

Reorientation and Translation of Individual Dye Molecules in a Polymer Matrix

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Abstract

The reorientational and translational motion of individual dye molecules embedded in a polymer matrix is studied in the temperature regime above the glass transition. The rotational diffusion close to the glass transition is heterogeneous on the single molecule level. Sudden changes in the reorientational speed of single molecules are found. The exchange between these reorientational speeds is found to be one order of magnitude slower than the reorientational time constant of the molecules. Translational motion can be clearly identified at about $1.2 T_g$. However, the translational diffusion shows no signs of heterogeneity on the timescale of our experiments, from which we conclude, that the timescale of the exchange process between microenvironments has become too fast.

Key words: polymer dynamics, single molecule

PACS:

1 Introduction

The physical properties of polymers, especially the dynamical properties close to the phase transition from the rubbery to the glassy state are complex and despite several decades of experimental and theoretical studies not completely understood [1]. The relaxation in polymers shows commonly a complex non-exponential pattern which is the result of a strong heterogeneity of the system [2]. While there was a debate on the nature of this heterogeneity, recent experiments favour the existence of dynamically fluctuating local physical properties - so called dynamic heterogeneities [3–5] - against a concept of a spacially static distribution of relaxation times. The details of the local dynamic changes are however not fully understood. Other still unsolved questions involve the processes behind the so called rotational translational paradoxon described i.e.

by Rössler [6]. Within a certain temperature regime above the glass transition ($T_g < T < T_c \approx 1.2T_g$), rotation and translation of molecules do not show the same viscosity dependence. While rotational diffusion follows the Debye-Stokes-Einstein relation with $D_{rot} \propto \eta^{-1}$, translational motion is enhanced and violates this relation with a $D_{rot} \propto \eta^{-\xi}$ dependence with $\xi < 1$ [6–11]. It was suggested that translation and rotation actually probe different domains of the glass [9] which requires the existence of a static heterogeneity too. Most experimental methods applied to polymer systems and the problems described above are limited to measure only mean values of observables and have to invert experimental data to obtain the distributions of the observables. It is especially this distribution of observables which is important in the study of heterogeneity. One experimental method, which is now applied more and more in the field of material studies is single molecule microscopy and spectroscopy [12,13]. Due to the fact that a highly sensitive detection allows the study of individual probe molecules, heterogeneity can be evaluated directly by measuring on many individual members of an ensemble. With the help of this method diffusion processes in lipid membranes [14–16] or heterogeneities in polymer systems [3,17–21] and other glass formers [5] as well as interface effect at solid liquid boundaries [22,23] have been studied with great success.

It is the purpose of this paper to apply single molecule microscopy to explore the transition from rotational to translational motion of individual dye molecules in polymer films and to study whether the existence of dynamical heterogeneities as shown in [3,4] shows up in translational diffusion too.

2 Experimental

Experiments are carried out on thin films of low molecular weight polymethylacrylate (PMA, $M_W=40000$, $T_g=281$ K) doped with Rhodamine 6G (R6G) molecules at a concentration of about 10^{-9} M. The films are deposited on a silicon substrate by spin coating a solution of PMA/R6G in toluene. The resulting films have been annealed at 350 K for 10 hours to remove residual solvent and to relax influences of the spin coating technique on the polymer conformations. The thickness of the polymer films were determined by reflectivity measurements. Film thicknesses of about 80 nm have been used. The experiments are carried out with silicon substrates from which the silicon oxide layer has been removed by etching in hydrofluoric acid. Due to the low lying band gap of silicon, emission from dye molecules in a region of about 10 nm distance from the substrate surface is quenched. This was checked by depositing 10 nm PMA/R6G films on the substrates. No dye emission has been observed from such samples. Thus we can avoid interface effects arising from the interaction of dye or polymer molecules with the solid surface. We neglect the influence of the free interface, since changes in the dynamics of

molecules close to the interface are expected up to a distance of 3 nm. Thus the fraction of molecules within this region is sufficiently small compared to the bulk region. To heat or cool the polymer film, the sample was mounted on a cryostat (Oxford Microstat He).

