

Two-step nucleation in a binary mixture of Patchy Particles

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Nucleation in systems with a metastable liquid-gas critical point is the prototypical example of a two-step nucleation process, in which the appearance of the critical nucleus is preceded by the formation of a liquid-like density fluctuation. So far, the majority of studies on colloidal and protein crystallization have focused on one-component systems, and we are lacking a clear description of two-step nucleation processes in multicomponent systems, where critical fluctuations involve coupled density and concentrations inhomogeneities. Here, we examine the nucleation process of a binary mixture of patchy particles designed to nucleate into a diamond lattice. By combining Gibbs-ensemble simulations and direct nucleation simulations over a wide range of thermodynamic conditions, we are able to pin down the role of the liquid-gas metastable phase diagram on the nucleation process. In particular, we show that the strongest enhancement of crystallization occurs at an azeotropic point with the same stoichiometric composition of the crystal.

INTRODUCTION

The ability to guide dilute solutions of properly designed colloidal particles to spontaneously turn into specific ordered structures is crucial for a large-scale application of the self-assembly paradigm [1, 2]. One relevant example of this strategy is provided by the DNA-origami nanotechnology, nowadays a mature methodology which allows the design and production of nanometric particles with controllable shape and interactions [3]. It has been demonstrated that these particles, once properly designed, are able to self-assemble into ordered crystalline lattices even starting from quite dilute conditions [4, 5], suggesting the exploitation of complex nucleation pathways that are still not fully understood.

One such pathway is the so-called *two-step nucleation pathway*, in which a metastable gas-liquid transition can spontaneously generate local regions with high particle concentration, thus greatly enhancing the nucleation rate [6–17]. In the last decade, this process has been observed in simple systems that are either one-component systems, or ionic compounds: examples include hard particle fluids [18], colloids [11, 19, 20], salt solutions [21], calcium carbonate [22, 23]. Importantly, two-step processes play an important role in the crystallization of globular proteins [19, 24–27]. For colloidal particles, globular proteins and non globular proteins, the short-range nature of the inter-particle interaction generates a metastable gas-liquid coexistence which provides a mechanism for locally enhancing the density and triggering crystal formation. Spinodal decomposition and/or liquid-phase nucleation are equally active mechanisms. In addition, ten Wolde and Frenkel [19] have called attention on the possibility to exploit critical fluctuations as a mechanism for locally increasing density. In all these cases, a two-step nucleation process takes place, the first

step being the local increase of density, and the second the formation of a crystal nucleus in one or several of such enhanced density regions.

The crystallization of specific crystal structures is not devoid of problems [28, 29]. Kinetic pathways can drive the assembly towards amorphous aggregates, or crystals different from the desired one. To address this last case, several strategies have been suggested, examples include: optimization of preparation protocols [30–32], the accurate design of the interparticle potential [29, 33–40], or geometrical approaches where the inter-particle interactions are made to match the geometric features of the target structure [41–48]. Rather than increasing the complexity of the inter-particle interactions, another successful strategy is to increase the number of building blocks. When the building blocks are modeled as patchy colloids, i.e. particles with specific directional interactions, the process of designing the bonding rules to assemble one specific crystal (and selectively avoid the formation of any other competing structure) can be turned into a set of boolean satisfiability (SAT) equations, which can be solved using modern SAT solver algorithms. This strategy, named *SAT-assembly*, was shown to lead to the successful assembly of several complex target structures, including the coveted colloidal diamond crystal [49–51].

Compared to the one-component case, crystal formation and two-step pathways in multi-component systems have received much less attention. In these systems, composition fluctuations couple with density fluctuations and it is not a priori clear if the stoichiometric properties of the spontaneously generated liquid phase are consistent with the corresponding properties of the selected crystal. Even when the components are fully miscible in the solid phase (such as Pd and Ag), it was shown that the competition between demixing and crystallization has a big impact on the growth process, causing large variations in the ra-

dial composition of the nuclei [52]. Multi-component systems also display thermodynamic behaviour that is not found in one-component systems, such as lines of binary critical points, azeotropic points, re-entrant condensation, etc. Experimental studies have already shown that phase separation in multi-component systems does not preserve the original ratio between the different species and that the phase behaviour strongly depends on the components ratio [53–55], but it is yet unclear whether some of the peculiar multi-component mixture features can affect crystal nucleation.

In this manuscript we use extensive computer simulations of a binary mixture of patchy particles that self-assemble in a diamond crystal with fixed stoichiometric ratio, with the goal of relating the phase behaviour of the mixture with its crystallization pathway. The manuscript is organized as follows. In the Methods section we introduce the N2c8 binary mixture and briefly summarize its phase behaviour. Numerically, we obtain the phase diagram with Gibbs ensemble simulations, while the crystallization behaviour is observed via direct Monte Carlo simulations. We make use of biased moves to accelerate diffusional times and observe crystallization for state points where the free-energy barrier can be overcome by thermal fluctuations during our simulation times. The Results section will superimpose the metastable phase diagram with histograms of the nucleation rate, confirming the two-step nucleation pathway and allowing us to study the relation between demixing and crystallization. In the final section we put our results in the wider context of Empty Liquids, and argue that while the two-step nucleation pathway is an effective strategy to overcome diffusional barriers, azeotropic points are the key to lower compositional barriers.

METHODS

We choose patchy particles as our model system: they describe systems with a hard-core isotropic repulsion and attractive directional interactions. Experimentally, the anisotropic interactions can be obtained either via the shape [56] or via chemical patterning of the surface [57–61], for example by attaching single strands of DNA to well-defined positions on their surface [61–66]. We represent them computationally via the Kern-Frenkel [67, 68] potential, in which hard-core spherical particles of diameter σ interact with a square well potential V_{SW} of depth ϵ and width δ , modulated by a term f depending on the relative orientation of the particles: in particular, two patchy particles can establish a *bond* if their centers are at distance between σ and $\sigma + \delta$, and if the line connecting their centres intersects the volume of both patches involved in the bond. In the following we measure energy in units of the square-well depth (ϵ) and distances in units of the patchy particle diameter (σ). Mathemati-

cally, the interaction between particle i and j is described by

$$V(\mathbf{r}_{ij}, \hat{\mathbf{r}}_{\alpha,i}, \hat{\mathbf{r}}_{\beta,j}) = V_{SW}(r_{ij})f(\mathbf{r}_{ij}, \hat{\mathbf{r}}_{\alpha,i}, \hat{\mathbf{r}}_{\beta,j}) \quad (1)$$

where $\hat{\mathbf{r}}_{\alpha,i}$ ($\hat{\mathbf{r}}_{\beta,j}$) indicates the position of patch α (β) of particle i (j), and

$$f(\mathbf{r}_{ij}, \hat{\mathbf{r}}_{\alpha,i}, \hat{\mathbf{r}}_{\beta,j}) = \begin{cases} \Upsilon_{(\alpha,i)(\beta,j)} & \text{if } \hat{\mathbf{r}}_{ij} \cdot \hat{\mathbf{r}}_{\alpha,i} > \cos(\theta_{max}) \\ & \hat{\mathbf{r}}_{ji} \cdot \hat{\mathbf{r}}_{\beta,j} > \cos(\theta_{max}) \\ 0 & \text{otherwise} \end{cases} \quad (2)$$

