

CROWDING EFFECT INDUCED PHASE TRANSITION OF AMPHIPHILIC DIBLOCK COPOLYMER IN SOLUTION*

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Abstract The crowding agent induced phase transition of amphiphilic block copolymers in solution was explicitly considered. The influence of the size and the volume fraction of the crowding agent on the phase separation of amphiphilic diblock copolymers is investigated by using self-consistent field theory (SCFT) method. The concentration of the disorder to order transition of the block copolymer decreases when the size of the crowding agent is larger than that of the solvent. The higher volume fraction of the crowding agent will induce the transition of the block copolymer from disorder to order state at a lower concentration. The relation between the size and the volume fraction of the crowding agent is elucidated. When the size of the crowding agent is larger, its volume fraction of the disorder to order transition of the block copolymer will be lower. The conformation of the crowding agent considered as a polymer chain is also studied and compared.

Keywords: Crowding agent; Disorder to order transition; Block copolymer.

INTRODUCTION

Amphiphilic block copolymers in solution can self-assemble into various complex microstructures, such as rod- and sphere-like micelles and vesicles. It has attracted much attention both experimentally and theoretically^[1–16]. In the microstructure formation of amphiphilic block copolymers, the property of the solution plays an important role. Discher and Eisenberg^[17] intensively studied polymer vesicles and observed the phase transitions by varying the solvent concentration. The solvent size effect is investigated in polymer solutions^[18–20], where the strengthened depletion effect by middle sized solvents occurs. Spontak *et al.*^[21] theoretically studied the nanoscale solvent driving the interfacial segregation and central accumulation of the ABA triblock copolymer. Li and Jiang^[19] studied the solvent size on the self-assembly of amphiphilic diblock copolymer, where the solvent size and the interaction parameter between the solvent and the hydrophobic block were considered. Huber and co-workers^[22] carried out an experiment on a polystyrene-silsesquioxane-toluene model system, where silsesquioxane (SILS) serves as a sub-colloidal or nanosized solvent.

From these work, we can see that the solution properties (component, size and interaction, *etc.*) are very important for the organization of polymers. The component with large molecular weight is always called crowding agent, which can also influence the protein folding and aggregation^[23–25]. As we know, the confinement and the crowding are two factors for the properties of biomolecules, such as, protein folding^[23–25],

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self-assembly of amphiphilic molecules^[26]. The crowding agent can have profound effects on a number of processes of biological importance, such as macromolecular crowding induced self-association of polynucleosome chains^[27], protein aggregation and amyloid fibril formation^[28]. Zhou^[23–25, 29, 30] has done much work about the macromolecular crowding effect on the protein folding. Zheng^[26] used Monte Carlo method to study the crowding effect on the self-assembly of the amphiphilic molecules. The conformation of a linear polymer chain is also studied as a function of the concentration of a macromolecular crowding agent F70 by neutron scattering^[31, 32]. Hong and Gierasch^[33] studied the macromolecular crowding remodeling the energy landscape of a protein and showed that the protein favored a more compact unfolded state in the crowded condition. How macromolecular crowding influences protein folding by effects on both unfolded state ensemble and unfolding kinetics is also elucidated. Even the macromolecular crowding induced elongation and compaction of single DNA molecules confined in a nanochannel was considered^[34]. The volume fraction occupied by the macromolecular crowding agent can be as large as 0.4^[26, 35, 36]. In recent years, the hydrophilic homopolymer or hydrophobic homopolymer^[14, 37] is used to study the vesicle or micelle formation theoretically. In these systems of mixtures of block copolymer and homopolymer, the size effect of the homopolymer is studied^[16, 38, 39]. The self-assembly behavior of amphiphilic block copolymer/nanoparticle mixture in dilute solution is studied by self-consistent field theory, where the nanoparticle is considered by the density functional theory^[40].

The excluded volume effect increases with the increasing of the crowding agent and the aggregation concentration of the amphiphilic AB diblock copolymer decreases^[41]. The volume fraction of the crowding agent is not explicitly considered. In many experiments, the volume fraction (concentration) of the crowding agent^[33, 34] is relatively easy to be considered, while the size of the crowding agent is seldom considered^[42, 43]. Therefore, the relation between the volume fraction and the size of the crowding agent is not elucidated previously. Continuing our previous work^[41], we explicitly consider the effect of the crowding agent on the self-assembly of the amphiphilic block copolymer. We add the crowding agent into the amphiphilic block copolymer solution, where the crowding agent deviates from the standard convention for polymer solutions of setting the reference volume, ρ_0^{-1} , equal to the volume of a solvent molecule, v_s . The crowding effect of the crowding agent is investigated in the real space implementation of the self-consistent field theory (SCFT), which is a good method and has been widely used to consider the morphology and phase diagram of block copolymers in melts or in solution^[44–49]. The size and the volume fraction of the crowding agent are considered, where the conformation of the crowding agent is studied both implicitly and explicitly in this work.

