Single-Molecule Studies of Heterogeneous Dynamics in Polymer Melts Near the Glass Transition

Laura A. Deschenes and David A. Vanden Bout*

Single-molecule spectroscopy was used to follow the orientation of a single probe molecule in a polymer film in real time. Broad spatially heterogeneous dynamics were observed on long time scales, which result from simple diffusive rotational motions on short time scales. This diffusive behavior persists for many rotations before the molecule's local environment changes to one characterized by a new time scale. This environmental exchange occurs instantaneously on the time scale of the experiment and may arise from large-scale collective motions. The distribution of exchange times for these environments was measured for several temperatures near the glass transition.

Understanding the nature of the glass transition has been a problem of interest for over half a century (*1–9*), especially the origin of the nonexponential relaxation that governs dynamics in supercooled systems at temperatures just above the glass transition temperature (T_g). The fundamental question is whether dynamics show multiple time scales because of heterogeneity in the system, where each molecule shows purely exponential reorientation (but with time scales that vary from molecule to molecule), or whether the dynamics are inherently nonexponential. Although recent works on related or model systems suggest that dynamics are heterogeneous, controversy remains regarding the critical issues of distribution, size, and lifetime of these heterogeneities (*1–3, 10–16*). These questions can be addressed definitively with single-molecule spectroscopy (*17–23*), a technique that measures the dynamics of the ultimate subensemble: the environment of one molecule (*24*). Studying individual molecular environments one at a time avoids the ensemble averaging that masks these dynamics in bulk studies. Single-molecule spectroscopy can measure the dynamics of any region where a probe molecule is found, thereby accessing the breadth of the distribution of environments, rather than emphasizing a particular subset of dynamic behaviors.

The single-molecule experiments were performed with an apparatus similar to that used previously (*25, 26*). Individual molecules were spatially isolated by diluting Rhodamine 6G molecules into 250-nm films of poly(methylacrylate) (PMA) (T_g = 8°C) (*27*). Films were stored under vacuum and imaged under nitrogen in order to minimize photooxidation. Care was taken to ensure that the studied molecules resided within the polymer film and not at the interfaces. Typical images of single molecules in a PMA film are shown in Fig. 1. Because the molecules rotate on the time scale of the data collection, their intensity in the two polarizations exchanges from line to line in the images. This rotational motion can be studied in more detail by centering one molecule in the laser focus and recording the transient fluorescence intensity in the two channels. We imaged single molecules over the course of 12 hours and found the translational diffusion constant to be $\sim 4 \times 10^{-18}$ m^2/s, which allows long transients to be recorded with a fixed microscope position. Transients for two molecules in PMA are shown in Fig. 2. The rotational motion of the molecules can be directly observed by the anticorrelation of the intensity on the two orthogonal detectors. The molecules rotate slowly, on the many-second time scale, permitting low excitation powers (0.02 μW) and long integration times (1 s). Short integration times (50 ms) showed no faster dynamics and were deemed unnecessary. The time-dependent intensities in the two polarization channels [$//(t)$ and $\perp(t)$] were then used to calculated the reduced linear dichroism $A(t)$ (*28–31*)

$$A(t) = \frac{//(t) - \perp(t)}{//(t) + \perp(t)} \tag{1}$$

Use of the reduced linear dichroism protects the experiments from artifacts that arise in overall fluctuations in the single-molecule fluorescence signal due to triplet blinking (*32*), spectral diffusion (*19*), out-of-plane rotation, translational diffusion, and laser fluctuations.

Although orientation changes for single molecules have been observed in previous single-molecule studies (*29, 33*), we measured rotational diffusion in real time by directly following the orientation of the probe transition dipole moment. Individual transients yield important information. Visual inspection resolves whether dynamics are spatially heterogeneous; different molecules show rotation on different time scales, an indication of spatial heterogeneity. A more quantitative picture of these differences can be obtained by looking at the autocorrelation function $C(t)$ of the dichroism. $C(t)$ was calculated with the standard method for discrete points in finite time series. Confidence limits are generated on the autocorrelations such that any values between the limits are consistent with zero (*34*)

$$C(t) = \frac{\sum\limits_{t'=0}^{T} A(t')A(t'+t)}{\sum\limits_{t'=0}^{T} A(t')A(t')} \tag{2}$$

where T is the total number of data points.

