Polymers

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Multimode Diffusion of Ring Polymer Molecules Revealed by a Single-Molecule Study**

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Diffusion processes of synthetic polymer molecules are crucial in deciding their rheological properties, and subsequently in polymer processing and fabrication of plastics, films, and fibers.[1] The topology of a polymer, whether linear, branched, or cyclic, can dramatically affect the motion in a dense entangled solution or in a melt. For linear and branched polymers, the reptation model[2–5] has been accepted as a valid diffusion mechanism and verified experimentally by light scattering, NMR, and viscoelastic measurements.[6–8] In the reptation model for linear and branched polymers, an entangled polymer diffuses in a dynamic tube confined by neighboring polymer chains. Single-molecule studies on naturally occurring macromolecules and also on synthetic polymers have demonstrated the reality of the tube and the diffusion scaling laws.[9–11] The number and structure of end groups is a critical factor in the dynamics of the diffusion of linear or branched polymers. Ring polymers are, on the other hand, topologically unique by the absence of free chain ends.[12,13] Therefore, their diffusion mechanism has attracted continuous attention,[14–17] but is still an important challenge.[18] Apart from cyclic DNA,[19,20] a variety of synthetic ring polymers of sufficiently long chains and of guaranteed purity have recently become accessible.[17,21] As a result, unequivocal topology effects have now been disclosed by using custom-made ring polymers with specific segment structures and optional functional groups.[21–26]

Herein, we show, at the single-chain level, the direct and real-time observation of diffusion dynamics of synthetic ring and linear polymers incorporating a fluorophore.[27–31] Single-molecule spectroscopy is recognized as a powerful tool for monitoring of polymer dynamics, and is capable of revealing topology effects in their diffusion process.[20,32]

We synthesized linear (**1**) and cyclic (**2**) poly(tetrahydrofuran)s (poly(THF)s) containing perylene diimide unit as a fluorophore by means of an electrostatic self-assembly and covalent fixation process[27,28] (Figure 1; see the Supporting Information and references therein for details on the synthesis and characterization). For single-molecule imaging experiments, polymers **1** and **2** were mixed with unlabeled linear poly(THF) in toluene in a semi-dilute concentration regime. The semi-dilute concentration of the unlabeled matrix polymer is about 20 times more than the critical concentration for chain overlap. The final concentrations of **1** and **2** in the matrix were on the order of 10^{-9} M. The sample solutions were sandwiched between two clean microscope cover slips, resulting in the sample thickness of 10 μm; their fluorescence images were measured using a fluorescence microscope and an EM-CCD camera.

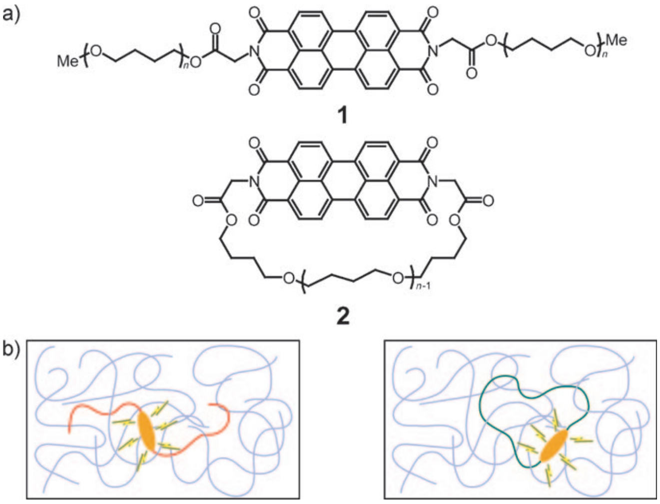

Figure 1. a) The linear (**1**) and cyclic (**2**) poly(THF) molecules containing a perylene diimide moiety. b) Left: linear polymer **1** surrounded by linear poly(THF)s. Right: cyclic polymer **2** surrounded by linear poly(THF)s.

