Towards Elucidating the Role of the Probe in Single Molecule Experiments in Supercooled Liquids[1]

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Summary

We present experimental and simulation results that aim to elucidate the information content of single molecule experiments in supercooled liquids. Experiments interrogating the dynamics of the probe Rhodamine 6G in the fragile glass former ortho-terphenyl show that this probe is capable of reporting on at least a subset of the heterogeneities present in this supercooled liquid. Simulations reveal a complicated picture in which large probes induce changes in the heterogeneous dynamics of supercooled systems, setting up persistent heterogeneities that would not otherwise exist. Moreover, the temperature dependence of the diffusion of such probes in simple supercooled liquids tracks that of the rest of the system even though the probe alters the heterogeneities in the system. Finally, we suggest that probe bearing single molecule experiments can interrogate the length scale of heterogeneity in supercooled liquids even when those probes are responsible for altering the dynamics of the system.

1. Introduction

We assess the information content of probe bearing single molecule (SM) experiments in supercooled liquids through experiments and molecular dynamics (MD) simulations. Previous SM experiments investigated the fluorophore Rhodamine 6G (R6G) in polymeric systems and ortho-terphenyl (OTP) just above their glass transition temperatures (T_g). Results of these experiments strongly implied the presence of spatial and dynamic heterogeneities in such systems [1, 2]. While these experiments directly measured heterogeneities in supercooled systems, they also raised new questions, including why the rotational relaxation times of the measured probes were many orders of magnitude longer than those of the host molecules themselves as measured in bulk probeless experiments. This question was addressed through a series of probe-bearing and probeless experiments that showed that the temperature dependences of the probe rotational relaxation and host α-relaxation times were the same. This result was taken to show that the probe does interrogate and report on the heterogeneous dynamics of interest, those of the host [3]. Several of these initial exciting SM experiments in supercooled systems have now been retracted [4,5]. This highlights the need for new SM experiments in supercooled systems. We have undertaken such experiments with an eye towards elucidating the role of the probe in, and thus the information content of, these experiments. We have performed complementary molecular dynamics (MD) simulations to address these same issues.

2. Experimental Details

Single molecule experiments are performed by collecting epi-fluorescence from dilute single molecule probes in supercooled systems. Here, we only discuss

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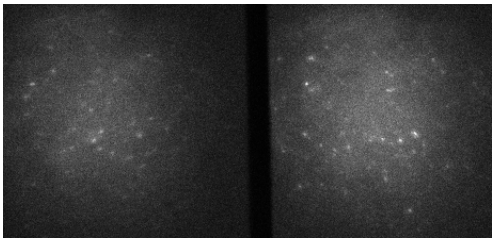

Fig.1 Raw data of two images of orthogonally polarized fluorescence of R6G molecules in OTP. Images are collected simultaneously on one CCD camera. The temperature is 248K, $T_g + 5$K. Integrated time is 400 ms. Note that some R6G molecules are evident in both channels, while some are evident in only one.

experiments on R6G in OTP. OTP was obtained from Sigma-Aldrich and was further purified by distillation and recrystallization. R6G was added at concentrations of 10^{-9}M. Samples were prepared by placing 0.2μl of liquid OTP on a cleaned silicon wafer. The OTP was then covered with a cleaned 0.5mm thick quartz coverslip. Final OTP film thicknesses between the silicon substrate and the quartz coverslip varied between 5-20μm, with most samples $\sim$10μm.

The sample was mounted inside a cryostat (Janis ST-500-LN) and imaged under vacuum with a high numerical aperture, long working distance air objective (Zeiss Neofluor, NA=0.75). Excitation was provided by the 488nm line of an Argon ion laser. Excitation light was circularly polarized. At the sample, the intensity of the 488nm light was $\sim$10kW/cm^2. Fluorescence was passed through a dichroic filter, a longpass filter, and a bandpass filter centered on the R6G emission (Chroma). The fluorescence is split into orthogonal polarizations with a Wollaston prism (Karl Lambrecht), and both polarizations are focused onto an EMCCD camera chip (Andor Technology) (Figure 1). Data were collected at 9 frames/s. Background autofluorescence was subtracted from the images, and total intensities in each polarization were determined for each molecule as a function of time. These intensities were used to calculate the linear dichroism, $A(t)$, a measure of the projection of the transition dipole into the XY plane:

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

Autocorrelation functions, $C(t)$, were then calculated for the linear dichroism of each molecule as a function of time to give information about the rotational dynamics of the R6G molecules.

$$C(t) = \frac{\sum_\tau (A(\tau + t) - \bar{A})(A(\tau) - \bar{A})}{\sum_\tau (A(\tau) - \bar{A})^2} \qquad (2)$$

