On the Interpretation of Quantitative Experimental Data on Nucleation Rates Using Classical Nucleation Theory

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We consider how experimental data for nucleation rates can be analyzed if it is assumed that classical nucleation theory is a reasonable description of the nucleation. In particular, we consider in detail experimental results for the nucleation of the protein lysozyme. Classical nucleation theory makes predictions for the functional form of the nucleation rate; physically reasonable bounds can be placed on the parameters of this functional form. The experimental data for lysozyme implies values for a parameter (the preexponential factor) that are outside the physically reasonable bounds for homogeneous nucleation but are consistent with heterogeneous nucleation. Also, the functional form of the rate suggests that there is a distribution of barrier heights. Such a distribution is likely for heterogeneous nucleation but not possible for homogeneous nucleation. Modeling experimental data with classical nucleation theory is a generally applicable way of distinguishing between homogeneous and heterogeneous nucleation.

1. Introduction

Nucleation is an activated process and it relies on a rare event, the formation of a critical nucleus. The critical nucleus has a high free energy and so a low probability of forming, and this makes the time scale for nucleation much larger than the characteristic time scale of the microscopic dynamics of the system. This nucleus may form in the bulk, in which case we have homogeneous nucleation. It may also form in contact with a foreign object, either something in solution or the walls of the container, in which case we have heterogeneous nucleation. The standard theory for nucleation is classical nucleation theory (CNT). Here we will assume CNT describes the nucleation in an experimental system in the sense that its assumptions about the basic physics of nucleation are correct. We do not require it to be quantitatively adequate. Having made this assumption, we see how experimental data can be analyzed with the help of CNT. We find that by fitting the data to expressions obtained within CNT, it is possible to distinguish between homogeneous and heterogeneous nucleation and between the presence of a single barrier height and a distribution of barrier heights. These are fundamental aspects of nucleation, and so it is crucial to be able to distinguish between these possibilities in order for the experimental findings to be understood.

Briefly, what we do is use the fact that CNT expresses the rate as an exponential factor times a prefactor that is proportional to the number density of sites for nucleation. This density of sites is orders of magnitude lower for heterogeneous nucleation than for homogeneous nucleation, and thus the size of the prefactor discriminates between the two. The prefactors obtained by fitting to experimental data[1,2] are orders of magnitude too small to be consistent with homogeneous nucleation. Thus we conclude that the experiments are observing heterogeneous nucleation. Having done this, we consider the possibility that there are a range of barrier heights. We find that this changes the functional form of the supersaturation dependence of the rate, taking it closer to the form found in experiment. A distribution of barrier heights is to be expected if the nucleation is heterogeneous. Then the nucleation occurs on impurities, which of course will not be all identical and so the barriers for nucleation will vary from impurity to impurity.

In the next section, we outline CNT for both homogeneous and heterogeneous nucleation and estimate some of the input parameters for the specific experimental system we consider here: the nucleation of crystals of the protein lysozyme from a dilute solution. In Section 3, we will consider the available experimental data for lysozyme and compare it with the CNT we have outlined above. Following that, we calculate the effect of having a distribution of barrier heights. Our last section is the conclusion.

2. Elements of the Classical Theory of Nucleation

CNT expresses the nucleation rate per unit volume as the product of an exponential factor and a prefactor. The exponential factor is $\exp(-\Delta F^*/kT)$, where ΔF^* is the free energy cost of creating the nucleus at the top of the barrier, called the critical nucleus. The prefactor ρZj is the product of three terms: ρ is the number density of molecules, Z is the Zeldovich factor, and j is the rate at which molecules attach to the nucleus. Physically, ρ is is the number density of sites for nucleation as the nucleus can form around any molecule, and Zj is the rate at which the nucleus at the top of the barrier actually crosses it. Thus, within CNT, the rate of homogeneous nucleation per unit volume is given by[3]

$$\text{rate} = \rho Zj \exp(-\Delta F^*/kT) \tag{1}$$

By definition, the probability of an event is proportional to the exponential of minus the free energy cost of the event over the thermal energy kT. Thus the exponential factor comes from the (very low) probability of forming a nucleus at the top of the barrier. CNT is described in a number of excellent books and reviews,[3−5] so we will not review it here. However, note that the exponential factor typically varies much faster with super-

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saturation than the prefactor, and so the prefactor is often taken to be a constant.

