O-GlcNAcylation inhibits the oligomerization of alpha-synuclein by declining intermolecular hydrogen bonds through a steric effect

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Abstract
Toxic abnormal aggregation of α-synuclein (α-Syn) is a feature of Parkinson’s disease. Several biochemical and biophysical studies have demonstrated that many post-translational modifications (PTM) of α-Syn could distinctly alleviate its oligomerization-mediated toxicity. Recently, a compelling link is emerging between the PTM O-GlcNAcylation (O-GlcNAc) and protein aggregation, yet the underlying molecular mechanism remains unclear. Based on the all-atom molecular dynamics simulations, we found that O-GlcNAc modifications can suppress the process of oligomerization of α-Syn aggregates via a steric effect—the additional O-linked glycosyl group disrupts the formation of hydrogen bonds (H-bonds) between α-Syn monomers. Besides, we proposed a theoretical model to further capture the physical mechanism of α-Syn aggregation/disaggregation in the absence/presence of O-GlcNAc-modified α-Syn. Our findings unveil the molecular mechanism of the O-GlcNAc-induced inhibition of α-Syn oligomerization, which may help to understand how O-GlcNAc prevents the oligomerization of other proteins and provides the guideline for the development of O-GlcNAc-based therapeutic strategies in neurodegenerative diseases.

1. Introduction
α-Synuclein (α-Syn), a presynaptic protein, is abundantly expressed throughout the central nervous system, sometimes in relatively high concentrations of up to 50 μM [1]. Existing as an intrinsically disordered monomer in aqueous solutions, α-Syn contains a conserved lipid-binding domain and forms an α-helical structure upon binding to membranes [2–4]. Although α-Syn can participate in transporting synaptic vesicles and plays a role in the release of second-messengers, [5–8] its aggregation is closely associated with Parkinson’s syndrome, and its aggregates are a hallmark of Parkinson’s disease and other neurodegenerative diseases [9–14]. Sheets rich in β-amyloid aggregates produced by the aggregation of α-Syn are toxic to cells [15–18], while normal α-Syn remains as monomers in cells [19]. Therefore, understanding how α-Syn maintains its monomer and resists aggregation, especially at high concentrations, may be pivotal in treating Parkinson’s disease.

A variety of post-translational modifications on α-Syn, e.g., acetylation, [20, 21] phosphorylation, [22] and O-GlcNAcylation (abbreviated hereafter as ‘O-GlcNAc’ [23]), confirmed in vivo may have important implications for both physiology and pathology. Among them, α-Syn O-GlcNAc is a widespread post-translational, intracellular modification occurring on serine and threonine residues of α-Syn. At least nine diverse O-GlcNAc sites have been identified on α-Syn (figure 1(a)), including five located in the aggregation-prone non-amyloid-β component (NAC) region corresponding to residues 61–95 [24–28]. Various recent experiments have shown that proper glycosylation can prevent the aggregation and fibrosis of α-Syn in a site-specific manner [23, 29, 30]. In particular, single O-GlcNAc modification at residue 81 and simultaneous modifications at
residues 72, 75, and 81 have demonstrated significant inhibitory effects in such experiments [30]. At the same time, those modifications have little effect on the binding of α-Syn to membrane or on the formation of its secondary structure [23, 29, 30]. Those findings suggest that O-GlcNAc modifications may be a cellular mechanism to prevent protein aggregation and, in turn, could provide a promising therapeutic strategy for Parkinson’s disease. However, to date, mechanistic insights into the O-GlcNAc-induced inhibition of α-Syn aggregation remain limited.

Here, we report all-atom molecular dynamics (MD) simulations of multiple α-Syn monomers in water conducted to explore the molecular mechanism behind the aforementioned O-GlcNAc-induced inhibitory effects. We found that O-GlcNAc modifications can inhibit the formation of hydrogen bonds (H-bonds) between α-Syn monomers due to a steric effect, thereby altering the process of oligomerization and the resulting structure of α-Syn aggregates. By extension, we proposed a theoretical model to understand the physical mechanism of α-Syn oligomerization with and without O-GlcNAc, which shows how oligomerization of α-Syn occurs and how O-GlcNAc modifications inhibit protein aggregation under different conditions (e.g., different degrees of glycosylation). Our study uncovered the molecular mechanism of the O-GlcNAc-induced inhibition of α-Syn oligomerization, which may help to clarify how O-GlcNAc prevents the oligomerization of other proteins and provide the guideline for the development of O-GlcNAc-based therapeutic strategies in neurodegenerative diseases.

