

Self-assembly of Amphiphilic Diblock Copolymers Induced by Liquid-Liquid Phase Separation

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 Electronic Supplementary Information

Abstract The liquid-liquid phase separation (LLPS) widely exists in biology, synthetic chemistry, crystallization kinetics and other fields, and it is very important to realize the related functions. The research on the competition between LLPS and micellization/vesiculation has made considerable progress. However, the way to effectively control the formation paths from homogeneous state to aggregates has not been completely solved, which is vital to determine its structure and properties and even its future functions. Here we describe the phenomenon of LLPS and its effect on the dynamic process of self-assembly of amphiphilic diblock copolymers (BCPs). Starting from the establishment of phase diagram, we explore the existence conditions of LLPS state, the internal morphology and external size of large droplets, and its significant implications to the dynamic path of vesicle formation. Vesicles formed via LLPS have larger sized outer dimensions and inner cavities, and contain more solvents during certain stages. The detailed research of LLPS and its self-assembly simulation has contributed to completing its theoretical basis and practical applications in the future in various fields.

Keywords Liquid-liquid phase separation; Amphiphilic diblock copolymers; Dissipative particle dynamics; Vesicle

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INTRODUCTION

Self-assembly is a common phenomenon in nature. In selective solvents, amphiphilic molecules spontaneously form stable, ordered, structurally determined aggregates in biological systems, e.g. cell membranes, lipids, some proteins, etc. Synthetically, such amphiphilic molecules can self-assemble into a series of various morphological structures, such as micelle structures with spherical, columnar, flaky and tubular shapes, as well as vesicles with spherical, ellipsoidal and oblate outer shapes and/or with complex inner cavities such as single-, multi-, annulated chambers and multilayered membrane structures.^[1,2] Therefore, they often have special physical, chemical and electrical properties not available in a single molecule, and are utilized in constructing new structures, realizing new functions and creating new materials, finding wide use in the most concerned biomedicine,^[3–6] containing biological diagnosis, tumor treatment, drug carrier, cell imaging, etc.

Phase separation is ubiquitous in polymer chemistry,^[7] and analogous phenomena are constantly being found in biolo-

gical macromolecules. Liquid-liquid phase separation (LLPS) is often observed in crystallization experiments, and the formation of droplets is beneficial.^[8–10] In recent experiments, Ianni et al. reported the LLPS of BCPs and polymer-rich liquid droplets act as a precursor in the bottom-up formation of spherical micelles and then vesicles.^[11] It is demonstrated that thermally-induced liquid-liquid separation is also one of the methods for preparing microporous membranes.^[12] Some proteins, such as hemoglobin, will undergo phase separation at high concentrations *in vitro*.^[13,14] The understanding that phase separation may control the formation of membrane-less organelles, which is often referred to as a biomolecular condensate and exists in the nucleus (nucleoli,^[15] Cajal body,^[16] etc.) and cytoplasm (stress granules,^[17,18] centrosomes,^[19] etc.), is an advance in recent years. Several pivotal proteins that cause neurodegenerative diseases are part of membrane-less organelles, and the maladjustment of its formation, maintenance or decomposition may be the origin of pathological aggregation.^[20,21] Many brain diseases are characterized by toxic aggregates (gelatinous or solid), such as plaques in Alzheimer's disease,^[22] Parkinson's disease,^[23] or in amyotrophic lateral sclerosis.^[24] Disease mutations accelerate the liquid-solid transition, and may affect the phase separation by producing aberrant proteins and RNA. In summary, the initial cell compartments or membrane-less organelles are

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formed by LLPS, while liquid-solid phase transition may generate the disease state. These facts give more prominence to the importance of phase transition to pathology.^[25–29]

Phase separation drives the components quickly concentrate in the cell to form condensates.^[30,31] This is a physical process. The supersaturated solution of components spontaneously separates into dense phase and dilute phase, and then stably coexists. Obviously, two complex mixtures are formed in this process, within which all components mix respectively, but they demix with each other.^[32,33] It is the energy reduction by demixing that opposes entropy-driven mixing. Within each phase, entropy still ensures that the components are well mixed.^[34]

