

Dynamic Critical Phenomena in Aqueous Protein Solutions

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We report our use of quasielastic light scattering to characterize concentration fluctuations in aqueous solutions of γ_{11} -crystallin along the critical isochore. We find that the wave number and temperature dependence of the average rate of decay of the concentration fluctuations are consistent with the theory for critical dynamics in binary liquid mixtures only if we allow both the background viscosity and the background contribution to the decay rate to be unusually large. Furthermore, in contrast to the behavior seen in binary liquid mixtures, we find that the concentration fluctuations exhibit very significant deviation from exponential decay.

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An aqueous protein solution is a binary mixture of large particles (protein molecules) and small particles (water molecules). In contrast, a binary liquid mixture is a mixture of two different kinds of particles that are approximately the same size. Thus, a comparison of the critical behavior in these two systems allows one to determine the effect on critical behavior of a relative difference of size in the two kinds of particles in a binary mixture.

The correlation length ξ , osmotic compressibility κ_T , and coexistence curve of aqueous protein solutions in the vicinity of the critical point for liquid-liquid phase separation have been reported recently [1–3]. These experimental results established that the static critical properties of these solutions are consistent with the behavior of members of the static universality class of three-dimensional systems with short range interactions and scalar order parameters. This universality class includes binary liquid mixtures.

In contrast to the equilibrium properties, the dynamic properties of aqueous protein solutions in the vicinity of the critical point have not been investigated as thoroughly. In this Letter, we report our use of quasielastic light scattering (QLS) to investigate the time decay of spontaneous concentration fluctuations, as a function of both wave number q of the fluctuations and temperature T , along the critical isochore of aqueous solutions of the bovine eye lens protein γ_{11} -crystallin.

The only previous study of critical dynamics in a protein solution of which we are aware is the work of Ishimoto and Tanaka who made QLS measurements at a single fixed wave number of the fluctuations using a 19 channel digital correlator on aqueous solutions of lysozyme [4] along the critical isochore for $(T - T_c)/T_c \geq 5 \times 10^{-3}$ ($q\xi < 0.1$), where T_c is the critical temperature. The present study is a significant experimental advance over the work of Ishimoto and Tanaka in that (i) we obtain data for fluctuations at up to 12 different wave numbers, (ii) we obtain data using a 144

channel digital correlator, and (iii) we obtain data much closer to the critical point [$(T - T_c)/T_c \geq 1.56 \times 10^{-4}$ and $q\xi \leq 3.18$].

The more thorough investigation of critical dynamics presented here has revealed new and unexpected behavior. Specifically, we find that the wave number and temperature dependence of the average rate of decay of the concentration fluctuations are consistent with the theory for critical dynamics in binary liquid mixtures [5] only if we allow both the background viscosity and the background contribution to the decay rate to be unusually large. Furthermore, in contrast to the behavior seen in binary liquid mixtures we find that the concentration fluctuations exhibit very significant deviation from exponential decay with time.

Of course, these protein solutions may be viewed as strongly interacting colloidal dispersions. The theory of the dynamics of colloidal dispersions has recently been advanced considerably by utilizing the generalized Smoluchowski equation. In particular, nonexponential decay of the concentration fluctuations has been predicted for dense suspensions of hard spheres [6]. When attractive interactions are included, strong deviation from exponential decay has been predicted, to first order in the concentration, for the time-dependent self-scattering function in the zero wave number limit [7]. However, this approach has yet to be extended to describe the dynamics in the vicinity of the critical point.

Bovine γ_{11} -crystallin is a compact globular protein with a molecular weight of 2.1×10^4 g/mole and a diameter of 35–55 Å [8]. In aqueous solution, the critical protein volume fraction ϕ_c is 0.19 ± 0.01 [2]. Previous work has revealed that the T_c of these solutions increases with time [9,10]. This drift in T_c has been studied carefully and a method for preparing the solutions has been developed that minimizes this drift [9,10]. Furthermore, this increase in T_c with time has been found to be completely prevented by the addition to the solution of the reducing agent dithiothreitol (DTT) [10]. The

addition of DTT to γ_{II} -crystallin in phosphate buffer does not change either ϕ_c or the shape of the coexistence curve [9].

