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ABSTRACT

We conducted molecular dynamics (MD) simulations in a binary Lennard-Jones system as a model system for molecular solutions and investigated the mechanism of liquid-liquid phase separation (LLPS), which has recently been recognized as a fundamental step in crystallization and organelle formation. Our simulation results showed that LLPS behavior varied drastically with the size ratio of solute to solvent molecules. Interestingly, increasing the size ratio can either facilitate or inhibit LLPS, depending on the combination of interaction strengths. We demonstrated that the unique behavior observed in MD simulation could be reasonably explained by the free energy barrier height calculated using our thermodynamic model based on the classical nucleation theory. Our model proved that the molecular size determines the change in number of interaction pairs through LLPS. Varying the size ratio changes the net number of solute-solvent and solvent-solvent interaction pairs that are either broken or newly generated per solute-solute pair generation, thereby inducing a complicated trend in LLPS depending on the interaction parameters. As smaller molecules have more interaction pairs per unit volume, their contribution is more dominant in the promotion of LLPS. Consequently, as the size ratio of the solute to the solvent increased, the LLPS mode changed from solute-related interaction-driven to solvent-related interaction-driven.

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I. INTRODUCTION

Liquid-liquid phase separation (LLPS), a demixing process into a coexisting state of dense and dilute liquid phases, is a ubiquitous phase transition process that is involved in the initial stage of crystallogenic and biological phenomena. In biology, it has been recently reported that LLPS is involved in the pathogenesis of diseases and the formation of membrane-less organelles.^{1–3} For example, LLPS precedes α -synuclein aggregation, which causes Parkinson's disease, and a mutant hemoglobin polymer nucleation, which causes sickle cell disease.^{4,5} In addition, during the self-assembly of amphiphilic polymers to form various higher-order structures such as micelles and vesicles, LLPS also precedes micelle formation and accordingly determines the structural properties of the resultant vesicles.⁶ In crystallization processes, LLPS is observed in several systems, including proteins,⁷ colloidal particles,⁸ and small organic molecules,^{9,10} and is recognized as a general crystal nucleation mechanism. In addition, the formation of prenucleation clusters (PNCs),^{11,12} which

have been discussed for their origin and role in biomineralization, is explained in the context of LLPS.¹³ These examples indicate the importance of LLPS in understanding and controlling crystallogenic and biological processes through the nucleation of liquid droplets.

From an engineering point of view, however, LLPS has often been considered undesirable because it can degrade the crystalline quality by introducing impurities and inducing aggregation.^{14,15} Consequently, in industrial crystallization processes, LLPS has been avoided by seeding,¹⁶ ultrasonic irradiation,¹⁷ and optimizing operation schemes based on phase diagrams^{18,19} to produce high-quality crystals. However, recent studies have emphasized the positive effects of LLPS on crystallization. By deliberately promoting LLPS in the crystallization processes, one can obtain crystals of different sizes,²⁰ shapes,^{21,22} and surface morphologies²³ that cannot be obtained using ordinary one-step crystallization. The LLPS-induced aggregation of crystals also enables the one-pot synthesis of spherical granules composed of crystals.²⁴

Owing to this, there has been a growing interest in the physical factors that drive LLPS processes. A previous study has demonstrated that short-range attractive interactions play a key role in the occurrence of LLPS for proteins before crystallization because they put a liquid–liquid phase boundary in a liquid–solid equilibrium region.²⁵ Various types of interactions owing to complicated protein structures are reported to cause LLPS, although their contributions are still ambiguous.²⁶ For small molecules, the lack of anchoring sites that bring a specific molecular conformation may be one of the major causes of LLPS, as it often occurs in solutions of hydrophobic molecules with low polarity.²⁷ However, the molecular mechanism of LLPS is not well understood, and there is still much room for further investigation of how molecular interactions and structural properties affect LLPS.

Simple model systems, such as coarse-grained molecules, have been utilized for systematic investigations to elucidate the universal molecular mechanisms of LLPS. A typical case is a simulation study using modified Lennard-Jones (LJ) molecules as models of globular proteins,²⁸ demonstrating that density fluctuations close to a metastable fluid–fluid critical point strongly enhance nucleation. Molecular simulations of LJ molecules also revealed that phase separation is promoted in crowded environments, such as inside cell nuclei.^{29,30} Simulation studies using model molecules equipped with isotropic and orientation-dependent attractive interactions^{31,32} demonstrated that strong isotropic interactions are crucial for phase separation before crystallization. Even for biological systems, which are naturally complex, a key factor that determines the morphology of domains formed through LLPS was clarified by simplifying the target system and referring to the well-known viscoelastic phase separation of soft matter systems.³³ Thus, simple model systems can extract the physical factors that determine LLPS behavior from a complicated system of interest.

Herein, we focus on the LJ system because it has been successfully employed to obtain molecular-level insights for a wide range of fundamental phenomena, such as crystal nucleation,^{34,35} solid solution formation,^{36,37} and solvation.³⁸ In our previous study, we took advantage of binary LJ systems to unveil the key factors governing nucleation pathway selection.³⁹ In this study, we conducted molecular dynamics (MD) simulations in the binary LJ systems to systematically investigate the effects of molecular size and interaction strength on LLPS behavior. Our simulation results show that the driving force for LLPS varies drastically with molecular size. Depending on the interaction strength, the size difference between the solute and solvent molecules either facilitates or suppresses

LLPS. We confirmed that the opposite dependence of LLPS behavior on molecular size can be explained using free energy analysis based on the classical nucleation theory (CNT). According to our analysis, the molecular size defines the number of interaction pairs per unit volume and, accordingly, determines the dominant interactions for LLPS promotion and inhibition. As the molecular size ratio of the solute to solvent increases, the LLPS mode transitions from solute-driven to solvent-driven interactions. In addition to the contribution of solute-related interactions, which have been primarily discussed in previous studies,^{26,40,41} our findings indicate that solvent–solvent interactions also play a significant role in determining the driving force of LLPS, providing novel strategies for controlling LLPS processes.