The aggregates formed by self-assembly of amphiphilic molecules are all in a certain phase state, or single, or multiple mixture, while certain conditions alter (*e.g.* temperature, concentration, pH)^[35–40] and phase transition subsequently occurs. The phase transition of amphiphilic molecules has its particularity compared with other substances based on the ordered aggregates of amphiphilic molecules. Heretofore, the research on the phase behavior of amphiphilic molecules mainly focuses on the phase stability, phase morphology and phase diagram construction.^[41–47] We have been doing research on the behavior and mechanism of the self-assembly of amphiphilic diblock copolymers (BCPs) in selective solvents, and probing into influence factors such as concentration, hydrophobic/hydrophilic region length and interaction parameters between diverse regions on the entire self-assembly process, which furnishes theoretical basis for this study.^[48–50]

However, the complexity of biological system makes it extremely difficult to verify and characterize the process of natural LLPS both *in vivo* and *in vitro*,^[51] so many researchers explored several approaches to incorporating LLPS as artificial cytoplasm and in artificial cells.^[52–56] Self-consistent field computations have been used to probe the self-assembly occurring in liquids with phase-separated domains, these domains will influence the assembly process, and hence these liquid-phase precursors may be important for controlling the resulting vesicles' structural properties,^[11] but LLPS is still not fully studied. Therefore, we attempted to build a simple model and applied the computational simulation method to understand the thermodynamics and dynamics of the LLPS process of amphiphilic BCPs at the molecular level, so as to provide a theoretical basis for the future design of special protein structure model, and pave the way for the realization of its precise control and research. It is predicted that LLPS can occur in a wide range of amphiphilic systems, and may affect the characteristics of the subsequent formed vesicles. Therefore, taking LLPS process into consideration in the design of the amphiphilic assemblies is expected to provide greater control and advantages over the structure and performance of the obtained target materials.

THEORY AND SIMULATION METHOD

In this work, the dissipative particle dynamics (DPD) method is utilized to elucidate the LLPS behavior of amphiphilic diblock copolymers (BCPs). DPD is a mesoscale meshless particle-based algorithm in continuous space and intermediate time, which

can overcome the mesoscopic issues unresolved by traditional molecular dynamics.^[57] The motion of DPD beads is described by the Newton's equation of motion and the conservation of momentum ensures the appropriate hydrodynamic behavior.^[58]

In the system of DPD simulations, there are three different types of coarse-grained beads including hydrophilic beads (A), hydrophobic beads (B) and solvent beads (S). The AB-type BCPs are modelled by the finitely extensible nonlinear elastic potential between the consecutive beads.^[59] The pair-wise interaction between beads is represented by a soft repulsive potential and the parameter a_{ij} between *i*- and *j*-type beads can be estimated by the Flory-Huggins interaction parameter χ_{ij} .^[58] For the number density of $\rho=3$, the repulsive interaction parameter between the same type of DPD beads is set as $a_{ii}=25$. Especially, the value of $a_{ij} \leq 25$ corresponds to $\chi_{ij} \leq 0$, indicating that the *i*- and *j*-type beads are compatible. More details about DPD method are provided in the electronic supplementary information (ESI).

The amphiphilic BCPs have a fixed hydrophilic block length and hydrophobic block length (*i.e.*, A₂B₇), where the subscripts 2 and 7 refer to the repeat units for the corresponding blocks. To ensure the occurrence of LLPS in the weak segregated regime, we fix the interaction parameter between A and S beads as $a_{AS}=30$ and alter the parameters a_{AB} and a_{BS} . The DPD simulations are mostly performed in a cubic box (30^3b , where *b* is the length unit) under periodic boundary conditions. Unless otherwise specified, the lengths such as distances or dimensions mentioned in this article all take *b* as the unit length. *b* is omitted latter for simplicity. Each simulation takes at least 2.0×10^6 timesteps so that the system can necessarily reach an equilibrium state, which is weakly dependent upon the initial random configurations.^[60]

RESULTS AND DISCUSSION

Inspired by the experimental findings,^[11] we theoretically propose a stepwise scheme to program the self-assembly of amphiphilic BCPs in selective solvents through the DPD simulations. The stepwise self-assembly includes two critical steps. In the first step, the incompatibility of hydrophobic blocks with the solvent beads drives the amphiphilic BCPs to self-organize into intermediate state of LLPS in selective solvents. Subsequently, by virtue of the change of solvent environment, the intermediate states are programmed to gradually evolve into the vesicles with controllable sizes. To elucidate the stepwise self-assembly behaviors of amphiphilic BCPs, this work includes the formation of LLPS and kinetically controlled vesicles as well as the tuneable size of vesicles.