Typical correlation functions are shown in Fig. 2. Most of the correlation functions exhibit nonexponential decays that can be fit with the Kohlrausch-Williams-Watt (KWW)

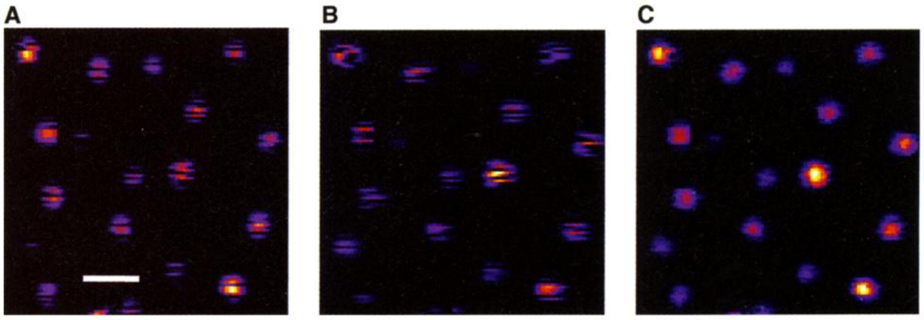

Fig. 1. Single molecules rotating in a PMA film. (**A** and **B**) These images were obtained simultaneously at orthogonal polarizations (scale bar is 1 μm). Bright and dark lines across the molecules in (A) and (B) result from molecular rotation on the time scale of a line scan (20 s), as the polarization flips from detector to detector. (**C**) The sum of (A) and (B), where the lines cancel out to form an unmodulated intensity image.

Department of Chemistry and Biochemistry, Center for Nano and Molecular Materials Science and Technology; and Texas Materials Institute, University of Texas, Austin, TX 78712–1167, USA.

*To whom correspondence should be addressed. E-mail: davandenbout@mail.utexas.edu

"stretched exponential" function KWW(t)

$$\mathrm{KWW}(t) = \exp(-\,t/\tau_{\mathrm{KWW}})^{\beta_{\mathrm{KWW}}} \qquad (3)$$

where β describes the breadth of the distribution of time scales. This functional form does an excellent job fitting the data and is convenient because the correlation time τ_C can be calculated directly from the KWW fitting parameters (*35*).

The two molecules shown in Fig. 2 exhibit very different behaviors; both are in PMA, but the molecule in Fig. 2A, whose correlation function is shown in Fig. 2D, has $\tau_{\mathrm{KWW}} = 104$, $\beta = 0.55$, and $\tau_C = 175$, whereas the molecule in Fig. 2C, whose correlation function is shown in Fig. 2E, shows $\tau_{\mathrm{KWW}} = 27$, $\beta = 0.62$, and $\tau_C = 39$, indicative of the broad distribution of time scales associated with systems near T_g. Quantifying the distributions of time scales from individual molecules is complicated by the rotational dynamics changing with time for each molecule. The molecule depicted in Fig. 2, A and B, makes a drastic jump after 3300 s to become dramatically faster. This switching behavior causes the dynamics of each molecule to depend on the molecule's photochemical lifetime. Longer lived molecules sample more environments and therefore show τ_C distributions that are different from those of short-lived molecules. It becomes necessary to examine not only the parameters derived from individual fits, but also the average behavior of the ensemble. Measurements in PMA at temperatures 5, 10, and 15 K above T_g give $\langle\tau_C\rangle = 140$, 106, and 43 s, respectively, demonstrating that this single-molecule technique probes polymer dynamics as it shows the expected slowing of dynamics closer to T_g. In order to ensure that dynamics were independent of excitation rate, we calculated correlation functions for dynamics measured at excitation powers that differ by a factor of 20; these calculations showed distributions of τ_{KWW} and β_{KWW} that were statistically indistinguishable.

