Evolution of Polymer Colloid Structure During Precipitation and Phase Separation

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Cite This: JACS Au 2021, 1, 936–944

ABSTRACT: Polymer colloids arise in a variety of contexts ranging from synthetic to natural systems. The structure of polymeric colloids is crucial to their function and application. Hence, understanding the mechanism of structure formation in polymer colloids is important to enabling advances in their production and subsequent use as enabling materials in new technologies. Here, we demonstrate how the specific pathway from precipitation to vitrification dictates the resulting morphology of colloids fabricated from polymer blends. Through continuum simulations, free energy calculations, and experiments, we reveal how colloid structure changes with the trajectory taken through the phase diagram. We demonstrate that during solvent exchange, polymer–solvent phase separation of a homogeneous condensate can precede polymer–polymer phase separation for blends of polymers that possess some degree of miscibility. For less-miscible, higher-molecular-weight blends, phase separation and kinetic arrest compete to determine the final morphology. Such an understanding of the pathways from precipitation to vitrification is critical to designing functional structured polymer colloids.

KEYWORDS: polymer colloid, precipitation, vitrification, phase separation

INTRODUCTION

The phase separation of polymer molecules plays a vital role in living and soft matter systems. In biology, phase separation has important health implications ranging from the formation and transformation of dynamic biomolecular condensates such as stress granules and P-bodies to fibril formation in liquid protein aggregates. In soft materials, phase separation can form structures that synergistically combine the properties of multiple components. In particular, polymer–polymer phase separation is widely exploited in the fabrication of structured polymer colloids. Morphology control is crucial to the utility of polymer colloids, as a particle’s structure largely dictates its physical properties and functionality. For example, the presence of distinct surface patches on colloids imparts them with the ability to selectively adsorb at liquid interfaces, forming Pickering emulsions. In drug delivery applications, compartmentalization within a colloid can control the release profile of internally sequestered drug molecules. Thus, an understanding of the mechanism and kinetics of phase separation is vital to controlling the internal and external structure of polymeric colloids.

A convenient method of producing polymer colloids is solvent exchange-induced precipitation. In this general approach, a dilute polymer solution is mixed with a miscible liquid in which the polymer is poorly soluble, i.e., the antisolvent. The addition of the antisolvent generates a high supersaturation of the polymers, forcing their precipitation. When the solvent contains a blend of at least two homopolymers that are immiscible with one another, phase separation between the polymers can lead to the formation of structured colloids. In such a case, the equilibrium microstructure is determined by a competition of interfacial energies, which can be tuned by end-group functionality or the addition of amphiphilic molecules. Resulting morphologies can range from Janus to partially and fully engulfed core–shell structures, depending on the species’ relative affinities for one another and for the antisolvent.

However, interfacial energy is not the only determinant of the final morphology in multicomponent polymer colloids. During precipitation, polymer phase separation kinetically competes with vitrification because of the solvent-induced glass transition: as good solvent is exchanged for antisolvent, the composition-dependent glass transition temperature eventually rises to room temperature and arrests polymer chain...
mobility.24−27 This can result in nonequilibrium colloid structures if the time scale of solvent exchange and thus vitrification is shorter than that of phase separation. For example, during flash nanoprecipitation (FNP) of polystyrene and polyisoprene blends, patchy rather than Janus particles form because of incomplete phase separation at the onset of polystyrene vitrification.28,29 The pathway to structure formation is difficult to assess experimentally due to the small relevant temporal and spatial length scales. Thus, our understanding of colloid formation by solvent-exchange-induced precipitation has been primarily inferred by analyzing the final colloid morphology. Alternatively, simulations can provide a detailed view into the evolution of components during precipitation. Molecular dynamics simulations of binary polymer blends have elucidated mechanisms of surface patch formation that are due to kinetic arrest under instantaneous solvent exchange, where various final morphologies were demonstrated for different initial component volume fractions.30 However, equilibrium conditions can shift gradually rather than instantaneously during solvent exchange, and an understanding of the specific pathway traversed by a colloid through the composition space and phase diagram during precipitation is crucial to further controlling structure.

Here, we demonstrate how the specific pathway from precipitation to vitrification dictates the resulting morphology of a polymer colloid. Through continuum simulations, free energy calculations, and experiments, we reveal how the colloid morphology changes with the trajectory taken through the phase diagram, all while the diagram itself evolves due to changing solvency conditions. In particular, we show that polymer−solvent phase separation of a homogeneous droplet can precede polymer−polymer phase separation for low-molecular-weight blends, resulting in internally structured particles, as spinodal decomposition is kinetically locked during vitrification. If kinetic arrest occurs prior to polymer−polymer phase separation, then a homogeneous colloid results. For less-miscible blends of higher molecular weight, we demonstrate that if polymer transport throughout the colloid is sufficiently faster than the rate of condensation from solution, then the equilibrium Janus morphology emerges. When polymer

