Using a reduced dimensionality model to compute the thermodynamic properties of finite polypeptide aggregates

Gustavo E. López · Anthony Cruz ·
Melyorise Sepulveda-Chervony · Juan López-Garriga ·
Madeline Torres-Lugo

Received: 8 July 2011 / Accepted: 21 December 2011 /
Published online: 2 February 2012
© Springer Science+Business Media B.V. 2012

Abstract By implementing a simple reduced dimensionality model to describe the interactions in finite systems composed of two seven-amino-acid peptides, the thermodynamic properties of ordered and disordered aggregates were computed. Within this model, the hydrophobicity of each amino acid was varied, and the stability of the systems computed. Accurate averages in the canonical ensemble were obtained using various replica exchange Monte Carlo algorithms. Low and high temperature regions were encountered where the ordered and disordered aggregates were stabilized. It was observed that as the degree of hydrophobicity increased, the stability of the aggregates increased, with a significant energetic stabilization obtained for the ordered aggregates. Upon decreasing the concentration of the solution, the stability of the amorphous aggregates increased when compared to the ordered systems.

Keywords Finite systems · Peptides · Monte Carlo · Replica exchange

1 Introduction

The study of the aggregation process in biomolecular systems has attracted the attention of scientists over the past years because of its relevance to degenerative diseases. It is well

G. E. López (✉) · A. Cruz · J. López-Garriga
Department of Chemistry, University of Puerto Rico, at Mayagüez,
Mayagüez, P.R. 00681, USA
e-mail: gustavo.lopez@upr.edu

G. E. López · A. Cruz
Department of Chemistry, Lehman College-CUNY, Bronx, NY 10468, USA
e-mail: gustavo.lopez1@lehman.cuny.edu

M. Sepulveda-Chervony · M. Torres-Lugo
Department of Chemical Engineering, University of Puerto Rico, at Mayagüez,
Mayagüez, P.R. 00681, USA

known that polypeptides undergo self-assembly, which causes the formation of ordered or disordered aggregates. Of particular interest is the formation of ordered aggregates, which are stabilized by atomistic interactions that extend the length of the chain. In particular, maximization of the hydrophobic interactions between side chains causes considerable stability in the initial stages of aggregation. These aggregates can form fibrillar structures, the precursors of amyloid fibrils. Under biological conditions, these fibrils are insoluble, thus causing accumulation in cellular tissues leading to organ failure and even death.

For a peptide/protein to aggregate into ordered structures, it has been postulated [1] that non-native unfolded or partially unfolded domains must be present within the protein. These domains will provide the proper intermolecular interactions that permit organized self-assembly of the monomeric species. However, no general trend has been observed related to structural changes that the protein undergoes before aggregation occurs, e.g., some peptides/proteins require total unfolding prior to fibril formation [2] while others require partial unfolding [3]. Factors such as temperature, solvent polarity, concentration, pH, ionic strength, to mention some, seem to play significant roles in favoring fibril formations [4]. For example, Chiti et al. [5] used trifluoroethanol (TFE), a solvent known to stabilize partially folded proteins, to control the growth of aggregated species in vitro. Bouchard et al. [6] achieved amyloid fibril formation by a successive step method, where partial denaturation was induced by first decreasing the pH to 2.3 and then heating the system. Efficient growth and propagation of amyloid structures were obtained under these conditions. Structural approaches, such as chemical modification, have also been successfully used in the preparation of aggregates [7]. In these experiments, the amino acid sequence of insulin was modified in order to induce amyloid fibril formation. Malisauskas et al. [8] prepared amyloid fibrils by changing the ionic strength of the solution with the addition of calcium ions to a low-pH mixture.

After a peptide/protein undergoes the necessary conformational changes, the mechanism of aggregation that follows is a complicated process [4], with various intermediates playing significant roles. Specifically, the formation of small oligomers (existing as small as dimeric species) seems to represent intermediate states in early stages of aggregation. If the oligomers form an ordered nucleus enriched with beta sheets, then prefibrillar material, a precursor of amyloid fibrils, could be formed. However, the oligomers can also form amorphous aggregates, which are less prone to form such prefibrillar intermediates.