Heterogeneous nucleation is also an activated process, and so if all nucleation sites on the impurities have identical nucleation barriers, then the functional form is the same as for homogeneous nucleation. However, the prefactor contains, not the number density of molecules, but the number density of sites for the nucleation, ρ_I: the number density of impurities times the number of places a critical nucleus can form on each impurity. Thus for heterogeneous nucleation with a single nucleation barrier, we have

$$\text{rate} = \rho_I Z j \exp(-\Delta F^*/kT) \tag{2}$$

where ΔF^* is now the barrier for heterogeneous nucleation, which will depend not only on the supersaturation but also on the nature of the surface of the impurity. Impurities are presumably rather variable, and so we would expect the barrier to vary from nucleation site to nucleation site. We will consider this possibility below, but for the moment we will assume that heterogeneous nucleation is characterized by a single barrier height.

Having obtained the functional forms of homogeneous and heterogeneous nucleation, we will consider the values of the parameters that are appropriate for the protein lysozyme. We will look at the prefactor and the barrier in turn.

An Estimate for the Prefactor for Lysozyme. For homogeneous nucleation, the prefactor is $\rho Z j$. For a dilute protein solution, $\rho = 10^{24}$ m^{-3}. The Zeldovich factor is approximately $[\partial^2(\Delta F/kT)/\partial n^2]^{1/2}$, evaluated at the top of the barrier, where ΔF is the free energy as a function of the number of molecules in the nucleus n. It is dimensionless. Within CNT, the second derivative of the free energy at the top of the barrier scales as $kT/(n^*)^{4/3}$, where n^* is the excess number of molecules in the critical nucleus. Thus within CNT $Z \simeq 1/(n^*)^{2/3}$, and so for $n^* = 10-100$, Z is of the order 0.1 or 0.01.

If the radius of the critical nucleus is R^*, then an upper bound on j is provided by the diffusion-limited flux onto a sphere of radius R^*. This upper bound is $j \simeq \rho D R^*$. The nucleus radius should be a few protein molecules and so of the order of 10 nm. The diffusion constant D for small proteins in dilute solution is around 100 μm^2 s^{-1}.[6] Thus we have an upper bound for $j \simeq 10^6$ s^{-1}. Combining this value for j with $Z = 0.1$, we obtain a prefactor of 10^{29} m^{-3} s^{-1}.

Now, Z is unlikely to be much below 0.01 unless the nucleus is huge. The density ρ is known. This leaves j as the major source of uncertainty in the prefactor. Unfortunately, the dynamics of attachment to the critical nucleus cannot be observed in experiment. However, the growth rate of large protein crystals can be measured.[7] These rates are sensitive to supersaturation, but at the supersaturations, we will consider below the rates are of the order of 1 nm s^{-1}.[7] These supersaturations are concentrations of approximately 10 times the concentration of the solution that coexists with the crystal. A growth rate of 1 nm s^{-1} corresponds to an attachment flux of less than one molecule per lattice site per second. To obtain a conservative lower bound on the nucleation rate, we take a j for the complete nucleus of 0.1 s^{-1}. We also set $Z = 0.01$. Then the estimated lower bound on the prefactor is 10^{21} m^{-3} s^{-1}. This is only a rough lower bound, but note that it assumes that the critical nucleus takes seconds to cross the barrier. The attachment flux j cannot be more than an couple of orders of magnitude lower than we assume, as that would suggest that the critical nucleus takes a hour to grow by a few molecules, yet nucleation can occur in an hour.

For heterogeneous nucleation, the prefactor is $\rho_I Z j$, i.e., a factor of ρ_I/ρ smaller than that for homogeneous nucleation. As the impurities are not controlled, we do not know ρ_I, but we expect ρ_I to be orders of magnitude lower than ρ. For example, if we have 1000 impurity sites in a 1 μl droplet, then $\rho = 10^9$ m^{-3} and the ratio $\rho_I/\rho = 10^{-15}$.