$\Upsilon_{(\alpha,i)(\beta,j)}$ is the (color) interaction matrix element and is equal to 1 if patch α on particle i interacts with patch β on particle j , and 0 otherwise. Υ is a matrix which encodes the interactions between both identical and distinct species. Finding the interaction matrix that will guide the system to self-assemble into a desired structure can be cast into a *inverse self-assembly* problem, whose solution can be found with the *SAT-assembly* algorithm [49, 50].

We run two types of simulations: Gibbs-ensemble simulations to determine the liquid-gas coexistence line, and NVT Monte Carlo simulations in an extensive range of densities (ρ) and relative concentrations (x , i.e. the fraction of species 2).

We fix the parameters of the Kern-Frenkel potential to $\cos(\theta_{max}) = 0.98$ and $\delta = 0.2$, for which nucleation is readily accessible within simulation times. Moreover, direct simulations take advantage of AVB biased moves [69] to accelerate the dynamics of bond-formation and bond-breaking. Both methods have been described in more detail in Ref. [70]. We confirmed with independent simulations that nucleation events are observed also in unbiased simulations, albeit requiring longer simulation times.

Our goal is to study the nucleation process in multi-component mixtures such as the binary mixture described by the N2c8 interaction matrix (so called because it has 2 species and 8 colored patches) which was originally introduced as a SAT-assembly solution to the problem of assembling the cubic diamond crystal, while at the same time avoiding the hexagonal diamond crystal [51]. The ability to suppress a competing polytype, thus avoiding the formation of stacking faults, makes this mixture a candidate for the experimental realization of photonic crystals. The N2c8 solution is graphically depicted in Fig. 1: two patchy particles species differing in the patch types (colors); patches with matchable colors that can bind with each others are reported in the bottom part of the table. This mixture crystallizes in the diamond-cubic phase, and notably cannot form the hexagonal diamond.

We highlight that the results presented in the following section for the N2c8 solution are general; we focus on this binary mixture since it is a meaningful example and since

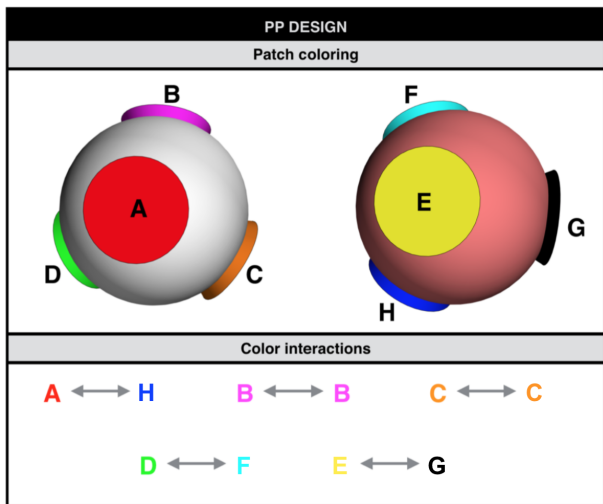


FIG. 1. 3D representation of the azeotropic N2c8 solution SAT-designed to self-assembly a cubic diamond crystal lattice. It consists of two patchy particles species (N2), the grey and the red one differing in the patch types. The patch types/different colors are eight (c8) referred to by the letters A through H. Patches that can bind with each other are reported in the bottom part of the table.

we already know its phase behaviour. Indeed the phase diagram of the mixture was studied within Wertheim thermodynamic perturbation theory in Ref. [70], which results we summarize in Fig. 2. The N2c8 mixture has a reentrant binary critical point line, which goes to zero temperature and pressure as the pure components are approached. This behaviour is immediately apparent from the color design of Fig. 1, where it can be seen that each species can form only two intra-species bonds, so when the system is made of only one component it can only form linear chains, and thus does not have a critical point at any finite temperature [71]. The non-ideal nature of the mixture manifests itself in a line of azeotropic points at $x = 1/2$. Along such line the system always demixes at the same composition, i.e. the liquid phase will have an equimolar composition of both species. As we will see, this is particularly relevant for the nucleation process, as the crystal also has an equimolar composition. In the results section we will look at the interplay between phase separation and nucleation.

To identify crystal particles we use local bond-order analysis [72]. A $(2l + 1)$ dimensional complex vector (\mathbf{q}_l) is defined for each particle i as $q_{lm}(i) = \frac{1}{N_b(i)} \sum_{j=1}^{N_b(i)} Y_{lm}(\hat{\mathbf{r}}_{ij})$, where we set $l = 12$, and m is an integer that runs from $m = -l$ to $m = l$. The functions Y_{lm} are the spherical harmonics and $\hat{\mathbf{r}}_{ij}$ is the normalised vector from particle i to particle j . The sum goes over the first $N_b(i) = 16$ neighbours of particle i . We then introduce a spatial coarse-graining step $Q_{lm}(i) = \frac{1}{N_b(i)} \sum_{k=0}^{N_b(i)} q_{lm}(k)$ [73]. The scalar product

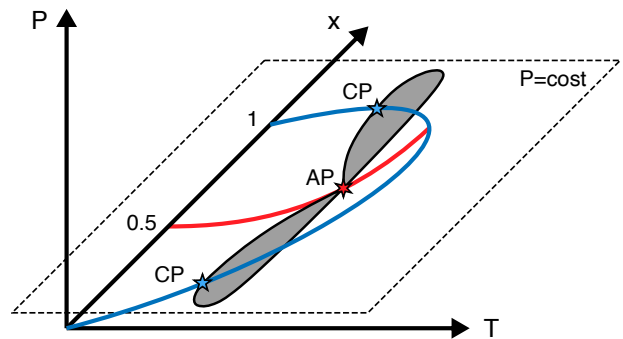


FIG. 2. Schematic PTx phase diagram of the N2c8 solution. The blue line is the binary critical point line which goes to $(P, T) \rightarrow 0$ for $x \rightarrow (0, 1)$. The red line, in the plane $x = 0.5$, represents the line of azeotropic points. The shaded area represents the coexistence region on a isobaric surface.