Liquid-liquid Phase Separation

In this section, we probe into the formation regime of liquid-liquid phase separation by constructing the morphological phase diagrams. The concentration of amphiphilic BCPs is fixed at $\phi=0.10$. The amphiphilic BCPs are randomly dispersed in the solution and quenched into the aggregated states. Next, we concentrate on the effect of interaction parameters a_{BS} and a_{AB} on the morphologies of amphiphilic BCPs in dilute solution.

From the DPD simulations, five kinds of equilibrium phase morphologies (*e.g.*, homogeneous polymer solution, phase-

separated polymer-rich liquid droplets, large compound micelles, multi-compartment vesicles and transition state) are identified by analyzing the profiles of number density of hydrophilic beads $\Phi_A(r)$, hydrophobic beads $\Phi_B(r)$ and solvents $\Phi_S(r)$, where r is the distance from the centroid of aggregates. Unlike the uniform distributions of beads for the homogeneous polymer solution (abbreviated as HS), the profiles of beads display a rugged characteristic (Fig. 1), indicating the non-uniform distributions of beads in the aggregates. Specifically, in the state of phase-separated polymer-rich liquid droplets (LL), the amphiphilic BCPs has the capability to form a polymer-rich droplet (i.e., a higher plateau of $\Phi_A(r)$ and $\Phi_B(r)$) and the free chains are simultaneously found in solvent-rich domains (i.e., long tails of $\Phi_A(r)$ and $\Phi_B(r)$), which are illustrated in Fig. 1(a). The large compound micelles (LCM) possess the similar features of $\Phi_A(r)$ and $\Phi_B(r)$, but the solvent molecules are excluded from the cores of aggregates, corresponding to the zero value of $\Phi_S(r)$ in the range of $r < 6$ (Fig. 1b). When the profiles of $\Phi_A(r)$ and $\Phi_B(r)$ are saliently bimodal or trimodal, the aggregates of amphiphilic BCPs are termed as multi-compartment vesicles (MCV), which are illustrated in Fig. 1(c). Considering the free chains of BCPs in the solvent-rich domains, the aggregates with multi-modal feature of $\Phi_{A/B}(r)$ are termed as transition state (Fig. 1d). What is more, the large droplets in transition state (TS) region rearrange and

are tighter under the influence of the interaction and the amplitude of solvents' curve is a little larger than that of MCV, whose internal morphologies correspond to the upper area, for instance, the droplets tend to be LCM when $a_{AB}=35$ and $a_{BS}=45$ (Fig. S1 in ESI). Hence the state of TS region is exactly a transition somewhere between the LL and micelles or vesicles, which just proves that the droplets go through a gradual maturation process during aggregate formation, the solvent beads release (forming LCM) or are partially trapped (forming MCV) and the building blocks are tightly closer integrated driven by the interaction.^[61–63]

Fig. 2 plots the morphological phase diagram of aggregates formed by diblock copolymer A_2B_7 in terms of the interaction parameters of a_{BS} and a_{AB} at $a_{AS}=30$ and polymeric concentration $\varphi=0.10$. Each point in the plot corresponds to a simulation result, and the dashed lines are drawn to highlight the resulting phase boundaries. The phase diagram is divided into five characteristic regimes: homogeneous polymer solution (HS), phase-separated polymer-rich liquid droplets (LL), transition state (TS), large compound micelles (LCM) and multi-compartment vesicles (MCV). For the smaller interaction parameter a_{BS} (e.g., $a_{BS} \leq 30$), the amphiphilic AB BCPs do not have sufficient attractive forces to associate into the aggregates and remain in homogeneous state, even when the incompatibility between A and B blocks is increased. With an