II. METHODS

A. Molecular dynamics simulation

Molecular dynamics simulations in the binary LJ system were conducted following the method reported in our previous study.³⁹ The outline is as follows: The system was composed of 1500 solute molecules A and 24 000 solvent molecules B. The two-body interaction of LJ molecules ϕ_{ij} is expressed as follows:

$$\phi_{ij} = 4\epsilon \left\{ \left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right\}, \quad (1)$$

where r_{ij} is the distance between molecules i and j , ϵ and σ are LJ parameters representing the interaction strength and the effective molecular size, respectively. The LJ parameters and the molecular weights of the solutes and solvents used in the MD simulations are summarized in Table I. We varied the size ratio of solute to solvent, σ_{AA}/σ_{BB} , under four parameter sets with different values of $\epsilon^* = \epsilon/kT$ (Table I parameter sets 1–4) and systematically investigated the effects of interaction parameters on LLPS behavior. The values of ϵ^* (solute–solute ϵ_{AA}^* , solvent–solvent ϵ_{BB}^* , and solute–solvent ϵ_{AB}^*) were chosen so that the values of $\Delta\epsilon^* = \epsilon_{AA}^* + \epsilon_{BB}^* - 2\epsilon_{AB}^*$ for the four parameter sets were approximately the same by adjusting the strength of solute–solvent interaction (ϵ_{AB}^*). This is because our previous study demonstrated that the value of $\Delta\epsilon^*$ primarily determines the LLPS behavior for a system with identical molecular size ($\sigma_{AA} = \sigma_{BB} = \sigma_{AB}$).³⁹ Because nucleation is a stochastic process, we performed at least ten simulation runs up to 200 ns with different initial solution configurations for a single condition. To detect the onset of LLPS, in which the solute molecules spontaneously form liquid clusters from the initial solution state, we calculated and traced the

TABLE I. Details of simulation parameters.

	$\epsilon^* = \epsilon/kT[-]$				σ (Å)			Molecular weight (g/mol)
	ϵ_{AA}^*	ϵ_{BB}^*	$\Delta\epsilon^*$	(ϵ_{AB}^*)	σ_{AA}	$\sigma_{AA}/\sigma_{BB}[-]$	σ_{AB}	
Set 1	1.34	1.17	0.73	(0.89)	3.0	0.85–1.15	$\frac{\sigma_{AA} + \sigma_{BB}}{2}$	5
Set 2	1.34	1.34		(0.97)				
Set 3	2.34	1.17	0.60	(1.45)				
Set 4	2.34	1.34		(1.54)				

number of solute molecules in the largest assembled structure, n_L , based on the procedure proposed in the work of Tribello *et al.*⁴²

All the simulation runs were performed in the *NPT* ensemble at 180 K and 0.77 kbar by using the velocity rescaling thermostat⁴³ and the Parrinello–Rahman barostat⁴⁴ of the open-source simulator GROMACS (version 2019.4). Periodic boundary conditions were applied in all three directions of a cubic cell with dimensions of $\sim 9 \times 9 \times 9 \text{ nm}^3$. The time step was set to 2 fs. The LJ-potential was cut and shifted at $4 \sigma_{\max}$ ($\sigma_{\max} = \max(\sigma_{AA}, \sigma_{BB})$). The calculation of n_L was performed using the PLUMED software package (version 2.5.4).

B. Calculation of free energy barrier for LLPS

CNT has been used as a theoretical platform to describe nucleation phenomena. We derived the free energy barrier of LLPS processes based on CNT. We applied CNT to the formation process of a spherical nucleus of a solute-rich liquid cluster with composition x_{is} ($i = A, B$) from the solution phase of an initial composition $x_{i0} = N_i/(N_A + N_B)$, where N_A and N_B indicate the number of solutes and solvents, respectively. We assume that the formation of a liquid cluster nucleus does not change the initial solution phase. The number of solute and solvent molecules in the nucleus is defined as n_L and $n_B (= n_L x_{Bs}/x_{As})$. The free energy change due to formation of the nucleus is expressed as the sum of the volume term ΔG_V , the stabilization term by forming a new stable phase, and the interface term ΔG_S , the energy penalty term by the interface formation:

$$\Delta G = \Delta G_V + \Delta G_S. \quad (2)$$

The volume term ΔG_V can be calculated based on the chemical potential, while the interface term ΔG_S is expressed as a product of the interfacial tension of the solution and the new phase, γ_L , and the interface area, S_L :

$$\Delta G = n_L \{ \mu_A(x_{As}) - \mu_A(x_{A0}) \} + n_B \{ \mu_B(x_{Bs}) - \mu_B(x_{B0}) \} + \gamma_L S_L, \quad (3)$$

where $\mu_i(x_i)$ is the chemical potential of component i in a solution with composition x_i . As derived in our previous study,³⁹ by assuming that the volume change due to mixing of solutes and solvents is negligible and considering a binary LJ system as a regular solution, we can express $\mu_i(x_i)$ and S_L as functions of the molecular properties (ϵ and σ). The resultant equation of ΔG is given as follows:

$$\Delta G = n_L kT \left[\frac{\sum x_{is} \ln(x_{is}/x_{i0})}{x_{As}} - \beta \frac{(\phi_{As} - \phi_{A0})^2}{\phi_{As}} \right] + \gamma_L a_L^{\frac{2}{3}} \left\{ n_L \left(1 + \frac{r x_{Bs}}{x_{As}} \right) \right\}^{\frac{2}{3}}, \quad (4)$$

with

$$\beta = Z_v \left\{ \epsilon_{AA}^* + \left(\frac{\sigma_{AA}}{\sigma_{BB}} \right)^3 \epsilon_{BB}^* - 2 \left(\frac{\sigma_{AA}}{\sigma_{AB}} \right)^3 \epsilon_{AB}^* \right\} - \left\{ 1 + \left(\frac{\sigma_{AA}}{\sigma_{BB}} \right)^3 - 2 \left(\frac{\sigma_{AA}}{\sigma_{AB}} \right)^3 \right\},$$

$$r = \left(\frac{\sigma_{BB}}{\sigma_{AA}} \right)^3, \quad \phi_A = \frac{x_A}{x_A + r x_B}, \quad \phi_B = \frac{r x_B}{x_A + r x_B},$$

where Z_v is the evaporation enthalpy of LJ molecules reduced by ϵ , $a_L = 6\sqrt{\pi}v_L$, and v_L is the occupied volume of solute molecules in the solution. Based on the equilibrium data of LJ molecule by Hansen and Verlet,⁴⁵ we set $Z_v = 6.62$ and $v_L = 1.143\sigma_{AA}^3$. The first and second terms in Eq. (4) represent the volume and interface terms, respectively. The contributions of interaction strength ϵ and molecular size σ on the volume term are represented by the interaction parameter β .