Although recreating the ensemble is a useful check for the single-molecule technique, additional information can be gleaned from individual transients. A long transient for a molecule that switches between several different environments is shown in Fig. 3. Initially, the molecular rotation is fast, and then it slows substantially before changing to a third, intermediate time scale. With the correlation function of these individual segments, the differences become clear; each segment shows dynamics with a β value that is nearer to unity than the entire transient. Most dramatically, the first section yields a correlation function with $\beta = 1$, which corresponds to the purely exponential relaxation indicative of diffusional rotation. The diffusional behavior at short times dramatically demonstrates that spatially varying environmental heterogeneities can be wholly responsible for the nonexponential dynamics.

Some of the most unexpected behavior exhibited by individual molecules is in their switching environments. The dynamics for individual molecules are well characterized by a single time scale that persists for long periods of time (many rotations) before rapidly switching to dynamics on a different time scale. This rapid switching can be seen in the transient plotted in Fig. 3.

The length of time of persistent dynamics or lifetime of the heterogeneity can be measured directly from individual transients. The angular change for the molecule as a function of time is shown in Fig. 4A. The transitions between different rotation rates are clear. The lengths of these segments are represented in the histograms of exchange times shown in Fig. 4, B through D, for molecules in PMA at T_g + 5 K, T_g + 10 K, and T_g + 15 K, respectively (*36*). The exchange time shows a distinct temperature dependence, ranging from 522 s (standard deviation σ = 357) to 1158 s (σ = 832) to 3420 s (σ = 2526). As the temperature decreases, the exchange times grow longer and their distribution broadens. We found that the exchange times range between 10 and 25 times the average correlation time at these temperatures, as calculated from the KWW fits to our correlation functions.

Because the molecules are switching local environments, the β value for an individual molecule depends on the length of the transient. The β values for a set of molecules as well as some short segments of transients in PMA at the lowest temperature probed are shown in Fig. 5. At very short times, the β values are near or equal to unity, but as the molecules sample more environments, the β values decrease to some limiting bulklike value. The longest lived molecules have β values that closely approach the bulk, a good indication of ergodic behavior.

The long lifetime of the heterogeneities and the rapid switching between time scales are molecular details that can be observed only in a single-molecule experiment. This combination of behaviors is indicative of highly cooperative molecular motion. During the lifetime of the heterogeneity at room temperature, if the Rhodamine 6G molecule were freely translationally diffusing, it would have moved more than 100 nm, and yet the dynamics remain nearly

