A Deep Learning Framework Discovers Compositional Order and Self-Assembly Pathways in Binary Colloidal Mixtures

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ABSTRACT: Binary colloidal superlattices (BSLs) have demonstrated enormous potential for the design of advanced multifunctional materials that can be synthesized via colloidal self-assembly. However, mechanistic understanding of the three-dimensional self-assembly of BSLs is largely limited due to a lack of tractable strategies for characterizing the many two-component structures that can appear during the self-assembly process. To address this gap, we present a framework for colloidal crystal structure characterization that uses branched graphlet decomposition with deep learning to systematically and quantitatively describe the self-assembly of BSLs at the single-particle level. Branched graphlet decomposition is used to evaluate local structure via high-dimensional neighborhood graphs that quantify both structural order (e.g., body-centered-cubic vs face-centered-cubic) and compositional order (e.g., substitutional defects) of each individual particle. Deep autoencoders are then used to efficiently translate these neighborhood graphs into low-dimensional manifolds from which relationships among neighborhood graphs can be more easily inferred. We demonstrate the framework on in silico systems of DNA-functionalized particles, in which two well-recognized design parameters, particle size ratio and interparticle potential well depth can be adjusted independently. The framework reveals that binary colloidal mixtures with small interparticle size disparities (i.e., A- and B-type particle radius ratios of \( r_A/r_B = 0.8 \) to \( r_A/r_B = 0.95 \)) can promote the self-assembly of defect-free BSLs much more effectively than systems of identically sized particles, as nearly defect-free BCC-CsCl, FCC-CuAu, and IrV crystals are observed in the former case. The framework additionally reveals that size-disparate colloidal mixtures can undergo nonclassical nucleation pathways where BSLs evolve from dense amorphous precursors, instead of directly nucleating from dilute solution. These findings illustrate that the presented characterization framework can assist in enhancing mechanistic understanding of the self-assembly of binary colloidal mixtures, which in turn can pave the way for engineering the growth of defect-free BSLs.

KEYWORDS: autoencoder, self-assembly, colloids, binary superlattice, DNA-functionalized particles, multiflavored particles, nucleation pathways, lattice defects

INTRODUCTION

Binary colloidal superlattices (BSLs), highly ordered crystalline structures that consist of two sublattices formed by two types of particles, have demonstrated significant potential for the design of multifunctional materials with applications in photonics,\textsuperscript{1} optical absorption,\textsuperscript{2} sensing,\textsuperscript{3,4} and catalysis.\textsuperscript{5,6} The spontaneous self-organization central to colloidal self-assembly can allow for “bottom-up” synthesis of BSLs with up to subnanometer precision in an inherently parallelizable and cost-effective manner.\textsuperscript{7−12} Many studies have demonstrated colloidal self-assembly as a viable synthetic route to achieve BSLs.\textsuperscript{13−15} However, colloidal self-assembly is prone to form defective structures that can impact the functional properties of BSLs.\textsuperscript{16} Some of the most commonly observed defective structures include kinetically trapped amorphous aggregates and crystals that contain substitutional defects (i.e., lattices in which A- and B-type particles occupy inconsistent lattice sites).\textsuperscript{13,17}

Particle size ratio and interparticle potential well depth have been postulated as two of the most important design parameters for influencing the self-assembly of BSLs.\textsuperscript{13−15,17} A natural question is whether these design parameters can promote the self-assembly of defect-free BSLs. The answer to this question has not been systematically explored, however, as creating a tractable framework for accurately characterizing the many complex and possibly defective two-component structures that can appear during colloidal self-assembly remains an open challenge. Although many methods for characterizing self-assembled colloidal structures exist in the
literature, the most common methods either (i) heavily rely on the concept of “cut-off” radii to determine local structure and are thus sensitive to thermal fluctuations, (ii) fail to provide quantitative information about particles whose local structure does not correspond to well-defined reference structures or templates, and/or (iii) rely on diffusion mapping methods that can become intractable for systems with large configurational phase spaces. Most importantly, to our knowledge, only two reported characterization methods explicitly account for particle type and, thus, can identify substitutional defects in BSLs. These two methods depend on cutoff radii, diffusion maps, and/or have only been shown to characterize either two-dimensional or very simple three-dimensional lattices.

In this work, we present a framework that employs branched graphlet decomposition with deep learning to address the above challenges for characterizing the self-assembly of three-dimensional BSLs. Branched graphlet decomposition evaluates local structure via “structural” and “compositional” neighborhood graphs that are robust to thermal fluctuations, provide quantitative information about particles whose local structure does not correspond to well-defined reference structures, and explicitly account for particle type. These neighborhood graphs quantify both structural order (i.e., the unit cells within BSLs such as FCC, BCC, and HCP) and compositional order (i.e., how A- and B-type particles are distributed among these unit cell sites). We then use deep autoencoders to efficiently translate the high-dimensional structural and compositional neighborhood graphs into low-dimensional structural and compositional spaces where it is easier to infer relationships among neighborhood graphs. As such, the presented framework can simultaneously characterize the thousands of unique and defective structures that can appear during self-assembly. The framework can distinguish defective, nearly defective, and ordered lattices and can thus precisely elucidate entire complex nucleation pathways.

We demonstrate the characterization framework by systematically investigating the self-assembly of an in silico system of 500 DNA-functionalized particles (DFPs) in which particle size ratios and attractive interactions between A-type and B-type particles can be varied independently. We observe that small increases in particle size disparity can drastically reduce the number of substitutional defects in FCC and HCP lattices while further increasing the size disparity leads to the formation of (nearly) defect-free BCC-CsCl, FCC-CuAu, and IrV crystals. Furthermore, we observe that mixtures of size-disparate colloids that form nearly defect-free BSLs can undergo nonclassical nucleation pathways in which a dense amorphous precursor is formed prior to the final binary crystalline phase. The fine control of colloidal self-assembly using (small) size disparity suggests a promising future research direction for synthesizing defect-free BSLs and the transformation pathway analysis provides a deeper mechanistic understanding of the self-assembly of binary colloidal mixtures.

