Revealing and resolving degeneracies in stretching exponents in temporally heterogeneous environments

Kevin Stokely, Alyssa S. Manz, and Laura J. Kaufman

Department of Chemistry, Columbia University, New York, New York 10027, USA

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Supercooled liquids are proposed to be dynamically heterogeneous, with regions exhibiting relaxation time scales that vary in space and time. Measurement of the distribution of such time scales could be an important test of various proposed theories of vitrification. Single molecule fluorescence experiments attempt to uncover this distribution, typically by embedding single molecule probes into these systems and monitoring their individual rotational relaxations from a computed autocorrelation function (ACF). These ACFs may exhibit stretched exponential decays, with the value of the stretching exponent assumed to report the set of dynamical environments explored by the probe. Here, we use simulated trajectories of rotation to investigate how the time scale of dynamic exchange relative to underlying relaxation time scales in the system affects probe ability to report the distribution of relaxation time scales present. We find that dynamically heterogeneous regions must persist for approximately 50 times the median relaxation time scale for a single molecule to accurately report the full distribution of time scales it has experienced. In systems with faster dynamic exchange, single molecule ACFs average over successive environments, limiting the reported heterogeneity of the system. This leads to degeneracies in stretching exponent for systems with different underlying relaxation time distributions. We show that monitoring single molecule median stretching exponent as a function of trajectory length or simultaneously measuring median stretching exponent and measured relaxation time distribution at a given trajectory length can resolve these degeneracies, revealing the underlying set of relaxation times as well as median exchange time.

I. INTRODUCTION

Despite over eighty years of research, a full quantitative theory of the glass transition remains lacking. In order for potential descriptions of glassy behavior to be compared and ultimately validated, they must make predictions of experimentally accessible quantities. One such candidate is the distribution of relaxation time scales displayed by a system approaching the glass transition.

The distribution of these time scales has been measured through a wide variety of approaches in both the frequency and time domain. Ensemble measurements of supercooled liquids in the time domain consistently show stretched exponential relaxations. A widely accepted explanation for such relaxations is that, though over a given time period each individual molecule exhibits exponential relaxation due to local Brownian motion, there exists a broad distribution of such relaxation time scales. This distribution of time scales reflects an underlying distribution of local environments that may vary not only as a function of position within the system (spatial heterogeneity) but also as a function of time (temporal heterogeneity).

In the presence of spatial heterogeneity, experimental measurements must be highly local to ensure a single environment is probed. Single molecule (SM) experiments represent the ultimate local measurements, and in recent years significant experimental effort has been devoted to SM measurements of the distribution and character of relaxations in supercooled liquids. An ideal SM experiment—one in which the probe molecules are identical in size and average relaxation time scale to the host molecules of the supercooled liquid—might record the exponential relaxation of a large number of individual probes, from which the full distribution of time scales may be ascertained. This requires, however, that a probe persists in a region of distinct dynamics sufficiently long to accurately report the time scale of that environment. If the SM probe does not persist in a given environment for the required time—due to either dynamic exchange into a different environment or due to probe photobleaching—the SM probe may demonstrate non-exponential relaxations that potentially obscure the true underlying time scales.

Here, we aim to understand the microscopic scenarios that are consistent with the measurement of a given stretched exponential decay of a SM probe experiencing different dynamic environments due to temporal heterogeneity. Distributions of relaxation time scales and exchange times as well as correlations between these time scales are varied, as is trajectory length. These scenarios are investigated by simulating the orientation of a SM probe undergoing Brownian motion through a succession of local environments and calculating rotational correlation functions analogous to those measured in experiments. While it is found that there is no unique set of underlying time scales and exchange times that yield a given

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1Author to whom correspondence should be addressed. Electronic mail: kaufman@chem.columbia.edu

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stretched exponential relaxation, monitoring the behavior of stretching exponents and probe-reported relaxation time distributions can resolve degeneracies in stretching exponents, revealing average exchange time relative to average host relaxation time.