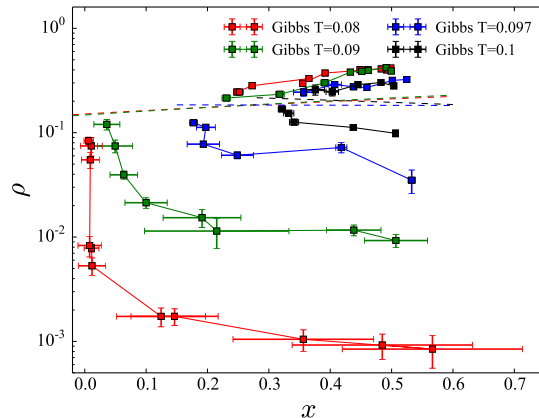


FIG. 3. SAT-designed N2c8 binary mixture density-concentration phase diagrams for different temperatures T expressed in unit of k_B/ϵ ; the coexistence region shrinks by increasing temperature. Each binodal curve is obtained by Monte Carlo simulations with AVB moves in the Gibbs ensemble [69]. In particular for temperatures $T = 0.08$ and $T = 0.09$, 500 particles were simulated while for temperatures $T = 0.097$ and $T = 0.1$, 1000 particles were considered. Dashed lines are rectilinear diameter lines indicating where critical points are located.

between $Q_{12,m}$ of two particles is defined as $\mathbf{Q}_{12}(i) \cdot \mathbf{Q}_{12}(j) = \sum_m Q_{12,m}(i) Q_{12,m}(j)$. If the scalar product $(\mathbf{Q}_{12}(i)/|\mathbf{Q}_{12}(i)|) \cdot (\mathbf{Q}_{12}(j)/|\mathbf{Q}_{12}(j)|)$ between two neighbours exceeds 0.75 then the two particles are deemed *connected*. We then identify particle i as crystalline if it is connected with at least 12 neighbours.

RESULTS

We run Gibbs ensemble simulations to determine the coexistence region at different temperatures. These are plotted in Fig. 3 for temperatures $T = 0.08$ (red),

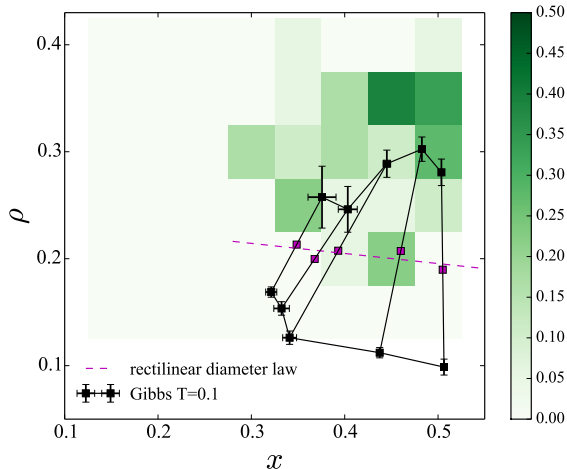


FIG. 4. Nucleation plot for temperature $T = 0.1$ (in unit of k_B/ϵ). The binodal black curve obtained via Monte Carlo simulations in the Gibbs ensemble is superimposed to a green colormap reporting, for each considered state point, the ratio between the number of trajectories that nucleate over the total number of trajectories analysed. We considered as successfully assembled the runs exhibiting a fraction of particles in the cubic diamond phase greater or equal to 0.2. Examined state points belong to the regular grid with $\rho \in [0.15, 0.4]$ and $x \in [0.15, 0.5]$ where $\Delta\rho = 0.05$ and $\Delta x = 0.05$. For each state point (centred in each rectangular of the colormap) several Monte Carlo simulations were run with 500 particles and AVB dynamics in the NVT ensemble. In particular we made 10 runs for each state point whose $x \leq 0.25$ and we increased them to 18 for state points with $x \geq 0.3$. Purple squares represent averages of each pair of coexisting points and, according to the rectilinear diameter law, the best straight line passing through them (the dashed line) provides an indication of where the critical point is.

$T = 0.09$ (green), $T = 0.097$ (blue), and $T = 0.1$ (black). The binodal line at each temperature is splitted in a liquid branch, at high densities, and a gas branch, at low densities. The two branches meet at the binary critical point, which location can not be estimated with Gibbs ensemble simulations [74]. The phase diagram is plotted only for $x < 0.5$, but it is symmetric with respect to the azeotropic line $x = 0.5$. The critical point is located at the intersection of the binodal line and the rectilinear diameter line (i.e. the line connecting the midpoint of the different tie-lines), which is represented for each temperature as a dashed line in Fig. 3. For temperatures $T = 0.08$ and $T = 0.09$ we run single trajectories, while for temperatures $T = 0.097$ and $T = 0.1$ simulations are averaged over at least 8 independent simulations; the statistical noise is due to the difficulty in equilibrating the mixtures at low temperatures.

In Fig. 4 we examine the nucleation behaviour by running simulation runs over a grid of state points at $T = 0.1$: $\rho \in [0.15, 0.4]$ with $\Delta\rho = 0.05$, and $x \in [0.15, 0.5]$

with $\Delta x = 0.05$. We run up to 10 (or 18 for state points where nucleation is more probable) independent simulation runs for more than $3 \cdot 10^8$ steps each, and measure the fraction of trajectories that have nucleated, which is a quantity proportional to the nucleation rate. In the figure we define a nucleation event as a run in which at least 20% of the particles are found in a crystalline state, according to the Q_{12} criteria described in the Methods section. In Fig. 4 this fraction is proportional to the color intensity of a tile centered around the state point, and the grid is superimposed on the $T = 0.1$ phase diagram. The figure shows that nucleation occurs in correspondence of the liquid-gas coexistence region, with the probability of nucleation being approximately the highest along the liquid binodal. We note in fact that no nucleation events are observed at low concentrations, from $x = 0.15$ to $x = 0.25$, i.e. outside the phase coexistence region. Visual inspection of the nucleation trajectories confirm that the nucleation event is preceded by the formation of a dense liquid phase, mostly via spinodal decomposition (i.e. liquid drops do not nucleate but instead a coarsening liquid networks appears at the start of the simulations).