### METHODS

#### Structural and Compositional Colloidal Characterization Framework

The proposed framework for characterizing the self-assembly of three-dimensional BSLs consists of three steps. In the first step, we implement branched graphlet decomposition to quantify local topology in the form of structural and compositional neighborhood graphs. The second step uses deep autoencoders to reduce the dimensionality of the neighborhood graphs and create structural and compositional low-dimensional spaces. The third step employs agglomerative hierarchical clustering to partition the low-dimensional spaces and assign physically meaningful classifications (e.g., FCC-CuAu, substitutionally defective HCP) to the resulting partitions (see Figure 1).
Neighborhood Graph Construction via Branched Graphlet Decomposition

The first step in characterizing the structure of a given colloidal particle is to establish that particle’s “neighborhood.” We define the neighborhood as a list of particles (and their relative positions) that are considered topologically adjacent to the particle of interest. We employ the methodology described in refs 20 and 31 to obtain particle neighborhoods. The gist of the method is that the convex hull formed by the set of neighboring atoms describes the local structure around an atom. The convex hull, which is represented as a Voronoi cell, is determined from a Delaunay triangulation of the particle of interest and its 18 nearest neighbors (or half the inner shell atoms in FCC and HCP lattices). Because this method avoids the concept of bonds or “cut-off radii” among particles and instead uses a geometry-based, fixed number of particles to establish the neighborhood, it is less sensitive to thermal fluctuations, density gradients, and structural anisotropy than many common structural characterization methods (e.g., Steinhardt bond order parameters, 51 common neighbor analysis). 25

The neighborhood is then used to construct one structural neighborhood graph and two compositional neighborhood graphs that quantify the structural and compositional local topology of the particle of interest. The structural neighborhood graph is composed of all particles in the neighborhood, while the compositional neighborhood graphs are composed of (i) all particles that are of the same species of the particle of interest and (ii) all particles that are of a different species than the particle of interest. Note that this work is tailored toward binary lattices in which only two types of particles exist (e.g., A- and B-type particles). Previous work has used Delaunay triangulation to construct structural neighborhood graphs 20,31 for colloidal characterization. A key contribution here is “branching” the structural neighborhood graphs created by Delaunay triangulation to create two compositional graphs for each particle’s structural neighborhood graph. 25

The structural neighborhood graph alone can be used to identify unit cells within a lattice as FCC, BCC, HCP, etc. The compositional neighborhood graphs, however, can be used to quantify how A- and B-type particles are distributed among the FCC, HCP, and BCC unit cell sites. The compositional neighborhood graphs can thus be used to identify substitutional defects within BSLs. To our knowledge, only two reported characterization methods can explicitly account for particle types within lattice sites. 19,25 In addition to using cutoff radii and only characterizing two-dimensional or simple three-dimensional structures, these methods did not explicitly identify substitutional defects. We evaluate the structural and compositional neighborhood graphs using a graphlet decomposition method that has been successfully implemented for analyzing local structure in various colloidal and biological networks. 20,31,36–39 Graphlets are small, connected, non-isomorphic induced subgraphs of a larger network that contain some number of nodes, k. The k nodes in each graphlet are topologically distinguished by their individual automorphism orbits that account for the symmetries among the nodes in said graphlet. Each graphlet thus contains 1 to k − 1 distinct automorphism orbits. The neighborhood graph is evaluated by computing the frequency of these orbits for a given neighborhood. Note that these frequencies are weighted to account for the fact that the appearance of more complex automorphism orbits can correlate with the appearance of simpler ones. 20,31 In this work, each node is a particle within a neighborhood graph established by the Delaunay triangulation described above. We evaluate the structural and compositional neighborhood graphs using graphlets with 2−5 nodes, as calculations involving larger graphlets quickly become intractable. 20,31 Graphlets with 2−5 nodes display 73 different automorphism orbits. 36 As a result, each particle’s structural and compositional neighborhood graphs is quantified by a 73 × 1 vector, where each entry in the vector refers to the weighted frequency of an automorphism orbit. The structural neighborhood graph thus becomes a 73 × 1 vector, while the two compositional neighborhood graphs are concatenated to form one 146 × 1 vector. From this point forward the term “structural neighborhood graph” will refer to the 73 × 1 vector and the term “compositional neighborhood graph” will refer to the 146 × 1 vector.

We note that heterogeneous graphlet decomposition methods have also been reported in the literature. 40,41 Although these methods explicitly take node type (or in this case, particle type) into account, they can be difficult to implement and computationally expensive, thus requiring several simplifying assumptions to become tractable. The branched graphlet decomposition strategy outlined above is simple, tractable, and does not require any (extra) simplifying assumptions.

Dimensionality Reduction via Deep Autoencoders

The high dimensionality of the neighborhood graphs and nonuniformity in the distances among their discrete entries indicate that dimensionality reduction must be performed to produce a continuous, low-dimensional manifold where relationships among neighborhood graphs can be more intuitively analyzed. We reduce the dimensionality of the structural and compositional neighborhood graphs using a self-supervised deep neural network called an autoencoder. 51,34,36 which is conceptually explained in more detail in Supporting Information (SI) section S1. Specifically, we train two separate autoencoders to create one structural and one compositional low-dimensional space that are subsequently used for structural and compositional classification. We note that diffusion maps are commonly employed for reducing the dimensionality of neighborhood graphs for colloidal characterization. 19,20,26–30,53,42 However, diffusion maps can be computationally intractable for large configurational phase spaces. Diffusion maps further do not provide an explicit functional mapping between the high- and low-dimensional spaces, thereby limiting physical interpretation of the low-dimensional space. As a result, deep-learning-based approaches to dimensionality reduction for colloidal characterization have become increasingly more common. 19,31,32,36–40

This work focuses on how the size ratio and interaction strengths between A- and B-type particles affect the self-assembly of FCC, HCP, BCC, IrVA, IrVB, DCsClA, and DCsC1B-like BSLs from an isothermal in silico system of binary DFPs, with particular attention to the substitutional defects within these target lattices. We seek to use the presented characterization framework to identify substitutionally defective (i.e., structurally ordered, yet compositionally disordered) and defect-free (i.e., structurally and compositionally ordered) versions of these lattices. That is, we look to identify particles whose local environments conform to target structures with and without substitutional defects. To this end, we collected particle position data for isothermal in silico self-assembly trajectories of DFPs over a range of size ratios and interaction potentials. We recorded structural and compositional neighborhood graphs for each particle in each simulation frame according to the branched graphlet decomposition method. We then used only the unique neighborhood graphs (45 032 unique structural neighborhood graphs and 4814 unique compositional neighborhood graphs) to train the structural and compositional autoencoders, respectively. We finally used the structural and compositional autoencoders to translate all unique structural and compositional neighborhood graphs into two separate three-dimensional spaces (see SI section S1 for justification of the size of the low-dimensional space). More details describing the pair potential and system simulations can be found later in the Methods section, while more details describing the autoencoder training can be found in SI section S1.