![Figure 1. Morphology evolution of simulated colloids and representative TEM images of PS−PBrS colloids prepared by dialysis precipitation. Time increases to the right, indicated by increasing \( x_{PS} \). Simulated (\( N_{A,B} \)) and experimental (\( N_{PS} \) and \( N_{PBrS} \)) degrees of polymerization: (a) \( N_{A,B} = 15, N_{PS} = 9, N_{PBrS} = 11 \), (b) \( N_{A,B} = 25, N_{PS} = 31, N_{PBrS} = 26 \), (c) \( N_{A,B} = 50, N_{PS} = 154, N_{PBrS} = 110 \), and (d) \( N_{A,B} = 50, N_{PS} = 3750, N_{PBrS} = 703 \). Red corresponds to polymer A, green to polymer B, blue to the solvent S, and gray to a homogeneous mixture of A and B. The simulation in panel d is performed with the same degree of polymerization as in panel c, \( N_{A,B} = 50 \), although with polymer mobility reduced 8-fold. In the TEM images, dark regions correspond to PBrS and light regions to PS. Scale bars are 500 nm.](https://pubs.acs.org/jacsau/937)
mobility within the condensate is low, then surface enrichment of the opposite polymer will occur, forming patchy particles.

## MATERIALS AND METHODS

We investigate a model binary blend comprising polystyrene (PS) and poly(4-bromostyrene) (PBrS), with tetrahydrofuran (THF) as the solvent and water the antisolvent. PS and PBrS are moderately immiscible with a Flory–Huggins interaction parameter $\chi_{\text{FS-brs}} \approx 0.15$ at room temperature. Both polymers are hydrophobic; the measured water contact angles for PS and PBrS are 92 and 93°, respectively. On the basis of interfacial energy considerations, the expected equilibrium morphology of a PS–PBrS colloid is the Janus structure. We employed dialysis precipitation to explore the resultant colloid structures, by which we dialyzed 0.5 mg/mL blend solutions of PS and PBrS in THF against water to precipitate stable suspensions under slow mixing conditions. Precipitation via dialysis increases the time scale of particle formation, allowing for a full exploration of the phase diagram during solvent exchange. By employing PS and PBrS, we eliminate the need to selectively stain one polymer for imaging by transmission electron microscopy (TEM), as the bromine inherently provides sufficient electron contrast relative to PS. Additional experimental details are presented in the Supporting Information.

In parallel, we perform continuum simulations and free-energy calculations to elucidate the pathways of particle formation which yield experimen tally observed morphologies. Ternary phase diagrams are calculated using the Flory–Huggins solution theory, in which we treat THF and water together as a single effective solvent, $S$, with degree of polymerization $N_S = 1$. Such a simplification disregards any effects that may arise due to nonequilibrium partitioning of the solvent and antisolvent within the condensed and dilute phases. However, such effects are primarily expected during rapid solvent exchange and may be minimal in these experiments due to the slow dialysis approach. Polymers A and B represent PS and PBrS, and the polymers are simulated with equal degrees of polymerization, $N_A = N_B = N_{AB}$ which we vary between 15, 25, and 50. We maintain $\chi_{AB} = 0.15$ as the interaction parameter between polymers A and B. The polymer–solvent interaction parameter, $\chi_{PS}$, is assumed to be equivalent for both A and B, $\chi_{AB} = \chi_{BS} = \chi_{PS}$ and is increased linearly with time. Such an approach is similar in spirit to multistep quench simulations, taken to the continuum step limit. This provides a simple model of the solvent exchange process: as solvent and antisolvent mix, the mixture’s effective solvent quality for the polymers decreases. We employ this model, together with Cahn–Hilliard gradient energy terms, to obtain a free energy density that describes the ternary mixture. Using this formalism, we solve the generalized diffusion equation in 2D to simulate the growth of a single particle via diffusive condensation from dilute solution. Simulations are initialized with an equivalent background concentration of both polymeric species, $\phi_{0A} = \phi_{0B} = 0.015$, to model the dilute polymer blend solution. We seed the simulation with a supercritical, spatially homogeneous nucleus of equivalent A and B, dilute polymer blend solution. Simulations are run until the polymer volume fraction within the condensed polymer-rich phase reaches the vitrification volume fraction, $\phi_{\text{vit}} = 0.66$, a concentration near which kinetic arrest is expected to occur. Additional computational details can be found in the Supporting Information.
RESULTS AND DISCUSSION