METHOD

We study a mixture of n_p linear AB diblock copolymers with n_s solvent molecules and n_c crowding agent. Each copolymer chain consists of N segments with compositions (average volume fractions) f_A and f_B ($f_B = 1 - f_A$), respectively. We assume the mixture is incompressible, with each polymer segment occupying a fixed volume ρ_0^{-1} and each solvent molecule taking the volume v_s . The crowding agent takes the volume v_c . Thus, the total volume of the system is $V = n_p N / \rho_0 + n_s v_s + n_c v_c$, the volume fraction of the AB diblock copolymer is $f_p = n_p N / V \rho_0$ and those of the solvents are $f_s = n_s v_s / V$ and $f_c = 1 - f_p - f_s$, respectively. The ratio of the size of the crowding agent to polymer segment is $\rho_0 = v_c \rho_0$, which means that the ratio of the crowding agent to polymer size is $\alpha = v_c \rho_0 / N$. Furthermore, we assume that the A and B segments have the same segment length a .

In the SCFT one considers the statistics of a single copolymer chain in a set of effective chemical potential fields w_i , where i represents block species A, B, solvent S or crowding agent C. These chemical potential fields, which represent the actual interactions between different components, are conjugated to the segment density fields, ϕ_i , of different species i . The free energy of the system is given by

$$\begin{aligned} \frac{NF}{k_B T \rho_0 V} = & -f_p \ln\left(\frac{Q_p}{f_p V}\right) - Nf_s \ln\left(\frac{Q_s}{Nf_s V}\right) - \frac{f_c}{\alpha} \ln\left(\frac{Q_c \alpha}{f_c V}\right) \\ & - \frac{1}{V} \int d\mathbf{r} \left[\sum_i w_i \phi_i + \xi \left(1 - \sum_i \phi_i\right) \right] + \frac{1}{2V} \int d\mathbf{r} \left[\sum_{i \neq j} \chi_{ij} N \phi_i \phi_j \right] \end{aligned} \quad (1)$$

where χ_{ij} is the Flory-Huggins interaction parameter between species i and j , and ξ is the Lagrange multiplier (which acts as a pressure). $Q_p = \int d\mathbf{r} q(\mathbf{r}, 1)$ is the partition function of a single chain in the effective chemical potential fields w_A and w_B . $Q_s = \int d\mathbf{r} \exp(-w_s(\mathbf{r})/N)$ and $Q_c = \int d\mathbf{r} \exp(-\alpha w_c(\mathbf{r}))$ are the partition functions of the solvent and the crowding agent in the effective chemical potential fields w_s and w_c , respectively. The fundamental quantity to be calculated in mean-field studies is the chain propagator $q(\mathbf{r}, s)$, which represents the probability of finding the end of a segment with contour length s at position $\mathbf{r}$. $q(\mathbf{r}, s)$ satisfies a modified diffusion equation using a flexible Gaussian chain model^[50]

$$\frac{\partial}{\partial s} q(\mathbf{r}, s) = \frac{Na^2}{6} \nabla^2 q(\mathbf{r}, s) - w q(\mathbf{r}, s) \quad (2)$$

where $w = w_A$ when $0 < s < f_A$ and w_B when $f_A < s < 1$. The initial condition of Eq. (2) satisfies $q(\mathbf{r}, 0) = 1$. Because the two ends of the AB block copolymer are different, a second chain propagator $q^+(\mathbf{r}, s)$ is needed which satisfies Eq. (2), with the right-hand side multiplied by -1 and the initial condition $q^+(\mathbf{r}, 1) = 1$. The density of each component is obtained by

$$\phi_A(\mathbf{r}) = \frac{f_p V}{Q_p} \int_0^{f_A} ds q(\mathbf{r}, s) q^+(\mathbf{r}, s) \quad (3)$$

$$\phi_B(\mathbf{r}) = \frac{f_p V}{Q_p} \int_{f_A}^1 ds q(\mathbf{r}, s) q^+(\mathbf{r}, s) \quad (4)$$

$$\phi_s(\mathbf{r}) = \frac{f_s V}{Q_s} \exp(-w_s(\mathbf{r})/N) \quad (5)$$

$$\phi_c(\mathbf{r}) = \frac{f_c V}{Q_c} \exp(-\alpha w_c(\mathbf{r})) \quad (6)$$

Minimization of the free energy with respect to density and pressure, $\delta F / \delta \phi = \delta F / \delta \xi = 0$, leads to another four equations.

