

How Does a Transient Amorphous Precursor Template Crystallization

Tian Hui Zhang and Xiang Yang Liu*

Contribution from the Department of Physics, National University of Singapore, 2 Science Drive 3, Singapore 117542

Received May 19, 2007; E-mail: phyluxy@nus.edu.sg

Abstract: Crystallization through metastable phases, such as polymorphism, plays an important role in chemical manufacture, biomineralization, and protein crystallization. However, the kinetics creating the final stable crystalline phase from metastable phases has so far remained unclear. In this study, crystallization via an amorphous precursor, the so-called multistep crystallization (MSC), is studied quantitatively in a colloidal model system. In MSC, amorphous dense droplets are first nucleated from the mother phase. Subsequently, a few unstable subcrystalline nuclei can be created simultaneously by fluctuation from the tiny dense droplets, which is different from previous theoretical predictions. It is necessary for these crystalline nuclei to reach a critical size N_{crys}^* to become stable. However, in contrast to subcrystalline nuclei, a stable mature crystalline nucleus is not created by fluctuation but by coalescence of subcrystalline nuclei, which is unexpected. To accommodate a mature crystalline nucleus larger than the critical size N_{crys}^* , the dense droplets have to first acquire a critical size N^* . This implies that only a fraction of amorphous dense droplets can serve as a precursor of crystal nucleation. As an outcome, the overall nucleation rate of the crystalline phase is, to a large extent, determined by the nucleation rate of crystals in the dense droplets, which is much lower than the previous theoretical expectation. Furthermore, it is surprising to see that MSC will promote the production of defect-free crystals. The knowledge acquired in this study will also significantly advance our understandings in polymorphism related processes.

Introduction

Crystallization is of fundamental importance in solid-state chemistry, biology, and material sciences. In chemistry, because the size, morphology, and structure of crystals can strongly affect chemical reactivity and the stability of suspensions, crystallization control is extremely desirable in industry fields including pharmaceuticals, photographic chemicals, agrochemicals, etc. Moreover, controlling crystal growth at the atomic level is also critical in nanotechnology where the properties of advanced devices are essentially determined by the size and shape of crystalline structures. To explore robust experimental strategies for crystallization control, much attention has been focused on studying crystallization mechanisms in the past decades.^{1–3} A major focus of such studies is crystallization through metastable phases. For instance, it has been found that polymorphism, the ability of a system to exist in different crystalline structures, is an important phenomenon in chemical manufacture including pharmaceuticals where crystalline structures will affect the bioavailability of drugs. According to Ostwald's rule,⁴ the first occurring phase in polymorphism is normally the one which is closest in free energy to the mother phase, that is, the least stable phase, followed by phases in order of increasing stability. An

intriguing example of Ostwald's rule is the so-called two-step crystallization (TSC) which has attracted much attention in the past decade.

TSC was originally suggested by ten Wolde and Frenkel.⁵ They found by simulations that, near the critical point of a liquid–liquid-phase separation (LLPS), proteins crystallize through a two-step process: at the first step, amorphous dense droplets nucleate from the mother phase through LLPS; subsequently, crystalline nuclei are created from the amorphous dense droplets. Due to its significant implications in science and technology, TSC has been studied both theoretically^{6–9} and experimentally^{10,11} in protein solutions. However, recent studies suggested that this mechanism occurs not only in protein solutions but also in typical atomic systems.^{12–14} Lutsko et al. argued that TSC may be a mechanism underlying most crystallization processes in atomic systems.¹⁴ Experimentally, this mechanism was actually observed during the growth of nanocrystals.¹³ Moreover, it has been observed that TSC occurs

- (1) Piana, S.; Gale, J. D. *J. Am. Chem. Soc.* **2005**, *127*, 1975.
- (2) Piana, S.; Jones, F.; Gale, J. D. *J. Am. Chem. Soc.* **2006**, *128*, 13568.
- (3) Anwar, J.; Boateng, P. K. *J. Am. Chem. Soc.* **1998**, *120*, 9600.
- (4) Ostwald, W. *Z. Phys. Chem.* **1897**, *22*, 289.

- (5) ten Wolde, P. R.; Frenkel, D. *Science* **1997**, *277*, 1975–1978.
- (6) Talanquer, V.; Oxtoby, D. W. *J. Chem. Phys.* **1998**, *109*, 223.
- (7) Haas, C.; Drenth, J. *J. Cryst. Growth* **1999**, *196*, 388.
- (8) Haas, C.; Drenth, J. *J. Phys. Chem. B* **2000**, *104*, 368.
- (9) Shiryayev, A.; Gunton, J. D. *J. Chem. Phys.* **2004**, *120*, 8318.
- (10) Kuznetsov, Y. G.; Malkin, A. J.; McPherson, A. *J. Cryst. Growth* **2001**, *232*, 30–39.
- (11) Vekilov, P. G. *Cryst. Growth Des.* **2004**, *4*, 671–685.
- (12) ten Wolde, P. R.; Frenkel, D. *Phys. Chem. Chem. Phys.* **1999**, *1*, 2191.
- (13) Chen, X.; Samia, A. C. S.; Lou, Y.; Burda, C. *J. Am. Chem. Soc.* **2005**, *127*, 4372–4375.
- (14) Lutsko, J. F.; Nicolis, G. *Phys. Rev. Lett.* **2006**, *96*, 046102.