The Barrier. Having considered the prefactor, let us consider the free energy barrier. For homogeneous nucleation, the free energy barrier, ΔF^*_{HOMO}, is given by

$$\Delta F^*_{\text{HOMO}} = \frac{16\pi}{3}\frac{\gamma^3}{(\rho_X \Delta\mu)^2} \tag{3}$$

if the nucleus is assumed to be spherical. In eq 3, $\Delta\mu$ is the chemical potential of the phase the nucleus is forming in minus the chemical potential of the phase of the nucleus. The interfacial tension between the nucleating phase and the surrounding phase is γ. The number density in the crystalline nucleus is ρ_X. For nucleation from a dilute solution, $\Delta\mu/kT = \ln(\rho/\rho_{\text{co}}) = s$ where ρ_{co} is the number density at coexistence with the crystal. This equation defines our reduced chemical potential s. Within CNT, the radius of the critical nucleus is $R^* = 2\gamma/(\rho_X \Delta\mu)$.

We can easily estimate the size of ΔF^*_{HOMO}. For molecules of diameter d, the number density of a crystalline phase will be $\rho_X \simeq d^{-3}$. Creating an interface should break one bond of free energy a few kT per protein molecule at the interface, thus $\gamma \simeq kTd^{-2}$. Putting these estimates into eq 3, we have a free energy barrier $\Delta F^*/kT \simeq (10-100)/s^2$. Note that the barrier is sensitive to γ as it varies as γ^3 but that it should not be much larger than $100/s^2$ unless the interfacial tension is very large. The barrier dominates the variation of the nucleation rate with supersaturation, and hence, in nucleation studies, usually the most effort is focused on calculating and understanding the barrier, with little effort devoted to the prefactor. However, here we will carefully consider the prefactor because, as we will see, the size of the barrier is unable to tell us much about the mechanism of the nucleation but the size of the prefactor does.

If the nucleation barrier ΔF^*_{HOMO} is many kT, then homogeneous nucleation will be slow. However, often the interaction between the surface of an impurity and the nucleus dramatically reduces the free energy of the nucleus. Then the rate near a surface can be many orders of magnitude higher than in the bulk, resulting in heterogeneous nucleation dominating and homogeneous nucleation being irrelevant. Impurities may be very variable; for simplicity, we will consider relatively large impurities, at least as large as the critical nucleus, which we expect to be perhaps 10 nm or a fews 10s of nm across. Smaller impurities will reduce the barrier by smaller amounts.

Having assumed the impurities provide a surface larger than the nucleus, we start by considering this surface to be smooth and flat. It is straightforward to extend CNT to nucleation on a smooth flat surface, and this was done by Volmer in the 1920s.[3,5,8] The result is that the barrier is

$$\Delta F^*_{\text{FLAT}} = \Delta F^*_{\text{HOMO}} f(\theta) \tag{4}$$

i.e., it differs from the barrier to homogeneous nucleation only by the presence of a function of θ. The contact angle θ is the angle the interface between the nucleus and the surrounding bulk phase makes with the surface. Attractions between the surface and the molecules that are stronger than those between the molecules in the nucleus will lead to a small angle θ as the nucleus spreads across the surface to maximize its contact area with the surface. However, if the surface tends to repel the

molecules, then the nucleus is pushed away from the surface, resulting in a contact angle $\theta > 90°$. The function $f(\theta)$ decreases monotonically as θ decreases.

There is no reason to expect the surface to be perfectly flat on the length scale of the nucleus R^*. As an example of a nonflat surface, we consider a wedge formed from two intersecting smooth planes. The CNT result for the barrier in such a wedge was calculated by Sholl and Fletcher.[9] It is that the barrier $\Delta F^*_{\mathrm{WEDGE}}$ is given by a generalization of eq 4, $\Delta F^*_{\mathrm{WEDGE}} = \Delta F^*_{\mathrm{HOMO}} f_W(\theta, \alpha)$,[9] where α specifies the angle at which the two planes meet. Again, the scaling of the nucleation barrier with s is identical to that in homogeneous nucleation. In all three cases, homogeneous nucleation, heterogeneous nucleation on a smooth plane, and heterogeneous nucleation in a wedge, the scaling of the nucleation barrier with supersaturation is the same; it is the standard CNT scaling of $1/s^2$. Thus the presence of such scaling in experimental data does not distinguish between homogeneous and heterogeneous nucleation with a single barrier height.