$$w_i(\mathbf{r}) = \sum_{j \neq i} \chi_{ij} N \phi_j(\mathbf{r}) + \xi(\mathbf{r}) \quad (i, j = A, B, S, C) \quad (7)$$

$$\sum_{i=A, B, S, C} \phi_i(\mathbf{r}) = 1 \quad (8)$$

If the crowding agent is considered as a polymer chain, the partition function of the crowding agent is $Q_c = \int d\mathbf{r} q_c(\mathbf{r}, 1)$, which means that the conformation of the crowding agent is considered explicitly. Then Eq. (6) is changed to $\phi_c(\mathbf{r}) = \frac{f_c V}{Q_c \rho_c} \int_0^{\rho_c} ds q_c(\mathbf{r}, s) q_c(\mathbf{r}, \rho_c - s)$ to obtain the volume fraction of the crowding agent.

We solve Eqs.(3)–(8) directly in real space by using a combinatorial screening algorithm proposed by Drolet and Fredrickson^[44, 45]. The algorithm consists of randomly generating the initial values of the fields $w_i(\mathbf{r})$.

The diffusion equations are then integrated to obtain q and q^+ , for $0 < s < 1$. Finally, the right-hand sides of Eqs. (3)–(6) are evaluated to obtain new values for the volume fractions of the blocks A, B, the solvent S and the crowding agent C, respectively. The numerical calculations were performed in three-dimensional space with $L_x \times L_y \times L_z$ grid points in order to further explore the morphologies of the amphiphilic block copolymer. The grid size $\Delta x = \Delta y = \Delta z = a$. Periodic boundary condition is used along x -, y -, z -direction. All calculations have been done with $L_x, L_y, L_z = 40\text{--}45$, which is large enough ($L \gg R_g$) to avoid boundary effects. The simulation is carried out until the phase is stable.

We selected A_3B_{17} diblock copolymers ($f_A = 0.15$) as a model polymer. The diblock copolymers are in selective solvents including crowding agents. The interaction parameters are set to be the followings: $\chi_{AB}N = 25$, $\chi_{AS}N = -0.5$, $\chi_{BS}N = 2.5$. Therefore, the solvent is good for block A, *i.e.* block A is hydrophilic. The size of the crowding agent is varied to consider the excluded volume effect on the self-assembly of the amphiphilic diblock copolymer. The internal structure and the mechanism of the crowding agent are not treated explicitly. But the conformation of the crowding agent can be included theoretically if the crowding agent is considered as a polymer chain. We mainly consider the average size of the crowding agent ranging from $\rho_C = 1$ to 10, which means that ρ_C is larger than 1. The volume fraction of the crowding agent is also considered. The present model captures the essential features of the crowding agent with different size and volume fraction. In this work, we only consider the disorder to order transition of the amphiphilic diblock copolymer which is determined by comparing the energy difference and the density difference.

RESULTS AND DISCUSSION

Continuously varying the size and the concentration of the crowding agent in amphiphilic diblock copolymer solution, the disorder to order transition will change, which means that the critical aggregation concentration can be tailored. The interactions between the crowding agent and the block copolymer are the same as those between the solvent and the block copolymer. The interaction between the solvent and the crowding agent is set to be zero. It means that the crowding agent has the similar properties with the solvent and the only difference is the molecular size. Therefore, we set the interaction parameters as $\chi_{AB}N = 25$, $\chi_{AS}N = \chi_{AC}N = -0.5$, $\chi_{BS}N = \chi_{BC}N = 2.5$, $\chi_{SC}N = 0$. We mainly consider the size, the volume fraction of the crowding agent, and the relation between its size and volume fraction where the conformation of the crowding agent is considered both implicitly and explicitly.

Size of the Crowding Agent

Figure 1 gives the concentration of the disorder to order transition of block copolymer as a function of the size of the crowding agent. When the size of the crowding agent is equal to the solvent size, the disorder to order transition of the amphiphilic block copolymer is $\phi_p^{\text{DOT}} = 0.071$, where the disorder to order transition corresponds to the critical micelle concentration (CMC) in the selective solvent. The aggregation concentration is higher when the size of the crowding agent is smaller. This result is also observed in Monte Carlo simulation^[26], where the aggregation concentration is higher than the bulk critical micelle concentration (CMC) when size of the crowding agent is very small. When the size of the crowding agent is larger, the block copolymer will aggregate at lower polymer concentration continuously. That is to say, the aggregate polymer concentration decreases in solution with larger sized crowding agent. The result is in agreement with Zhou and Shi's work, where they considered the mixture of block copolymer and homopolymer and the size ratio of homopolymer/copolymer is included^[39]. We hold the view that the larger solvent has the larger depletion or larger excluded volume interaction^[18, 41]. When the concentration of the crowding agent is high, the disorder to order transition of the block copolymer easily occurs (lower polymer concentration), see the spheres in Fig. 1.