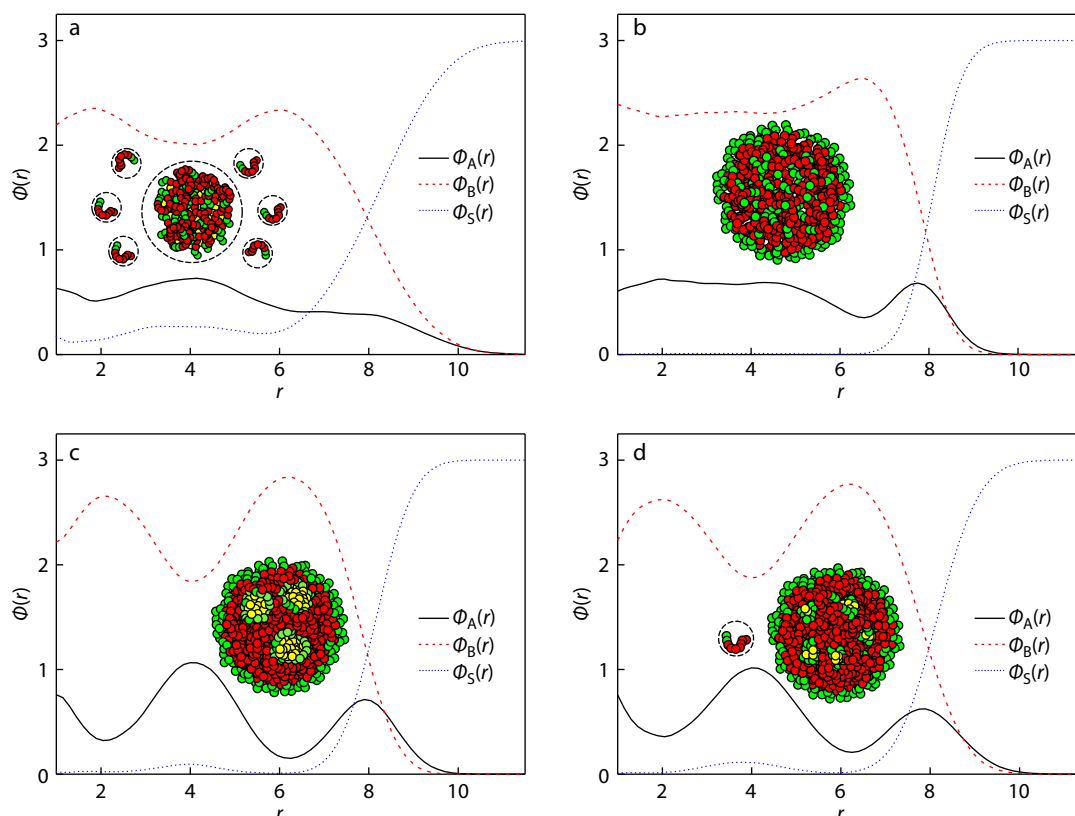


Fig. 1 Radial distribution of number density of hydrophilic beads $\Phi_A(r)$, hydrophobic beads $\Phi_B(r)$ and solvents $\Phi_S(r)$ as a function of the distance r from the centroid of aggregates (a) LL at $a_{AB}=50$, $a_{BS}=40$; (b) LCM at $a_{AB}=30$, $a_{BS}=60$; (c) MCV at $a_{AB}=50$, $a_{BS}=60$; (d) TS at $a_{AB}=50$, $a_{BS}=50$ with $a_{AS}=30$ and $\varphi=0.10$. Note that normalizations are $4\pi\int_0^R\Phi(r)r^2dr=N_{agg}$. Insets correspond to the morphologies of aggregates at parameters highlighted by the dot-dashed circles in Fig. 2. The hydrophilic blocks A and hydrophobic blocks B are represented by the green and red spheres, respectively. The dashed circles signify the interface between the polymer-rich liquid droplet and the bulk environment.

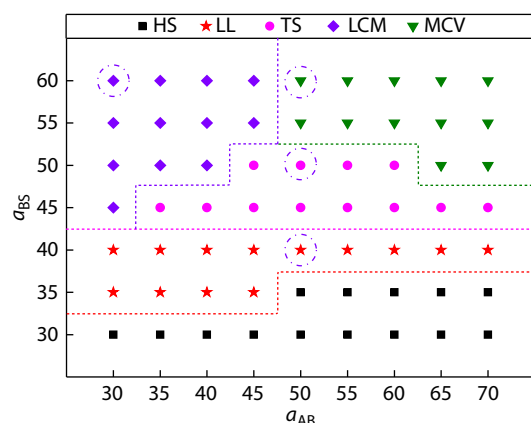


Fig. 2 Morphological phase diagram of aggregates formed by diblock copolymer A_2B_7 in terms of the interaction parameters a_{BS} and a_{AB} at $a_{AS}=30$ and polymeric concentration $\phi=0.10$. The homogeneous polymer solution (HS), phase-separated polymer-rich liquid droplets (LL), transition state (TS), large compound micelles (LCM) and multi-compartment vesicles (MCV) are identified in the DPD simulations.

increase of a_{BS} , the incompatibility between the hydrophobic B blocks and S beads induce the occurrence of LLPS, leading to the coexistence of polymer-rich droplets and free polymer chains in solvent-rich domain. Note that the regime of this morphology is weakly dependent on the parameter of a_{AB} . With a further increase of a_{BS} , the regimes of micelles, vesicles and transition state are tuned by varying the parameter of a_{AB} . Especially, in the range of $55 \leq a_{BS} \leq 60$ and $50 \leq a_{AB} \leq 70$, the amphiphilic AB BCPs can self-assemble into the MCVs. The above observations manifest the fact that the regime of LLPS can be effectively tailored by the thermodynamic properties of amphiphilic BCPs such as the incompatibility between the A and B blocks or the B blocks and S beads.