Partitioning the Low-Dimensional Spaces for Structural and Compositional Classification

We implemented agglomerative hierarchical clustering (with Ward’s linkage) on the low-dimensional data in order to partition the structural and compositional low-dimensional spaces. Although the strategy produces a cluster tree (known as a dendrogram) that shows the hierarchical structure of all 1 to N possible cluster distributions, the process of choosing the “best” number of clusters is somewhat subjective; 31,46,47 see SI section S2 for the justification of the choice of the number of clusters in each low-dimensional space. Since the
Figure 2. Classified colloidal binary superlattices. The A- and B-type particles within a perfect spherical FCC-CuAu lattice are manually swapped over time (i.e., simulation frames). Swap attempts are only accepted if the potential energy of the new configuration is higher than that of the current configuration. (a) The number of like (A-A, B-B) and unlike (A-B) nearest neighbors ($#N_{ij}$) is plotted against the simulation frame number. (b) The presented characterization framework identifies the number of structurally and compositionally ordered (CO) and structurally ordered, yet compositionally disordered (CD) particles in each frame. Note that both the number of CO particles and A-B nearest neighbors $N_{AB}$ decrease over time. (c) Snapshots of lattices where A-type particles are colored blue and B-type particles are colored orange sit above snapshots of lattices that are classified by the characterization framework. In the latter case, CO particles are colored dark red, CD particles are colored light red, and particles that are not structurally ordered are transparent. Frame 0 contains the perfect FCC-CuAu configuration. Frames 20 and 100 contain lattices that have gone through several swapping attempts.

We note that the characterization framework classifies ≈10 near-surface particles in the defect-free lattice in frame 0 in Figure 2b as compositionally disordered. This “misclassification” is a reflection of the fact that the 14 discrete classes used in this work are defined based on the bulk structures. This choice in turn can lead the framework to occasionally misclassify particles near the surface (as these particles can have different neighborhood topologies than bulk particles). To reduce this imprecision, we can define more classes (i.e., label more clusters) that identify surface or near-surface ordered and disordered particles. In fact, we identified such near-surface order in a previous iteration of this work that only focused on structural order.13 We did not view identifying near-surface structural and compositional order as necessary for this work; see SI section S2 for more details.

Colloidal Self-Assembly System Description

The purpose of this work is to use the presented characterization framework to investigate the self-assembly of an in silico system of binary DFPs under various interparticle size ratios and interaction potentials. We describe the pair potential model and resulting molecular dynamics simulations used throughout this work in more detail below.
**Pair Potential Model.** DFPs interact with each other through complementary Watson—Crick base-pairing interactions. As a means of achieving selective binding among DFPs, particles can be functionalized with a blend of two types of DNA strands with complementary concentrations on each particle. By changing the blending ratio of these two types of DNA strands, these “multi-flavored” particles can exhibit a tunable attraction between the like particles while maintaining interactions between unlike pairs. This approach has been shown to induce the crystallization of equally sized particles into BCC, HCP, and FCC structures.

We model the tunable and independent pairwise interactions of DFP colloidal mixtures in this work using the Fermi—Jagla pair potential (see eq 1), which previously has been used to represent binary DFPs effectively in both two and three dimensions. 50,53

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\text{Figure 3. (a) Example pair potentials with independent and tunable pairwise interactions } E_{AA}, E_{BB}, \text{ and } E_{AB} \text{ for identically sized particles at } \sigma = 1.0. \text{ The red, blue, and green curves represent } E_{AA} = -0.3\epsilon, E_{BB} = -0.5\epsilon, \text{ and } E_{AB} = -\epsilon. \text{ These epsilon values are achieved by tuning } B_0 \text{ to values of } 0.56, 0.8, \text{ and } 1.32 \text{ respectively. (b) Example pair potential with different particle sizes. Red, blue, and green curves represent particle sizes of } r_A = 0.9, r_{AB} = 0.95, \text{ and } r_B = 1.0. \text{ These sizes are achieved by tuning values of } \sigma \text{ to } 0.9, 0.95, \text{ and } 1.0, \text{ respectively. All parameter values used to create these plots are provided in SI Table S3.}
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\text{Figure 4. (a) Crystallization order diagram as a function of particle size ratio, } r^* = r_A/r_B, \text{ and relative like interaction strength, } E_{AA}^* = E_{AA}/E_{AB} (E_{BB}^* = E_{BB}^*/E_{AA}). \text{ MD simulations (see Methods section) are performed at a variety of size ratios and interaction strengths that are indicated by the gray dots. The characterization framework classifies each particle in the final snapshot of each simulation according to SI Table S1. The color bar represents the fraction of structurally ordered (SO) particles in these final snapshots; the fraction calculation is normalized by the number of SO particles in a perfect FCC spherical lattice. Each region within the order diagram is labeled based on the specific classifications of the SO particles. In the compositionally disordered close-packed (CD-CP) region, structurally ordered, yet compositionally disordered (CD) FCC and HCP particles are observed, which form polymorphic and randomly packed lattices. In the FCC-CuAu and HCP-straight region, structurally and compositionally ordered (CO) FCC and HCP particles are observed, which form FCC-CuAu lattices and polymorphic HCP-straight/FCC-CuAu lattices. CO BCC particles are observed in the BCC-CsCl region. In the IrV and DCScl regions, CO IrVA, IrVB, DCSClA, and DCSClB particles are observed, which form CD/CO and amorphous or amorphous particles that are not explicitly identified by the characterization framework. (c) The ratio of the total number of CO particles (N_{CO}) to the total number of SO particles (N_{SO}) is plotted for different size ratios r_A/r_B at E_{AA}/E_{AB} = 0.3. The red, green, pink, and orange bars quantify FCC-CuAu, HCP-straight, CO IrVA/B, and DCSClA/B, respectively. N_{CO}/N_{SO} = 1.0 suggests that all particles within BSLs are structurally and compositionally ordered particles (i.e., defect-free).}
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strength of these interactions, while \( A_1 \) and \( B_1 \) control the interaction range. \( A_2 \) and \( B_2 \) control the separation distance.

