

Homogeneous Crystal Nucleation Near a Metastable Fluid-Fluid Phase Transition

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Several scenarios exist for the protein crystallization and aggregation in solutions near a metastable fluid-fluid phase separation below the solubility line. Based on computations, it was proposed that the fluid-fluid critical point enhances the crystallization rate by many orders of magnitude, while, based on experiments, it was proposed that the fluid-fluid spinodal controls the crystallization rate. Using molecular dynamic simulations for an isotropic model with sticky interaction, we show that neither of these scenarios adequately describes the crystallization mechanism near a metastable fluid-fluid phase separation. We find that the emergence of the high-density fluid inside the spinodal drastically enhances the crystal nucleation in the subcritical region following Ostwald's rule of stages.

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Protein aggregation is believed to cause neurodegenerative diseases, such as Alzheimer's, Huntington's chorea, and Parkinson's; prion diseases, such as bovine spongiform encephalopathy; blood diseases, such as sickle-hemoglobin (HbS) anemia; and less fatal but widespread pathologies, such as eye cataracts—a common cause of blindness. The repercussions of protein aggregation, together with the need for methods to produce better protein crystals for structural studies, has increased scientific interest in understanding the mechanisms of nucleation of solidlike clusters of proteins. Studies [1–5] suggest that the fluid-fluid separation between protein-poor and protein-rich phases could play a relevant role in this process. Thus, one strategy to control the process could be to find the fluid-fluid phase boundaries and develop a protocol to modify them to reduce their triggering effect. Following Rosenbaum, Zamora, and Zukoski [6], we model globular proteins with a short-range attractive interaction potential, as those used for uncharged spherical colloids. When the range of attraction is less than 25% of the colloid diameter, only gas and crystal phases are stable, and the gas-liquid coexistence becomes metastable below the sublimation line [7]. In this approach, the colloid density ρ replaces the protein concentration and the sublimation line $T_m(\rho)$ replaces the solubility line.

According to the classical nucleation theory (CNT) [8], the nucleation rate I is defined as

$$I \equiv \kappa \exp\left(\frac{-\Delta G}{k_B T}\right), \quad (1)$$

where κ is the kinetic prefactor, $\Delta G = (16\pi\gamma^3)/(3\rho_c^2|\Delta\mu|^2)$ is the activation free energy barrier, ρ_c is the number density of the crystal, $\Delta\mu < 0$ is the difference in chemical potential between the crystal and the liquid, γ is the crystal-liquid interfacial free energy density, k_B is the

Boltzmann constant, and T is the absolute temperature. In the CNT picture, increasing the supersaturation, i.e., increasing $|\Delta\mu|$, lowers the nucleation barrier. If the surface free energy γ were independent of $\Delta\mu$, then ΔG would always decrease with increasing $|\Delta\mu|$. Criticisms of standard approximations of the CNT have been presented by several authors [9].

According to the Becker-Döring formalism to nucleation in condensed systems, Turnbull and Fisher [10] proposed an approximate expression for the nucleation time $\tau \equiv 1/I \equiv A \exp(\Delta G/k_B T)$, where $A \equiv 1/\kappa$,

$$\frac{\Delta G}{k_B T} = B \frac{T_m^2}{T(T_m - T)^2} \equiv \chi B, \quad (2)$$

$B \equiv (16\pi\gamma^3)/(3k_B\rho_c^2\Delta h^2)$, Δh is the enthalpy difference between the crystal and the fluid at coexistence, and $\chi \equiv \chi(\rho, T) \equiv T_m^2(\rho)/\{T[T_m(\rho) - T]\}$ is the CNT scaling parameter. A standard assumption is that A and B weakly depend on ρ ; hence, the nucleation rate I is dominated by the degree of supercooling $T_m - T$.

However, this conclusion is inconsistent with simulation results for narrow attractive potentials. In the vicinity of the metastable gas-liquid critical point at a temperature T_c , ten Wolde and Frenkel (tWF) found that ΔG is lower than expected from the CNT Eq. (2) [2]. It was concluded that there is an enhancement of crystal nucleation by critical density fluctuations near a metastable fluid-fluid critical point. This is consistent with experiments showing that large density fluctuations associated with fluid-fluid demixing could facilitate protein crystal nucleation. Specifically, Vaiana *et al.* [4] established for various hemoglobin concentrations a direct quantitative link between HbS fiber nucleation kinetics and the temperature T_s of the fluid-fluid spinodal. This result suggests that the crystallization rate at temperature T outside the spinodal region is

governed by the temperature distance $T - T_s$ [4]. However, both Vaiana *et al.* and tWF analyzed only specific paths to approach the spinodal. Vaiana *et al.* considered only data for subcritical protein concentration, while tWF considered paths along iso-CNT lines in the T - ρ phase diagram, i.e., loci at a constant value of χ , at $T \geq T_s$. Here, we ask whether these interpretations can be extended for any metastable fluid-fluid critical point.

