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Metastable cluster intermediates in the condensation of charged macromolecule solutions

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The authors examine the possibility of a two-step nucleation to the bulk condensation transition that proceeds via a metastable liquid cluster intermediate having some preferred size. The metastable intermediate is stabilized by electrostatic repulsion, which becomes screened by small mobile ions at sufficiently large cluster sizes, thus allowing the eventual condensation to a bulk phase. Our calculation employs a capillary model for the cluster and the electrostatic interactions are treated using the Poisson-Boltzmann approach. Condensation via this metastable intermediate may be a very general phenomenon which applies not only to solutions of charged particles (e.g., proteins, colloidal particles, and polyelectrolytes) but to any system involving short-range attraction and long-range repulsion undergoing macrophase separation in which a metastable microphase separation is also possible. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2761891]

I. INTRODUCTION

Weakly charged polyelectrolytes and colloidal particles are of great interest due to their rich phase behavior and relevance to biopolymers. For example, weakly charged polymer solutions below the coil/globule transition and charged colloidal suspensions share many properties with proteins in solution. Because protein structural determination still largely relies on x-ray diffraction from high quality protein crystals, protein crystallization from solution has been an area of active study. Recent work by Vekilov *et al.* on several protein solution systems[1–4] suggests a two-step nucleation mechanism, whereby the nucleation of the crystalline phase is preceded by the formation of a dense liquidlike precursor. This two-barrier scenario predicts different features than implied by the more common single-barrier model, such as the temperature dependence of the nucleation rate.[2,5] The nature of the dense liquidlike clusters is not clear. However, in order for there to be two nucleation barriers in the formation of the bulk crystal phase, these clusters must correspond to a local free energy minimum with respect to the size (which is one of the key reaction coordinates). In this paper we explore one physical scenario due to long-ranged Coulomb repulsion which can give rise to metastable cluster intermediates in the transition between a dilute solution of monomers and a bulk, condensed phase.

The existence of large equilibrium clusters stabilized by charge has been demonstrated previously for a charged colloidal system in the absence of excess ions by Groenewold and Kegel.[6] In this case, the driving force for condensation due to short-range van der Waals interactions is checked by the unfavorable long-range electrostatic repulsion between the particles that increases superlinearly with the number of particles in a cluster, thus preventing the formation of a bulk condensed phase. In contrast, we are interested in clusters that can further nucleate a bulk condensed phase. We propose that the presence of excess ions that neutralize and screen charges on the monomers in solution provides the needed mechanism for allowing the formation of a bulk liquid phase. We find that for some combinations of parameters, a bulk condensation transition proceeds by going through a metastable cluster state with a well-defined cluster size, and that the metastable cluster itself is formed through nucleation, i.e., the overall process involves two nucleation barriers. While our model may not be directly applicable to the crystallization of proteins from solution, we believe the mechanism for condensation via metastable cluster intermediates to be quite general, though possibly confined to a small region of the phase diagram, in systems having short-range attraction and long-range repulsion, such as solutions of polyelectrolytes,[7] charged colloids,[8] and globular proteins,[9] which can exhibit both microphase and macrophase separations,[10] with the former involving clusters of finite sizes.

Conditions giving rise to the metastable intermediate cluster behavior as well as the nature of cluster formation are analyzed in the following sections. Our analysis employs a simple capillarity model for the cluster that is commonly used in classical nucleation theory, combined with the Poisson-Boltzmann equation for treating the electrostatic interactions. In Sec. II A, we briefly review the classical nucleation theory and describe its application to our system. In Sec. II B, the electrostatic contribution to the cluster free energy is calculated using the Poisson-Boltzmann approach. We present the results of our calculation in Sec. III: we show the free energy of cluster formation as a function of size and demonstrate several scenarios with respect to the stability of the intermediate clusters for a specific set of parameters. The formation of clusters with preferred size is shown to be similar to the formation of micelles and a concentration similar to

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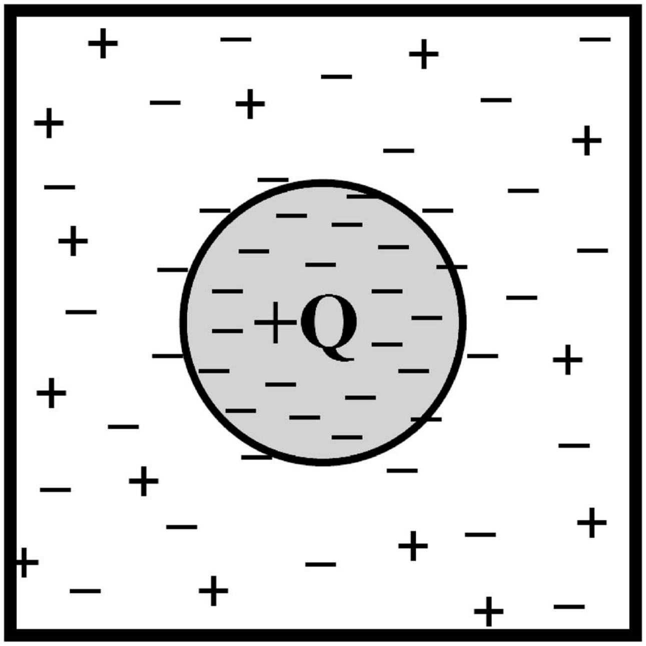

FIG. 1. Schematic of a cluster of charge $+Q$ within a monovalent coion and counterion reservoir.

the critical micelle concentration can be identified. Section IV provides a summary of our results with some concluding remarks.

II. MODEL

We consider a dilute solution of weakly charged particles (henceforth called unimers in their unclustered state), which can be globular proteins, polyelectrolyte globules, or colloidal particles, each carrying an electric charge q. Without loss of generality, we take the charge on the unimers to be positive. The system is maintained electrically neutral by counterions which we assume to be monovalent for simplicity. In addition, the system contains ions from salt, also assumed to be monovalent. Short-range attractions, e.g., van der Waals or hydrophobic interactions, provide the driving force for the formation of clusters and for potential condensation into a bulk phase; a schematic is shown in Fig. 1. As the focus of this work is on the metastable liquidlike clusters, we will not address issues directly related to the formation of a crystal phase.