also widely in biorelated crystallizations.¹⁵ For example, during the formation of calcite in sea urchin larvae, a transient amorphous phase is first formed, before the final crystal phase is reached.^{16,17} Similarly, a transient amorphous phase is also identified during the formation of aragonite controlled by mollusk bivalve larvae.^{17,18} It is widely believed that the development of crystalline structures with a well-defined shape and size in biological systems is essentially facilitated by the occurrence of transient amorphous phases.^{16,17,19}

A remarkable drawback of previous studies concerning TSC is that no direct investigation of the kinetic processes of TSC has been conducted. Therefore, in which way the occurrence of a transient amorphous precursor will affect and modify the formation of a crystalline structures has so far not been addressed. A comprehensive understanding of this issue will advance our knowledge on multistep crystallization and provide a guideline in identifying robust experimental strategies for the engineering of crystalline structures. Despite its importance, however, the kinetic study of stepwise crystallization such as TSC has so far been absent.

In this study, the kinetics of crystallization via an amorphous precursor was studied using a colloidal model system. Our results were obtained from in situ observations at the single-particle level. The major purpose of this study is to address the kinetics of the creation of crystalline nuclei and their subsequent growth in an amorphous precursor. Furthermore, the kinetic advantages of crystallization via an amorphous precursor in producing high quality crystals are addressed. This information will provide an in-depth understanding of the effect of amorphous precursors on crystal growth.

Experimental Section

Figure 1a shows the experimental setup. Monodisperse colloidal particles (polystyrene spheres of diameter $0.99\mu\text{m}$, polydispersity $<5\%$, Bangs Laboratories) were dispersed uniformly in deionized water. The colloidal suspension was then sealed between two parallel horizontal conducting glass plates coated with indium tin oxide (ITO). In this system, fluid flow induced by an alternating electric field (AEF) transports the colloidal particles to the surface of the glass plates where two-dimensional crystals are formed under certain conditions.²⁰ The processes of crystallization were recorded for analysis by a digital camera (CoolSNAP cf, Photometrics) which was mounted on an Olympus BX51 microscope. This system has proven to be a good model system for studies of nucleation,²¹ phase transitions,²² and crystal growth.^{23,24} Unlike previous studies, we used the significantly lower volume fraction of 0.03%, because in experiments a high volume fraction tends to lead the system to gel. The surface potential of the colloidal particles was adjusted to -72mV by adding Na_2SO_4 with a concentration of 10^{-4}M . The pH of the suspension was measured to be 6.35. In this system, colloidal particles are brought together by the electrodynamic fluid flow and aggregate on the surfaces of the glass plates. Two-dimensional crystals (2DC) are obtained under certain conditions, as Figure 1b shows. Three-dimensional liquid (3DL) and

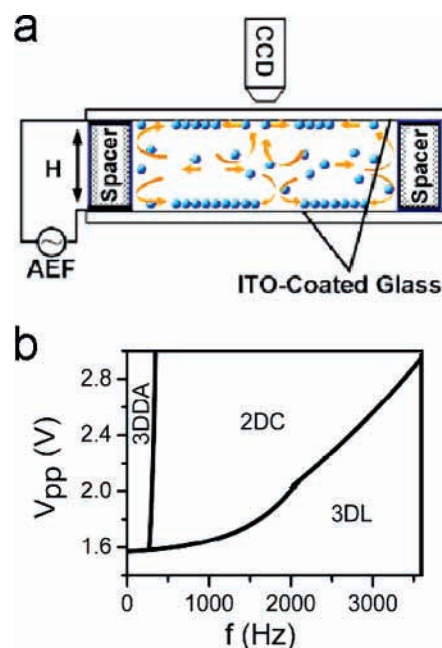


Figure 1. (a) Experimental setup. Colloidal suspension is sealed between two pieces of ITO-coated conducting glass plates separated by insulating spacers. The gap between the two glass plates is $H = 120 \pm 5\mu\text{m}$. The dynamic process is recorded by a digital camera for analysis. (b) Phase diagram of the colloidal suspension.

three-dimensional disordered aggregation (3DDA) were also observed in this system.

Results and Discussion

In our experiments, the most interesting phenomenon observed in the 2DC region is crystallization via an amorphous dense phase. A typical process is shown in Figure 2. In our system, the mother phase is a dilute solution as shown in Figure 2a. As the system is supersaturated by applying an AEF ($f = 800\text{ Hz}$, $E = 167\text{ V/cm}$), two-dimensional amorphous dense droplets are first formed on the glass surfaces as shown in Figure 2b. Two-dimensional crystals are subsequently formed from the droplets as shown in Figure 2c. Consistently with previous theoretical studies of TSC,^{6–9} the crystals are covered by a liquid film (Figure 2c). In this process, as suggested by Ostwald's rule, the amorphous dense droplets are first nucleated because their lower interfacial free energy (the 1D analogue of the surface free energy) results in a lower nucleation energy barrier as compared with the crystals. Nevertheless, the subsequent nucleation of the crystalline phase in the droplets indicates that the amorphous phase is metastable with respect to the crystalline phase.