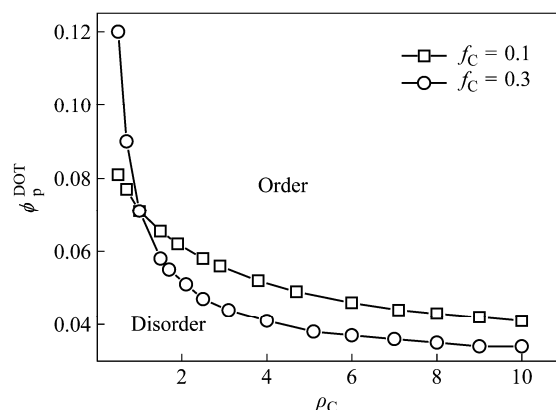


Fig. 1 Disorder to order transition of the amphiphilic block copolymer as a function of the size of the crowding agent with the volume fraction of the crowding agent f_C of 0.1 (squares) and 0.3 (spheres)

Volume Fraction of the Crowding Agent

From the above result, we can see that the concentration of the crowding agent also affect the disorder to order transition of the amphiphilic block copolymer. The effect of the volume fraction of the crowding agent was also studied in some biosystems^[33, 34]. When the volume fraction of the crowding agent increases, the crowded environment forms and the biomolecules have to adopt a compact conformation, therefore, the local concentration of the biomolecules will increase. In this part, we intensively consider the volume fraction of the crowding agent. The size of the crowding agent is set as $\rho_C = 2$ and 4. The concentration of the amphiphilic block copolymer for the disorder to order transition as a function of the volume fraction of the crowding agent is shown in Fig. 2. When the size of the crowding agent is equal to the solvent size, the disorder to order transition of the amphiphilic block copolymer is $\phi_p^{DOT} = 0.071$. When more crowding agents are added into the system, the concentration of the disorder to order transition of the amphiphilic block copolymer decreases. If the size of the crowding agent is larger than the solvent size, the disorder to order transition occurs at a lower polymer concentration, such as $\rho_C = 4$ (spheres) in the figure.

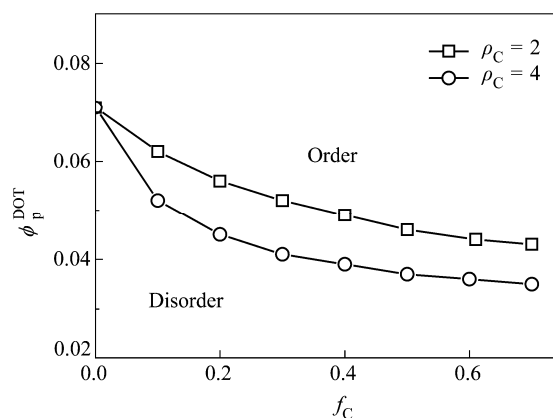


Fig. 2 Disorder to order transition of the amphiphilic block copolymer ϕ_p^{DOT} as a function of the volume fraction of the crowding agent f_C (The size of the crowding agent ρ_C is 2(squares) and 4(spheres).)

Relation between the Size and the Volume Fraction of the Crowding Agent

In this part, we consider the relation between the size and the volume fraction of the crowding agent. The crowding agent has the same interaction as that of the solvent, *i.e.*, $\chi_{AB}N = 25$, $\chi_{AS}N = \chi_{AC}N = -0.5$, $\chi_{BS}N = \chi_{BC}N = 25$, $\chi_{SC}N = 0$. The only difference is the molecular size. Here, we consider two polymer concentrations,

$f_p = 0.04$ and 0.05 . Figure 3 gives the disorder to order transition of the volume fraction of the crowding agent as a function of the size of the crowding agent. From the figure, we can see that the concentration of the disorder to order transition of the crowding agent will decrease with increasing the size of the crowding agent. It means that the disorder to order phase transition can occur when adding less larger or more smaller molecular sized crowding agent. This phenomenon is in agreement with Zhang's work, where the third component (here we called it crowding agent) is considered as hard particles^[40]. The transition easily occurs in solution with higher polymer concentration (see the spheres in Fig. 3). Analyzing the curves, we can obtain the scaling about the relation between the size ρ_C and the volume fraction f_C , $f_C \propto \rho_C^{-\beta}$ (β ca. 1.2), see the inserted lg-lg figure in Fig. 3. Here, we can see that β is greater than 1, which probably shows that the crowding agent with larger size has a stronger excluded volume effect on the self-assembly of the amphiphilic block copolymer.

The conformation of the crowding agent is also considered explicitly. The results are also shown in Fig. 3. The similar result is obtained. The difference is that a little higher concentration of the crowding agent is needed with the same size of the crowding agent and vice versa under this consideration. This means that the excluded volume effect is a little weaker when the crowding agent is considered as a polymer chain in the model.