Figure 1 depicts the simulated spatiotemporal evolution of polymer colloids with varying degrees of polymerization. Relevant experimentally observed particle morphologies from dialysis precipitation are shown in the rightmost column, and additional examples are provided in the Supporting Information. Figure 1a–c is simulated with $N_{AB} = 15, 25$, and 50, respectively, to model increases in the experimental polymer molecular weights. In Figure 1d, we maintain the simulated degree of polymerization at $N_{AB} = 50$ to avoid numerical artifacts, and we model a further increase in experimental molecular weight by reducing polymer mobility 8-fold compared to Figure 1c. Solvent exchange, indicated by increasing $\chi_{PS}$ occurs as time progresses. Figure 1a shows the simulation of a blend with degree of polymerization $N_{AB} = 15$. As expected, the particle diameter increases with time because of the condensation of polymers from dilute solution. While $\chi_{PS}$ increases, so too does the polymer volume fraction within the condensed droplet as it follows the evolving binodal composition. This is the only change in this low $N_{AB}$ blend: the morphology of the growing colloid remains homogeneous as A and B remain miscible up to the point of vitrification, at $\phi_{vit} = 0.66$ (Figure S3). This homogeneous morphology is experimentally shown in the right panel of Figure 1a, which depicts structureless precipitated colloids of PS with $N_{PS} = 9$ ($M_n = 0.95$ kg/mol) and PBrS with $N_{PBrS} = 11$ ($M_n = 2.5$ kg/mol).

An increase in molecular weight expands the region of immiscibility between A and B due to the reduced entropy of mixing. As a result, the spinodal region and critical point are moved to smaller polymer volume fractions. Figure 1b shows the simulated morphology with a degree of polymerization $N_{AB} = 25$, and Figure 2a depicts the evolution of the corresponding ternary phase diagram. The initial simulated composition of the polymer solution is marked by the cyan circle near the apex of the phase diagram. Good solvent conditions are shown for $\chi_{PS} = 0.5$, at which the starting composition is stable within a one-phase region. At $\chi_{PS} = 0.83$, the solubility limit is reached. The starting composition intersects the two-phase coexistence region and liquid–liquid phase separation into solvent-rich and polymer-rich domains proceeds, following the vertical gray tie line in the central panel of Figure 2a. The initial precipitated polymer-rich phase is homogeneous since its composition at the limit of solubility, $\phi_{P0}$, which is indicated by the purple triangle, has not yet reached the critical volume fraction required for polymers A and B to phase separate, $\phi_c$, which is indicated by the yellow square in Figure 2a. In the window of A–B miscibility between $\chi_{PS} = 0.83$ and 0.92, the polymer droplet remains homogeneous while it grows via condensation from solution. At $\chi_{PS} = 0.92$, $\phi_c$ is reached and a three-phase coexistence region develops. The homogeneous precipor now undergoes spinodal decomposition, with domains enriched in either polymer A or B creating a bicontinuous internal structure. The green dashed outline at $\chi_{PS} = 1.00$ is the location of the binodal for A–B phase separation, depicting the depth of the quench. Phase separation and coarsening proceed until $\chi_{PS} = 1.05$, at which the vitrification boundary at $\phi_{vit} = 0.66$ is reached and kinetic arrest ensues. The bicontinuous internal structure resulting from spinodal decomposition is confirmed by PS–PBrS colloids with $N_{PS} = 31$ ($M_n = 3.2$ kg/mol) and $N_{PBrS} = 26$ ($M_n = 5.6$ kg/mol), where light and dark regions are enriched with PS and PBrS, respectively.

As $N$ is further increased, the pathway of morphology evolution changes dramatically. Figure 1c depicts the simulated colloid morphology for $N_{AB} = 50$, where a Janus particle with two compositionally distinct hemispheres is observed at all times. The slightly noncircular shape of the simulated colloids in Figures 1c,d compared to experimental results is caused by smaller relative values of $\chi_{PS}$ vs $\chi_{AB}$ in simulations than encountered in experiments. In addition, a curved PS–PBrS interface is observed in the experimental cases of Figure 1c,d. This deviates from the planar interface of our simulated morphologies due to the simplified model assumption of $\chi_{PS} = \chi_{AB}$ which may not be the case, experimentally. In experiments, the Janus morphology with two distinct lobes is observed at increased molecular weights of $N_{PS} = 154$ ($M_n = 16$ kg/mol) and $N_{PBrS} = 110$ ($M_n = 24$ kg/mol), in the rightmost panel of Figure 1c. Figure 2b demonstrates how the pathway through the ternary phase diagram crucially determines the colloid structure. Here, the greater degree of polymerization further expands the region of immiscibility between polymers A and B. When the solubility limit is reached at $\chi_{PS} = 0.72$, the polymer volume fraction of the precipitated particle is already greater than $\phi_c$. Precipitation thus proceeds directly into a three-phase coexistence region, as seen in the center panel of Figure 2b. Polymer–solvent and polymer–polymer phase separation are concomitant: the initially homogeneous seed nucleus immediately undergoes phase separation as it grows, forming the distinct Janus morphology, i.e., the equilibrium structure for this system. The phase-separated structure then becomes kinetically arrested when $\phi_{vit}$ is reached, at $\chi_{PS} = 1.00$. Movies of all simulated morphologies evolving alongside their changing ternary phase diagrams can be found in the Supporting Information.