3. Nucleation in Protein Solutions

The nucleation rate of the protein lysozyme has been studied quantitatively by a number of groups.[1,2,10−14] The results obtained by these groups do not agree quantitatively.[15−17] The first results were obtained by Galkin and Vekilov.[1] They fitted their results to an equation of the CNT form and obtained

$$\text{rate} = 10^9 \exp(-65/s^2)\ \text{m}^{-3}\ \text{s}^{-1} \tag{5}$$

which is their eq 5 converted into S. I. units. The nucleation occurred at concentrations around 50 mg/ml. This rate was ascribed to homogeneous nucleation, and they also observed the formation of a few nuclei that formed very rapidly, which they ascribed to heterogeneous nucleation. They did not count those nuclei in the rate of eq 5. First, let us consider the factor in the exponent. Assuming that eq 3 applied, they obtained a crystal−solution interfacial tension γ of the order of 1mJ m^{-2}. Taking lysozyme to be 3 nm across, a surface tension of kT per area of 9 nm^2 is 0.4 mJ m^{-2}. So their value is physically very reasonable.

Bhamidi et al.[2] also used an expression of the form of eq 5 and obtained a very similar interfacial tension. However, in what they refer to as the "homogeneous region", they obtain a prefactor of approximately 10^{11} m^{-3} s^{-1}, see their Figure 4a[2]. This is 2 orders of magnitude larger than Galkin and Vekilov's prefactor[1].

In the previous section, we obtained an estimated lower bound on the prefactor of 10^{21} m^{-3} s^{-1}. This was if the nucleation was homogeneous. We see that the prefactors found by fitting to the experimental data are 10 or 12 orders of magnitude smaller than this lower bound. By comparison, fitting an expression of the CNT form to experimental data for hard-sphere colloids gave prefactors between 1 and 3 orders of magnitude smaller than a simple approximation to the prefactor.[18] Also, comparison of the CNT prediction for the prefactor with accurate computer simulation predictions of this factor[19,20] show that CNT's estimate for the prefactor for hard spheres actually gives the right order of magnitude. It is not known why fitting to experimental data gives a prefactor up to 3 orders of magnitude smaller than that found via simulation and CNT.

Size of the Critical Nucleus. Galkin and Vekilov[1] also used the nucleation theorem[4,21,22] to estimate the size of the critical nucleus. The nucleation theorem is

$$\frac{\partial \Delta F^*}{\partial \Delta \mu} = -n^* \tag{6}$$

where n^* is the excess number of molecules in the critical nucleus. Now, the derivative of the log of the rate of eq 1 with respect to $s = \Delta\mu/kT$ is

$$\frac{\partial \ln(\text{rate})}{\partial s} = \frac{\partial \ln(\rho Z j)}{\partial s} - \frac{\partial \Delta F^*/kT}{\partial s} \tag{7}$$

If we assume that the chemical potential dependence is dominated by the term coming from the barrier, then we can neglect the first term on the right-hand side. The second term is simply n^*. Thus we have

$$\frac{\partial \ln(\text{rate})}{\partial s} \simeq n^* \tag{8}$$

which also holds for heterogeneous nucleation. Thus, if in an experiment the rate is measured over a range of supersaturations and the variation of the chemical potential with supersaturation is known, then an estimate of the excess number of molecules in the critical nucleus can be obtained.

Galkin and Vekilov used eq 8 to obtain an n^* of approximately 10, which was essentially constant over the range of supersaturations they were able to cover. Note that this disagrees with the CNT prediction. Inserting the CNT ΔF^*, eq 3, into eq 6, we obtain

$$n^* = \frac{32\pi}{3} \frac{\gamma^3}{\rho_X^{\,2} (\Delta\mu)^3} \tag{9}$$

This is far from constant, it varies as $1/s^3$.

Conclusions of the Analysis of the Experimental Data. We conclude that CNT for homogeneous nucleation does not describe the experimental data well, in particular: (i) when the CNT form for the rate is fit to experimental data, the prefactor is orders of magnitude too small, and (ii) the CNT prediction of an n^* that varies rapidly with s disagrees with the experimental finding.