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U(r/\sigma) = \frac{A_i}{\sigma^3} \left( \frac{\sigma}{r - \sigma/\sqrt{15}} \right)^6 + \frac{A_{ij}}{\sigma^6} \left( 1 + \exp(A_{ij}(r/\sigma - A_2)) \right) - \frac{B_{ij}}{r^6 (1 + \exp(B_i(r/\sigma - B_2)) \right)
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To tune interparticle potentials, we keep unlike pair interaction \( E_{AB} \) fixed and vary like interactions \( E_{AA} \) and \( E_{BB} \) independently. The relative like interaction strength \( E_{AA}^r = E_{AA}/E_{AB} \) and \( E_{BB}^r = E_{BB}/E_{AB} \) can thus be adjusted independently from 0.0 to 1.0. We set \( E_{AA}^r = E_{BB}^r = 1.0 \) throughout all simulations unless otherwise noted. Note that setting \( E_{AA}^r = E_{BB}^r = 1.0 \) reduces the multiflavoring to single flavoring, where all particles are identical. Setting \( E_{AA}^r = E_{BB}^r = 0.0 \) makes the system a conventional binary mixture, where A–A and B–B interactions are purely repulsive and only A–B interactions are attractive. On the other hand, the particle size ratio \( r_A/r_B \) is tuned by varying the size of A-type particles within a range of \( r = 0.8–1.0 \) while maintaining the size of B-type particles at \( r = 1.0 \). Setting \( r_A/r_B = 1.0 \) makes the system the same size. Setting \( r_A/r_B = 0.8 \) means B particles are 20% larger than A particles. Figure 3 shows the pair potentials of multiflavored, binary micrometer-sized DFPS with parameter choices similar to those used in this work. These DFPS interact with each other via tunable and independent pairwise interactions \( E_{AA}, E_{BB}, \) and \( E_{AB} \).

Simulation Details. Molecular dynamic (MD) simulations are performed using LAMMPS\textsuperscript{53} in the canonical ensemble. The system contains 500 total particles with a 1:1 mixture ratio of A-type and B-type particles. The interaction strength and size ratio are varied using the pair potential model discussed above. Simulations are performed in a cubic box with periodic conditions applied to all three dimensions, under dilute conditions with number density \( \rho = 0.02\sigma^{-3} \), and using a Langevin thermostat with a time constant \( \tau = 2m\sigma^{-2} \). Each simulation involves \( 1 \times 10^6 \) total time steps where each time step is \( \Delta t = 10^{-3} m \sigma^{-1} \). Each MD simulation is performed at a constant, predetermined temperature suitable for crystallization starting from a random dilute liquid phase, where particles are allowed to evolve spontaneously to form crystals. The entire self-assembly process can be tracked and quantified by the characterization framework demonstrated above. The trajectories generated from these simulations are visualized using Open Visualization Tool (OVITO).\textsuperscript{54}

## RESULTS AND DISCUSSION

### Small Size Disparity Promotes Compositional order of BSLs

In this section, we illustrate that slightly tuning particle size ratio and attractive interaction strength can change the structural order of self-assembled BSLs and can promote the formation of structurally and compositionally ordered (defect-free) BSLs. We demonstrate the effects of small size disparity and interaction strength on mediating the self-assembly of BSLs with the binary in silico system of 500 DFPS discussed in the Methods section. In this system, particle size (i.e., \( r_A \) and \( r_B \)) and interparticle interaction strengths (i.e., \( E_{AA}, E_{BB}, \) and \( E_{AB} \)) can be tuned independently, which provides additional flexibility and control over the self-assembly process in comparison to standard binary colloidal systems.

We use the presented characterization framework to classify each particle within self-assembled BSLs over a range of relative attractive interaction strengths \( E_{AA}^r = E_{AA}/E_{AB} \) (\( E_{BB}^r = E_{BB}/E_{AB} \)) and size ratios \( r^* = r_A/r_B \). Figure 4a shows how size ratio can change the structural order of self-assembled BSLs. At size ratio \( r^* = 1.0 \), the formed BSLs are polymorphic, primarily CD, randomly close-packed FCC/HCP structures. Slightly increasing the size disparity results in the formation of FCC-CuAu or polymorphic FCC-CuAu/HCP-straight BSLs. More interestingly, further increasing the size disparity leads to the formation of two more different BSL structures, IrV and distorted CsCl (DCsCl). These two structures can be viewed as deformed versions of BCC-CsCl, in which the increasing size difference and interaction strength of the two species forces some particles to lie either too far apart or too close to one another (see SI Figure S1). The impact of interaction strength on the formation of IrV or DCCsCl structures suggests that the presence of enthalpic driving forces from pairwise interactions adds a degree of freedom (in addition to size ratio) for mediating the self-assembly of BSLs. Note that the self-assembled BSLs listed here are much richer than systems of hard spheres\textsuperscript{56,57} as well as sticky spheres\textsuperscript{58,59} at similar size ranges, where substitutionally disordered FCC is the most dominant structure.

Figure 4a also shows that an amorphous zone, where particles are trapped in disordered amorphous states, exists for systems of size-disparate particles. We note that polydispersity/ bidispersity are two commonly used parameters for inhibiting crystallization. Crystallization is usually suppressed for systems above 5% polydispersity (or 15% bidispersity).\textsuperscript{59,60} As shown in Figure 4a, crystallization suppression is widely observed at extremely high \( E_{AA}^r \) and becomes more pronounced for colloidal mixtures with larger size disparities. However, well-ordered BSLs can still be assembled at relatively weak \( E_{AA}^r \). These observations reinforce the importance of proper selection of \( E_{AA}^r \) for adopting different structural ordering of BSLs and demonstrate \( E_{AA}^r \) as an important design parameter for promoting or inhibiting crystallization.