We study the formation of clusters and the eventual condensation into a bulk phase by taking a nucleation perspective, i.e., by constructing the appropriate free energy of formation as a function of the size of the clusters. Since a cluster is formed from the surrounding solution of unimers and in the presence of small mobile coions, and counterions, it is convenient to treat the volume enclosing the cluster as an open system, with chemical potentials μ, μ_+, and μ_-, respectively, for the unimers, the coions, and the counterions. The appropriate free energy is the change in the grand potential upon the formation of a cluster from a uniform solution, defined as

$$\begin{aligned}\Delta W &= W_c(m;\mu,\mu_+,\mu_-,V) - W_s(\mu,\mu_+,\mu_-,V)\\ &= F_c(m,n_+,n_-,V) - m\mu - n_+\mu_+ - n_-\mu_-\\ &\quad + (p_s + p_+ + p_-)V. \end{aligned} \tag{1}$$

In this expression, $F_c(m,n_+,n_-,V)$ is the Helmholtz free energy of a charged cluster of size m, with n_+ coions and n_- counterions, and $W_s(\mu,\mu_+,\mu_-,V)=-(p_s+p_++p_-)V$, with p_s, p_+, p_-, the (osmotic) pressure of the unassociated unimers, the coions, and counterions, respectively; we have implicitly assumed that these pressures are additive for the purpose of calculating W_s, which amounts to making an ideal solution (in the sense of Henry's law) approximation for these species. Since we do not explicitly consider the solvent, for simplicity of terminology, we will often refer to the dilute solution as the vapor, and the osmotic pressure as just the pressure.

We treat the cluster using a simple capillary model, i.e., taking it as a spherical of liquid of uniform density with a sharp interface. While this is clearly a crude approximation and there are many recognized pitfalls in its application to nucleation, for our present purpose, the model is sufficient to capture most of the essential physics of interest without complications that would be introduced in a more realistic but mathematically more involved representation and solution.

With the use of the capillary model, it is convenient to separate the free energy change into two parts: a contribution due to the short-range interactions in a hypothetical uncharged system and a contribution due to the electrostatic effects. Hereafter we will use the word "uncharged" when referring to the hypothetical uncharged system lacking coions and counterions. We write Eq. (1) as

$$\begin{aligned}\Delta W &= \Delta W^{(0)} + \Delta W^{(e)}\\ &= [F_c^{(0)}(m,V) - m\mu^{(0)} + p_sV] + [F_c^{(e)}(m,n_+,n_-,V)\\ &\quad - n_+\mu_+ - n_-\mu_- + (p_+ + p_-)V - m\mu^{(e)}], \end{aligned} \tag{2}$$

where $F_c^{(0)}$ and $F_c^{(e)}$ are, respectively, the uncharged and electrostatic parts of the Helmholtz free energy of the cluster (the translational entropy of the cluster is not included), and $\mu^{(0)}$ and $\mu^{(e)}$ are, respectively, the uncharged and electrostatic parts of the chemical potential of the unimers $[\mu^{(0)}+\mu^{(e)}=\mu]$, with the translational contribution contained in $\mu^{(0)}$.

We now proceed to calculate these two free energy changes separately. We treat the uncharged part using the classical nucleation theory and the electrostatic part using the Poisson-Boltzmann approach.

A. Free energy without electrostatic interaction

We calculate the free energy of formation for an m-sized, uncharged cluster using classical nucleation theory. The classical nucleation theory makes the simplistic assumption that the free energy of a cluster can be dissected into a negative bulk free energy and a positive interfacial free energy. Thus the grand free energy of a dense cluster of volume V_c having interfacial area A_c is

$$W_c = - p_c(\mu^{(0)}) V_c + A_c \gamma,$$

where p_c is the pressure (the negative of the grand potential density) inside the cluster and γ is the interfacial tension. The grand potential change upon creating a cluster of volume V_c in an observational volume V from a homogeneous solution of unimers with pressure p_s is then

$$\begin{aligned}\Delta W^{(0)} &= - V_c p_c(\mu^{(0)}) + A_c\gamma - (V - V_c)p_s(\mu^{(0)}) + V p_s(\mu^{(0)}) \\ &= - V_c[p_c(\mu^{(0)}) - p_s(\mu^{(0)})] + A_c\gamma. \end{aligned} \qquad (3)$$

We now write the pressure difference in terms of the supersaturation relative to the phase coexistence pressure of the hypothetical uncharged system.

To do so, we utilize the thermodynamic relation $\partial\mu/\partial p|_T = 1/\rho$, where ρ is the density. It follows that the pressure difference is related to the difference in chemical potential through $p_c - p_s = (\rho_c - \rho_s)(\mu^{(0)} - \mu^{(0)}_{\text{coex}}) \approx \rho_c(\mu^{(0)} - \mu^{(0)}_{\text{coex}})$, where $\mu^{(0)}_{\text{coex}}$ is the chemical potential of the hypothetical uncharged system at vapor-liquid coexistence at which $p_c = p_s$. Finally, assuming ideal gas behavior for the vapor phase, the chemical potential difference can be written as $\mu^{(0)} - \mu^{(0)}_{\text{coex}} = kT\ln(p_s/p_{\text{coex}}) \equiv kT\ln(S)$. The resulting free energy of formation is

$$\Delta W^{(0)} = - kT\rho_c V \ln(S) + A_c\gamma = - mkT\ln(S) + A_c\gamma. \qquad (4)$$

To use Eq. (4) for the free energy of formation for a cluster of m unimers, the dependence of the surface area on m needs to be specified. In the case of coalescing globular polyeletrolytes, if we assume that the globules are uniform spheres of radius R_1 and that there is no change in the monomer density (as the polymers can deform and interpenetrate) upon the formation of a cluster of radius R_c, then clearly

$$m = (R_c/R_1)^3. \qquad (5)$$

On the other hand, hard spherical colloids pack less densely and therefore this expression would need to be modified by inclusion of a packing factor. Throughout the rest of the paper, we assume the former so that

$$A_c(m) = 4\pi R_1^2 m^{2/3}. \qquad (6)$$

The expression for $\Delta W(m)$ as given by Eqs. (4) and (6) contains one of the well-known inconsistencies of classical nucleation theory: the existence of a finite free energy of formation for a cluster of one monomer. Resolution of this inconsistency is subtle and has been discussed intensely by Lothe and Pound,[11] Reiss and Katz,[12,13] and more recently by Kusaka.[14] Rather than make use of these more rigorous methods, which are unnecessary given the nature of the approximations we are making, we utilize a simple corrected form that has been artificially shifted by taking the free energy relative to a cluster of size one,

$$\Delta W^{(0)} = - kT\ln(S)(m-1) + 4\pi R_1^2\gamma(m^{2/3} - 1). \qquad (7)$$

We note that this shift has no consequence on the main results of this study.

Equation (7) is the expression for the free energy of formation of a cluster of m unimers for an uncharged system. For $S > 1$, this expression predicts the well-known behavior for nucleation of a dense liquid phase through a single nucleation barrier. No metastable cluster is allowed. We next discuss the electrostatic interaction which provides the necessary balancing force that makes the metastable clusters possible.

B. Free energy of electrostatic interactions

It is easy to see how Coulomb repulsion can lead to a preferred cluster size. We first consider the case of no counterions and no added salt.

