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Direct Observation of Rotational Motion of Fluorophores Chemically Attached to Polystyrene in Its Thin Films

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Glass transition of polymers under confinement is a very interesting topic in polymer physics.[1−8] The significance of the researches in this field also lies in the fact that it is related to the fundamental knowledge underlying numerous technologies and applications, such as coating, adhesion, membrane technology, polymer nanocomposites, microelectronic industry, nanofabrication, etc. Polymer films with very small thickness comparable to the polymer chain dimension (for example, the radius of gyration, R_g) offers the ideal model systems for the investigation of glass transition of confined polymers. According to previous studies,[9−13] systems of this kind exhibit very unique glass transition behavior compared with the bulk states. The most noticeable feature is the shift of glass transition temperature (T_g) compared with the bulk values. Studies show that this behavior may originate from a number of reasons: spatial confinement, free surface effect, polymer−substrate interactions, etc. Various methods have been employed to explore the physical origins of glass transition under confinement, by investigating a number of different properties of polymer thin film. For example, ellipsometry,[1] X-ray, and neutron reflectivity[14,15] have been used to measure the thermal expansion coefficient; atomic force microscopy[16] and microrheology[9] to study the viscoelastic properties at polymer surface; nonlinear optical spectroscopy (sum-frequency generation spectroscopy[17] and second harmonic generation spectroscopy[18]) to study the segmental orientation and reorientation; differential scanning calorimetry (DSC) to investigate thermodynamic properties;[19] positron annihilation spectroscopy to explore the free volume of the systems;[20] nuclear magnetic resonance spectroscopy to study local motion of chemical groups on the polymer chains;[21] dielectric relaxation spectroscopy to study the segmental motion of polymer chains;[22] Brillouin scattering spectroscopy[2,23] and fluorescence spectroscopy[24−26] to study the microenvironment of the polymer systems, etc.

Theoretical analysis shows that the glass transition is a collective process involving contributions from multiple chains or segments.[27] This brings up the importance of studies focusing on the behavior of individual chains or segments and their collective behavior during the glass transition, allowing the exposure of the microscopic information at single molecular level. However, because of the limitation of experimental method, study on the fundamental mechanism related to single chains or segments has been challenging. Thanks to the development of sensitive imaging techniques, single molecule fluorescence microscopy has been applied successfully to study polymer systems.[28] With its high sensitivity and spatial resolution, single molecule fluorescence microscopy is able to visualize individual fluorescent molecules and track the molecular motion. "Defocus fluorescence microscopy" has been demonstrated to be very powerful in studying the orientation of individual fluorescence dipoles and tracking the rotational motions, by recording and analyzing the diffraction patterns of the fluorescence emission of single dye molecule.[29−33] By this method, the three-dimensional orientation of fluorescence emission dipoles can be explicitly determined and the rotational motion of individual molecules can be monitored. Besides, this method can image and track the motion of multiple dipoles simultaneously. According to previous studies, the rotational motions of guest fluorophores and chromophores in polymer systems are coupled to the segmental motion of the polymer chains and can be used to study the segmental motion, especially the α-relaxation process.[29,34,35] Considering the fact of multisegmental contribution in the glass transition of polymers, defocus fluorescence microscopy should be a very suitable method to monitor the rotational motion of multiple chain segments at single molecular level, especially when the fluorescence dipoles are chemically attached to the polymer chain.

In this study, defocus fluorescence microscopy method is adopted to study the segmental motion in polystyrene (PS) thin films. According to the results of previous researches on PS thin film systems, the T_g value of PS thin film is much lower than the bulk value.[1−4,9−12,20,22,23] On the basis of this fact, it is expected that the segmental motion of PS chain should be activated at temperature well below the bulk T_g value. It is based on this thought that the current study is initiated. Attention is paid to the fraction of rotating fluorophores chemically attached to the PS chain ends, as a function of the sample's temperature. The results show an abrupt increase of rotating molecule fraction at temperature well deep below the T_g of bulk PS, and a thickness dependence of this behavior is found.

The PS samples were purchased from Polymer Source (Québec, Canada). One sample (M_w = 355 000 g mol^{-1}, M_w/M_n < 1.18) was terminated with an amino group at one of its chain end. In our laboratory, chemical reactions were conducted so that a bright and stable fluorescence molecule (Alexa 532, Invitrogen) was attached to the chain end. The labeled sample was carefully purified by size exclusive chromatography. This labeled sample served as the probe in the experiments and another PS sample (M_w = 392 000 g mol^{-1}, M_w/M_n < 1.09) served as the matrix. DSC measurement showed that the glass transition of this sample occurred at 100 °C (the heating rate was 10 °C/min).