In our work, the simulation results shown in Figs. 1 and 2 suggest that the aggregation behaviors of amphiphilic BCPs exhibit a well-defined droplet owing to the characteristic of LLPS of hydrophobic B blocks in selective solution. Our simulations also show that the vesicles appear to be straightforwardly dependent upon their thermodynamic parameters, so careful selection of solvent and self-assembly pathway of amphiphilic BCPs is necessary to yield the vesicles with controlled sizes. Therefore, the LL can act as well-defined intermediate state for the construction of vesicles with predictive shape and size. From the experimental findings in conjunction with our simulation results, LLPS plays an important role in determining the final external size and internal structure of vesicles, and significantly affects the dynamic path of the formation process.

Kinetic Pathway of Vesicle Formation

As shown in Fig. 2, the formation of well-defined droplets and vesicles can be tuned by the interaction parameters of a_{BS} and a_{AB} . Inspired by the observations, we propose the stepwise scheme to program the self-assembly of amphiphilic BCPs. First, the randomly dispersed BCPs form the intermediate states of LL under the conditions of $a_{AB}=50$ and $a_{BS}=40$. Secondly, by tuning the interaction parameters (e.g., $a_{AB}=70$ and $a_{BS}=60$), the intermediate states are programmed to gradually evolve into

the target morphologies. The self-assembly pathway is designated as from LL. For comparison, we also implement the DPD simulations of amphiphilic BCPs, where the homogeneous state is quenched into the vesicle state at $a_{AB}=70$ and $a_{BS}=60$ (from HS pathway).

Fig. 3(a) shows the vesicle formation of amphiphilic BCPs from HS pathway. Vesicles developed through bilayer membrane closure mechanism (mechanism I), after the instant contact and combination of polymers, the aggregates of numerous small micelles consistently conduct mutual collision and fusion to form larger spherical micelles, and gradually adjust their shape to oblate micelles, then indentation occurs to obtain bowl-shaped micelles, and finally the membrane closes so that the solvent particles are wrapped, forming spherical vesicles. In sum, block copolymers are like containers of holding solvents, starting with plates (Figs. 3a-a' and 3a-b'), then bowls (Figs. 3a-c', 3a-d' and 3a-e') and finally closed spheres (Figs. 3a-f'). For the sake of more regular and beautiful shape of aggregates, the results in a simulation box