In this Letter, we report the results of our measurements on two different samples of γ_{II} -crystallin in 50 mM phosphate buffer solution (ionic strength 120 mM, pH 7.1, with 3 mM sodium azide). All solutions were prepared as described previously [9,10]. One solution was γ_{II} -crystallin in 50 mM phosphate buffer (protein volume fraction $\phi = 0.199 \pm 0.001$) in a cylindrical scattering cell (5.6 mm diam). All measurements on this sample were performed within 100 h of its preparation, over which time T_c was found to increase by less than 0.1° . The other sample was γ_{II} -crystallin in 50 mM phosphate buffer with 20 mM DTT ($\phi = 0.192 \pm 0.001$) in a rectangular scattering cell (1.85 mm optical path length). The addition of DTT to the second sample assured us that no significant change in T_c would occur during the course of measurements on this sample. A comparison of the results obtained on the two different samples thus allows us to demonstrate that the small increase in T_c that occurred during the course of our measurements on the first sample does not affect our results. In addition, since the two samples were studied in different size scattering cells, a comparison of the results obtained on the two different samples helps us to confirm that multiple light scattering does not affect our results.

Both static and quasielastic light scattering measurements were made using an apparatus which is described in detail elsewhere [11] and is based on the design of Haller, Destor, and Cannell [12]. This instrument is able to maintain the sample temperature constant to within $\pm 0.002^\circ$. We used a vertically polarized helium-neon laser operating at a wavelength λ of 6328 Å. The light scattered by the sample was detected at up to 12 fixed angles ($11.50^\circ \leq \theta \leq 163.36^\circ$).

The static light scattering measurements were used to obtain κ_T and ξ along the critical isochore using a procedure described elsewhere [3,9]. These measurements also provided us with very accurate values for T_c for each of the solutions studied. We found that the osmotic compressibility for both γ_{II} -crystallin solutions was consistent with $\kappa_T = \kappa_T^0[(T - T_c)/T_c]^{-\gamma}$. For the solution in the 5.6 mm cell, we found $T_c = 4.00 \pm 0.05^\circ\text{C}$, $\kappa_T^0 = (7 \pm 1) \times 10^{-8} \text{ cm}^2/\text{g}$, and $\gamma = 1.19 \pm 0.05$. For the solution in the 1.85 mm cell, we found $T_c = 2.057 \pm 0.005^\circ\text{C}$, $\kappa_T^0 = (7.3 \pm 0.5) \times 10^{-8} \text{ cm}^2/\text{g}$, and $\gamma = 1.17 \pm 0.05$. For the solution in the 1.85 mm cell, we determined ξ and found that it was consistent with $\xi = \xi_0[(T - T_c)/T_c]^{-\nu}$ with $\xi_0 = 7 \pm 1 \text{ Å}$ and $\nu = 0.61 \pm 0.06$. These values for κ_T^0 , γ , ξ_0 , and ν are consistent with those of Schurtenberger *et al.* [3]. We used the above results in our analysis of the experimental data for the dynamics of these solutions in the vicinity of the critical point.

For the QLS measurements, we obtained the time autocorrelation function of the photocounts $\langle n(q, t)n(q, 0) \rangle$, using a Langley-Ford (Amherst, MA) model 1096 correlator with 144 channels. Here, the photocount $n(q, t)$ is the number of photons counted by the detector in a time interval between t and $t + \Delta t$ where Δt is the sample time. The wave number $q = (4\pi m/\lambda) \sin(\theta/2)$, where m is the refractive index of the sample and θ is the scattering angle. The last 16 channels of the correlator were delayed by 1024 sample times. The sample time was chosen so that the first 128 channels would span two average decay times of the correlation function ($3.0 \times 10^{-6} \leq \Delta t \leq 2.8 \times 10^{-2} \text{ s}$). It can be shown [13] that $\langle n(q, t)n(q, 0) \rangle$ is related to the decay of spontaneous fluctuations in the protein concentration ρ by

$$\frac{\langle n(q, t)n(q, 0) \rangle}{\langle n(q) \rangle^2} = 1 + \beta \left[\frac{\langle \hat{\rho}(q, t)\hat{\rho}(q, 0) \rangle}{\langle |\hat{\rho}(q)|^2 \rangle} \right]^2, \quad (1)$$

where β is a constant between 0 and 1 that depends on the number of coherence areas subtended by the detector and $\hat{\rho}(q, t)$ is the Fourier component of ρ with wave number q . The above relation holds provided that the scattering volume is much larger than ξ^3 , which was always the case for the experiments presented here.

For each correlation function, we have determined the value of $\langle n(q) \rangle^2$ in two ways. First, we obtained $\langle n(q) \rangle^2$ by taking an average of the number of counts in the delayed last 16 channels of the correlator. Second, we calculated $\langle n(q) \rangle^2$ as the square of the average of the photocounts detected during the acquisition of the correlation function. The two methods always provided values for $\langle n(q) \rangle^2$ that agreed within experimental uncertainty. In the analysis of the correlation functions, the second method of determining $\langle n(q) \rangle^2$ was always used.