We consider the case of a short-range square-well potential

$$U(r) \equiv \begin{cases} \infty & r < a \\ -U_0 & a < r < b, \\ 0 & r > b, \end{cases} \quad (3)$$

where a is the hard core diameter, b is the attractive well diameter, and U_0 is the attraction energy. The ratio b/a determines whether the fluid has a metastable liquid phase [11], $b/a = 1.25$ being the maximum value to have a metastable critical point, and $b/a = 1.005$ being the lower limit for a detectable metastable critical point [12]. We choose $b = 1.06a$ for which we can reliably detect the metastable fluid and observe hundreds of nucleation events in the vicinity of the fluid-fluid critical point in a relatively small system. We perform constant $\rho \equiv N/V$ and constant T simulations for $N = 1728$ particles of mass m in a cubic box of volume V with periodic boundary conditions. We adopt standard discrete molecular dynamics simulations with a modified Berendsen thermostat, calibrating the thermostat heat-exchange coefficient in such a way as to attain the desired T within a time $\Delta t_N = 200t_0$, where $t_0 \equiv a\sqrt{m/U_0}$ is the unit of time, and within a tolerance $\Delta T = 0.001U_0/k_B$ [13].

For each T - ρ state point, we perform 100 independent runs, quenching the system to a desired T at fixed ρ from fluid configurations at $T = 0.5U_0/k_B$, and find a phase diagram with a metastable fluid-fluid spinodal ending in a critical point (Fig. 1). The pressure $P_m(T)$ of sublimation is calculated as the time average over $25000t_0$ of the equilibrium pressure achieved at constant T in the gaseous phase in contact with the face-centered-cubic (fcc) crystal made of $N = 16 \times 16 \times 16$ particles, cut along the [001] plane in an elongated simulation box of fixed total V . The equilibrium density of gas, $\rho_m(T)$ was computed in the simulations of pure phases at constant $N = 4096$, $P = P_m(T)$, and T . Crystal nucleation times τ faster than the fluid equilibration time τ_e do not allow us to calculate the spinodal line for $T < 0.382U_0/k_B$ at high ρ and for $T < 0.354U_0/k_B$ at low ρ . We test the finite size effect on the spinodal by comparing results for $N = 1728$ and $N = 850$, finding no significant difference. For $T \geq 0.384U_0/k_B$, we can calculate both the low- and high- ρ branch of the spinodal, allowing us to estimate the coexistence line.

To determine the equilibration time τ_e for the metastable fluid after the quench, we find the time of first local

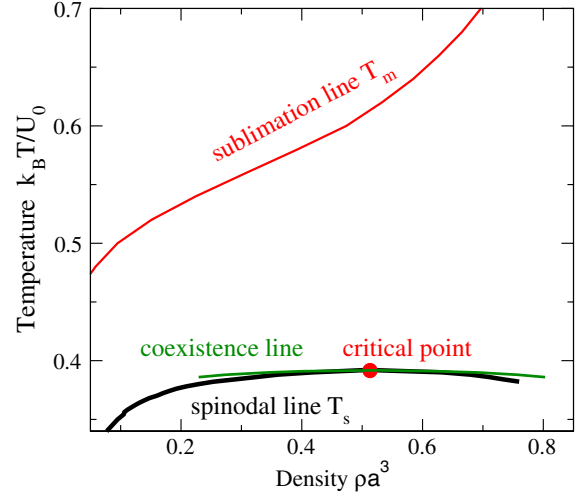


FIG. 1 (color online). T - ρ phase diagram for the potential in Eq. (3). Below the sublimation line, we calculate the metastable gas-liquid spinodal line by connecting minima and maxima along the isotherms in the P - ρ plane and the coexistence line from the equal-area Maxwell construction in the P - V plane. The point of merging of these two lines corresponds to the metastable critical point. By interpolating the minima of the inverse compressibility for the isotherms at $T = 0.392U_0/k_B$ and $T = 0.391U_0/k_B$, we locate the critical point at $T_c = (0.3916 \pm 0.0005)U_0/k_B$, $\rho_c = (0.523 \pm 0.005)(1/a^3)$, and $P_c = (0.0519 \pm 0.0005)U_0/a^3$, with $T_c/T_m \approx 0.64$ at ρ_c .