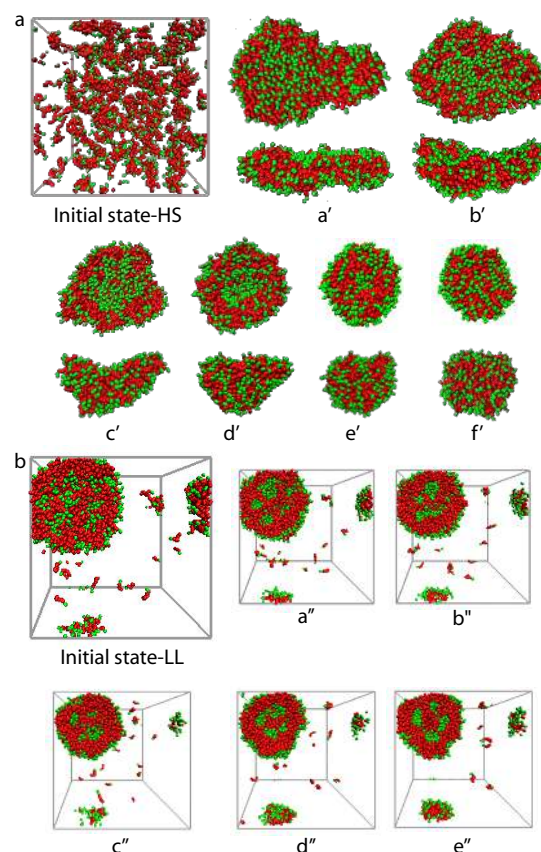


Fig. 3 (a) The top view and front view of the aggregates formed from HS by A_2B_7 BCPs at different time: (a') 1710τ; (b') 1800τ; (c') 1890τ; (d') 1980τ; (e') 2040τ; (f') 2100τ according to the mechanism I of bilayer membrane closure with interaction parameters $a_{AB}=70$, $a_{AS}=30$, $a_{BS}=60$ and $\phi=0.10$. (b) The front view of the aggregates formed from the LL ($a_{AB}=50$, $a_{AS}=30$, $a_{BS}=40$), has experienced a prenucleation time of $6.0 \times 10^4 \tau$ by A_2B_7 diblock copolymers at different time: (a'') 300τ; (b'') 600τ; (c'') 1500τ; (d'') 2100τ; (e'') 3000τ according to the mechanism II of nucleation growth with the same parameters as above. Green and red beads represent block A and block B, respectively.

of 40^3 are selected for presentation in Fig. S2 (in ESI).

Fig. 3(b) depicts the vesicle formation of amphiphilic BCPs from LL pathway. Vesicles formed through nucleation and growth mechanism (mechanism II), begin with phase-separated polymer-rich liquid droplets. Thanks to the existing large droplet that can be treated as the core, a more convenient growth and evolution occurring in unimer addition processes is preferred. The free polymers directly incorporate with large droplets or form small micelles by colliding with each other before merging into the collective. The large droplets are incompact and not tightly packed, and therefore a small number of solvent particles are originally wrapped in, and then they will gather to the center driven by the interaction, that is, to form vesicles ultimately. All these actions happen in a short period of time, during the detailed observation of the internal structure of the MCVs. It is found that although the small clusters of hydrophilic particles are dispersed, they can be generally distributed on a virtual spherical shell with a certain distance from the center (sometimes also exist around the center), and a small number of solvent particles are wrapped in these small clusters.

To evaluate the formation pathway of vesicles, we carry out the statistic of solvent number in the aggregates. Fig. 4 shows the temporal evolution of percentage of solvents in the aggregates via the LL and HS pathways. The percentage at the time t is defined as $p_{LL(HS)}(t) = n_{t,LL(HS)}/n_{f,HS} \times 100\%$. Herein, $n_{t,LL(HS)}$ and $n_{f,HS}$ are the number of solvent beads at time t via the LL (HS) pathway and the final solvent number via the HS pathway, respectively. The HS pathway has a steep rise and then gradually declines with time. In the front view at $6.0 \times 10^4 \tau$ in the insets, there is a certain amount of solvents inside the vesicle, while this number is obviously larger via the LL pathway.

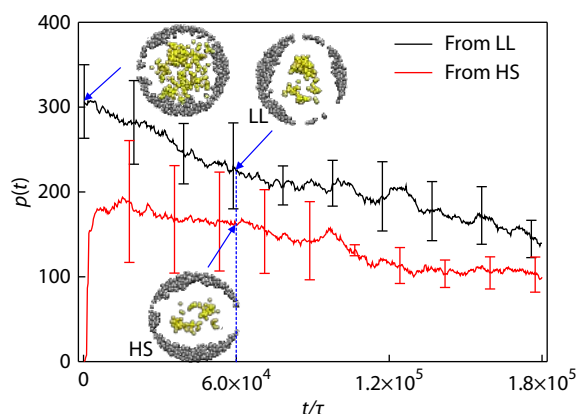


Fig. 4 Temporal evolution of percentage $p(t)$ of solvents in the aggregates formed from LL (experienced a prenucleation time of $6.0 \times 10^4 \tau$) and HS. Insets depict the distribution of solvent beads in the aggregates. The silver spheres highlight the outer part of aggregates.

The LL pathway is also applied to program the self-assembly behavior of amphiphilic BCPs at various interaction parameters a_{BS} and a_{AB} . At the initial time of LL pathway, the large droplet of BCPs here itself is like a sponge having a large capacity of solvents, evenly and dispersed, so the fact that

vesicles formed by this path at some stage can carry much more solvents is predictable and reasonable in the focused context of our study. The morphological phase diagram of aggregates in terms of the interaction parameters a_{BS} and a_{AB} via LL pathway is provided in Fig. S3 (in ESI). In comparison with the case of HS pathway, the phase diagram of aggregates is extremely similar. This fact implies that the final morphologies of aggregates are only related to thermodynamic parameters.