The position and orientation of single dye molecules is studied using a home built wide field fluorescence microscope. Circular polarized laser light (514 nm line of an Ar⁺ laser) is coupled into the microscope with an dichroic beamsplitter. The beamsplitter reflects the light into a microscope objective (100x/0.9NA, Zeiss Epiplan Neofluar) which illuminates the sample. The emission of individual molecules is then collected by the same objective and imaged onto an intensified CCD camera (Pentamax, Roper Scientific) by a lens. In front of the CCD we insert a Wollaston prism to split the emitted light into its two orthogonal polarization components. Thus we obtain two images on the CCD which represent s- and p-polarized light as shown in Figure 1. For the rotational motion we recorded the two polarizations over up to 4000 frames with exposure times between 30 ms and 1 s. The large variation in the exposure time was necessary to cover all possible rotational speeds. Two temperatures $T_g+10\text{K}$ and $T_g+15\text{K}$ were used to observe rotational motion. Translational motion was studied at temperatures reaching from 339 K to 373 K with a time resolution of up to 30 ms (33 frames per second).

3 Data Analysis

3.1 Rotational Motion

Data analysis was performed using software developed in our lab and is done mostly automatically which allows us to study a large number of individual molecules at the same time. To analyze the rotational diffusion we follow mainly the analysis in [4] and calculate the fluorescence polarization of each intensity trace by

$$A(t) = \frac{I_p - I_s}{I_p + I_s} \quad (1)$$

The fluorescence polarization $A(t)$ varies between -1 and 1 which corresponds to a change in the orientation of the molecule by an angle of π . To characterize the rotational dynamics we further calculate the fluorescence polarization autocorrelation function

$$C(\tau) = \frac{\langle A(t)A(t+\tau) \rangle}{\langle A(t)A(t) \rangle} \quad (2)$$

The decay of the correlation function $C(\tau)$ is then fitted with a stretched exponential function

$$D(t) = e^{-\left(\frac{t}{\tau_s}\right)^\beta} \quad (3)$$

where τ_s is the time constant and β is the stretching exponent. The stretching exponent can be interpreted in terms of a distribution of relaxation times. An stretching exponent of $\beta = 1$ shrinks the distribution to a delta function and thus to a single relaxation time, which is expected for normal brownian rotational diffusion. The width of the distribution will increase with decreasing β ($0 < \beta < 1$). Since β and τ_s vary between different molecules, we further calculate the mean relaxation time of equation 3

$$\tau_c = \frac{\tau_s}{\beta} \Gamma\left(\frac{1}{\beta}\right) \quad (4)$$

to compare different molecules.

3.2 Translational Motion

In order to analyze diffusional motion first the positions of the molecules are determined for each frame. Using that information trajectories of diffusing molecules are constructed and analyzed by calculating mean square displacements (MSD) as a function of time as

$$\langle \underline{r}^2(\tau) \rangle = \langle [\underline{r}(t) - \underline{r}(t + \tau)]^2 \rangle \quad (5)$$

for each single molecule trajectory. Finally we calculate diffusion coefficients from MSD using a weighted fit to the MSD-data where the weights reflect the significance of each datapoint according to [24]. The resulting slope of the linear fit equals $4D$, with D being the diffusion coefficient.

4 Results and Discussion

Figure 2 shows the distribution of relaxation times τ_c obtained for the reorientational diffusion of molecules at $T = 290$ K (left) and $T = 295$ K (right). The mean relaxation time of both distributions clearly changes from $\langle \tau_{290K} \rangle = 3.3$ s at $T = 290$ K to $\langle \tau_{295K} \rangle = 380$ ms at $T = 295$ K and changes therefore by one order of magnitude within a temperature interval of 5 K. The