To confirm the results of Fig. 4, we run nucleation trajectories in a density-concentration grid at a lower temperature, $T = 0.097$, which makes the coexistence region wider. The results are presented in Fig. 5(a). Compared to the case at $T = 0.1$ now nucleation events occur over a much wider region of the phase diagram, following the widening of the coexistence region.

These results confirm the observations that were made for one-component systems, i.e. that density fluctuations promote nucleation [11, 15, 19, 75]. But compared to one-component systems, in a multi-component system there are both density and concentration fluctuations. Since the crystal has a fixed stoichiometric composition, the formation of a liquid phase at concentrations different from equimolarity is expected to slow down and limit the growth of the crystal. We show this effect in the four panels of Fig. 5, where the colored grid plots the fraction of independent trajectories whose crystalline particles include at least the 20% (a), 50% (b), 70% (c), and 90% (d) of the whole system. Fig. 5 shows that, while small crystals can form along coexistence regions, the formation of larger crystals is progressively shifted towards the $x = 0.5$ line, i.e. towards the azeotropic point. At the azeotropic point, in fact, the liquid phase forms with the same stoichiometric composition of the crystal.

As demonstrated originally in Ref. [19], critical fluctuations are able to trigger crystal nucleation. We find here that in a binary mixture, it is the azeotropic point which more strongly promotes crystallization. The azeotropic point and the critical point coincide where the two corresponding thermodynamic lines meet (see Fig. 2), which corresponds to the maximum critical temperature at $x = 0.5$. In Fig. 6 we study the nucleation behaviour in the density-temperature plane at $x = 0.5$. The nu-

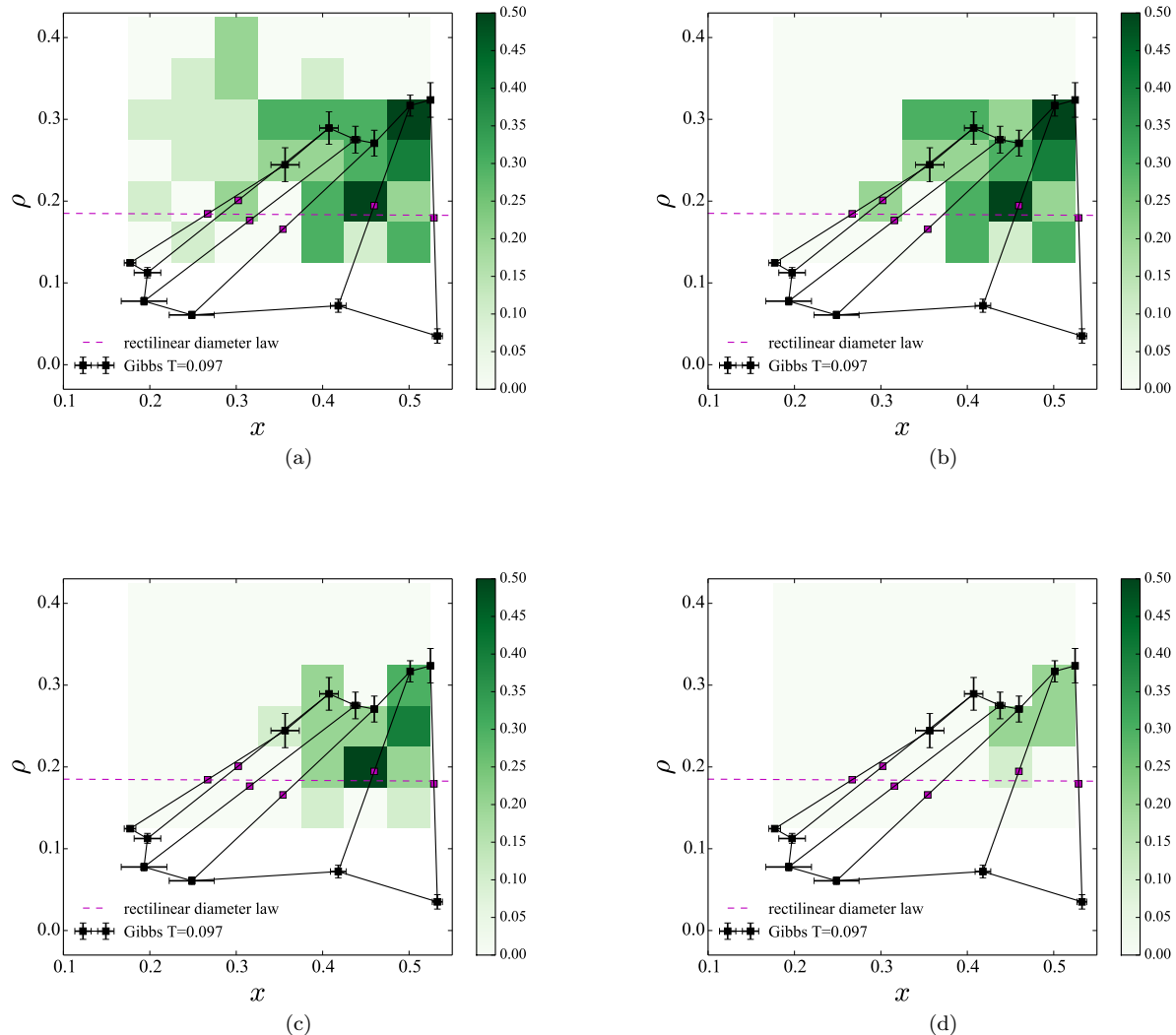


FIG. 5. Nucleation plots for temperature $T = 0.097$ (in unit of k_B/ϵ). The binodal black curve obtained via Monte Carlo simulations in the Gibbs ensemble is superimposed to a green colormap reporting, for each considered state point, the ratio between the number of trajectories that nucleate over the total number of trajectories analysed. We considered as successfully assembled the runs exhibiting a fraction of particles in the cubic diamond phase (t) greater or equal to 0.2 in a), 0.5 in b), 0.7 in c) and 0.9 in d). Examined state points belong to the regular grid with $\rho \in [0.15, 0.4]$ and $x \in [0.2, 0.5]$ where $\Delta\rho = 0.05$ and $\Delta x = 0.05$. For each state point (centred in each rectangular of the colormap) were run 10 Monte Carlo simulations with 1000 particles and AVB dynamics in the NVT ensemble. Purple squares represent averages of each pair of coexisting points and, according to the rectilinear diameter law, the best straight line passing through them (the dashed line) provides an indication of where the critical point is. Nucleation occurs for almost each considered state point, but extended cubic diamond lattice ($t \geq 0.7$) can appear only close to the liquid branch and to the azeotropic condition.

cleation of the diamond cubic occurs again in correspondence of the phase separation, confirming that nucleation is aided by the formation of dense liquid regions during the phase-separation process. We observe that nucleation not only occurs in correspondence of the critical point, but, interestingly, we observe nucleation events at supercritical conditions. The system thus shows two-step nucleation aided both by spinodal decomposition at sub-critical temperatures, and by criti-

cal fluctuations at super-critical conditions, just as predicted by Ref. [19]. While the prediction was made for isotropic one-component systems (globular proteins), at the azeotropic conditions of Fig. 6 the system is indeed thermodynamically equivalent to a one component system [70].