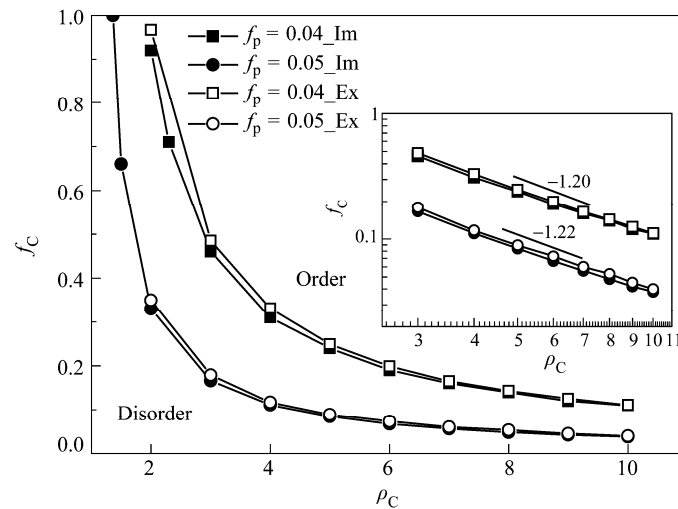


Fig. 3 Volume fraction of the crowding agent for disorder-to-order transition of the amphiphilic block copolymer as a function of the size of the crowding agent at $\chi_{AB}N = 25$, $\chi_{AS}N = \chi_{AC}N = -0.5$, $\chi_{BS}N = \chi_{BC}N = 25$, $\chi_{SC}N = 0$ (The hollow squares and circles represent the result from the explicit consideration of the conformation of the crowding agent.)

Figure 4 shows the volume fractions of the crowding agent for the disorder to order transition as a function of the size of the crowding agent for $f_p = 0.05$ at case (1) $\chi_{AB}N = 25$, $\chi_{AS}N = \chi_{AC}N = -0.5$, $\chi_{BS}N = \chi_{BC}N = 25$, $\chi_{SC}N = 0$ (squares) and case (2) $\chi_{AB}N = 25$, $\chi_{AS}N = -0.5$, $\chi_{BS}N = \chi_{AC}N = \chi_{BC}N = 25$, $\chi_{SC}N = 0$ (circles). The difference is the interactions between the crowding agent and the block copolymer. In case (1) $\chi_{AC}N = -0.5$, $\chi_{BC}N = 25$ while in case (2) $\chi_{AC}N = \chi_{BC}N = 25$. The case (2) is relatively unfavorable, *i.e.*, the crowding agent has the repulsion interaction with the block copolymer. From the figure, we can see the disorder to order transition in the case (2) can occur early. This means that the polymers easily aggregate in a non good environment with a lower polymer concentration. It is in agreement with our previous study^[18]. The explicit conformation consideration shows the same behavior with the above case. It confirms that the excluded volume effect is a little weaker when the crowding agent is considered as a polymer chain.

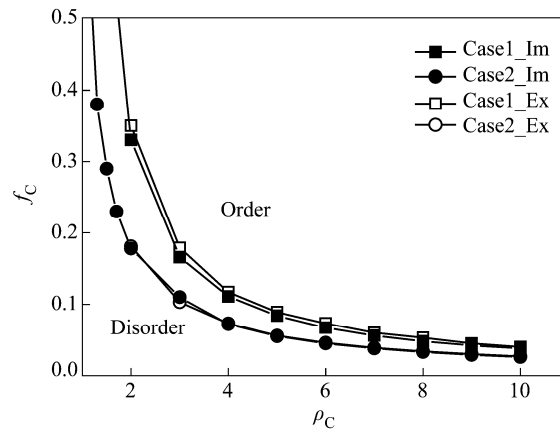


Fig. 4 Volume fraction of the crowding agent for disorder to order transition of the amphiphilic block copolymer as a function of the size of the crowding agent for $f_p = 0.05$ at case (1) $\chi_{AB}N = 25$, $\chi_{AS}N = \chi_{AC}N = -0.5$, $\chi_{BS}N = \chi_{BC}N = 25$, $\chi_{SC}N = 0$ and case (2) $\chi_{AB}N = 25$, $\chi_{AS}N = -0.5$, $\chi_{BS}N = \chi_{AC}N = \chi_{BC}N = 25$, $\chi_{SC}N = 0$ (The hollow squares and circles represent the result from the explicit consideration of the conformation of the crowding agent.)