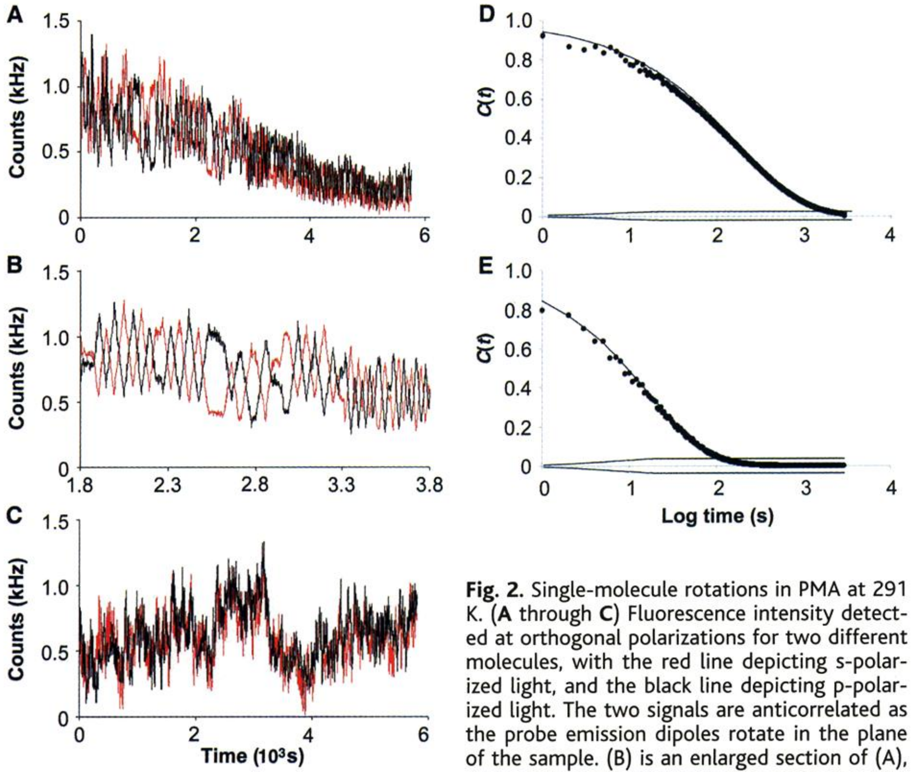

Fig. 2. Single-molecule rotations in PMA at 291 K. (**A** through **C**) Fluorescence intensity detected at orthogonal polarizations for two different molecules, with the red line depicting s-polarized light, and the black line depicting p-polarized light. The two signals are anticorrelated as the probe emission dipoles rotate in the plane of the sample. (B) is an enlarged section of (A), emphasizing the anticorrelation of the two signals. The slow change in total intensity in (A) is attributed to translational diffusion of the probe molecule toward the edge of the focused laser spot, whereas the intensity fluctuations in (C) result from the out-of-plane rotation of the dye molecule freely rotating in three dimensions. (**D** and **E**) The autocorrelation functions for the linear dichroism calculated from (A) and (C), respectively. Both are fit to the KWW function, with (D) giving $\tau_{\mathrm{KWW}} = 104$, $\beta_{\mathrm{KWW}} = 0.55$, and $\tau_C = 175$ and (E) giving $\tau_{\mathrm{KWW}} = 27$, $\beta_{\mathrm{KWW}} = 0.62$, and $\tau_C = 39$. The correlation function for the total intensity of (C) has similar behavior.