II. SIMULATION METHODS

The rotation of a SM probe is modeled as a diffusive process in which the local environment sets the probe’s rotational diffusion constant $D_r$. At each simulation step, a unit vector $\phi$ in $\mathbb{R}^3$ is rotated through an angle $\delta$ chosen from a Rayleigh distribution of width $\sqrt{2D_r}$. The rank-$l$ rotational autocorrelation function (ACF) is given by

$$C_l(t) = \langle P_l(\hat{\phi}(t_0) \cdot \hat{\phi}(t_0 + t)) \rangle_0,$$  \hspace{1cm} (1)

where $P_l(x)$ is the rank-$l$ Legendre polynomial, and the average is performed over the trajectory. Within an environment characterized by $D_r$, for isotropic probes undergoing homogeneous rotational diffusion through small angular displacements, all ranks exhibit exponential relaxation,

$$C_l(t) = e^{-t(l+1)D_r} \equiv e^{-t/\tau_l},$$  \hspace{1cm} (2)

where $\tau_l \equiv (l(l + 1)D_r)^{-1}$ is the relaxation time scale of the local environment. SM probes may experience relaxations that are non-exponential not only because they are typically anisotropic but also because most SM experiments monitoring rotation measure linear dichroism, which has components from all even rank rotational correlation functions. In practice, linear dichroism measurements are strongly dominated by $C_l(t)$ and effects due to anisotropy are small. As such, in this study, we compute and discuss $\tau_r \equiv \tau_2$.

We assume the system comprises a broad distribution of local environments and always choose $\tau_r$ from a log-normal distribution with median 100 steps. The full-width-at-half-maximum (FWHM) of the log($\tau_r$) distribution is varied between 0.2 and 2.2. Because dynamic exchange may be a sudden process, initially, we model temporal heterogeneity via abrupt random exchange: after a given exchange time, $\tau_x$, the molecule is assumed to switch local environments, trading the current value of $\tau_r$ for a new value from the same distribution. Exchange times, $\tau_x$, are either held constant throughout the simulation or are chosen from a log-normal distribution with FWHM of the log($\tau_x$) distribution equal to 1.0. Previous studies modeled similar environmental exchanges and their connection to reports of dynamic heterogeneity. Our approach emphasizes the persistence of local environments, allows the study of correlations between local environments and dynamic exchange, and assesses the importance of trajectory length in probe reports of dynamic heterogeneity.

For each combination of parameters studied, both very long and experimentally realistic trajectory length simulations were performed and analyzed. For very long (effectively infinite) trajectories, 30-100 simulations of $10^7$ steps were performed. For experimentally realistic trajectories, 500-1500 simulations of $10^6$ steps were performed. Each was then cut to the appropriate number of steps prior to fitting for trajectory length analysis. In all cases, ACFs were computed and fit until they decayed to a value of 0.02, with the resulting fit parameters averaged over simulations. In the presence of heterogeneity, relaxation is non-exponential, instead found to be well fit by a stretched exponential form,

$$C_l(t) = Ae^{-(t/\tau_x)} \beta_l \cdot \beta_0$$  \hspace{1cm} (3)

Pure exponential relaxation is recovered for $\beta_l = 1$, and stretching exponents $\beta_l < 1$ may be regarded as quantifying the deviation from this homogeneous behavior. Consistent with our choice of $\tau_r \equiv \tau_x$, we focus on stretching exponent $\beta \equiv \beta_2$.

III. RESULTS AND DISCUSSION

A. Infinite trajectories

We first investigate the simplest case, that of a SM probe experiencing a succession of different environments with a median relaxation time, $\tau_r$, of 100 steps and FWHM of the log($\tau_r$) distribution of 1.0. The blue points in Fig. 1(a) show how the reported stretching exponent $\beta$ varies as a function of
a fixed exchange time, $\tau_x$. For short exchange times ($\tau_x \to 1$), $\beta$ approaches unity, indicating a lack of reported heterogeneity. Here, $\tau_x$ is at least an order of magnitude less than $\tau_r$, and the local environment shifts before significantly affecting relaxation of the probe. The probe instead reports only an average local environment throughout the entire simulation, and relaxation of the probe is found to be purely exponential. This is similar to the case of a liquid at high temperature, in which fluctuations are both localized and short-lived.