In Fig. 7 we show the evolution of a typical nucleation trajectory at the azeotropic point at $T = 0.097$ and $\rho = 0.3$. Panel (a) shows the evolution of the en-

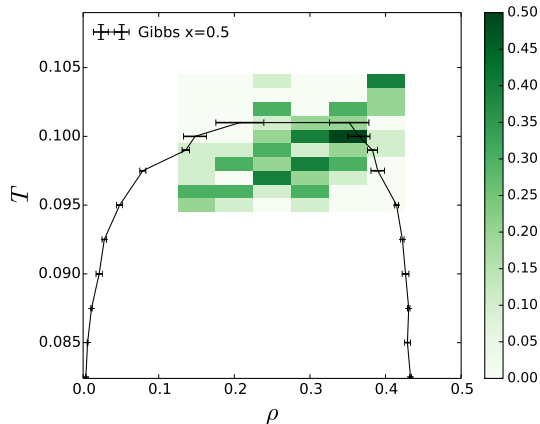


FIG. 6. Equimolar nucleation plot showing that nucleation occurs in two steps where there is a previous locally increasing density phenomenon. The binodal black curve obtained via Monte Carlo simulations in the Gibbs ensemble is superimposed to a green colormap reporting, for each considered state point, the ratio between the number of trajectories that nucleate over the total number of trajectories analysed. We considered as successfully assembled the runs exhibiting a fraction of particles in the cubic diamond phase greater or equal to 0.2. Examined state points belong to the regular grid with $\rho \in [0.15, 0.4]$ and $T \in [0.095, 0.105]$ where $\Delta\rho = 0.05$ and $\Delta T = 0.001$. For each state point (centred in each rectangular of the colormap) 10 Monte Carlo simulations were run with 500 particles and AVB dynamics in the NVT ensemble.

ergy, displaying a two-step drop: initially the system is prepared in a metastable gas phase, which then forms a percolating liquid phase; in the second step (from $1.9 \cdot 10^8$ MC sweeps) the nucleation occurs within the liquid drop and a diamond cubic crystal replaces all the liquid phase, finally coexisting with a low-density gas. Panel (b) shows a snapshot at the end of the first stage, where a liquid-gas coexistence is established in the box. Panel (c) shows a snapshot at the final stage of the trajectory, visually confirming the formation of a large crystal of the diamond cubic phase. The absence of the hexagonal diamond phase and stacking fault defects is a property embedded into the color interactions of the N2c8 mixture, which was designed with this property via the SAT-assembly framework [51].

DISCUSSION AND CONCLUSIONS

Recent experimental advances hold the promise to fully unlock the potential of systems with directional and specific interactions. In particular DNA nanotechnology already allows the fabrication of patchy particles in the form of wireframe DNA origami [4, 5, 66, 77] and gold nanoparticles with selective patterning [78]. DNA strands naturally encode sequence complementary and

thus allow the assembly of structures with the desired interactions: different interacting patches correspond to complementary single DNA strands as well as the self-interacting ones correspond to palindromic DNA strands. This class of systems goes under the name of *Empty Liquids* [79], which have unique properties compared to simple (hard-sphere like) systems, such as the existence of low-density stable liquid phases [80], the ability to crystallize in open crystals [81], and the potential to model the phase behaviour of biological molecules [82–85].

To obtain a complex target structure (such as a photonic crystal) requires either a complex interaction potential carefully optimized to have the target as a free-energy minima, or a mixture of simpler building blocks that make the target structure accessible by reducing its overall symmetry (crystal sites are occupied by different species). Recent advances in DNA-nanotechnology, allowing the design of building blocks with arbitrary interaction rules, are opening the door to this last approach.

Several strategies have been proposed to design the interaction potential in multi-component system. One such strategy is the SAT-assembly framework, which translates the design problem in a Boolean satisfiability problem [49, 50]. The ability to design interaction matrices to self-assemble arbitrary structures is a necessary but not sufficient condition for observing the nucleation of such structures under experimental conditions. These often involve preparing the system in very dilute conditions, from which the classical nucleation rate is severely suppressed by both diffusional and compositional barriers.

Regarding the diffusional barrier, probably the most promising strategy to overcome it is *two-step* nucleation, where the crystal nucleation is aided by the formation of dense liquid drops. This nucleation pathway has been the subject of intense research, especially in the context of one-component systems.

So far not much work has been devoted to the second barrier, i.e. the compositional barrier, which controls the rate at which a particle of the correct species enters in contact with the incipient nucleus. In this work we have presented a first study in this direction. We have examined a two-step nucleation process in a binary mixture of patchy particles designed to assemble into cubic diamond. In particular we have focused on the role of the metastable liquid-gas phase diagram on the nucleation process. Comparing Gibbs ensemble simulations with direct nucleation runs, we have established that nucleating state points coincide with the locus of metastability of the mixture, confirming the relevant role played by the thermodynamic instabilities, generating local environments with a density enhanced in comparison to the average value. We have shown that composition effects in multi-component systems play a big role in the growth of the crystal, and that crystal growth is enhanced at state points where the emerging liquid phase has a composition close to the stoichiometric composition of the crystal.