To calculate γ_L , we utilized the following simple estimation formula for interfacial tension that we developed in our previous study:³⁹

$$\gamma_L = \gamma_I + \gamma_{II} - 2C_{I,II}(\gamma_I \gamma_{II})^{\frac{1}{2}}, \quad (5)$$

where γ_I and γ_{II} are the surface tension of the initial solution and liquid cluster phases, respectively, and $C_{I,II}$ is a parameter related to the affinity of these two phases. $C_{I,II}$ and the surface tension of a phase with solute composition x_A are expressed as follows:

$$\gamma(x_A) = \kappa \frac{kT(\epsilon_{AA}^* \phi_A^2 + 2\epsilon_{AB}^* \phi_A \phi_B + \epsilon_{BB}^* \phi_B^2)}{\sigma_{AA}^2 x_A + \sigma_{BB}^2 x_B}, \quad (6)$$

$$C_{I,II} = 1 - \left(1 - \frac{\epsilon_{AB}^*}{\sqrt{\epsilon_{AA}^* \epsilon_{BB}^*}} \right) (X_{AI} - X_{AII}), \quad (7)$$

where

$$X_A = \frac{\sigma_{AA}^2 x_A}{\sigma_{AA}^2 x_A + \sigma_{BB}^2 x_B}.$$

For liquid–liquid interface, κ is 1.72. By substituting the compositions of the initial solution and liquid cluster phases, x_{i0} and x_{is} , into Eqs. (5)–(7), we calculated γ_L .

Figure 1 shows a typical profile of ΔG with respect to n_L calculated using Eq. (4). As the volume and interface terms are proportional to n_L and $n_L^{2/3}$, respectively, the interface term is dominant for a small nucleus, which increases the free energy of the system, whereas a large nucleus enhances the contribution of the volume term and decreases the free energy. Thus, the system must

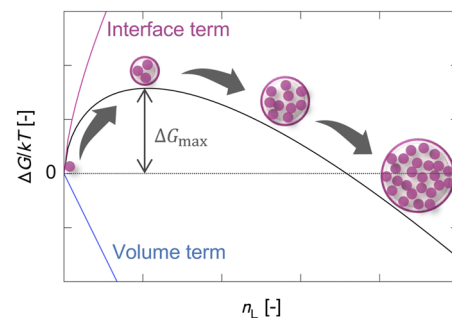


FIG. 1. Typical free energy profile with n_L based on CNT.

overcome an energy barrier, $\Delta G_{\max}$, to initiate nucleation. By differentiating Eq. (4) with n_L , we obtain an analytical solution for the energy barrier height as follows:

$$\frac{\Delta G_{\max}}{kT} = \frac{4}{27} \frac{\left(\gamma_L a_L^{\frac{2}{3}} \left\{ \left(1 + \frac{r x_{Bs}}{x_{As}} \right) \right\}^{\frac{2}{3}} / kT \right)^3}{\left[\sum \frac{x_{is} \ln(x_{is}/x_{i0})}{x_{As}} - \beta \frac{(\phi_{As} - \phi_{A0})^2}{\phi_{As}} \right]^2}. \quad (8)$$

In Eq. (8), the contribution of the interface term appears in the numerator and that of the volume term in the denominator.

III. RESULTS AND DISCUSSION

A. Simulation results

Figure 2(a) shows typical snapshots of the simulation for parameter set 1, $(\epsilon_{AA}^*, \epsilon_{BB}^*, \Delta\epsilon^*) = (1.34, 1.17, 0.73)$, with $\sigma_{AA}/\sigma_{BB} = 1$ and the corresponding time course of n_L . In the initial solution state [Fig. 2(a-I)], n_L was nearly zero because the solute molecules were randomly dispersed in the solution and there were no assembled structures. After the induction time t_L , n_L rapidly increases, indicating the nucleation of a single solute-rich liquid cluster, as shown in Fig. 2(a-II), which is an LLPS process. Owing to the stochasticity of nucleation, the induction time t_L varied from run to run. We thus calculated an average induction time, τ_L , of 10 or more runs (see “Appendix” section; a detailed definition of t_L and τ_L is available). The calculated τ_L under various values of σ_{AA}/σ_{BB} for the parameter sets 1–4 are shown in Fig. 2(b). For parameter set 1, τ_L monotonically decreases by four orders of magnitude with an increase in σ_{AA}/σ_{BB} from 0.95 to 1.15, indicating that a slight increase in σ_{AA}/σ_{BB} significantly facilitates nucleation. At $\sigma_{AA}/\sigma_{BB} = 0.95$, nucleation was not observed in all of the simulation runs within the simulation time up to 200 ns, as indicated by the open

circle in Fig. 2(b). For $\sigma_{AA}/\sigma_{BB} \leq 0.90$, the phase-separated state was no longer stable compared with the solution state, which was confirmed by control simulations conducted from either a crystal or a liquid cluster as initial configurations. Thus, under parameter set 1, LLPS was less likely to occur for smaller values of σ_{AA}/σ_{BB} . We then conducted the same series of simulations for parameter set 2, $(\epsilon_{AA}^*, \epsilon_{BB}^*, \Delta\epsilon^*) = (1.34, 1.34, 0.73)$, in which the solvent–solvent interaction intensified while keeping the value of $\Delta\epsilon^* = \epsilon_{AA}^* + \epsilon_{BB}^* - 2\epsilon_{AB}^*$ constant. Although increasing the value of ϵ_{BB}^* shortened τ_L , the same trend of σ_{AA}/σ_{BB} vs τ_L was observed as with parameter set 1.