3. Simulation Details

The 3D system modeled consists of a probe surrounded by a supercooled liquid. The model for the supercooled liquid is a binary mixture with a 4:1 ratio of A and B particles: the Kob Andersen (KA) mixture [6-8]. The particles interact via a Lennard Jones (LJ) potential, $V_{\alpha\beta}(r) = 4\varepsilon_{\alpha\beta}[(\sigma_{\alpha\beta}/r)^{12} - (\sigma_{\alpha\beta}/r)^6]$, with $\sigma_{AA} = 1$ and $\varepsilon_{AA} = 1$ for interactions between the A particles and $\sigma_{BB} = 0.88\sigma_{AA}$ and $\varepsilon_{BB} = 0.5\varepsilon_{AA}$ for interactions between the B particles. The LJ parameters between the A and B particles, $\varepsilon_{AB} = 1.5\varepsilon_{AA}$ and $\sigma_{AB} = 0.80\sigma_{AA}$, ensure the system does not crystallize. The masses of the mixture particles were taken to be $m_A = m_B = 1.0$. The simulations were carried out, and the results were reported, in terms of the reduced variables $r^* = r/\sigma_{AA}$, $m^* = m/m_A$, $T^* = k_BT/\varepsilon_{AA}$, $t^* = t(k_BT/m_A\sigma_{AA}^2)^{1/2}$.

Both smooth and rough probes have been investigated [9], though here we concentrate only on smooth probes. The smooth probe is represented by a single particle with LJ parameters $\varepsilon_P = 1.0$ and different values of σ_P. A set of seven simulations with $\sigma_P = 1.0$, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 were performed to model probes with different sizes but the same mass, $m_P = 4.0$. Since the value of σ_P was as large as 7.0, the interparticle potentials and the corresponding forces in the range of $4.0 \leq r^* \leq 5.0$ were interpolated from their LJ values at $r^* = 4.0$ to zero at $r^* = 5.0$ by a third degree polynomial. The same number of mixture particles are taken, $N_A = 2400$. $N_B = 600$, and $N_P = 1$, for the simulations with different σ_P; however, the volume of the cubic simulation box was adjusted so that the measured pressure was similar. The resulting reduced density, $\rho^* = N/V^*$, is $\rho^* = 1.188$ at $T^* = 0.48$ for the system with $\sigma_P = 1$. More details are presented in Reference 9. The LJ parameters of the probe with the mixture particles were calculated from the geometric combination rule of the parameters associated with the mixture particles $(\sigma_{AA}, \sigma_{BB}, \varepsilon_{AA}, \varepsilon_{BB})$ and the LJ parameters of the probe.

The MD simulations were performed using the GROMACS package version 3.2.1 [10]. For each simulation the system was equilibrated for at least 10^7 MD

steps. The simulations were performed in the micro-canonical ensemble (N, V, E), except for the preparation stage where the Berendsen thermostat was applied to obtain the desired temperature of $T^* = 0.48$ [11]. The data collection step consisted of short and long simulations. The short simulations were run for 1.6 x 10^5 MD steps with time step $\Delta t^* = 0.0025$ where the trajectory was saved every 8 MD steps. The long simurations were run for 10^7 MD steps with $\Delta t^* = 0.01$, and the trajectory was saved every 500 MD steps.

4. Results and Discussion

4.1 Experimental Results

We endeavor to interrogate a variety of SM probes in a single small molecule glass former and to interrogate a single SM probe in a variety of small molecule glass formers. These sets of experiments will clarify the information content of SM experiments in supercooled systems. However, thus far only R6G in OTP has been investigated, and that system will be discussed here.

Figure 1 shows raw data for R6G in OTP. Tracing the intensities of each visualized molecule over time allows calculation of the autocorrelation, $C(t)$, of the linear dichroism, $A(t)$. While we are still working to increase signal to noise in our measurements and collect longer trajectories, preliminary results do indicate that R6G samples spatial heterogeneities in supercooled OTP. The results show that multiple individual R6G molecules in OTP exhibit different dynamics simultaneously (Figure 2). However, at this time we have not observed any individual R6G molecules that appreciably change their dynamics over time. Such results would be expected if R6G molecules probe dynamic heterogeneity in OTP. Such behavior may be observed when longer trajectories are collected. Collecting more trajectories demonstrating the first (and potentially second) behavior described will allow characterization of the breadth (and potentially the lifetime) of the heterogeneities that R6G samples in OTP. Such measurements will first be completed for R6G in OTP and then for a variety of other probe/host systems.