For long exchange times ($\tau_x \to \infty$), $\beta$ plateaus to a constant value. Here, each environment is explored fully, with the probe completely decoupling from its original orientation before exchanging environments. In this case, the ACF of the probe is given by an average over the purely exponential relaxations experienced in each local environment. Such a correlation function may be calculated numerically for any given $\tau_r$ distribution as

$$C(t) = \int p(\tau_r) e^{-t/\tau_r} d\tau_r. \quad (4)$$

This may be fit to a stretched exponential form, with the resulting stretching exponent, denoted $\beta^{\tau_x=\infty}$, providing a lower limit to values of $\beta$ with temporal heterogeneity present. Note that the situation $\tau_x \to \infty$ is identical to that of an ensemble measurement of many probes, each within a distinct local environment, but without temporal heterogeneity, i.e., with spatial heterogeneity only. The value of $\beta^{\tau_x=\infty}$ depends strongly on the width of the $\tau_x$ distribution, with a wider distribution leading to a lower $\beta^{\tau_x=\infty}$ value. When the time scales across environments are more similar, the average of the pure exponential relaxations experienced will itself be closer to a pure exponential; hence, the full correlation function will demonstrate $\beta$ closer to unity. The horizontal dashed line in Fig. 1(a) represents $\beta^{\tau_x=\infty}$ for the given $\tau_r$ distribution.

For $\tau_x$ comparable to the median rotational time scale, an intermediate case is observed. Slow local environments are averaged over while faster environments are fully reported. The measured correlation function is no longer equivalent to an average of the exponential relaxations of each environment, as the probe does not always have time to fully relax before exchange. Due to this partial averaging over local environments, reporting of the full distribution of local environments is suppressed. Thus, although it is possible for a given $\tau_r$ distribution to lead to a value of $\beta$ as low as $\beta^{\tau_x=\infty}$, in the presence of temporal heterogeneity, measured values of $\beta$ are typically higher. This is perhaps a counterintuitive result, as more (temporal) heterogeneity in the system leads to a seemingly less heterogeneous system.

The red points in Fig. 1(a) again show the effect of the median exchange time on $\beta$, now for a distribution of exchange times, with a FWHM of the log($\tau_x$) distribution of 1.0. The overall shape of the curve is similar to the case of constant exchange. In this case, however, due to the distribution of exchange times, some slower environments will be fully explored. This leads to lower values of $\beta$, an increase in the reported heterogeneity, over a range of $\tau_x^{med}$. At short $\tau_x^{med}$, the deviation of $\beta$ from unity now occurs at shorter median exchange time. The inset in Fig. 1(a) explores the effect of the width of the log($\tau_x$) distribution for the case $\tau_x^{med} = \tau_r^{med} = 100$ steps (vertical dashed line in main panel). A wider distribution of exchange times leads to an increase in the apparent heterogeneity on that time scale. It is also evident from the main panel of Fig. 1(a) that at long $\tau_x^{med}$, $\beta$ reaches $\beta^{\tau_x=\infty}$ at approximately the same median exchange time value as in the case of constant exchange. Indeed, we find that regardless of the width of the exchange time distribution, the median exchange time governs whether a SM probe can report the full heterogeneity of a system. We find that the exchange time must be approximately 50 times the median relaxation time scale to return a stretching exponent that is consistent with the full width of the underlying $\tau_x$ distribution.

The results shown in Fig. 1(a) hold qualitatively when varying the width of the underlying $\tau_x$ distribution, though each individual curve plateaus at a different value of $\beta^{\tau_x=\infty}$. We define $\beta^* = (\beta - \beta^{\tau_x=\infty}) / (1 - \beta^{\tau_x=\infty})$, for which $0 \leq \beta^* \leq 1$ for any distribution of $\tau_r$. Fig. 1(b) plots $\beta^*$ as a function of constant $\tau_x$ for log normal distributions of relaxation times with different widths. At short exchange times, a wider underlying distribution leads to lower values of $\beta^*$. However, $\beta^*$ reaches 0 at approximately the same $\tau_x$ for all $\tau_r$ distributions. This reinforces that regardless of the breadth of actual relaxation time scales in the system, it is the median exchange time that governs whether a SM probe can fully report that distribution.

