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Revisiting particle formation beyond classical nucleation: A unified classification framework for nonclassical pathways

Yuya Iida [a,*], Satoshi Watanabe [b,*]

[a] Faculty of Environmental, Life, Natural Science and Technology, Okayama University, 3-1-1, Tsushima-naka, Kita-ku, Okayama 700-8530, Japan
[b] Department of Chemical Science and Engineering, Kyoto University, Katsura, Nishikyo, Kyoto 615-8510, Japan

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ABSTRACT

Particle formation is pervasive in both nature and technology, shaping environmental and biological phenomena while underpinning a broad range of industrial products. A mechanistic understanding of particle formation is therefore important both for fundamental science and for the design of particle properties. It is now well established that particle formation can proceed through diverse pathways beyond classical one-step nucleation. At the same time, the proliferation of reported nonclassical pathways has blurred the distinctions among mechanistic categories and obscured their underlying relationships. In this review, we revisit the development of concepts in particle formation pathways and present a unified perspective on this increasingly complex mechanistic landscape. To this end, we classify the nonclassical features of particle formation along three conceptual axes: stepwise transitions between phases or phase-like fluctuations, chemically driven formation of structural units, and aggregation-dominant nucleation and growth processes. This framework places diverse pathways within a common three-dimensional space referenced to classical nucleation, providing a basis for comparing apparently distinct mechanisms within a single picture. We further discuss representative theoretical descriptions in relation to these axes and outline future challenges.

Contents

* Corresponding authors.
E-mail addresses: iiday@okayama-u.ac.jp (Y. Iida), nabe@cheme.kyoto-u.ac.jp (S. Watanabe).

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1. Introduction

Particle formation occurs in virtually all natural environments, including the atmosphere, aquatic systems, and living organisms. Elucidating the mechanisms of particle formation in these diverse settings is essential for addressing pressing environmental and health challenges, such as aerosol-driven climate impacts [1–3], water-quality deterioration caused by aquatic colloids [4,5], and diseases associated with particle formation in living systems [6,7]. In addition, particles are estimated to account for approximately 80% of chemical products across industrial sectors and play central roles in a wide range of applications, including pharmaceuticals, cosmetics, food, energy, and electronics [8–10]. If the structural properties of particles could be designed with the same degree of control as molecular structures in organic chemistry, on the basis of a mechanistic understanding of particle formation, this would open new possibilities for transformative advances in industry.

Over the past few decades, it has become clear that particle formation can proceed through a wide variety of pathways. In particular, nonclassical pathways that cannot be adequately explained by classical theory have been identified [11,12]. Such nonclassical pathways are not restricted to a particular class of materials, but have been reported across diverse systems, including inorganic compounds [13,14], organic molecules [15,16], proteins [17,18], polymers [19,20], and colloidal systems that serve as experimentally accessible model platforms for atomic and molecular nucleation [21,22]. These findings have provided important insights into the control of particle properties. At the same time, however, the growing number of proposed pathways has made the distinctions among them and their underlying relationships increasingly unclear. In this review, we trace the historical development of the understanding of particle formation pathways and propose a unified framework for their classification.

This review is organized as follows. In Section 2, we briefly summarize the classical view of particle formation based on classical nucleation theory (CNT). In Section 3, we review three representative nonclassical pathways, tracing their historical development and identifying the defining features of the processes described by the corresponding terminology. We then propose a unified classification framework for particle formation pathways based on three conceptual axes and discuss theoretical models that capture the key features represented by each axis. Finally, in Section 4, we discuss remaining challenges and future directions.

2. Classical perspective of particle formation

Particle formation begins with nucleation process, which is the initial process of a stable new phase formation from a metastable phase. It is widespread in nature and related to various phenomena, ranging from common occurrences such as water boiling and freezing to extraordinary events like the formation of black holes [23]. Living organisms rely on crystallization to form skeletal components such as bones and teeth [24–27] and on liquid–liquid phase separation to form cell organelles [28–30]. Thus, the nucleation of liquid and solid phases plays a crucial role in the formation of living organisms' structure and the manifestation of their functions. In addition, the crystallization and abnormal deposition of proteins in living organisms can cause various diseases [30–32]. Understanding the nucleation mechanism of these protein crystals should provide effective strategies to treat these diseases. From an engineering perspective, the nucleation process is critical in the fabrication of particles because it determines their geometrical properties, including shape, particle size distribution, and crystalline polymorphism, and thus governs the optical, electrical, thermal, and mechanical properties of particles. For example, quantum dots, which are semiconductor nanoparticles, require precise size control of particles in the single nanoscale because the optical properties significantly depend on the particle size [33,34]. In crystallization for solid purification and separation, the size and shape of the particles affect not only the characteristics of the particles as products but also the ease of subsequent processes such as filtration [35]. Consequently, controlling the nucleation process is essential for rational particle design.

The great demand for controlling nucleation has persistently required a theoretical framework to describe and understand nucleation processes. The most popular and successful one is the classical nucleation theory (CNT), which originated from a thermodynamic consideration by Gibbs [36]. The basic idea of CNT is to express the free energy change of nucleation, ΔG, as a summation of two effects as follows:

$$\Delta G = n\Delta\mu + \gamma(an)^{2/3}, \tag{1}$$

where n is the number of monomers in the nucleus, $\Delta\mu$ is the chemical potential difference between the new phase and the initial phase, γ is the interfacial tension between the two phases, and a is a coefficient to convert n to the interfacial area. The first term is the volume term, which represents the free energy gain due to the formation of a new stable phase, and the second term is the interface term, which is the energy penalty due to the formation of the interface between the new phase and the initial phase. As Eq. (1) clearly shows, the nucleus size n is the only reaction coordinate. In CNT, n varies only through monomer attachment and detachment. Because the dominant term shifts from the interface term to the volume term with increasing nucleus size, the variation of ΔG with n results in a profile with a maximum as illustrated in Fig. 1a. This means the system must surpass an energy barrier, $\Delta G_{\max}$, at the critical nucleus size, $n_{\max}$, to initiate nucleation. Nuclei smaller than the critical size tend to decay, and only nuclei above the critical size can proceed to further growth. Based on the above thermodynamics of nucleation, Volmer and Weber proposed the first kinetic theory of nucleation in 1926 [37]. It was further refined by Farkas [38], Becker and Döring [39], and Zeldvich [40], and has become CNT of today.

Despite its simplicity, CNT can well depict qualitative characteristics of nucleation, such as the concentration and temperature dependence of the nucleation rate. It has also been a useful analytical tool for determining physical quantities that are difficult to measure directly, such as crystal/liquid interfacial tension [41]. However, quantitative prediction of nucleation rates is rarely successful. In fact, the nucleation rates obtained from experiments and simulations often differ by orders of magnitude from those predicted by CNT [17,42–44], challenging the validity of some of the assumptions of CNT.

3. Nonclassical pathway

A paradigm shift in the understanding of nucleation has occurred by revisiting the validity of the "one-step pathway assumption" in CNT. Because the nucleus size is the only reaction coordinate in CNT, it presumes that the nucleus has an identical structure to the bulk of the new phase. However, such a direct one-step nucleation pathway from the initial phase to the final stable one is not always valid. This is intuitive if one considers the formation of crystalline particles from dilute phases such as solution or gas phase. To form a crystalline phase from a dilute phase, the system must evolve two order parameters: the density and orientational order of monomers. The one-step pathway assumption in CNT requires the simultaneous development of both order parameters. It is more plausible that each order parameter evolves independently. Indeed various alternative pathways have been proposed and demonstrated over the past few decades. These include multistep pathways leading to the new stable phase via one or more intermediate states, such as a dense liquid phase and metastable polymorphs [12,45,46] (Fig. 1b,c). Furthermore, in contrast to the classical perspective that nucleation and subsequent growth proceed through monomer attachment and detachment, substantial evidence has suggested that larger units than monomers, such as oligomers and nano-sized particles, can play a central role in the nucleation and growth process through aggregation

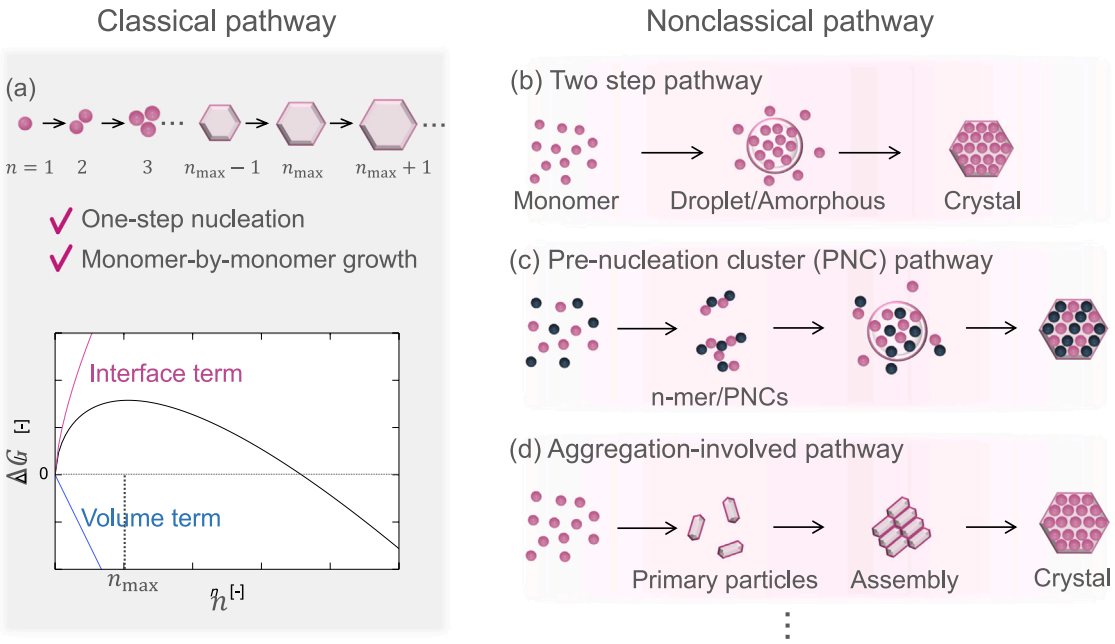

Fig. 1. Schematic illustration of particle formation pathways: (a) classical pathway and nonclassical (b) two-step pathway, (c) pre-nucleation cluster (PNC) pathway, and (d) aggregation-involved pathway.

and coalescence [11,47–49] (Fig. 1c,d). Thus, the pathway from the initial to the stable phase is considerably more diverse than previously considered.