The thin film samples were prepared by spin-coating from their 1-chloropentane solutions with different concentrations (such as 2, 6, and 10 mg/mL). [The film's surface morphology is comparable to films prepared with other commonly used solvents such as toluene.[37] One advantage of using 1-chloropentane as the solvent is that it is a nonsolvent for most other polymers such as poly(methyl methacrylate).] A tracer amount of florescence-labeled PS was mixed in this solution at the concentration of 5×10^{-10} M (in the 2 mg/mL PS solution), 4×10^{-10} M (in the 6 mg/mL PS solution) and 1.0×10^{-10} M (in the 10 mg/mL PS solution). Glass coverslips (Thermo Fisher) were used as substrates. Before use, the substrates were treated in acetone and deionized water under ultrasonication copiously and then treated in oxygen plasma for 30 min. After the substrates were cleaned and dried, the spin-coating was performed at the speed of 6000 rpm. The thin film samples were incubated for 24 h in vacuum at the temperature of 100 °C. Before microscopic observation, the samples were cooled

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down to room temperature at the rate of ~0.5 °C/min. The film thickness determination was conducted by AFM measurements (Nanoscope IIIA, Vecco) on the samples scratched with a razor blade after all microscopic observations were accomplished.

Fluorescence defocus microscopic measurements were conducted on an Olympus IX-71 inverted microscope equipped with an Andor 887 EMCCD camera. The excitation laser beam (532 nm output of a solid laser) was introduced through an oil-immersion objective lens (100× PlanApo, numerical aperture = 1.45), and wide-field fluorescence images were recorded at controlled timing. The excitation light was adjusted to be circularly polarized in order to guarantee equal excitation probability for fluorophores with all orientation. Optimized filtering optics (Chromatech) was installed to the microscope to guarantee high enough signal-to-noise ratio. A nanopositioner (PI, Germany) was mounted under the objective lens so that the focus of the objective lens was precisely controlled. In the experiments, an optimal defocus distance of 0.6 μm was achieved to generate the well-defined diffraction patterns and the best image contrast. The temperature control was achieved by controlling the temperature of both the sample and the objective lens. The samples were housed inside a commercial hot-cold stage for inverted microscopes (HCS60, Instec). In order to guarantee uniform distribution of temperature, the temperature of the objective lens was also controlled by a home-built device. Efforts were made to minimize the temperature difference between the sample and the objective lens, which were closely positioned to each other. By doing this, the temperature of the sample was well stabilized.

Figure 1 shows a typical defocus fluorescence microscope image of the PS film with the thickness of 11.5 nm. The dumbbell-shaped patterns are the diffraction patterns of the fluorescence emission of individual fluorophores attached to the PS chain ends. Such well-fined dumbbell-shaped patterns indicate the emission dipoles of separated single fluorophores. Because the exact dimension is not reflected in the defocus images, the features provide information on the orientation of the emission dipoles: the direction of the dark line in the middle of the dumbbell-shaped pattern is the orientation of the emission dipole. Movies of successive images under different temperatures (40 and 44 °C, as two examples) are provided in the Supporting Information. Careful analysis of the images and movies shows that most molecules (>95%) were oriented parallel to the substrate's surface, as demonstrated by the symmetrical dumbbell-shaped patterns in the defocus fluorescence images. Only a small portion (<5%) had noticeable nonzero out-of-plane orientation angle by exhibiting symmetric and nonsymmetric donut-shaped patterns (an example is indicated by arrow A in Figure 1).

The results show that all of the molecules were immobilized under the temperature studied, and no translational diffusion was observed. Attention is paid to the observation of the rotational motion, as demonstrated by the change of orientation angle of fluorophores with time. As an example, the time sequence of typical images of one specific fluorophore is shown in Figure 2, together with the data of its in-plane orientation angle (ψ). The rotational motion of the fluorophore is clearly demonstrated. During the observation time of 16.5 s, the ψ value of this fluorophore fluctuated drastically: the angle difference within a time interval of 2.5 s was as big as 94°. Such a jumping variation of the orientation angle demonstrated the sharp change of the microenvironment surrounding the fluorophore in the polymer thin film and clearly demonstrated the dynamical heterogeneities in polymer systems. Analysis of rotation rate has been conducted, and the data showed very wide distributions, from one fluorophore to another, as well as from time to time for one specific fluorophore. Also, the data did not provide a clear tendency of variation when the temperature was tuned. This behavior is similar to what has been observed in other polymer systems,