In a cluster of m unimers each having a point charge q, the total Coulomb interaction is given by

$$E_c = \frac{1}{2}\sum_{i,j\neq i}\frac{q^2}{4\pi\varepsilon_0\epsilon R_{ij}},$$

where R_{ij} is the distance between charges i and j, ϵ is the dielectric constant of the medium (assumed uniform throughout V here), and ε_0 is the vacuum permittivity. Since the average distance between the charges scales as the size of the cluster R_c, the electrostatic interaction in a cluster of m charged unimers scales as

$$E_c \sim \frac{q^2}{8\pi\varepsilon_0\epsilon R_c} m(m-1) \sim \frac{q^2}{8\pi\varepsilon_0\epsilon R_1} m^{5/3}, \qquad (8)$$

where the second part follows from the relationship between the size of the cluster and the size of the unimer, Eq. (5), and we have assumed m to be much larger than one.

When combined with the neutral part of the free energy $\Delta W^{(0)}$ from Eq. (4), we see that as the cluster size (number of unimers) increases, the free energy first increases due to the positive surface free energy term, then decreases because of the negative bulk free energy term, but increases again due to the positive superlinear electrostatic energy term, creating a free energy minimum at some intermediate size

$$\Delta W \sim 4\pi R_1^2\gamma m^{2/3} - mkT\ln(S) + \frac{q^2}{8\pi\varepsilon_0\epsilon R_1} m^{5/3};$$

this is, in fact, the same argument as given by Groenewold and Kegel.[6]

While this simple scaling analysis predicts the existence of clusters with a preferred size, the superlinear dependence of the free energy on m for large clusters excludes the possibility of a dense bulk phase. Thus these mesoscopic clusters are fully stable. A dense bulk phase is made possible by the combined neutralization and screening by the coions and counterions. It is expected that bare Coulomb repulsion is dominant when the cluster size is small, while neutralization and screening become effective when the cluster size becomes sufficiently large, making the electrostatic free energy extensive again. Figure 2 shows the crossover from the $m^{5/3}$ scaling to the linear scaling of the electrostatic free energy as a function the cluster size. We now proceed with a more detailed description of the calculation for the electrostatic part of the free energy of cluster formation.

Consistent with our use of the uniform liquid model for the cluster, we assume the charge distribution due to the clustering unimers to be continuous and uniform inside the cluster with a constant charge density ρ_0. This *jellium* model is similar to that used by Sear and Warren[15] in their calcula-

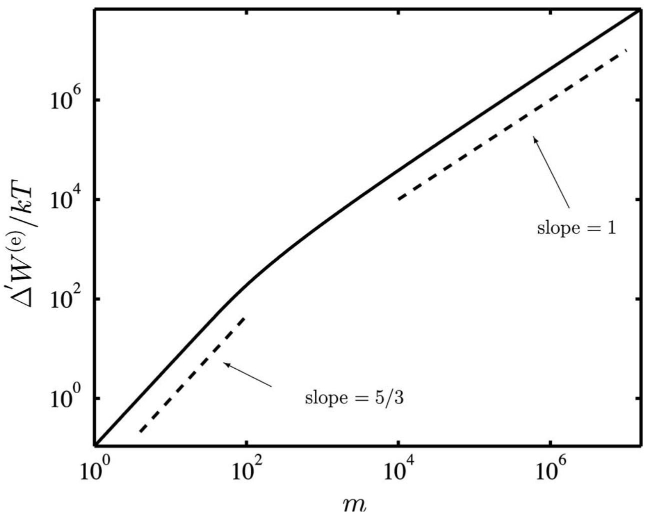

FIG. 2. Example of the size dependence of the free energy of electrostatic interaction $[\Delta' W^{(e)}]$ depicting $m^{5/3}$ and m behavior for small and large clusters, respectively. This curve was obtained using cluster charge density, $\rho_0 l_B^3 = 0.08$, Debye screening length, $\lambda / l_B = 0.15$, and dielectric constant ratio, $\epsilon_1/\epsilon_2 = 0.2$.

tion of the electrostatic contribution to planar surface tension. We then include coions and counterions that equilibrate with the charged cluster with ion density profiles c_+ and c_-. (We make the simplifying but inconsequential assumption that the cations from the salt are the same as the counterions for the charged clusters of interest.) Superposition of these charge density profiles with the step-function charge density profile of the cluster results in an overall charge density profile $\rho(\mathbf{r}) = \rho_0 e \theta(R_c - r) + ec_+(r) - ec_-(r)$, where $\theta(R_c - r)$ is a step function equal to one inside the cluster and zero outside and e is the elementary unit of charge. Re-expressing Coulomb's law in terms of a variational integral by introducing the electrostatic potential $\psi(\mathbf{r})$ leads to the following expression for the electrostatic energy:

$$E_c[\rho(\mathbf{r}), \psi(\mathbf{r})] = \int d\mathbf{r} \left\{ \rho(\mathbf{r}) \psi(\mathbf{r}) - \frac{\varepsilon_0 \epsilon}{2} [\nabla \psi(\mathbf{r})]^2 \right\}. \tag{9}$$

The total electrostatic free energy must include the translational entropy of the mobile free ions. Since the volume surrounding the cluster is semiopen: closed with respect to the condensed cluster unimers, but open with respect to the coions and counterions, we add the grand free energy due to these mobile ions. Treating the ions as volumeless, noninteracting particles in contact with a reservoir of chemical potential μ_2, we have the total electrostatic free energy of the cluster as

$$\begin{aligned} W^{(e)} = & \int_V d\mathbf{r} \left\{ \rho(\mathbf{r}) \psi(\mathbf{r}) - \frac{\varepsilon_0 \epsilon}{2} [\nabla \psi(\mathbf{r})]^2 \right\} \\ & + kT \int_V d\mathbf{r} \{ c_+ \ln(c_+ a^3) - c_+ - c_+ \beta \mu_2 \} \\ & + kT \int_V d\mathbf{r} \{ c_- \ln(c_- a^3) - c_- - c_- \beta \mu_2 \}. \end{aligned} \tag{10}$$

The system volume (V) is taken to be large enough to include the cluster and any variations in ion concentration. The length scale, a, assumed to be the same for both types of ions, is of no consequence in the results because of cancellation with its appearance in the reservoir chemical potential, $\mu_2 = kT \ln(c_\infty a^3)$.