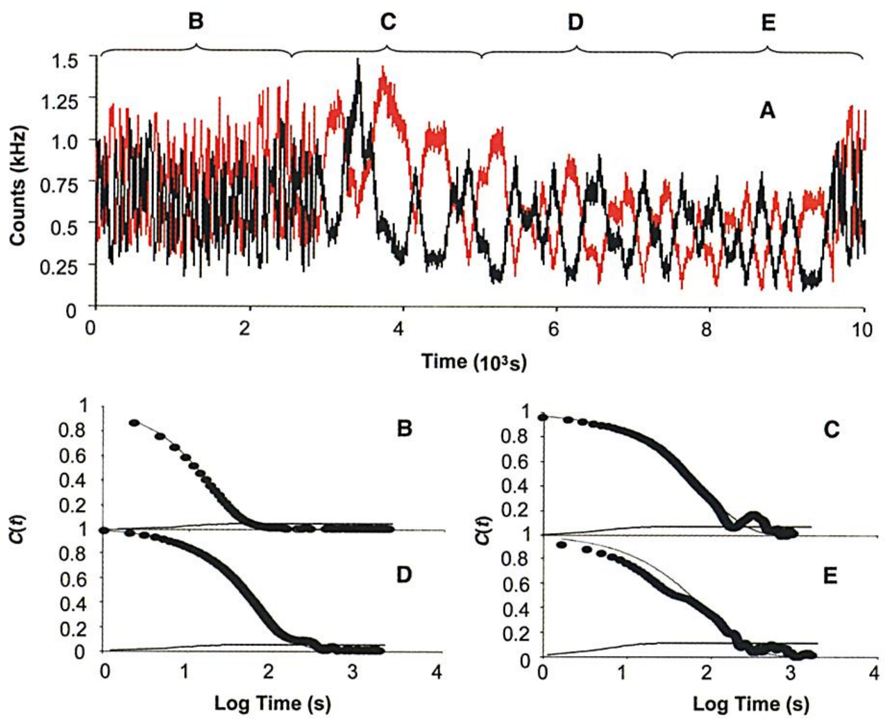

Fig. 3. Heterogeneous single-molecule dynamics at 291 K. (**A**) Fluorescence intensity detected at orthogonal polarizations for a long-lived single molecule in PMA. Its correlation function (not shown) yields $\tau_{KWW} = 88$, $\beta_{KWW} = 0.46$, and $\tau_C = 211$. (**B** through **E**) The correlation functions of the corresponding subsets of the full transient. (B) $\tau_{KWW} = 15$, $\beta_{KWW} = 1.0$, and $\tau_C = 15$. (C) $\tau_{KWW} = 112$, $\beta_{KWW} = 0.77$, and $\tau_C = 130$. (D) $\tau_{KWW} = 90$, $\beta_{KWW} = 0.59$, and $\tau_C = 139$. (E) $\tau_{KWW} = 64$, $\beta_{KWW} = 0.59$, and $\tau_C = 98$.

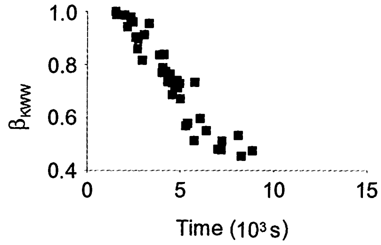

Fig. 5. Values of β_{KWW} as a function of photochemical survival time in PMA at T_g + 5 K.

constant on that time scale. This would suggest that the molecule resides in a local polymer environment that maintains its average viscosity for a long period of time as the entire environment is either stationary or slowly diffuses. This collective retention of local viscosity is reminiscent of the "cage" predicted by mode-coupling theory. When the environment changes, it does so in a drastic and nearly instantaneous step, revealed by the distinct change in rotation rate featured in Fig. 4. Our experiment was unable to distinguish between changes in dynamics due to diffusion of the probe molecule between members of a static distribution of heterogeneous environments and changes in measured rotation rate from evolving dynamics within a single-polymer environment. However, similarly abrupt motions have been observed in simulations of polymer melts, although at temperatures far above T_g (*37*), and also in experiments in model colloid systems (*11*). Motion in these systems is characterized by particles that show slow fluctuations punctuated by occasional sudden cooperative long-range translational cage-escape motions. Such collective jumps would explain the long-lived environments and the rapid changes in local environment observed in our single-molecule experiments near T_g.

Dynamics that occur on multiple time scales have long been considered a hallmark of systems near T_g. Single-molecule studies of rotational dynamics in polymer melts confirm that spatial heterogeneities give rise to the characteristically nonexponential dynamics in these systems. At short times, the polymers may be characterized by domains that each have a particular rotation time. The data suggest that these heterogeneities suddenly exchange in large collective motions to form new domains characterized by new time scales. At long times, the molecules have experienced enough different environments to give nonexponential rotational relaxation similar to the bulk. The average lifetime of these heterogeneities varies with temperature even relatively far (+15 K) from the glass transition, but many questions remain as to the relation between this exchange lifetime and the onset of the glass transition.