Figure 2 shows single-molecule fluorescence images of **1** (Figure 2 a) and **2** (Figure 2 b) mixed with linear poly(THF) in toluene. The positions of the molecules were determined using a two-dimensional Gaussian fitting (see Supporting Information for details on the particle tracking analysis).[33] Figure 2 c,d shows examples of diffusion trajectories of **1** and **2**, respectively, obtained by plotting the measured molecular positions. The length of the trajectories was limited primarily by the three-dimensional character of the diffusion of the

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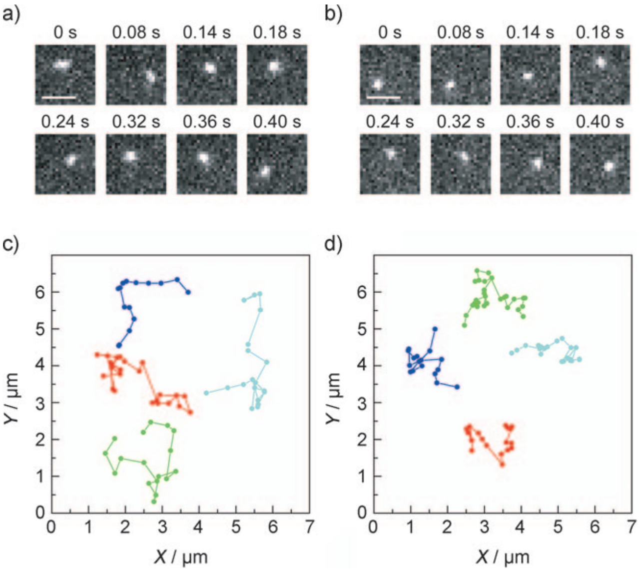

Figure 2. a,b) Single-molecule fluorescence imaging of self-diffusion: a) **1** and b) **2** each mixed with linear poly(THF) in toluene. Scale bars = 2 µm. c,d) 2D trajectories of self-diffusing molecules of c) **1** and d) **2**. The red trajectories in (c) and (d) correspond to the images displayed in (a) and (b), respectively. Each trajectory contains on average 20 data points.