The pathway of colloid formation through the evolving phase diagram can be characterized by a comparison between the precipitate’s initial composition, $\phi_{P0}$, the critical volume fraction for A–B phase separation, $\phi_c$, and the vitrification volume fraction, $\phi_{vit}$. Using Flory–Huggins solution theory, we construct a phase diagram predicting the expected pathway of colloid formation as a function of polymer degree of polymerization and $\chi_{AB}$. This phase diagram is depicted in Figure 3, calculated for initial solution polymer volume fractions $\phi_{0A} = \phi_{0B} = 0.015$. Figure S4 depicts $\phi_A$, $\phi_{P0}$ and $\phi_{AB}$ as a function of $N_{AB}$ for the specific case of $\chi_{AB} = 0.15$. In the blue region, the volume fraction of the initial precipitate is greater than the critical volume fraction required for polymer–polymer phase separation: $\phi_c < \phi_{P0} < \phi_{vit}$. Thus, the polymer solution undergoes precipitation within a three-phase coexistence region. Colloids precipitated from such blends are expected to form and maintain a phase-separated morphology with distinct A- and B-rich domains at all times, ultimately yielding either Janus or patchy particles. The yellow and purple regions denote blends for which the precipitate’s initial polymer volume fraction is smaller than that required for polymer–polymer phase separation. In these regions, a homogeneous droplet is predicted to form and persist for some duration during solvent exchange. The yellow region encompasses low-molecular-weight blends that are kinetically trapped in the initial homogeneous state because of vitrification prior to phase separation: $\phi_{P0} < \phi_{vit} < \phi_c$. For these blends, the final morphology is a structureless colloid. The purple domain indicates blends which do eventually reach...
the three-phase coexistence regime and can undergo spinodal decomposition prior to vitrification, forming bicontinuous colloids: \( \phi_P < \phi_c < \phi_{\text{vit}} \). In cases of high polymer mobility and slow solvent exchange, the bicontinuous structure may have the ability to further coarsen into the equilibrium Janus morphology. The location of the dashed line delineating the yellow and purple regions depends on the vitrification polymer volume fraction and is calculated here for \( \phi_{\text{vit}} = 0.66 \). In polymer blends with low \( \chi_{AB} \), a homogeneous condensate can form over a wide range of degrees of polymerization. At higher \( \chi_{AB} \), this range is substantially reduced because of the reduced miscibility of the blends favoring phase separation.

So far, we have only considered thermodynamic aspects of colloid formation during solvent exchange and precipitation. However, kinetic factors can also strongly influence the final colloid morphology. In particular, polymer diffusion within the condensates can be substantially reduced in high-molecular-weight blends. The colloid morphology will be kinetically trapped in a nonequilibrium structure if the time scale of molecular diffusion within the condensate is longer than that required for vitrification. This is evidenced by the formation of colloids exhibiting multiple, distinct surface patches formed by dialysis precipitation in Figure 1d, as well as by flash nanoprecipitation.28,29 During precipitation, the fluxes of both polymeric species arriving at the colloid surface are uniform due to their equal solution concentration and mutual aversion to the solvent. However, the equilibrium Janus morphology inherently presents two compositionally distinct faces at which polymeric species arrive. Thus, a competition must exist between material condensation onto the colloid and mass redistribution within the colloid.

To understand this competition, we simulate different polymer diffusion coefficients to emulate the reduction in mobility associated with increased molecular weight in experiments.28 The composition-dependent diffusivity employed in the simulations is modeled using a volume-fraction weighted geometric mean of the polymer and solvent diffusion coefficients, \( D_{A,B} \) and \( D_S \), where \( D_S \) is maintained at 1024. In the Flory–Huggins free energy, we maintain the degree of

Figure 3. Phase diagram for precipitating blends of different \( N_{A,B} \) and \( \chi_{AB} \) depicting blends for which a homogeneous condensate is predicted to arise, yellow and purple, and for which phase separation occurs directly into a three-phase coexistence region in which A and B demix, the blue region. Insets depict particle morphology evolution pathways. Within the blue region, diffusion and vitrification compete to determine the final structure, with \( \phi_{\text{c}} < \phi_P < \phi_{\text{vit}} \). The purple region indicates colloids that can spinodally decompose into a bicontinuous structure, \( \phi_P < \phi_{\text{v}} < \phi_{\text{c}} \), and the yellow region indicates colloids that vitrify in the homogeneous state, \( \phi_P < \phi_{\text{v}} < \phi_{\text{c}} \).