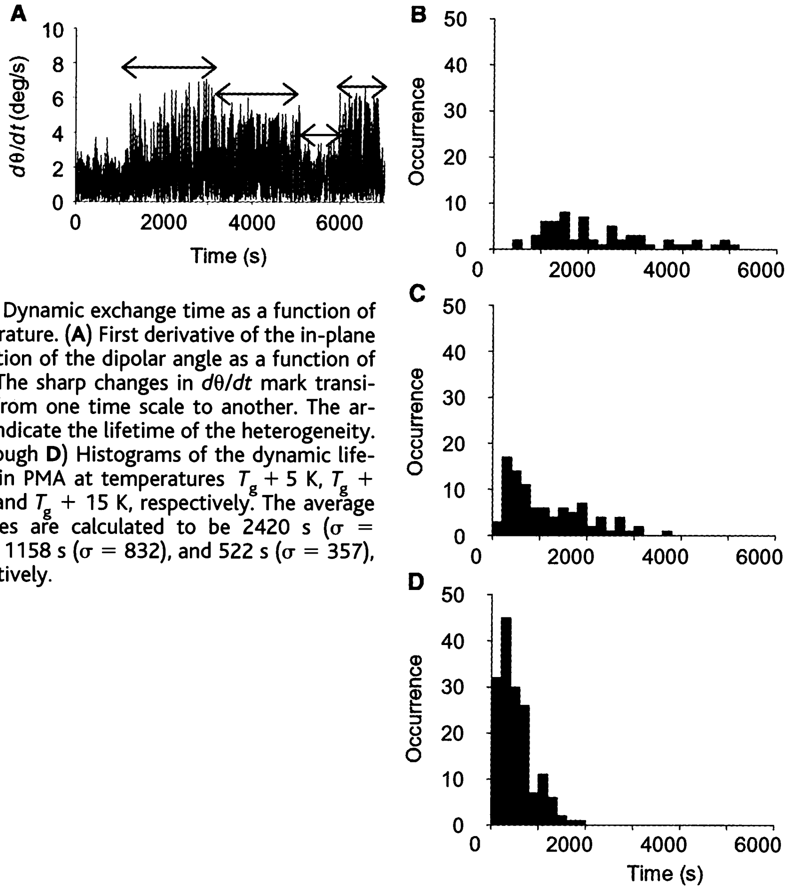

Fig. 4. Dynamic exchange time as a function of temperature. (**A**) First derivative of the in-plane projection of the dipolar angle as a function of time. The sharp changes in $d\theta/dt$ mark transitions from one time scale to another. The arrows indicate the lifetime of the heterogeneity. (**B** through **D**) Histograms of the dynamic lifetimes in PMA at temperatures T_g + 5 K, T_g + 10 K, and T_g + 15 K, respectively. The average lifetimes are calculated to be 2420 s (σ = 1807), 1158 s (σ = 832), and 522 s (σ = 357), respectively.