2. Results and discussion

O-GlcNAc shows an inhibitory effect on the oligomerization of α-Syn

To study the mechanism of O-GlcNAc of preventing the formation of α-Syn oligomers, we performed MD simulations to analyze the structure and dynamics of the modified α-Syn monomers. The initial structure contained three monomers of α-Syn (residues 1–96, PDB 1XQ8). The overall structures were shown in figure S1(a) (https://stacks.iop.org/ PB/18/016002/mmedia) in the supporting information, whereas the molecular structures of three threonine residues before and after O-GlcNAc were shown in figures 1(b) and (c), respectively. We selected two modified schemes focusing on the NAC region, including the single modification of residue 81 and the triple modifications of residues 72, 75, and 81 (referred to as models 2 and 3 respectively, see figure S1; all the three residues 72, 75, and 81 are threonine). The wild-type case (i.e., model 1) was set as the control model.

Consistent with previous experimental results [23, 29, 30], 300 ns equilibrium simulations of the three models revealed that O-GlcNAc inhibited α-Syn oligomerization, among which the triple O-GlcNAc case (model 3) had a more dramatic inhibitory effect (figure 2). To describe the state of the oligomers in the various models, we calculated the radius of gyration ($R_g$) of multiple α-Syn monomers in each model and examined their evolution over time, as shown in figure 2(a). Compared to the wild-type case, O-GlcNAc increased the $R_g$ of the entire protein system; that is, the oligomers became looser packing. Based on the data from the 100 to 300 ns simulation among which the systems were in equilibrium, we found that the average $R_g$ of multiple α-Syn monomers increased by ∼0.7 nm in model 2 and by ∼1.9 nm in model 3, comparing to the wild-type case (see table S1 in supporting information). The $R_g$ of monomeric α-Syn in the oligomers for all three models were also calculated, showing that O-GlcNAc had a weak impact on the conformation of monomeric α-Syn, for rarely affecting the $R_g$ of monomeric α-Syn (see table S1). Further, we calculated the average intermolecular distance (AID) between two individual α-Syn monomers in the oligomer. The AID values of models 1–3 were 1.93 nm, 3.14 nm, and 5.12 nm, respectively. All of those AID values far exceeded the increase in the monomer α-Syn’s $R_g$ under the corresponding modification, which suggests that O-GlcNAc modification may reduce the oligomerization of α-Syn and weaken the interaction between monomers of α-Syn. To confirm the results, we also performed the MD simulations starting from the disordered coiled structures of α-Syn, which showed that the results of O-GlcNAc modifications reducing the oligomerization of α-Syn are independent of the initial structures (see figure S2 in the supporting information).

To address how O-GlcNAc limits the oligomerization of α-Syn, we investigated the alteration of network patterns for intermolecular H-bonds during α-Syn oligomerization. An H-bond is considered to be formed if the distance between the donor and acceptor is less than 3.5 Å and the bond angle is less than 30° [31]. All the H-bonds we considered are the intermolecular ones between monomers, rather than the intramolecular H-bond within one monomer. Figure 2(b) showed the probability distribution of the number of H-bonds between each two monomers in the three models, identified via a Gaussian fitting. This distribution indicated the occurrence probability for the given H-bonds number after the simulations reach equilibrium. For instance, it was shown that there are 25 intermolecular H-bonds with the highest probability for Model 1 during 100 ns to 300 ns in the simulation (see figure 2(b)). The results show that O-GlcNAc modification can significantly reduce the number of H-bonds between monomers. For instance, compared with model 1 there are about on average 12 H-bonds less in model 2 and 22 bonds less in model 3. To gain further insight into the formation and structure of the oligomers, we obtained the 2D statistical distribution of the oligomers with
respect to the number of intermolecular H-bonds and the $R_g$ (see figure 2(c)). The case of model 1 showed the most populated structure with wild-type $\alpha$-Syn represented a small $R_g$ and a large number of H-bonds (figure 2(c)), indicating that the wild-type $\alpha$-Syn monomers well interact with each other. In contrast, although contact with monomers remained possible, the oligomer in model 2 was looser and more flexible than that in model 1 (see model 2 in figure 2(c)). Comparing to the wild-type case, the most populated structure of the triple O-GlcNAc modifications exhibits a larger $R_g$ and fewer H-bonds (model 3 in figure 2(c)), indicating that the interaction between the monomers was fragile, and stable contact between molecules was nearly nonexistent. It is known that the H-bond network is critical to stabilize the protein–protein interactions [32–37]. The simulations showed that the O-GlcNAc modifications inhibit H-bonds formation, thus weakening intermolecular interactions between monomers of $\alpha$-Syn, which is consistent with the experimental findings [23, 29, 30]. Accordingly, the above results indicate that O-GlcNAc inhibits monomeric $\alpha$-Syn oligomerization or nucleation by limiting the formation of H-bonds between $\alpha$-Syn monomers.