Energy Analysis

The size of the crowding agent, its volume fraction, even the interaction between the crowding agent and the blocks contribute to the depletion effect^[18]. In general, the energy can be spitted into two parts by internal free energy U_{int} and entropic free energy $-TS$, which are shown as below

$$U_{\text{int}} = \frac{1}{V} \int d\mathbf{r} [\chi_{AB}N\phi_A\phi_B + \chi_{AS}N\phi_A\phi_S + \chi_{BS}N\phi_B\phi_S + \chi_{AC}N\phi_A\phi_C + \chi_{BC}N\phi_B\phi_C + \chi_{SC}N\phi_S\phi_C] \quad (9)$$

$$-TS = -f_p \ln\left(\frac{Q_p}{f_p V}\right) - Nf_s \ln\left(\frac{Q_s}{Nf_s V}\right) - \frac{f_c}{\alpha} \ln\left(\frac{Q_c \alpha}{f_c V}\right) - \frac{1}{V} \int d\mathbf{r} [w_A\phi_A + w_B\phi_B + w_S\phi_S + w_C\phi_C] \quad (10)$$

Then $F = U_{\text{int}} - TS$. The first one, Eq. (9), represents the interfacial free energy and the second one, Eq. (10), the sum of the entropic free energy. In Fig. 5, the energy distribution of the system is given as a function of the size ρ_C (a) and the volume fraction f_C (b) of the crowding agent. Before the disorder to order

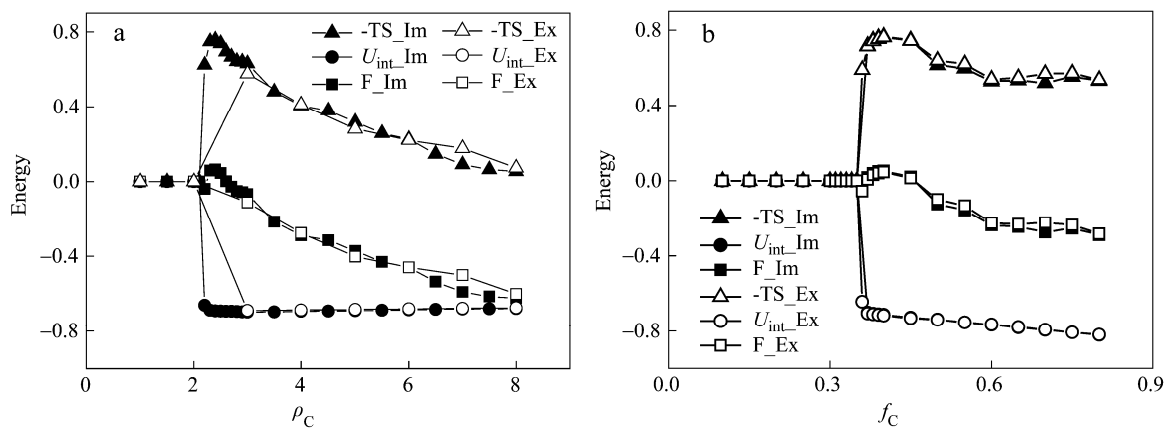


Fig. 5 Energy variation of the system as a function of (a) the size of the crowding agent ρ_C at $f_C = 0.3$, $f_p = 0.05$ and (b) the concentration of the crowding agent f_C at $\rho_C = 2$, $f_p = 0.05$ with $\chi_{AB}N = 25$, $\chi_{AS}N = \chi_{AC}N = -0.5$, $\chi_{BS}N = \chi_{BC}N = 25$, $\chi_{SC}N = 0$ (The hollow squares, circles and triangles represent the result from the explicit consideration of the conformation of the crowding agent.)

transition, the energy is constant. When the phase transition occurs, there is a sharp energy change, which is in agreement with other disorder to order transition of block copolymer in solution^[41, 51, 52]. The interfacial free energy and the entropic free energy contribute to the phase transition after the phase separation of the diblock copolymer occurs.

In Fig. 5(a), we only change the size of the crowding agent. So the transition is from the excluded volume effect of the crowding agent. This excluded volume effect widely exists in many bio-systems, which can be used to separate bio-molecules. The crowding agent with the larger molecular size has the larger excluded volume effect on the polymer, which will make the local concentration of the polymer higher than the average concentration, so the block copolymer will aggregate at the lower concentration with larger molecular size of the crowding agent. The crowding agent with the larger size will gain more translation entropy to lower the total free energy. It dominantly contributes to the total free energy of the polymer solution. The free energy corroborates the simple physical origin of the depletion interaction^[53–56]. Figure 5(b) presents the energy as a function of the volume fraction of the crowding agent. The similar results are obtained. The depletion effect will become stronger when the volume fraction of the crowding agent is higher.

