

FROM VAN DER WAALS TO PROTEIN CRYSTALLISATION

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The range of the intermolecular potential determines whether or not stable liquid-vapour coexistence is possible. We argue that for short range attraction, the liquid-vapour transition may be located below the sublimation line. Under those conditions, meta-stable critical fluctuations may enhance the rate of crystal nucleation

1. Introduction

Why do liquids exist? We are so used to the occurrence of phenomena such as boiling and freezing that we rarely pause to ask ourselves if things could have been different. Yet the fact that liquids must exist is not obvious *a priori*. This point is eloquently made in an essay by V. F. Weisskopf¹:

...The existence and general properties of solids and gases are relatively easy to understand once it is realized that atoms or molecules have certain typical properties and interactions that follow from quantum mechanics. Liquids are harder to understand. Assume that a group of intelligent theoretical physicists had lived in closed buildings from birth such that they never had occasion to see any natural structures. Let us forget that it may be impossible to prevent them to see their own bodies and their inputs and outputs. What would they be able to predict from a fundamental knowledge of quantum mechanics? They probably would predict the existence of atoms, of molecules, of solid crystals, both metals and insulators, of gases, but most likely not the existence of liquids. However, although Weisskopf argues that liquids are "unexpected", he seems to take it for granted that the liquid phase must exist. In fact, he even appears to apologize for the fact that his essay does not prove this:

[This] essay does not show how the existence of liquids necessarily follows from quantum mechanics. Its aims are much less ambitious...

The words are important: Weisskopf states that he will not show *how* the existence of liquids follows from quantum mechanics. The implication seems to be that he takes it for granted *that* such a causal relationship exists and that, given more time, he would have given the proof. This sounds a bit like the famous lines in Lewis Carroll's "The Hunting of the Snark" :

The method employed I would gladly explain,
While I have it so clear in my head,

If I had but the time and you had but the brain –
But much yet remains to be said.

In fact, the existence of liquids is not inevitable (apart, maybe, from the fact that we would not be here to ask the question if liquid-vapor interfaces did not exist). Depending on the range of the intermolecular forces, coexistence between a liquid and a vapor in thermodynamic equilibrium may, or may not, be possible. Below, we briefly review the effect of the intermolecular potential on the liquid-vapor transition. In addition, we shall show that the presence of a *meta-stable* liquid-vapor coexistence curve may have a pronounced effect on the rate of crystal nucleation.

2. The liquid-vapor transition

In his 1873 thesis, van der Waals presented the first molecular theory of the ‘Continuity of the Gaseous and Liquid States’. In his thesis, van der Waals gave the correct explanation for a well known yet puzzling feature of liquids and gases, namely that there is no essential distinction between the two: above a critical temperature T_c , a vapor can be compressed continuously all the way to the freezing point. Yet below T_c , a first-order phase transition separates the dilute fluid (vapor) from the dense fluid (liquid)².

The thermodynamic perturbation theory that lies at the root of the van der Waals equation, becomes exact in the limit of weak, long-ranged intermolecular interactions³. From the work of Longuet-Higgins and Widom⁴, we know that the van der Waals model (molecules are described as hard spheres with an infinitely weak, infinitely long-ranged attraction) is even richer than originally expected: it exhibits not only the liquid-vapor transition but also crystallization. The liquid-vapor transition is possible between the critical point and the triple point, and in the van der Waals model, the temperature of the critical point is about a factor two large than that of the triple point. There is, however, no fundamental reason why this transition should occur in every atomic or molecular substance, nor is there any rule that forbids the existence of more than one fluid-fluid transition.