In sum, Fig. 1 shows that a SM probe that cannot fully relax within each environment is unable to report the full breadth of variation in time scales among those environments. The degree to which a probe fails to do so depends on the median and FWHM of the exchange time distribution. If the median exchange time of the system is less than ~50 times the median relaxation time scale, some degree of environmental averaging will occur.

The results presented thus far refer only to cases in which the full distribution of $\tau_x$ and $\tau_r$ is accessed in an unbiased manner. It is also possible that various correlations within or between these times exist. For example, the time a molecule spends in a given environment may depend on the characteristic time scale of that environment, i.e., a molecule may spend a longer time in an environment in which it finds it difficult to rotate. To capture this effect, we introduce correlations between the time scale of a given environment ($\tau_x$) and the time spent in that environment ($\tau_r$), defining the correlation between these two variables by $\rho_x$. Here, the correlation between any two variables $A$ and $B$ is defined as

$$\rho = \frac{\langle (A - \langle A \rangle)(B - \langle B \rangle) \rangle}{\sigma_A \sigma_B}, \quad (5)$$

where $\langle \cdot \rangle$ denotes a time average and $\sigma$ denotes the standard deviation. Hence for $\rho_x = 0$, $\tau_x$ and $\tau_r$ are completely independent (as in the above results) while $\rho_x = 1$ implies full correlation, such that when $\tau_x$ deviates from its mean by a given fraction of its standard deviation, $\tau_r$ is guaranteed to do the same. Simulations are performed such that each simultaneous choice of $\tau_x$ and $\tau_r$ has the same correlation $\rho_x$.

Fig. 2(a) shows how $\beta$ depends on log($\tau_x^{med}$) for several values of $\rho_x$, with FWHM of the log($\tau_x$) and log($\tau_r$) distributions equal to 1.0. The inset depicts how $\beta$ changes with $\rho_x$ for the case $\tau_x^{med} = \tau_r^{med} = 100$ steps (vertical dashed line in main panel). An increase in correlation leads to a higher value of $\beta$. 


i.e., a decrease in the observed heterogeneity. To understand this, we imagine four possible cases: (i) short $\tau_r$ with short $\tau_x$, (ii) short $\tau_r$ with long $\tau_x$, (iii) long $\tau_r$ with short $\tau_x$, and (iv) long $\tau_r$ with long $\tau_x$. With perfect correlation, only cases (i) and (iv) are present, and the probe decouples from its original orientation by the same amount within each local environment, irrespective of $\tau_r$. The degree of decoupling, the degree of averaging over local environments, and the resultant observed value of $\beta$ all depend on the particular relationship between $\tau_r$ and $\tau_x$. If the amount of correlation between $\tau_r$ and $\tau_x$ then decreases, cases (ii) and (iii) also manifest. In case (ii), the probe completely decouples from its original orientation before exchange, and the time scales $\tau_r$ associated with the environments sampled will be reported, pushing $\beta$ down relative to the fully correlated case. On the other hand, in case (iii), the probe does not depart substantially from its original orientation before exchange and the values of $\tau_x$ associated with the original environments will not be reported, pushing $\beta$ up relative to the fully correlated case. Given the large $\tau_x$ values in case (ii), we expect this case to weigh more heavily in calculation of the correlation function of a probe over its entire trajectory. This is consistent with the observation that as correlation $\rho_x$ decreases, $\beta$ decreases. It is also observed that at long exchange times, $\beta$ does not reach its lowest possible value, $\beta_{\kappa \rightarrow \infty}$. Even with very long exchange times in the system, these are preferentially attached to the slower environments, and the probe is not able to fully relax within every environment.