The explicit conformation consideration shows the similar behavior. From the figure, we can see that the interfacial free energy is almost the same. The difference is the entropic free energy, which is the cause of the difference of the disorder-to-order transition in Figs. 3 and 4. Due to considering the crowding agent as a polymer chain, the data in the curve of Fig. 5(a) is not enough because the polymerization of the crowding agent as a polymer is an integer. Therefore, we can fix the polymer concentration and the size of the crowding agent, and continuously change the volume fraction of the crowding agent to accurately obtain the disorder to order transition curve in Figs. 3 and 4. From these figures, we can see energy changing by varying the volume fraction or the size of the crowding agent.

CONCLUSIONS

In this paper, we study the effect of the crowding agent on the self-assembly of the amphiphilic block copolymer by using the self-consistent field theory. The crowding effect of the crowding agent is intensively studied by continuously varying the size and the volume fraction of the crowding agent. When the polymer concentration is low, the disordered phase is stable. The block copolymer will aggregate by adding the crowding agent with larger size into the solution, which means that the aggregation concentration decreases in solution having crowding agent. The aggregation concentration of the amphiphilic block copolymer will also decrease with the increasing of the volume fraction of the crowding agent when the size of the crowding agent is larger than the solvent size or that of the polymer segment. Due to the crowding agent with larger molecular size depleting the block copolymer, the CMC of amphiphilic AB diblock copolymer will decrease. At the fixed polymer concentration, the disorder to order transition can occur with larger size or higher volume fraction of the crowding agent. The same result is obtained if the conformation of the crowding agent is explicitly considered. The higher concentration of the crowding agent is evaluated at the fixed polymer concentration and the same volume of the crowding agent, which is due to the entropic free energy difference. If the size of the crowding agent is large, we only need to add less crowding agent into the system to cause the disorder to order transition. The relation between the size and the volume fraction of the crowding agent is also elucidated. This finding is very useful for understanding the excluded volume effect/crowding effect in biomolecules.