For both protein solutions, we found that, in the vicinity of the critical point, $\langle n(q, t)n(q, 0) \rangle$ exhibited a significant deviation from exponential decay with time at all wave numbers studied. This deviation from exponential decay is much larger than the very small deviation expected for binary liquid mixtures [14]. However, it is interesting that large deviations from exponential decay of concentration fluctuations in the vicinity of the critical point have also been reported for a water-in-oil microemulsion [15].

Nonexponential correlation functions may be characterized in terms of a distribution of decay rates $G(\Gamma_i)$, so that $\langle \hat{\rho}(q, t)\hat{\rho}(q, 0) \rangle / \langle |\hat{\rho}(q)|^2 \rangle = \sum_i G(\Gamma_i) \exp(-\Gamma_i t)$, where $\sum_i G(\Gamma_i) = 1$. In order to obtain an estimate for $G(\Gamma_i)$ for each correlation function, we have used a constrained regularization algorithm [16], which is described in more detail elsewhere [9]. Representative distributions of decay rates that we obtain from the correlation functions are shown in Fig. 1. We found, for both solutions, that the distributions are extraordinarily broad at all wave numbers studied. In addition, as the critical point is approached a second component in the distribution appears with an average decay rate approxi-

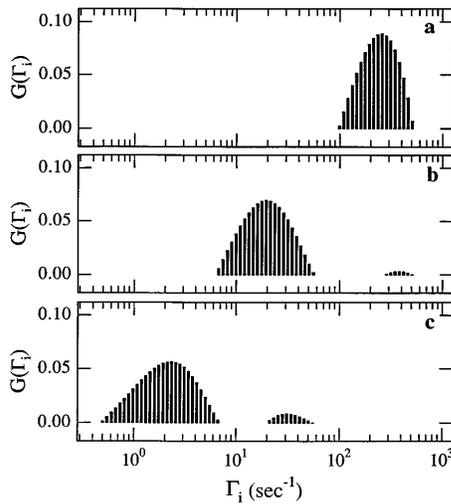


FIG. 1. Distribution of decay rates Γ obtained from representative autocorrelation functions using the regularization algorithm. All correlation functions were obtained at $q = 1.04 \times 10^5 \text{ cm}^{-1}$ ($\theta = 44.36^\circ$) using the solution in the 1.85 mm cell. (a) $T - T_c = 7.943^\circ$, $q\xi = 0.06$; (b) $T - T_c = 0.943^\circ$, $q\xi = 0.23$; (c) $T - T_c = 0.043^\circ$, $q\xi = 1.53$.

mately an order of magnitude larger than that of the major peak in the distribution. This second component never constitutes more than 5% of the total distribution. It is important to bear in mind that this small second component may arise because the regularization procedure erroneously separated a very broad distribution into two peaks. Interestingly, a double-peaked distribution of decay rates is predicted for a dense suspension of hard spheres [6].

It is evident in this figure that the average rate of decay decreases significantly as the critical point is approached. To characterize this critical slowing down, we have

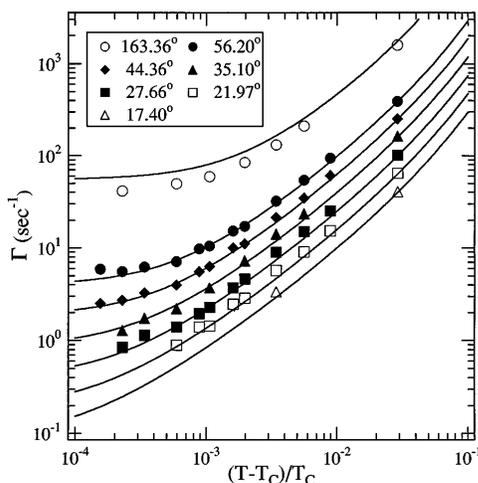


FIG. 2. The average decay rate Γ vs $(T - T_c)/T_c$ at various scattering angles θ for the solution in the 1.85 mm cell. The solid curves were obtained using Eqs. (2) and (3) with $\eta_B = (1.3 \pm 0.4) \times 10^2 \eta_{\text{H}_2\text{O}}$ and $q_C^{-1} = (0.7 \pm 0.3) \times 10^3 \text{ \AA}$.

determined the average decay rate Γ of the concentration fluctuations for each of the correlation functions that we obtained. Figure 2 contains a plot of Γ vs $(T - T_c)/T_c$ for all of the scattering angles θ studied for the solution in the 1.85 mm cell.

