Crystallization of Membrane Protein Nanoparticles

Andrey Shiryayev*, Edward F. Holby** and J. D. Gunton*

* Department of Physics, Lehigh University
Bethlehem, PA 18015
** Carleton College, Northfield, MN 55057

ABSTRACT

The self-assembly of membrane proteins from solution into two-dimensional crystals is studied numerically using a continuum model proposed by Talanquer and Oxtoby (J. Chem. Phys. **109**, 223 (1998)). Although the mean field theory presented here is only qualitatively accurate in two dimensions, we obtain the main features of the crystal nucleation process, such as the nucleation free energy barrier and the number of particles and crystalline particles, respectively, in the critical nucleating droplet. Particular emphasis is given to the region near the metastable fluid-fluid coexistence curve, where the free energy barrier is small. The free energy barrier that separates a protein rich metastable fluid phase from its stable crystalline phase is studied for a variety of nucleation pathways in the metastable region. As in the three dimensional case of globular proteins, the nucleation barrier is smallest in the vicinity of the fluid-fluid critical point.

Keywords: nanoparticle, protein, crystallization, nucleation

1 INTRODUCTION

Membrane proteins are an important component of the cell. These proteins are associated with the cell membrane and serve a variety of important functions. They help in the transport of ions across the cell membrane, in cell-cell communication and as cell receptors. In order to determine the function of these membrane proteins, it is necessary to determine their structure, which traditionally is done via x-ray crystallography. This requires the growth of high quality membrane protein crystals, which is notoriously difficult and which is very sensitive to the initial conditions of the proteins in solution. While in the case of globular proteins three dimensional crystallization is a common step toward the determination of the atomic structure, success of growing three dimensional crystals for membrane proteins is still infrequent [1]. It is difficult to maintain a crystal lattice with the sole interactions between the hydrophilic domains of the proteins. A two dimensional crystallization in the presence of lipids, when the membrane proteins reconstruct into lipid membranes to form crystals, is an entire new way to determine the structure of the membrane proteins [2] [3].

Recently some attention has been given to understanding from a theoretical perspective the conditions for optimal crystal nucleation from solution, based on phenomenological microscopic models [8], [9]. In this work we present a brief summary of a recent, complementary approach, based on a continuum, density functional approach developed for the study of the crystallization of globular proteins from solution. As in the case of globular proteins, we focus on the region near the metastable two phase fluid-fluid coexistence, as this is presumably the region for optimal crystal nucleation. The two fluid phases correspond to protein-poor and protein-rich solutions. A prior simulation study of a two dimensional modified Lennard-Jones model of membrane proteins found that the lifetime of this two fluid metastable coexistence state was too short to be studied [9]. Hence it remains to be seen if this is also the case for the continuum model presented here.

2 MODEL

In this study we model membrane proteins as two dimensional nanoparticles, using a density functional model proposed by Talanquer and Oxtoby [4] for the study of globular proteins. Their model is in the class of the phase field models and is based on the well known van der Waals free energy density of the fluid phase and a corresponding phenomenological van der Waals like free energy density of the solid phase. The resulting free energy density is the minimum of these two free energy branches. The free energy functional is given by

$$\Omega\left[\rho, m\right] = \int d\vec{r}\Big[f(\rho, m) - \mu\rho + \\ + \frac{1}{2}K_\rho(\nabla\rho)^2 + \frac{1}{2}K_m\rho_s^2(\nabla m)^2\Big] \quad (1)$$

where f is the Helmholtz free energy density, μ is the chemical potential. The free energy depends on two order parameters: the (conserved) local density $\rho(\mathbf{r}, t)$ and a (non-conserved) local structural order parameter that shows whether the system is in a solid or fluid phase $m(\mathbf{r}, t)$. A critical cluster is a saddle-point of the functional (1); therefore in order to determine its size and

profile we must solve the Euler-Lagrange equations with appropriate boundary conditions:

$$\frac{\delta\Omega}{\delta\rho} = 0 \quad \text{and} \quad \frac{\delta\Omega}{\delta m} = 0 \tag{2}$$

Using the saddle-point solutions for $\overline{\rho}(\mathbf{r})$ and $\overline{m}(\mathbf{r})$ we can obtain such properties of the inhomogeneous system as the free energy barrier for nucleation, $\Delta\Omega = \Omega\{\overline{\rho}(\mathbf{r}), \overline{m}(\mathbf{r})\} - \Omega\{\rho_0, 0\}$, the surface tension of the nucleating droplet and the number of particles in the critical cluster.

3 RESULTS AND DISCUSSION

We solve the saddle-point equation (2) numerically using the shooting method. The boundary conditions correspond to the metastable disordered fluid state at infinity (an infinite distance from the center of the droplet) and an ordered solid state at the center of the droplet. We also assume that the system has a polar symmetry. The solutions to the equations (2) are very sensitive to the choice of shooting parameters and thus diverge easily. In order to avoid this divergence we use a shooting method with a fitting point which we choose to be at the intersection of the solid and the fluid branches of the free energy density. This allows us to decrease the numerical error. The starting point for shooting from infinity is chosen in agreement with the implementation of Sear's approximation [7] in two dimensions. Further details of this numerical approach are described in [5].