The spherically symmetric equilibrium ion concentration profiles c_+ and c_- and the electrostatic potential are obtained by extremization of the free energy in Eq. (10) with respect to these variables. Variation with respect to the ion concentrations yields

$$c_+(r) = c_\infty \exp[-\beta e \psi(r)],$$

$$c_-(r) = c_\infty \exp[\beta e \psi(r)],$$

where β is the inverse thermodynamic temperature $1/kT$. Substitution of these relations back into the free energy Eq. (10) and subsequent variation with respect to $\psi(\mathbf{r})$ results in the Poisson-Boltzmann equation,

$$\varepsilon_0 \epsilon \nabla^2 \psi(r) = -\rho_0 e \theta(R_c - r) + 2kTc_\infty \sinh[\beta e \psi(r)]. \tag{11}$$

To reduce the number of independent parameters and gain better insight into the relative importance of the various physical effects, it is convenient to nondimensionalize the equations. To this end, we introduce the Debye screening length, $\lambda = (8\pi b c_\infty)^{-1/2}$, and the Bjerrum length, $l_B = \beta e^2/(4\pi\varepsilon_0\epsilon)$. We elect to express the length r in units of l_B as opposed to λ to avoid the complication of an ion concentration dependent length. Similarly, the charge density ρ_0 is nondimensionalized as $\rho_0 l_B^3$. Finally, $\beta e \psi$ is the resulting dimensionless electrostatic potential, ξ. The free energy of a charged cluster in an ion reservoir expressed in terms of the above dimensionless variables is

$$\begin{aligned} W^{(e)} = -kT \int_V r^2 dr \Bigg\{ & \frac{1}{2} [\nabla \xi]^2 + \left(\frac{l_B}{\lambda} \right)^2 \cosh[\xi(r)] \\ & - 4\pi \rho_0 l_B^3 \xi(r) \theta(R_c - r) \Bigg\}. \end{aligned} \tag{12}$$

The corresponding nondimensionalized Poisson-Boltzmann equation is

$$-\nabla^2 \xi + \left(\frac{l_B}{\lambda} \right)^2 \sinh[\xi(r)] = 4\pi \rho_0 l_B^3 \theta(R_c - r). \tag{13}$$

The ratio l_B/λ is seen to be a natural parameter measuring the importance of screening. It can be easily verified that in the limit of $l_B/\lambda = 0$ or $\lambda / l_B \to \infty$, we recover the electrostatic energy of a uniformly charged sphere.

For simplicity of presentation, thus far we have considered the dielectric constant to be uniform. In reality, the two regions inside and outside the cluster will have different dielectric constants, ϵ_1 and ϵ_2, respectively. Apart from modifying the energy due to change in the electrostatic potential, the difference in dielectric constant also results in a preferred solubility of the ions within the region of higher dielectric constant due to difference in the reference chemical potential in the two different media. This difference may be approximated using the Born solvation energy (see Appendix A). An analogous derivation can be performed that treats the effect of a difference in the dielectric constant between the cluster

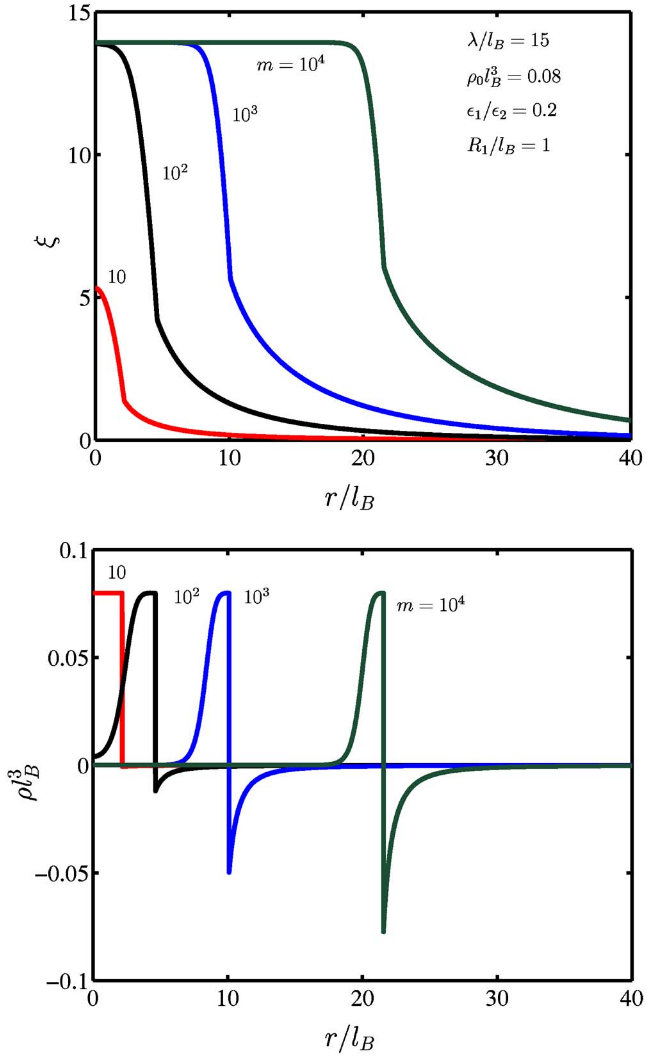

FIG. 3. (Color online) Electrostatic potential profiles (a) and total charge density profiles (b) obtained from the Poisson-Boltzmann equation [Eq. (B2)] for cluster radii of increasing radius.

and the solvent. It requires two additional dimensionless groups, the ratio of the dielectric constants ϵ_1/ϵ_2, and the difference in reference chemical potentials, $\beta\Delta\mu^* = \beta\mu_1^* - \beta\mu_2^*$. The expressions for this derivation are given in Appendix B. It is the solutions to these equations that are presented in this section and Sec. III.

The Poisson-Boltzmann equation is solved numerically using the Runga-Kutta method. Boundary conditions arise from neutrality at an infinite distance from the cluster, $\xi \to 0$ as $r \to \infty$, and spherical symmetry, $\partial\xi/\partial r \to 0$ as $r \to 0$. Additionally, across the cluster boundary, we have the continuity of the electric potential, $\xi(R_c^-) = \xi(R_c^+)$, and the electric displacement field, $-\epsilon_1 \nabla \xi(R_c^-) = -\epsilon_2 \nabla \xi(R_c^+)$.

Solution of the differential equation that includes the dielectric constant discontinuity [Eq. (B2)] results in potential profiles and corresponding total charge density profiles such as those shown in Fig. 3 for clusters of different sizes, m. It is clear from these figures that for small cluster sizes, e.g., $m = 10$, the charge of the cluster is essentially the bare charge carried by the aggregating molecules, and the corresponding electrostatic potential is barely distinguishable from that obtained from solving the simple unscreened Poisson equation (with an inverted parabolic potential profile). For $m = 100$, we find that there is now appreciable neutralization of the charge by the counterions in the center of the cluster and the potential in the center nearly reaches the bulk asymptotic value. For the larger clusters, $m = 10^3$ and $m = 10^4$, the neutralization of charge in the interior of the cluster is almost complete. The charge that is not neutralized is distributed primarily near the edge of the cluster with an oppositely charged region in the immediate vicinity outside the cluster. The potential profile becomes essentially flat inside the cluster for these larger sized clusters and decays to zero with a length scale on the order of the Debye screening length. For clusters whose radii are much larger than the Debye screening length, it is possible to write the electrostatic free energy of the cluster as a volume term and an interfacial term, with a negative interfacial tension.[15,16]

The crossover from the small cluster to the large cluster behavior in the electrostatic potential and charge density is consistent with the crossover in the free energy shown in Fig. 2. It is this crossover that is largely responsible for the two-barrier nucleation scenario in the condensation of the molecules.