In contrast, intensifying solute–solute interaction results in the completely opposite trend of σ_{AA}/σ_{BB} vs τ_L as shown by the plots of parameter set 3, $(\epsilon_{AA}^*, \epsilon_{BB}^*, \Delta\epsilon^*) = (2.34, 1.17, 0.60)$, where we slightly varied $\Delta\epsilon^*$ so that τ_L at $\sigma_{AA}/\sigma_{BB} = 1$ is similar to that of parameter set 1. A smaller value of σ_{AA}/σ_{BB} induced a rapid LLPS, whereas a larger value of σ_{AA}/σ_{BB} inhibited the nucleation. Moreover, increasing the value of ϵ_{BB}^* from parameter set 3 lengthened τ_L while the trend of σ_{AA}/σ_{BB} vs τ_L continued to monotonically increase (parameter set 4, $(\epsilon_{AA}^*, \epsilon_{BB}^*, \Delta\epsilon^*) = (2.34, 1.34, 0.60)$).

Therefore, the induction time of LLPS varies in a complex manner depending on the molecular size ratio σ_{AA}/σ_{BB} and interaction strengths ϵ^* . We observed the following two types of unique trends: First, two opposite variation trends of σ_{AA}/σ_{BB} vs τ_L appear, with a monotonic decrease of τ_L by several orders of magnitude for a smaller ϵ_{AA}^* and a monotonic increase for a larger ϵ_{AA}^* . Second, strengthening the solvent interaction facilitates or inhibits LLPS, depending on the combination of parameters.

B. Free energy barrier of LLPS

To explain the complicated LLPS trends observed in our MD simulations, we calculated the free energy barrier $\Delta G_{\max}$ of the LLPS

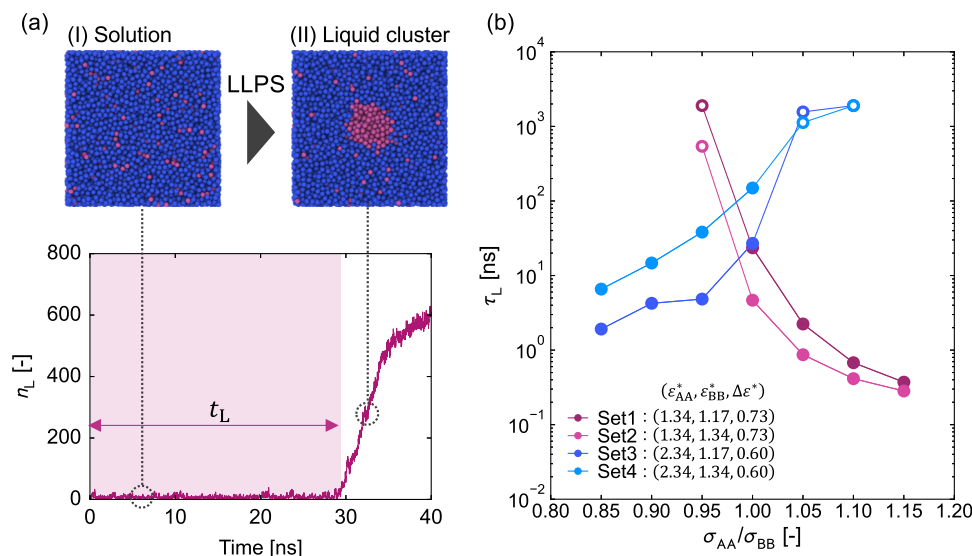


FIG. 2. Observed LLPS behavior for different parameter sets. (a) Snapshots from MD simulation and time courses of n_L for parameter set 1, $(\epsilon_{AA}^*, \epsilon_{BB}^*, \Delta\epsilon^*) = (1.34, 1.17, 0.73)$, with $\sigma_{AA}/\sigma_{BB} = 1$. (b) Average induction time τ_L obtained from simulations under various size ratios σ_{AA}/σ_{BB} for parameter sets 1–4. The open circles indicate that LLPS was not observed within the simulation time of 200 ns in at least one simulation run.

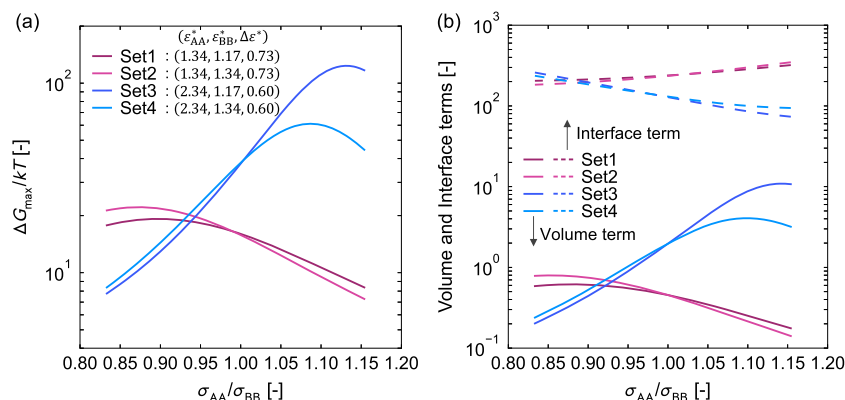


FIG. 3. Variation of (a) energy barrier height $\Delta G_{\max}$ and (b) volume [the denominator of Eq. (8)] and interface [the numerator of Eq. (8)] terms with size ratio σ_{AA}/σ_{BB} calculated for parameter sets 1–4 of the MD simulation.