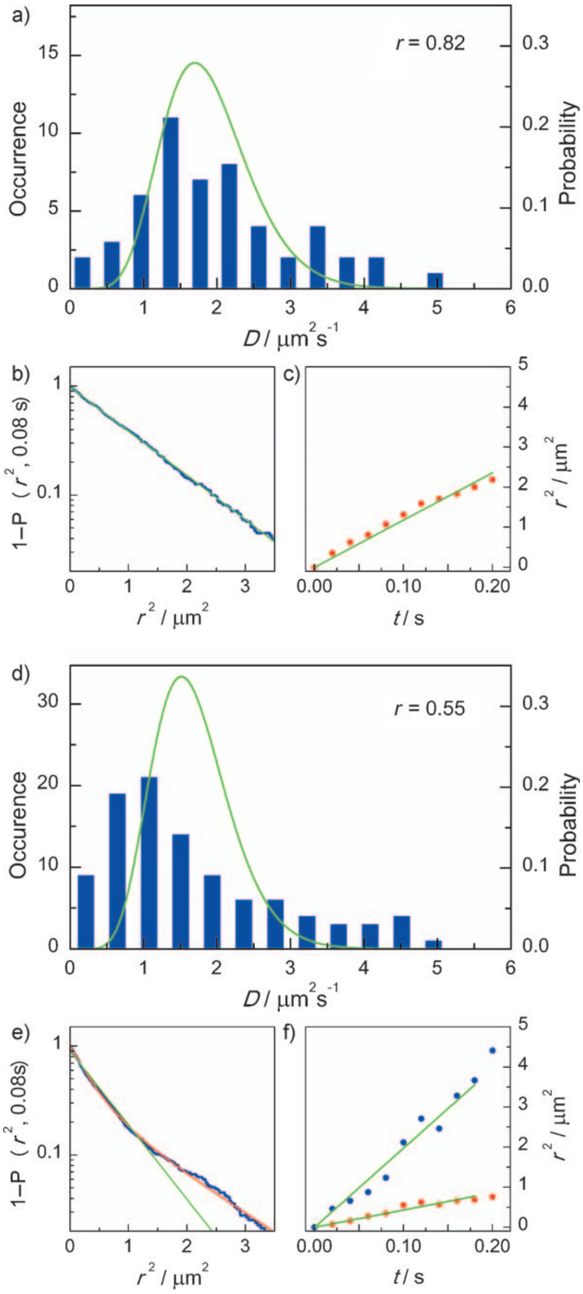

Figure 3. Analysis of the single-molecule self-diffusion. a,d) Frequency histograms (blue bars) of the diffusion coefficient determined for a) **1** and d) **2**. The green lines show calculated theoretical statistical distributions corresponding to diffusion of single molecules in a homogeneous environment, with the diffusion coefficient given by means of the respective histograms.[36] b,e) Experimentally obtained cumulative distribution functions (CDFs) in the form of 1−*P* (blue lines) for b) **1** and e) **2**. The green and red lines show single- and double-exponential fittings. c,f) CDF coefficients at different time lags for c) **1** and f) **2**. The green lines show linear fittings (see text for details).

molecules; that is, the positions of the molecules can be determined only when they are in the focal plane. Nevertheless, we were able to obtain trajectories long enough to calculate the diffusion coefficients D for statistically relevant ensembles of single chains.

The diffusion coefficients were determined by mean square displacement (MSD) analysis of the trajectories (see Supporting Information for details), and plotted in frequency histograms (Figure 3, blue bars). The distributions of D are broad, with ranges for **1** of 0.25–5.1 $\mu m^2 s^{-1}$ and for **2** of 0.17–5.2 $\mu m^2 s^{-1}$, and with similar mean values (2.0 and 1.74 $\mu m^2 s^{-1}$ for **1** and **2**, respectively). However, the positions of their peaks differ significantly, with the peak for the linear poly(THF) **1** ($1.66 \pm 0.12\ \mu m^2 s^{-1}$) being 60 % larger than that for the cyclic poly(THF) **2** ($1.04 \pm 0.12\ \mu m^2 s^{-1}$).[34]

Generally, distribution of D measured by single-molecule tracking consists of a statistical contribution owing to a limited number of measured points (molecular positions) in a single trajectory, and of a contribution due to the physical heterogeneity of the system.[35] We calculated statistical distributions corresponding to the theoretical diffusion of single molecules in a homogeneous environment, with single diffusion coefficients given by the mean values of the histograms.[36,37] The distributions are shown in Figure 3 a,d as green lines. The measured distribution of D for linear poly(THF) **1** (Figure 3 a) is reproduced reasonably well by the calculated distribution (Pearson's correlation coefficient $r = 0.82$), with only a small portion of molecules showing slower or faster diffusion. This result demonstrates that linear poly(THF) chains diffuse in a homogeneous environment with a single-mode diffusion coefficient. On the other hand, the measured histogram of D for the cyclic poly(THF) **2** (Figure 3 d) deviates significantly from the calculated homogeneous statistical distribution ($r = 0.55$). The deviation reflects the inhomogeneous nature of the system.

The diffusion of both **1** and **2** was further analyzed using a cumulative distribution function (CDF) *P*, which is the cumulative probability of finding a diffusing molecule within a radius r from the origin at a given time lag (see Supporting Information for details).[38] The CDF analysis and plots of $1-P$ versus r^2 are useful tools to extract the values of diffusion coefficients from trajectories that contain multiple diffusion modes. Fast decay of $1-P$ reflects a rapidly decreasing probability that a molecule diffuses into larger distances and thus corresponds to slow diffusion. The CDF shows a single-exponential behavior for **1** (Figure 3b), which suggests the presence of single diffusion mode. The diffusion coefficient calculated from the CDF analysis was 2.9 $\mu m^2 s^{-1}$ (Figure 3c). These results are consistent with the homogeneous diffusion suggested by the D distribution (Figure 3a). In contrast, the CDF of **2** shows a multi-exponential behavior (Figure 3e), indicating multimode diffusion. Using a double-exponential fitting, two diffusion coefficients (1.1 and 4.9 $\mu m^2 s^{-1}$, with fractions of 0.66 and 0.34) were obtained (Figure 3f). These results are also consistent with the inhomogeneous diffusion suggested by the D distribution (Figure 3d).