C. Total free energy

By construction, we have written the total free energy of cluster formation as composed of an uncharged part and an electrostatic part. Referring to Eq. (2), the free energy expression derived in Sec. II A accounts for the terms in the first bracket of the second equality in Eq. (2), while the electrostatic free energy described in the last section corresponds to the first three terms in the second bracket. It is straightforward to include the remaining bulk osmotic pressure terms due to the coions and counterions in a uniform solution by defining a free energy difference $\Delta' W^{(e)}$ using $W^{(e)}$ from Eq. (10) yielding

$$\Delta' W^{(e)} = W^{(e)} - kT \int_V d\mathbf{r}\ 2c_\infty, \tag{14}$$

which has no effect on solution of the governing differential equations. To complete our total free energy, we need to account for the last term in the second bracket of Eq. (2). By our definition, $\mu^{(e)}$ is the excess chemical potential of the unassociated unimers due to the electrostatic self-interaction within the globular cluster. We treat the unimer in a manner identical to that for the cluster, i.e., regarding it as a uniformly charged sphere surrounded by mobile coions and counterions. The excess chemical potential is then logically identified as the special case of Eq. (14) when $m = 1$. The total electrostatic free energy that accounts for all the terms in the second equality of Eq. (1) is then

$$\Delta W^{(e)} = \Delta' W^{(e)}(m) - m\Delta' W^{(e)}(1) \tag{15}$$

and the total free energy of cluster formation is

$$\Delta W = \Delta W^{(0)} + \Delta W^{(e)}. \tag{16}$$

This expression yields the free energy of formation of an m-sized cluster for a given set of parameters: λ/l_B, R_1/l_B,

$\rho_0 l_B^3$, ϵ_1/ϵ_2, the interfacial tension γ, and the supersaturation S. Its behavior as a function of m provides information about the thermodynamic stability of the clusters, their size distribution, and their role in the formation of a dense bulk phase.

D. Bulk phase coexistence

In Sec. II A, the supersaturation S is introduced with reference to the coexistence vapor pressure of a hypothetical uncharged system. For this hypothetical system, a bulk condensation occurs at $S=1$. The addition of electrostatic interactions shifts the value of the coexistence supersaturation from one, for an uncharged system, to a value greater than one, for the charged system. Its new value is determined by the condition that the extensive terms ($\propto m$) in the total free energy of cluster formation must sum to zero in the limit of $m \to \infty$; this is equivalent to the equality of the grand potential between the dilute solution of unimers and the condensed phase (within the same volume), i.e., the equality of (osmotic) pressure. From our expression for the total free energy, it is straightforward to show that the coexistence "supersaturation" (that is, the supersaturation relative to the coexistence pressure of a hypothetical uncharged system) is given by

$$S_{\mathrm{coex}} = \exp\left[\frac{1}{kT}\left(w_{\mathrm{bulk}}^{(e)} - \Delta' W^{(e)}(1)\right)\right],$$

where $w_{\mathrm{bulk}}^{(e)} = \lim_{m\to\infty} \Delta' W^{(e)}(m)/m$. The full algebraic expression for $w_{\mathrm{bulk}}^{(e)}$ is somewhat cumbersome and is given in Appendix C. Note that while there exists an algebraic form for $w_{\mathrm{bulk}}^{(e)}$, we still must solve the Poisson-Boltzmann equation to obtain the electrostatic energy of a unimer, $W^{(e)}(1)$.

We now have a reference with which to judge whether any clusters formed are metastable with respect to the dense-liquid phase, and therefore can take part as an intermediate in bulk phase formation.

III. RESULTS AND DISCUSSION

Before presenting and discussing the main results of this study, we first revisit the physics leading to cluster formation and discuss the limits of their existence and their relation to bulk phases. As discussed in Sec. II B and illustrated in Fig. 2, there are two main regimes associated with the electrostatic part of the free energy of formation, the superlinear ($m^{5/3}$) and the linear (m) regimes, corresponding to highly charged and largely neutralized clusters, respectively. The interplay between the location and magnitude of these regimes, the surface tension, and the solution supersaturation gives rise to several possible behaviors involving preferred-size clusters.

In order to obtain the two-barrier free energy curve we discuss here, the electrostatic and the uncharged free energies must be of the same order of magnitude. This results in rich phase behavior, but limited cluster sizes. The size of clusters is limited by the size range of the $m^{5/3}$ region in Fig. 2. In other words, clusters acting as metastable intermediates can be no larger than the size (m) at which the $\Delta W^{(e)}$ dependence changes from $m^{5/3}$ to linear. While very large clusters ($m > 10^4$) are predicted under certain conditions, we find that these clusters do not exist in a state metastable to the bulk liquid, i.e., the bulk liquid state does not exist for these conditions. It may be possible that these large clusters are metastable with respect to a more stable crystalline state; this possibility will be examined in future work.

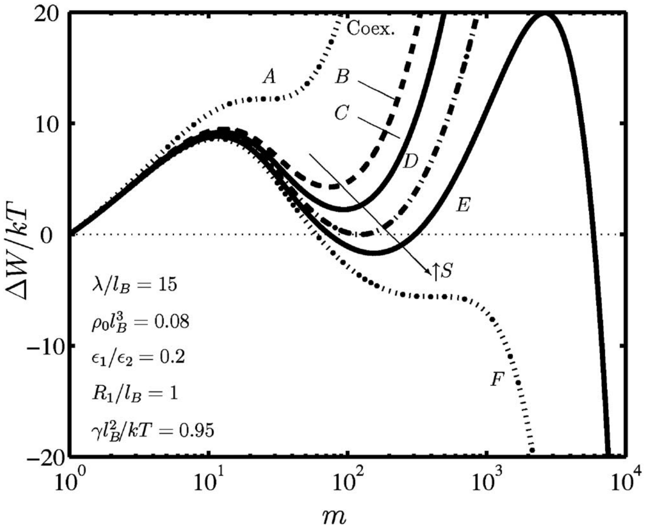

FIG. 4. Total free energy of cluster formation for six increasing values of supersaturation (S).