The manner in which the initial crystalline nuclei are created from the metastable amorphous phases has never been addressed^{6–14} due to the difficulty of directly observing the nucleation process in real space. In contrast to previous studies,^{6–14} in our system, the motion of individual colloidal particles can be traced in real time and real space, allowing us to follow the dynamic processes of the creation of the crystalline nuclei at the single-particle level. Taking advantage of this, the dynamics for the dense droplets to create crystalline nuclei was investigated in detail. A typical process is exhibited in Figure 3. From Figure 3a, it is found that, initially, a few crystalline nuclei are created simultaneously from the dense droplets. Nevertheless, these small nuclei are unstable, namely, they are

- (15) Addadi, L.; Weiner, S. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 153.
- (16) Benish, E.; Aizenberg, J.; Addadi, L.; Weiner, S. *Proc. R. Soc. London, Ser. B* **1997**, *264*, 461.
- (17) Addadi, L.; Raz, S.; Weiner, S. *Adv. Mater.* **2003**, *15*, 959.
- (18) Weiss, I. M.; Tuross, N.; Addadi, L.; Weiner, S. *J. Exp. Zool.* **2002**, *293*, 478.
- (19) Weiss, I. M.; Tuross, N.; Addadi, L.; Weiner, S. *J. Exp. Zool.* **2002**, *293*, 478.
- (20) Liu, Y.; Narayanan, J.; Liu, X. Y. *J. Chem. Phys.* **2006**, *124*, 124906.
- (21) Zhang, K.-Q.; Liu, X. Y. *Nature* **2004**, *429*, 739.
- (22) Zhang, K.-Q.; Liu, X. Y. *Phys. Rev. Lett.* **2006**, *96*, 105701.
- (23) Zhang, T. H.; Liu, X. Y. *J. Phys. Chem. C* **2007**, *111*, 1342.
- (24) Zhang, T. H.; Liu, X. Y. *Appl. Phys. Lett.* **2006**, *89*, 261914.

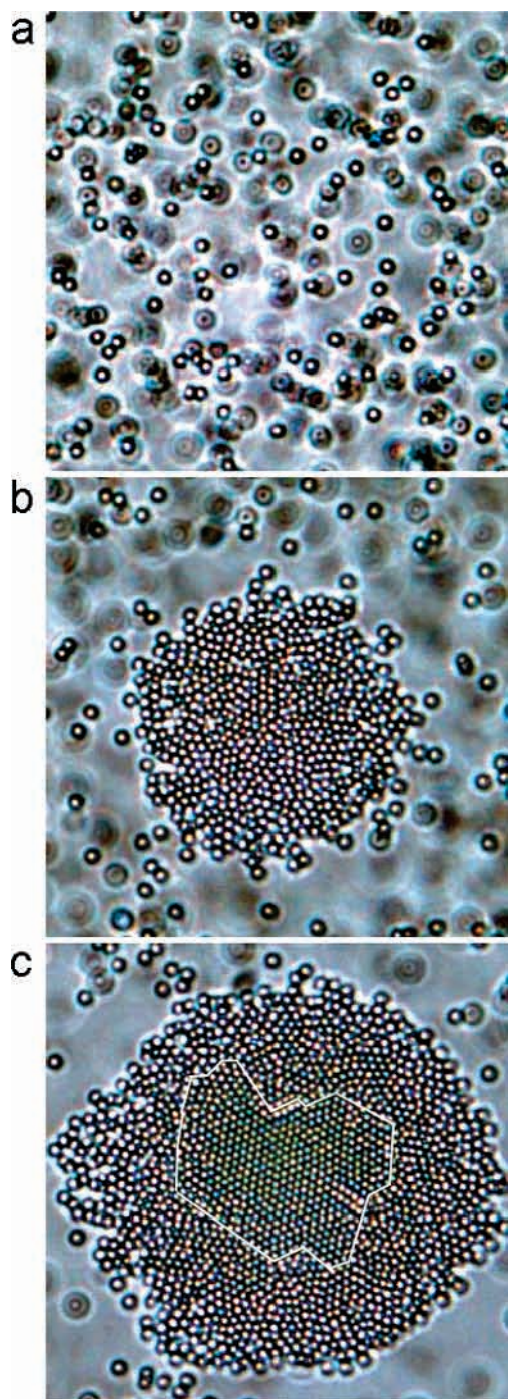


Figure 2. Multistep crystallization observed at 800 Hz and 167 V/cm: (a) Initial dilute liquid phase. (b) Amorphous dense droplets are first created from the mother phase. (c) Crystalline nuclei are created from the amorphous phase.