First, let us consider the prefactor. It is essentially a number density over a time. The number density is that of protein molecules, which is 10^{24} m^{-3}. Thus, to obtain a prefactor of 10^{11} m^{-3} s^{-1}, the time must be of the order of 10^{13} s, an extremely long time scale for a protein solution. This is hard to understand. However, if we assume that the nucleation is heterogeneous, then the prefactor contains the number density of nucleation sites on impurities ρ_I, not that of protein molecules, see eq 2. The impurity density will be orders of magnitude lower than the density of protein molecules, and so this automatically gives us the dramatic reduction in the prefactor we need to get agreement with experiment. If the density of impurities upon which the nucleation is occurring is a factor of at least 10^{10} smaller than the density of protein molecules, we obtain a prefactor of the correct size to agree with the experimental data.[1,2,10] Galkin and Vekilov[1] observed the very rapid (faster than they could measure) nucleation of a few nuclei, followed by a nucleation at a slower rate. It was this slower rate, which they ascribed to homogeneous nucleation, that they measured, leading to their fit, our eq 5. If our CNT modeling is correct, both the fast and slower nucleation are heterogeneous but occur on different impurities. The fast occurs on impurities that lower the barrier so much that the nucleation is effectively instant on the time scale of the experiment, whereas for the slower

nucleation, the impurities reduce the barrier but nucleation is still slow enough for the rate to be measured.

The need to reduce the prefactor by a factor of 10^{10} or more implies impurities with a density ρ_I, which is at least 10 orders of magnitude lower than the number density of protein molecules. This is why, when we considered CNT for heterogeneous nucleation, we considered large impurities, $>R^*$ across, as these will exist at low density but may still contribute to the nucleation rate as they can lower the free energy barrier dramatically. Note that protein solutions also inevitably contain small impurities such as other proteins, dimers etc.[24] Lysozyme dimers are known to affect the nucleation rate,[11] but although they may be contributing to both nucleation in the bulk of the solution and heterogeneous nucleation at the surface of a large impurity, they are present at too high a concentration to be able to rationalize the small prefactor.

Thus, if we assume the nucleation is heterogeneous, we can explain (i), but what about (ii)? If the nucleation is heterogeneous and so is occurring in contact with the surfaces of impurities, then variability in these surfaces will cause variability in the nucleation barrier, i.e., we will have a distribution of barrier heights, not a single barrier as in homogeneous nucleation. Let us examine how the presence of a distribution of barriers affects the functional form of the nucleation rate and hence that of n^*.

4. Heterogeneous Nucleation with a Distribution of Barrier Heights

If the nucleation rate is dominated by barriers with a single height and if the variation of this rate with s is correctly given by CNT, we have for the nucleation rate a function of the form

$$\text{rate} = A \exp(-B/s^2) \tag{10}$$

with A and B constants. Now, if for example, the nucleation is heterogeneous and is occurring on surfaces with variable contact angles θ, then the $1/s^2$ scaling of the individual barriers remains, but instead of there being one value for B, there is a distribution of B's. Surfaces with different contact angles θ will have different values of B, eq 4.

As we do not know what these impurities are, we cannot calculate the B's. In earlier work, we encountered this problem and simply assumed that we could model the B's as random variables.[23,25] We do this here and assume that the B's at different sites are independent random variables with a Gaussian probability distribution of mean $\bar{B}$ and standard deviation σ. Thus the probability $p(B)$ that a nucleation site has a barrier B/s^2 is

$$p(B) = \frac{1}{(2\pi\sigma^2)^{1/2}} \exp[-(B-\bar{B})^2/2\sigma^2] \tag{11}$$

and the total nucleation rate is given by

$$\text{rate} = A \int \mathrm{d}B p(B) \exp(-B/s^2) \tag{12}$$

where we have assumed that the prefactor does not vary from impurity to impurity. Using eq 11 and performing the integration, we obtain

$$\text{rate} = A \exp(-\bar{B}/s^2 + \sigma^2/2s^4) \tag{13}$$

A distribution of barrier heights always results in a rate larger than if all the barriers were of the mean height. This expression is valid so long as $\bar{B} \gg \sigma$ and results in a different, flatter, functional form to that predicted by CNT with a single barrier height, see Figure 1. Note that, on the log-linear scale of this figure, the rate for the distribution of barrier heights is straighter, closer to a simple exponential.