We now briefly discuss the parameters used in our model. An ion concentration c_∞ in the range of $0.8M$–8 mM in water (physiological salt concentration $=0.2M$) with a dielectric constant of 80 (ϵ_2 in our notation) results in the parameter λ/l_B ranging from 0.25 to 25, the Bjerrum length being 7 Å. We choose $\lambda/l_B=15$ for our representative calculations. The dielectric constant within proteins, ϵ_1 is difficult to estimate; however, values from 4 to 20 (Ref. 17) have been theoretically estimated and used, resulting in corresponding ϵ_1/ϵ_2 values of 0.05–0.25. In our representative calculations, we set $\epsilon_1/\epsilon_2=0.2$. Decreasing ϵ_1/ϵ_2 increases the electrostatic free energy $W^{(e)}$ as well as results in a slight increase in the cluster size at which crossover between the $m^{5/3}$ and m behavior occurs. For the interfacial tension, we use $\gamma=0.95kT/l_B^2=8$ mN/m, which is intermediate between a clean oil/water interface (50 mN/m) and tensions measured in colloidal phases (1–20 μN/m).[15] We use a low charge density $\rho_0 l_B^3=0.08$. This charge density is on the same order of magnitude as many globular proteins. Low charge density is necessary in order to still allow for a bulk condensation. In the case of polyelectrolytes, the low charge density is also necessary to ensure that the collapsed polymer is in the globular regime, not in the necklace regime.[18] Finally, we choose $R_1/l_B=1$ for the unimer size. We choose this size because it most clearly illustrates the behavior we wish to highlight. This size is on the smaller end even for proteins. For larger unimer size, the range of the superlinear dependence of the free energy electrostatic interaction decreases, thus decreasing the aggregation number of the metastable clusters, other conditions being equal. On the other hand, smaller sizes for charged unimers are relevant for other systems, e.g., in zeolite or nanoparticle synthesis.[19,20]

The main result of this study is summarized in Fig. 4, which shows the free energy of formation as a function of the cluster size for increasing values of the supersaturation (or equivalently the unimer concentration). At small supersaturation, the only minimum of the free energy curve occurs at $m=1$. As supersaturation increases beyond some critical value (curve A), a local minimum in the free energy for a finite-sized cluster first begins to develop. As the concentration of unimers increases, the free energy of the finite-sized cluster decreases, with an attendant shift in the location of the minimum to larger cluster size. When the supersaturation exceeds that corresponding to the vapor-liquid coexistence (curve B, indicated by Coex.), the global free energy minimum is that of the bulk liquid phase (i.e., infinite cluster size) and the vapor phase becomes metastable with respect to the stable bulk liquid. However, the formation of the bulk liquid phase requires overcoming two barriers, the first for the formation of the finite-sized clusters and the second for the clusters at the free energy minimum to grow into the bulk liquid phase. We thus have the scenario of a two-step nucleation process with the clusters of finite sizes acting as long lived metastable intermediates. The lifetime of these metastable clusters is determined by the barriers on the two sides of the minimum. For the range of parameters we use in our study, the typical free energy barrier for the dissolution of the clusters is on the order of $10kT$, depending on the supersaturation. The barrier to the formation of the bulk liquid phase can be quite large (which also implies large critical nuclei), again depending on the supersaturation, due to the strong electrostatic interactions. In reality, these large clusters are more likely to take other shapes, such as disks or rods,[6] to decrease the electrostatic repulsions; this would lead to a decrease in the second nucleation barrier. For sufficiently large supersaturation, the second nucleation barrier disappears (curve F) and the condensation into a liquid bulk phase proceeds through a simple single-barrier scenario.

In addition to the metastable liquidlike clusters that can be intermediates in the bulk phase transition, in other words, that result in two nucleation barriers such as those shown in Fig. 4, our model also predicts the existence of clusters that are metastable or stable with respect to the dilute solution, with no possibility for bulk liquid formation. [We clarify that the terms "stable" and "metastable" are used in the sense of Fig. 4: the clusters are considered stable with respect to the solution phase if the minimum in their free energy of formation from the solution phase is negative (curve E, for example), and metastable if the minimum of the free energy of formation is positive (curve C). Curve D denotes the limit of this stability.] The case of metastable clusters with respect to the unimer solution phase, whether a bulk liquid exists or not, may be reasonably described by our model, as the number density of the clusters formed will be small due to the positive free energy required for their formation. In the case where the clusters are more stable than the solution of unimers, we expect that the clusters will appear in large numbers; cluster-cluster as well as unimer-cluster interactions become important and our picture of noninteracting clusters is no longer valid. In all likelihood this latter case would correspond to microphase separation, which is beyond the scope of this work.

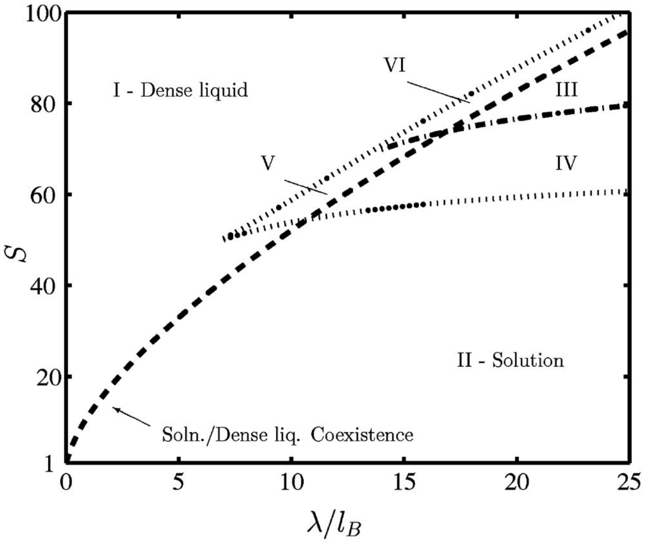

FIG. 5. "Phase diagram" for a unimer radius of $1l_B$, dielectric ratio, ϵ_1/ϵ_2, of 0.2, charge density, $\rho_0 l_B^3$, of 0.08, and surface tension, $\gamma=0.95kT/l_B^2$.

Figure 4 demonstrates that several phase transition scenarios are possible as the supersaturation varies. Clearly the transition scenarios will depend significantly on the extent of screening. Since the unimer concentration is proportional to S, and the reservoir ion concentration is proportional to $(l_B/\lambda)^2$, these serve as the natural controlling parameters in experiments. We thus present in Fig. 5 generalized "phase diagram" in S and λ/l_B that illustrates the different phase transition scenarios. In this "phase diagram," the dash line designates the vapor-liquid coexistence. Region II corresponds to stable dilute solutions, while in region I a supersaturated dilute solution phase will turn into the bulk liquid phase via the normal one barrier nucleation. Note that region I corresponds to high screening and/or high supersaturation. The region bounded by the two dotted lines is the region where finite-sized clusters have a free energy minimum. With reference to Fig. 4, the lower dotted line corresponds to the first appearance of a shoulder on the left (curve A), while the upper dotted line corresponds to the disappearance of the barrier on the right (curve F). The dash-dot line denotes the supersaturation at which the free energy of formation of the clusters becomes zero (curve D). Thus, regions III and VI correspond to clusters that have lower free energy than the unimers. As alluded to in the last paragraph, the energetic favorability for forming the clusters will lead to a high concentration of clusters which is outside the regime of validity of this study. Regions IV and V are the most interesting from the perspective of this work. Region IV corresponds to metastable preferred-size clusters that cannot grow into bulk liquid, while in region V the metastable clusters serve as intermediates to the formation of the bulk liquid. A two-barrier nucleation mechanism operates in region V.