subcrystalline nuclei. A detailed investigation shows that the subcrystalline nuclei tend to shrink or dissolve soon after their creation and, subsequently, other subcrystalline nuclei are formed randomly again. It is evident that the creation as well as the dissociation of the subcrystalline nuclei in the dense droplets is governed by thermal fluctuations. As the number of subcrystalline nuclei increases with the growth of the dense droplets, a stable mature crystalline nucleus is finally created (Figure 3c). It is interesting to note that although a few subcrystalline nuclei are created simultaneously at an early stage as shown in Figure 3a, only one stable mature crystalline nucleus

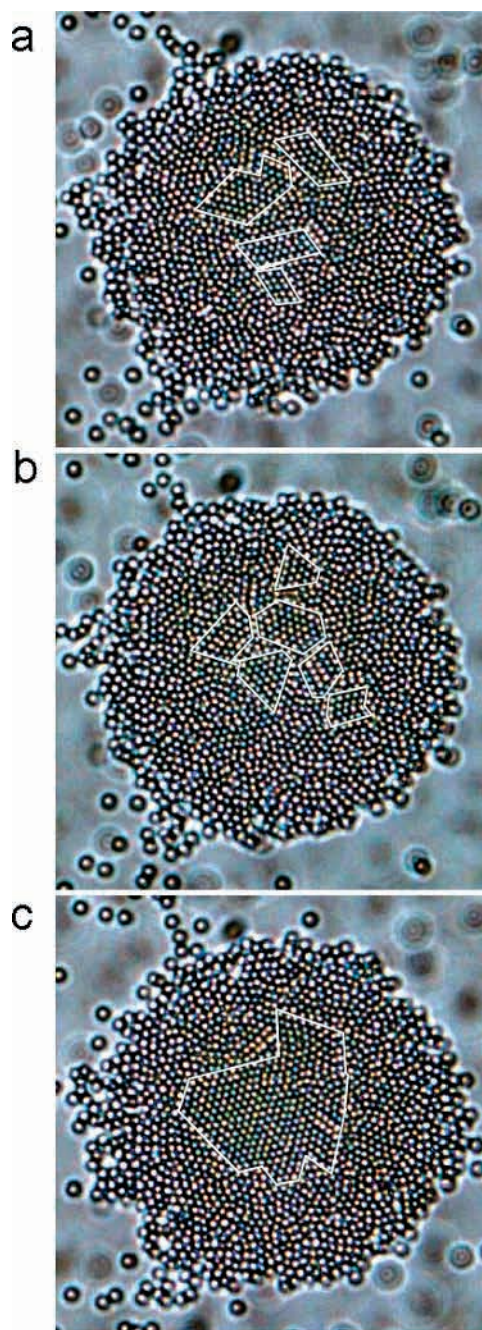


Figure 3. The evolution of crystalline structures inside the amorphous phase. (a) Small subcrystalline nuclei are initially created in the droplets. (b) More subcrystalline nuclei are created as the droplet grows. (c) Stable mature crystalline nuclei are created from the dense droplets.

is possible in a dense droplet. It is clear that, in the dense droplets, crystalline nuclei have to get large enough in order to become stable.

The fact that the formation of crystalline nuclei in the tiny droplets is subjected to thermal fluctuations, and several subcrystalline nuclei can be created simultaneously, was not realized in previous studies of TSC.^{6–11} On the contrary, although not clearly stated, it was assumed^{6–11} that a crystalline nucleus is formed and thus grows continuously from the beginning. Based on this assumption, the so-called mononuclear mechanism was developed.²⁵ It follows that once a crystalline nucleus is created from the dense droplets, it grows so fast that the formation of the second crystalline nucleus is suppressed.

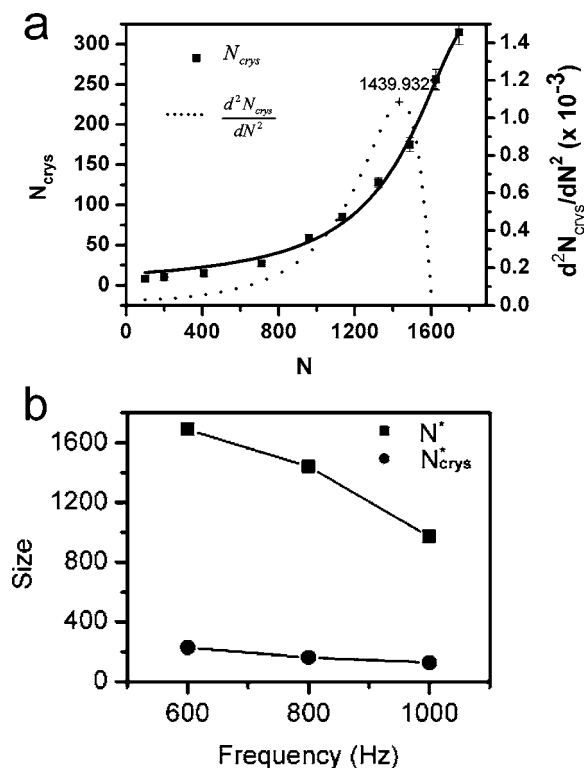


Figure 4. (a) Dependence of N_{crys} on N in a typical MSC ($f = 800$ Hz and $E = 167$ V/cm). The critical size of crystalline nuclei is identified from the maximum of d^2N_{crys}/dN^2 . (b) Dependence of N^*_{crys} and N^* on frequency under condition of $E = 167$ V/cm.

