bulky aromatic group. In our synthesis **1** is first condensed with a commercially available amino acid, and alkoxyamines are then esterified onto one or both ends of the perylene diimide (Scheme 1). As the alkoxyamines are initiators for living free radical polymerizations, this strategy provides a means of generating well-defined polymer architectures with a single perylene diimide at the center or end of the chain. This synthetic method is flexible and rapid and allows a variety of polymerization initiators to be attached to the perylene diimide through the carboxylic acid.

Free-radical polymerizations mediated by nitroxides provide a controlled method for generating well-defined architectures; new alkoxyamines were recently reported that extended this method to include the polymerization of acrylates and dienes,[20−23] in addition to styrenes. The alkoxyamine polymerizations are stable to a variety of functional groups including carboxylic acids, epoxides, amines, nitriles, alcohols, and anhydrides and provide accurate control over the molecular weights, end groups, polydispersities, and architectures of the polymers synthesized.[21,22]

The perylene-labeled initiators were used to polymerize styrene, butyl acrylate, butadiene, and isoprene into homopolymers and block copolymers (Table 1).[24] In entries 1a and 5a we demonstrate that we can place the perylene diimide at the end of diblock copolymers or the middle of triblock copolymers of similar composition. Entries 6b and 7b are triblock polymers of the type B−A−P−A−B where P is the perylene and A and B are the polymer blocks. The bifunctional initiator provides a route to triblock polymers that are not readily accessible by sequentially growing the blocks from one alkoxyamine since styrene can be blocked onto poly(butyl acrylate), but butyl acrylate cannot be as readily blocked onto polystyrene.[21,22]

To demonstrate that the perylene diimide was in the center of the polymer for entries 5−9, we hydrolyzed the ester bonds of the polymer in entry 9a. Entry 9a was refluxed in methylene chloride for 4.5 h with trimethylsilyl iodide, and a white polymer was isolated. The GPC of the isolated polymer had a molecular weight of 20 000 g/mol and a PDI of 1.07. This reaction demonstrated that the arms had molecular weights

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Scheme 1. Synthesis of the Polymers Labeled with Perylene Diimides

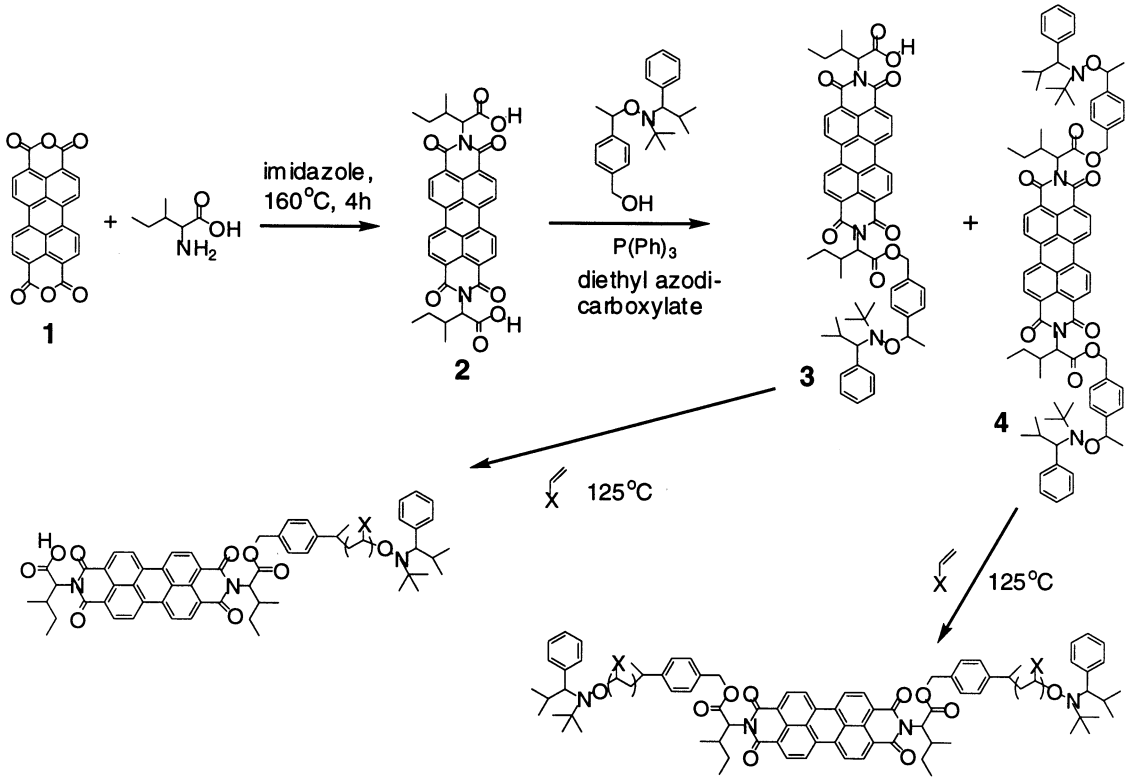
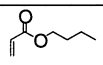
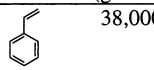
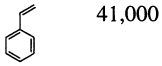
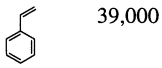
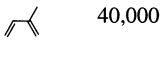