processes because the induction time was directly related to the height of the energy barriers. Figure 3(a) shows the variation of $\Delta G_{\max}$ as a function of the size ratio σ_{AA}/σ_{BB} calculated using Eq. (8) for parameter sets 1–4. The calculated profiles of $\Delta G_{\max}$ are basically decreasing functions for parameter sets 1 and 2 and increasing functions for those of 3 and 4 within the molecular size range of our investigation; however, they have maxima at σ_{AA}/σ_{BB} values close to the ends of the investigated range. Based on the calculated $\Delta G_{\max}$ profiles, LLPS is more likely to occur with larger solutes (or smaller solvents) for parameter sets 1 and 2 and smaller solutes (or larger solvents) for those of sets 3 and 4, which are consistent with the two opposite variation trends of σ_{AA}/σ_{BB} vs τ_L observed in our MD simulations [Fig. 2(b)]. Furthermore, the $\Delta G_{\max}$ values for parameter set 2 are smaller than those for parameter set 1 in the range of $\sigma_{AA}/\sigma_{BB} > 1$, and those for parameter set 4 are larger than those for parameter set 3 for $\sigma_{AA}/\sigma_{BB} < 1$, which consistently explains the simulation results obtained by varying the solvent–solvent interaction. Although, in ranges with higher barriers for each parameter set (sets 1 and 2: $\sigma_{AA}/\sigma_{BB} < 1$, sets 3 and 4: $\sigma_{AA}/\sigma_{BB} > 1$) the profiles [Fig. 3(a)] appear to contradict the simulation results [Fig. 2(b)], this is possibly because a statistically sufficient number of nucleation events to accurately calculate τ_L was not observed within the simulation time up to 200 ns due to the limitation of computational cost. Overall, our thermodynamic model based on CNT successfully captured two unique physical trends in the simulated system.

We then investigated the physical factor that causes the above unique trends by decomposing $\Delta G_{\max}$ into the volume and interface terms. Although we focused on the reversal trend of σ_{AA}/σ_{BB} vs τ_L primarily caused by differences in the solute–solvent interaction, the key factor for the two types of unique physical trends was identical, as discussed later. Figure 3(b) shows how the volume term [the denominator of Eq. (8)] and interface term [the numerator of Eq. (8)] vary with the size ratio σ_{AA}/σ_{BB} . The $\Delta G_{\max}$ profiles in Fig. 3(a) are more similar to those of the volume term than those of the interface term, indicating that the volume term predominantly determines the barrier height trend.

The volume term is composed of the enthalpic term $\Delta h = -\beta(\phi_{As} - \phi_{A0})^2/\phi_{As}$ and the entropic term $\Delta s = \sum x_{is} \ln(x_{is}/x_{i0})/x_{As}$. To further determine which component

in the volume term caused the reversal trend of the barrier height between parameter sets 1 and 2, as well as 3 and 4, we investigated the relationship between the size ratio σ_{AA}/σ_{BB} and the components Δh and Δs . Figure 4(a) clearly shows that Δh governs the trend of the volume term and accordingly determines that of the barrier height,

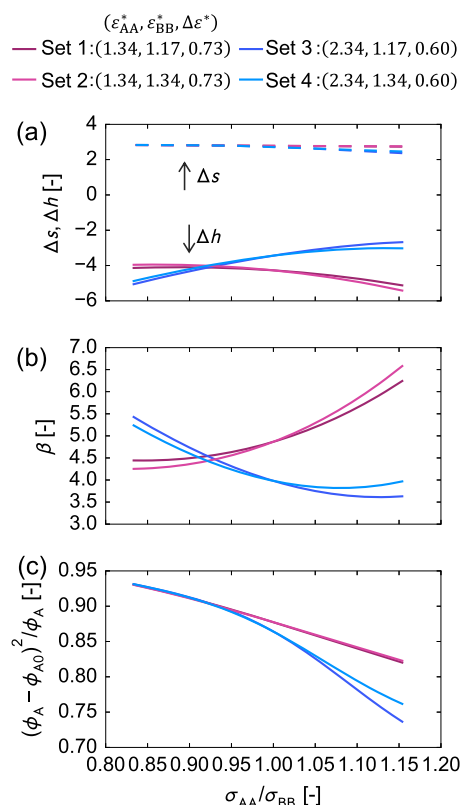


FIG. 4. Relationship between size ratio σ_{AA}/σ_{BB} and (a) entropic term $\Delta s = \sum x_{is} \ln(x_{is}/x_{i0})/x_{As}$, and enthalpic term $\Delta h = \beta(\phi_{As} - \phi_{A0})^2/\phi_{As}$, (b) β , and (c) volume fraction term for parameter sets 1–4.

while Δs hardly varies with σ_{AA}/σ_{BB} . Further decomposition of Δh reduces to the interaction parameter, β , and the volume fraction term, $(\phi_{As} - \phi_{A0})^2/\phi_{As}$. As shown in Figs. 4(b) and 4(c), the profiles of β show opposite trends between parameter sets 1 and 2, as well as 3 and 4, whereas the variation of the volume fraction term shows a monotonical decrease for all four parameter sets. Therefore, the characteristic trend of the enthalpic term stems from the interaction parameter β , which accordingly indicates that β governs the barrier height of LLPS.

C. Mechanism—How molecular size affects LLPS

Now that we know that β is the key factor in determining LLPS behavior, we discuss the role of interaction parameters (ϵ and σ) in the LLPS processes by considering the physical meaning of β . To simplify our discussion, we consider an approximate form of β as follows because the first term is much larger than the second one:

$$\begin{aligned}\beta &= Z_v \left\{ \epsilon_{AA}^* + \left(\frac{\sigma_{AA}}{\sigma_{BB}} \right)^3 \epsilon_{BB}^* - 2 \left(\frac{\sigma_{AA}}{\sigma_{AB}} \right)^3 \epsilon_{AB}^* \right\} \\ &\quad - \left\{ 1 + \left(\frac{\sigma_{AA}}{\sigma_{BB}} \right)^3 - 2 \left(\frac{\sigma_{AA}}{\sigma_{AB}} \right)^3 \right\} \\ &\approx Z_v \left\{ \epsilon_{AA}^* + \left(\frac{\sigma_{AA}}{\sigma_{BB}} \right)^3 \epsilon_{BB}^* - 2 \left(\frac{\sigma_{AA}}{\sigma_{AB}} \right)^3 \epsilon_{AB}^* \right\}.\end{aligned}\quad (9)$$