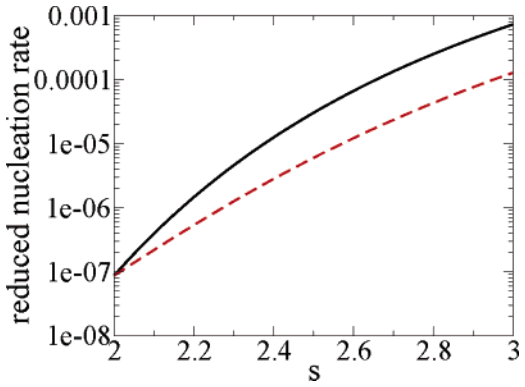

Figure 1. Predictions for the variation of the reduced rate with supersaturation, $s = \ln(\rho/\rho_{co})$. In each case, the reduced rate is for heterogeneous nucleation and is the ratio: rate/$(\rho_I Z j)$. The solid black curve is the reduced rate for a single barrier of height $65/s^2$. The dashed red curve is the reduced rate for a distribution of barrier heights B/s^2, where B is modeled by a random variable with mean $\bar{B} = 93.125$ and standard deviation $\sigma = 15$. At $s = 2$, this combination of mean and standard deviation gives the same nucleation rate as for a single barrier of height $65/s^2$.

Experimental data is also analyzed[1] by taking the derivative of the log of the rate and assuming eq 8 applies. If there is a distribution of barriers, there will also be a distribution of critical nuclei with a distribution of sizes. So, let us see what the derivative of the log of the rate is when there is a distribution of barriers. The rate is defined by eq 12 and its derivative is

$$\frac{\partial \ln(\text{rate})}{\partial s} = \frac{\int \mathrm{d}B p(B)(2B/s^3) \exp(-B/s^2)}{\int \mathrm{d}B p(B) \exp(-B/s^2)}$$

$$\langle n^* \rangle = \frac{\int \mathrm{d}B p(B) n^*(B) \exp(-B/s^2)}{\int \mathrm{d}B p(B) \exp(-B/s^2)} \tag{14}$$

where $n^*(B) = 2B/s^3$ is the size of nuclei at a barrier height B/s^2. The equation of the second line defines our average of the excess number of molecules in the critical nuclei, $\langle n^* \rangle$. The average is taken by weighting critical nuclei by their contribution to the overall rate, $\propto \exp(-B/s^2)$.

To obtain the s dependence of the average number of molecules in the critical nuclei for our Gaussian distribution of barrier heights, we simply take the s derivative of the log of the rate of eq 13 and obtain

$$\langle n^* \rangle = \frac{2\bar{B}}{s^3} - \frac{2\sigma^2}{s^5} \tag{15}$$

The second term comes from the distribution of barrier heights and has the opposite sign to the first term. Hence the distribution of barrier heights tends to flatten out the curve, to reduce the variation of the size of the critical nucleus with supersaturation. This is clearly seen in Figure 2, where over the same range of supersaturation, n^* drops by almost a factor of 3 when there is only a single barrier height, while when there is a distribution, n^* only drops from 9 to 5. Thus a distribution of barrier heights can explain the observation of Galkin and Vekilov[1] that the size of the nucleus was essentially independent of supersaturation.

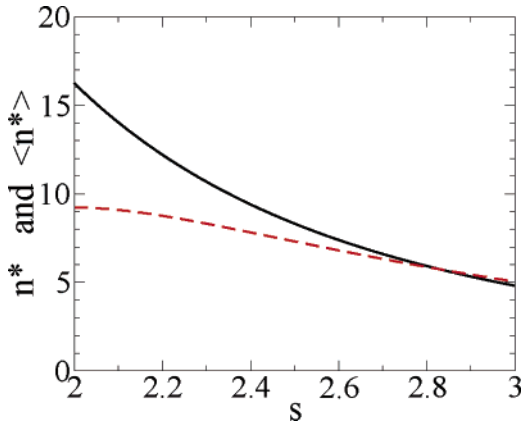

Figure 2. Excess number n^* and average excess number $\langle n^* \rangle$ of molecules in the critical nucleus, as a function of supersaturation, $s = \ln(\rho/\rho_{co})$. The solid black curve is n^* for a single barrier of the CNT form, $65/s^2$. The dashed red curve is $\langle n^* \rangle$ for a distribution of barrier heights B/s^2, where B is modeled by a random variable with mean $\bar{B} = 93.125$ and standard deviation $\sigma = 15$.