strong dependence of the mean reorientation time is a sign for being close to the glass transition, which should occur at $T = 281$ K. Measurements carried out at $T = 285$ K showed only slow rotational motion (timescale of several 100 seconds) of a few molecules and allowed no sufficient statistics of time constants to characterize the motion. The values obtained in this study are despite the use of the same polymer quite different from the values in reference [4]. Since the glass transition is sensitive to the thermal polymer treatment and studies carried out on different samples treated by the same procedure give reproducible results, we suppose that a different thermal history might be the origin. In a few cases we observe clear changes in the rotational speed of the molecules as shown in figure 3 and reported i.e. in [4]. These changes are apparently connected to dynamic changes of the local environment of the dye and commonly regarded as dynamical heterogeneities. The characteristic time constant of the rotational motion is thus a function of time and the overall autocorrelation $C(\tau)$ of the fluorescence polarization will thus lead to a non-exponential behavior. In all cases we find that $C(\tau)$ is best fitted with a stretched exponential function with stretching exponents considerably below one, which reflects this dynamic heterogeneity. This holds even for the cases when the trajectory can be separated into different periods (such as Figure 3) and $C(\tau)$ can be calculated for these different periods. The obtained distributions of stretching exponents β are displayed in figure 4. Values of β start at about 0.3 and average values of $\langle \beta_{290K} \rangle = 0.64$ at $T = 290$ K and $\langle \beta_{295K} \rangle = 0.72$ at $T = 295$ K are found. Both distributions are similar, which suggests, that mainly the timescale of the motion but not the available spread of micro-environments has changed.

To further quantify the rotational motion in the polymer we try to calculate an additional time constant τ_x , which reflects the speed of the environmental fluctuations in the polymer. Since the autocorrelation $C(\tau)$ is non-exponential even for shorter fractions of fluorescence polarization time traces, the change in the rotational time constant must be rather continuous than discrete and cannot be simply identified directly from the single molecule trajectory. However, when regarding these changes of τ_c as a walk through a space of rotational time constants, molecules should sample an increasing part of this space with increasing observation time. To quantify τ_x we therefore calculate first a trajectory for the rotational time constants τ_c by shifting a time window of 400 time steps over each polarization trajectory and calculating a correlation time τ_c for each window position. An example for such a time trace of the correlation function is shown in Figure 5, calculated for the polarization time trace in Figure 3. Clearly, a drastic change in τ_c between 1400 s and 2000 s is found as expected from the polarization time trace. We then further calculate a histogram of time constants that occurred in the time constant trajectory until a certain time. The width of the histogram averaged over all molecular time constant trajectories as a function of the trajectory length gives then a measure of the time constant space explored in the experiment. As shown in

Figure 5 the explored time constant space increases with the trajectory length and then saturates after a certain time. Clearly this walk through the available rotational time constants is faster at higher temperature and slower than the rotational motion itself. By fitting with

$$W(t) = 1 - e^{-t/\tau_x} \quad (6)$$

we obtain an exchange time of $\tau_x = 13$ s for $T = 290$ K and $\tau_x = 2$ s for $T = 295$ K. The time constant of exchange defined in this way is thus apparently about one order of magnitude slower than the value of τ_c , which is consistent with recent experimental work [4].

If such a dynamical heterogeneity in the rotational motion exists, we suppose, that this dynamic heterogeneity should also show up in the translational diffusion of molecules at higher temperature. In a similar way this heterogeneity may cause a time dependence of the translational diffusion coefficient. The main questions to be asked are then how fast the fluctuations in the environment occur and if the translation is to some extent confined to cages of certain physical properties. Specially the latter one would correspond to a picture of domains which keep their properties for a certain lifetime, which we have quantified with the exchange time. To verify this, we heated our sample up to 373 K ($\approx 1.3T_g$) and measured the translational motion of dye molecules in the same polymer film. At each temperature about 150 trajectories were recorded and analyzed by calculating diffusion coefficients as described in section 3. Figure 6 shows the distributions of diffusion coefficients at three different temperatures ($T = 339$ K, $T = 360$ K and $T = 373$ K). The distribution of the diffusion coefficients gets clearly wider with increasing temperature and the mean diffusion coefficient shifts from $D_{339K} = 0.03 \cdot 10^{-8} \text{ cm}^2/\text{s}$ at $T = 339$ K ($\approx 1.2T_g$) to $D_{373K} = 0.35 \cdot 10^{-8} \text{ cm}^2/\text{s}$ at $T = 373$ K. Figure 6 shows all measured mean diffusion coefficients at different temperatures. The data can be well fitted by a modified Williams Landel Ferry equation [25,26], which we have shown for completeness as the dashed line in the Figure 6b. Since we are interested in dynamic changes in the diffusion process we skip a more detailed discussion of this fit within this paper. The identification of such changes from single molecule diffusion trajectories is difficult. Translational trajectories are usually short (20 to 200 frames) and are analyzed by calculating the MSD as a function of a lag time t_{lag} (see section 3). This calculation involves an average over all pairs in the trajectory, which have a certain temporal spacing t_{lag} . For large t_{lag} only few pairs are available from the trajectory and the error for the MSD increases with increasing t_{lag} . Thus the MSD is unsuitable for an analysis of dynamic changes in the diffusion coefficient. A way to unravel such changes is given by calculating the statistics of distances $\Delta x = x(t+t_{lag}) - x(t)$ a molecule has traveled within a time t_{lag} . According to diffusion theory [27]