Based on the mononuclear mechanism, the overall nucleation rate of crystals in TSC was established. However, our observation shows that this assumption is not applicable in real experiments.

To quantify the crystallization process and identify the critical size for the crystalline nuclei, a local two-dimensional bond-order parameter is defined by

$$\psi_6(r_i) = M^{-1} \left| \sum_j e^{i6\theta_{ij}} \right| \quad (1)$$

where r_i is the center of particle i , and θ_{ij} is the angle subtended between the vector from particle i to its j th nearest neighbor and the arbitrarily chosen x -axis. M is the number of nearest neighbors of particle i . The mean value $\langle \psi_6 \rangle$ in typical crystals obtained from our experiments is 0.80 which is taken as the criterion for a crystal-like particle in this study.

Using ψ_6 , the crystallization process illustrated by Figures 2 and 3 was analyzed quantitatively. The dependence of the number N_{crys} of crystal-like particles on the size N of the dense droplets is plotted in Figure 4a. It is clear that N_{crys} increases with N . However, the N_{crys} vs N plot consists of three regimes in terms of N . Regime I (linear regime): $N < 1000$. The increase of N_{crys} is essentially proportional to the increase of N , giving rise to a small slope $dN_{crys}/dN \approx 0.03$. Regime II (nonlinear regime): $1000 < N < 1400$. The plot N_{crys} vs N is a curve, dN_{crys}/dN rising continuously with N . Regime III (linear regime): $N > 1400$. dN_{crys}/dN remains constant and N_{crys} becomes linearly dependent on N again, giving rise to a relatively much larger dN_{crys}/dN (~ 0.45). Further investigation

reveals that as N increases beyond 1400, the crystalline structure in the dense droplets is represented by a mature crystalline nucleus as shown in Figure 3c. It is clear that the fast steady increase of N_{crys} as $N > 1400$ is due to the existence of the mature crystalline nucleus, which acts as a stable core for subsequent crystal growth, while the slow steady growth of N_{crys} when N is less than 1000 should be attributed to the co-occurrence of dissociation and creation of crystalline nuclei. Accordingly, the creation of a critical crystalline nucleus should be responsible for the termination of the continuous rise of dN_{crys}/dN in Regime II. Based on the above reasoning, d^2N_{crys}/dN^2 should approach its maximum when the amorphous dense droplets reach a critical size N^* and thus are able to form a crystalline critical nucleus with size N^*_{crys} . d^2N_{crys}/dN^2 was derived as a function of N by differentiating the fit of the dependence of N_{crys} on N . As shown in Figure 4a, d^2N_{crys}/dN^2 approaches its maximum at $N^* \sim 1440$. The corresponding N^*_{crys} is measured to be ~ 161 . Here, it is clear that to accommodate a critical crystalline nucleus, an amorphous dense droplet needs to first acquire a critical size N^* . Compared with previous results of TSC,^{6–14} the stepwise crystallization in our experiments is characterized by the fluctuation-governed dynamic of creating crystalline nuclei and the critical sizes N^*_{crys} and N^* . To notice this difference, multistep crystallization (MSC) is used to establish the special crystallization processes observed in our experiments.

In our experiments, both of the critical sizes N^*_{crys} and N^* in MSC are a function of supersaturation which in our system is dependent on both frequency f and the strength of electric field E .²¹ Figure 4b shows that increasing frequency leads to a decrease of both N^*_{crys} and N^* . However, compared with the decrease of N^* , the change of N^*_{crys} is minor, indicating that, at high frequencies, the dense droplets are likely to create a mature crystalline nucleus at an earlier stage in terms of N . This indicates that the relative supersaturation in the dense droplets for the nucleation of the crystalline phase is enhanced by increasing the frequency.

We have discussed above that the subcrystalline nuclei in the metastable amorphous droplets are created by thermal fluctuations. However, it is surprising to find that the stable mature crystalline nuclei are not created by thermal fluctuations but by the coalescence of the subcrystalline nuclei. As the time sequence in Figure 3 shows, accompanying the sharp increase of dN_{crys}/dN in the region $1000 < N < 1400$, the average number of subcrystalline nuclei increases as can be seen from Figure 3a and b. As a consequence, the subcrystalline nuclei approach one another in the dense droplets. Finally, these subnuclei meet and coalesce. However, in the early stage, the result of the coalescence is usually a crystalline nucleus smaller than N^*_{crys} , which normally breaks up again into subcrystalline nuclei. Once a crystalline nucleus larger than N^*_{crys} is coalesced, it will remain stable, functioning as a growth center for the subsequent growth.

