NUCLEATION OF CRYSTALS IN SOLUTION

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Solution crystallization is an essential part of processes in the chemical and pharmaceutical industries and a major step in physiological and pathological phenomena. Crystallization starts with nucleation and control of nucleation is crucial for the control of the number, size, perfection, polymorphism and other characteristics of the crystalline materials.

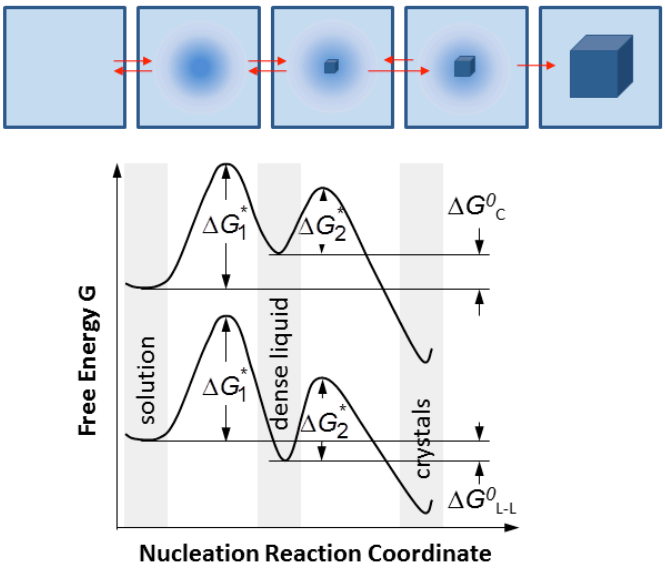

Figure 1. Schematic illustration of the two-step mechanism of nucleation of crystals. A dense liquid cluster forms. A crystal nucleus may form inside the cluster. Top: Macroscopic viewpoint of events. Bottom: The free-energy ΔG along two possible versions of the two step nucleation mechanism. ΔG^0_{L-L} *is the standard free energy of formation of dense liquid phase and* ΔG^0_C*—that of formation of clusters. If the dense liquid is unstable and* $\Delta G^0_{L-L} > 0$*, the dense liquid exists as mesoscopic cluster,* ΔG^0_{L-L} *transforms to* ΔG^0_C*, and upper curve applies; if dense liquid is stable,* $\Delta G^0_{L-L} <$ *; this case is depicted by lower curve.* ΔG_1^* *is the barrier for formation of a cluster of dense liquid,* ΔG_2^* *– for a formation of a crystalline nucleus inside the dense liquid.*

The starting point for the recent advances in the understanding of the nucleation mechanisms was the realization that the classical nucleation theory failed to provide understanding of several features of measured kinetic curves: nucleation rates, which are orders of magnitude lower than the classical prediction; nucleation kinetics curves which exhibit saturation [1], or, even more puzzling, maxima and decreasing branches [2],

with increasing supersaturation; as well as the role of the other, stable and unstable, phases possible in solution [3].

We show that these features of the nucleation kinetics reflect the action of two factors, which are unaccounted by the classical nucleation theory: the existence of a spinodal for the solution to crystal phase transition, and the action of a two-step nucleation mechanism. As the spinodal is reached upon supersaturation increase, the barrier for nucleation of crystals vanishes and further increases in supersaturation do not yield faster nucleation rate [4]. According to the two-step mechanism, as illustrated in Figure 1, the nucleation of a crystal, step two, occurs within mesoscopic clusters of dense liquid, formed during step one [4]. While the initial thought provoking results on the nucleation kinetics were obtained for the nucleation of protein crystals, and, correspondingly, the two-step mechanism was first proposed for these types of crystals only, further investigations have shown the validity of this mechanism to several organic [5], inorganic and colloid [6] materials, including the important class of biominerals [7].

Two intermediate states for the two-step mechanism are possible: the stable dense liquid and the metastable clusters. The stable dense liquid is relatively well understood [8]. The metastable liquid clusters, illustrated in Figure 2, have been discovered relatively recently [8] and their underlying causes are still a subject of intense work [9].