In figure 1 the density and order parameter profiles are shown for the case of the system close to the critical point, while in figure 2 the profiles are shown for the system further from the critical point. As we can see, the density profile near the critical point has a longer tail. This shows that the correlation length is larger as we approach the critical point and diverges at the critical point. This long tail is in agreement with Sear's results [7].

Next, we calculated the free energy barrier at constant supersaturation versus reduced temperature T/T_c. As in the three dimensional case [4], [6], [5], the free energy barrier has a minimum in the vicinity of the critical point. This can be explained in terms of the second derivative of the free energy density with respect to the number density of nanoparticles in the metastable disordered state $f_{\rho\rho}$. The free energy barrier decreases as one goes away from the liquidus line and increases as one approaches the liquidus line. The slope of the paths with constant supersaturation is proportional to $f_{\rho\rho}$, which vanishes at the critical point. Thus these paths become horizontal near the critical point [5] and the system changes its behavior from going away from the liquidus line to approaching it. Therefore the free energy barrier has a minimum near the critical point. Because the mean field theory breaks down near the

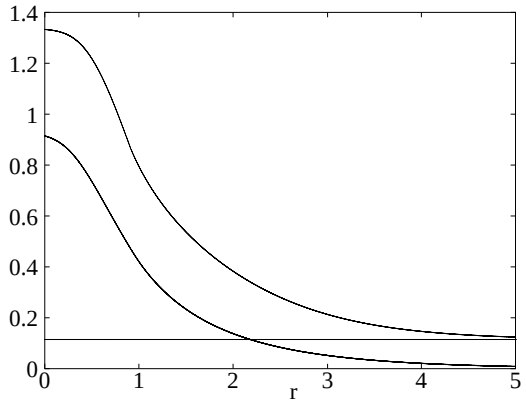

Figure 1: Density and order profiles of critical droplet close to the critical point. Higher profile is the dependence of the density on the distance from the center of the cluster. Lower profile is the dependence of the order parameter on the distance from the center of the cluster. The horizontal line shows the background fluid density.

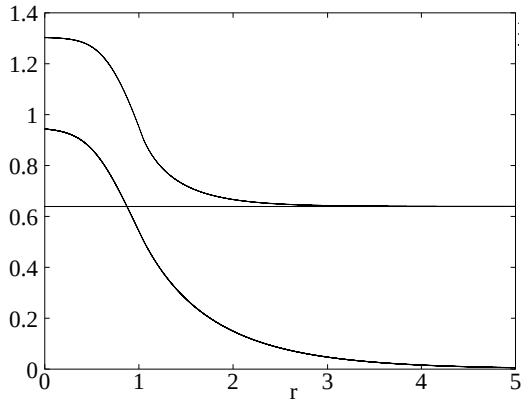

Figure 2: Density and order profiles of the critical droplet further from the critical point. Higher profile is the dependence of the density on the distance from the center of the cluster. Lower profile is the dependence of the order parameter on the distance from the center of the cluster. The horizontal line shows the background fluid density.

critical point, the details of the free energy barrier minimum can be explained only qualitatively by these calculations.

We also calculated the dependence of the number of particles in the critical cluster on the number of crystalline particles. One can see that as in the case of globular proteins, the number of particles in a two-dimensional critical cluster diverges as we approach the metastable critical point. This result is a mean field theory result. However ten Wolde and Frenkel [6] also obtained a similar liquid-like structure of the critical cluster in the vicinity of the critical point in their Monte-Carlo simulations. Further details of our results for the free energy barrier and the nature of the critical droplet will be published elsewhere.

4 CONCLUSION

Frenkel and Noro [9] were unable to determine the the fluid-fluid binodal for a modified Lennard-Jones model in two dimensions, as the barrier between the fluid-fluid metastable state and stable crystalline state was very small. However, they determined the metastable spinodal (defined as the locus of points where the barrier to self-assembly vanishes). There is thus a metastable critical point in the two-dimensional case. Although the decay time of the metastable binodal curve might be too short to be observed, the existence of the metastable critical point still affects the nucleation barrier and therefore the kinetics of self-assembly of the membrane proteins.

This work was supported by NSF grant DMR-0302598. One of us (EFH) was supported by a summer REU grant from Lehigh University.

REFERENCES

[1] C. Ostermeier and H. Michel, Curr. Opin. Struct. Biol. **7** 697 (1997).
[2] R.M. Garavito and S. Ferguson-Miller, J. Biol. Chem. **276**, 32403 (2001).
[3] D. Levy, M. Chami and J.-L. Rigaud, FEBS Letters. **504**, 187 (2001).
[4] V. Talanquer and D. Oxtoby, J. Chem. Phys. **109**, 223 (1998).
[5] A. Shiryayev and J.D. Gunton, J. Chem. Phys. to be published.
[6] P.R. ten Wolde and D. Frenkel, Science **277**, 1975 (1997).
[7] R. Sear, J. Chem. Phys. **114**, 3170 (2001).
[8] M. A. Bates, M. G. Noro and D. Frenkel, J. Chem. Phys. **116**, 7217 (2002)
[9] M.G. Noro and D. Frenkel J. Chem. Phys. **114**, 2477 (2001).