Figure 4. Simulated growth of colloids with \( N_{A,B} = 50 \). Insets depict the upper left quadrant from which the volume fraction and flux of polymer A, the red component, is shown. (a) Growing particle for which the polymer diffusivity, \( D_{A,B} = 512 \), is sufficiently large to preclude the formation of a surface patch. (b) \( D_{A,B} = 64 \) and material redistribution to the pre-existing Janus face is sluggish, allowing for surface enrichment and the eventual formation of a surface patch of polymer A. Time is measured in units of \( \tau \) as described in the Supporting Information.
polymerization at $N_{AB} = 50$. Figure 4 depicts the evolution of the upper-left quadrant of a simulated colloid, where the concentration depicted is that of polymer A, which is the red component in the insets. Arrows indicating the flux of polymer A demonstrate that two primary fluxes are present: that of A arriving at the surface of the colloid from solution and that of A traversing the colloid to join the existing A-rich Janus face. Material redistribution within the colloid can occur either along the polymer–solvent interface or through the B-rich bulk. In Figure 4a, $D_{AB} = 512$ and the flux of polymer A along the interface as well as through the B-rich bulk is sufficiently greater than that of material arriving at the surface, thus preventing a buildup of A on the surface. In contrast, $D_{AB} = 64$ in Figure 4b and material redistribution at the surface of the growing particle is substantially slower. As a result, the surface becomes enriched in polymer A and subsequently develops into a well-defined surface patch which itself acts as a diffusion sink, drawing material in from adjacent regions. Animated movies of this process can be found in the Supporting Information.

The patchy morphology is reflected experimentally in Figure 1d with $N_{PS} = 3750$ ($M_e = 390 \text{ kg/mol}$) and $N_{PBrS} = 703$ ($M_e = 153 \text{ kg/mol}$). Here, multiple dark PBrS patches appear in the light PS domain, as opposed to the Janus case of Figure 1c in which only two compositionally distinct lobes are present. The lack of light PS patches can be attributed to the lower glass transition temperature of PS, ~100 °C,42 vs PBrS, ~140 °C,43 which permits PS chain mobility for a greater duration of time during solvent exchange.

The simulated time evolution of this surface enrichment process is shown in Figure 5. The peak volume fraction within a growing patch, $\phi_{patch}$, is plotted for polymer diffusion coefficients ranging from $D_{AB} = 64$ to 512 are simulated. Diffusivities greater than 371 yield the Janus morphology, whereas smaller diffusivities result in patchy particles, as illustrated by the insets. Vitrification occurs at $\phi_{vitr} = 0.66$, indicated by the black dashed line.

Figure 5. Temporal evolution of polymer A’s peak volume fraction, $\phi_{patch}$, on the surface of polymer B’s Janus face during precipitation. Polymer diffusion coefficients ranging from $D_{AB} = 64$ to 512 are simulated. Diffusivities greater than 371 yield the Janus morphology, whereas smaller diffusivities result in patchy particles, as illustrated by the insets.

After material depletion, $\phi_{patch}$ tends toward a near-zero equilibrium value, yielding the Janus morphology. When the polymer diffusivity is reduced below $D_{AB} = 371$, surface patches develop. At first, $\phi_{patch}$ rises slowly. During this incubation period, the surface concentration gradually builds up as condensation competes with surface redistribution to the pre-existing Janus face. When a threshold volume fraction is reached, around $\phi_{patch} \approx 0.2$, the patch begins to act as a diffusion sink. It draws material in from adjacent locations on the colloid surface and increases rapidly in volume fraction. Once a patch forms, it continues to grow in size so long as material continues to condense from solution. Over time, $\phi_{patch}$ also continues to rise due to solvent exchange until the onset of vitrification at $\phi_{vitr} = 0.66$, the black dashed line. If a complete surface patch has formed but material fluxes from solution are depleted prior to vitrification, then curvature-driven fluxes will continue to redistribute material to the larger Janus face until kinetic arrest occurs. Thus, the presence of patchiness in the final morphology ultimately depends on several competing factors. Our simulations identify a clear transition from Janus to patchy morphologies as a function of $D_{AB}$ at fixed solvent exchange rate and initial solution concentration. However, in experiments all these parameters will conspire to dictate patch formation. With a greater initial solution concentration, more mass redistribution is required to form the equilibrium Janus particle, and when the solvent exchange rate is increased, there is less time for this redistribution to occur. Employing a polymer with a higher glass transition temperature also reduces the available time, as vitrification occurs at lower polymer volume fraction.44 Finally, an increased molecular weight will reduce polymer diffusivity, increasing the requisite time for Janus formation. Therefore, the specific molecular weight at which polymer blend colloids will transition from Janus to patch will ultimately depend on a competition between vitrification and phase separation time scales.