minimum, t_* , of the graph of the potential energy $\langle U(t) \rangle$ averaged over 20 independent runs and then average t_* over five independent blocks of 20 runs. When computing $\langle U(t) \rangle$, we make sure that the system remains in the fluid phase, i.e., checking that (i) the root mean square displacement of the particles in $100t_0$ is greater than the typical interparticle distance and (ii) the system does not crystallize. As a criterion to define the crystal phase, we calculate the t dependence of the size S of clusters of nearest neighbors (NN) particles, i.e., particles within a distance $r < b$, which have local crystalline environment. Namely, for each particle i , we compute vectors $\vec{r}_{i,j} \equiv (r_{i,j}, \theta_{i,j}, \phi_{i,j})$ to its $n = 12$ closest particles $j = 1, \dots, 12$ in terms of spherical coordinates and define

$$Q_6^i \equiv \sqrt{\frac{4\pi}{13} \sum_{m=-6}^6 |\langle Y_m^i \rangle|^2}, \quad (4)$$

where

$$\langle Y_m^i \rangle \equiv \frac{1}{n} \sum_{j=1}^n Y_6^m(\theta_{i,j}, \phi_{i,j}), \quad (5)$$

and $Y_\ell^m(\theta, \phi)$ are orthonormal spherical harmonics. For a particle i in a perfect fcc crystal $Q_6^i = 0.575$, while for a particle in a perfect hexagonal-close-packed crystal $Q_6^i = 0.485$. We find that the probability of each value of Q_6^i shows large peaks at the fcc and hexagonal-close-packed

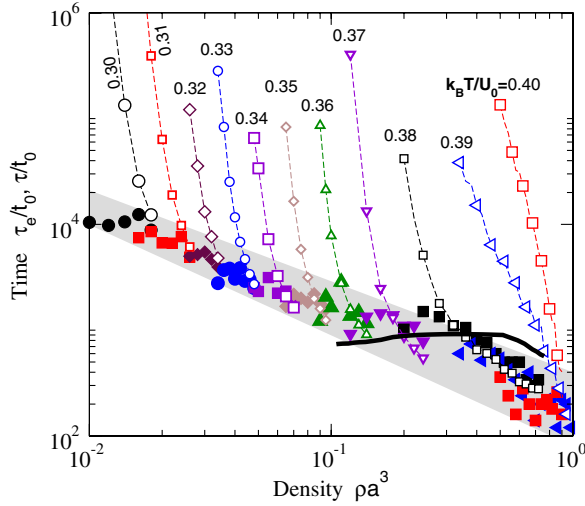


FIG. 2 (color online). Equilibration time τ_e (closed symbols) and nucleation time τ (open symbols) as function of density ρ for different temperatures T (labels near symbols) in units of U_0/k_B . At each ρ , the metastable fluid is defined only when $\tau > \tau_e$, above the gray area; otherwise, the crystallization is instantaneous compared to τ_e . Along the spinodal line, the values of τ (connected by a solid line) are larger than τ_e only for $0.38 \leq k_B T/U_0 \leq 0.39$ and $\rho > 0.3/a^3$.

values only when the particle i has at least $z_i = 11$ particles in the NN shell. Therefore, we classify particle i as fluid-like if $z_i < 11$ and crystal-like if $z_i \geq 11$ and $Q_0^i \geq 0.475$ [14]. We observe that when a crystal-like cluster reaches size $S = 5$, the system irreversibly crystallizes [15]. Hence, $S = 5 \geq S_c$ is the size of the critical nucleus for the crystallization, and τ_5 is the time at which the system has at least one crystal-like cluster with $S = 5$. When the crystallization starts, S increases very sharply with time; therefore, we define the crystal nucleation time $\tau \equiv \langle \tau_5 \rangle$, averaged over 100 independent simulations at the same T and ρ . When $\tau_e < t < \tau$, the system is in its metastable fluid phase (Fig. 2) [16].

To test the theory of Vaiana *et al.* [4], we plot τ along isochores as a function of $T - T_s(\rho)$ (Fig. 3). The theory predicts that all the data should collapse on a single curve because τ only depends on $T - T_s(\rho)$. We observe that the scaling holds for a limited range of $\rho < \rho_c$, corresponding to the subcritical case considered by Vaiana *et al.* [4], but breaks down for $\rho > \rho_c$ and for $\rho \ll \rho_c$.