Although the entire protein is involved in the formation of amyloid fibrils, the study of peptides provides useful insight regarding the aggregation process of specific regions within a protein. Previous evidence demonstrated [9] that certain short sequence fragments contained within specific protein domains can form amyloid fibrils even in isolated form, with the formed fibrils being essentially similar to those obtained by the full-length protein.

Computer simulations have provided an extremely useful tool in the study of protein aggregation. Proteins and peptides that are prone to form amyloid aggregates have been studied using either reduced dimensionality (termed coarse-grained) [10–14] or fully atomistic models [15–19]. In the present study, a reduced dimensionality model was used to characterize the interparticle interactions of dimeric polypeptide aggregates. The thermodynamic properties of ordered and amorphous aggregates were obtained from the computed average constant volume heat capacity as a function of temperature. The variation in the stability of the aggregates as a function of the hydrophobicity of the residues forming the peptides was studied in detail. The reason for centering our study on the hydrophobicity of the residues is based on previous studies [20, 21] on the A β _{16–22} amyloid peptide. Specifically, it has been shown that the initial stages of fibril formation are determined by the formation of hydrophobic interactions between the residues in the backbone of

the peptide. Moreover, backbone interpeptide hydrogen bonding, which could stabilize the formation of ordered aggregates, is less relevant to the stability of the peptide because of possible disruption in an aqueous solvent.

All calculations were performed using various replica-exchange Monte Carlo algorithms in the canonical ensemble. The techniques used have been well characterized for identifying conformational changes in finite systems. We shall see that this approach provided useful thermodynamic information related to the conditions that are necessary for the aggregation to occur in an ordered or disordered system, as well as how the primary sequence of the peptide plays a crucial role in the behavior of aggregates. By considering a dimeric finite system interacting by long-range hydrophobic forces, the early stages of amyloid fibril formation were simulated, and relevant information associated with how prone ordered aggregates are formed.

2 Computational methods

In this study, the Sorenson/Head-Gordon model was implemented to describe the interaction between residues. In this model, the total potential energy is written as [22, 23]:

$$\begin{aligned}
 V = & \sum_i 0.5k_b (r - r_0)^2 + \sum_i 0.5k_a (\theta - \theta_0)^2 \\
 & + \sum_i \{A(1 + \cos \phi) + B(1 - \cos \phi) + C(1 + \cos 3\phi) + D[1 + \cos(\phi + \pi/4)]\} \\
 & + 4\epsilon \sum_{i < j} [H_1 \left(\frac{\sigma}{r_{ij}}\right)^{12} - H_2 \left(\frac{\sigma}{r_{ij}}\right)^6]
 \end{aligned} \tag{1}$$

where the first two terms are harmonic oscillators describing the bond (r) and angles (θ), respectively. The third term describes the dihedral angle (ϕ) and is mainly responsible for the secondary structure of biomolecular systems. The last term describes long-range forces, which in this study represent the hydrophobicity of the amino acids. Specifically, the hydrophobic–hydrophobic interactions are represented by a simple Lennard-Jones potential ($H_1 = H_2 = 1$) that mimics the potential of mean force between hydrophobic spheres in water. For the hydrophilic–hydrophilic and hydrophobic–hydrophilic interactions, $H_1 = 1/3$ and $H_2 = -1$, which represents a purely repulsive potential caused by the hydration shells around the hydrophilic residues. All the parameters (r_0 , k_b , k_a , A , B , C , D , H_1 , H_2 , ϵ , σ) used in this force field were available in the literature [22]. The systems considered (Table 1) were two similar peptides, each with seven coarse-grained amino acids, and a well-defined sequence that was characterized by the hydrophobicity of each residue. For example, what we labeled as system 1 (S1) consisted of two peptides, each with seven hydrophobic residues. It has been shown [24, 25] that such peptides, containing only hydrophobic residues, are linked to amyloidogenesis, e.g., the fragments of prion proteins with sequence AGAAAAGA. The different sequences used are shown in Table 2. It is interesting to notice that system 7 (S7) consisted of a sequence that had hydrophilic residues in positions 1 and 7 (terminal residues), with all other residues being hydrophobic. S7 represents the fragment 16–22 of the α -beta amyloid protein that has been widely studied because of its unambiguous formation of ordered fibrils. For each peptide, the hydrophobicity of the system was calculated in terms of what we called the number of hydrophobic bonds (HB), namely, the number of bonds between hydrophobic residues in a given peptide, i.e., in