References and Notes

1. C. A. Angell, K. L. Ngai, G. B. McKenna, P. F. McMillan, S. W. Martin, *J. Appl. Phys.* **88**, 3113 (2000).
2. M. D. Ediger, *Annu. Rev. Phys. Chem.* **51**, 99 (2000).
3. H. Sillescu, *J. Non-Cryst. Solids* **243**, 81 (1999).
4. M. D. Ediger, C. A. Angell, S. R. Nagel, *J. Phys. Chem.* **100**, 13200 (1996).
5. J. T. Fourkas, D. Kivelson, U. Mohanty, K. A. Nelson, Eds., *Supercooled Liquids: Advances and Novel Applications*, vol. 676 of *ACS Symposium Series* (American Chemical Society, Washington, DC, 1997).
6. G. Harrison, *The Dynamic Properties of Supercooled Liquids* (Academic Press, New York, 1976).
7. T. Gleim, W. Kob, K. Binder, *Phys. Rev. Lett.* **81**, 4404 (1998).
8. D. B. Hall, A. Dhinojwala, J. M. Torkelson, *Phys. Rev. Lett.* **79**, 103 (1997).
9. G. Adam, J. H. Gibbs, *J. Chem. Phys* **43**, 139 (1965).
10. H. Wendt, R. Richert, *Phys. Rev. E.* **61**, 1722 (2000).
11. E. R. Weeks, J. C. Crocker, A. C. Levitt, A. Schofield, D. A. Weitz, *Science* **287**, 627 (2000).
12. C.-Y. Wang, M. D. Ediger, *J. Phys. Chem. B* **104**, 1724 (2000).
13. I. Chang, H. Sillescu, *J. Phys. Chem. B* **101**, 8794 (1997).
14. K. L. Ngai, *J. Phys. Chem. B* **103**, 10684 (1999).
15. R. Richert, *J. Phys. Chem. B* **101**, 6323 (1997).
16. E. V. Russell, N. E. Israeloff, *Nature* **408**, 695 (2000).
17. W. E. Moerner, *Science* **265**, 46 (1994).
18. P. F. Barbara, W. E. Moerner, *Acc. Chem. Res.* **29**, 561 (1996).
19. H. P. Lu, X. S. Xie, *Nature* **385**, 143 (1997).
20. S. Nie, D. T. Chiu, R. N. Zare, *Science* **266**, 1018 (1994).
21. D. A. Vanden Bout *et al.*, *Science* **277**, 1074 (1997).
22. R. M. Dickson, A. B. Cubitt, R. Y. Tsien, W. E. Moerner, *Nature* **388**, 355 (1997).
23. T. Basche, S. Kummer, C. Braeuchle, *Nature* **373**, 132 (1995).
24. The single-molecule environment is probe dependent, with its size determined by the dye used.
25. J. J. Macklin, J. K. Trautman, T. D. Harris, L. E. Brus, *Science* **272**, 255 (1996).
26. The sample is held atop a closed loop piezo scanning stage and illuminated with the second harmonic of a Nd:yttrium-aluminum-garnet laser through a 1.25 numerical aperture (NA) oil immersion objective in the epi configuration. By scanning the sample in the focus of a laser beam, we can observe the fluorescence from individual molecules. The fluorescence signal from the molecules is passed through a dichroic beam splitter to remove excitation light and split into two orthogonal polarizations, which are imaged onto the front of two single-photon-counting avalanche photodiodes. For temperature-dependent data, the sample was held at constant temperature in an Oxford Microstat-N cryostat, and the fluorescence was excited and collected through a 0.6-NA long working distance air objective. All other aspects of the experiment remain unchanged.
27. The T_g of the polymer was measured by differential scanning calorimetry.
28. T. Ha, T. A. Laurence, D. S. Chemla, S. Weiss, *J. Phys. Chem. B.* **103**, 6839 (1999).
29. R. M. Dickson, A. P. Bartko, *J. Phys. Chem. B.* **103**, 3053 (1999).
30. R. M. Dickson, D. J. Norris, Y.-L. Tzeng, W. E. Moerner, *Science* **274**, 966 (1996).
31. Measurement of the full three-dimensional orientation (*27, 28*) is not required because the dynamics are isotropic in three dimensions and thus revealed fully in the fluctuations of the dichroism. Additionally, the use of a high NA objective (NA = 1.25) leads to a slight contamination of the dichroism signal due to the out-of-plane component of the fluorescence that manifests itself as an additional background but does not adversely affect the measured dynamics (*28*).
32. J. A. Veerman, M. F. Garcia-Parajo, L. Kuipers, N. F. van Hulst, *Phys. Rev. Lett.* **83**, 2155 (1999).
33. T. Ha, J. Glass, T. Enderle, D. S. Chemla, S. Weiss, *Phys. Rev. Lett.* **80**, 2093 (1998).
34. G. E. P. Box, G. M. Jenkins, *Time Series Analysis Forecasting and Control* (Holden-Day, San Francisco, 1976).
35. For all correlation functions, the first point containing the noise is not shown and not included in the fit. Fits of correlation functions yield coefficient of determination R^2 values greater than 0.99 in most cases. Standard errors for the fitting parameters tend to be extremely small (1 part in 10^5) but provide little meaningful basis for comparison because of the large covariance of β and τ. A useful measure of this error determines that a 1% variation in β or a 5% variation in τ is sufficient to reduce the R^2 value from 0.99 to 0.98. The rotational correlation time τ_C is defined as the integral from zero to infinity of the correlation function, but for a stretched exponential function, this integral can be conveniently written in terms of the τ and β parameters as