More importantly, we observe that a slight increase in size disparity will also change the compositional order of formed BSLs. We first observe that, in the case of identically sized particles, substitutionally defective CD-CP lattices are formed over nearly the entire parameter space (\( E_{AA}^r > 0.2 \)). As \( E_{AA}^r \) increases, the fraction of CO particles within BSLs further decreases (see SI Figure S3). This suggests that the bulk crystals become more and more substitutionally defective, despite the fact that the primary crystals remain structurally ordered. These observations agree well with previous simulation and experimental work that shows that FCC-CuAu crystals change to substitutionally defective FCCs with increasing like-particle interaction strength.\textsuperscript{59} While colloidal mixtures with identically sized particles are prone to form CD BSLs, our simulation results reveal size disparity as an exclusive design parameter that can promote the formation of defect-free BSLs. For mixtures with small size disparity, the CO BSL structure FCC-CuAu can be formed within a much larger parameter space of \( E_{AA}^r \) extended from \( E_{AA}^r = 0.2 \) to \( E_{AA}^r = 0.7 \). Figure 4c quantifies the fraction of identified CO particles within different types of BSLs as a function of size ratio. Defect-free FCC-CuAu BSLs form at a size ratio of \( r^* = 0.95 \) and \( E_{AA}^r = 0.3 \), while substitutionally defective CD-CP lattices form at size ratio \( r^* = 1.0 \). As the size disparity further increases, IrV and DCCsCl, two BSLs that are structurally different than the BCC/FCC/HCP-like lattices, were formed. However, the particles within these lattices are usually CO particles, illustrating the universality of the impact of size disparity for reducing the number of CD particles within BSLs. All of these observations indicate the importance of size disparity, and how small changes in this parameter (at certain
interaction strengths) can radically change the structural and compositional ordering of self-assembled BSLs. Antisite formation penalties provide a plausible explanation for slight size disparity leading to a reduced number of substitutional defects in self-assembled BSLs. It has been previously reported that the antisite formation penalty decreases as $E_{AA}^{*}$ increases for systems of identically sized sticky colloidal particles. In these systems, the relative interaction strength is the main parameter that drives BSL self-assembly. As $E_{AA}^{*}$ increases, less enthalpic penalty is introduced when an A-type particle occupies a site where a B-type particle should be present. We calculated the antisite formation penalty for both identically sized and size-disparate particles (SI Figure S4) and found that a slight size disparity significantly raises the antisite formation penalty. We thus hypothesize that particle size disparity could guide the formation of BSLs at the early stage of nucleation. We use the presented characterization framework to investigate this hypothesis in the next section. Here, we show that size disparity can assist in promoting the formation of defect-free BSLs through unique pathways, such as nonclassical transformations during colloidal self-assembly.

**Figure 5.** (a) Schematic illustration of self-assembly pathways for forming BSLs. The self-assembly of BSLs can occur via either a one-step or two-step nucleation process. (b) Example of a one-step nucleation pathway observed at $E_{AA}^{*} = 0.3$ and $r^* = 0.95$. (c) Example of a two-step nucleation pathway (amorphous-crystal) observed at $E_{AA}^{*} = 0.6$ and $r^* = 0.95$. The self-assembly process is quantified by plotting the fraction of identified structurally ordered (SO) particles (blue curves), structurally and compositionally ordered (CO) particles (red curves), and largest cluster size (dashed gray curves) as a function of time. The inset snapshots show identified crystalline particles at the single-particle level at different times. The particle coloring scheme is same as that of Figure 4b. (d) Quantification of self-assembly pathways for size-disparate systems at size ratio $r_A/r_B = 0.95$. (e) Quantification of self-assembly pathways for identically sized systems at size ratio $r_A/r_B = 1.0$. Plots (d) and (e) show the fraction of SO particles within the largest cluster for different $E_{AA}^{*}$ (color bar), while the insets show the fraction of SO particles ($X_{SO}$) as a function of the fraction of CO particles ($X_{CO}$).

**Probing Self-Assembly and Structural Evolution Processes**

We use the presented characterization framework to show the mechanistic details of how BSLs evolve (or nucleate) from dilute solutions. The characterization framework reveals that BSL nucleation pathways either occur via one-step (classical) or two-step (nonclassical) processes (Figure 5a–c). Figure 5b demonstrates how BSLs can self-assemble by classical one-step nucleation. Here, the fraction of identified SO crystalline particles, the fraction of identified CO particles, and the total largest cluster size are plotted over time. First, a small crystalline nucleus with an FCC-CuAu (CO) structure is formed. This small crystal nucleus then grows into a larger size, and the final stabilized crystal is identical in structure with the initially formed nuclei. Figure 5c shows how the nucleation of BSLs can also proceed by nonclassical two-step nucleation. Here, instead of forming a small crystal nuclei, the particles rapidly form large disordered amorphous aggregates with very few crystalline particles within these clusters. Subsequently, these disordered amorphous clusters evolve into an ordered BSL, as indicated by the continuous growth of identified SO particles.
Figure 5d,e further demonstrates the differences between the nucleation processes of size-disparate and identically sized particles over a broader parameter space of $E^*$.

For colloidal mixtures in which one-step nucleation occurs, the SO crystal fraction grows linearly with the size of the largest cluster. In contrast, for colloidal mixtures in which two-step (amorphous-solid) nucleation occurs, no crystal is identified until the largest cluster size reaches about 80% of the total system size. An abrupt increase in crystal fraction is then observed after this initial amorphous state. Interestingly, we note that the two-step nucleation usually occurs for size-disparate particles and relatively high $E^*$ close to the boundary of amorphous states (other size ratios are provided in SI Figure S5). For this relatively high interaction range ($E^*_A = 0.3$ to $E^*_A = 0.7$), however, identically sized particles usually nucleate via a one-step process and form highly CD crystals. Unlike size-disparate particles that can transform from disordered amorphous clusters into BSls via a diffusionless process, the identically sized particles usually form CD crystalline nuclei quickly at the initial nucleation stage. Such CD nuclei then continuously grow larger in size and remain trapped in substitutionally disordered crystalline phases.