Whether a given compound will have a liquid phase, depends sensitively on the range of the intermolecular potential: as this range is decreased, the critical temperature approaches the triple-point temperature, and when T_c drops below the latter, only a single stable fluid phase remains. This phenomenon is well known in mixtures of spherical colloidal particles and non-adsorbing polymer, where the range of the attractive part of the effective colloid-colloid interaction can be varied by changing the size of the polymer^{5,6,7,8,9}. Experiment, theory and simulation all suggest that when the width of the attractive well becomes

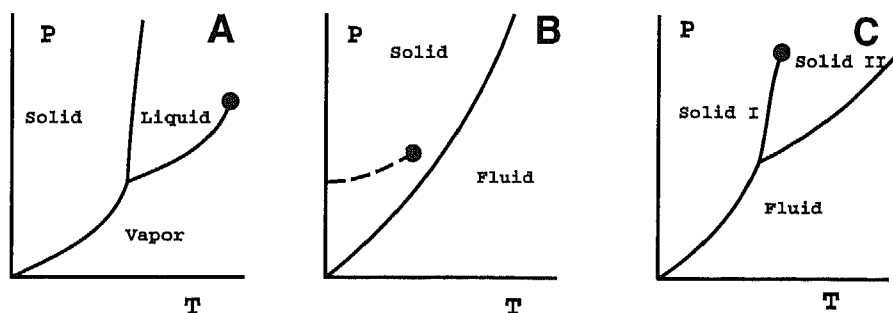


Figure 1: Schematic drawing of the PT phase diagram of a system of spherical particles with (A): an attractive interaction with a range larger than one third of the hard-core diameter. (B): range of attractive interaction between 5 and 20 % of the hard-core diameter. (C) range of the attractive interaction less than 5% of the hard-core diameter.

less than approximately one third of the diameter of the colloidal spheres, the colloidal 'liquid' phase disappears. Figure 1 shows schematically the evolution of the phase-diagram of a system of spherical particles with a variable ranged attraction. As the range of attraction decreases, the liquid-vapor curve moves into the meta-stable regime. For very short-ranged attraction (less than 5% of the hard-core diameter), a first-order iso-structural solid-solid transition appears in the solid phase¹⁰. It should be stressed that phase diagrams of type B in figure 1 are common for colloidal systems, but rare for simple molecular systems. A possible exception is C_{60} ¹¹. Phase diagrams of type C have, thus far, not been observed in colloidal systems, but iso-structural solid-solid transitions in metals (Cs and Ce ,¹²) have been known for a long time.

The fact that the liquid-vapor transition may become meta-stable, has important consequences for crystal nucleation. This will be discussed in the next section, in the context of the crystallization of globular proteins¹³.

3. Protein crystallization

The human genome codes for some hundred thousand proteins. To understand the function of these proteins, we need to know their three-dimensional structure. At present, only some four thousand protein structures have been determined (almost all of them globular proteins – less than ten membrane-protein structures have been resolved). One of the bottlenecks in the determination

of the three-dimensional structures of proteins by X-ray crystallography is the difficulty of growing good protein crystals. In his book on this subject, McPherson¹⁴ wrote "*The problem of crystallization is less approachable from a classical analytical standpoint, contains a substantial component of trial and error, and draws more from the collective experience of the past century. [...] It is much like prospecting for gold*". The experiments clearly indicate that the success of protein crystallization depends sensitively on the physical conditions of the initial solution^{14,15}. It is therefore crucial to understand the physical factors that determine whether a given solution is likely to produce good crystals.

During the past few years, evidence has accumulated that not just the strength, but also the range of the interactions between protein molecules is of crucial importance for crystal nucleation. In 1994, George and Wilson¹⁶ showed that the success of protein crystallization appears to correlate with the value of B_2 , the second osmotic virial coefficient of the protein solution. George and Wilson measured B_2 for a number of proteins in various solvents. They found that for those solvent conditions that are known to promote crystallization, B_2 was always restricted to a narrow "slot". If B_2 was too large, crystallization did not occur at all, while for large negative values of B_2 protein aggregation, rather than crystallization, took place.