We plot the loci of equal τ in the T - ρ phase diagram and do not observe any special feature when these lines approach the metastable critical point. To determine whether this is consistent with the tWF theory [2], we calculate the iso-CNT lines from Eq. (2) for constant A and B (Fig. 4). Along the iso-CNT line around T_c , tWF predict that the $\Delta G/k_B T$ has a minimum at T_c , which corresponds to a minimum of τ at T_c for constant A . However, in the present case we observe that τ decreases monotonically for decreasing T along the iso-CNT line around T_c . The decrease

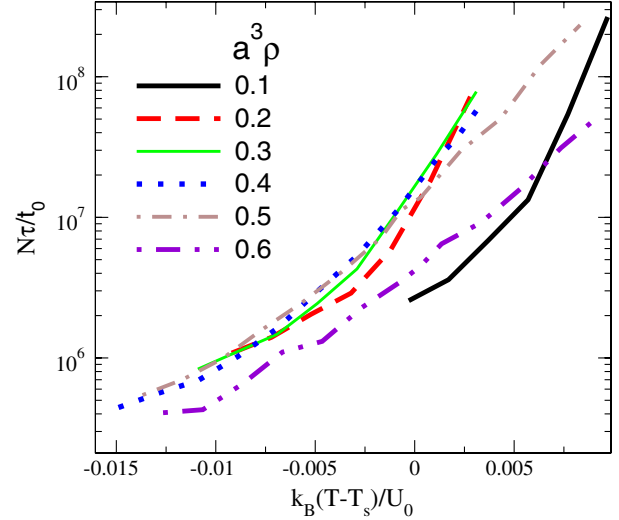


FIG. 3 (color online). The scaling of the crystal nucleation time τ with respect to the spinodal temperature T_s , proposed by Vaiana *et al.*, only holds for the subcritical range $0.2 < a^3 \rho < 0.5$ but fails outside of it. Lines correspond to different densities.

is even more dramatic along iso-CNT lines at subcritical densities. Therefore, either the tWF theory does not describe our results or A is not constant in the vicinity of T_c .

It is clear that the presence of the spinodal affects the loci of constant τ , with one approaching the other at $a^3 \rho < 0.15$. At this low- ρ range, the loci of constant τ seems to follow the iso-CNT lines. However, a closer look of the plot shows that along the iso-CNT line τ decreases by one order of magnitude within a density range $\Delta \rho = 0.03 a^3$, with a large deviation from the CNT prediction.

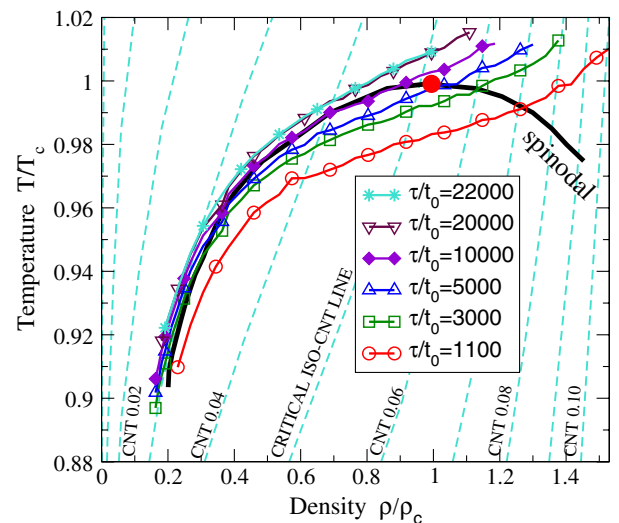


FIG. 4 (color online). The loci of equal τ (symbols) are compared with the iso-CNT lines (dashed lines) for different values of CNT parameter χ (labels near dashed lines, expressed in units of U_0/k_B) and the spinodal (solid) line, the vertex of which is the metastable critical point (full circle).