The final mature crystals in the dense droplets are, to a large extent, free from defects, such as grain boundaries, as can be seen in Figures 2c and 3c. However, since the subcrystalline nuclei as shown in Figure 3a and b usually do not match one another's orientations before the coalescence, grain boundaries are expected to appear in the final crystals. In fact, grain boundaries actually exist in the initial mature crystals, but they

(25) Kashchiev, D.; Vekilov, P. G.; Kolomeisky, A. B. *J. Chem. Phys.* **2005**, *122*, 244706.

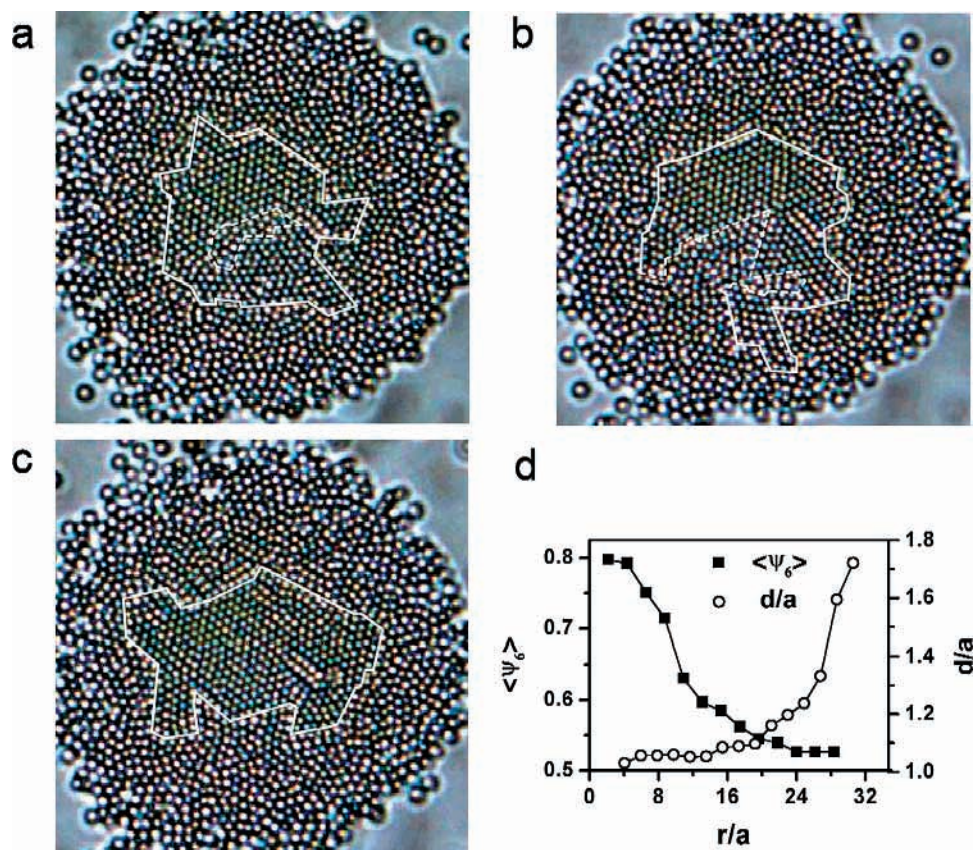


Figure 5. Elimination of grain boundaries. (a) Grain boundaries (encircled by a dashed line) are created during the coalescence. (b) A local transformation from crystalline phase to amorphous phase is adopted to release the strain. (c) Defect-free crystalline structure after the elimination. (d) The order degree in terms of $\langle \psi_6 \rangle$ and the average center-to-center distance d as a function of the distance r to the mass center of the colloidal cluster shown in Figure 5c. The gradual increase of d is a direct reflection of the gradual decrease of density. a is the diameter of the colloidal particles.

are eliminated gradually during the following growth. The kinetics of the defect elimination is illustrated by a time sequence shown in Figure 5. Initially, a grain boundary exists in the mature crystal as shown in Figure 5a. However, in the following process, part of the crystalline structure adjacent to the grain boundary melts and becomes amorphous as shown in Figure 5b. We suggest that this local transition from the crystalline phase to the amorphous phase is triggered by the strain contained in the grain boundary. By this local crystal-liquid transition, the strain is released, and most importantly, the particles involved get a chance to rearrange themselves along the orientations defined by the remaining crystalline structure. As a result, the orientations are unified considerably (Figure 5c). Normally, the removal of grain boundaries needs to break the surface bonds of crystals due to the interfacial tension, and thus energy is needed to initiate this process. Nevertheless, during the process shown in Figure 5, no additional energy is input. It seems that the local crystal-liquid transition shown in Figure 5 is free of an energy barrier.