To the best of our knowledge, no theory has been formulated specifically for the critical dynamics of a binary mixture of large and small particles. However, sufficiently close to the critical point, the correlation length becomes much larger than both the size of the proteins and the distances between them. Under these conditions, it is reasonable to expect that not only the static but also the dynamic properties of the system will be universal. Therefore, we compare our data with the accepted theory for critical dynamics in binary liquid mixtures, which is based on the mode coupling approach of Kawasaki [5]. In this theory, the decay rate Γ may be written as $\Gamma = \Gamma_B + \Gamma_C$, where Γ_B and Γ_C are the background and the critical contributions, respectively, to Γ . The accepted expressions for Γ_B and Γ_C are [17]

$$\Gamma_B = \frac{k_B T q^2}{6\pi \eta_B \xi} \left(\frac{1 + q^2 \xi^2}{q_C \xi} \right), \quad (2)$$

$$\Gamma_C = \frac{k_B T q^2}{6\pi \eta_B \xi} \mathcal{K}(q\xi) \left(1 + \frac{q^2 \xi^2}{4} \right)^{z_\eta/2}, \quad (3)$$

with $\mathcal{K}(x) = (3/4x^2)[1 + x^2 + (x^3 - x^{-1}) \arctan(x)]$, and q_C a system-dependent constant with dimensions of wave number that characterize the relative contribution of Γ_B to the total decay rate. The viscosity $\eta = \eta_B(Q_0 \xi)^{z_\eta}$, where η_B is the background viscosity,

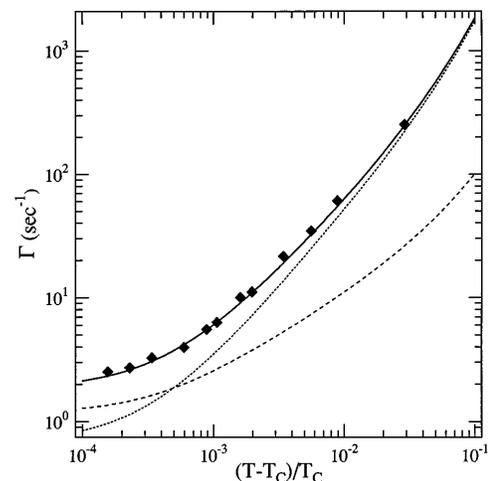


FIG. 3. The background Γ_B (dotted curve) and critical Γ_C (dashed curve) contributions to the decay rate Γ for $q = 1.04 \times 10^5 \text{ cm}^{-1}$ ($\theta = 44.36^\circ$). The curves were obtained using Eqs. (2) and (3) with $\eta_B = (1.3 \pm 0.4) \times 10^2 \eta_{\text{H}_2\text{O}}$ and $q_C^{-1} = (0.7 \pm 0.3) \times 10^3 \text{ \AA}$. The solid curve is the total decay rate $\Gamma = \Gamma_B + \Gamma_C$.

Q_0 is a system-dependent constant with dimensions of wave number, and z_η is an exponent that characterizes the divergence of the viscosity. The two system-dependent quantities Q_0 and q_C are related by $q_C \approx [4e^{4/3}/3\pi]Q_0$ [17,18]. We compare the data for Γ with the theoretical predictions by treating q_C and η_B as adjustable parameters. We find that the best fit to the data for the solution in the 5.6 mm cell is obtained with $\eta_B = (3 \pm 2) \times 10^2 \eta_{\text{H}_2\text{O}}$ (where $\eta_{\text{H}_2\text{O}}$ is the viscosity of water) and $q_C^{-1} = (1.2 \pm 0.5) \times 10^3 \text{ \AA}$. For the solution in the 1.85 mm cell, we find $\eta_B = (1.3 \pm 0.4) \times 10^2 \eta_{\text{H}_2\text{O}}$ and $q_C^{-1} = (0.7 \pm 0.3) \times 10^3 \text{ \AA}$. In Fig. 2, the predictions of this theory are compared with the data for the solution in the 1.85 mm cell. The individual background and critical contributions to the decay rate are shown, for one particular scattering angle, in Fig. 3. The values for η_B that we find for both solutions are 2 orders of magnitude larger than $\eta_{\text{H}_2\text{O}}$. The value of q_C^{-1} for both solutions is more than an order of magnitude larger than the size of an individual protein molecule. By comparison, in binary liquid mixtures q_C^{-1} is only several angstroms [19,20], which is comparable to the range of the intermolecular potential. In a water-in-oil microemulsion, it has been found that q_C^{-1} is on the order of the size of the droplets [21].

In summary, the critical dynamic behavior of aqueous protein solutions differs from that of binary liquid mixtures in that (i) the concentration fluctuations exhibit significant deviation from exponential decay with time, and (ii) unusually large η_B and q_C^{-1} are required to fit Eqs. (2) and (3) to the experimental data for Γ . Thus, our work implies that the present theory for critical dynamics does not provide an adequate description of the behavior in aqueous protein solutions.

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