It is also possible that the variation of the local environment around a given molecule is not random or abrupt. This may be captured by correlation between successive environments, in which the characteristic relaxation time scale of a given local environment bears similarity to that of the previous environment. We characterize the correlation between the current and previous values of $\tau_x$ via $\rho_r$, defined analogously to $\rho_x$. Fig. 2 shows $\beta$ as a function of (b) $\log(\tau_x)$ or (c) $\log(\tau_x)$ for various values of $\rho_r$, for FWHM of the $\log(\tau_x)$ distribution equal to (b) 0.0 and (c) 1.0. Insets depict how $\beta$ changes with $\rho_r$ for the case $\tau_{\text{med}} = \tau_{\text{med}}^{\kappa \rightarrow \infty} = 100$ steps (vertical dashed line in main panel). In both cases, an increase in $\rho_r$ manifests in a clear decrease in $\beta$, i.e., an increase in the reported heterogeneity. Because the environment around a probe evolves more gradually, probes without adequate time to fully relax within a given local environment may continue to relax within a similar local environment, effectively increasing the exchange time and the ability to report that time scale. This effect is slightly less pronounced for a distribution of exchange times than for constant exchange. Correlations between successive $\tau_r$ have a larger effect on $\beta$ than correlations between $\tau_r$ and $\tau_x$. This is likely due to the presence of higher order correlations in the former case, as any correlation between the current and next values of $\tau_r$ also implies a correlation between the current and all future values of $\tau_r$. Though this correlation falls off exponentially in the number of exchanges, the effect of these further correlations is clear.

For infinite trajectories, we thus find that given a system with a distribution of relaxation time scales, an ideal SM probe can report relaxation with widely varying degrees of non-exponentiality depending on the time scale of environmental exchange relative to the other time scales in the system. In all cases, in the presence of temporal heterogeneity, the degree of non-exponential relaxation is less than that allowed for by the underlying distribution of sampled environments. This is due to time averaging and akin to motional narrowing. This result is summarized in Fig. 3(a), where contours of constant $\beta$ are plotted in the plane defined by the FWHM of the $\log(\tau_r)$ distribution and constant exchange time $\tau_x$. The
FHWM sets the underlying degree of heterogeneity among local environments, while the value of $\beta$ reflects the reported heterogeneity. Fig. 3(a) clearly demonstrates that a measured value of $\beta$ does not uniquely identify the underlying set of time scales present in the system. For example, a SM probe reporting $\beta = 0.9$ may result from (i) a narrow distribution of environments (FWHM of $\log(\tau_r) \sim 0.4$) and moderate exchange times ($\tau_r^{med} \sim 10^{\tau_r^{med}}$) or (ii) a wider distribution of environments (FWHM of $\log(\tau_r) \sim 1.0$) and shorter exchange times ($\tau_r^{med} \sim 10^{\tau_r^{med}}$).

**B. Finite trajectories**

The above results demonstrate a fundamental limit on information about the distribution of relaxation time scales extracted from ACFs in systems with dynamic exchange, whether by ensemble or SM measurements. However, SM techniques allow access not only to a system’s average relaxation but also to individual probe relaxations. Measuring the distribution of SM probe relaxations may resolve degeneracies such as those depicted in Fig. 3(a).

Consider a system with spatial heterogeneity but lacking temporal heterogeneity; here, each single molecule ACF will be exponential. In cases with temporal heterogeneity with exchange occurring on time scales longer than other relaxation time scales in the system, each single molecule ACF would instead yield a $\beta$ value consistent with the full heterogeneity of the system. As environmental exchange becomes faster, overlapping with other relaxation time scales in the system, single molecule ACFs will yield a $\beta$ value that does not report the full distribution of relaxation time scales in the system due to time averaging over different environments. For very rapid exchange, faster than approximately 0.1 times the median $\tau_r$, all rotational correlation function measurements will report exponential relaxation regardless of width of the underlying $\tau_r$ distribution due to extensive averaging over environments. As such, single molecule probes will relax exponentially in the two extreme cases of no environmental exchange or very fast environmental exchange. These two cases may be distinguished, however, by whether all single molecule probes report the same relaxation time scale, as they would in the case of fast exchange, or a distribution of time scales, as they would in the case of no exchange. While such distinction is possible given the effectively infinite trajectories studied above, it is not necessarily the case for experimentally relevant finite trajectories, which are limited in time due to probe photobleaching.