These pathways that deviate from the assumptions of the classical theory are often referred to as "nonclassical pathways" or "nonclassical crystallization" [11,12]. However, the term "nonclassical" is rather vague and used somewhat subjectively by researchers to describe the pathways they observe. In addition, the term "nonclassical pathway" is often used to describe both situations where only the nucleation process (nucleation pathway) leading to the post-critical nucleus is nonclassical and those where the entire particle formation process (particle formation pathway) is nonclassical, which blurs the distinction between the two. As noted in the literature [50], a pathway should be clearly defined by "what it is", not by "what it is not".

To address these ambiguities, in the following section, we first summarize the representative nucleation and growth pathways reported thus far, including (1) the two-step nucleation pathway, (2) the pre-nucleation cluster (PNC) pathway, and (3) the aggregation-involved pathway, taking into account their historical background. Subsequently, we introduce three conceptual axes for understanding the differences between these pathways. Additionally, several theoretical models formulated to depict these pathways are outlined.

3.1. Two-step nucelation pathway

The two-step nucleation pathway, commonly observed in protein crystallization, is one of the so-called "nonclassical pathways", in which the nucleation process deviates from the classical picture. In this section, we review the historical development of research on the two-step nucleation pathway and provide an overview of the current usage of the term "two-step nucleation pathway".

Origin of two-step nucleation pathway (-2000)

Studies on the two-step nucleation pathway originated from protein crystallization for structural analysis, as well as from theoretical and experimental research on colloidal phase diagrams. In the 1990s, it was found that a solution with a certain range of the second virial coefficient, which represents the interaction state of a protein in solution, easily undergoes successful crystallization regardless of the type of protein [51]. This specific range of the second virial coefficient was termed "crystallization slot". Meanwhile, in colloidal systems with short-range interactions, it was demonstrated that the fluid–fluid (vapor–liquid or liquid–liquid) equilibrium line sinks below the solid-fluid equilibrium line and becomes metastable with respect to the solid-fluid transition [52–54], as shown in Fig. 2. Ten Wolde and Frenkel first proposed the concept of a two-step pathway by focusing on the similarity between proteins and colloidal particles [55]. Using molecular simulations of a simple model of a globular protein in solution with short-range interactions, they found that increased density fluctuations above the critical point of the metastable fluid–fluid phase equilibrium line enhance crystal nucleation (point A in Fig. 2). As illustrated by the free energy landscapes in Fig. 3, their model system favored a direct one-step pathway to a crystalline cluster at temperatures well below the fluid–fluid critical temperature (the dotted line in Fig. 3a, where N_ρ and N_{crys} increase simultaneously), whereas at the critical temperature it favored an indirect pathway, proceeding first to a dense liquid-like cluster and then to a crystalline cluster (the dotted line in Fig. 3b). The nucleation barrier along this indirect pathway was significantly lower than that of the direct one-step pathway. Ten Wolde and Frenkel suggested that the enhancement of crystallization by critical density fluctuations might correspond to the "crystallization slot" observed in protein crystallization.

This research spurred further studies on the relationship between molecular interactions, the position of the metastable fluid–fluid phase equilibrium line, and nucleation behavior [56–68]. The term "two-step nucleation" was first used in one of these studies [58]. However, it should be noted that the definition of "two-step nucleation" was ambiguous at the time. The two-step pathway originally proposed by ten Wolde and Frenkel stems from critical density fluctuations above the critical point of the metastable fluid–fluid phase equilibrium line, i.e., a phenomenon occurring outside the metastable fluid–fluid region (area A in Fig. 2). Nevertheless, some researchers argued that two-step nucleation could only occur below the line (area B in Fig. 2), as they believed that fluid–fluid phase separation was the only mechanism to form dense liquid-like clusters [57,58]. It was unclear whether the formation of clusters in the two-step pathway was due to density fluctuations or fluid–fluid phase separation that could lead to a macroscopic liquid phase.

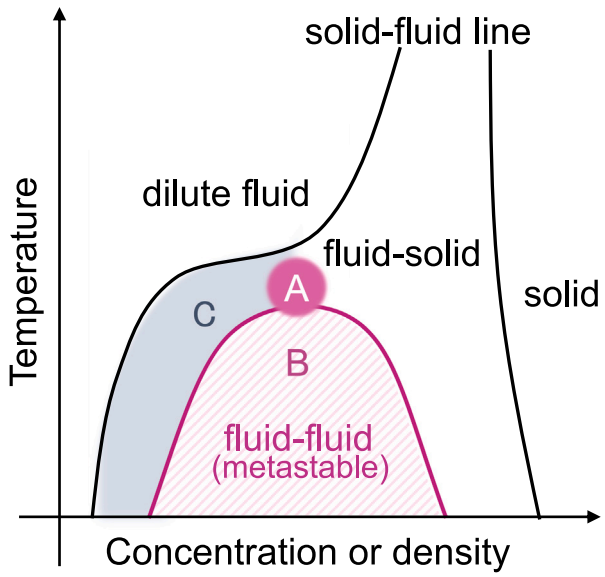

Fig. 2. Phase diagram with metastable fluid–fluid phase equilibrium line. The characters A, B, and C indicate the region where specific mechanisms of the two-step nucleation pathway of proteins were proposed (A: critical density fluctuations, B: fluid–fluid phase separation, C: mesoscopic clusters).

Two-step nucleation pathway in protein systems (2000–2007)

Since 2000, two-step nucleation has been experimentally confirmed [69–78]. Vekilov et al. have led the study of two-step nucleation in protein systems. They conducted crystallization experiments on various proteins and demonstrated that two-step nucleation occurs across a wide region of the phase diagram, including both the upper and lower sides of the metastable liquid–liquid phase equilibrium line apart from the liquid–liquid critical point [69–71]. The nucleation rate of crystals peaked at the temperatures slightly above the metastable liquid–liquid phase equilibrium line. Near the critical point, gelation predominated over crystallization due to the high concentration. Vekilov et al. argued that the enhancement of crystallization above the metastable liquid–liquid phase equilibrium line is not attributable to density fluctuations, as suggested by ten Wolde and Frenkel [55] and other studies [79–81], but to relatively long-lived (several hours to days) mesoscopic clusters that mediate crystallization [73,76–78]. The existence of these clusters has been experimentally confirmed in several protein systems using optical methods and atomic force microscopy (AFM). Below the metastable liquid–liquid phase equilibrium line, macroscopic liquid droplets form through liquid–liquid phase separation (LLPS). The crystallization of these droplets largely depends on their viscosity. Macroscopic droplets with low viscosity promote crystallization, while those with high viscosity inhibit it [82–84].

At this point, the two-step nucleation pathway of proteins differentiated into the following two subtypes:

1. a crystallization pathway mediated by mesoscopic clusters created by some mechanism in solution. This subtype of the pathway occurs mainly outside the metastable liquid–liquid phase equilibrium line (area C of Fig. 2).
2. a pathway in which droplets of macroscopic phase created by LLPS affect crystal nucleation and growth. This subtype of the pathway occurs inside the metastable liquid–liquid phase equilibrium line (area B of Fig. 2).

The role and formation mechanism of mesoscopic clusters (2007-present)

For subtype (1), the mechanism of cluster formation in crystallization became one of the main research topics in protein crystallization. Initially, the combination of short-range attraction and long-range repulsion was considered a strong candidate for the mechanism underlying the formation of mesoscopic clusters [85–87]. Systems with such interactions form numerous finite-size clusters under conditions above the liquid–liquid phase equilibrium line. This equilibrium state is referred to as the cluster phase [88,89]. Hutchens and Wang have theoretically demonstrated that the cluster phase can mediate macroscopic phase separation states [90]. However, clusters resulting from the combination of short-range attraction and long-range repulsion are at most a few dozen nanometers, which is significantly smaller than the experimentally observed size of protein mesoscopic clusters (several hundred nanometers) [73,76–78]. It is currently believed that the combination of short-range attraction and long-range repulsion does not play a role in the formation of mesoscopic clusters in many protein systems.

Subsequently, the “complex hypothesis”, based on the formation of transient complexes such as oligomers, was proposed [91–93]. A schematic illustration of this hypothesis is shown in Fig. 4. In dilute solution, monomers are assumed to be in chemical equilibrium with complexes formed from the monomers. Because these complexes favor a condensed phase, they can give rise to cluster formation. In the condensed phase, however, the equilibrium shifts toward monomers because of the high concentration of the complexes. As a result, the complexes tend to convert back to monomers within the cluster, thereby suppressing further cluster growth. Although the validity of this hypothesis remains controversial [94,95], Lubchenko et al. recently showed that, under the assumption that transient dimers form from monomers, the thermodynamic conditions required for cluster formation are indeed satisfied for specific dimer binding strengths [96]. In addition, they reported a reaction–diffusion model incorporating the concept of finite-lifetime complexes, which successfully reproduced characteristic features of mesoscopic clusters, including the independence of the steady-state cluster size from solute concentration and an Ostwald-like ripening process [97].

The role of mesoscopic clusters in crystallization is another critical issue [73,98–103]. In many cases, mesoscopic clusters play a central role in crystal nucleation and growth. Sleutel et al. confirmed the involvement of clusters in crystallization by filtering supersaturated solutions [100]. They found that the rate of crystal formation was significantly lower in filtered lysozyme solutions than in unfiltered ones. Gilko et al. used AFM to confirm that the growth of crystals under low supersaturation conditions proceeds solely through the attachment of clusters to the crystal surface [73]. On the other hand, in olanzapine systems, the mesoscopic clusters promote only the crystal nucleation process. During the subsequent crystal growth process, the cluters incorporate in the crystal as foreign bodies and do not contribute to the growth of the crystal [104]. Mesoscopic clusters have now been reported not only in aqueous protein solutions but also in systems involving organic small molecules and organic solvents [16,104,105]. Although they are therefore increasingly recognized as a universal phenomenon, their roles may vary somewhat depending on the system.

It has been implicitly assumed that mesoscopic clusters are liquid-like and that crystallization occurs within them. Several experimental studies and successful nucleation rate prediction models based on internal crystallization support this assumption [101,106–109]. However, some studies have called this assumption into question. Direct transmission electron microscopy (TEM) observations by Yamazaki et al. suggest that mesoscopic clusters in aqueous lysozyme solutions are solid amorphous particles and act as sites for heterogeneous nucleation [103]. Similarly, van Driessche et al. reported that, in the crystallization of glucose isomerase, the precursor phase present in the early stage of the process is a nanoscale crystal with the same lattice symmetry as the mature crystals observed at the end of the crystallization experiment [110]. Further studies are needed to clarify the true nature of mesoscopic clusters and their role in crystallization.