These results illustrate that size-disparate particles can form BSls via a two-step process: particles first aggregate into disordered amorphous clusters and then rearrange into crystalline BSls structures. Note that the observation of two-step nucleation pathways directly contradicts the well-recognized classical nucleation theory (CNT)\(^\text{61,62}\), suggesting a more complex picture of the transformation mechanism for BSls. While CNT is a widely used rule for characterizing nucleation of particles from the solution phase, more and more evidence now supports two-step nucleation’s occurrence in nature.\(^\text{63-67}\) One commonly believed reason for the emergence of two-step nucleation pathways is the supercooling/supersaturation that can occur within colloidal self-assembly systems. In colloidal self-assembly, two-step nucleation has been reported once the liquid is deeply quenched.\(^\text{68}\) In such cases, amorphous aggregates are formed initially before they sluggishly transform into crystals depending on the temperature and cooling rate. Previous work has also suggested that slow particle mobility brought on by supercooling can inhibit crystallization and promote the formation of amorphous aggregates.\(^\text{69}\) The successful transformation from amorphous to crystalline phases is assumed to be caused by an interplay between thermodynamics and kinetics. While the full picture of the emergence of two-step nucleation requires more theoretical calculations, our framework provides a way to efficiently quantify the emergence of well-ordered crystalline nuclei from many defective crystalline nuclei or amorphous aggregates at the particle level. This capability allows us to probe into the self-assembly details of BSls at early nucleation stages under supercooling. Such probing is otherwise not achievable in experiments, as nucleation is usually a rare event that is generally difficult to capture and quantify.

We thus further investigate BSL nucleation to determine the role of supercooling in influencing the observed nucleation pathways. Our simulation results show that, for size-disparate systems that previously underwent two-step nucleation, raising the temperature causes a tendency toward the observation of one-step nucleation pathways that result in well-defined CO crystals (Figure 6). Two-step nucleation usually occurs under moderate supercooling. Under deeper supercooling, more amorphous particles were identified during the self-assembly process. However, even under these highly undercooled conditions, size-disparate particles within dense amorphous aggregates still tend to rearrange into more ordered (but not “well-ordered”) BSL structures, despite the process becoming slower with further lowering of the temperatures. The reduced mobility of the particles in the clusters must significantly impact the kinetics and inhibit the transformation from amorphous to well-defined binary crystalline structures. A similar tendency of suppression of crystallization is observed for identically sized particles (SI Figure S7). However, neither raising nor lowering the temperature improves the formation of defect-free BSls. Mixtures of identically sized particles rather tend to be kinetically trapped in structures that are formed early on during self-assembly, either in highly compositionally disordered BSls or more structurally disordered amorphous aggregates at lower temperatures.

The above analysis has shown that particle size disparity can assist in the formation of defect-free BSls through two unique nucleation pathways: particles either can rearrange from amorphous aggregates into BSls under moderate supercooling or can directly nucleate and grow into larger BSls at higher temperatures. We note that raising the temperature can drive crystallization mechanisms from two-step (nonclassical) to one-step (classical). The observation of such transitions is similar to those previously observed in a NaCl solution\(^\text{70}\) or a Lennard-Jones fluid system.\(^\text{71}\) In the NaCl solution, single-step nucleation is observed before the solution reaches the spinodal regime and two-step nucleation is observed after the solution reaches the spinodal regime. Similarly, in the Lennard-Jones fluid system, a crossover from a classical nucleation regime to a more collective mechanism of freezing is observed, influenced by the existence of a spinodal singularity at higher supercooling. Experimentally, it is also reported that two-step nucleation is widely observed, especially in the DFP systems.\(^\text{72,73}\) Nonetheless, the cooling rate, the quench temperature window, as well as the details of particles can all impact the self-assembly pathways. For instance, the presence of DNA molecules around particles could result in
dramatic sluggishness of rearrangement of amorphous aggregates into crystalline structures due to the presence of hybridization kinetics.\textsuperscript{2} Accordingly, it may be valuable to further investigate the relationship between these parameters and crystallization transition mechanisms to build a proper interpretation of the nucleation of BSLs.

\section*{CONCLUSIONS}

We presented a framework for characterizing the self-assembly of binary colloidal mixtures based on branched graphlet decomposition and deep learning. The characterization framework was demonstrated by investigating the self-assembly of binary mixtures of DNA-functionalized particles while varying two well-recognized design parameters, i.e., particle size ratio and pairwise interaction potential. Our investigation revealed that size disparity at certain interaction potentials can improve the structural diversity of self-assembled BSLs, leading to the formation of BCC-CsCl, FCC-CuAu, IrV, DCsCl, and CD-CP lattices. As a comparison, systems of hard spheres (without presence of pairwise interactions) assemble a limited range of rFCC-like structures. We also found that small A/B particle size ratios can drastically reduce the number of substitutional defects within BSLs and, thus, promote the formation of defect-free BSLs.

The proposed characterization framework can pave the way for systematic and computationally efficient investigation of the underlying mechanisms of the self-assembly of BSLs. Our analysis showed that size-disparate colloidal mixtures can undergo two-step, nonclassical nucleation pathways where BSLs evolve from dense amorphous precursors, instead of directly nucleating from dilute solution in one step. Interestingly, size-disparate mixtures tend to form (nearly) defect-free BSLs, regardless of their adopted nucleation pathway. On the other hand, systems of identically sized particles always follow one-step classical nucleation pathways, but often become kinetically trapped in substitutionally defective structures in the early stage of nucleation. Thus, the fine control of self-assembly of defect-free BSLs using size-disparate particles under given conditions can facilitate potential approaches to engineer defect-free BSLs. The proposed framework can be easily adapted to investigate the underpinning mechanisms of other colloidal self-assembly systems.

\section*{ASSOCIATED CONTENT}

\subsection*{Supporting Information}

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.2c00111.

General background information and figures regarding dimensionality reduction via deep autoencoders and classification via agglomerative hierarchical clustering, several supporting figures for the analysis of observed colloidal self-assembly trends, and a table that clarifies the abbreviations used throughout the main text (PDF).

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Notes

The authors declare no competing financial interest.

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\section*{REFERENCES}

(1) Sun, L.; et al. Polarization-dependent optical response in anisotropic nanoparticle–DNA superlattices. \textit{Nano Lett.} 2017, 17, 2313–2318.

(2) Redl, F. X.; Cho, K.-S.; Murray, C. B.; O’Brien, S. Three-dimensional binary superlattices of magnetic nanocrystals and semiconductor quantum dots. \textit{Nature} 2003, 423, 968–971.

(3) Barnaby, S. N.; et al. Modular and chemically responsive oligonucleotide “bonds” in nanoparticle superlattices. \textit{J. Am. Chem. Soc.} 2015, 137, 13566–13571.

(4) Lee, J.-S.; Han, M. S.; Mirkin, C. A. Colorimetric detection of mercuric ion (Hg2+) in aqueous media using DNA-functionalized gold nanoparticles. \textit{Angew. Chem., Int. Ed.} 2007, 46, 4093–4096.