The effect of time-limited trajectories has been studied previously for the case of no exchange.\textsuperscript{23–25} In these studies, exponential relaxations were expected, and any observed non-exponentiality was entirely due to statistical effects. Here, we extend such analysis to systems with dynamic exchange. In ACFs calculated from time-limited trajectories in systems with dynamic exchange, two effects are present. First, as for systems with no dynamic exchange, statistical effects will lead to a loss of accuracy in determining best-fit parameters for relaxation functions. Second, for time-limited trajectories with exchange, depending on the exchange time and trajectory length, no or few exchange events may occur before photobleaching, leading to variation among SM ACFs, as each single molecule probe has sampled a different subset of the full distribution of relaxation rates.

Figure 3(b) shows how systems with the relaxation time distributions and exchange times depicted in Fig. 3(a) are affected by finite trajectory length. For each point with a given $\tau_x$ and FWHM of $\log(\tau_r)$ in Fig. 3(a), we perform 500 simulations with $\tau_r^{med}$ trajectories, showing how the sets of parameters that lead to ACFs with nearly identical $\beta$ values at effectively infinite trajectory length ($10^6 \tau_r^{med}$) do not do so at the experimentally typical trajectory length of $100 \tau_r^{med}$. Scenarios that lead to high $\beta$ values at very long trajectory length tend to yield lower $\beta$ values at shorter trajectory length, with the most prominent for systems with relatively short exchange times. For sets of parameters that lead to lower $\beta$ values at very long trajectory length, deviations both above and below the infinite trajectory limit $\beta$ are seen. While the degeneracies obvious in Fig. 3(a) and the left axis of Fig. 3(b) are broken for the $100 \tau_r^{med}$ trajectory length cases, unfortunately new degeneracies are introduced.

To examine the manner in which the full information available from SM studies may be able to break degeneracies...
present for finite trajectories, we examine in detail systems with FWHM of $\log(t_r) = 1.0$ with a single exchange time $-\log(t_x) = 2.0$, 3.0, or 4.0—for trajectory lengths ranging from 10 to 1000 times the median relaxation time in the system. This covers the range of trajectory lengths that have been reported in SM rotational measurements in supercooled liquids to date.\textsuperscript{13} The corresponding systems of infinite trajectory are those shown in Fig. 1(a) in open symbols. For each trajectory, the ACF is computed and fit with a stretched exponential, with the resulting distributions of parameters $\beta$ and $\tau_{\text{ff}}$ then fit to normal distributions. The median of the $\beta$ distribution and FWHM values of the fits to the $\beta$ and $\tau_{\text{ff}}$ distributions are shown in Fig. 4 as a function of trajectory length. To ascertain how effects from statistics and those from limited number of exchanges affect the data, two additional cases are considered and included on these plots: a heterogeneous system with FWHM of $\log(t_r) = 1.0$ and no exchange and a homogeneous system, both with $\tau_{\text{ff}} = 100$ steps. In each panel, the black line represents the homogeneous system, where any deviation from $\beta = 1$ and delta function distributions for $\beta$ and $\tau_{\text{ff}}$ are due solely to poor statistics. The orange line represents the heterogeneous system without exchange, where any deviation from $\beta = 1$, delta function distribution for $\beta$, and known relaxation distribution width of 1.0 is also due to statistics.

Fig. 4(a) shows the dependence of $\beta_{\text{med}}$ on trajectory length. Both the homogeneous system (black) and heterogeneous system without exchange (orange) show very limited effects even at the shortest trajectory length studied, with $\beta_{\text{med}} = 1$ for all trajectory lengths, showing this quantity is unaffected by statistical fluctuations associated with short trajectories. Thus, in systems with exchange and short trajectory lengths, effects due to the limited number of exchanges are expected to dominate median $\beta$. When trajectory length is shorter than the exchange time, only one environment has been sampled and $\beta_{\text{med}} \sim 1$. With many exchanges, $\beta_{\text{med}}$ is independent of trajectory length, as seen for $\log(t_x) = 2.0$: here, 10 exchanges have occurred even at the shortest trajectory. For $\log(t_x) = 3.0$, $\beta_{\text{med}}$ decreases from $\sim 1$ to a plateau after approximately 15 exchanges, showing that relatively few exchanges are necessary to accurately sample the distribution of relaxation times on the SM level. At $\log(t_x) = 4.0$, the reported $\beta_{\text{med}}$ continues to decrease with trajectory length for the longest trajectories studied, as only 10 exchanges have taken place by this point.