O-GlcNAc groups inhibit intermolecular H-bond formation via a steric effect.

Since the O-GlcNAc scheme in this study (residues 72, 75, and 81) is concentrated in the NAC region (residues 61–95), which is highly involved in protein aggregation, the number of H-bonds formed among these regions in the three models should change accordingly. We analyzed the variation of the pattern of the H-bond network in the modified region to gain insight into the prevention of large oligomers of $\alpha$-Syn by O-GlcNAc. Figure 3(a) showed that the O-GlcNAc modification caused more than half of the reduction in the number of H-bonds in the NAC region in model 2, compared with that in model 1. As the number of O-GlcNAc modifications increased (model 3), the number of H-bonds formed between the NAC region and other monomers decreased more significantly (see figure 3(a)).

Unlike acetylation, O-GlcNAc did not cause $\alpha$-Syn to lose H-bond donors. From the structural view (figure 2(d)), it is reasonable to suppose that the massiveness of the O-linked glycosyl group may limit the formation of H-bonds within a range nearby. The steric effect could become increasingly evident as the number of modification sites increases. We found that the reduction in the number of H-bonds by O-GlcNAc modification mainly occurred in the region residues 71 to 83, as shown in figure 3. Remarkably, in model 3 (triple modifications of residues 72, 75, and 81)
Figure 2. O-GlcNAcylation (O-GlcNAc) has an inhibitory effect on the oligomerization of α-Syn. (a) Evolution of the radius of gyration ($R_g$) of three wild-type α-Syn monomers (i.e., model 1), and the models with the single modification of residue 81 and the triple modifications of residues 72, 75, and 81 (models 2 and 3, respectively). The initial conformation is shown in figure S1. (b) Probability distribution of the number of H-bonds between the monomers of models 1–3. (c) Population landscape of the three models in the 2D plane spanned by the number of H-bonds and $R_g$. (d) The most populated structures of the three models during simulations indicated by (c). The sites of O-GlcNAc are highlighted in the atomic structure to show the steric effect. Different monomers are represented by different colors.

75, and 81), the formation of H-bonds by residues 71 to 83 was nearly blocked (see figure 3). However, the probability of H-bond formation at the modification site (e.g., residue 81) did not change remarkably. For instance, the probability of H-bond formation at residue 81 only showed a decrease of approximately 5% and 10% in models 2 and 3, respectively, compared with the case of model 1 (see figure 3). Such results imply that instead of altering the probability of H-bond formation at the modification sites, O-GlcNAc tends to reduce the likelihood of nearby H-bond formation.

The O-GlcNAc modifications are located in the NAC region that associates with the aggregation of α-Syn. In the wild-type case (model 1), we can see that the residues 72, 75 and 81 are buried inside the oligomer and well interact with each other (see figure 2(d)). In contrast, after modifications the O-GlcNAc groups are more likely to be exposed to the solution (model 2 and 3 in figure 2(d)) that inhibits the interaction between the NAC regions. Therefore, the steric effect caused by O-GlcNAc modifications may be the primary factor in preventing α-Syn oligomerization.

The change in intermolecular interaction (induced by a steric effect of O-GlcNAc) could affect protein aggregation remarkably.