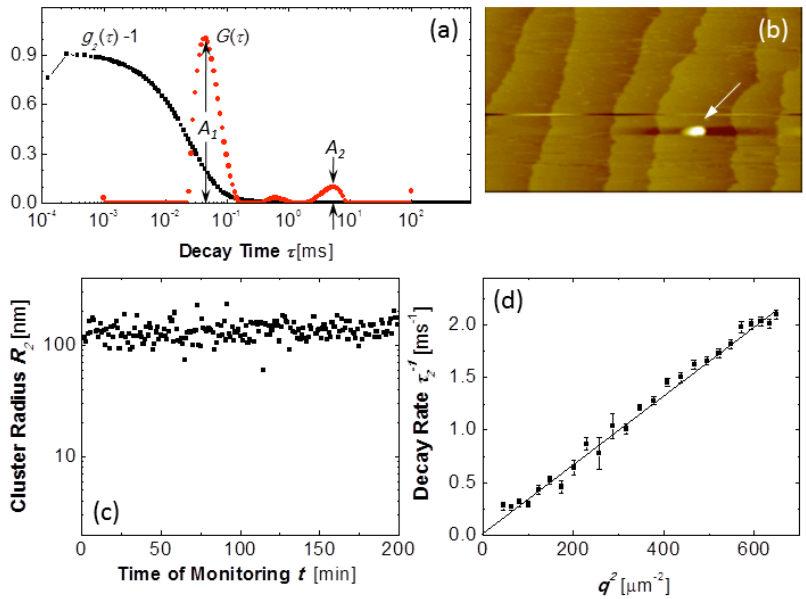

Figure 2. Evidence of dense liquid clusters. (a) Examples of correlation function of the scattered intensity $g_2(\tau)$ and the respective intensity distribution function $G(\tau)$ of a lysozyme solution with C = 148 mg ml^{-1} in 20 mM HEPES buffer; data collected at angle 145°. (b) Atomic force microscopy imaging of liquid cluster landing on the surface of a crystal in a lumazine synthase solution. Tapping mode AFM imaging, scan width 20 μm. Apparent lateral cluster dimensions are misleading, cluster height is 120 nm (c) Time dependence of the radius of dense liquid clusters in the same lysozyme solution as in a. (d) The dependence of the decay rate $\Gamma_2 = \tau_2^{-1}$ of the cluster peak in the correlation function on the squared wave vector q^2 for a lysozyme solution as in (a).

To assess the applicability of the two-step mechanism to the overwhelming majority of untested systems, we note that its action relies of the availability of disordered liquid or

amorphous metastable clusters in the homogeneous solutions prior to nucleation. These two precursors have distinct mechanisms: the discrepancy of the lengthscale of the intermolecular interactions in the solution and the size of the crystallizing molecules for the stable dense liquid [10], and the existence of limited lifetime complexes for the clusters [9]. Thus, for a given system the availability of any of these two intermediate states is independent of the other; both of them depend on the exact physicochemical characteristics of the system.

While metastable clusters have been demonstrated for several protein systems and for calcium carbonate solutions it is likely that not all solutions would support the existence of such clusters with properties allowing the nucleation of crystals in them. In such systems the action of the direct nucleation mechanism might be the only option. On the other hand, an intriguing hypothesis is presented by one of the theories discussed above: that a stabilized intermediate state, as a stable dense liquid, or as a metastable mesoscopic cluster is not needed and the two-step mechanism will act even if the intermediate step is just a density fluctuation. Thus, the two-step mechanism may in fact operate in systems where no intermediate is independently found.

The applicability of the concept of the solution-crystal spinodal appears more straightforward: the nucleation of numerous crystals in industrial and laboratory practice is carried out at such high supersaturations that the nucleation occurs either in the spinodal regime or in the immediate vicinity of this regime, where the nucleus consist of just a few molecules.

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