The formation of the metastable clusters with preferred sizes is akin to the formation of micelles in surfactant solutions. To explore this connection, we calculate the cluster size distribution and the partitioning of the molecules between unimers and clusters. Assuming no cluster-cluster and unimer-cluster interactions, the cluster size distribution, i.e., the number density of m-sized clusters is directly related to the free energy of formation, $\Delta W(m)$,[21]

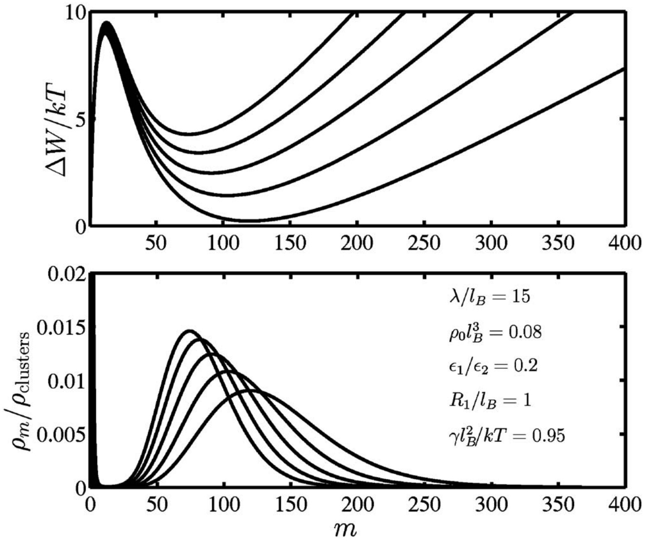

FIG. 6. Cluster density profiles for varying supersaturations. The lower figure is obtained by dividing the cluster density as obtained by Eq. (17) at a given cluster size by the total number of clusters, obtained by integrating the profile generated by Eq. (17) over the entire cluster size range, in other words from barrier to barrier in the energy profiles shown in the upper figure.

$$\rho_m \propto e^{-\beta \Delta W(m)}. \tag{17}$$

The proportionality involves a volume scale that arises from evaluation of the full partition function of the clusters, including shape fluctuations. As such fluctuations are not included in our mean-field calculation, we simply set the volume to be the volume of a unimer $4\pi R_1^3/3$. As the size distribution is dominated by the exponential term, uncertainties in this volume factor should have little consequence on the results.

Figure 6 shows an example of the cluster size distribution (normalized by the total number of clusters) for several values of the supersaturation (unimer concentration). The bimodal nature of the distribution, with one peak for the unimers and one at some preferred size, is very similar to the behavior of micellar solutions. However, the width of the distribution is fairly broad and also the cluster peak shifts significantly to larger sizes as the supersaturation increases, in contrast to micellar solutions. In the case of micelles, the size is determined primarily by local packing constraints,[22] which severely limit the range of size variations.

A quantity similar the critical micelle concentration (CMC) can be defined for the cluster formation by examining the partitioning of molecules between the unimers and clusters. Because of the broad nature of the size distribution, we include the entire distribution of clusters between the two free energy barriers. A typical plot is shown in Fig. 7.

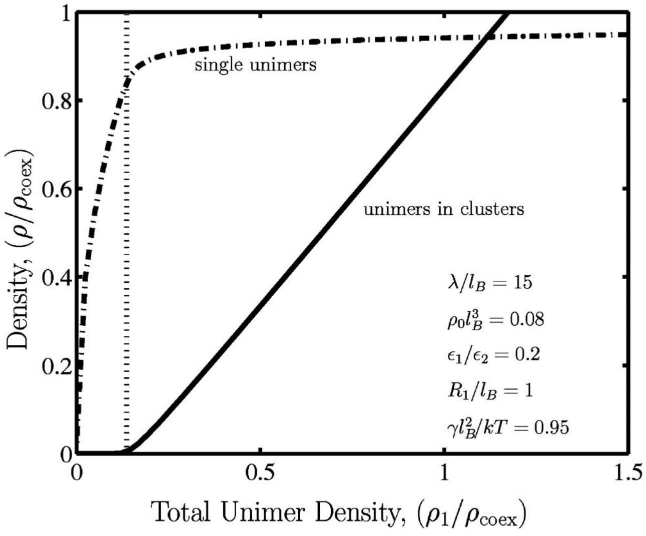

FIG. 7. Illustration of the critical micellelike behavior of cluster formation. The micellar (cluster) dissolution concentration (MDC) is denoted here by the dotted line.

In the case of surfactant solutions, CMC is usually defined to correspond to the condition at which the surfactant molecules are equally distributed as unimers and micelles. In Fig. 7, this occurs at a scaled density $\rho/\rho_{\text{coex}} \approx 1.15$. [Here, ρ_{coex} is the unimer density at which coexistence occurs for the hypothetical uncharged system.] In our case, however, a more dramatic change of behavior occurs at $\rho/\rho_{\text{coex}} \approx 0.16$. This condition corresponds to the first appearance of a local minimum in the free energy (curve A). Since the condition corresponding to curve A sets the limit of metastability for clusters of finite sizes, i.e., clusters with finite sizes can exist as entities with finite lifetime only beyond this supersaturation, it should be logically termed the micellar (cluster) dissolution concentration (MDC), following a similar concept (the micellar dissolution temperature) introduced in Ref. 23 in the study of micellar formation in diblock copolymer melts. For our current system, the MDC is a theoretically more meaningful concept than the CMC.

For concentrations beyond the MDC, adding more molecules to the system essentially only increases the number of clusters, with only small increases in the unimer density, similar to the behavior seen in micelles. Thus, once the clusters form, they provide a regulating mechanism for the unimer concentration and hence for the value of the supersaturation, thus limiting the driving force for nucleation of the bulk liquid phase. Therefore, for the same *nominal* relative supersaturation, condensation through metastable cluster intermediates will take much longer than the simple vapor-liquid nucleation.

IV. CONCLUSIONS

In this paper we have examined a simple model of liquidlike clusters with preferred sizes that act as intermediates in the formation of a bulk liquid phase in solutions of charged macromolecules. Such clusters arise as a consequence of competition between the short-range attractions that cause condensation and the long-range Coulomb interactions. The presence of these clusters can lead to a two-barrier nucleation scenario, with the first barrier associated with the formation of these clusters and the second associated with the growth of these preferred-sized clusters into the bulk liquid phase. However, in order that these clusters are only metastable and will eventually grow into a bulk liquid phase, charge neutralization by counterions and screening by both coions and counterions are crucial. The size of the clusters and the nucleation barrier from these clusters to the bulk liquid phase are determined by a combination of these effects.