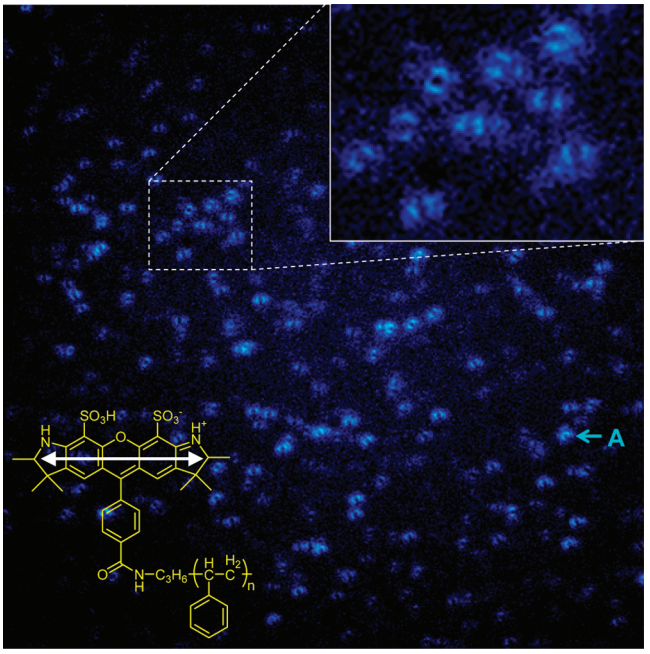

Figure 1. A typical fluorescence defocus image of PS film (thickness: 11.5 nm) at 40 °C. Most of the molecules show orientation parallel to the substrate's surface, demonstrated by the symmetrical dumbbell-shaped patterns. A small portion has noticeable out-of-plane orientation, denoted by arrow A as an example. The size of the image is 50.6 μm × 50.6 μm. The inset shows a magnified section of the original image. The corresponding movie is provided in the Supporting Information. The chemical structure of fluorophore (Alexa 532)-labeled polymer is also shown. The double-ended arrow in white denotes the direction of emission dipole moment of the fluorophore.

for example, the fluorescence molecules doped in poly(methyl acrylate) (PMA) above its T_g.[32,33]

The detailed comparison between the movies taken at 40 and 44 °C exposed one important feature: the number of rotating fluorophore at 44 °C is much lager than that at 40 °C. At 40 °C (movie 1 in the Supporting Information), most molecules were found to be stationary, and only a small portion was observed to undergo rotational motion (during the observation time of 399 s). We believe that the observation of rotational motion at such a low temperature shows the dynamic heterogeneity of polymer glassy state. It is believed that the glassy state of polymers is not homogeneous, in both space and time.[29,38] This observation shows the very wide distribution of the microenvironment for different fluorophores so that rotational motion is still enabled at temperature way below its glass transition temperature.

However, when the temperature was elevated over 44 °C (movie 2 in the Supporting Information), almost all of the molecules were found to rotate (during the observation time of 389.5 s). In order to quantify the vast difference of number of rotating fluorophores, a quantity was defined as the faction of rotating fluorophores (f_R)—the ratio of number of rotating fluorophores to the total number of fluorophores in the field of view. The data analysis was conducted with all fluorophores in the field view of the same sample. The rotating fluorophores were defined as those which showed more than 10° changes in orientation angle. The effect of photobleaching was considered—the counting of rotating and stationary fluorophores included all fluorophores with and without photobleaching. The results show that the f_R value for the 11.5 nm thick sample at 40 °C was ~2%, and it increased sharply to 95% at 44 °C. The data analysis of f_R was conducted for all temperatures investigated, and the temperature dependence is shown in Figure 3, in which the sharp increase of f_R was seen when temperature was elevated crossing