In these two processes, the divergence between the downward trend and the steep rise and subsequent smooth of solvent fluctuations is obvious. The degree of decline has little to do with the time points of the selected LL (Fig. S4 in ESI) and the fluctuations during the ascent are also independent of random numbers (Fig. S5 in ESI). From the perspective of the quantity of solvents inside the early vesicles, the origin LL has an obvious numerical advantage over the HS departure until they reach the thermodynamically stable state over a sufficiently long period of time (Fig. S6 in ESI). We continue to pay attention to LL itself and the impact of LLPS on the resulting vesicles' structural properties, especially internal solvents.

Effect of Concentration on the Vesicle Size and Structure

Concentration is undisputedly very important as a direct impact on the size of aggregates. We explore the sensitivity of large droplets to concentration under LLPS, including their difference of internal structure and external dimensions. The radial distribution of the hydrophilic and hydrophobic blocks and the radius of large droplets are presented in Fig. 5. As the curves show, the A beads are more evenly distributed in the droplets from the center to a certain range, and the trend of the curve is also roughly opposite to that of the B beads, that is, one increases and the other decreases. As the concentration increases, particles will also appear farther from the center, which qualitatively explains the fact that the size enlarges. Compared with MCV, the shape of droplet is not regularly spherical, so that in order to improve the accuracy, we first sum the total number of particles A, B and S to make up the large droplet. We can get the volume of the aggregate and the effective radius R of the large droplet, which is approximately proportional to the concentration (Fig. 5b) as a function of $\phi^{1/3}$. The concentration also affects the number of solvents in the large droplets, but the density of the solvents seems constant, as shown in Fig. 5(c), suggesting that the solvent in the droplet has the definite density.

We also consider the stepwise self-assembly of amphiphilic BCPs from LL and HS at different concentrations. As for the radial distribution of both the hydrophilic and hydrophobic blocks and the solvents in Figs. 6(a)–6(c), the opposite trend between A beads and B beads appears as well. Although the internal morphology of MCVs formed with HS and LL as the initial states is roughly the same, it can be seen that the situation starting from LL in the radial distribution of $\Phi_A(r)$ is slightly different from that of HS. The two peaks painted by the red curve have a certain degree of right shift, that is, the hydrophilic particles are wrapped in the outermost layer of the vesicle and the outer layer of the solvents are more likely to appear at positions farther from the center of mass. In oth-

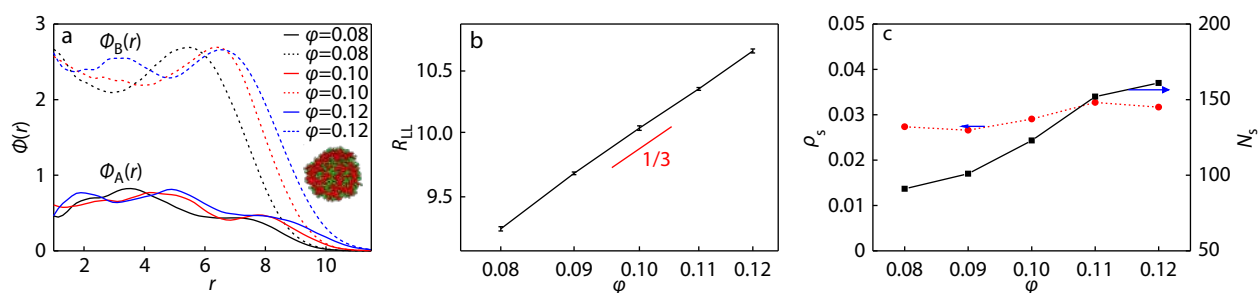


Fig. 5 (a) Radial distribution of hydrophilic block $\Phi_A(r)$ and hydrophobic block $\Phi_B(r)$ as a function of the distance r from the centroid of the large droplets under different concentrations at $a_{AB}=50$, $a_{AS}=30$, $a_{BS}=40$. Inset is the large droplet formed by A_2B_7 at $\phi=0.10$; (b) Radius of large droplets in LL (R_{LL}) at different concentrations; (c) Density ($\rho_s=N_s/V_{agg}$) and number of solvents (N_s) in the large droplets at different concentrations.