While this study is motivated by the experimental work of Vekilov *et al.* on protein crystallization, direct application of our results does not explain the experimental observations. Their more recent work reveals that the clusters that are involved in the protein nucleation contain on the order of 10^5 or more protein molecules per cluster.[3,4] This size is much too large to explain with our electrostatic model: the size in our case is limited by the Debye screening length, which under our subphysiological conditions is 100 Å, corresponding to 10^2-10^3 protein molecules. Experiments on protein solutions and charged colloidal particles with depletion attraction show stable clusters with aggregation number of order 10.[24] Also, the typical charge on proteins is larger than the small charge necessary to make the clusters metastable with respect to a bulk liquid (however, they can be metastable to the crystalline phase). Therefore, our study seems to suggest that the clusters in the protein nucleation are not due to electrostatic interactions. At present, the nature of the intermediate clusters in the protein crystallization is still unclear, although Pan *et al.* have recently suggested that the clusters are purely kinetic in nature as a result of slow dynamics.[3] Liu *et al.* suggest that patchiness is an important feature in protein-protein interactions[25] and can give rise to slow relaxation dynamics at high densities,[26] leading to a delay in the crystal formation. In these scenarios, the cluster intermediates observed in the work of Vekilov *et al.* would be some long-lived amorphous dense regions in the protein solution. However, if that is the case, it is difficult to envision a two-barrier scenario for nucleation, which necessarily implies a free energy minimum between two barriers.

Although our results appear to exclude electrostatic interaction as the explanation behind the intermediate dense-liquid clusters in protein crystallization, the behavior predicted in this paper should be relevant and observable for many weakly charged macromolecular and colloidal systems, based on our estimates of the parameter ranges for this type of behavior. It is therefore desirable to refine and extend this initial study using more realistic models and more sophisticated approaches. For example, the use of molecular density functional theory that properly accounts for the excluded volume, short-range attractions, as well as electrostatic effects beyond the simple Poisson-Boltzmann approach, avoids the capillary approximations and also allows the inclusion of a crystalline phase.

At the phenomenological level, our model shares many common features with several systems that exhibit both macroscopic phase separation and aggregation into finite-size clusters, such as diblock copolymer-homopolymer mixtures,[27] and solutions of amphiphilic copolymers.[28] For example, Wu and co-workers have reported stable aggregates with aggregation number ranging from 10 to several thousands (depending on temperature and concentration) in aqueous solutions of the nonionic hydrophobically modified poly (N-isopropylacrylamide), between single-chain globules and macroscopic precipitation.[29,30] It is quite probable that these mesoglobules can also exist in a metastable state relative to the dilute single-globule solution phase and macroscopically condensed phase, in which case condensation will occur through a two-step mechanism similar to the one proposed in our work.

Another interesting phenomenon that is hinted at by our results but cannot be addressed due to our use of the single cluster picture is the relationship between microphase separation and macroscopic condensation. In particular, the existence of a metastable microphase separated state and its effects on the nucleation of a bulk dense liquid phase from a dilute solution phase appear to be a problem worthy of investigation.

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APPENDIX A: BORN SOLVATION ENERGY

The free energy change for transferring an ion with charge q and radius a from a medium with dielectric constant ϵ to a medium with dielectric ϵ_0 is

$$-\frac{q^2}{8\pi a\varepsilon_0}\left(\frac{1}{\epsilon_0}-\frac{1}{\epsilon}\right).$$

Thus the difference in chemical potential between an ion in dielectric medium one versus an ion in dielectric medium two is given by

$$\begin{aligned}\Delta\mu^* &= \mu_1-\mu_2\\ &= -\frac{q^2}{8\pi a\varepsilon_0}\left(\frac{1}{\epsilon_2}-\frac{1}{\epsilon_1}\right) = -\frac{q^2}{8\pi a\varepsilon_0\epsilon_2}\left(\frac{\epsilon_2}{\epsilon_1}-1\right).\end{aligned} \tag{A1}$$

In our case, ϵ_1/ϵ_2 is always less than one. Therefore, $\Delta\mu^*$ is always positive and it is energetically unfavorable for the coions and counterions to enter the lower dielectric medium within the cluster.

APPENDIX B: FREE ENERGY EXPRESSIONS FOR DIFFERING DIELECTRIC CONSTANTS

Defining the Bjerrum length using the dielectric constant of the solvent, ϵ_2, the free energy expression for a cluster with dielectric constant ϵ_1 within a reservoir with dielectric constant ϵ_2 is

$$\begin{aligned}W^{(e)} = -kT\int_0^\infty r^2dr\Bigg\{&\frac{1}{2}\left(1+\left[\frac{\epsilon_1}{\epsilon_2}-1\right]\theta(R_c-r)\right)[\nabla\xi]^2\\ &+\left(\frac{l_B}{\lambda}\right)^2(1+[e^{-\beta\Delta\mu^*}-1]\theta(R_c-r))\cosh[\xi(r)]\\ &-4\pi\rho_0 l_B^3\xi(r)\theta(R_c-r)\Bigg\}.\end{aligned} \tag{B1}$$

The corresponding Poisson-Boltzmann equation is

$$- \left(1 + \left[\frac{\epsilon_1}{\epsilon_2} - 1\right]\theta(R_c - r)\right)\nabla^2\xi + \left(\frac{l_B}{\lambda}\right)^2 \times (1 + [e^{-\beta\Delta\mu^*} - 1]\theta(R_c - r))\sinh[\xi(r)] = 4\pi\rho_0 l_B^3 \theta(R_c - r). \tag{B2}$$

APPENDIX C: FREE ENERGY OF A BULK LIQUID

In the limit of cluster size approaching infinity, ξ is constant ($=\sinh^{-1}[4\pi\rho_0 b^3(\lambda/l_B)^2 e^{-\beta\Delta\mu^*}]$), and the free energy of electrostatic interaction becomes

$$\frac{w_{\text{bulk}}^{(e)}}{m} = -kT\left(\frac{l_B}{\lambda}\right)^2 \frac{R_1^3}{3}\left[e^{-\beta\Delta\mu^*}\sqrt{1 + \left(4\pi\rho_0 l_B^3\left(\frac{\lambda}{l_B}\right)^2 e^{\beta\Delta\mu^*}\right)^2} - 4\pi\rho_0 l_B^3\left(\frac{\lambda}{l_B}\right)^2 \sinh^{-1}\left(4\pi\rho_0 l_B^3\left(\frac{\lambda}{l_B}\right)^2 e^{-\beta\Delta\mu^*}\right)\right].$$

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