5. Conclusion

It is important to be able to distinguish between homogeneous and heterogeneous nucleation, both from the point of view of understanding the process and from a purely practical point of view. For example, if the nucleation is heterogeneous, then the rate will be sensitive to impurity levels. It might be reduced by purifying the sample but could be increased if surfaces were added where the nucleation barrier is particularly low.[23] As the nucleation of protein crystals, and crystals of small molecules, cannot be observed directly, we cannot directly see whether the nucleus forms in the bulk or at a surface. This surface can belong to a particle that is comparable in size to the nucleus[26] or even smaller,[27] and if so, then we will not even be able to see the particle. Thus if we want to find out whether the nucleation is heterogeneous or homogeneous, we will need to work it out from indirect experimental evidence, such as the variation of the nucleation rate with supersaturation. This may be difficult and will typically involve the use of a model for the nucleation, e.g., CNT. Our CNT model assumes that the rate can be expressed as the product of a factor of $\exp(-\Delta F^*/kT)$ and a prefactor, where the prefactor is the product of the number density of nucleation sites and the rate at which a nucleus at the top grows via the addition of protein molecules.

Here we found that within CNT, the functional form of the rate as a function of supersaturation depends only on whether a single barrier or barriers with a distribution of heights contribute to the rate. Thus, the functional form is the same for homogeneous nucleation and for heterogeneous nucleation at a single type of impurity nucleation site. However, for dilute impurities, the prefactor will be orders of magnitude lower for heterogeneous nucleation. Thus, the size of the prefactor can be used to discriminate between homogeneous and heterogeneous nucleation.

We also found that, if there is a distribution of nucleation barriers, then the functional form of the rate differs from the standard CNT form; for a Gaussian distribution of barriers, it is given by eq 13. This functional form is more slowly varying than the function for a single barrier and leads to an n^* estimated from eq 8 that varies slowly with supersaturation. This allows us to discriminate between nucleation via a single barrier and via a distribution of barriers. Also, note that, in this case, eq 8 yields a weighted average of the excess number of molecules in the critical nuclei. These nuclei vary in size with those at impurity sites with lower barriers being smaller.

Other Theoretical Work on Nucleation in Lysozyme Solutions. Lysozyme has a fluid−fluid transition within the fluid−crystal coexistence region.[36] This has inspired theoretical work on attempting to understand how this second, fluid−fluid, phase transition affects the nucleation of the crystal. Starting with the work of ten Wolde and Frenkel,[31] there have been a number of studies of the effect of the critical point of the fluid−fluid transition.[31−35] Note that this critical point occurs at densities significantly above those studied in the experiments[1,2] we have considered. We do not expect the critical point to affect nucleation at the densities we are considering.

Also, Vekilov and co-workers have studied in experiment[37] and theoretically[28−30] nucleation in the vicinity of the transition from a protein-poor to a protein-rich phase. This is below the critical point considered by ten Wolde and Frenkel and by others. The study of Pan et al.[29] in particular aimed at using a two-step nucleation model to explain the maximum in the nucleation rate as a function of temperature. Indeed, they were able to reproduce the experimental behavior. However, their prefactor, their U_2 of eq 7, contained a parameter they fitted to the experimental data. The result was a prefactor comparable to that in their earlier work[1]. This small value suggests that they too are studying heterogeneous nucleation.