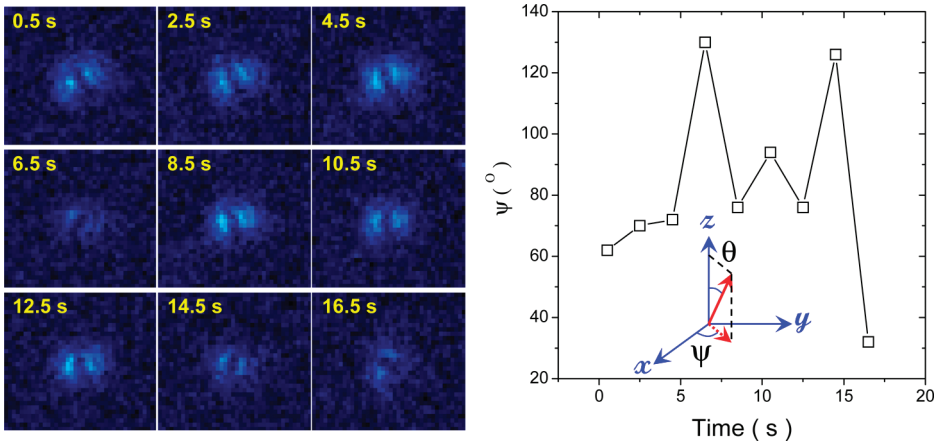

Figure 2. Left panel: a series of typical defocus fluorescence images of one specific fluorophore at different time of observation. The sample is 11.5 nm thick PS film, and the temperature is 44 °C. Right panel: the in-plane dipole orientation angle (ψ) of this fluorophore as a function of observation time. The inset shows the definition of the angles.

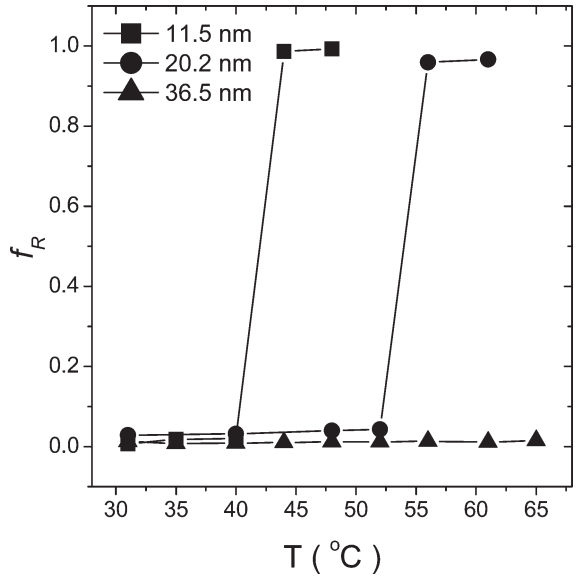

Figure 3. Temperature dependence of fraction of rotating fluorophore (f_R) in PS thin film. Data with thicknesses 11.5 nm (■), 20.2 nm (●), and 36.5 nm (▲) are presented.

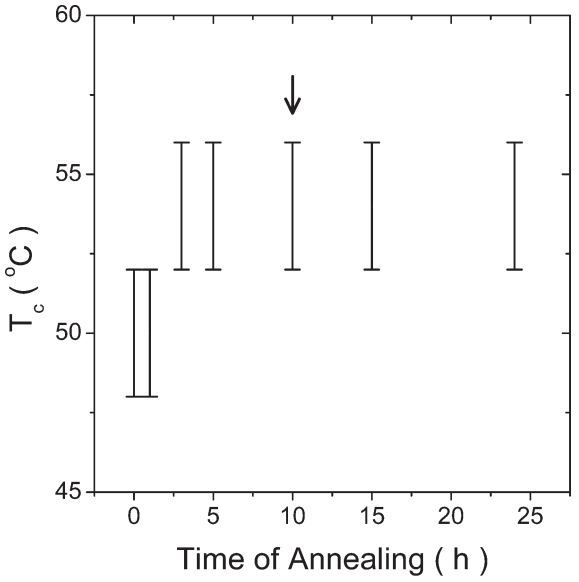

Figure 4. The "critical temperature", T_c, of the sudden change of f_R for the 20.2 nm thick film as a function of the time of sample annealing at 100 °C. The data denoted by the arrow is for the sample annealed at 150 °C. The temperature is shown as value ranges instead of data points due to the step of the temperature rise in the experiment.

the region between 40 and 44 °C. As a comparison, the data for a thicker film (20.2 nm thick) shows such a prompt increase of f_R between 52 and 56 °C. An even thicker film (36.5 nm thick) exhibited a constant low level of f_R value around 2% up to 65 °C. These data clearly demonstrate a thickness dependence of f_R change with temperature—the temperature of the sudden change of f_R increases with the film thickness. It is expected that the change of f_R for 36.5 nm thick film should happen at even higher temperature. However, because of the instability of the fluorescence signal at elevated temperature, data for this sample are not available at the current stage.