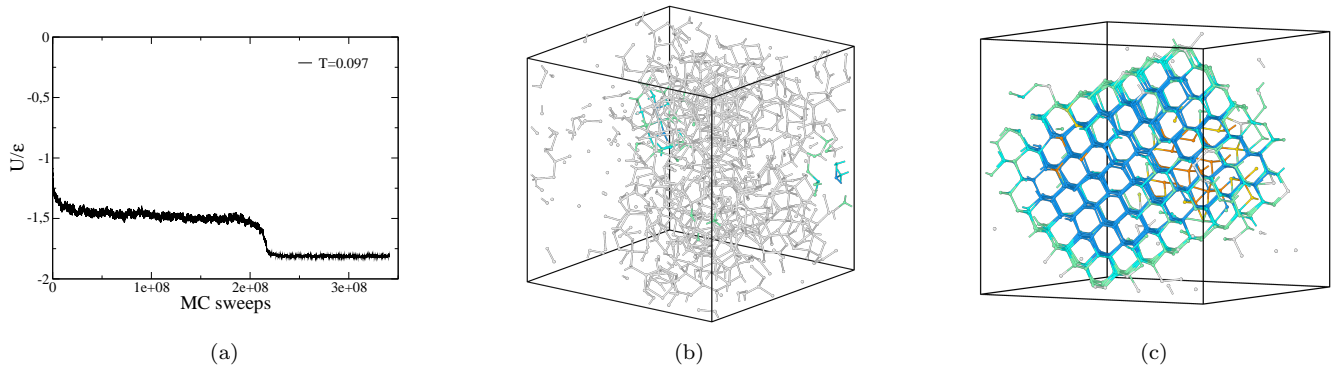


FIG. 7. Overview of Monte Carlo simulation with AVB moves in the NVT ensemble run at $T = 0.097$, $x = 0.5$ and $\rho = 0.3$ where T is in unit of k_B/ϵ and ρ is in unit of $1/\sigma^3$. a) Potential energy per particle as a function of MC sweeps: the energy drop signals nucleation event. b) Snapshot of a metastable configuration before the energy drop showing that we deal with a two step nucleation since crystalline nucleus arise in the denser liquid phase. c) Snapshot of the last configuration showing that the N2c8 binary mixture eventually self-assembles into a cubic diamond crystal. Neither patches nor the two patchy particles species are shown while blue/green colors indicate particles belonging to the cubic diamond phase. Snapshots and crystal identification are obtained with the Ovito software [76].

In the studied system, this corresponds to an azeotropic point at equimolar concentration, $x = 0.5$. We find evidence of critical-like enhanced nucleation along the binary critical line when this intersects the azeotropic line.

To unlock the promises of nanotechnology to self-assemble new materials with desired mechanical, optical and thermal properties, a big role will be played by the search of general principles behind the crystallization of multi-component systems. We believe that two-step nucleation, augmented with azeotropic conditions, will be one of such guiding principles.

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- [1] S. Whitelam and R. L. Jack, The statistical mechanics of dynamic pathways to self-assembly, *Annual review of physical chemistry* **66**, 143 (2015).
- [2] S. K. Kumar, G. Kumaraswamy, B. L. Prasad, R. Bandyopadhyaya, S. Granick, O. Gang, V. N. Manoharan, D. Frenkel, and N. A. Kotov, Nanoparticle assembly: a perspective and some unanswered questions, *Current Science*, 1635 (2017).
- [3] N. C. Seeman and H. F. Sleiman, Dna nanotechnology, *Nature Reviews Materials* **3**, 1 (2017).
- [4] W. Liu, M. Tagawa, H. L. Xin, T. Wang, H. Emamy, H. Li, K. G. Yager, F. W. Starr, A. V. Tkachenko, and O. Gang, Diamond family of nanoparticle superlattices, *Science* **351**, 582 (2016).
- [5] T. Zhang, C. Hartl, K. Frank, A. Heuer-Jungemann, S. Fischer, P. C. Nickels, B. Nickel, and T. Liedl, 3d dna origami crystals, *Advanced Materials* **30**, 1800273 (2018).
- [6] D. Kashchiev, P. G. Vekilov, and A. B. Kolomeisky, Kinetics of two-step nucleation of crystals, *The Journal of chemical physics* **122**, 244706 (2005).
- [7] D. Erdemir, A. Y. Lee, and A. S. Myerson, Nucleation of crystals from solution: classical and two-step models, *Accounts of chemical research* **42**, 621 (2009).
- [8] P. G. Vekilov, The two-step mechanism of nucleation of crystals in solution, *Nanoscale* **2**, 2346 (2010).
- [9] G. I. Tóth, T. Pusztai, G. Tegze, G. Tóth, and L. Gránásy, Amorphous nucleation precursor in highly nonequilibrium fluids, *Phys. Rev. Lett.* **107**, 175702 (2011).
- [10] R. P. Sear, The non-classical nucleation of crystals: microscopic mechanisms and applications to molecular crystals, ice and calcium carbonate, *International Materials Reviews* **57**, 328 (2012).
- [11] T. K. Haxton, L. O. Hedges, and S. Whitelam, Crystallization and arrest mechanisms of model colloids, *Soft matter* **11**, 9307 (2015).
- [12] J. Russo and H. Tanaka, Nonclassical pathways of crystallization in colloidal systems, *MRS Bulletin* **41**, 369 (2016).
- [13] G. C. Sosso, J. Chen, S. J. Cox, M. Fitzner, P. Pedevilla, A. Zen, and A. Michaelides, Crystal nucleation in liquids: Open questions and future challenges in molecular dynamics simulations, *Chemical reviews* **116**, 7078 (2016).