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REFERENCES

- 1 Luo, L.B. and Eisenberg, A., *J. Am. Chem. Soc.*, 2001, 123: 1012
- 2 Yuan, J.M., Chyan, C.L., Zhou, H.X., Chung, T.Y., Peng, H.B., Ping, G.H. and Yang, G.L., *Protein Sci.*, 2008, 17: 2156
- 3 Luo, L.B. and Eisenberg, A., *Langmuir*, 2001, 17: 6804
- 4 Liu, F.T. and Eisenberg, A., *J. Am. Chem. Soc.*, 2003, 125: 15059
- 5 Wang, J.F., Guo, K.K., An, L.J., Muller, M. and Wang, Z.G., *Macromolecules*, 2010, 43: 2037
- 6 Burke, S., Shen, H.W. and Eisenberg, A., *Macromol. Symposia*, 2001, 175: 273
- 7 Bryskhe, K., Jansson, J., Topgaard, D., Schillen, K. and Olsson, U., *J. Phys. Chem. B*, 2004, 108: 9710
- 8 van Vlimmeren, B.A.C., Maurits, N.M., Zvelindovsky, A.V., Sevink, G.J.A. and Fraaije, J., *Macromolecules*, 1999, 32: 646
- 9 Jiang, Y., Chen, T., Ye, F.W., Liang, H.J. and Shi, A.C., *Macromolecules*, 2005, 38: 6710
- 10 Du, B., Mei, A., Yang, Y., Zhang, Q., Wang, Q., Xu, J. and Fan, Z., *Polymer*, 2011, 51: 3493
- 11 Jiang, N., Jiang, S., Hou, Y., Yan, S., Zhang, G. and Gan, Z., *Polymer*, 2011, 51: 2426
- 12 Li, W., Nakayama, M., Akimoto, J. and Okano, T., *Polymer*, 2011, 52: 3783
- 13 Wang, H., Liu, Y.T., Qian, H.J. and Lu, Z.Y., *Polymer*, 2011, 52: 2094
- 14 Zhu, Y., Yang, Q., Tong, C., Li, M. and Yu, X., *Polymer*, 2010, 51: 702
- 15 Pan, Q.Y., Tong, C.H., Zhu, Y.J. and Yang, Q. H., *Polymer*, 2010, 51: 4571
- 16 Zhuang, Y., Wang, L. and Lin, J., *Acta Polymerica Sinica (in Chinese)*, 2011, (11): 1320
- 17 Discher, D.E. and Eisenberg, A., *Science*, 2002, 297: 967
- 18 Hu, J.L., Wang, R. and Xue, G., *J. Phys. Chem. B*, 2006, 110: 1872
- 19 Li, W. and Jiang, W., *Macromol. Theor. Simul.*, 2009, 18: 434
- 20 Li, Y.Q., Huang, Q.R., Shi, T.F. and An, L.J., *J. Chem. Phys.*, 2006, 125: 044902
- 21 Spontak, R.J., Shankar, R., Bowman, M.K., Krishnan, A.S., Hamersky, M.W., Samseth, J., Bockstaller, M.R. and Rasmussen, K.O., *Nano Lett.*, 2006, 6: 2115
- 22 Kramer, T., Schweins, R. and Huber, K., *Macromolecules*, 2005, 38: 151
- 23 Zhou, H.X., *J. Mol. Recogn.*, 2004, 17: 368
- 24 Zhou, H.X., *Arch. Biochem. Biophys.*, 2008, 469: 76
- 25 Zhou, H.X., *Proteins-Struct. Func. Bioinform.*, 2008, 72: 1109
- 26 Zheng, F.X., Chen, G.J., Zhang, X.R. and Wang, W.C., *J. Chem. Phys.*, 2009, 130: 204701
- 27 Hancock, R., *European Biophys. J. Biophys. Lett.*, 2008, 37: 1059
- 28 Munishkina, L.A., Cooper, E.M., Uversky, V.N. and Fink, A.L., *J. Mol. Recogn.*, 2004, 17: 456
- 29 Zhou, H.X., *J. Phys. Chem. B*, 2009, 113: 7995
- 30 Zhou, H.X., Rivas, G.N. and Minton, A.P., *Annu. Rev. Biophys.*, 2008, 37: 375
- 31 Le Coeur, C., Deme, B. and Longeville, S., *Phys. Rev.E*, 2009, 79: 031910
- 32 Le Coeur, C., Teixeira, J., Busch, P. and Longeville, S., *Phys. Rev. E*, 2010, 81: 061914
- 33 Hong, J.A. and Gierasch, L.M., *J. Am. Chem. Soc.*, 2010, 132: 10445
- 34 Zhang, C., Shao, P.G., van Kan, J.A. and van der Maarel, J.R.C., *Proceed. National Acad. Sci. USA*, 2009, 106: 16651
- 35 Ellis, R.J., *Trends in Biochem. Sci.*, 2001, 26: 597
- 36 Ellis, R.J. and Minton, A.P., *Nature*, 2003, 425: 27
- 37 Xu, G.K., Feng, X.Q. and Li, Y., *J. Phys. Chem. B*, 2010, 114: 1257
- 38 Cavallo, A., Mueller, M. and Binder, K., *Macromolecules*, 2006, 39: 9539
- 39 Zhou, J. and Shi, A.C., *Macromol. Theor. Simul.*, 2011, 20: 690
- 40 Zhang, L., Lin, J. and Lin, S., *Macromolecules*, 2007, 40: 5582
- 41 Wang, R., Jiang, Z.B. and Xue, G., *Polymer*, 2011, 52: 2361
- 42 Zhou, D.S., Li, L., Che, B., Cao, Q., Lu, Y. and Xue, G., *Macromolecules*, 2004, 37: 4744
- 43 Chen, J.L., Xue, G., Li, Y.H., Wang, L. and Tian, G.H., *Macromolecules*, 2001, 34: 1297
- 44 Drolet, F. and Fredrickson, G.H., *Phys. Rev. Lett.*, 1999, 83: 4317

- 45 Drolet, F. and Fredrickson, G.H., *Macromolecules*, 2001, 34: 5317
- 46 Pan, Q.Y., Tong, C.H. and Zhu, Y.J., *Acs Nano*, 2011, 5: 123
- 47 Song, W.D., Tang, P., Qiu, F., Yang, Y.L. and Shi, A.C., *Soft Matt.*, 2011, 7: 929
- 48 Vorselaars, B., Kim, J.U., Chantawansri, T.L., Fredrickson, G.H. and Matsen, M.W., *Soft Matt.*, 2011, 7: 5128
- 49 Wang, R., Zhang, S.N. and Qiu, Y.D., *Polymer*, 2011, 52: 586
- 50 Helfand, E., *J. Chem. Phys.*, 1975, 62: 999
- 51 Wang, R., Chen, Y.L., Hu, J.L. and Xue, G., *J. Chem. Phys.*, 2008, 129: 044907
- 52 Wang, R., Jiang, Z.B., Chen, Y.L. and Xue, G., *J. Phys. Chem. B*, 2006, 110: 22726
- 53 Asakura, S. and Oosawa, F., *J. Polym. Sci.*, 1958, 33: 183
- 54 Asakura, S. and Oosawa, F., *J. Chem. Phys.*, 1954, 22: 1255
- 55 Poon, W.C.K., *J. Phys. Condens. Matter*, 2002, 14: R859
- 56 Yodh, A.G., Lin, K.H., Crocker, J.C., Dinsmore, A.D., Verma, R. and Kaplan, P.D., *Phil. Trans. R. Soc. Lond. A*, 2001, 359: 921