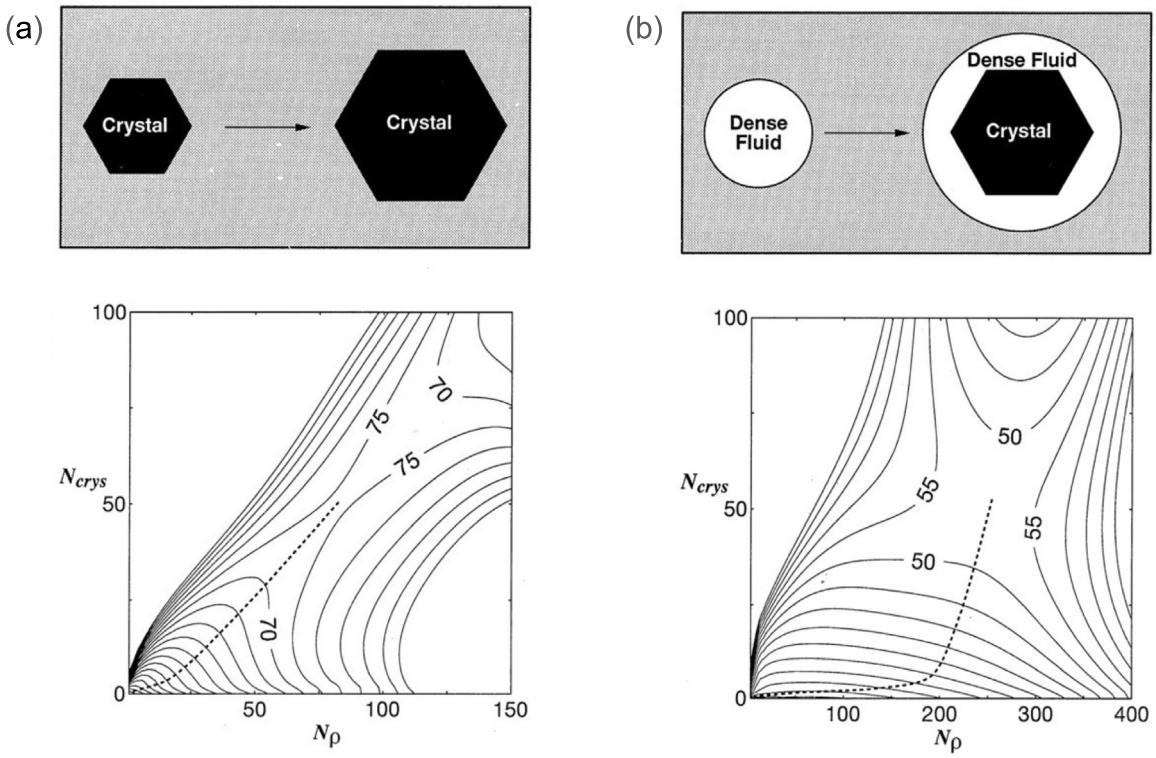

Fig. 3. Contour plots of the free-energy landscape for the model system of ten Wolde and Frenkel, calculated using umbrella sampling. The free energy is shown as a function of the number of connected particles, N_ρ, and the number of solid-like particles, N_{crys}, with contour intervals of $5k_BT$. The upper panels schematically illustrate the corresponding nucleation pathways. (a) Well below the metastable fluid–fluid critical temperature, nuclei form directly as crystalline clusters. (b) At the metastable fluid–fluid critical temperature, nuclei first form as dense liquid-like clusters and subsequently transform into crystalline clusters.
Source: Adapted with permission from Ref. [55].

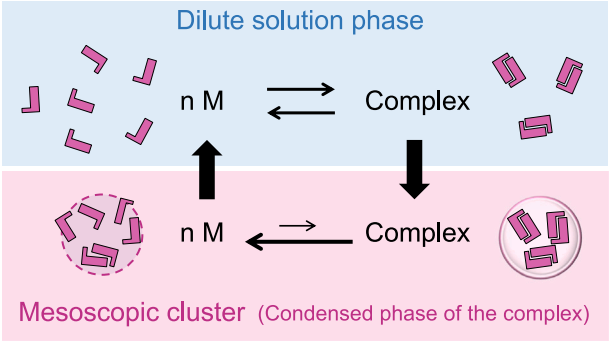

Fig. 4. Schematic illustration of the complex hypothesis for the formation of mesoscopic clusters.

Liquid–liquid phase separation and two-step nucleation (2000-present)

For subtype (2) of the two-step nucleation pathway, the role of macroscopic liquid droplets in crystallization has been frequently discussed. Since the solute-rich droplet phase and the solute-lean continuous phase, formed through LLPS, have the same chemical potential, crystallization does not necessarily occur from within the droplet. In some cases, crystals formed in the continuous phase [111,112], while in others, they formed near the droplet surface and grew outward into the continuous phase [113]. Droplets also affect crystal growth in various ways. For glucose isomerase observed by Vivarès et al. crystal growth proceeded only when the crystal was enclosed within a droplet [75]. Once the crystal consumed the droplet, regions of solute depletion were observed around the crystal, suggesting that the enhanced rate of solute monomer supply due to wetting was essential for crystal growth in this system. In contrast, other studies observed that crystals could grow in the continuous phase [112,114]. As the crystals grew in the continuous phase, the droplets dissolved, acting as monomer reservoirs to maintain the monomer concentration in the solution. Furthermore, macroscopic droplets acting as monomer reservoirs can coexist with mesoscopic clusters. Zhang et al. proposed a combined mechanism of subtypes (1) and (2) of the two-step nucleation pathway, where mesoscopic clusters form in the continuous phase, and crystals originating from these clusters grow as they consume the macroscopic droplets [98].

The discovery of the two-step nucleation pathway of subtype (2) in proteins has particularly stimulated research in industrial crystallization. In this field, LLPS during crystallization processes such as cooling and mixing is called "oiling-out" [115–117]. While initially recognized as a peculiar phenomenon mainly in polymers and proteins [118–120], oiling-out was also directly observed in small organic molecules in 2003 [121]. Despite the similarities between crystallization through oiling-out and two-step nucleation, research on oiling-out seldom cited studies on the two-step nucleation pathway until around 2015. This would be because oiling-out was considered an undesirable phenomenon in crystallization. It often degrades crystalline quality by introducing impurities, inducing aggregation, and inhibiting crystallization [122–124]. Therefore, researchers primarily focused on preventing LLPS by adding seeds [125], employing ultrasonic waves [126], and predicting the liquid–liquid phase equilibrium line using thermodynamic theories [127,128].

However, some studies have emphasized the positive aspects of oiling-out on crystallization. By deliberately promoting oiling-out in the crystallization processes, one can obtain crystals of different sizes, shapes, and surface morphologies [129–132], which are unattainable through conventional one-step crystallization. The oiling-out-induced

aggregation of crystals also enables the one-pot synthesis of spherical granules composed of crystals [133,134]. Consequently, recent papers have increasingly used the term "two-step nucleation" to refer to crystallization via oiling-out [117,131,135–137].

Furthermore, LLPS is involved in the pre-nucleation cluster (PNC) pathway [138,139], a typical pathway for biominerals, and in the formation of organelles in cells [28–30,140]. The ubiquity and significance of LLPS in the formation processes of various structures are increasingly being recognized.

Two-step nucleation pathway in other systems (2000-present)

Research on the two-step nucleation pathway in proteins has led to the hypothesis that similar pathways may exist in other systems. In 2006, Lustko and Nicolis employed the density functional method [141] to examine nucleation barriers for both spherical protein model molecules, which had the same short-range interactions as the ten Wolde and Frenkel's simulations [55], and molecules with standard Lennard-Jones (LJ) 12-6 interactions. Their findings indicated that the pathway involving the formation of droplets followed by crystallization is thermodynamically favorable for both protein and LJ molecules. They suggested that the absence of experimentally observed droplet-like states in small molecules stems from the lower crystallization barrier. Around the same time, experiments and simulations of glycine [15], AgBr [142], two-dimensional colloidal crystals [21,22,143,144], polymer melts [20], and LJ molecules [145] also indicated a pathway where crystallization occurs via a disordered state. By 2010, the two-step pathway was widely recognized as a universal phenomenon, not unique to proteins [17,45,146]. Subsequently, the transition pathway to the final stable structure via an intermediate structure has been observed in a wide range of systems, including electrolytes [147,148], organic small molecules [149–152], metal nanoparticles [153,154], zeolites [155], clathrate hydrates [156,157], metal–organic frameworks (MOFs) [158], quantum dots [159], and solid–solid transitions [160–162]. All these are labeled "two-step nucleation" or "multistep nucleation". It should be noted that, compared to typical examples such as proteins, the terms "two-step pathway" and "multistep nucleation" are used somewhat loosely in other systems.

Many of the pathways currently labeled as two-step or multistep pathways are **pathways where phases or fluctuations with structural features of those phases transition in stages to reach the final phase.** For example, crystallization via LLPS is a typical case in which the phase changes in stages [158,163,164]. In bismuth nanoparticles, the most stable phase varies depending on the nucleus size [165], leading to multistep nucleation where the most stable phase is sequentially followed based on size. Conversely, in an aqueous solution, urea forms a crystalline nucleus from dense regions induced by density fluctuations rather than metastable liquid phase droplets corresponding to a local minimum in the free energy landscape [149]. In this pathway, the fluctuations, rather than the phases, transition from a disordered to an ordered state. James et al. termed this transition below the critical nucleus size as a "fluctuation phase transition" and demonstrated its universality through theoretical considerations and simulations in lattice gas systems [166]. In addition, as a variant of these two-step pathways, some research has reported pathways where composite nuclei, consisting of a stable phase core and a metastable phase shell, surpass a single energy barrier [167–170].

In recent years, the two-step nucleation pathway is often described using a CNT-like model, which has two reaction coordinates (See Section 3.5) [171–173]. Consequently, some researchers believe that two-step nucleation should no longer be considered a "nonclassical pathway" but a "classical pathway" [50,174]. However, the transition mechanism from metastable to stable structures is diverse, and some of these transitions involve processes that do not include nucleation, such as the coalescence of crystals within a droplet or the coherent sliding of atoms in parallel rows [22,175]. A theory that extends beyond CNT is necessary to explain these phenomena.

3.2. Pre-Nucleation Cluster (PNC) pathway

In biominerals such as calcium carbonate and calcium phosphate, which form the skeletons of organisms like bones, teeth, and shells, pathways involving solute species known as PNCs have been observed. The presence of PNCs enables both the nucleation and growth processes to deviate from classical assumptions. In this section, we first provide the historical development of research on the PNC pathway in the same way as the two-step nucleation pathway in the previous section. We then highlight the important aspects that the emergence of the PNC pathway has brought to particle synthesis research.