Equation (9) implies that β is expressed as the difference between the strength of like-pair interactions (ϵ_{AA}^* and ϵ_{BB}^*) and that of unlike-pair interaction (ϵ_{AB}^*), which accurately reflects the following microscopic molecular exchanges involved in an LLPS process, as shown in Fig. 5. In the initial solution state, solvent molecules surround solute molecules, and most solute-related interactions in the solution are solute–solvent interactions. During LLPS, the positions of solute and solvent molecules are exchanged; accordingly, these solute–solvent interactions are replaced by solute–solute and solvent–solvent interactions. Therefore, β represents the degree of stabilization by this replacement, that is, the driving force of LLPS from a molecular point of view. More specifically, $(\sigma_{AA}/\sigma_{ij})^3$ appears as the coefficient of each ϵ^* in Eq. (9), which indicates that the volume ratio of the solute and solvent molecules determines the contribution of each interaction to the driving force β . Physical pictures based on the meaning of the coefficients $(\sigma_{AA}/\sigma_{ij})^3$ are also shown in Fig. 5. A solute must break solute–solvent interactions to generate a solute–solute interaction for liquid cluster formation, which negatively contributes to the β value to prevent the system from undergoing LLPS. This process simultaneously generates new solvent–solvent interactions with the replaced solvent molecules, which contributes positively to the β value and facilitates LLPS. For a system of $\sigma_{AA} = \sigma_{BB}$, the net variations in the number of solvent–solvent and solute–solvent interaction pairs are plus one and minus two, respectively, per solute–solute pair generation, as

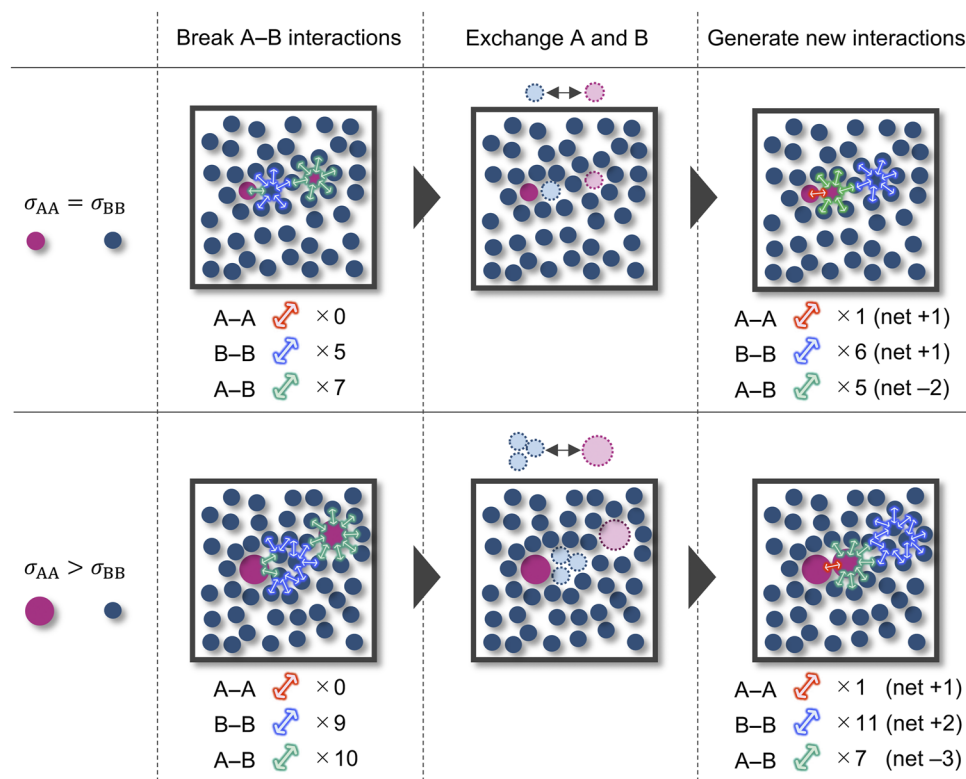


FIG. 5. Schematic of microscopic molecular exchanges involved in a LLPS process.

indicated by the coefficients of each ε^* in Eq. (9) with $(\sigma_{AA}/\sigma_{ij})^3 = 1$. Compared with a system of $\sigma_{AA} = \sigma_{BB}$, a larger solute ($\sigma_{AA} > \sigma_{BB}$) is surrounded by more solvents. As a result, a larger solute must break more solute–solvent interactions to generate a single solute–solvent interaction, as represented by a larger value of the coefficient $2(\sigma_{AA}/\sigma_{AB})^3 (>2)$. Simultaneously, a larger solute is replaced with more solvents to form a larger number of solvent–solvent pairs, as expressed by a larger value of the coefficient $(\sigma_{AA}/\sigma_{BB})^3 (>1)$. Thus, the molecular size σ defines the net number of solute–solvent and solvent–solvent interaction pairs that are either broken or newly generated by a single solute–solvent pair generation through LLPS, thereby determining the contribution of each interaction.

Based on the above discussion, we determine the interactions that effectively facilitate or inhibit LLPS by referring to Eq. (9). In the case of $\sigma_{AA} > \sigma_{BB}$, ε_{BB}^* and ε_{AB}^* have a larger contribution to the value of β than ε_{AA}^* because of the magnitude order of $1 < \sigma_{AA}/\sigma_{AB} < \sigma_{AA}/\sigma_{BB}$. Consequently, solvent-related interactions dictate whether LLPS is promoted or inhibited. The observed differences in τ_L between parameter sets 1 and 2, which occur for $\sigma_{AA}/\sigma_{BB} > 1$ [Fig. 2(b)], can be reasonably attributed to the difference between ε_{BB}^* and ε_{AB}^* . Conversely, in cases where $\sigma_{AA} < \sigma_{BB}$, the progress of LLPS is primarily governed by the solute-related interaction, as indicated by the condition $1 > \sigma_{AA}/\sigma_{AB} > \sigma_{AA}/\sigma_{BB}$. The discrepancies in τ_L between parameter sets 3 and 4, which can be observed for $\sigma_{AA}/\sigma_{BB} < 1$ [Fig. 2(b)], can be reasonably explained by the difference between ε_{AA}^* and ε_{AB}^* . Generally, it can be concluded that LLPS is driven by interactions between smaller molecules, which exhibit a larger number of interaction pairs per unit volume. This indicates that not only the solute-related interactions, which have been primarily discussed in previous studies,^{26,40,41} but also the solvent–solvent interactions play a significant role in the formation of a “solute”-rich liquid cluster through LLPS.