More recently, Rosenbaum *et al.*^{17,18} established a link between the work of George and Wilson and earlier studies of the phase behavior of spherical, uncharged colloids^{5,19,20,21}. As explained in the previous section, for sufficiently short-ranged attractive forces, the liquid-vapor critical point crosses the triple point (see figure 1B) and only a meta-stable liquid-vapor coexistence curve survives below the equilibrium sublimation curve. This fact is relevant for protein solutions, because globular proteins often have relatively short-ranged attractive interactions. In fact, a series of studies by Benedek and Broide and collaborators^{22,23,24} show that the phase diagram of a wide variety of proteins is of the kind shown in figure 1B (see figure 2). Moreover, the range of the effective interactions between proteins can be changed by the addition of non-ionic polymer (e.g. poly-ethylene glycol) to the solution (see ref. ^{21,25}).

The interesting observation presented by Rosenbaum *et al.*¹⁷, is that the conditions under which a large number of globular proteins can be made to crystallize, map onto a narrow temperature range – or, more precisely, a narrow range in the value of the osmotic second virial coefficient – of the computed fluid-solid sublimation curve of colloids with short-ranged attraction²⁰. Starting with the work of Kose and Hachisu, several authors had already noted that a similarly narrow crystallization window exists for colloidal suspensions²⁶. The question that we address here is: what is the physical origin of this nucle-

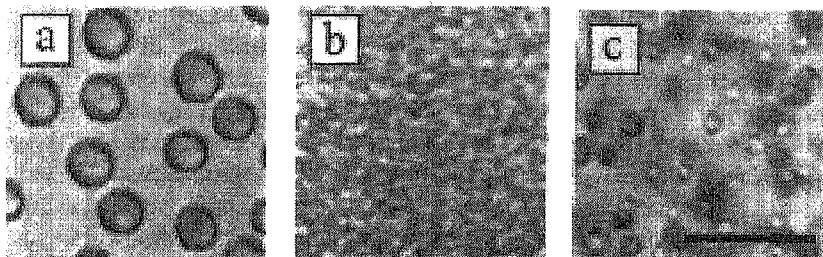


Figure 2: Optical micro-graphs of liquid-liquid phase separation of calf γ IVa-crystallin solutions for three different protein concentrations quenched below the coexistence curve between solid and dilute solution. (a): droplets of protein-rich phase in protein poor solution. (b): bi-continuous structure of protein-rich and protein poor phase, near the meta-stable critical point, (c): Droplets of protein-poor phase in a protein-rich solution. For details, see ref. ²². Photograph by courtesy of Michael Broide.

ation window?

The rate-limiting step in crystal nucleation is the crossing of a free-energy barrier. In our simulations we compute the barrier for homogeneous crystal nucleation for a model “globular protein”. Our model system exhibits some of the essential features needed to get a “protein-like” phase diagram of the type shown in figure 1B: the particles repel strongly at short distances and attract at larger distances.

For the interaction between the particles we chose a suitable generalization of the Lennard-Jones potential:

$$v(r) = \frac{4\epsilon}{\alpha^2} \left(\frac{1}{[(r/\sigma)^2 - 1]^6} - \alpha \frac{1}{[(r/\sigma)^2 - 1]^3} \right) \quad (1)$$

where σ denotes the hard-core diameter of the particles. This potential has a well depth ϵ . The potential in Eqn. 1 provides a highly simplified description of the interaction between real proteins in solution. It should be thought of as an effective interaction: it accounts both for direct and for solvent-induced interactions between the globular proteins. The width of the attractive well can be adjusted by varying the parameter α . We found that for $\alpha \approx 50$, the system reproduced the phase behavior of protein solutions studied in ^{22,23,24}, i.e. the fluid-fluid coexistence curve is located in the meta-stable region some 20 % below the equilibrium crystallization curve.

Conventional Molecular Dynamics simulations cannot be used to study crystal nucleation under realistic conditions. The reason is that the formation