An important feature of the colloidal clusters as shown in Figure 5a–c is that both the order degree and density in the dense droplets decrease continuously from the crystalline core to the amorphous fringe. A quantitative description of the continuous changes of the degree of ordering and density in the cluster is presented in Figure 5d. By noticing this continuous change in the order degree and the density, the “free” local crystal-liquid transition can be then well understood. Interfacial tension is normally induced by a sharp change in density or in

structure on the interface. However, in our experiments, due to the continuous change in the density and the order degree, there is an intermediate region in the dense droplets between the crystalline core and the amorphous fringe. In the intermediate region, the density in the interior adjacent to the crystalline core is almost identical to the crystalline phase, and the arrangement of colloidal spheres there is also ordered or at least partially ordered. In fact, it is hard to define an interface between the crystalline phase and the amorphous phase in the dense droplets due to the existence of the intermediate region. The particles in this region are just as likely to become crystal-like as well as liquid-like by fluctuations. Therefore, the intermediate region is ready to allow a crystal-liquid transition whereby the grain boundaries are eliminated. Moreover, through the transition from crystal to liquid, crystal-like particles incorrectly incorporated in the intermediate region can easily become liquid-like again and obtain one more chance to incorporate itself. This advantage can significantly inhibit the creation of defects caused by incorrect incorporations.

Another advantage of MSC proposed by previous studies of TSC^{6–11} is that the nucleation of amorphous dense droplets occurs much faster than the nucleation of a crystalline phase due to its lower free energy barrier. And subsequently, the larger density in the dense droplets will in turn enhance the nucleation of the crystalline phase. Consequently, the overall nucleation of the crystalline phase would be enhanced. To quantify the enhancement of the nucleation of crystals, the overall nucleation rate J_c of crystals in TSC was calculated by Kashchiev et al.²⁵

with the so-called mononuclear assumption. It was found that the overall nucleation rate J_c of the crystal at a stationary stage was equal to the nucleation rate J of the dense droplet. Since J is much larger than that of the crystals nucleated directly from the mother phase, it follows that the overall nucleation rate of the crystalline phase is actually enhanced. However, the mononuclear mechanism does not hold in real experiments as we discussed above. Another important assumption contained in the conclusion of Kashchiev et al. was that every dense droplet is able to develop a mature crystalline nucleus. However, in contrast to this assumption, it was found that although a good deal of dense droplets were created at an early stage, most of them disappeared gradually, and only a few succeeded in developing a stable crystal.¹¹ Similarly, in our system, due to the existence of a critical size N^* for dense droplets, under conditions $f = 800$ Hz and $E = 167$ V/cm, normally only 3 or 4 out of 20 dense droplets can grow beyond the critical size N^* and thus create a stable crystalline nucleus. Other droplets disappear gradually, and their matter is transported to the droplets containing a stable crystal. Therefore, the conclusion in previous studies^{6–11} that the presence of an amorphous precursor can enhance the overall nucleation of the crystal becomes questionable. It is possible that, although only a part of the dense droplets can allow the nucleation of a crystal, the effective overall nucleation rate of the crystal is still much higher than that of the crystals nucleated directly from the mother phase. However, it is also possible that the nucleation of the crystal in the dense droplets is not fast enough to support an enhancement of the overall nucleation rate of the crystal. Therefore, whether or not the overall nucleation rate of the crystal in MSC is enhanced will, to a large extent, be determined by the crystal nucleation processes in the dense droplets, as well as the nucleation rate of the dense droplets. However, since every droplet can produce only one mature crystalline nucleus, the conventional method of measuring nucleation rates by counting the number of nuclei as a function of time is no longer applicable. Thus, a special method has to be developed, and it will be addressed in another manuscript.

TSC or MSC in protein solutions has been believed to be induced by short-range attractions.^{6–11} However, experimentally, TSC or MSC had previously not been observed in colloids where only short-range attractions operate. In practice, when TSC was expected to take place, colloids with only short-range attractions were usually confined in gel-like states.^{26–28} Noro et al. found that an additional long-range attraction can shift the critical point of LLPS of colloids with short-range attractions out of the gel region.²⁸ Consequently, they argued that long-range attractions may be important for the mechanism of TSC, and the experimentally observed TSC in protein solutions,^{10,11} as Noro et al. argued, should be attributed to some less-known long-range attractions. This argument is supported by a recent experimental study, in which it was found that a weak long-range attraction does indeed exist between protein molecules in solutions.²⁹ Moreover, the latest simulation carried out by Lutsko et al.¹⁴ suggested that, in typical atomic systems, where attractions are relatively long range, TSC is favored and may underlie most

of the crystallization processes. All these studies suggested that the relation between TSC and short-range attractions may be incompletely understood, and that long-range attractions may play an important role in the mechanism of TSC.

In our system, a long-range attraction exists between colloidal particles. This long-range attraction is induced by an electrohydrodynamic (EHD) mechanism. Both the range and the strength of this long-range attraction are dependent on the frequency as well as on the strength of the electric field.^{30–33} Experimentally, this long-range attraction has to be mediated by fluid flow. Therefore, it should no longer work inside the droplets or the crystals where fluid flow becomes negligible.³⁴ In this case, we suggest that a short-range attraction exists between colloidal particles, working to balance the electrostatic repulsion between colloidal particles inside the droplets. This short-range attraction may arise from an electrostatic ion–ion correlation.³⁵ According to the argument of Noro et al.,²⁸ the short-range attraction in our system plays a role in inducing the metastable LLPS and maintaining the presence of the metastable dense droplets, while the long-range EHD attraction works to shift the LLPS from a possible gelation region.