So far, the contribution of ε^* with fixed values of σ_{AA}/σ_{BB} has been discussed. The boundary values of σ_{AA}/σ_{BB} between LLPS promotion and inhibition with fixed interaction strengths are then discussed. Because the barrier height is governed by the value of β , the maximum of the barrier height in Fig. 3(a) approximately corresponds to the minimum of β in Fig. 4(b). This minimum of β comes from the trade-off between the number of broken solute–solvent interactions expressed by $2(\sigma_{AA}/\sigma_{AB})^3$ and that of new solvent–solvent interactions corresponding to $(\sigma_{AA}/\sigma_{BB})^3$. We thus derived the critical size ratio of solute and solvent molecules, $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$, defined as the ratio satisfying the condition stating that the derivative of β by $(\sigma_{AA}/\sigma_{BB})$ is zero ($\partial\beta/\partial(\sigma_{AA}/\sigma_{BB}) = 0$), as follows:

$$\left(\frac{\sigma_{AA}}{\sigma_{BB}}\right)_{\text{critical}} = 2 \left(\frac{Z\varepsilon_{AB}^* - 1}{Z\varepsilon_{BB}^* - 1} \right)^{\frac{1}{4}} - 1. \quad (10)$$

This equation can be simplified by assuming $Z\varepsilon^* \gg 1$:

$$\left(\frac{\sigma_{AA}}{\sigma_{BB}}\right)_{\text{critical}} = 2 \left(\frac{\varepsilon_{AB}^*}{\varepsilon_{BB}^*} \right)^{\frac{1}{4}} - 1. \quad (11)$$

The value of $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$ in Eq. (11) gives a critical size ratio at which the dependence of LLPS on σ_{AA}/σ_{BB} inversely changes for fixed interaction strengths. Under conditions of

$\sigma_{AA}/\sigma_{BB} < (\sigma_{AA}/\sigma_{BB})_{\text{critical}}$, LLPS is suppressed by increasing σ_{AA}/σ_{BB} (increasing the solute size or decreasing the solvent size) because the contribution of the broken solute–solvent interactions becomes dominant. In contrast, for $\sigma_{AA}/\sigma_{BB} > (\sigma_{AA}/\sigma_{BB})_{\text{critical}}$, LLPS is promoted by increasing the solute size (or decreasing the solvent size) because the contribution of the newly generated solvent–solvent interactions prevails over that of broken solute–solvent interactions. These discussions reasonably explain the results shown in Figs. 2(b) and 3(a), in which τ_L and ΔG_{max} are decreasing functions with σ_{AA}/σ_{BB} for parameter sets with a small value of $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$ [parameter sets 1 and 2 in Figs. 2(b) and 3(a): $(\sigma_{AA}/\sigma_{BB})_{\text{critical}} = 0.85, 0.82$]. Meanwhile, they are increasing functions for parameter sets with a large value of $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$ [parameter sets 3 and 4 in Figs. 2(b) and 3(a): $(\sigma_{AA}/\sigma_{BB})_{\text{critical}} = 1.13, 1.08$]. By using Lorentz–Berthelot combining rules ($\varepsilon_{AB} = \sqrt{\varepsilon_{AA}\varepsilon_{BB}}$), Eq. (11) can be simplified to

$$\left(\frac{\sigma_{AA}}{\sigma_{BB}}\right)_{\text{critical}} = 2 \left(\frac{\varepsilon_{AA}^*}{\varepsilon_{BB}^*} \right)^{\frac{1}{8}} - 1, \quad (12)$$

by which we can calculate $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$ using only the interaction parameters of pure substances. In practice, it is true that a real system is not as simple as the model system used in this study because the

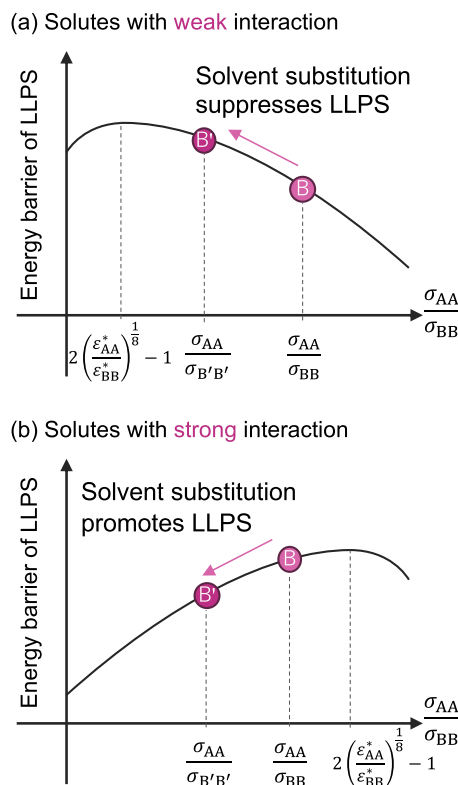


FIG. 6. Prediction of LLPS behavior based on Eq. (12) in two opposite cases with (a) solutes with weak interaction and (b) those with strong interaction.

change in molecular species of different sizes is generally accompanied by a change in the interaction strength. Nevertheless, Eqs. (12) and (9) offer a simple and convenient approach to *a priori* prediction of LLPS behavior. By using Eq. (12), we can easily predict any change in the probability of LLPS occurrence when we substitute a certain solvent B with another larger solvent B' that has a similar interaction with B (namely, $\epsilon_{BB}^* \approx \epsilon_{B'B'}^*$ and $\sigma_{BB} < \sigma_{B'B'}$, for example, substitution from benzene to toluene). Because $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$ increases with ϵ_{AA}^* and the magnitude of $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$ relative to σ_{AA}/σ_{BB} changes accordingly depending on ϵ_{AA}^* , as shown in Fig. 6, solvent substitution from B to B' tends to suppress LLPS for solute molecules with strong interactions [Fig. 6(a)] and promote LLPS for solute molecules with weak interactions [Fig. 6(b)]. Even for component molecules with completely different interactions, Eq. (9) can be used to estimate the LLPS trend. Therefore, β and the critical size ratio $(\sigma_{AA}/\sigma_{BB})_{\text{critical}}$ provide useful guidelines for developing strategies to suppress or promote LLPS.