The values of $\beta_{\text{med}}$ for the situations depicted in Fig. 4(a) also highlight degeneracies in $\beta_{\text{med}}$ values that may be seen in experimentally realistic trajectory length experiments as depicted in Fig. 3(b). For a trajectory length of $100 \tau_{\text{rr}}$, the long exchange case ($\tau_x = 4.0$) appears indistinguishable from both the homogeneous case and the spatially heterogeneous case. At the slightly longer, still experimentally reasonable trajectory length of $125 \tau_r$, the measured $\beta_{\text{med}}$ is very similar for the systems with the fastest ($\tau_x = 2.0$) and slowest ($\tau_x = 4.0$) exchange times. Fig. 4(a) shows that this degeneracy can be resolved by assessing $\beta_{\text{med}}$ as a function of trajectory length. This is a viable experimental approach, as longer trajectories may be achievable through lowering laser power and extending probe life to photobleaching if signal:noise is acceptable, while shorter trajectories can be obtained by truncating the longest trajectories achieved in experiment. For the case described above, where systems with fast ($\tau_x = 2.0$) and slow ($\tau_x = 4.0$) exchange with the same underlying distribution ($\log(t_r) = 2.0$; FWHM ($\log(t_x) = 1.0$)) yield the same $\beta_{\text{med}} \sim 0.89$ at $125 \tau_r$, altering trajectory length by a factor of less

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**FIG. 4.** Various quantities as a function of trajectory length in units of $\tau_{\text{med}} = 100$ steps. The FWHM of the $\log(t_r)$ distribution is 1.0 and $\log(t_x) = 2.0$ (blue circles), 3.0 (green triangles), or 4.0 (red squares). These scenarios are also identified by open symbols of the same shape and color in Fig. 1(a) for the infinite trajectory case. The orange line represents a system with no exchange, while the black line represents a homogeneous system (FWHM of the ($t_{\text{rr}}$) distribution is 0.0). (a) Median $\beta$, with dashed horizontal lines indicating values of $\beta$ expected for these simulations at infinite trajectory length as obtained from Fig. 1(a), (b) FWHM of normal fit to $\beta$, values, and (c) FWHM of normal fit to $\log(t_{\text{ff}})$ distribution. Data are obtained from 1500 simulations for each data point.
than two in either direction would clarify the relative median relaxation and exchange times. For the case with $\tau_r > \tau_x$, a clear decay in $\beta_{\text{med}}$ would be evident until the trajectory length reaches $\sim 15 \tau_r$ as exchanges accumulate, while no trajectory length dependence of $\beta_{\text{med}}$ would be evident in the case of fast exchange. We also note that it is only in the case of exchange similar to or faster than median relaxation time $\tau_r$ that $\beta_{\text{med}}$ will not vary over the full range of experimentally obtainable trajectory lengths. Indeed, a finding of $\beta_{\text{med}} < 1$ and independent of trajectory length is a clear indication of exchange similar to or faster than $\tau_r$ with the value of $\beta_{\text{med}}$ then reflecting the width of the underlying $\tau_r$ distribution.

In addition to evaluating $\beta_{\text{med}}$ as a function of trajectory length to distinguish between possible scenarios that lead to the same $\beta_{\text{med}}$ at a given trajectory length, evaluating the full distributions of obtained $\beta$ and $\tau_{\text{fin}}$ values may also resolve $\beta_{\text{med}}$ degeneracies. Fig. 4(b) shows the FWHM of the $\beta$ distributions for the same systems shown in Fig. 4(a). It is immediately apparent that scrutinizing this quantity is not particularly helpful. As can be appreciated from trends seen in all systems, including those with no heterogeneity or no exchange, statistical effects dominate this measure, and all systems show a very similar decrease of FWHM with increasing trajectory length. On top of this effect, the effect of limited exchange is apparent for the system with $\log(\tau_x) = 4.0$, where a sudden increase in FWHM occurs after one exchange, as each single molecule has sampled the distribution of relaxation time scales differently, yielding ACFs that are variously well fit by the stretched exponential form and increasing the distribution relative to that expected statistically. This result suggests that a set of trajectories of given trajectory length with FWHM of the $\beta$ distribution larger than expected due to statistical effects implies the presence of $\tau_r$ longer than $\tau_x$ and somewhat shorter than (between 1 and 1/15) the trajectory length.