this distance should be distributed by a Gaussian

$$p(\Delta x, t_{lag}) = p_0(t_{lag})e^{-\Delta x^2/\sigma(t_{lag})} \quad (7)$$

with the amplitude $p_0(t_{lag})$ and a width $\sigma(t_{lag})$ for a defined diffusion coefficient D . The width of the Gaussian is directly related to the diffusion coefficient by

$$\sigma(t_{lag}) = 4Dt_{lag} \quad (8)$$

If we consider one component of the position of the molecule in the plane (i.e. the x component), then the amplitude $p(t_{lag})$ is given by

$$p_{lag}(t_{lag}) = \frac{1}{\sqrt{4\pi Dt_{lag}}} \quad (9)$$

If the diffusion coefficient is now a function of time, the distribution $p(\Delta x, t)$ at a time t_{lag} will become a convolution of a gaussian with a distribution for the values of D that exist. Depending on the distribution of diffusion coefficients, the $p(\Delta x, t_{lag})$ will then deviate from a simple gaussian. We have done this type of analysis for all temperatures for six different lag time. An example of the results is shown in Figure 7a-c for $T = 339$ K. Apparently all three observables (distribution of distances $p(\Delta x, t_{lag})$, amplitude $p_0(t_{lag})$ and width $\sigma(t_{lag})$) fit nicely to the theoretical predictions mentioned above and show apparently no heterogeneity of the translational diffusion. There might be several reasons for this. First, the distribution of diffusion coefficients is a Gaussian distribution itself and therefore the convolution results in a Gaussian too. Since we do not know the exact type of distribution which is caused by the heterogeneities in the sample we cannot decide weather this is the reason. However, from the above consideration of the rotational motion, it is rather likely that the exchange between different environmental properties has become already to fast even at $T = 339$ K. In this case the diffusion constant changes rapidly within the exposure time of our measurements and therefore the experiments shows only the mean diffusion coefficient of the distribution.

5 Conclusions

We have measured the rotational and translational diffusion of individual dye molecules in a polymer matrix. The results show a rotational diffusion, which dynamically changes its characteristic time scale due to local fluctuations of the environment. The fluctuations in the environment occur on a timescale

which is about one order of magnitude slower than the timescale of the rotational diffusion. Translational diffusion measured between $1.2 T_g$ and $1.3 T_g$ shows no signs of heterogeneity. This suggests that the timescale of the local environmental fluctuations have already become faster than our time resolution of about 30 ms and we observe only averages of the translational diffusion constant.