IV. CONCLUSION

We conducted MD simulations to systematically investigate the effects of interaction strength ϵ and molecular size σ on LLPS processes. We adopted a binary LJ system as a model system of molecular solutions to avoid the molecular complexity and extract the physical essence behind the LLPS phenomena. Our simulation results showed that increase in the size ratio of solute to solvent molecules, σ_{AA}/σ_{BB} , can either facilitate or inhibit LLPS, depending on the combination of interaction strengths. For parameter sets with a weaker solute-solute interaction, LLPS was more likely to occur with an increase in σ_{AA}/σ_{BB} , whereas it was suppressed for parameter sets with a stronger solute-solute interaction. The solvent-solvent interactions also either facilitate or suppress LLPS, depending on the molecular size and strength of the other interactions.

These unique behaviors observed in MD simulations can be reasonably explained by the free energy barrier height calculated using our CNT-based model. Our model demonstrates that the complicated dependence of LLPS on interaction parameters is directly related to variations in the number of interaction pairs per volume against the molecular size. Increasing σ_{AA}/σ_{BB} increases the net number of solute-solvent and solvent-solvent interaction pairs that are either broken or newly generated per solute-solute pair generation, which means that the solute-solute interaction becomes less dominant in the promotion of LLPS. Therefore, the increase in σ_{AA}/σ_{BB} shifts the LLPS mode from the solute-related interaction-driven into solvent-related interaction-driven mode. Alternatively, LLPS is primarily driven by interactions between smaller molecules that exhibit a larger number of interaction pairs per unit volume. Even in our simplified system using LJ molecules, the LLPS behavior was demonstrated to be highly sensitive to slight variations in the interaction parameters. Elucidation of the key physical factors that govern the LLPS process through investigation of real systems of molecules with complex shapes and interactions is a challenging task, and an approach that builds up insights from simple systems is necessary, as demonstrated in this study. As the insights obtained in this study are based on a simple and universal molecular model, they would be of great help in understanding the LLPS processes of more complex molecules.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Y. I. and S. W. designed the study and prepared the manuscript. Y. I. conducted the simulations and analyses. S. W., S. H., and M. M. provided instructions as supervisors.

Yuya Iida: Conceptualization (equal); Investigation (lead); Writing – original draft (lead). **Shotaro Hiraide:** Supervision (equal). **Minoru T. Miyahara:** Supervision (equal). **Satoshi Watanabe:** Conceptualization (equal); Supervision (lead); Writing – review & editing (lead).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

APPENDIX: DEFINITION OF THE INDUCTION TIME t_L AND ITS AVERAGE τ_L

We defined the induction time of LLPS, t_L , as the period for a resulting liquid cluster to reach a certain size without redissolving, which corresponded to the time t that satisfied $n_L(t) \geq 30$ and $n_{L,\min} > 0.6n_L(t)$, where $n_{L,\min}$ is the minimum value of n_L among all n_L after time t . Because nucleation processes are stochastic, t_L varies from run to run, even for an identical parameter set. We accordingly calculated the average induction time, τ_L , of N ($= 10b$ or b more) runs by the survival probability of the nuclei-free state, which is defined as follows:

$$P(t) = \frac{(\text{Total number of runs without nucleation until } t)}{N}. \quad (\text{A1})$$

Assuming that a stochastic nucleation event follows a Poisson process, $P(t)$ can be expressed as

$$P(t) = \exp\left(-\frac{t}{\tau_L}\right). \quad (\text{A2})$$

We can calculate the average induction time τ_L by fitting the plots of simulated $P(t)$ using Eq. (A2). However, it is not always feasible to obtain the simulated values of $P(t)$ because some simulation runs did not form a liquid cluster within a maximum simulation time of 200 ns. We accordingly calculated τ_L in the following three cases, depending on the number of runs, n ($\leq N$), in which a liquid cluster formation by LLPS was observed.

Case 1, $2 \leq n \leq N$:

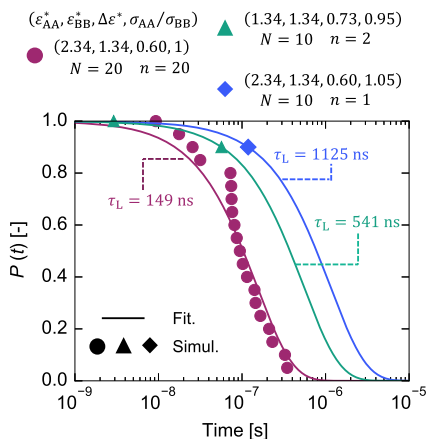


FIG. 7. Fitting of survival probability $P(t)$ to calculate average induction time τ_L .

In this case, we obtain n data points for $P(t)$ using Eq. (A1) and then calculated τ_L by fitting the n data points using Eq. (A2), as shown in Fig. 7 (red-purple and green lines).

Case 2, $n = 1$:

In this case, we obtain only one data point for $P(t) = 1$ at $t = t_L$ using Eq. (A1). This data point for $P(t_L) = 1$ is not suitable for fitting because Eq. (A2) provides $P(t_L) = 1$ only in the case of $\tau_L \rightarrow \infty$. Instead of fitting using Eq. (A2), we compute the simulated $P(t_L)$ as follows:

$$P(t_L) = 1 - \frac{1}{N}, \quad (\text{A3})$$

which implies that we interpreted the simulation results as a liquid cluster generated within time t_L with a probability of $1/N$. We calculated τ_L as a value such that Eq. (A2) satisfies Eq. (A3), as shown in Fig. 7 (blue line).

Case 3, $n = 0$:

In this case, no data points were obtained for $P(t)$. The only known observation is that no nuclei were produced within the maximum simulation time of 200 ns. We estimated a lower limit of τ_L using the same procedure as Case 2 by assuming that a liquid cluster formed at $t = 200$ ns in one of the simulation runs.

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