4.2 Simulation Results

Complementary MD simulations have been undertaken to examine the role of probes of different size in simple supercooled systems [9]. Examining 2D projections of 3D trajectories of spheres comprising a supercooled probe-bearing KA system reveals that particles in the first several shells around a large probe exhibit behavior dissimilar from particles far from the probe or in systems lacking a large probe. Recently, particle Debye-Waller factors,

$$DW_i = \left\langle \left\langle (\boldsymbol{r}_i(t) - \langle \boldsymbol{r}_i \rangle_\tau)^2 \right\rangle_\tau \right\rangle, \qquad (3)$$

interrogating short time dynamics were shown to well predict a particle's long time dynamics in supercooled systems [12]. Calculating "shell" Debye-Waller factors,

$$DW(\boldsymbol{r}) = \left\langle DW_i \cdot \delta(\boldsymbol{r} - (\boldsymbol{r}_i - \boldsymbol{r}_{probe})) \right\rangle, \qquad (4)$$

in the caged, or β-regime for particles in the presence of a large probe confirms that the dynamics of particles surrounding a large smooth probe are accelerated relative to particles far from the probe or in probeless systems (Figure 3) [9].

In addition to investigating the dynamics of the host particles in a supercooled probe-bearing system, we also investigate probe dynamics via simulation, as these are the only dynamics that can be directly probed by SM experiments. In particular, previous experimental work had suggested that if the probe and host exhibit the same rotational and α-relaxation temperature dependences, respectively, then the probe and host behavior must be reflecting the same underlying dynamics [3]. Thus, we have examined the temperature dependence of probe diffusion in systems with two different probes and host diffusion in a probeless system. The probes investigated have $\sigma_p = 2$ and

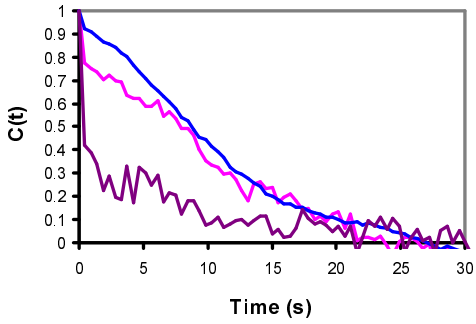

Fig.2 Autocorrelation functions describing rotational relaxation as derived from simultaneous linear dichroism measurements of three R6G molecules in OTP at $T_g + 5$K. The three molecules behave differently, although only the fluorophore represented by the blue trace is long lived enough to lead to a smooth $C(t)$.

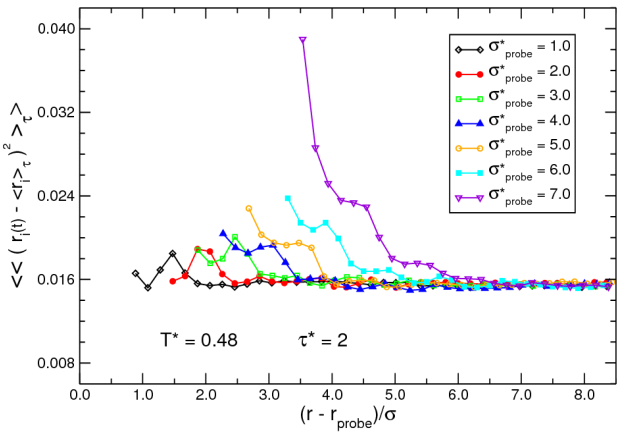

Fig.3 Shell Debye-Waller factors ($DW(\boldsymbol{r})$) for smooth probes from $\sigma_P = 1.0 - 7.0$ at $T^* = 0.48$ and $\tau^* = 2$. Adapted from Reference 9.

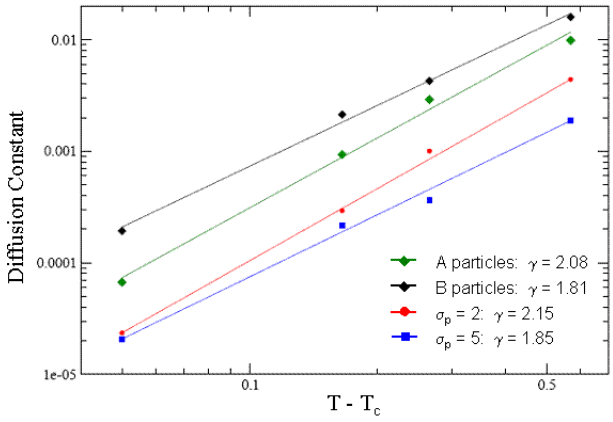

Fig.4 Temperature dependence of the diffusion of the A (green) and B (black) particles in a probeless KA mixture and of a $\sigma_p = 2$ probe (red) and a $\sigma_P = 5$ probe (blue) in KA systems at the same temperature and density.