Until now, we have only considered symmetric polymer blends. Asymmetric mixtures will follow the same principles, with morphology evolution controlled by precipitation pathway as well as kinetics, but further subtleties can be introduced. In particular, if the polymer molecular weights are sufficiently low to form a homogeneous condensate prior to polymer–polymer phase separation, then the asymmetric nature of the ternary phase diagram predicts phase separation of a condensate enriched in the higher molecular weight component. In a sufficiently asymmetric blend, the minority component’s volume fraction can be forced below a requisite threshold for percolation, forming isolated droplets within the majority phase.45 We demonstrate this with asymmetric PS–PBrS blends of equal mass concentration in Figure 6. Figure 6a shows a colloid precipitated from a mixture of PS with $N_{PS} = 9$ ($M_e = 0.95 \text{ kg/mol}$) and PBrS with $N_{PBrS} = 110$ ($M_e = 24 \text{ kg/mol}$). In the TEM image, the darker component, PBrS, forms a continuous matrix while the lighter PS forms isolated domains. The alternate occurs in Figure 6c, with PS, $N_{PS} = 58$ ($M_e = 6 \text{ kg/mol}$), forming the light, continuous phase and PBrS, $N_{PBrS} = 26$ ($M_e = 5.6 \text{ kg/mol}$), forming dark occlusions. The asymmetric case, $N_{PS} = 31$ ($M_e = 5.2 \text{ kg/mol}$) and $N_{PBrS} = 26$ ($M_e = 5.6 \text{ kg/mol}$), is illustrated in Figure 6b, with the expected bicontinuous internal structure. The influence of asymmetric mixtures on final morphology thus allows one to...
tune the internal structure and potential functionality of the colloid.

**CONCLUSION**

An understanding of how processing affects the morphology of a polymer colloid is critical to any application in which a particle’s structure determines its physical properties. These results demonstrate the coupling of colloid morphology to the precipitation pathway and kinetic constraints. During solvent exchange, polymer–solvent phase separation of a homogeneous condensate can precede polymer–polymer phase separation for blends of sufficiently low molecular weight. Depending on the onset conditions for vitrification, this can result in either a homogeneous or bicontinuous structure. For higher-molecular-weight blends, the equilibrium Janus morphology emerges if polymer diffusion is sufficiently fast; otherwise, patchy particles are formed. These dependencies are exemplified here for a model binary homopolymer blend, and these results can be applied in other contexts. In the cell, precipitation of a homogeneous phase accompanied by subsequent phase separation can occur for coprecipitating proteins, and oligomerization can drive the phase separation of initially miscible, low-molecular-weight components. Subsequent vitrification of such protein condensates due to changing physicochemical conditions within the cell may yet be a pathway to the formation of structured biomolecular aggregates. Industrially, structured colloids have a vast variety of applications, ranging from drug delivery and biosensing vehicles to self-assembled photonic colloidal crystals to colloids for heterogeneous catalysis. The ability to generate complex internal and external structures is paramount to these colloids’ functionality. Future studies exploiting the pathway to particle formation in conjunction with microphase-separating, charged, or inorganic components such as hydrophobic or amphiphilic block copolymers, polyelectrolytes, and inorganic nanoparticles may lead to structures with novel functionalities.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.1c00110.
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by the National Science Foundation (NSF) Materials Research Science and Engineering Center Program through the Princeton Center for Complex Materials (PCCM) (DMR-2011750). The authors acknowledge the use of Princeton’s Imaging and Analysis Center (IAC), which is partially supported by the NSF via PCCM. This work used the Extreme Science and Engineering Discovery Environment’s (XSEDE) Comet cluster at the San Diego Supercomputer Center (DMR-200053), which is supported by National Science Foundation grant number ACI-1548562. The authors gratefully acknowledge the use of molecules from the Data Molecule Explorer (DME) project, which is supported by the National Science Foundation through the Chemistry Division’s Coordination Chemistry Infrastructure (CCI) program (DMR-0907787). The authors also acknowledge the use of the Center for Molecular Imprints, which is supported by the NSF via PCCM.