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6 Figures

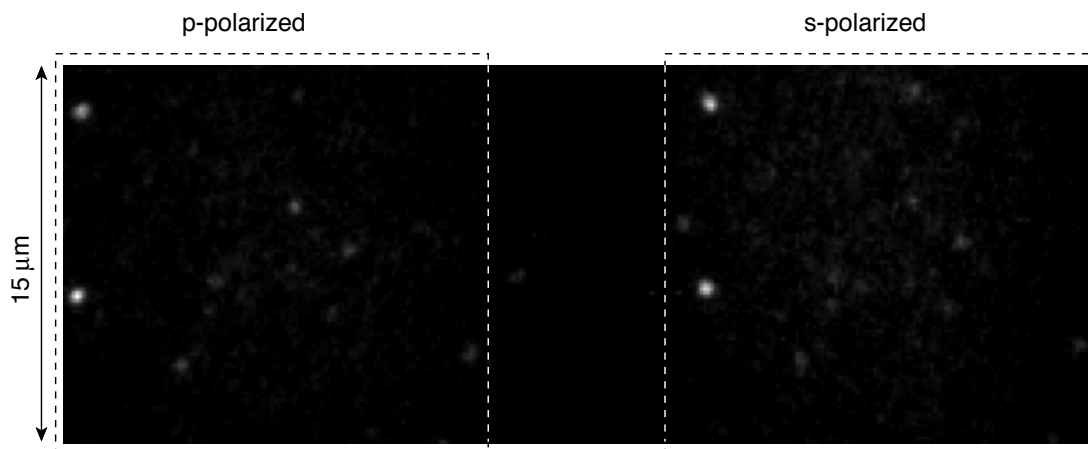


Fig. 1. Sample image of the single molecule rotation measurements with a wide field fluorescence microscope. The image is composed of a p- and s-polarized channel. Both parts are imaged at the same time. For the analysis of rotational diffusion, pairs of single molecule spots are localized and tracked throughout the image.

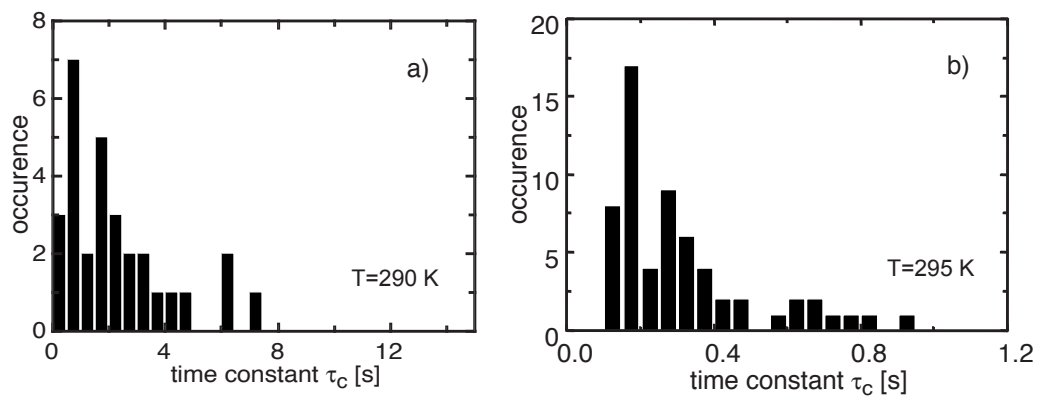


Fig. 2. Distribution of single molecule rotational correlation times at $T=290\text{K}$ (a) and $T=295\text{ K}$ (b). The mean correlation times are $\langle \tau_{290K} \rangle = 3.3\text{ s}$ and $\langle \tau_{295K} \rangle = 0.38\text{ s}$

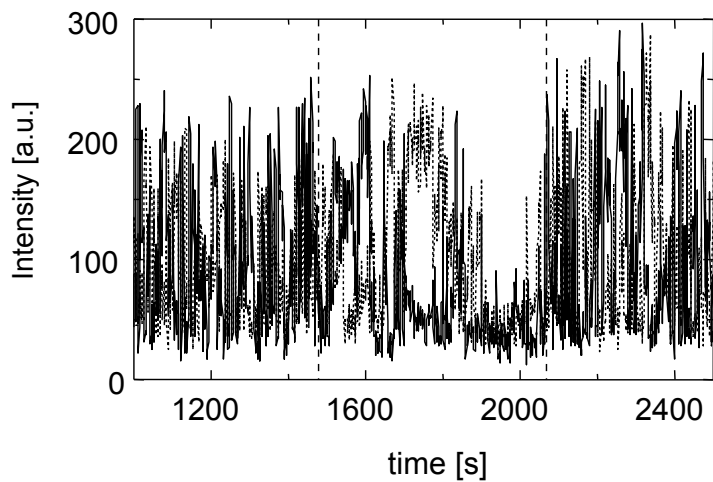


Fig. 3. Sample emission intensity trajectory of the s (solid) and p-polarization (dashed) channel of a single molecule at $T=290$ K. The intensity time trace clearly shows two drastic changes in the rotational speed of the molecule. The resulting three periods can be characterized by mean correlation times of $\tau_c^1 = 4$ s, $\tau_c^2 = 34$ s and $\tau_c^3 = 4.6$ s.