As aforementioned, the steric effect caused by site-specific O-GlcNAc modifications weakens the interaction between two α-Syn monomers. The more the
modifications, the weaker the intermolecular interaction should be. In the process of α-Syn oligomerization, the intermolecular interaction should play a critical role. In previous experiments, it has been observed that despite differences in the strength of intermolecular interactions by modifications, many modification schemes significantly reduce or delay the formation of oligomers [23, 29, 30]. The oligomerization has also been reduced or been postponed significantly when modified (e.g., residues 72, 75, and 81) and wild-type α-Syn monomers were mixed in equal proportions, in which the intermolecular interaction was decreased [30]. Hereby, we developed a theoretical model (see methods for details) to understand the phenomena of the oligomerization (i.e., phase separation [14]) of α-Syn that results from strengthening or weakening the intermolecular interaction between α-Syn monomers. We formulated a three-component solution model involving water and two types of solutes: the wild-type α-Syn monomers, denoted by \( a \), and the O-GlcNAc-modified α-Syn monomers, denoted by \( b \). We used \( \varepsilon_{ij} \) to represent the dimensionless interaction energy between two protein molecules, \( i \) and \( j \) denote the wild-type \( a \) and O-GlcNAc-modified α-Syn monomers \( b \), respectively. In the model, we included three types of interactions \( \varepsilon_{ij} \), i.e., \( \varepsilon_{ab} \) for the wild-type \( a \) and the O-GlcNAc-modified monomers \( b \), \( \varepsilon_{aa} \) for the wild-type \( a \) themselves, and \( \varepsilon_{ab} \) for the O-GlcNAc-modified monomers \( b \) themselves, respectively. The larger the absolute value of \( \varepsilon_{ij} \) is, the stronger the intermolecular interaction would be. Taking the volume fraction of the two solutes (i.e., \( \phi_a \) and \( \phi_b \)) as independent variables, we plotted the surface of the dimensionless free energy density of the solution with two different sets of \( \varepsilon_{ij} \) (see supporting information for detailed descriptions of the sets of \( \varepsilon_{ij} \)), as shown in figure 4. Clearly, the resulting free energy landscape depended on the strength of the intermolecular interaction.

We examined three conditions involving different components: the pure wild-type \( (\phi_b = 0) \), the equal-proportion mixed type \( (\phi_a = \phi_b) \), and the pure modified type \( (\phi_i = 0) \), as shown in figures 4(b) and (d). In the case of \( \phi_b = 0 \), i.e., there are only wild-type α-Syn monomers, there is one energetic barrier in the free energy curve, as shown in the upper panels of figures 4(b) and (d). According to the theory of thermodynamics of solutions [38], if the free energy density \( f(\phi) \) has an upper convex part where \( \partial^2 f(\phi) / \partial \phi^2 < 0 \), a homogeneous solution cannot be stable. In other words, if there is an obvious energetic barrier (bulge) in the free energy curve, then phase separation will occur—a homogeneous solution will minimize its free energy by separating into two solutions with the high-density phase and the low-density phase, respectively [38]. In the present case, the solution separates into the concentrated phase \( (\phi_a \approx 1, \text{ corresponding to the protein oligomerization}) \) and the dilute phase \( (\phi_a \approx 0) \). In the case of \( \phi_a = 0 \), since all α-Syn monomers are O-GlcNAcylated, the intermolecular interactions between proteins are weakened. Therefore, due to no energetic barrier in the lowest panels of figures 4(b) and (d), the phase separation will not occur. In the case of \( \phi_a = \phi_b \) by assuming this condition also works in any local region of the solution, the intermolecular interaction of modified proteins is profoundly weakened, corresponding to high levels of O-GlcNAcylation, e.g., the triple O-GlcNAc modifications (see the comparison of interaction strengths between figures 4(a) and (c)). Therefore, the solution will change from a phase-separation state to a non-phase-separation state, corresponding to the changes in the middle panel of figure 4(b) and figure 4(d). Because of the energetic barrier in the middle panel of figure 4(b), the solution will separate into a dilute phase \( (\phi_a \approx 0.07) \) and a concentrated phase \( (\phi_a \approx 0.45) \) if considering the assumption that two types of proteins can be fully mixed in the equal proportions. This concentrated phase corresponds to the protein oligomerization. On the other hand, there is no energetic barrier in the middle panel of figure 4(d), indicating the solution is homogeneous and protein oligomerization will not occur. This phenomenon suggests that O-GlcNAc weakens the intermolecular interactions and the mixing of a certain proportion of protein with high levels of O-GlcNAcylation (such as triply modified) with wild-type proteins in the solution may greatly decelerate oligomerization.