Unlike the $\beta$ distributions, the distributions of measured relaxation time scales, $\tau_{\text{fin}}$, provide additional information that may help distinguish between systems with identical $\beta_{\text{med}}$ values (and FWHM of $\beta$ distributions). Distributions of such time scales have commonly been used to assess degree of heterogeneity in single molecule rotational experiments in supercooled liquids.26,27 Here, we show this is an important quantity to consider in assessing the underlying relaxation time distribution, but it should not be considered in isolation. Fig. 4(c) shows that in a system with heterogeneity but no exchange, the measured $\tau_{\text{fin}}$ is consistent with the relaxation breadth in the system, while for a homogeneous system, where a delta function distribution is expected, poor statistics lead to a $\log(\tau_{\text{fin}})$ distribution with FWHM as large as 0.3, which decreases with increasing trajectory length. This effect is mirrored in the heterogeneous system with fast exchange, where a delta function in relaxation times—representing an average of those felt by each single molecule—is also expected. For systems with longer exchange, where systems with zero to many exchanges are represented in the trajectory lengths assessed, the spread in $\tau_{\text{fin}}$ values is large, and expected to transition from the case of no exchange (orange) to the case of a single relaxation time (black), another manifestation of motional narrowing. As this limit is approached at long trajectory lengths, the measured $\tau_{\text{fin}}$ distribution is again dominated by statistics. As with the FWHM of the $\beta$ distribution, given known trajectory length, a wider distribution of $\tau_{\text{fin}}$ than expected from statistics points to heterogeneity and, if it changes with trajectory length, can be used in determining exchange times.

Taking the information from all panels of Fig. 4 together, it is apparent that either considering $\beta_{\text{med}}$ as a function of trajectory length or simultaneously considering $\beta_{\text{med}}$ and the FWHM of the $\tau_{\text{fin}}$ distribution at a given trajectory length can resolve degeneracies for systems with identical $\beta_{\text{med}}$ at a given trajectory length. For the example described above, a system with $\log(\tau_{\text{fin}}) = 2.0$, FWHM of $\log(\tau_r) = 1.0$, and $\tau_{\text{ex}} = 2.0$ or 4.0, each system has $\beta_{\text{med}} \sim 0.89$ at the experimentally reasonable trajectory length of 125 $\tau_r$. However, $\beta_{\text{med}}$ is constant as a function of trajectory length for the fast exchange case and rapidly changing with trajectory length for the slow exchange case. Additionally, these systems yield radically different widths of the measured $\tau_{\text{fin}}$ distributions, with only the system with slow exchange having a $\tau_{\text{fin}}$ FWHM significantly greater than that which would be expected from statistical fluctuations associated with short trajectories.

**IV. CONCLUSIONS**

A key result of this work is that a probe must fully relax within a given environment in order to report the relaxation time scale of that environment. In many scenarios, including ones with various correlations between relaxation times and/or exchange times, temporal heterogeneity leads to reported environmental diversity that is less than the true underlying distribution. This time averaging applies to both probes measured at the SM level and in ensemble measurements. While the situation would be exacerbated for large, slow probes, it readily occurs even for the ideal probes studied here that match the relaxation time scale of the host. In cases of very long trajectories, regardless of particular underlying distributions of relaxation times, we find that the median exchange time of the system sets the fundamental limit on the stretching exponent values that may be obtained from measured autocorrelation functions. This inherent limit leads to degeneracies of scenarios consistent with a given measured $\beta$. Using the full information available from typical single molecule experiments allows discrimination between various scenarios that yield identical stretching exponents. Investigating either how median stretching exponent changes with trajectory length or the value of the median stretching exponent and the breadth of the relaxation time distribution at a particular trajectory length allows resolution of such degeneracies and identification of the relative time scales of relaxation and exchange.

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