of a critical nucleus is a rare event – crystallization in real protein solutions may take days or weeks. In a simulation, the situation would be worse because the volume that can be conveniently studied by simulation is some fifteen orders of magnitude smaller, and the probability of forming a crystal is decreased by the same amount. Moreover, the computational costs of Molecular Dynamics simulations that cover more than 10^{-8} s becomes prohibitive. Hence, the problem has to be approached in a different way. In the present work, we used a technique that we have developed to study crystal nucleation in simple liquids²⁷. In general, we can write the nucleation rate as the product of a kinetic prefactor, ν , and the part that describes the probability that a spontaneous fluctuation will bring the system to the top of the nucleation barrier: $\exp(-\delta G^*/k_B T)$, where ΔG^* is the height of the (gibbs) free-energy barrier separating the meta-stable liquid from the crystal phase. Unless the system is close to a glass or gel transition, the temperature-dependence of the nucleation rate is dominated by the variation of $\exp(-\delta G^*/k_B T)$. The model that we study is not close to gelation, and hence we can safely focus on the variation of the free energy barrier. A rough estimate of ΔG^* is given by classical nucleation theory²⁸,

$$\frac{\Delta G^*}{k_B T} = \frac{16\pi\gamma^3}{3k_B T \rho^2 \Delta\mu^2} \quad (2)$$

where γ is the free-energy density per unit area of the solid-fluid interface, ρ is the number density of the solid phase, and $\Delta\mu$ is the difference in chemical potential between the fluid and the solid – it is the thermodynamic driving force for crystallization.

To compute the free energy barrier that the system must overcome to form a critical crystal nucleus, we use a suitable modification of the so-called umbrella-sampling Monte Carlo scheme of Valleau^{27,29}. In order to compute the free energy as a function of the size of the incipient crystal nucleus, we must be able to identify which particles belong to the crystal nucleus. As discussed in ref.²⁷, we can classify each particle as either solid-like or liquid-like by analysing the local symmetry of its surroundings. If two solid-like particles are neighbors, they belong to the same cluster. We denote the number of solid-like particles belonging to a given crystal nucleus by N_{cryst} . As we discuss below, crystallization near the meta-stable fluid-fluid critical point is strongly influenced by the large density fluctuations that occur in the vicinity of such a critical point. We should therefore also consider the free energy barrier associated with formation of dense, liquid-like droplets. Hence, for every particle, we determine not just the structure of its surroundings, but also the local density. In this way, we can define the size of a high-density cluster (be it solid or liquid-like), as the number N_ρ of (connected³⁰) particles that

have a significantly denser local environment than particles in the remainder of the system. In our simulations, we determine the free-energy “landscape” of the system, as a function of the two coordinates N_{cryst} and N_ρ . In a crystal nucleation event, we start from the homogeneous liquid ($N_{cryst} \approx N_\rho \approx 0$) - the free energy then increases, until it reaches a saddle-point (the critical nucleus). From there on, the crystal will grow spontaneously.

We first computed the phase diagram of our model system, using the techniques described in ref.²⁰. Subsequently, we studied the free-energy barrier for crystal nucleation at four different points in the phase diagram: one well above the meta-stable critical point ($T = 2.23T_c$), one at T_c , and the remaining two at $0.89T_c$ and $1.09T_c$. All systems were prepared at a comparable degree of super-cooling - to be more precise, we chose the degree of super-cooling such that classical nucleation theory would predict the same value of $\Delta G^*/(k_B T)$ for all systems. To estimate ΔG^* from Eqn. 2, we used

$$\Delta\mu \approx \Delta H \frac{T_m - T}{T_m} \quad (3)$$

where ΔH is the enthalpy of melting at the coexistence temperature T_m . To estimate the surface free-energy γ , we used Turnbull’s empirical rule²⁸ which states that γ is proportional to ΔH . For all points, we studied the free-energy landscape and the lowest free-energy path to the critical nucleus.