**REFERENCES**

(1) Shin, Y.; Brangwynne, C. P. Liquid phase condensation in cell physiology and disease. Science 2017, 357, 8aa4382.
(2) Brangwynne, C. P.; Tompa, P.; Pappu, R. V. Nat. Phys. 2015, 11, 899–904.
(3) Feric, M.; et al. Coexisting Liquid Phases Underlie Nucleolar Subcompartments. Cell 2016, 165, 1686–1697.
(4) Banani, S. F.; Lee, H. O.; Hyman, A. A.; Rosen, M. K. Biomolecular condensates: Organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol. 2017, 18, 285–298.
(5) de Oliveira, G. A. P.; Cordeiro, Y.; Silva, J. L.; Vieira, T. C. R. G. Liquid-liquid phase transitions and amyloid aggregation in proteins related to cancer and neurodegenerative diseases. In Advances in Protein Chemistry and Structural Biology; Elsevier, 2019; Vol. 118, Chapter 9.
(6) Grundy, L. S.; et al. Rapid Production of Internally Structured Colloids by Flash Nanoprecipitation of Block Copolymer Blends. ACS Nano 2018, 12, 4660–4668.
(7) Lee, V. E.; Sosa, C.; Liu, R.; Prud’homme, R. K.; Priestley, R. D. Scalable Platform for Structured and Hybrid Soft Nanocolloids by Continuous Precipitation in a Confined Environment. Langmuir 2017, 33, 3444–3449.
(8) Iaino, A.; et al. Liquid-liquid phase separation during amphiphilic self-assembly. Nat. Chem. 2019, 11, 320–328.
(9) Yang, S. M.; Kim, S. H.; Lim, J. M.; Yi, G. R. Synthesis and assembly of structured colloidal particles. J. Mater. Chem. 2008, 18, 2177–2190.
(10) Kietzke, T.; et al. Phase separation of binary blends in polymer nanoparticles. Small 2007, 3, 1041–1048.
(11) Morozova, T. I.; et al. On the Stability of Polymeric Nanoparticles Fabricated through Rapid Solvent Mixing. Langmuir 2019, 35, 709–717.
(12) Ruhland, T. M.; et al. Influence of Janus particle shape on their interfacial behavior at liquid-liquid interfaces. Langmuir 2013, 29, 1388–1394.
(13) Bradley, L. C.; Chen, W. H.; Stebe, K. J.; Lee, D. Janus and patchy colloids at fluid interfaces. Curr. Opin. Colloid Interface Sci. 2017, 20, 25–33.
(14) Morozova, T. I.; Nikoubashman, A. Surface Activity of Soft Polymer Colloids. Langmuir 2019, 35, 16907–16914.
(15) Čejková, J.; Štěpánek, F. Compartmentalized and Internally Structured Particles for Drug Delivery - A Review. Curr. Pharm. Des. 2013, 19, 6298–6314.
(16) Röslar, A.; Vandermeulen, G. W. M.; Klok, H. A. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. Adv. Drug Delivery Rev. 2012, 64, 270–279.
(17) Zhang, C.; Chung, J. W.; Priestley, R. D. Dialysis nanoprecipitation of polystyrene nanoparticles. Macromol. Rapid Commun. 2012, 33, 1798–1803.
(18) Yabu, H.; Higuchi, T.; Ijiri, K.; Shimomura, M. Spontaneous formation of polymer nanoparticles by good-solvent evaporation as a nonequilibrium process. Chaos 2005, 15, 047505.
(19) Yabu, H. Fabrication of Nanostructured Composite Microspheres Based on the Self-Assembly of Polymers and Functional Nanomaterials. Part. Part. Syst. Charact. 2019, 36, 1900178.
(20) Saad, W. S.; Prud’homme, R. K. Principles of nanoparticle formation by flash nanoprecipitation. Nano Today 2016, 11, 212–227.
(21) Mao, S.; Kuldinow, D.; Haataja, M. P.; Košmrlj, A. Phase behavior and morphology of multicomponent liquid mixtures. Soft Matter 2019, 15, 1297–1311.
(22) Mao, S.; Chakraverti-Wuerthwein, M. S.; Gaudio, H.; Košmrlj, A. Designing the Morphology of Separated Phases in Multi-component Liquid Mixtures. Phys. Rev. Lett. 2020, 125, 1–6.
(23) Morozova, T. I.; et al. In Silico Design Enables the Rapid Production of Surface-Active Colloidal Amphiphiles. ACS Cent. Sci. 2020, 6, 166–173.
(24) Wodo, O.; GnanapathySubramanian, B. Modeling morphology evolution during solvent-based fabrication of organic solar cells. Comput. Mater. Sci. 2012, 55, 113–126.
(25) Kouijzer, S.; et al. Predicting morphologies of solution processed polymer:Fullerene blends. J. Am. Chem. Soc. 2013, 135, 12057–12067.
(26) Arnauts, J.; Berghmans, H.; Koningsveld, R. Structure formation in solutions of atactic polystyrene in trans-decalin. Macromol. Chem. 1993, 194, 77–85.
(27) Li, S.-G.; van den Boomgaard, T.; Smolders, C. A.; Strathmann, H. Physical gelation of amorphous polymers in a mixture of solvent and nonsolvent. Macromolecules 1996, 29, 2053–2059.
(28) Sosa, C.; et al. Soft Multifaced and Patchy Colloids by Constrained Volume Self-Assembly. Macromolecules 2016, 49, 3580–3585.
(29) Sosa, C.; et al. Combining Precipitation and Vitrification to Control the Number of Surface Patches on Polymer Nanocolloids. Langmuir 2017, 33, 5835–5842.
(30) Li, N.; Nikoubashman, A.; Panagiotopoulos, A. Z. Controlled production of patchy particles from the combined effects of nanoprecipitation and vitrification. Soft Matter 2017, 13, 8433–8441.
(31) Gorga, R. E.; Jablonski, E. L.; Thiyyagarajan, P.; Seifert, S.; Narasimhan, B. Quantifying phase behavior in partially miscible polystyrene/polystyrene-co-4-bromostyrene) blends. J. Polym. Sci., Part B: Polym. Phys. 2002, 40, 255–271.
(32) Strobl, G. R.; Benjedl, J. T.; Kambour, R. P.; Shultz, A. R. Thermally Reversible Phase Separation in Polystyrene/Polystyrene-co-4-bromostyrene) Blends. Macromolecules 1986, 19, 2683–2689.
(33) Kambour, R. P.; Bendler, J. T.; Bopp, R. C. Phase Behavior of Polystyrene, Polystyrene-co-2,6-dimethyl-1,4-phenylene oxide), and Their Brominated Derivatives. Macromolecules 1983, 16, 753–757.
(34) Berry, J.; Brangwynne, C. P.; Haataja, M. Physical principles of intracellular organization via active and passive phase transitions. Rep. Prog. Phys. 2018, 81, 046601.
(35) Morozova, T. I.; Nikoubashman, A. Colloid - Globule Collapse of Polystyrene Chains in Tetradecylofuoran - Water Mixtures. J. Phys. Chem. B 2018, 122, 2130.
(36) Henderson, I. C.; Clarke, N. Two-Step Phase Separation in Polymer Blends. Macromolecules 2004, 37, 1952–1959.
(37) De Castro, P.; Sollich, P. Phase separation of mixtures after a second quench: Composition heterogeneities. Soft Matter 2019, 15, 9287–9299.
(38) Kessler, S.; Schmid, F.; Dresse, K. Modeling size controlled nanoparticle precipitation with the co-solvency method by spinodal decomposition. Soft Matter 2016, 12, 7231–7240.
(39) Cahn, J. W. Free energy of a nonuniform system. II. Thermodynamic basis. *J. Chem. Phys.* 1959, 30, 1121–1124.