Future Work. Determining the nucleation mechanism for lysozyme will require further quantitative experiments. In particular, systematic quantitative studies of systems of varying purities would be useful, as would quantitative studies of nucleation rates in systems where surfaces are deliberately added to increase the nucleation rate.[23,38,39]

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References and Notes

(1) Galkin, O.; Vekilov P. G. *J. Phys. Chem. B* **1999**, *103*, 10965.
(2) Bhamidi, V.; Varanasi, S.; Schall, C. A. *Langmuir* **2005**, *21*, 9044.
(3) Debenedetti, P. G. *Metastable Liquids*; Princeton University Press: Princeton, NJ, 1996.
(4) Oxtoby, D. W. *Acc. Chem. Res.* **1998**, *31*, 91.
(5) García-Ruiz, J. M. *J. Struct. Biol.* **2003**, *142*, 22.
(6) Albright, J. G.; Annunziata, O.; Miller, D. G.; Paduano, L.; Pearlstein, A. J. *J. Am. Chem. Soc.* **1999**, *121*, 3256.
(7) Gorti, S.; Forsythe E. L.; Pusey, M. L. *Cryst. Growth Des.* **2005**, *5*, 47.
(8) Turnbull, D. *J. Chem. Phys.* **1950**, *18*, 198.
(9) Sholl, C. A.; Fletcher, N. H. *Acta Metall.* **1970**, *18*, 1083.
(10) Galkin, O.; Vekilov, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 156.
(11) Burke, M. W.; Leardi, R.; Judge, R. A.; Pusey, M. L. *Cryst. Growth Des.* **2001**, *1*, 333.
(12) Kulkarni, A.; Zukoski C. F. *J. Cryst. Growth* **2001**, *232*, 156.
(13) Kulkarni, A. M.; Zukoski, C. F. *Langmuir* **2002**, *18*, 3090.
(14) Filobelo, L. F.; Galkin, O.; Vekilov, P. G. *J. Chem. Phys.* **2005**, *123*, 014904.
(15) Dixit, N. M.; Kulkarni, A. M.; Zukoski, C. F. *Colloids Surf., A* **2001**, *190* 47.
(16) Vekilov, P. G.; Galkin, O. *Colloids Surf., A* **2003**, 215, 125
(17) Zukoski, C. F.; Kulkarni, A. M.; Dixit, N. M. *Colloids Surf., A* **2003**, *215*, 137.
(18) Palberg, T. *J. Phys.: Condens. Matter* **1999**, *11*, R323.
(19) Auer, S.; Frenkel, D. *Nature* **2001**, *409*, 120.
(20) Auer, S.; Frenkel, D. *J. Chem. Phys.* **2004**, *120*, 3015.
(21) Oxtoby, D. W.; Kashchiev, D. *J. Chem. Phys.* **1994**, *100*, 7665.
(22) Viisanen, Y.; Strey, R.; Reiss, H. *J. Chem. Phys.* **1993**, *99*, 4680.
(23) Chayen, N. E.; Saridakis, E.; Sear, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 597.
(24) Rosenberger, F.; Vekilov, P. G.; Muschol, M.; Thomas, B. R. *J. Cryst. Growth* **1996**, *168*, 1.
(25) Sear, R. P. *Phys. Rev. E* **2004**, *70*, 021605.
(26) Cacciuto, A.; Auer, S.; Frenkel, D. *Nature* **2004**, *428*, 404.
(27) Sear, R. P. *J. Phys. Chem.* **2006**, *110*, 4985.
(28) Vekilov, P. G. *Cryst. Growth Design* **2004**, *4*, 671.
(29) Pan, W.; Kolomeisky, A. B.; Vekilov, P. G. *J. Chem. Phys.* **2005**, *122*, 174905.

(30) Kashchiev, D.; Vekilov, P. G.; Kolomeisky, A. B. *J. Chem. Phys.* **2005** *122*, 244706.
(31) ten Wolde, P. R.; Frenkel, D. *Science* **1997**, *277*, 1975.
(32) Talanquer, V.; Oxtoby, D. W. *J. Chem. Phys.* **1998**, *109*, 223.
(33) Shiryayev, A.; Gunton, J. D. *J. Chem. Phys.* **2004**, *120*, 8318.
(34) Sear, R. P. *J. Chem. Phys.* **2001**, *114*, 3170.
(35) Sear, R. P. *J. Chem. Phys.* **2002**, *116*, 2922.
(36) Muschol, M.; Rosenberger, F. *J. Chem. Phys.* **1997**, *107*, 1953.
(37) Galkin, O.; Vekilov, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6277.
(38) McPherson, A.; Shlichta, P. *Science* **1988**, *239*, 385.
(39) Falini, G.; Fermani, S.; Conforti, G.; Ripamonti, A. *Acta Crystallogr., Sect. D* **2002**, *58*, 1649.