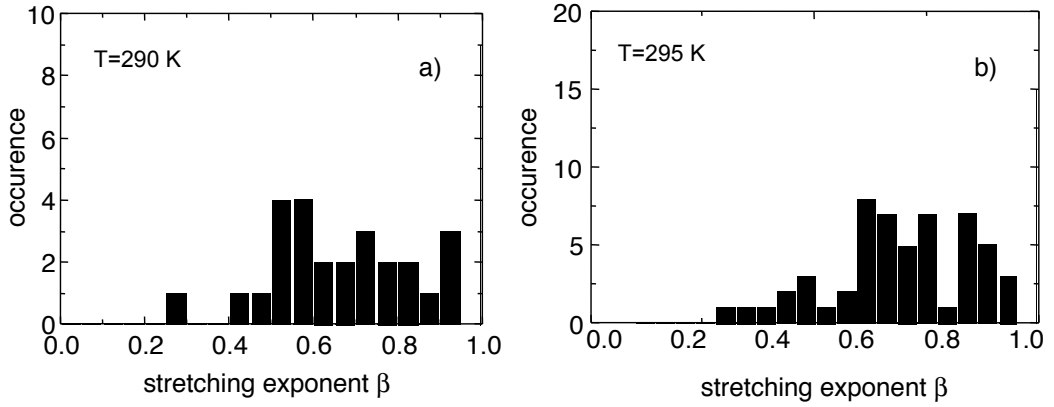


Fig. 4. Distribution of stretching exponents for $T = 290\text{ K}$ (a) and $T = 295\text{ K}$ (b) resulting from the fit of the fluorescence polarization autocorrelation function $C(\tau)$ with a stretched exponential function as given in eq. 3.

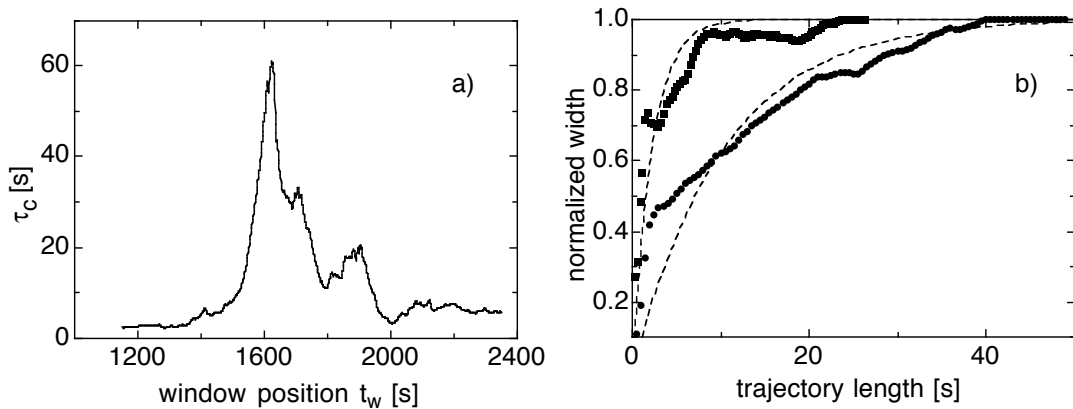


Fig. 5. (a) Example of the time dependence of the rotational correlation time τ_c for the single molecule time trace of Figure 3. (b) Normalized width of the rotational time constant histogram of individual τ_c traces of all measured molecules as a function of the length of the time trace (see text for further details on the calculation).