$$\tau_C = \int_0^{\infty} C(t)dt = \frac{\tau_{KWW}}{\beta_{KWW}} \Gamma\left(\frac{1}{\beta_{KWW}}\right)$$

where Γ is the gamma function, defined as $\Gamma(n) = \int_0^{\infty} e^{-x} x^{n-1}\, dx$.
36. The orientational transient can be calculated from the polarized fluorescence data with

$$\theta(t) = \tan^{-1}\left[\sqrt{\frac{//(t)}{\perp(t)}}\right]$$

Molecules were determined to have exchanged environments when the average angle jump changes by more than 2 SD from the previous average angle jump.
37. C. Bennemann, C. Donati, J. Baschnagel, S. C. Glotzer, *Nature* **399**, 246 (1999).
38. D.A.V.B. is a Research Corporation Cottrell Scholar and a Camille and Henry Dreyfus Foundation New Faculty Awardee. This work is supported by grants from the Research Corporation.

10 October 2000; accepted 28 February 2001

Real-Space Imaging of Nucleation and Growth in Colloidal Crystallization

U. Gasser,[1]* Eric R. Weeks,[1]† Andrew Schofield,[2] P. N. Pusey,[2] D. A. Weitz[1]

Crystallization of concentrated colloidal suspensions was studied in real space with laser scanning confocal microscopy. Direct imaging in three dimensions allowed identification and observation of both nucleation and growth of crystalline regions, providing an experimental measure of properties of the nucleating crystallites. By following their evolution, we identified critical nuclei, determined nucleation rates, and measured the average surface tension of the crystal-liquid interface. The structure of the nuclei was the same as the bulk solid phase, random hexagonal close-packed, and their average shape was rather nonspherical, with rough rather than faceted surfaces.

The study of the structure, growth, and properties of crystals is one of the most important areas of solid state physics (*1*). Although a great deal is known about the behavior of bulk crystals, considerably less is known about their earliest stage, when they nucleate and grow from a liquid. Homogeneous nucleation occurs when small crystalline regions form from structural fluctuations in a liquid cooled below its freezing point. The growth of these regions depends on a competition between a decrease in bulk energy, which favors growth, and an increase in surface energy, which favors shrinkage. The smallest crystals continually form by fluctuations but then typically shrink away because of the high surface energy. Growth becomes energetically favorable only when the crystallites reach a critical size. The competition between the surface energy and bulk energy is reflected in the free energy for a spherical crystallite

$$\Delta G = 4\pi r^2\gamma - \frac{4\pi}{3} r^3 \Delta\mu n \qquad (1)$$

where r is the radius, γ is the free energy of the crystal-liquid interface per unit area, $\Delta\mu$ is the difference between the liquid and solid chemical potentials, and n is the number density of particles in the crystallite (*2*). The size of the critical nucleus is $r_c = 2\gamma/(\Delta\mu n)$, corresponding to the maximum of ΔG (Eq. 1). Despite its crucial role, very little is known about this nucleation process, primarily because of the difficulty of directly observing the nuclei in real space.

No experiment has ever directly measured the size of the critical nucleus. It cannot be determined theoretically—even the surface tension is unknown. Furthermore, an additional important yet unresolved question involves the internal structure of critical nuclei, because nucleation need not occur via the stable bulk phase (*3*). General arguments based on symmetry suggest that nucleation may proceed by a body-centered cubic (bcc) structure as an intermediate phase, with the final equilibrium solid phase having a differ-

---

[1] Department of Physics and Division of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA. [2] Department of Physics and Astronomy, University of Edinburgh, Edinburgh, Scotland EH9 3JZ, UK.

*To whom correspondence should be addressed. E-mail: gasser@deas.harvard.edu

†Present address: Physics Department, Emory University, Atlanta, GA 30322, USA.