(40) Cogswell, D. A. A Phase-Field Study of Ternary Multiphase Microstructures. *PhD Thesis*, Massachusetts Institute of Technology, Cambridge, MA, 2010.

(41) Huang, C.; de la Cruz, M. O.; Swift, B. W. Phase Separation of Ternary Mixtures: Symmetric Polymer Blends. *Macromolecules* 1995, 28, 7996–8005.

(42) Burroughs, M. J.; Napolitano, S.; Cangialosi, D.; Priestley, R. D. Direct measurement of glass transition temperature in exposed and buried adsorbed polymer nanolayers. *Macromolecules* 2016, 49, 4647–4655.

(43) Malhotra, S. L.; Lessard, P.; Blanchard, L. P. A Thermal Decomposition and Glass Transition Temperature Study of Poly(p-bromostyrene). *J. Macromol. Sci., Chem.* 1981, 15, 1577–1599.

(44) Keshavarz, L.; Khansary, M. A.; Shirazian, S. Phase diagram of ternary polymeric solutions containing nonsolvent/solvent/polymer: Theoretical calculation and experimental validation. *Polymer* 2015, 73, 1–8.

(45) Brunini, V. E.; Schuh, C. A.; Carter, W. C. Percolation of diffusively evolved two-phase systems. *Phys. Rev. E - Stat. Nonlinear, Soft Matter Phys.* 2011, 83, 1–9.

(46) Kaur, T. et al. Sequence-encoded and Composition-dependent Protein-RNA Interactions Control Multiphasic Condensate Topologies. *bioRxiv* 2020, 2020.08.30.273748.

(47) Shin, Y.; et al. Spatiotemporal Control of Intracellular Phase Transitions Using Light-Activated optoDroplets. *Cell* 2017, 168, 159–171.

(48) Yi, Y.; Sanchez, L.; Gao, Y.; Yu, Y. Janus particles for biological imaging and sensing. *Analyst* 2016, 141, 3526–3539.

(49) He, M.; et al. Colloidal diamond. *Nature* 2020, 585, 524–529.

(50) Kim, S. H.; Lee, S. Y.; Yang, S. M.; Yi, G. R. Self-assembled colloidal structures for photonics. *NPG Asia Mater.* 2011, 3, 25–33.

(51) Lim, C. W.; Lee, I. S. Magnetically recyclable nanocatalyst systems for the organic reactions. *Nano Today* 2010, 5, 412–434.

(52) Gross, E.; Dean Toste, F.; Somorjai, G. A. Polymer-Encapsulated Metallic Nanoparticles as a Bridge Between Homogeneous and Heterogeneous Catalysis. *Catal. Lett.* 2015, 145, 126–138.

(53) Gijis, M. A. M.; Lacharme, F.; Lehmann, U. Microfluidic applications of magnetic particles for biological analysis and catalysis. *Chem. Rev.* 2010, 110, 1518–1563.

(54) Towns, J.; et al. XSEDE: Accelerating Scientific Discovery. *Comput. Sci. Eng.* 2014, 16, 62–74.