![Figure 3](image)

**Figure 3.** Probability distribution of the number of H-bonds between the specific residues on one monomer and residues belonging to other α-Syn monomers in models 1–3, averaged over all three monomers.
Figure 4. Relationship between dimensionless free energy density $f_c$ of the solution (free energy per unit volume of the solution) and the volume fraction of wild-type and O-GlcNAc modified α-Syn ($\phi_a$ and $\phi_b$, respectively) at various conditions. (a) Free energy density surface of the solution with the relatively strong interactions between protein monomers $\epsilon_{ij}$ is the dimensionless interaction strength between two protein monomers with the subscripts $a$ and $b$ denoting wild-type and modified proteins, respectively. (b) The free energy profile along with the protein fraction when $\phi_b = 0$, $\phi_a = \phi_b$, and $\phi_a = 0$ (from upper to lower, respectively). (c) and (d) Same as (a) and (b) with the weaker interactions between the modified protein monomers, corresponding to a stronger effect of glycosylation.

This can explain the experimental observation that the α-Syn bearing three O-GlcNAc modifications can completely prevent the aggregation of wild-type proteins when the two types of proteins are mixed in equal proportions [30]. Our model showed that when the interaction between α-Syn proteins decreases to some degree due to glycosylation, the aggregated proteins prefer to be dispersed and form a homogeneous solution.

3. Conclusion

In summary, O-GlcNAc is a post-translational, intracellular modification widespread in animals and plants. Although the physiological role of O-GlcNAc is multifaceted, a variety of in vivo [39–41] and in vitro [42, 43] experiments support that such modification plays a vital role in neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease. O-GlcNAc has recently attracted attention owing to its an essential role in early protein aggregation and nucleation [35, 36, 44] by reducing the nucleation and toxicity of α-Syn, despite its relatively slight impact on α-Syn’s ability to bind to phospholipid membranes [23, 29, 30, 45]. However, due to the unstable, transitive properties of amyloid oligomers, it remains difficult to detect structural changes formed in amyloid oligomers at the atomic level in experimental settings. Therefore, we analyzed the outcome of the O-GlcNAc modifications on the oligomerization and nucleation of α-Syn monomers via simulations and theoretical analysis. We showed that the O-linked glycosyl group had a steric effect, to reduce the possibility of H-bond formation by the residues around the modification sites. The steric effect reduces the interaction between the regions around the modification sites, thereby reduces the interaction between protein monomers. Moreover, the steric effect tended to exert a synergistic effect that more O-GlcNAc modifications would cause a more significant decline in protein–protein interaction, preventing the residues around the modification sites from interacting with other monomers. The weakening of the intermolecular interaction results in the inhibiting or postponement of oligomer formation. By using a theoretical model, we found...
that variation in the strengths of intermolecular interaction may induce qualitative changes in the phase-separation state of α-Syn. Theoretical analysis showed that as long as the interaction was reduced to a certain degree (mimicking a certain steric effect by O-GlcNAc), the formation of oligomers can be suppressed. In addition, modified proteins (with weaker interactions between monomers) can also prevent α-Syn from oligomerization when wild-type and modified proteins were mixed in equal proportions. Our results shed light on the mechanism of the experimental evidence that O-GlcNAc inhibits monomeric α-Syn’s nucleation with site-specific differences [23, 29] and that the modified (O-GlcNAc residues 72, 75, and 81) α-Syn monomers inhibit the aggregation of wild-type proteins [30]. In that sense, our study furnished insights into the regulation of modified interaction schemes such as O-GlcNAc and acetylation [20, 21, 46] as a possible way to inhibit oligomer formation. Moreover, O-GlcNAc may affect the entropy barrier of conformational changes during α-Syn fibrosis, thereby affecting the growth, morphology, and toxicity of the fibers. Since α-Syn oligomers can be the seeds of protein aggregation that are closely related to the pathology of early-onset Parkinson’s disease, our results suggest that the O-GlcNAc modifications can be a potential treatment strategy to alleviate the oligomerization-mediated toxicity of α-Syn.

4. Methods

Simulation models. In aqueous solutions α-Syn exhibits an intrinsically disordered structure [47] and forms a helical structure in association with a phospholipid membrane [48]. O-GlcNAc exerts little effect on the formation of any secondary structure [23, 29]. To reduce the computational demand, we chose the residues 1–96 for simulations to clarify the effect of O-GlcNAc on the NAC region, which plays the key role in the aggregation of α-Syn [49]. This selection is widely used in experiments and simulations to study the aggregation of α-Syn [50–52]. To study the influence of the secondary structure during oligomerization, two types of initial structures were used: one started with the structure of PDB: 1XQ8 [48] (residues 1–96) with two α-helices in each α-Syn (see figure S1(a) in the supporting information), whereas the second one started with a disordered coiled structure in each α-Syn (figure S1(b)). We also tested the influence of initial placement of α-Syn monomers. The results showed that the initial configuration has little influence on the aggregation process (figure S3). The structure of the O-GlcNAc modifications α-Syn was built using the GLYCAM-Web tools [53]. Three α-Syn monomers were randomly solvated with water in a cubic simulation box. The distance from the protein molecules to the box boundary is larger than 12 Å.