$\sigma_p = 5$, the latter of which is large enough to significantly change the environment surrounding the probe (see Fig. 3). Figure 4 shows the diffusion constant at four temperatures in the supercooled regime for the A and B particles in a probeless KA system and for $\sigma_p = 2$ and $\sigma_p = 5$ probes in a KA system at the same temperature and density. It is found that the temperature dependence of the diffusion of probes, as ascertained by a fit to $D = A(T - T_C)^\gamma$ with $T_C = 0.435$ [7], yields $\gamma = 2.08$ for the A particles, $\gamma = 1.81$ for the B particles, $\gamma = 2.15$ for the $\sigma_p = 2$ probe, and $\gamma = 1.85$ for the $\sigma_p = 5$ probe. Clearly, the difference between the exponent for the probes relative to the A particles in the probeless system is smaller than is the difference between the exponents for the A and B particles comprising the probeless system. This result, together with the result depicted in Figure 3, shows that even when the temperature dependence of the diffusion of a probe tracks that of a probeless system of the same type, the probe does not necessarily sample the heterogeneous dynamics of the host system as they would exist in the absence of a probe.

Finally, we have begun simulations that will clarify how probe bearing SM experiments report on a length scale of heterogeneity in supercooled systems even if the probes alter the heterogeneities in the system to a certain extent. To do so, we are exploring how the Stokes Einstein (SE) relation,

$$D\eta = \frac{k_B T}{c\pi a}, \tag{5}$$

breaks down in probe-bearing systems as a function of probe size. It is known from both experiment and simulations that the zero frequency limit of the SE relation does not generally hold in supercooled systems [13-17], although it is true that mode coupling theory does not predict the breakdown of the SE relation in such systems [18].

A thought experiment suggests that probes significantly smaller than the particles that comprise the rest of a supercooled system can not report on heterogeneities because the probe will not experience caging. Thus, if the diffusion constant of such a probe is used as the diffusion constant in the SE equation, no SE breakdown will be apparent. Similarly, if a probe much larger than the rest of the particles, and larger than the size of the heterogeneities in the system, is employed, this probe will average over the heterogeneities and again not report on them. A probe of intermediate size should sample at least a subset of heterogeneities in the system. Exploring (both experimentally and in simulations) the breadth of heterogeneities sampled as a function of probe size, taking into account that probes of certain size will alter the heterogeneities in the system, will allow determination of a length scale of heterogeneity in supercooled systems. Here, we employ simulations to explore both this length scale and the lifetimes of heterogeneities in the system simultaneously, by interrogating the *generalized* (frequency-dependent) SE relation,

$$D(\omega)\eta(\omega) = \frac{k_B T}{c\pi a}, \tag{6}$$

as a function of probe size. For these simulations, D(ω) and a will be values of the probe.

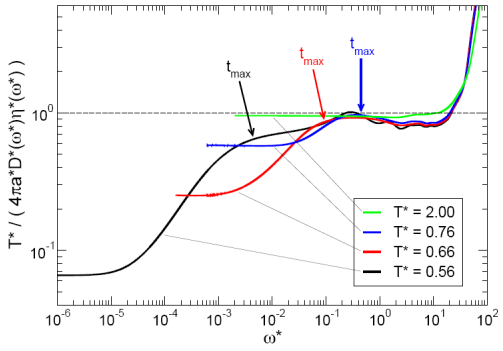

Fig.5 Deviations of the frequency dependent SE relation. The value of the expression $T^*/(4\pi a^* D^*(\omega)\eta^*(\omega^*))$ is plotted as a function of frequency. Adapted from Reference 19.

As a preliminary simulation, we have examined the generalized SE relation for a probeless supercooled KA system, which could also be thought of as a probe-bearing system with $\sigma_P = 1$ [19]. Figure 5 shows that such a system does not exhibit SE breakdown in the β-regime. Instead, SE breakdown is only apparent at the smaller frequencies, or the longer times, associated with α-relaxation. Interestingly, the breakdown is maximized at the lowest frequencies probed even though behavior in that time regime is stochastic.

In conclusion, preliminary SM experiments show that the relatively large probe R6G embedded in supercooled OTP can sample at least a subset of the environments in OTP. Because simulations suggest that large probes embedded in supercooled samples may set up non-representative environments that are persistent and that the probe preferentially samples, it remains unclear whether R6G can explore the full breadth of heterogeneities in OTP. Simulations suggest that monitoring the temperature dependence of the rotational relaxation of R6G molecules relative to the α-relaxation of OTP is not a good measure of R6G's ability to report on heterogeneities in OTP. Simulations exploring how the size of a probe in such a supercooled system may reveal a length scale associated with heterogeneity even when the probe alters the heterogeneities in the system will guide us in designing SM experiments that will reveal the lifetimes and breadth of heterogeneities in supercooled molecular systems.

Acknowledgments

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