- [14] J. F. Lutsko, How crystals form: A theory of nucleation pathways, *Sci. Adv.* **5**, eaav7399 (2019).
- [15] D. James, S. Bearsto, C. Hartt, O. Zavalov, I. Saika-Voivod, R. K. Bowles, and P. H. Poole, Phase transitions in fluctuations and their role in two-step nucleation, *J. Chem. Phys.* **150**, 074501 (2019).
- [16] C. Desgranges and J. Delhommelle, Can ordered precursors promote the nucleation of solid solutions?, *Phys. Rev. Lett.* **123**, 195701 (2019).
- [17] D. Kashchiev, Classical nucleation theory approach to two-step nucleation of crystals, *Journal of Crystal Growth* **530**, 125300 (2020).
- [18] S. Lee, E. G. Teich, M. Engel, and S. C. Glotzer, Entropic colloidal crystallization pathways via fluid–fluid transitions and multidimensional prenucleation motifs, *Proceedings of the National Academy of Sciences* **116**, 14843 (2019).
- [19] P. R. ten Wolde and D. Frenkel, Enhancement of protein crystal nucleation by critical density fluctuations, *Science* **277**, 1975 (1997).
- [20] P. Tan, N. Xu, and L. Xu, Visualizing kinetic pathways of homogeneous nucleation in colloidal crystallization, *Nature Physics* **10**, 73 (2014).
- [21] H. Jiang, P. G. Debenedetti, and A. Z. Panagiotopoulos, Nucleation in aqueous nacl solutions shifts from 1-step to 2-step mechanism on crossing the spinodal, *The Journal of chemical physics* **150**, 124502 (2019).
- [22] D. Gebauer, A. Völkel, and H. Cölfen, Stable prenucleation calcium carbonate clusters, *Science* **322**, 1819 (2008).
- [23] E. M. Pouget, P. H. H. Bomans, J. A. C. M. Goos, P. M. Frederik, G. de With, and N. A. J. M. Sommerdijk, The initial stages of template-controlled caco3 formation revealed by cryo-tem, *Science* **323**, 1455 (2009).
- [24] D. Fusco and P. Charbonneau, Crystallization of asymmetric patchy models for globular proteins in solution, *Physical Review E* **88**, 012721 (2013).
- [25] S. James, M. K. Quinn, and J. J. McManus, The self assembly of proteins; probing patchy protein interactions, *Physical Chemistry Chemical Physics* **17**, 5413 (2015).
- [26] J. J. McManus, P. Charbonneau, E. Zaccarelli, and N. Asherie, The physics of protein self-assembly, *Current opinion in colloid & interface science* **22**, 73 (2016).
- [27] D. Fusco and P. Charbonneau, Soft matter perspective on protein crystal assembly, *Colloids and Surfaces B: Biointerfaces* **137**, 22 (2016).
- [28] A.-Y. Jee, K. Lou, H.-S. Jang, K. H. Nagamanasa, and S. Granick, Nanoparticle puzzles and research opportunities that go beyond state of the art, *Faraday discussions* **186**, 11 (2016).
- [29] M. Dijkstra and E. Luijten, From predictive modelling to machine learning and reverse engineering of colloidal self-assembly, *Nature Materials* **20**, 762 (2021).
- [30] S. Whitelam and I. Tamblyn, Learning to grow: Control of material self-assembly using evolutionary reinforcement learning, *Physical Review E* **101**, 052604 (2020).
- [31] A. Bupathy, D. Frenkel, and S. Sastry, Temperature protocols to guide selective self-assembly of competing structures, *Proceedings of the National Academy of Sciences* **119**, e2119315119 (2022).
- [32] S. Whitelam and I. Tamblyn, Neuroevolutionary learning of particles and protocols for self-assembly, *Physical review letters* **127**, 018003 (2021).
- [33] M. C. Rechtsman, F. H. Stillinger, and S. Torquato, Optimized interactions for targeted self-assembly: application to a honeycomb lattice, *Physical review letters* **95**, 228301 (2005).
- [34] E. Marcotte, F. H. Stillinger, and S. Torquato, Optimized monotonic convex pair potentials stabilize low-coordinated crystals, *Soft Matter* **7**, 2332 (2011).
- [35] E. Marcotte, F. H. Stillinger, and S. Torquato, Communication: Designed diamond ground state via optimized isotropic monotonic pair potentials, *The Journal of Chemical Physics* **138**, 061101 (2013).
- [36] G. Zhang, F. Stillinger, and S. Torquato, Probing the limitations of isotropic pair potentials to produce ground-state structural extremes via inverse statistical mechanics, *Physical Review E* **88**, 042309 (2013).
- [37] M. Z. Miskin, G. Khaira, J. J. de Pablo, and H. M. Jaeger, Turning statistical physics models into materials design engines, *Proceedings of the National Academy of Sciences* **113**, 34 (2016).
- [38] B. A. Lindquist, R. B. Jadrich, and T. M. Truskett, Communication: Inverse design for self-assembly via on-the-fly optimization, *The Journal of Chemical Physics* **145**, 111101 (2016).
- [39] D. Chen, G. Zhang, and S. Torquato, Inverse design of colloidal crystals via optimized patchy interactions, *The Journal of Physical Chemistry B* **122**, 8462 (2018).
- [40] R. Kumar, G. M. Coli, M. Dijkstra, and S. Sastry, Inverse design of charged colloidal particle interactions for self assembly into specified crystal structures, *The Journal of chemical physics* **151**, 084109 (2019).
- [41] É. Ducrot, M. He, G.-R. Yi, and D. J. Pine, Colloidal alloys with preassembled clusters and spheres, *Nature materials* **16**, 652 (2017).
- [42] D. R. Nelson, Toward a tetravalent chemistry of colloids, *Nano Letters* **2**, 1125 (2002).
- [43] V. N. Manoharan, M. T. Elsesser, and D. J. Pine, Dense packing and symmetry in small clusters of microspheres, *Science* **301**, 483 (2003).
- [44] Z. Zhang, A. S. Keys, T. Chen, and S. C. Glotzer, Self-assembly of patchy particles into diamond structures through molecular mimicry, *Langmuir* **21**, 11547 (2005).
- [45] F. Romano, J. Russo, and H. Tanaka, Influence of patch-size variability on the crystallization of tetrahedral patchy particles, *Physical review letters* **113**, 138303 (2014).
- [46] J. D. Halverson and A. V. Tkachenko, Dna-programmed mesoscopic architecture, *Physical Review E* **87**, 062310 (2013).
- [47] F. Romano and F. Sciortino, Patterning symmetry in the rational design of colloidal crystals, *Nature communications* **3**, 975 (2012).
- [48] D. F. Tracey, E. G. Noya, and J. P. K. Doye, Programming patchy particles to form complex periodic structures, *The Journal of Chemical Physics* **151**, 224506 (2019).
- [49] F. Romano, J. Russo, L. Kroc, and P. Šulc, Designing patchy interactions to self-assemble arbitrary structures, *Physical Review Letters* **125**, 118003 (2020).
- [50] J. Russo, F. Romano, L. Kroc, F. Sciortino, L. Rovigatti, and P. Šulc, Sat-assembly: A new approach for designing self-assembling systems, *Journal of Physics: Condensed Matter* (2022).