(5) Kang, Y.; et al. Design of pt–pd binary superlattices exploiting shape effects and synergistic effects for oxygen reduction reactions. \textit{J. Am. Chem. Soc.} 2013, 135, 42–45.

(6) Kang, Y.; et al. Engineering catalytic contacts and thermal stability: gold/iron oxide binary nanocrystal superlattices for co-oxidation. \textit{J. Am. Chem. Soc.} 2013, 135, 1499–1505.

(7) Paulson, J. A.; Mesbah, A.; Zhu, X.; Molaro, M. C.; Braatz, R. D. Control of self-assembly in micro-and nano-scale systems. \textit{Journal of Process Control} 2015, 25, 38–49.

(8) Liddle, J. A.; Gallatin, G. M. Nanomanufacturing: a perspective. \textit{ACS Nano} 2016, 10, 3095–3104.

(9) Furst, E. M. Directed self-assembly. \textit{Soft Matter} 2013, 9, 9039–9045.

(10) Rogach, A. L. Binary superlattices of nanoparticles: Self-assembly leads to “metamaterials.” \textit{Angew. Chem., Int. Ed.} 2004, 43, 148–149.

(11) Bodnarchuk, M. I.; Kovalenko, M. V.; Heiss, W.; Talapin, D. V. Energetic and entropic contributions to self-assembly of binary nanocrystal superlattices: temperature as the structure-directing factor. \textit{J. Am. Chem. Soc.} 2010, 132, 11967–11977.

(12) Yu, Y.; Bosoy, C. A.; Smilgies, D.-M.; Korgel, B. A. Self-assembly and thermal stability of binary superlattices of gold and...
silicon nanocrystals. journal of physical chemistry letters 2013, 4, 3677–3682.
(13) Knorowski, C.; et al. Dynamics of DNA-programmable nanoparticle crystallization: gelation, nucleation and topological defects. Soft Matter 2012, 8, 12053.
(14) Shevchenko, E. V.; Talapin, D. V.; Kotov, N. A.; O’Brien, S.; Murray, C. B. Structural diversity in binary nanoparticle superlattices. Nature 2006, 439, 55.
(15) Unnissen, M. E.; et al. Ionic colloidal crystals of oppositely charged particles. Nature 2005, 437, 235–240.
(16) Ross, M. B.; Ku, J. C.; Blaber, M. G.; Mirkin, C. A.; Schatz, G. C. Defect tolerance and the effect of structural inhomogeneity in plasmonic DNA-nanoparticle superlattices. Proc. Natl. Acad. Sci. U. S. A. 2015, 112, 10292–10297.
(17) Bartlett, P.; Campbell, A. I. Three-dimensional binary superlattices of oppositely charged colloids. Phys. Rev. Lett. 2005, 95, 128302.
(18) Boattini, E.; Dijkstra, M.; Filion, L. Unsupervised learning for local structure detection in colloidal systems. J. Chem. Phys. 2019, 151, 154901.
(19) Reinhart, W. F.; Long, A. W.; Howard, M. P.; Ferguson, A. L.; Panagiotopoulos, A. Z. Machine learning for autonomous crystal structure identification. Soft Matter 2017, 13, 4733–4745.
(20) Reinhart, W. F.; Panagiotopoulos, A. Z. Automated crystal characterization with a fast neighborhood graph analysis method. Soft Matter 2018, 14, 6083–6089.
(21) Steinhardt, P. J.; Nelson, D. R.; Ronchetti, M. Bond-orientational order in liquids and glasses. Phys. Rev. B 1983, 28, 784.
(22) Lechner, W.; Delgado, C. Accurate determination of crystal structures based on averaged local bond order parameters. J. Chem. Phys. 2008, 129, 114707.
(23) Larsen, P. M.; Schmidt, S.; Schiøtz, J. Robust structural identification via polyhedral template matching. Modell. Simul. Mater. Sci. Eng. 2016, 24, 055007.
(24) Ackland, G.; Jones, A. Applications of local crystal structure measures in experiment and simulation. Phys. Rev. B 2006, 73, 054104.
(25) Stukowski, A. Structure identification methods for atomistic simulations of crystalline materials. Modell. Simul. Mater. Sci. Eng. 2012, 20, 045021.
(26) Long, A. W.; Ferguson, A. L. Nonlinear machine learning of patchy colloid self-assembly pathways and mechanisms. J. Phys. Chem. B 2014, 118, 4228–4244.
(27) Long, A. W.; Zhang, J.; Granick, S.; Ferguson, A. L. Machine learning assembly landscapes from particle tracking data. Soft Matter 2015, 11, 8141–8153.
(28) Long, A. W.; Ferguson, A. L. Landmark diffusion maps (l-dmaps): Accelerated manifold learning out-of-sample extension. Applied and Computational Harmonic Analysis 2019, 47, 190–211.
(29) Ferguson, A. L.; Panagiotopoulos, A. Z.; Kevrekidis, I. G.; Debenedetti, P. G. Nonlinear dimensionality reduction in molecular simulation: The diffusion mapping approach. Chem. Phys. Lett. 2011, 509, 1–11.
(30) Ferguson, A. L. Machine learning and data science in soft materials engineering. J. Phys.: Condens. Matter 2017, 30, 043002.
(31) O’Leary, J.; et al. Deep learning for characterizing the self-assembly of three-dimensional colloidal systems. Soft Matter 2021, 17, 989–999.
(32) DeFever, R. S.; Targonski, C.; Hall, S. W.; Smith, M. C.; Sarupria, S. A generalized deep learning approach for local structure identification in molecular simulations. Chemical science 2019, 10, 7503–7515.
(33) Reinhart, W. F.; Panagiotopoulos, A. Z. Multi-atom pattern analysis for binary superlattices. Soft Matter 2017, 13, 6803–6809.
(34) Baldi, P. Autoencoders, unsupervised learning, and deep architectures. In Proceedings of ICML workshop on unsupervised and transfer learning, Guyon, I.; Dror, G.; Lemaire, V.; Taylor, G.; Silver, D., Eds.; PMLR: Bellevue, Washington, USA, 2012, pp 37–49.
(35) Wang, Y.; Yao, H.; Zhao, S. Auto-encoder based dimensionality reduction. Neurocomputing 2016, 184, 232–242.
(36) Milenković, T.; Pržulj, N. Uncovering biological network function via graphlet degree signatures. Cancer Inform. 2008, 6, CIN.5680.
(37) Pržulj, N.; Corneil, D. G.; Jurisica, I. Modeling interactome: scale-free or geometric? Bioinformatics 2004, 20, 3508–3515.
(38) Pržulj, N. Biological network comparison using graphlet degree distribution. Bioinformatics 2007, 23, e177–e183.
(39) Hočevar, T.; Demšar, J. A combinatorial approach to graphlet counting. Bioinformatics 2014, 30, 559–565.
(40) Gu, S.; Johnson, J.; Faisal, F. E.; Milenković, T. From homogeneous to heterogeneous network alignment via colored graphlets. Sci. Rep. 2018, 8, 12524.
(41) Rossi, R. A.; et al. Heterogeneous graphlets. ACM Trans. Knowl. Discov. Data 2021, 15, 1–43.
(42) Howard, M. P.; et al. Evaporation-induced assembly of colloidal crystals. J. Chem. Phys. 2018, 149, 094901.
(43) Kadulka, S.; Howard, M. P.; Truskett, T. M.; Ganasan, V. Prediction and optimization of ion transport characteristics in nanoparticle-based electrolytes using convolutional neural networks. J. Phys. Chem. B 2021, 125, 4838–4849.
(44) Spelling, M.; Glotzer, S. C. Machine learning for crystal identification and discovery. AIChE J. 2018, 64, 2198–2206.
(45) Jadrich, R. B.; Lindquist, B. A.; Truskett, T. M. Recent advances in accelerated discovery through machine learning and statistical inference. arXiv (Chemical Physics), June 16, 2017, 1706.05405. ver. 1.
(46) Sasirekha, K.; Baby, P. Agglomerative hierarchical clustering algorithm-a. Int. J. Sci. Res. Publications 2013, 83, 83.
(47) Jain, A. K.; Murty, M. N.; Flynn, P. J. Data clustering: a review. ACM computing surveys (CSUR) 1999, 31, 264–323.
(48) O’Leary, J. Characterizing colloidal self-assembly system states. https://github.com/toearly/colloid_cha1s (accessed 2021-10-01).
(49) Casey, M. T.; et al. Driving diffusionless transformations in colloidal crystals using DNA handshaking. Nat. Commun. 2012, 3, 1209.
(50) Pretti, E.; et al. Assembly of three-dimensional binary superlattices from multi-flavored particles. Soft Matter 2018, 14, 6303–6312.
(51) Scarlett, R. T.; Ung, M. T.; Crocker, J. C.; Sinno, T. A mechanistic view of binary colloidal superlattice formation using dna-directed interactions. Soft Matter 2011, 7, 1912–1925.
(52) Macfarlane, R. J.; et al. Nanoparticle Superlattice Engineering with DNA. Science 2011, 334, 204–208.
(53) Song, M.; Ding, Y.; Zerze, H.; Snyder, M. A.; Mittal, J. Binary superlattice design by controlling dna-mediated interactions. Langmuir 2018, 34, 991–998.
(54) Plimpton, S. Fast parallel algorithms for short-range molecular dynamics. J. Comput. Phys. 1995, 117, 1–19.
(55) Stukowski, A. Visualization and analysis of atomistic simulation data with ovito—the open visualization tool. Modell. Simul. Mater. Sci. Eng. 2010, 18, 015012.
(56) Bommilneni, P. K.; Klement, M.; Engel, M. Spontaneous crystallization in systems of binary hard sphere colloids. Phys. Rev. Lett. 2020, 124, 218003.
(57) Eldridge, M.; Madden, P.; Frenkel, D. Entropy-driven formation of a superlattice in a hard-sphere binary mixture. Nature 1993, 365, 35–37.
(58) Tkachenko, A. V. Generic phase diagram of binary superlattices. Proc. Natl. Acad. Sci. U. S. A. 2016, 113, 10269.
(59) Lee, G. H.; Kim, J. B.; Choi, T. M.; Lee, J. M.; Kim, S.-H. Structural coloration with nonclose-packed array of bidisperse colloidal particles. Small 2019, 15, 1804548.
(60) Auer, S.; Frenkel, D. Suppression of crystal nucleation in polydisperse colloids due to increase of the surface free energy. Nature 2001, 413, 711–713.
(61) Debenedetti, P. G. Metastable liquids; Princeton University Press, 2021.
(62) Pruppacher, H. R.; Klett, J. D. *Microphysics of Clouds and Precipitation: Reprinted 1980*; Springer Science & Business Media, 2012.