Table 1. Perylene-Labeled Polymers[a]

entry	alkoxy amine	first monomer	M_n (g/mole)	PDI	entry	second monomer	M_n (g/mole)	PDI
1a	3		25,000	1.11	1b		38,000	1.14
2a	3		22,000	1.06	2b		57,000	1.30
3a	3		38,000	1.08				
4a	3		12,000	1.04				
5a	4		20,000	1.08	5b		41,000	1.33
6a	4		8,600	1.10	6b		39,000	1.16
7a	4		18,000	1.05	7b		40,000	1.12
8a	4		33,000	1.22				
9a	4		42,000	1.08				

[a] The M_n and PDI refer to the entire polymer.

approximately one-half the value of the original polymer and that the polymerization was well controlled.

We verified that entry 8a could be seen on the single-molecule level in a PMMA host (Figure 1). The dye-labeled polymer was dissolved in a solution of PMMA and toluene (1% by mass) which was then spun onto a glass coverslip, and the toluene was allowed to evaporate. The PMMA sample contained $\sim 10^{-11}$ mol of dye-labeled polymer per mole of host and was approximately 100 nm thick. This sample was continuously excited by laser light modulated between *x* and *y* polarizations (Figure 1a,b) and imaged in epifluorescence. For each single-molecule spot, the fluorescence intensities at each pumping polarization were extracted and plotted as a function of time (Figure 1c). From these intensities, the value of the in-plane dipole angle of the perylene diimide can be calculated. Information can also be obtained from the overall brightness of the molecule, which can reflect the relative angle with respect to the *z*-axis in the absence of changes in the molecular photophysics. The polar plot in Figure 1d shows the trajectory of the in-plane angle and brightness for the molecule in Figure

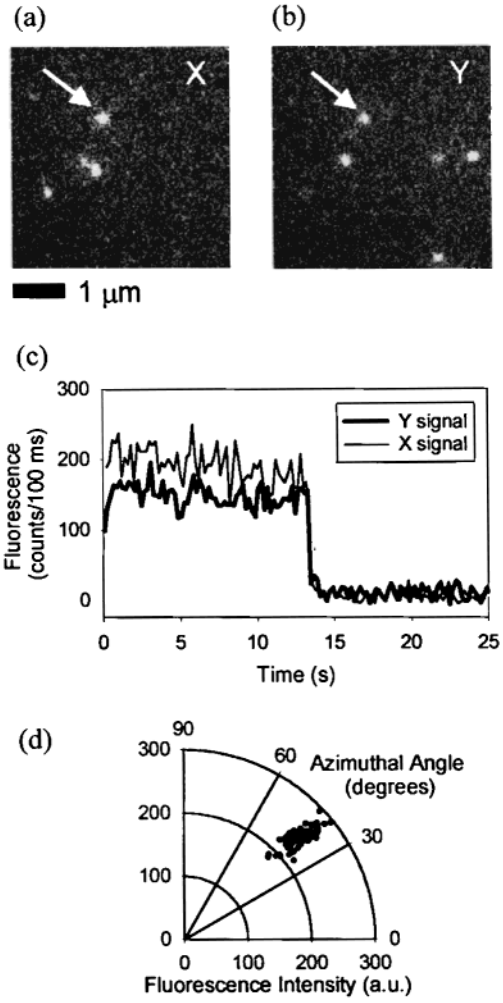


Figure 1. Wide field epifluorescence images of entry 8a from Table 1 in a poly(methyl methacrylate) host excited with light polarized in the (a) *x* and (b) *y* directions. (c) The intensity of the fluorescence of the molecule indicated by the arrows in (a) and (b). (d) A plot of intensity vs angle of orientation for the molecule demonstrates that it was not rotating in the *xy* plane during the course of this experiment.

1c. The molecule no longer fluoresced after approximately 13 s. This digital photobleaching is characteristic of a single molecule. An unavoidable source of noise arises from Poisson counting statistics scaling as the square root of the number of photons detected. The fluctuations in the plotted signal in Figure 1c have an approximate component of 12−14 ADC counts rms from this source. In this case the conservative conclusion is to regard the dipole as fixed; that is, we do not have specific evidence that the molecule in Figure 1 is reorienting.