Both the MSD and CDF analyses point to heterogeneous multimode diffusion of the cyclic poly(THF). The heterogeneity is related to the cyclic nature of the polymer itself, because the diffusion of the linear polymer (Figure 3a) indicates that the semi-dilute solution of the unlabeled linear chains creates a homogeneous environment. The observed heterogeneity can be interpreted as partial threading of the cyclic chains with the linear matrix,[39] resulting in slower diffusion and in a shift of the peak of the distribution to smaller values of D compared to the linear counterpart. Even a small amount of linear chain impurities in a ring polymer melt can change the rheological properties of the melt dramatically owing to threading.[17,40,41] On the other hand, those molecules that are not threaded diffuse slightly faster because they have a more compact conformation, and they contribute to the long tail of the distribution in Figure 3d. The observed deviation from the calculated homogeneous statistical distribution of single-molecule diffusion coefficients may thus help to understand some of the controversial experimental observations on dynamics of ring polymers.[18]

Apart from static distributions of diffusion coefficients, the single-molecule technique also offers the unique possibility to follow dynamical changes in diffusion occurring on individual molecules. For diffusion of ring polymers, analyzing individual traces could provide insight into the timescales of the persistence of the two diffusion modes, that is, threaded and unthreaded. The two diffusion modes would appear as a switching of the diffusion coefficient (between the 1.1 and 4.9 $\mu m^2 s^{-1}$ components) during the observation interval. We found no evidence of such switching in the diffusion trajectories of **2**. The result thus indicates that threading persists on timescales longer than the experimental time window (200 ms).

In summary, single-molecule spectroscopy has a potential to unravel information that is hidden in observations by ensemble techniques. One example of such information is the distribution of diffusion coefficients; others are dynamical changes in the diffusion process on the level of individual molecule. Our results on synthetic ring polymers in a linear polymer matrix demonstrate that the single-molecule approach is capable of revealing different modes in the diffusion mechanism and that the modes are associated with specific molecules for timescales longer than the timescale of the experiment. The method holds great promise to provide new insight into the diffusion dynamics of ring polymers which is a hotly debated topic in polymer physics.

Experimental Section

Linear (**1**) and cyclic (**2**) poly(tetrahydrofuran) (poly(THF)) containing a perylene diimide unit as a fluorophore were synthesized by the process described before[27,28] and characterized by ^{1}H NMR spectroscopy, MALDI-TOF, SEC, and FTIR (see Supporting Information and references therein for details). Molecular weights: **1**, $M_n = 4200$ ($M_w/M_n = 1.12$); **2**, $M_n = 3800$ ($M_w/M_n = 1.19$). The ring structure of **2** thus consists, on average, of as many as 250 atoms. Unlabeled linear poly(THF) was obtained from Aldrich (10^3 gL^{-1}, $M_n = 3000$). Absorption and fluorescence spectra of **1** and **2** were virtually identical, with absorption maxima at 528 nm, and fluorescence maxima at 536 nm (Supporting Information, Figure S9). Fluorescence quantum yields ϕ_{fl} in toluene of **1** (0.53) and **2** (0.51) are comparable to the precursor perylene diimide derivative **4** ($\phi_{fl} = 0.54$ in ethanol; see the Supporting Information).

Fluorescence images were recorded using an inverted microscope (Olympus, IX71) equipped with a high numerical aperture objective lens (Olympus, ×100, N.A. = 1.3) and an EM-CCD camera (Andor technology, iXonEM+). A circularly-polarized 488 nm line from an argon ion laser with typical power of 190 $W cm^{-2}$ was used as an excitation light source (see Supporting Information for details).[33,42] The images were recorded with a 20 ms integration time to avoid movement of the molecule within the exposure.

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