Origin of the PNC pathway (-2012)

The study of the PNC pathway has completely different origins from the two-step nucleation observed in proteins. As early as the 1960s, the presence of a transient amorphous state was suggested [176]. From the late 1990s to the early 2000s, experimental evidence emerged that this amorphous state was the precursor to crystals [177,178], leading to the rapid recognition of crystallization through the amorphous state as a common phenomenon in biomineral systems. The historical context of this field is explained in detail in other review [26]. Although crystallization from disordered states resembles the two-step pathway in proteins, there were few instances where studies of the two-step pathway and those in the biomineral field referenced each other at that time.

With regard to the nucleation pathway of biomineral systems, researchers focused not only on the formation of crystalline particles via amorphous but also on the pathway leading to the formation of the amorphous particles. In 2008, Gebauer et al. presented experimental results suggesting the existence of PNCs that act as precursors to aggregate and form amorphous calcium carbonate (ACC) particles [13]. Their study investigated the precipitation process of ACC by adding a calcium chloride solution to a carbonate buffer solution while maintaining a constant pH. They monitored the amount of free calcium ions throughout the process, from the unsaturated state to the precipitation of ACC. Notably, they observed that the amount of free calcium ions was lower than added even during the initial unsaturated phase, suggesting the formation of stable clusters of Ca^{2+} and CO_3^{2-} ions in the solution. The existence of these clusters, with a dynamic diameter of approximately 2 nm, was further confirmed through ultracentrifugation. The PNCs formation was enhanced at high pH levels, where the concentration of carbonate ions is high, indicating that PNCs are thermodynamically stable species that form according to equilibrium thermodynamics. Gebauer et al. emphasized that PNCs are solute species that form through chemical equilibrium in solution. Unlike the clusters with an interface considered in CNT, PNCs do not have any interface [12].

The validity of the concept of PNCs has been reinforced by various methods, including experimental observations and simulations [179–183]. Pouget et al. utilized cryo-TEM to observe the formation of calcium carbonate particles, employing a stearic acid monolayer as a template [179]. They observed clusters of 0.6 to 1.1 nm in size in the supersaturated solution (Fig. 5a), as well as amorphous particles of 30 nm in size that appeared to have formed due to the aggregation of these clusters. Similar experimental findings have been reported in the calcium phosphate system [180]. Moreover, it has been shown that PNCs are stabilized in the presence of silicates [184]. At high pH levels, silicate-stabilized PNCs do not aggregate due to electrostatic repulsion, preventing the formation of ACC. This suggests that the aggregation of PNCs is a critical step in ACC formation. Additionally, molecular dynamics (MD) simulations by Demichelis et al. revealed that PNCs of calcium carbonate consist of polymer chains with calcium and carbonate ions arranged alternately [182]. Their simulations demonstrated that the size of these clusters increased over time, eventually reaching a certain limiting size, suggesting that the clusters are stable rather than metastable compared to solvated ions or ion pairs in the solution.

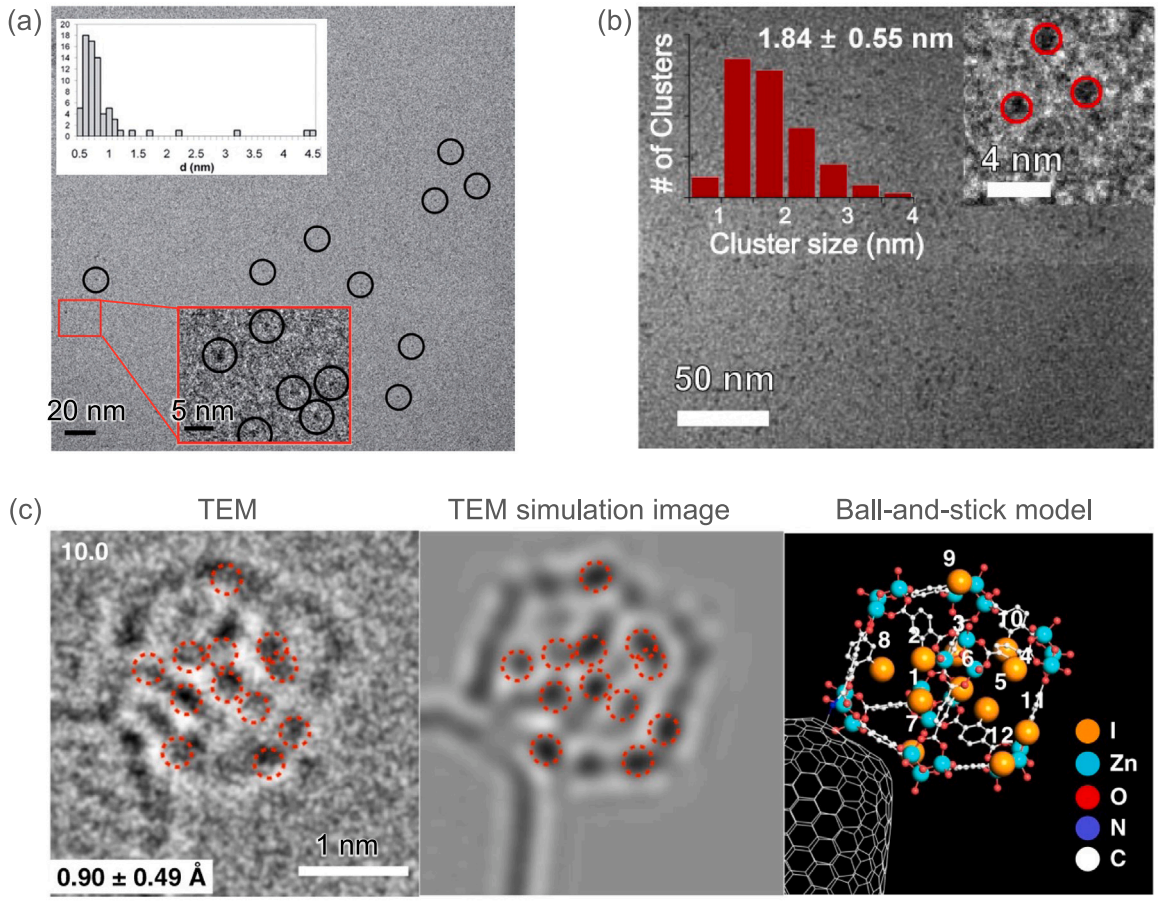

Fig. 5. TEM images of PNCs observed in various systems. (a) Calcium carbonate. Adapted with permission from Ref. [179]. Copyright 2009 American Association for the Advancement of Science. (b) InAs. Adapted with permission from Ref. [185]. Copyright 2016 American Chemical Society. (c) MOF-5. The red circles indicate 12 spots of iodine atoms. The number in the lower left shows the average deviation between the simulated and experimental iodine positions. Adapted from Ref. [186], licensed under CC BY 4.0.

PNC pathway and liquid–liquid phase separation (2012-present)

Some researchers expressed skepticism toward the concept of PNCs for the formation process of calcium carbonate [138,187–189]. They proposed alternative explanations based on LLPS. Wallace et al. conducted molecular simulations to calculate the free energy of ACC particles and found that the free energy decreased monotonically with increasing ACC size [138]. They interpreted this behavior as spinodal decomposition and proposed a phase diagram for calcium carbonate. To bolster their argument, they performed simulations using the Ising gas model, demonstrating that various sizes of nanoparticles, like PNCs, emerge when the energy barrier for nucleation decreases to approximately k_BT. This finding suggests that the appearance of the PNCs is in the middle of spinodal decomposition. Another study employed molecular simulations and equilibrium speciation models to argue that only free ions or ion pairs predominantly exist in solution, attributing the formation process of ACC to a CNT-like binodal LLPS of these entities [187,188]. Conversely, simulation studies incorporating more detailed interaction contributions have reported that larger chemical species beyond ion pairs can also exhibit relative stability [190].

The assertion that PNCs exist even in unsaturated solutions is incompatible with the CNT-like LLPS or spinodal decomposition. However, in recent years, the concepts of PNCs and LLPS have been conflated, with the PNC pathway being explained as follows (Fig. 6): [191–193]

1. Monomer molecules or ions in the solution form PNCs made up of highly dynamic polymer chains. PNCs are solute species formed by chemical equilibrium and lack a distinct interface with the solution phase.
2. The partial dehydration and reduced mobility of PNCs cause them to form nano-droplets, which create an interface with the mother phase. This process is considered to be LLPS in this system. LLPS occurs not because the cluster size exceeds a critical value but because the dynamics of the clusters decrease.
3. The resulting nano-droplets undergo further dehydration and coalescence to form an amorphous solid.

The idea that links LLPS with the reduction in PNCs dynamics is currently supported by both experimental evidence and theoretical models [139,194,195]. Sebastiani et al. analyzed water dynamics during the precipitation of ACC using terahertz absorption spectroscopy [139], observing a nonlinear change in absorbance well before the solid precipitation. This change, which cannot be explained by the effects of single ions or ion pairs alone, is most likely attributable to LLPS driven by alterations in the hydration state of PNCs. Additionally, Avaro et al. successfully predicted the position of the experimental liquid–liquid equilibrium line using the PNC model based on the thermodynamics of solute association [194]. These studies suggest that the concepts of PNCs and LLPS can coexist without contradiction. Furthermore, LLPS driven by reduced precursor-species dynamics has also been observed in organic molecules, supporting the broader generality of this concept [196].