The different temperature dependence of f_R with PS films of different thickness should show the different behavior of segmental motion in these films, especially the α-relaxation motion. First of all, the fluorophore is chemically attached to the PS chain through the chain end. Any rotational motion of this rigid conjugate fluorophore is directly connected to the motion of the segments at or near the chain end. Second, it should not be related to the β-relaxation process. The fluorophore's dimension is ~1.2 nm, much bigger than the side group of PS. Therefore, the time scale of its rotation cannot be related to the motion of the side groups.[36] Third, control experiments proved that the observed changes of f_R is not related to the orientation relaxation from the highly nonequilibrated state frozen in the spin-coating process. The experiments were conducted with 20.2 nm thick films under different annealing conditions—the annealing time and temperature. The results show that the temperature of f_R change for samples with short annealing time was ~4 °C lower than those with longer annealing time (Figure 4). Because of the step size of the temperature increase in the current experiment, the temperature of f_R change is denoted as a temperature range, named as "the temperature of change, T_c". The results show that the values T_c stabilized after annealing exceeding 3 h. The result for the sample annealed at 150 °C shows similar values (data indicated by the arrow in Figure 4). All of these data demonstrate that the sample annealing provided long enough time for the polymer chain to relax and the observed change of f_R is not related to the relaxation from the possible stretched state from spin-coating.

On the basis of the discussion and analysis above, we believe that the motion of the fluorophores is related to the α-relaxation process of PS chain. In a number of researches by different

experimental methods, such as second harmonic generation spectroscopy,[35] dielectric spectroscopy,[34] and single molecule fluorescence microscopy,[29] it has been proved that the rotational motion of guest chromophores and fluorophores, both doped and chemically attached to polymer chains, are coupled to the segmental motion and served as the probe of the α-relaxation of polymer chains.

As observed in many previous studies,[9−12] the T_g value of PS thin film on solid substrates is reduced when the film thickness gets smaller. The results of the current study agree well with this tendency. The results demonstrate the collective feature of the segmental motion with respect to the glass transition, as described by the Adam−Gibbs theory.[27] Although it is not clear whether the temperature at which the f_R value changes corresponds to the T_g value of the sample, it does provide a direct observation that the excitation and unfreezing of segmental motion inside the polymer thin film.

The difference in the temperature dependence of f_R for these samples also agrees with the predicted and measured values of T_g. Empirically, the thickness dependence of T_g value of polymer thin films can be expressed by the equation of $T_g(h) = T_g(\infty)[1 - (a/h)^{\delta}]$, where the $T_g(\infty)$ is the bulk value of T_g, h is the film thickness, and a and δ are characteristic parameters of materials.[1] By using 3.2 nm and 1.8 for a and δ as scalable values, respectively, the T_g values of these PS thin films were estimated as 63 °C for the 11.5 nm thick film, 86 °C for the 20.2 nm thick film, and 95 °C for the 36.5 nm thick film. These values by this empirical equation agree with the T_g value by experimental measurements.[9,11,12] Therefore, defocus fluorescence microscopy results reported here demonstrate a direct observation of individual segmental motion related to the glass transition of PS film. The results indicate that the activation energy of the segmental motion has a dependence on film thickness. Because the temperature of f_R change is ∼20−30 °C lower than the reported T_g of PS film with similar thickness, this observation by defocus microscopy demonstrates the early activation of segmental motion before the glass transition effect sets in. Detailed researches on the thickness dependence, polymer molecular weight dependence, free surface effect, and polymer−substrate interaction are being conducted.

The orientation-sensitive single molecule defocus fluorescence microscopy helps to visualize and monitor the orientation of individual fluorophores chemically attached to the PS chain end. The segmental motion observed is believed to be related to the α-relaxation of the chain and demonstrates the early activation of segmental motion before the glass transition effect sets in. The sharp change in fraction of rotational fluorophores at specific temperature and the thickness dependence of the transition temperature demonstrates the collective molecular feature of the glass transition and agrees well with the shift of T_g value with the reduction of film thickness. The high sensitivity of defocus microscopy enables the observation of the motion of individual segments and help to shed light on the molecular mechanism of glass transition process of polymers.

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Supporting Information Available: Videos of defocus fluorescence microscopy of fluorophores chemically attached to polystyrene chain ends. This material is available free of charge via the Internet at http://pubs.acs.org.

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