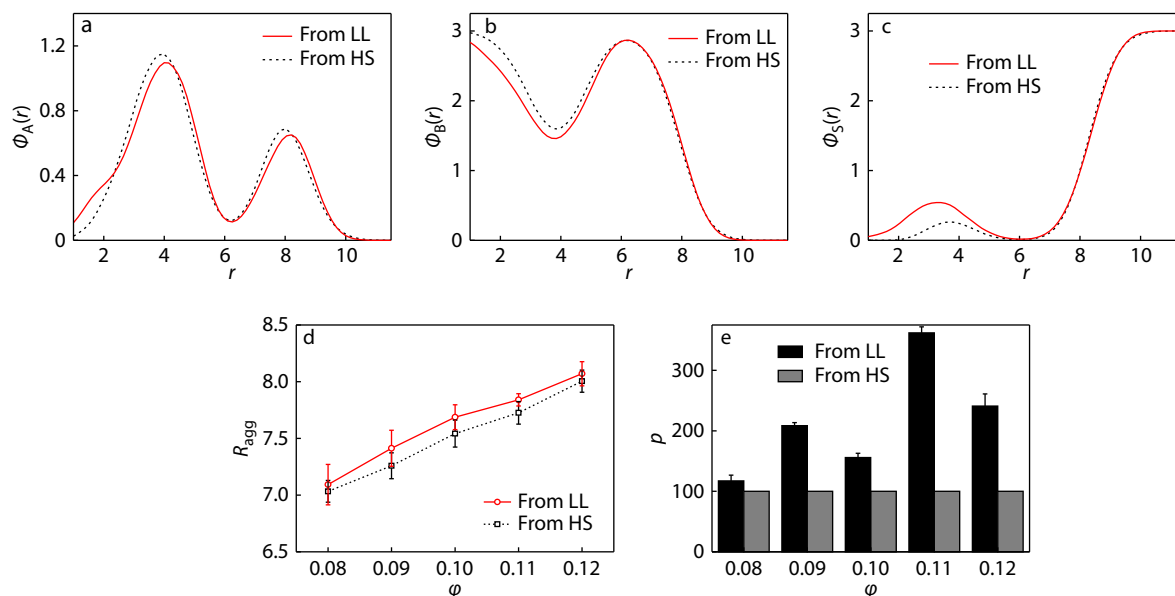


Fig. 6 Radial distribution of (a) hydrophilic block $\Phi_A(r)$, (b) hydrophobic block $\Phi_B(r)$ and (c) solvents $\Phi_S(r)$ with the distance r from the mass center of the vesicles at $a_{AB}=70$, $a_{AS}=30$, $a_{BS}=60$ and $\phi=0.10$ formed by A_2B_7 and self-assembled from LL ($a_{AB}=50$, $a_{AS}=30$, $a_{BS}=40$) and HS. (d) Radius of the aggregates (R_{agg}) formed from different paths under different concentrations. (e) Histogram of the percentage p of solvents in the hydrophobic inner cavity of MCVs generated by different pathways at different concentrations.

er words, the outer size and inner cavity size of the vesicle became larger. In response to the curve of $\Phi_S(r)$, the obvious bulge with initial state of LL again supports the fact of more solvents.

We now consider the size of MCVs formed by the different pathways shown in Fig. 6(d). Error bars are added and the average of multiple sets of data are taken, in order to lessen accidental errors. Obviously, the size of vesicles formed from LL is larger than that from HS. We take the solvent number in the MCV from HS as a reference state and get the percentage of solvents in MCV shown in Fig. 6(e). It can be seen that the final amount of solvent in vesicles formed from LL is much more than that from HS, which is particularly prominent when the concentration is 0.11.

CONCLUSIONS

In summary, when the interaction between the hydrophobic blocks and the solvents is at low values, a LLPS region appears, enriching the known phase morphology system of amphiphilic BCPs. The division of each typical region is clarified by analyzing

the radial distribution of the aggregates in the simulation system. Five characteristic morphologies are found: HS, LL, TS, LCM and MCV. The self-assembly of amphiphilic BCPs can be regulated and controlled. We quench HS into an unstable two-phase region with respect to copolymer-solvent demixing, and after a prenucleation time, stable nuclei (LL) emerge, which then grow steadily into vesicles until almost all the copolymers in solution are exhausted. We make the stepwise self-assembly with a start from LL abides by nucleation and growth mechanism, differing from the experience throughout bilayer membrane closure mechanism begun with HS. Many metastable states appear during the latter process, while the former makes the uniquely affirmatory situation of the system. It is demonstrated that early vesicles formed via this path have a larger size and a significantly larger cavity due to the quantitative statistics regarding to internal solvents. Concentration is the most direct factor affecting the vesicle size. A linear relationship with the size of large droplets is established. We examined the application of LLPS state to control the self-assembly path of vesicles, and its impact on the formed vesicles itself is expected to create a good start for its development and

application in biomedicine, food science, separation science and template synthesis as transport vehicles and reaction vessels.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <https://doi.org/10.1007/s10118-021-2563-6>.

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