PNC pathway in other systems (2009-present)

After Gebauer et al. first reported the PNC pathway in a calcium carbonate system [13], similar pathways were subsequently observed

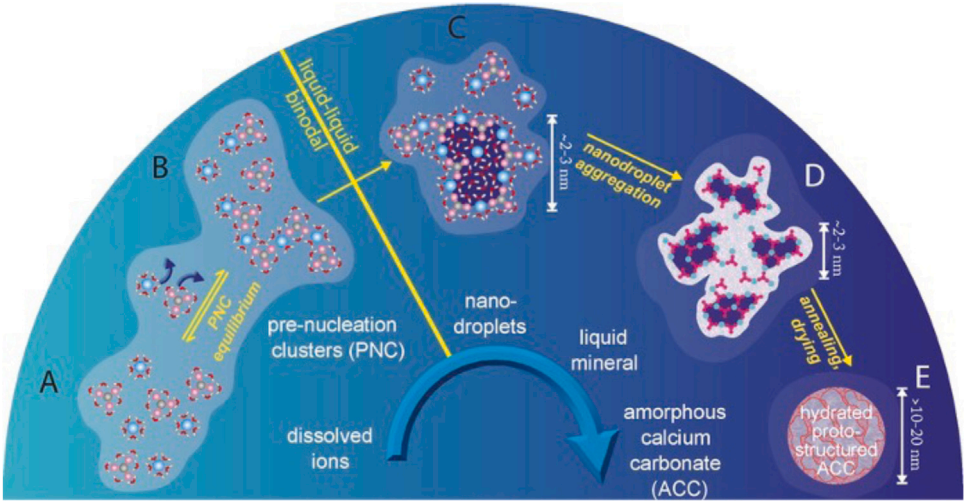

Fig. 6. Schematic illustration of the PNC pathway. *Source:* Reprinted with permission from Ref. [139]. © 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

in various mineral systems, including calcium phosphate [180,197], calcium oxalate [198], iron oxide [192], calcium hydroxide [199], and strontium sulfate [200]. In the case of calcium oxalate, the addition of citric acid stabilizes PNCs and suppresses their aggregation [198], thereby inhibiting particle formation.

Some other systems also follow a particle formation mechanism similar to the PNC pathway. For example, gold chloride forms molecular clusters in a hexane solution in the presence of oleylamine [201]. These non-reduced clusters undergo progressive reduction before abruptly shrinking to form nuclei of gold nanoparticles. Another example can be found in quantum dots. Tamang et al. observed amorphous InAs clusters approximately 1.8 nm in diameter, similar to PNCs (Fig. 5b), at room temperature when commonly used InAs precursors were reacted under a nitrogen atmosphere [185]. Similar PNC-like intermediate pathways have also been reported for other quantum dots, such as CdS [14], CdTe [202], PbS [203], and InP [204]. Although these examples can all be termed "PNC pathways", it is important to understand that the nature of the clusters can differ significantly from one system to another. The formation mechanism and function of clusters are highly dependent on the unique chemical interactions within each system. Some PNC-like clusters found in quantum dots showed function as a monomer reservoir [203], which is akin to the role of a macroscopic liquid phase in a two-step nucleation pathway of protein crystallization. In MOF systems, secondary building units (SBUs), which are structural units composed of metal ions and organic ligands, are a PNC-like concept used to describe the formation processes of MOF crystals [186,205–209]. During these processes, SBUs are assembled like building blocks into MOF crystals. TEM observations [186] (Fig. 5c) and pair distribution function analysis [207] have confirmed the presence of such cluster structures in solution for certain MOFs. Interestingly, the formation of different structured clusters can occur depending on the synthesis conditions even when using the same metal ions and organic ligands. These variations in cluster structures ultimately lead to different final crystal structures [186,208].

"Chemistry of nucleation" emphasized by the PNC pathway

The PNC pathway highlights the significant role of chemical interactions between monomer molecules, ions, and their complexes, which are ignored in the CNT's simplified nucleation model. The recognition of the PNC pathway has consequently led researchers in the field of particle synthesis to focus more closely on the "chemistry of nucleation". Several reviews have suggested that it is time to shift away from approaches that aim to develop universal nucleation models, like CNT, and instead adopt methodologies grounded in atomic-scale chemistry [193,210].

Indeed, numerous studies have underscored the influence of chemical factors on nucleation and growth processes [210–212]. For instance, in the liquid-phase reduction synthesis of metal nanoparticles, the strength of the reductant plays a crucial role in determining the particle formation process [213,214]. When a strong reductant is used, metal precursor ions are fully reduced to zero-valent atoms in a single step, leading to the aggregation of these atoms into spherical particles. Conversely, the use of a relatively mild reductant results in the aggregation of partially reduced complexes into clusters, which are further reduced to form particles with a different shape. In some cases, chemical reactions drive the entire process of particle formation without a distinct separation between nucleation and growth. Chang et al. investigated the formation of iron oxide nanoparticles synthesized from iron-oleate complexes with strongly chelating carboxylate moieties [215]. Their findings revealed a continuous growth process of the nanoparticles without a clearly defined nucleation step. This continuous growth was attributed to the sequential development from trinuclear oxoclusters to larger iron oxoclusters, and eventually to iron oxide nanoparticles, driven by the controlled organic reaction between the strongly binding ligands on the clusters and the alcohol added to the solution. Moreover, chemical species that do not directly participate in the phase separation process can still affect the nucleation rate and its dependence on operational variables, such as concentration and temperature, by altering the chemical equilibrium concentration of active species [216]. In this way, chemistry plays a vital role in determining the components involved in the phase separation process.

3.3. Aggregation-involved pathway

Particle formation processes involving aggregation of structural units larger than the solute monomer contrast with the monomer-by-monomer growth assumed in the classical theory. In this sense, such aggregation-involved pathways are also classified as "nonclassical pathways". The term "Crystallization by Particle Attachment (CPA)" is often used to describe this pathway [49], broadly defining "particle" to include entities such as liquid droplets and oligomeric species. These aggregation-involved pathways are not exclusive to the two-step or PNC pathways mentioned above. Instead, they can be integrated into these pathways, leading to even more complex nucleation and

growth processes. Since aggregation can occur both upstream and downstream in the particle formation process, it affects the particle formation pathway in multiple ways. For a better understanding of the aggregation-involved pathways, it is helpful to classify how aggregation is involved in the pathway. Some reviews divide the effects of aggregation into nucleation and particle growth processes, labeling them as "nonclassical nucleation" and "nonclassical growth", respectively [174,217–219]. While this framework is useful, there are cases in which distinguishing between "nucleation" and "growth" is not straightforward in the context of aggregation. For example, when the aggregation of primary particles leads to the formation of a single-crystal particle, the process can be interpreted either as nucleation from the primary particles or as growth through their aggregation. In this section, we therefore introduce an alternative classification based on the stability of the aggregating species and provide an overview of the corresponding categories.

1. Aggregation of kinetic species that are stable against dissolution and melting, i.e., aggregation of post-critical nuclei with various sizes.
2. Aggregation of kinetic species that are unstable against dissolution and melting, i.e., aggregation of sub-critical nuclei with various sizes.
3. Aggregation of intermediate species that are kinetically trapped, i.e., aggregation of "super-monomers" with uniform sizes formed by specific thermodynamic or kinetic mechanisms.

(1) Aggregation of post-critical nuclei

Post-critical nuclei, formed through the nucleation process, are kinetic species growing toward the bulk phase, which represents the system's most stable state. When their number density is high, these nuclei can collide with each other and aggregate. This type of aggregation is a well-established phenomenon that has been studied for a long time. As early as 1917, Smoulchouski proposed a population balance model to describe this process [220].

However, the coexistence of aggregation and monomer-by-monomer growth leads to complex particle formation processes, which are difficult to describe by theoretical models [221–223]. Polte et al. investigated the mechanism of gold nanoparticle formation in the classical citrate synthesis method using various in-situ measurement techniques, including UV–Vis, SAXS, and XANES [224]. Their experimental results suggested the following four-step mechanism: fast initial formation of small nuclei, coalescence of the nuclei into bigger particles, slow growth of particles sustained by ongoing reduction of gold precursor, and subsequent fast reduction possibly because of an autocatalytic reduction on the surface of the formed nanoparticles. In this system, aggregation and monomer-by-monomer growth are sequential processes. In contrast, Zheng et al. observed a more intricate growth process during the formation of Pt nanoparticles, combining both aggregation and monomer-by-monomer growth [225]. Their TEM observations revealed that polycrystalline particles formed through aggregation and eventually transformed into single crystals through structural relaxation. Surprisingly, this relaxation process temporarily halts the particles' growth, allowing other particles that grow solely by monomer attachment to "catch up" with the aggregated particles.

Among the aggregation-involved pathways driven by post-critical species, oriented attachment (OA) is one of the major research topics [11,226]. OA is a process where nanoparticles or small crystallites align and fuse along specific crystallographic orientations to form larger, single-crystal structures. First identified by Penn and Banfield in the late 1990s [47,227], OA garnered attention due to its ability to create a wide variety of crystal structures that cannot be formed through the conventional monomer addition growth mechanism [228]. The diverse structures and patterns observed in biologically derived crystals are often attributed to pathways involving OA [27]. Initially, research on OA primarily focused on inorganic systems, particularly biominerals. However, it is now recognized as a universal phenomenon that can occur across vaious systems, including proteins [18,229]. Recent reports have indicated that MOFs can also form higher-order structures of primary crystals through an OA-like process [230]. The spaces between these primary crystals subsequently fuse, forming single crystals. Because particle interactions are central to this phenomenon, traditional theories of colloidal science offer a good starting point for its explanation. However, nanoscale-specific interactions, which are overlooked in those theories, also play a significant role [231–233]. A framework that extends beyond conventional theories is necessary to fully understand the principles of OA.

(2) Aggregation of sub-critical nuclei

Under the assumptions of CNT, the only way to overcome the nucleation barrier is the successive addition of monomers to a cluster until it reaches the critical size. However, the system can bypass this nucleation barrier through the aggregation of sub-critical nuclei. Although sub-critical nuclei are generally unstable and prone to dissolution or melting, if the characteristic time for their collision is shorter than that for dissolution or melting, the aggregation and coalescence of these sub-critical nuclei may become the dominant pathway for the formation of post-critical nuclei. Furthermore, the aggregation and coalescence of liquid droplets that remain uncrystallized due to their small size can induce crystallization by steep droplet size increase. The advancement of direct observation techniques using microscopes has enabled real-time, in situ observation of these phenomena in various metal nanoparticle synthesis processes [234–236]. The aggregation of sub-critical nuclei can also be integrated with the two-step and PNC pathways mentioned earlier, as seen in colloidal suspension and calcium carbonate systems [22,179]. In these systems, multiple sub-critical crystals form within droplets or amorphous particles that serve as precursor structures, subsequently aggregating to create stable crystals (Fig. 7). A similar process has been observed in simulations of LJ molecules [237], where droplets that do not crystallize independently begin to crystallize upon contact with a seed crystal. The small contact area between the seed crystal and the newly formed crystal nucleus may be mechanically sheared by stirring or other disturbances, potentially serving as the primary mechanism of secondary nucleation. Whether nuclei are subcritical or postcritical also influences the manner in which they fuse. Postcritical nuclei tend to fuse via OA when rotational alignment is possible, whereas subcritical nuclei tend to merge through a process involving the transient formation of an amorphous phase [238].