The initial distances between each monomeric α-Syn are larger than 10 Å, ensuring no contact between individual α-Syn (figure S1). The total charge of the system was neutralized by adding appropriate number of Na+ or Cl− ions as required.

Molecular dynamics (MD) simulation. We performed MD simulations using the GROMACS 5.1.2 package [54, 55] with a time step of 2 fs. Short-range van der Waals interactions were switched off between 10 and 12 Å. The PME algorithm [56] was used to treat long-range electrostatic interactions, and all the covalent bond with hydrogen atoms were constrained with the LINCS algorithm [57]. Production runs were performed in the NPT ensemble at 300 K at a pressure of 1 bar. The AMBER99SB force field [58], the (GLYCAM)06g force field [59], and the TIP3P model [60] were used to describe proteins, glycoligands, and water, respectively.

The radius of gyration $R_g$ of the oligomerization structure was calculated by

$$R_g = \left( \frac{\sum m_i |r_i|^2}{\sum m_i} \right)^{\frac{1}{2}}$$  

where $m_i$ is the mass of atom $i$, and $r_i$ is the position of atom $i$ in relation to the molecule’s center of mass. The $R_g$ has often been used to distinguish the oligomerization state of multiple proteins [21, 47, 61, 62].

Theoretical models. We established the following three-component-solution model by using the lattice model [38]:

$$f(\phi_a, \phi_b) = \frac{k_B T}{V_C} [\phi_a \ln \phi_a + \phi_b \ln \phi_b + (1 - \phi_a - \phi_b) \ln (1 - \phi_a - \phi_b)] + \frac{1}{2} \omega_{ab}(1 - \phi_a) \phi_a + \frac{1}{2} \omega_{bb}(1 - \phi_b) \phi_b + \phi_a \phi_b \omega_{ab}$$  

where $f$ is the free energy density (free energy per unit volume of the solution), and the term in the square bracket is the mixing entropy of two types of proteins with other terms denoting the interaction energy between proteins. The subscripts $a$ and $b$ denote wild-type and modified proteins, respectively; $\phi$ is the volume fraction of two types of proteins; $\omega$ is the interaction constant between protein monomers. $V_C = 1 \text{nm}^3$ is the reference volume [63].

Rerendering equation (2) to a dimensionless form, we yielded:

$$f_C(\phi_a, \phi_b) = f(\phi_a, \phi_b) \frac{V_C}{k_B T \omega_{ab}} = \frac{k_B T}{\omega_{ab} V_C} [\phi_a \ln \phi_a + \phi_b \ln (1 - \phi_a - \phi_b) \ln (1 - \phi_a - \phi_b)] + \frac{1}{2} \epsilon_{aa}(1 - \phi_a) \phi_a + \frac{1}{2} \epsilon_{bb}(1 - \phi_b) \phi_b + \phi_a \phi_b \epsilon_{ab}$$
where $\varepsilon_{ij}$ is the corresponding $\omega_{ij}$ after the dimensionless treatment $\varepsilon_{ij} = (\omega_{ij} V_{C_i})/(\omega_{aak} k_B T)$, $i$ and $j$ denote the subscripts $a$ and $b$. For simplicity, $\varepsilon_{aak}$, $\varepsilon_{bkb}$, $\varepsilon_{abk}$ are considered to be constant in our calculations. The theoretical model describes the competition between entropy and enthalpy of the protein solution [38]—when the interactions between the solutes are weak, the entropy effect will be dominant in the system that leads to a homogeneous solution. In contrast, when the interactions between the solutes are stronger than the entropy contribution, the solutes are likely to contact with each other that leads to phase separation (e.g. protein oligomerization) [38, 63]. The lattice model we used in this study has been widely applied to study the aggregation and phase separation of soft matter [64, 65]. Detailed descriptions of the sets of $\varepsilon_{ij}$ can be found in the supporting information.

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Author contributions

DL, PX, and JD contributed to concept and design. KW and PX performed MD simulations and the theoretical analysis. All authors discussed results and wrote the manuscript.

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