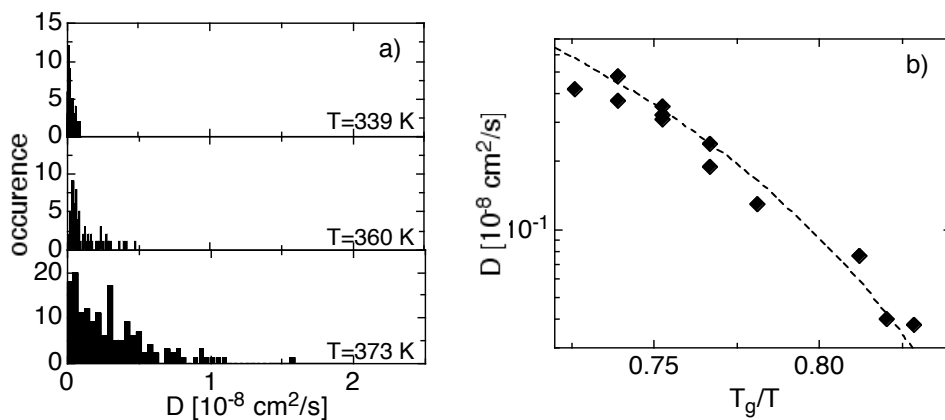


Fig. 6. (a) Distribution of diffusion coefficients obtained for the temperatures $T = 339$ K, $T = 360$ K and $T = 373$ K. The mean diffusion coefficients are $D_{339K} = 0.03 \cdot 10^{-8} \text{cm}^2/\text{s}$, $D_{360K} = 0.13 \cdot 10^{-8} \text{cm}^2/\text{s}$ and $D_{373K} = 0.35 \cdot 10^{-8} \text{cm}^2/\text{s}$ (b) Mean diffusion coefficients for all measurements plotted over T_g/T ($T_g=281$ K) (b). The dashed line corresponds to a fit with a modified Williams-Landel-Ferry equation as described i.e. in [25,26]

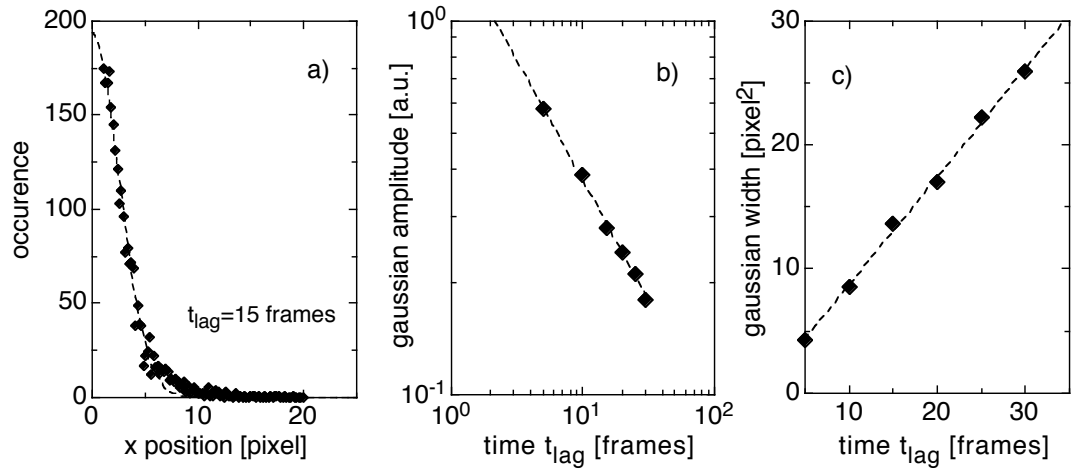


Fig. 7. (a) Distribution of distances $x(t + t_{lag}) - x(t)$ of translational diffusion for a given time lag $t_{lag} = 1.5$ s. The dashed line corresponds to a fit with a gaussian corresponding to equation 7. (b) Amplitude of the gaussian fit as a function of the lag time t_{lag} . The dashed line corresponds to a fit with a power law $t^{-\alpha}$ with $\alpha = 0.57$. (c) Width of the gaussian fit as a function of t_{lag} . The dashed line is the result of a linear regression.