(3) Aggregation of kinetically trapped species

The aggregating species may be primary particles or clusters with uniform characteristics. Due to their uniformity, these particles and clusters function as "monomers". These primary particles may correspond to local minima on the free energy landscape due to size, shape, chemical interactions, and other factors, or they may be generated by kinetic factors. PNCs of biomineral follow this type of aggregation. Habraken et al. demonstrated that PNCs in the calcium phosphate system are actually calcium phosphate complexes, using a combination of cryo-TEM, various in-situ analytical techniques, and first-principles calculations [197]. Particle formation in this system progresses through the aggregation of these complexes. The magnetite system shows similar behavior [239]. In this system, primary particles with a uniform size of approximately 2 nm, likely representing the amorphous ferrihydrite phase, first form and then aggregate into branched networks. These aggregates subsequently densify locally, leading to the formation of crystalline magnetite particles.

An "extended nucleation theory" has been proposed for these systems [197,239], which leverages the uniformity of primary particles, treating them as monomers within the framework of CNT (See Section 3.5). Unlike the monomers in the original CNT, primary particles have an interface with the solution phase, contributing to excess free energy. As these primary particles aggregate, the excess free energy

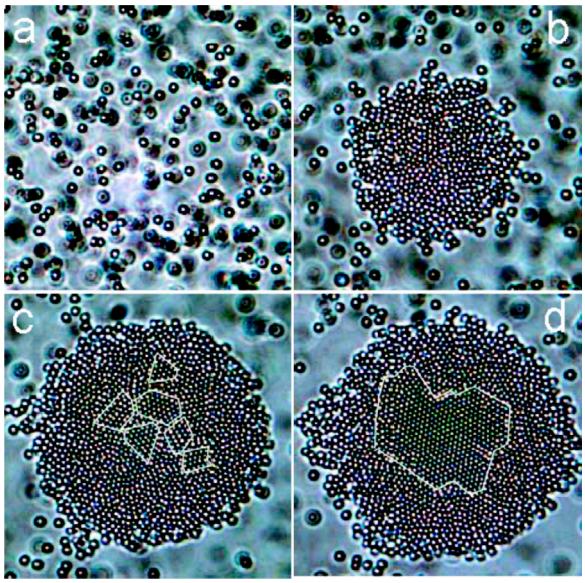

Fig. 7. Two-step nucleation pathway involving the aggregation of sub-critical nuclei observed in a colloidal suspension.
Source: Adapted with permission from Ref. [22].

decreases, significantly lowering the nucleation barrier compared to that predicted by CNT. The competition between amorphous and crystalline phases has also been analyzed using extended nucleation theory. Within this framework, the stability of primary particles, along with the relative bulk and surface energies of the two phases, determines which phase is preferentially formed. A subsequent model further incorporates the process of cluster formation through the aggregation of primary particles and their eventual transformation into crystals [240].

3.4. Proposed pathway classification with three conceptual axes

As discussed earlier, the pathways of particle formation are highly diverse, making it increasingly difficult to accurately categorize observed pathways under the previously reported "two-step nucleation pathway" or "PNC pathway". Even in pathways involving mesoscopic clusters of proteins [73,76], which are typically classified as two-step nucleation, the formation of molecular complexes is necessary to initiate clustering [91,97], highlighting the chemical aspects similar to those in the PNC pathway. On the other hand, the concept of LLPS is also used to describe the PNC pathway [139,193]. In this sense, the PNC pathway exhibits characteristics similar to the two-step nucleation pathway. Moreover, aggregation is frequently observed during the two-step or PNC pathway. Based on these facts, we propose to focus on the following three conceptual axes in order to classify pathways:

- **Axis 1**: Stepwise transitions between phases or between fluctuations that possess structural features of those phases.
- **Axis 2**: Chemically driven formation of structural units larger than monomers.
- **Axis 3**: Aggregation-dominant nucleation and growth processes.

As shown in Fig. 8, these three axes represent distinct vectors of nonclassical characteristics in particle formation pathways. The classical pathway assumed in CNT corresponds to the origin, with each nonclassical pathway described by the relative contributions of these three axes. For instance, crystallization via LLPS exemplifies a pathway where the characteristics of axis 1 are particularly emphasized. Crystallization via mesoscopic clusters of proteins involves both axes 1 and 2 due to the phase-like properties of the clusters and the formation mechanism based on the presence of molecular complexes. The PNC pathway in biomineral systems is a complex process involving all three axes: the formation of PNCs (axis 2), LLPS and amorphous phase formation (axis 1) through the aggregation (axis 3) of dehydrated PNCs. In some cases, as with the continuous growth of iron oxide nanoparticles [215], axis 2 dominates the entire particle growth process. In this way, some pathways can be effectively classified along a single axis, while others require a combination of two or three axes to describe the characteristics of the pathways.

Considering pathways in terms of the conceptual axes described above not only facilitates a clearer understanding of the differences between pathways but also helps determine the appropriate theoretical framework for describing each pathway. In the next section, we outline several theoretical models formulated to depict each axis of particle formation pathways.

3.5. Theoretical models for describing particle formation pathways

Model for stepwise transition between phases (model for axis 1)

Although CNT cannot directly describe multistep nucleation, its fundamental concepts have been adapted in various studies by introducing the idea of core–shell nuclei [161,171–173,241–245]. These nuclei consist of a core representing the most stable phase and a shell composed of a metastable phase. Iwamatsu was the first to formulate the free energy change associated with the formation of such a core–shell nucleus for systems that have a metastable phase in addition to the initial and most stable phases [171]. The free energy required to form a core–shell nucleus with a radius R, comprising a solid-phase core with a radius r_c and a liquid-phase shell of width $R - r_c$, originating from the vapor phase, is expressed by the following equation:

$$\Delta G = n\left(\mu_L - \mu_V\right) + \left(n - n_s\right)\left(\mu_S - \mu_L\right) + 4\pi R^2\left[\gamma_{LV} + \gamma_{SL}\left(r_c^2/R^2\right) + Se^{-(R-r_c)/\delta_s}\right], \quad (2)$$

where n and n_s are the number of monomers in the whole core–shell nucleus and in the liquid phase shell, respectively, μ_L, μ_V, and μ_S are the chemical potentials of the liquid, vapor, and solid phases, respectively, γ_{LV} and γ_{SL} are the interfacial tensions between the liquid-vapor and solid–liquid phases, respectively, S is the spreading coefficient, and δ_s is the range of interaction between the two interfaces. This formula represents multistep nucleation by applying CNT to each stage of liquid-phase nucleus formation from the gas phase and solid-phase formation within it. The term $Se^{-(R-r_c)/\delta_s}$ captures the effects of the proximity between the liquid-vapor and solid–liquid interfaces due to crystallization within droplets. Using this approach, Iwamatsu demonstrated that core–shell nuclei, pure liquid nuclei, and pure solid nuclei can serve as critical nuclei structures, depending on the wettability of the solid core to the liquid shell and the degree of supersaturation. Kashchiev et al. employed a similar model to express the free energy change associated with core–shell nucleus formation as an explicit function of the number of molecules [172,241]. By using equations where the densities of the core and shell are equal and the proximity effect of the two interfaces is ignored, they outlined the thermodynamic conditions under which the two-step pathway is favored and formulated a master equation. These core–shell-type CNT models have successfully explained and predicted nucleation pathways observed in a variety of simulations. For example, in lattice-gas systems, the two-dimensional free energy profiles calculated using this model showed excellent agreement with those obtained by umbrella sampling in Monte Carlo (MC) simulations [242]. Similarly, in aqueous NaCl solutions, the model successfully accounted for changes in pathway characteristics by fitting the free energy profiles obtained from molecular simulations [243]. Another successful example is found in binary Lennard-Jones systems [173]. Fig. 9 shows the free energy landscape calculated by the core–shell-type CNT model parameterized on the basis of molecular interaction parameters. The pathways observed in MD simulations (black solid lines) were consistent with those that bypass the local maximum in the landscapes.

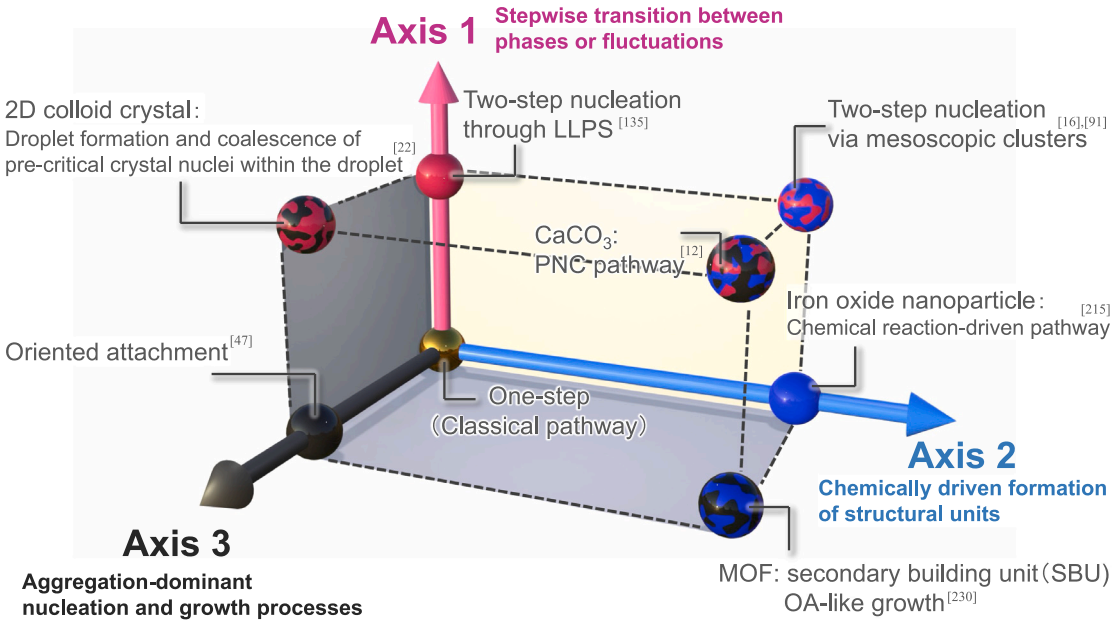

Fig. 8. Classification of particle formation pathways based on three conceptual axes.