Another important characteristic of this study is that the system under investigation is two-dimensional. In practice, two-dimensional (2D) nucleation is an important mechanism underlying aggregations occurring on interfaces.^{36,37} Moreover, for dislocation-free crystals, the growth of faceted crystal faces is governed by a 2D nucleation mechanism.^{38,39} Similarly to 3D systems, both homogeneous and heterogeneous nucleation can occur in 2D systems.^{38,40} The studies of 2D homogeneous and heterogeneous nucleation have revealed that the mechanisms underlying 2D nucleation are essentially similar to those for 3D nucleation.^{21,40,41} In other words, the general conclusions obtained in 2D nucleation are normally readily applicable to 3D nucleation. In this study, MSC is examined in a two-dimensional system. However, three-dimensional MSC has already been observed in simulations and experiments.^{17,18} Comparing our results with previous studies, it is found that the mechanisms of the 2D MSC observed in our experiments are similar to 3D MSC in several characteristics: First, a metastable liquid-like precursor is first nucleated; Second, crystalline nuclei are subsequently created from the metastable precursor; Finally, the density decreases gradually from the crystalline core to the liquid-like fringe. Based on the above knowledge, it is reasonable to conclude that the key results of this study about MSC, including the mechanism of creating subcrystalline nuclei from the metastable liquid-like precursor, the kinetics underlying the formation of the critical crystalline nuclei, the continuous change of concentration and structure in the metastable liquid clusters, and its advantages in eliminating defects, are readily applicable to a 3D MSC.

- (26) Soga, K. G.; Melrose, J. R.; Ball, R. C. *J. Chem. Phys.* **1999**, *110*, 2280.
 (27) Anderson, V. J.; Lekkerkerker, H. N. W. *Nature* **2002**, *416*, 811.
 (28) Noro, M. G.; Kern, N.; Frenkel, D. *Europhys. Lett.* **1999**, *48*, 332–338.
 (29) Liu, Y.; Frattini, E.; Baglioni, P.; Chen, W.-R.; Chen, S.-H. *Phys. Rev. Lett.* **2005**, *95*, 118102.

- (30) Yeh, S.-R.; Seul, M.; Shraiman, B. I. *Nature* **1997**, *386*, 57.
 (31) Trau, M.; Saville, D. A.; Aksay, I. A. *Langmuir* **1997**, *13*, 6375.
 (32) Sides, P. J. *Langmuir* **2003**, *19*, 2745.
 (33) Nadal, F.; Argoul, F.; Hanusse, P.; Poulligny, B.; Ajdari, A. *Phys. Rev. E* **2002**, *65*, 061409.
 (34) Santana-Solano, J.; Wu, D. T.; Marr, D. W. M. *Langmuir* **2006**, *22*, 5932.
 (35) Hansen, J. P.; Löwen, H. *Annu. Rev. Phys. Chem.* **2000**, *51*, 209.
 (36) Zeng, X. C. *J. Chem. Phys.* **1996**, *104*, 2699.
 (37) Richter, R. P.; Him, J. L. K.; Tessier, B.; Tessier, C.; Brisson, A. R. *Biophys. J.* **2005**, *89*, 3372.
 (38) Liu, X. Y.; Maiwa, K.; Tsukamoto, K. *J. Chem. Phys.* **1997**, *106*, 1870.
 (39) Kashchiev, D. *J. Cryst. Growth* **2004**, *267*, 685.
 (40) Liu, X. Y. *J. Chem. Phys.* **1999**, *111*, 1628; **2000**, *112*, 9949.
 (41) Liu, X. Y.; Maiwa, K.; Tsukamoto, K. *J. Chem. Phys.* **1997**, *106*, 1870.

Summary

In this study, two-dimensional crystallization via a metastable amorphous phase was studied in a colloidal model system. It is found that the crystalline nuclei in the metastable dense droplets are initially created by fluctuation, and it is necessary for them to acquire a critical size before they can remain stable and grow steadily. Every droplet can produce only one mature crystalline nucleus. In contrast to the creation of subcrystalline nuclei, the mature crystalline nuclei may be created by the coalescence of subcrystalline nuclei. Initially, a mature crystalline nucleus is created with grain boundaries. To eliminate the grain boundaries, a local structure transition from crystal to liquid is adopted to release the strain contained by grain boundaries. This kind of transition is supported by the continuous change of the density and the structure in the dense droplets.

It is reasonable to suggest that the kinetics observed in this study may also govern protein crystallization because globular proteins can be well modeled by colloidal hard spheres. Furthermore, our observations of MSC may serve as an illustration of how biomineralization may proceed with the presence of an amorphous metastable phase. We believe that the results presented in this article may offer a basis for further critical study.

Acknowledgment. The research is supported by ARF Project R-144-000-148-112. The authors are much indebted to Dr. C. Strom for her valuable suggestions and critical reading of the manuscript.

JA073598K