(63) Vekilov, P. G. The two-step mechanism of nucleation of crystals in solution. *Nanoscale* **2010**, *2*, 2346–2357.

(64) De Yoreo, J. Crystal nucleation: More than one pathway. *Nat. Mater.* **2013**, *12*, 284–285.

(65) Myerson, A. S.; Trout, B. L. Nucleation from solution. *Science* **2013**, *341*, 855–856.

(66) Pan, W.; Kolomeisky, A. B.; Vekilov, P. G. Nucleation of ordered solid phases of proteins via a disordered high-density state: Phenomenological approach. *J. Chem. Phys.* **2005**, *122*, 174905.

(67) Dai, W.; Kumar, S. K.; Starr, F. W. Universal two-step crystallization of DNA-functionalized nanoparticles. *Soft Matter* **2010**, *6*, 6130.

(68) Wang, Y.; et al. Crystallization of DNA-coated colloids. *Nat. Commun.* **2015**, *6*, 7253.

(69) Debenedetti, P. G.; Truskett, T. M.; Lewis, C. P.; Stillinger, F. H. Theory of supercooled liquids and glasses: Energy landscape and statistical geometry perspectives. *Advances in Chemical Engineering* **2001**, *28*, 21–79.

(70) Jiang, H.; Debenedetti, P. G.; Panagiotopoulos, A. Z. Nucleation in aqueous NaCl solutions shifts from 1-step to 2-step mechanism on crossing the spinodal. *J. Chem. Phys.* **2019**, *150*, 124502.

(71) Trudu, F.; Donadio, D.; Parrinello, M. Freezing of a Lennard-Jones fluid: From nucleation to spinodal regime. *Phys. Rev. Lett.* **2006**, *97*, 1–4.

(72) Macfarlane, R. J.; et al. Assembly and organization processes in DNA-directed colloidal crystallization. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 10493–10498.