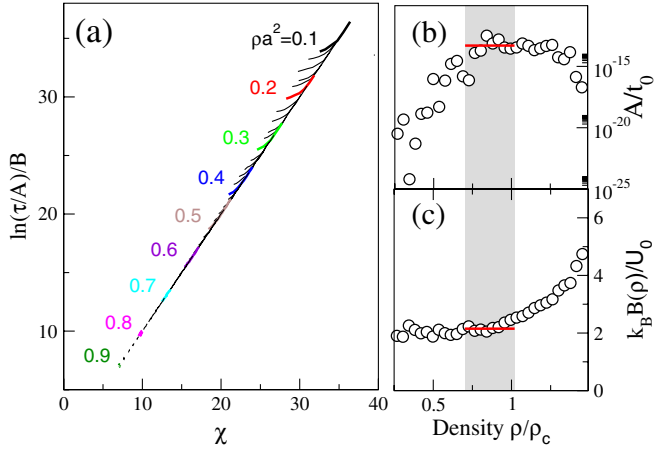


FIG. 5 (color online). (a) Scaling plot of nucleation time τ along different isochores (with labels near lines for a selected number of them) following Eq. (2) with strongly ρ -dependent A and B parameters. Deviations from the straight line occurs for $\tau \approx \tau_e$ (gray region in Fig. 2). (b) The ρ dependence of A , inverse of the kinetic prefactor κ , as resulting from our fitting to collapse data in (a). The shaded region corresponds to the density range of the critical iso-CNT line, where $A \approx 5 \times 10^{-14} t_0$ (horizontal line). Around the critical density, A reaches a maximum plateau and κ is minimum. (c) The ρ dependence of B , in units of U_0/k_B , from the fitting in (a). B increases approaching ρ_c from subcritical densities. The red line (at $B = 2.15 U_0/k_B$) is a guide for the eyes, the shaded region is as in (b).

Nevertheless, if we allow both parameters A and B to be strongly dependent on ρ , but independent of T , we achieve a satisfactory collapse of τ with respect to χ (Fig. 5).

We find that A is approximately constant within the density range of the critical iso-CNT line [Fig. 5(b)]. This implies, according to the above discussion, that the tWF theory does not describe our results. According to Eq. (2) and along an iso-CNT line (constant $T_m - T$), $\Delta G/k_B T \sim B(\rho(T))$. Thus, the decrease of B within the density range of the critical iso-CNT line implies a decrease of $\Delta G/k_B T$ for $T < T_c$ along the critical iso-CNT line, at variance with the tWF theory. The saturating behavior of B for $\rho < \rho_c$ is consistent with the fact that for low ρ the slopes of the CNT lines approach the slopes of the equal- τ lines in Fig. 4.

For subcritical densities, the rapid decrease of A is consistent with the large decrease of τ along iso-CNT lines (Fig. 4), considering the fact that B is approximately constant in the subcritical region as shown in Fig. 5(c). We note that this decrease of A (increase of the kinetic prefactor κ) occurs within the spinodal region, where the fluid separates into a low-density fluid and a high-density fluid. Therefore, the dominating mechanism for the crystallization within the spinodal region at this range of densities is the large increase of the collision rate due to the formation of the high-density fluid.

For supercritical region, e.g., $\rho > 1.25\rho_c$, we find that A decreases with increasing density, opposite to the behavior of B . Thus, according to Eq. (2), the large increase of τ along iso-CNT at $\rho > \rho_c$ shows that the increase of B overpowers the decrease of A . We understand the maximum of $A(\rho)$ in the vicinity of ρ_c to be a consequence of the critical slowing down of the dynamics near the critical point.

The behavior of B allows us to predict up to a 50% decrease of the free energy barrier for the crystal nucleation approaching the fluid-fluid critical point along the iso-CNT line from the supercritical region, favoring the increase of the crystallization rate. However, the rate has no maximum around the critical point. Specifically, we find that the nucleation time τ monotonically decreases with decreasing density along iso-CNT lines. In the subcritical region below the spinodal, τ rapidly decreases as a consequence of the increase of rate of collisions due to the high- ρ phase. At the critical point, the slowing down is maximal, determining the minimum kinetic prefactor κ . Nevertheless, the increase of κ in the supercritical region is overpowered by the increase of the free energy barrier, here proportional to B , determining an increase of τ . Hence, we conclude that the presence of a metastable fluid-fluid phase transition does not necessarily imply an enhancement of the nucleation rate in the vicinity of the critical point, clarifying that the tWF theory [2] does not hold for all metastable fluid-fluid phase transition. On the other hand, the effect of the metastable spinodal line is evident but cannot be interpreted as in the scaling theory proposed by Vaiana *et al.* [4], which we show holds only for subcritical conditions. However, the metastable fluid-fluid phase transition drastically favors the crystal nucleation process due to the high- ρ fluid phase formation following Ostwald's rule of stages on the route to the crystallization. A similar result has been found recently in simulations for silicon [17] and in experiments for water-glycerol mixture in the vicinity of a liquid-liquid phase transition [18].

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- [14] We find that our system has no relevant amount of body-centered-cubic crystal-like clusters, corresponding to a characteristic value $Q_6 = 0.511$.
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