Although the majority of the labeled polymer molecules were stationary in the PMMA host, we also observed molecules that reoriented on the time scale of the measurement (100 ms). Several types of reorientation were observed. Figure 2a shows a continuous change of the in-plane dipole angle of a single molecule in which the initially dominant emission signal from the *y* polarized pumping decreases as the emission signal from the *x* polarized pumping increases. The trajectory of the in-plane angle and overall brightness shown in Figure 2b illustrates that the molecule samples a number of angles during the time in which it is fluorescent. Another type of observed reorientation is

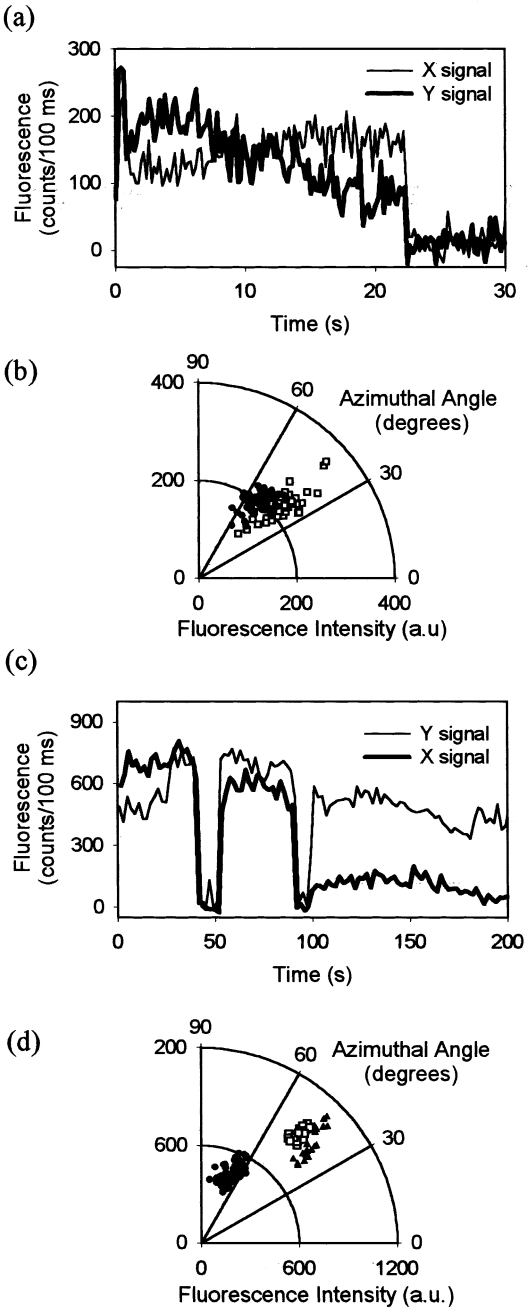


Figure 2. Examples of labeled single polymer chains that reorient in PMMA. (a) Fluorescence emission with *x* and *y* polarized pumping as a function of time, illustrating a continuously changing in-plane dipole angle. (b) The trajectory of the in-plane dipole angle and the overall brightness of the molecule shown in (a), which shows the range of angles sampled by the dipole. The open squares and filled circles correspond to the early half and last half of the trajectory, respectively. (c) For a different molecule, fluorescence emission as a function of time for a dipole angle that changes while the molecule is in a dark "off" state. (d) The trajectory of the in-plane dipole angle and the overall brightness of the molecule shown in (c). The filled triangles, open squares, and filled circles correspond to the first, second, and third "on" times, respectively.

shown in Figure 2c. Here the labeled polymer switches between a bright "on" state and a dark "off" state. After emerging from each dark state, the fluorescence intensities at each pumping polarization have changed magnitude relative to one another, demonstrating that a change in the in-plane dipole angle has occurred some-

time during the "off" time. The polar plot in Figure 2d shows the trajectory for each "on" state of the molecule and provides a clear picture that the dipole of the labeled polymer has shifted to various positions during the observation time. Reorientation occurs for about 5% of the labeled polymer molecules in PMMA, regardless of pumping intensity. (Of the 717 total molecules studied over a range of three different pumping intensities, 40 were observed to reorient.) Since the dye is intimately attached to the polymer backbone at both ends, the orientation of the dye reflects the motion of the local portion of the polymer chain. The extent to which the dye orientation can be used to infer the overall motion of the entire polymer molecule will be the subject of future work. This study demonstrates the ability of the dye-labeled polymer to be used in fluorescence polarization experiments to measure quantities such as the in-plane dipole moment and the overall brightness of the fluorophore. Following these parameters over time and versus temperature can lend insight into dynamic processes that occur within the polymer host; thus, these labeled polymers act as probes of the local environment and will be useful in studying the heterogeneity of the host. Further studies of these polymers in various hosts are underway.

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Supporting Information Available: Experimental procedures and characterization data. This information is available free of charge via the Internet at http://pubs.acs.org.

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(24) In a typical polymerization the alkoxyamine was dissolved in the monomer in a glass vial; the contents of the vial were frozen in liquid nitrogen, evacuated, and thawed three times. The vial was flame-sealed under nitrogen. The vial was heated to 125 °C for 8 h (for the styrene), 16 h (for the acrylate), and 32 h (for the isoprene). The vial was allowed to cool after the polymerization, and the contents were added to methanol.

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