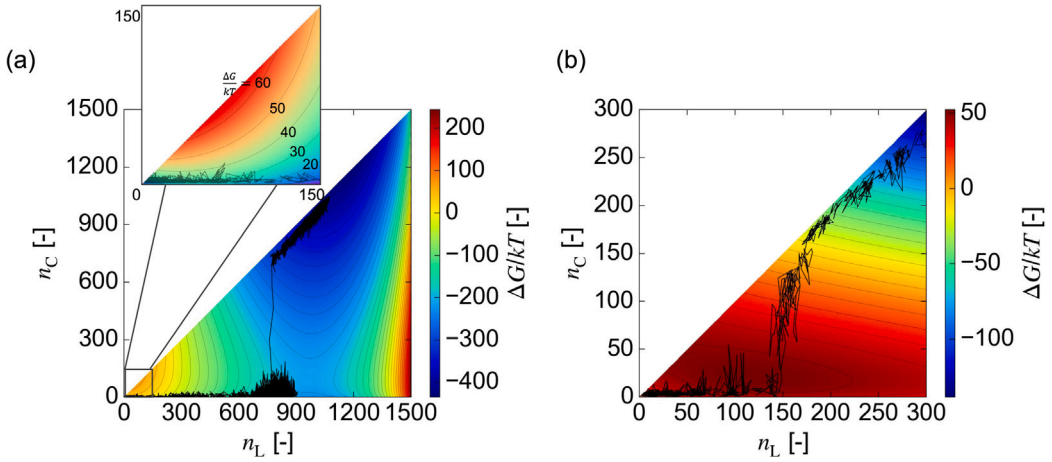

Fig. 9. Free energy landscapes calculated for binary Lennard-Jones systems using the core–shell-type CNT model parameterized on the basis of molecular interaction parameters. Here, n_L and n_C denote the numbers of solute molecules in the largest assembled structure and the largest crystal, respectively. (a) A condition with weak solute–solute interactions, resulting in two-step nucleation. (b) A condition with strong solute–solute interactions, resulting in the immediate crystallization of the assembled structure and hence one-step nucleation.
Source: Adapted with permission from Ref. [173].

As a non-CNT-based model for calculating nucleation rates, Pan et al. proposed a phenomenological approach that addresses the two-step nucleation of proteins [109]. Their model assumes the formation of the most stable crystalline phase within droplets of an intermediate phase, which are reversibly generated and dissociated in the initial solution phase. The rates of droplet formation, droplet dissociation, and crystallization are denoted by u_0, u_1, and u_2, respectively, and are expressed in an Arrhenius form as $u_i(T) = U_i \exp(-E_i/k_BT), i = 0, 1, 2$, where U_i and E_i are kinetic prefactors and energy barriers, respectively. Pan et al. derived the following equation for the rate of crystal nucleation based on the average first passage time from the initial phase to the most stable phase:

$$J = \frac{U_0 U_2 \exp\left(-\frac{E_0+E_2}{k_BT}\right)}{U_0 \exp\left(\frac{-E_0}{k_BT}\right) + U_1 \exp\left(\frac{-E_1}{k_BT}\right) + U_2 \exp\left(\frac{-E_2}{k_BT}\right)}. \quad (3)$$

Assuming that crystallization inside the liquid droplet is the rate-limiting process and explicitly expressing the temperature dependence of U_2 based on viscosity, Eq. (3) was simplified as follows:

$$J = \frac{k_{in} C_{in} T \exp\left(\frac{-E_2}{k_BT}\right)}{\eta(C_{in}, T)\left[1 + \frac{U_1}{U_0}\exp\left(\frac{-(E_0-E_1)}{k_BT}\right)\right]}, \quad (4)$$

where k_{in} is the rate constant for crystallization inside the droplet, C_{in} is the monomer concentration in the droplets, and $\eta(C_{in}, T)$ is the viscosity in the droplet. This model successfully explains the unique temperature dependence of nucleation rates observed in lysozyme, where the nucleation rate exhibits a maximum near the metastable liquid–liquid phase equilibrium line. The decrease in nucleation rate at low temperatures is attributed to the significant increase in droplet viscosity.

Some studies have proposed models that provide the time evolution of the particle characteristics following multistep nucleation [107,246–248]. Kashchiev et al. derived the following general formula for the time dependence of the total number of crystals, $N_c(t)$, during the two-step nucleation process, assuming that each droplet of the intermediate phase generates one crystal: [246]

$$N_c(t) = N_p(t) - \int_0^t \exp\left[-\int_\tau^t J_c^{in}(t')\, V_l(t',t)dt'\right]\left[dN(\tau)/d\tau\right] d\tau \quad (5)$$

where $N_p(t)$ represents the total number of intermediate phase droplets and crystals at time t, $J_c^{in}(t)$ is the nucleation rate of crystals in the intermediate phase, and $V_l(t',t)$ is the volume of the droplet formed at time t'. $N_p(t)$ can be calculated using the system volume V and the nucleation rate of droplets, $J_l(t)$, as follows:

$$N_p(t) = V\int_0^t J_l(t')\, dt' \quad (6)$$

Numerical results obtained from Eq. (5) indicated that the overall crystal nucleation rate can be significantly delayed if the growth rate of a droplet or $J_c^{in}(t)$ is low. Kashchiev et al. also suggested that it may be possible to distinguish between two-step and one-step pathways based on the supersaturation dependence of the delay time for nucleation. Other models that describe the time evolution of particle characteristics include the kinetic MC model developed by Hsieh et al. [248], the population balance model proposed by Alexandrov et al. which accounts for growth-mode transitions during crystallization [247], and the population balance model developed by Iida and Watanabe, which incorporates pathway variation on the basis of the free energy barrier for crystallization of intermediate droplets [249]. These models also enable calculation of the time evolution of particle size distributions.

Model for the chemically driven nucleation process (model for axis 2)

When slight chemical differences lead to distinct pathways, formulating a universal model of the phenomenon becomes challenging, as detailed chemical mechanisms are required for accurate modeling. Consequently, many researchers have adopted an approach that involves listing the reactions and chemical equilibrium relationships among the expected chemical species within a system, then estimating the formation rates and fractions of these species under various assumptions [194].

A recent study by Wall et al. attempted to generalize the effects of chemical reactions by proposing a reaction-driven nucleation theory that incorporates the progress of chemical reactions into CNT [250]. Although their model assumes that clusters form through the sequential addition of monomer molecules, similar to the original CNT, the state of the monomer molecules can include any intermediate states from the precursor complex ML_x to the state M in the final product crystal. The reaction equation is expressed as follows, where the state of the monomer is defined by the reaction progress variable ξ:

$$r_1R_1 + r_2R_2 + \cdots + ML_x \rightleftharpoons p_1P_1 + r_2P_2 + \cdots + M, \quad (7)$$

where r_i and p_i are the stoichiometric coefficients of the reactants and products, respectively. The work required to form clusters, $W(n)$, accounting for the change in free energy due to reaction progress, is given by:

$$W(n) = nk_BT\ln\left(\frac{\overline{Q_{bulk}}}{K_{eq}}\right) + \phi(n,\zeta,\xi). \quad (8)$$

The first term represents the bulk contribution, while the second term ϕ is the excess free energy. $\overline{Q_{bulk}}$ is the geometric mean of the reaction quotient of n monomers in the state of reaction progress ξ, K_{eq} is the equilibrium constant, and ζ is a parameter related to the structure of the cluster. This model was tested on InP quantum dots and Au nanoparticles, successfully predicting the formation of magic-sized clusters.

Model for the aggregation-involved pahtways (model for axis 3)

Since aggregation has long been a focus of colloid science, numerous sophisticated models have been developed to describe this phenomenon [251–254]. Consequently, when the aggregation of post-critical nuclei is dominant, the particle formation process can be effectively explained using these models. Indeed, OA has been reviewed in several studies, where various models based on traditional models, such as Smoluchowski's cohesion equation, have been extensively summarized [228,255,256].

When aggregation coincides with nucleation and monomer-by-monomer growth or is coupled with other complex nucleation mechanisms, theoretical descriptions become increasingly challenging. However, when the number of aggregating species is limited, it is possible to model the particle formation process as a series of sequential and parallel reactions. For example, Leffler et al. modeled the formation process of iron oxide nanoparticles, including precursor reactions, transient amorphous state formation, and particle aggregation, using a set of simultaneous differential equations for each relevant species [257].

If the properties of the aggregating species are uniform, extended nucleation theory can be applied [197,239]. As mentioned in Section 3.3, the aggregating species are treated as monomers possessing excess free energy in this theory. Denoting the average energy density of the bulk and interfacial energies of the aggregating species as f_p, the free energy change associated with the formation of particle nuclei with radius R from these "aggregating species monomers" can be expressed as follows:

$$\Delta G = \frac{4}{3}\pi R^3\Delta\mu_v + 4\pi R^2\gamma + \frac{4}{3}\pi R^3 f_p, \quad (9)$$

where $\Delta\mu_v$ is the change in chemical potential per unit volume upon nucleus formation, γ is the interfacial tension, and the aggregating species and nucleus are assumed to be spherical. As evident from the equation, the absence of the f_p term reduces the equation to that of CNT. If the aggregating species are metastable with respect to the solution ($f_p < 0$), ΔG is lower than the value predicted by CNT. In cases where the aggregating species are more stable than the solution ($f_p > 0$), the pathway via aggregation is not thermodynamically favored and can only dominate due to kinetic factors. This point has been highlighted in several studies [258,259]. Therefore, the value of f_p has a significant impact on ΔG, and accurate application of this theory requires a precise understanding of the thermodynamic state of the aggregating species.

4. Challenges and future directions

As described above, a variety of particle formation pathways beyond the classical view have been identified, and steady progress has been made in elucidating their mechanisms and developing theoretical descriptions. Nevertheless, particle synthesis still remains largely an art guided by trial and error. In this section, we discuss two major challenges that must be addressed to achieve a more comprehensive understanding of particle formation pathways and to enable the rational design of particle synthesis processes.

4.1. Pathway selection and competition

The particle formation pathway can vary with synthesis conditions, even for the same substance. It is often observed that a system following a classical one-step pathway under one set of conditions shifts to a different pathway under another. Table 1 summarizes representative examples of pathway variation under various experimental and simulation conditions. Factors affecting pathway selection include the initial phase [142], solvent [150], temperature [165,260–263], template [235,264–266], monomer concentration [143,147,170,200,267–269], molecular interactions [270–272], system dynamics [271,273], additives [203,274–276], and others [19,213]. Although many factors governing pathway selection have been identified, theoretical models

Table 1
Factors on particle formation pathway variations.