We find that away from T_c (both above and below), the path of lowest free energy is one where the increase in N_ρ is proportional to the increase in N_{cryst} (see figure 3A). Such behavior is expected if the incipient nucleus is simply a small crystallite. However, we observe a striking change in the free-energy landscape around T_c (see figure 3B). First, the route to the critical nucleus leads through a region where N_ρ increases while N_{cryst} is still essentially zero - in words: the first step towards the critical nucleus is the formation of a *liquid-like* droplet. Then, beyond a certain critical size, the increase in N_ρ is proportional to N_{cryst} - a crystalline nucleus forms inside the liquid-like droplet. Near the fluid-fluid critical point, the wetting of the crystal nucleus by a liquid-like layer results in a value of the interfacial free-energy γ , and therefore of the barrier height ΔG^* , that is much lower than would be estimated on the basis of Turnbull’s rule (see Table 1). In fact, in a recent paper, Haas and Drenth³¹ note that the experimentally determined interfacial free energy of small protein crystals³² is much smaller than the value predicted on the basis of their version of Turnbull’s rule. Clearly, the presence of large density fluctuations close to a fluid-fluid critical point, has a pronounced effect on the route to crystal nucleation. But, more importantly, the nucleation barrier close to T_c is much lower than at either higher or lower temperatures.

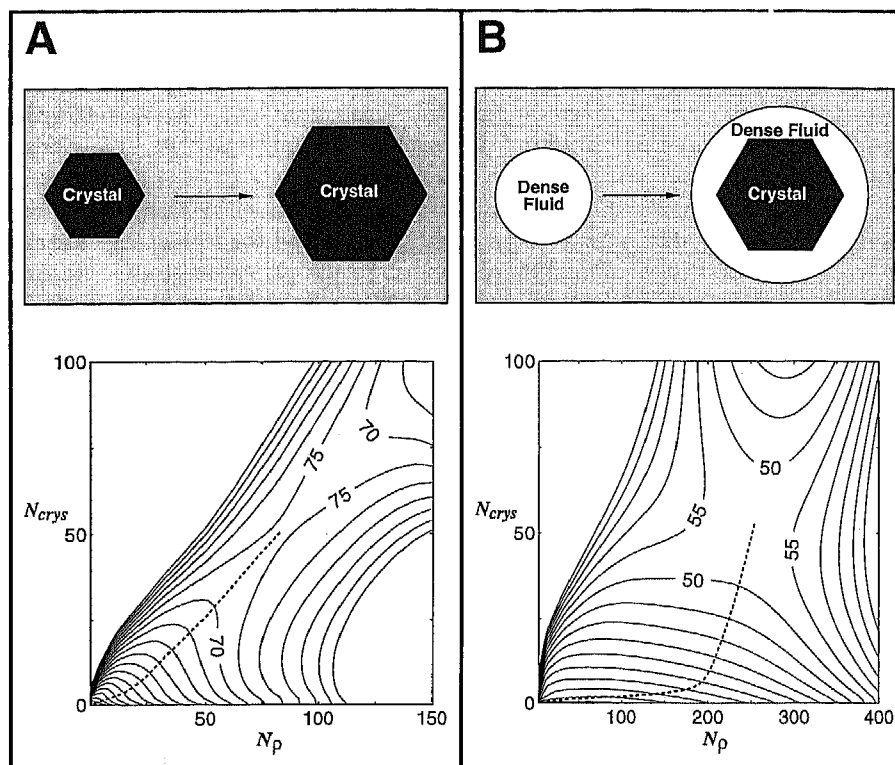


Figure 3: Contour plots of the free-energy landscape along the path from the metastable fluid to the critical crystal nucleus, for our system of spherical particles with short-ranged attraction. The curves of constant free energy are drawn as a function of N_ρ and N_{crys} (see text) and are separated by $5k_B T$. If a liquid-like droplet forms in the system, we expect N_ρ to become large, while N_{crys} remains essentially zero. In contrast, for a normal crystallite, we expect that N_ρ is proportional to N_{crys} . Figure 3A shows the free energy landscape well below the critical temperature ($T/T_c = 0.89$). The lowest free-energy path to the critical nucleus is indicated by a dashed curve. Note that this curve corresponds to the formation and growth of a highly crystalline cluster. Figure 3B: Idem, but now for $T = T_c$. In this case, the free-energy valley (dashed curve) first runs parallel to the N_ρ axis (formation of a liquid-like droplet), and moves towards a structure with a higher crystallinity (crystallite embedded in a liquid-like droplet). The free energy barrier for this route is much lower than the one shown in figure 3A.