- [51] L. Rovigatti, J. Russo, F. Romano, M. Matthies, L. Kroc, and P. Sulc, A simple solution to the problem of self-assembling cubic diamond crystals, *Nanoscale* **14**, 14268 (2022).
- [52] C. Desgranges and J. Delhommelle, Unraveling the coupling between demixing and crystallization in mixtures, *Journal of the American Chemical Society* **136**, 8145 (2014).
- [53] Y. Wang, A. Lomakin, J. J. McManus, O. Ogun, and G. B. Benedek, Phase behavior of mixtures of human lens proteins gamma d and beta b1, *Proceedings of the National Academy of Sciences* **107**, 13282 (2010).
- [54] Y. Wang, A. Lomakin, R. F. Latypov, and G. B. Benedek, Phase separation in solutions of monoclonal antibodies and the effect of human serum albumin, *Proceedings of the National Academy of Sciences* **108**, 16606 (2011).
- [55] M. Heidenreich, J. M. Georgeson, E. Locatelli, L. Rovigatti, S. K. Nandi, A. Steinberg, Y. Nadav, E. Shimon, S. A. Safran, J. P. Doye, and E. D. Levy, Designer protein assemblies with tunable phase diagrams in living cells, *Nature Chemical Biology* **16**, 939 (2020).
- [56] G. van Anders, N. K. Ahmed, R. Smith, M. Engel, and S. C. Glotzer, Entropically patchy particles: engineering valence through shape entropy, *Acs Nano* **8**, 931 (2013).
- [57] Z. Zhang and S. C. Glotzer, Self-assembly of patchy particles, *Nano Letters* **4**, 1407 (2004).
- [58] A. B. Pawar and I. Kretzschmar, Fabrication, assembly, and application of patchy particles, *Macromolecular rapid communications* **31**, 150 (2010).
- [59] E. Bianchi, R. Blaak, and C. N. Likos, Patchy colloids: state of the art and perspectives, *Physical Chemistry Chemical Physics* **13**, 6397 (2011).
- [60] F. Romano and F. Sciortino, Colloidal self-assembly: patchy from the bottom up, *Nature materials* **10**, 171 (2011).
- [61] K. Suzuki, K. Hosokawa, and M. Maeda, Controlling the number and positions of oligonucleotides on gold nanoparticle surfaces, *Journal of the American Chemical Society* **131**, 7518 (2009).
- [62] J.-W. Kim, J.-H. Kim, and R. Deaton, Dna-linked nanoparticle building blocks for programmable matter, *Angewandte Chemie International Edition* **50**, 9185 (2011).
- [63] Y. Wang, Y. Wang, D. R. Breed, V. N. Manoharan, L. Feng, A. D. Hollingsworth, M. Weck, and D. J. Pine, Colloids with valence and specific directional bonding, *Nature* **491**, 51 (2012).
- [64] L. Feng, R. Dreyfus, R. Sha, N. C. Seeman, and P. M. Chaikin, Dna patchy particles, *Advanced Materials* **25**, 2779 (2013).
- [65] P. W. K. Rothmund, Folding DNA to create nanoscale shapes and patterns, *Nature* **440**, 297 (2006).
- [66] Y. Tian, J. R. Lhermitte, L. Bai, T. Vo, H. L. Xin, H. Li, R. Li, M. Fukuto, K. G. Yager, J. S. Kahn, *et al.*, Ordered three-dimensional nanomaterials using dna-prescribed and valence-controlled material voxels, *Nature materials* **19**, 789 (2020).
- [67] W. Bol, Monte Carlo simulations of fluid systems of waterlike molecules, *Molecular Physics* **45**, 605 (1982).
- [68] N. Kern and D. Frenkel, Fluid–fluid coexistence in colloidal systems with short-ranged strongly directional attraction, *The Journal of chemical physics* **118**, 9882 (2003).
- [69] L. Rovigatti, J. Russo, and F. Romano, How to simulate patchy particles, *The European Physical Journal E* **41**, 59 (2018).
- [70] C. Beneduce, F. Sciortino, P. Sulc, and J. Russo, How to include azeotropy in the design of self-assembling patchy particles systems, *arXiv preprint arXiv:2208.09856* (2022).
- [71] M. Rubinstein, R. H. Colby, *et al.*, *Polymer physics*, Vol. 23 (Oxford university press New York, 2003).
- [72] H. Tanaka, H. Tong, R. Shi, and J. Russo, Revealing key structural features hidden in liquids and glasses, *Nature Reviews Physics* **1**, 333 (2019).
- [73] W. Lechner and C. Dellago, Accurate determination of crystal structures based on averaged local bond order parameters, *The Journal of chemical physics* **129**, 114707 (2008).
- [74] D. Frenkel and B. Smit, *Understanding molecular simulation: from algorithms to applications*, Vol. 1 (Elsevier, 2001).
- [75] L. Xu, S. V. Buldyrev, H. E. Stanley, and G. Franzese, Homogeneous crystal nucleation near a metastable fluid–fluid phase transition, *Physical Review Letters* **109**, 095702 (2012).
- [76] A. Stukowski, Visualization and analysis of atomistic simulation data with OVITO—the Open Visualization Tool, *MODELLING AND SIMULATION IN MATERIALS SCIENCE AND ENGINEERING* **18**, 10.1088/0965-0393/18/1/015012 (2010).
- [77] I. Chakraborty, D. J. Pearce, R. W. Verweij, S. C. Matysik, L. Giomi, and D. J. Kraft, Self-assembly dynamics of reconfigurable colloidal molecules, *ACS nano* **16**, 2471 (2022).
- [78] Y. Xiong, S. Yang, Y. Tian, A. Michelson, S. Xiang, H. Xin, and O. Gang, Three-dimensional patterning of nanoparticles by molecular stamping, *ACS nano* **14**, 6823 (2020).
- [79] J. Russo, F. Leoni, F. Martelli, and F. Sciortino, The physics of empty liquids: from patchy particles to water, *Reports on Progress in Physics* (2021).
- [80] E. Bianchi, J. Largo, P. Tartaglia, E. Zaccarelli, and F. Sciortino, Phase diagram of patchy colloids: Towards empty liquids, *Physical Review Letters* **97**, 168301 (2006).
- [81] F. Romano, E. Sanz, and F. Sciortino, Crystallization of tetrahedral patchy particles in silico, *The Journal of chemical physics* **134**, 174502 (2011).
- [82] I. Altan and P. Charbonneau, Obtaining soft matter models of proteins and their phase behavior, in *Protein Self-Assembly* (Springer, 2019) pp. 209–228.
- [83] N. Gnan, F. Sciortino, and E. Zaccarelli, Patchy particle models to understand protein phase behavior, in *Protein Self-Assembly* (Springer, 2019) pp. 187–208.
- [84] J. J. McManus, *Protein Self-Assembly* (Springer, 2019).
- [85] T. V. Hvozď, Y. V. Kalyuzhnyi, V. Vlady, and P. T. Cummings, Empty liquid state and re-entrant phase behavior of the patchy colloids confined in porous media, *The Journal of Chemical Physics* **156**, 161102 (2022).