Factors	System/Substance	Method	Pathway variations & Notes	Year
Initial phase	AgBr	MD	Vacuum: 1-step/Solution: 2-step via disorderd cluster	2000 [142]
Solvent	Urea	MD	Methanol or ethanol: 1-step/Water or acetonitrile: 2-step	2015 [150]
Supercooling	2D colloidal crystal	Experiment	High: 1-step/Low: 2-step via disorderd cluster	2009 [144]
Temperature	Potts lattice gas	MD	Low temp.: 1-step/High temp: 2-step via liquid-like phase	2009 [260]
Temperature	Phase field crystal	DFT	Low temp.: 1-step. Mid-low temp.: 2-step(amorphous →nucleation of □ structure) Mid-high temp.: 2-step(amorphous →deformation into □ structure) High temp.: 3-step(amorphous →△ structure →hetero. nucleation of □)	2016 [261]
Temperature	Zeolites	MD	Low temp.: 1-step/High temp.: 2-step via metastable gyroid	2018 [262]
Temperature	Methan hydrates	MD	Low temp.: 2-step/Middle temp.: competition/High temp.: 1-step.	2019 [263]
Temperature	Bi nanocrystal	MD	Low temp.: 2-step(half-ordered cluster →crystal) Middle temp.: 3-step(droplet →half-ordered cluster →crystal) High temp.: 2-step(droplet →crystal)	2019 [165]
Template	Ice nucleation	MD	Nucleation at wurtzite structure surface Low temp.: competition of 1-step and 2-step/High temp.: 1-step	2021 [264]
Template	Glucose isomerase	MD	Nucleation at mica surface: 1-step	2014 [265]
Template	NaCl	MD	Nucleation in graphen liquid cell: 2-step via hexagonal structure	2021 [266]
Template	Bi nanocrystal	MD	Nucleation at crystalline & amorphous surface Crystalline: 2-step via droplets formation Amorphous: droplets →coalescence followed by crystallization	2022 [235]
Monomer conc.	2D colloidal crystal	Experiment	Low conc.: 1-step/High conc.: 2-step.	2009 [143]
Monomer conc.	$CaCO_3$	Experiment	Nucleation at organic interfaces Low conc.: 1-step instead of PNC pathway	2012 [267]
Monomer conc.	Amyloid fiblil	MD	Coarse-grained model Low conc.: 2-step via disordered oligomers/High conc.: 1-step.	2014 [268]
Monomer conc.	KH_2PO_4	MD	Low conc: 1-step High conc: multistep(metastable polymorph →stable crystal)	2016 [269]
Monomer conc.	NaCl	MD	Low conc.: 1-step/High conc.: 2-step	2016 [147]
Monomer conc.	Amyloid-β(16-21)	MD	Low conc.: 1-step(core–shell structured nuclei)/High conc.: 2-step	2017 [170]
Monomer conc.	$SrSO_4$	MD	Co-titration of $SrCl_2$ and Na_2SO_4 Slow titration rate : PNC pathway/Fast titration rate: 2-step	2018 [200]
Interaction	Rectangular particle	MD	Particle with isotropic and orientation-dependent interacition Strong isotropic interaction: 2-step Strong orientation-dependent interacition: 1-step	2010 [270]
Interaction	Lattice model	MC	Isotropic and orientation-dependent(OD) interacition Strong isotropic interaction: 2-step Strong OD interacition: 1-step Requirement for exact orientation match for OD interaction: 2-step	2011 [271]
Interaction	Patchy particle	MC	Asymmetric patchy models for globular proteins Isotropic interactions: 2-step(multiple small crystals in a droplet) Anisotropic interacition: 2-step(single crystal in a droplet)	2013 [272]
MC trials	Charged colloids	MC	With charge inversion: CsCl structure (equilibrium structure). Without charge inversion: FCC structure(kinetically trapped structure)	2009 [273]
MC trials	Lattice model	MC	System with orientation-dependent interactions. Low rotational ratio (slow rotational dynamics): 2-step	2011 [271]
Additive	Polyoxometalate	Experiment	Na^+ addition: 1-step. Cs^+ addition: 2-step.	2017 [274]
Additive	Glucose isomerase	Experiment	Low PEG and AS: 1-step. High AS: nanorod formation and aggregation. Medium PEG: aggregation of fibrous particles.	2018 [275]
Additive	PbS nanoparticles	Experiment	Amine addition: suppression of PNCs formation results in 1-step.	2020 [203]
Additive	LJ system	MD	Without additive: 2-step. Additive1 having strong affinity for the monomers: 2-step. Additive 2 having strong affinity for Additive 1: 2-step	2025 [276]
Reductant conc.	Pt nanoparticles	Experiment	High conc.: assembly of atoms (fully reduced species). Low conc.: assembly partially reduced complex.	2012 [213]
Hydrophobicity	Polymer	Experiment	No hydrophobic groups: 1-step/Hydrophobic groups introduced: 2-step	2017 [19]

MD: Molecular Dynamics simulation, DFT: simulation based on Density Functional Theory, MC: Monte Carlo simulation.

capable of predicting the dominant pathway directly from these variables remain limited [173,242,276]. In some cases, the factors that determine pathway selection are still poorly understood. For instance, what distinguishes cases of multistep nucleation, in which crystallization occurs within intermediate structures such as droplets [101,106], from those in which heterogeneous nucleation occurs on the surface of an intermediate? [103,155] Furthermore, when the thermodynamic or kinetic barriers associated with different pathways are comparable, multiple pathways may compete and coexist [277–279]. To achieve selective particle formation, strategies for suppressing competing pathways are therefore required. In experiments, local inhomogeneities in experimental conditions may also promote such competition. Accordingly, elucidating the relationships among molecular properties, environmental conditions, and the resulting pathways remains a central challenge.

The proposed three-axis framework offers a useful perspective for considering how particle formation pathways can be controlled. For pathways dominated by axis 1, namely stepwise transitions between phases, pathway selection is often governed by the position of the system in the phase diagram, which is determined by operating conditions such as temperature, concentration, and composition. In such cases, knowledge of phase behavior, including metastable regions, is central to prediction and control. This class of pathways is likely to

be the most amenable to description by natural extensions of existing theories, such as CNT-based models for core–shell nuclei. However, further understanding is still needed, particularly for metastable regions and multicomponent systems, where experimentally and theoretically accessible phase diagrams are often incomplete. For pathways in which axis 2, the chemically driven formation of structural units, plays the primary role, specific interactions or chemical reactions exert a controlling influence. A basic strategy is therefore to promote or suppress the relevant interactions or reactions. This strategy underlies several reported examples of additive-mediated pathway control [203,276]. For such strategies to be effective, however, the key interactions or reactions governing particle formation must be identified. As emphasized elsewhere [210], this requires the accumulation and classification of knowledge across a broad range of reactions, analogous to the systematic knowledge base developed in organic chemistry. In many systems, contribution of axis 3 (aggregation) is coupled with those from axes 1 and 2, giving rise to more complex pathways. This coupling makes it difficult to isolate the specific role of aggregation in the overall pathway. A useful first step is therefore to establish a clearer understanding of pathways in which aggregation is the dominant contribution and can be analyzed in relative isolation. From this perspective, OA, in which the contribution of axis 3 is dominant, represents an important model pathway for investigating the factors governing this axis. In recent years, direct TEM observations of OA processes, combined with DFT calculations, have provided deeper mechanistic insight into OA [280,281]. These studies have identified dipolar interactions and the binding energies of ligand molecules adsorbed on particle surfaces as key determinants of the OA process, highlighting the role of microscopic interactions that are not fully captured by conventional theories of colloidal interactions. Molecular simulations may therefore also provide a useful approach for elucidating the physical factors governing axis 3. This potential has been demonstrated by Chen et al. who used enhanced-sampling techniques, such as umbrella sampling, to analyze OA processes in molecular simulations [282]. Integrating these approaches to clarify the factors governing axis 3 would provide a basis for analyzing more complex particle formation pathways in which aggregation is coupled with contributions from axes 1 and 2.

4.2. Pathway as a control variable for particle properties

Although the diversity of particle formation pathways complicates the mechanistic understanding of particle formation processes, it also offers opportunities for more versatile particle synthesis. For example, the size distribution and morphology of particles produced via a two-step pathway involving LLPS can differ substantially from those obtained through the classical one-step pathway [130,131]. In addition, several studies have suggested that the structures of intermediate PNCs determine the final crystal structure in the PNC pathway [13,186,208]. If the conditions under which specific pathways emerge can be systematically identified, together with the particle states that they tend to produce, the pathways themselves could serve as powerful control variables in particle synthesis.

To this end, numerical simulations based on models that incorporate pathway selection and competition are particularly useful. Recently, Iida et al. developed a population balance model that describes the time evolution of particle size distributions while accounting for pathway selection [249]. Their simulation results showed that size focusing, a phenomenon in which the particle size distribution narrows during growth, can occur in a two-step pathway because of differences in the growth rates of intermediate droplets and stable crystals. Because numerical models capable of describing the time evolution of particle characteristics across different pathways remain limited [247,248], further development of such models is needed to achieve a systematic understanding of the relationship between pathways and particle properties.

Particle formation pathways can be exploited not only to control the properties of primary particles but also to construct higher-order particle structures. One example is the one-pot synthesis of spherical granules via LLPS. Liu et al. developed a spherical agglomeration technology by exploiting the strong agglomeration tendency associated with crystallization via LLPS [134]. They further extended this approach to high-melting-point drugs by using additives prone to LLPS [283]. In the future, further advances can be expected in a wide range of particle design and control techniques that leverage the distinctive characteristics of different pathways.

5. Summary

Particle formation pathways are highly diverse and exhibit a wide range of characteristics that depart from the classical nucleation theory. In this review, we summarized the characteristics and historical development of the most important pathways reported to date, namely two-step pathways, PNCs pathways, and aggregation-involved pathways. Because the pathways observed in experimental systems are often complex, they cannot be simply assigned to any one of these three categories. To provide a unified perspective on these diverse and often difficult-to-classify pathways, we proposed organizing the "nonclassical" features of particle formation pathways along three axes: (1) stepwise transitions between phases or phase-like fluctuations, (2) chemically driven formation of structural units larger than monomers, and (3) aggregation-dominant nucleation and growth processes. Within this framework, diverse pathways can be represented as points in a three-dimensional space defined by these axes, with the classical one-step nucleation pathway taken as the origin. We also summarized previously reported models describing particle formation processes in relation to these three axes. Finally, as future perspectives, we discussed the issues of pathway selection and competition, as well as the control of particle properties through the exploitation of these pathways. We hope that this review will contribute to a clearer understanding of the diverse and often confusing landscape of particle formation pathways and stimulate further progress in the field.

CRediT authorship contribution statement

Yuya Iida: Conceptualization, Investigation, Writing – original draft. **Satoshi Watanabe:** Conceptualization, Supervision, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process.

During the preparation of this work the authors used ChatGPT in order to refine language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors have no conflicts to disclose.

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Data availability

No data was used for the research described in the article.

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