T/T_c	T_m/T_c	$(T_m - T)/T_m$	$\Delta H(T_m)/(k_B T_m)$	$\rho_{cr}(T_m)$	$\Delta G^*/k_B T$
0.89	1.04	0.136	-12.59	0.877	75
1.0	1.14	0.123	-10.35	0.872	53
1.09	1.22	0.109	-8.153	0.869	83
2.23	2.39	0.069	-2.996	0.814	128

Table 1: Table I: Parameters characterizing the simulations described in the text. Meaning of symbols – T : absolute temperature, T_c : critical temperature. T_m : melting temperature. $\Delta H(T_m)/(k_B T_m)$: ratio of solid-fluid enthalpy difference and thermal energy at coexistence. $\rho_{cr}(T_m)$: density of the crystal phase at coexistence (in units σ^{-3}). $\Delta G^*/k_B T$: nucleation barrier height divided by $k_B T$. If we estimate γ using Turnbull’s phenomenological rule²⁸, Eqn. 2 predicts a constant nucleation barrier. The lowering of the barrier at T_c is due to the fact that, near a (meta-stable) critical point, large liquid-like clusters can be formed at little free-energy cost.

Note that the observed reduction in ΔG^* near T_c by some $30k_B T$ corresponds to an increase in nucleation rate by a factor 10^{13} . The crucial point to note is that, at near the critical point, the incipient crystallite is wetted by a nearly critical liquid. This has a pronounced effect on the surface free energy of the nucleus. Let us denote the free energy density of the solid surface in contact with the “vapor” phase by γ_{sv} . This is the interfacial free-energy density to which Turnbull’s rule should apply. However, if the crystallite is covered by a thin liquid layer, then (ignoring curvature effects) the surface free-energy density becomes $\gamma_{sl} + \gamma_{lv}$, where γ_{sl} is the solid-liquid interfacial free-energy density and γ_{lv} the liquid-vapor surface tension. The first point to note is that γ_{sl} is typically an order of magnitude less than γ_{sv} . But, in addition, at the critical point γ_{lv} vanishes. Hence it is not surprising that the surface free energy of a crystallite embedded in a critical droplet is much less than that the same crystallite in contact with the dilute vapor phase.

Let us consider the implications of this reduction of the crystal nucleation barrier near T_c . An alternative way to lower the crystal nucleation barrier would be to quench the solution deeper into the meta-stable region below the solid-liquid coexistence curve. However, such deep quenches often result in the formation of amorphous aggregates^{16,17,18,21,33,24}. Moreover, in a deep quench, the thermodynamic driving force for crystallization ($\mu_{liq} - \mu_{cryst}$) is also enhanced. As a consequence, the crystallites that nucleate will grow rapidly and far from perfectly¹⁵. In contrast, by adjusting the solvent conditions (e.g. by the addition of non-ionic polymer) such that a meta-stable fluid-fluid critical point is located just below the sublimation curve, we can selectively speed up

the rate of crystal *nucleation*, but not the rate of crystal growth, nor the rate at which amorphous aggregates form.

Clearly, our description of the early stages of protein crystallization is highly simplified. Yet, we believe that the mechanism for enhanced crystal nucleation that we find is quite general. We conclude by noting that the phase diagram shown in figure 1B is likely to be the rule, rather than the exception for compact macromolecules. Moreover, it occurs both in the bulk and in (quasi) two-dimensional systems (e.g. in membranes). There is also evidence that a similar phenomenon occurs in the crystal nucleation of certain metal alloys³⁴. It is therefore tempting to speculate that nature already makes extensive use of "piggy-back riding" on critical density fluctuations to facilitate the formation of ordered structures.

Acknowledgments

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References

1. V.F. Weisskopf, Trans. NY Acad. Sci. II, **38**(1977)202.
2. See, e.g. J.S. Rowlinson, **Studies in Statistical Mechanics**, Vol. XIV, J.L. Lebowitz (editor), North Holland, Amsterdam, 1988.
3. P.C. Hemmer and J.L. Lebowitz, in *Critical Phenomena and Phase Transitions 5b*, C. Domb and M. Green (editors), Academic Press, New York, (1976)
4. H.C. Longuet-Higgins and B. Widom, Mol. Phys. **8**(1964)549.
5. A.P. Gast, C.K. Hall and W.B. Russel, J. Coll. Inter. Sci. **96**(1983)251.
6. S. Asakura and F. Oosawa, J. Pol. Sci. **33**(1958)183.
7. P.N. Pusey, in *Liquids, freezing and glass transition*, J.P. Hansen, D. Levesque and J. Zinn-Justin (editors), North-Holland, Amsterdam, 1991, p. 763
8. E.J. Meijer and D. Frenkel, Phys. Rev. Lett. **67**(1991)1110.
9. E.J. Meijer and D. Frenkel, J. Chem Phys. **100**(1994)6873.

10. P.G. Bolhuis and D. Frenkel, Phys. Rev. Lett. **72**(1994)221.
P.G. Bolhuis, M.H.J. Hagen and D. Frenkel, Phys. Rev. E **50**(1994)4880.
11. M.H.J. Hagen et al., Nature, **365**(1993)425.
12. A. Jayaraman, Phys. Rev. **137**(1965)A179.
13. P. R. ten Wolde and D. Frenkel, Science **277**(1997)1975.
14. A. McPherson *Preparation and analysis of protein crystals*, Krieger Publishing, Malabar, 1982.
15. S.D. Durbin and G. Feher, Ann. Rev. Phys. Chem. **47**(1996)171, F. Rosenberger, J. Crystal Growth **166**(1996)40.
16. A. George and W.W. Wilson, Acta Crystallogr. D **50**(1994)361.
17. D. Rosenbaum, P.C. Zamora, and C.F. Zukoski, Phys. Rev. Lett. **76**(1996)150.
18. D.F. Rosenbaum and C.F. Zukoski, J. of Cryst. Growth **169**(1996)752.
19. H.N.W. Lekkerkerker et al., Europhys. Lett. **20**(1992)559.
20. M.H.J. Hagen and D. Frenkel, J. Chem. Phys. **101**(1994)4093.
21. S.M. Ilett, A. Orrock, W.C.K. Poon, and P.N. Pusey, Phys. Rev. E **51**(1995)1344.
22. M.L. Broide et al., Proc. Natl. Acad. Sci. USA **88**(1991)5660. C.R. Berland et al., Proc. Natl. Acad. Sci. USA **89**(1992)1214. N. Asherie, A. Lomakin, and G.B. Benedek, Phys. Rev. Lett. **77**(1996)4832.
23. M.L. Broide, T.M. Tominc, and M.D. Saxowsky, Phys. Rev. E **53**(1996)6325.
24. M. Muschol and F. Rosenberger, *J. Chem. Phys.* **107**(1997)1953.
25. A. McPherson, J. Biol. Chem. **251**(1976)6300.
26. A. Kose and S. Hachisu, J. Coll. Interface Sci. **55**(1976)487. C. Smits et al., Phase Transitions **21**(1990)157.
27. P.R. ten Wolde, M.J. Ruiz-Montero, and D. Frenkel, Phys. Rev. Lett. **75**(1995)2714, and J. Chem. Phys. **104**(1996)9932.
28. See e.g.: K.F. Kelton, in *Crystal Nucleation in Liquids and Glasses*, H. Ehrenreich and D. Turnbull editors, (Academic, Boston, 1991), Vol. 45, pp.75-177.
29. G.M. Torrie and J.P. Valleau, Chem. Phys. Lett. **28**(1974)578.
30. Two particles belong to the same cluster if their distance is less than 1.5σ .
31. C. Haas and J. Drenth, J. Crystal Growth, **154**(1995)126.
32. A.J. Malkin and A. McPherson, J. Crystal Growth, **128**(1992)1232, *ibid.* **133**(1993)29.
33. W.C.K. Poon, A.D. Pirie and P.N. Pusey, Faraday Discuss. **101**(1995)65.
W.C.K. Poon, Phys. Rev. E **55**(1997)3762.
34. F. G. Shi, preprint.