Table 1 Systems considered in this study, with 1 and 2 symbolizing hydrophobic and hydrophilic residues, respectively

System	Sequence	Number of HB
S1	1111111	6
S2	1111112	5
S3	1111122	4
S4	1111222	3
S5	1112222	2
S6	1122222	1
S7	2111112	4
S8	1212121	0

an all-atom representation this quantity is the number of peptide bonds formed between hydrophobic residues. For example, in the peptide composed of seven hydrophobic residues, the number of HB is equal to six: all bonds are between hydrophobic residues. This quantity is depicted in the third column of Table 1 for each peptide. We shall see that the number of HB seems to be a significant factor in the stability of ordered aggregates.

A continuous spherical constraining potential, V_c , was implemented to consider peptide aggregation as a function of concentration. Namely, a constraining radius, R_c , was defined as initial input, and the constraining potential computed by [26]:

$$V_c = \sum_{i=1}^N \left[\frac{(R_i - R_{cm})}{R_c} \right]^{20} \quad (2)$$

where R_i and R_{cm} are the position of the i^{th} residue and the center of mass of the system, respectively. As the constraining radius increases, the concentration of the peptide in the solution decreases. The concentration of the peptide, $[P]$, was calculated using the following equation

$$[P] = \frac{NB}{\frac{4}{3}\pi R_c^3} \quad (3)$$

where NB is the number of beads in the system, and the volume of the system is assumed to be spherical. All quantities were reported in reduced units, with ε and σ in the long-range part of the potential being set to 1.0.

Table 2 Fragments of various proteins that have been considered experimentally

System	Sequence	Calculated fraction of HB
Alpha-beta amyloid	KLVFF	0.76
Prion	AGAAAAGA	0.43
Tau	VQIVYK	0.60
Beta2-microglobulin	A: IQRTPLIQVYSRMPAE*	0.13
	B: NGKSNFLNCYVSG*	0.17
	C: FMPSDIEWDLLE*	0.18
	D: NGERIEKVENSDLSFSKD*	0.06
	E: DWSFYLLYYTEFT	0.42
	E': DWSFYLLYYTEFTPTGKDETA	0.30
	F: DWSFYLLYYTEFTPTGKDETA*	0.20
	D: LSQPKIVKWDRDM*	0.0

For the characterization of possible conformational transitions, the constant volume heat capacity as a function of temperature was calculated using the standard energy fluctuation expression [27]:

$$\langle C_V \rangle = \frac{\langle V^2 \rangle - \langle V \rangle^2}{k_B T^2} \quad (4)$$

where V is the potential energy, T is the absolute temperature, and k_B is the Boltzmann constant. As in our previous studies [28, 29], we computed the amount of heat absorbed, ΔU , by the system during the conformational transitions by numerical calculation of the area under the $\langle C_V \rangle$ vs. T curve. Moreover, because in the transition region $\Delta A = 0$, the change in entropy can be obtained from $\Delta S = \Delta U/T_{\max}$, where $T_{\max}$ is the temperature at which $\langle C_V \rangle$ shows a maximum value (also called the transition temperature). This approach is similar to that used in experimental differential scanning calorimetric (DSC) techniques [30, 31], which have been extensively applied to conformational changes in proteins.

Additionally, the thermodynamic stability of the dipeptides was computed in terms of the change in the Gibbs free energy, ΔG , for the process

The method of thermodynamic integration [32] was used for the computation of ΔG . In this method the interparticle potential was rescaled by a constant λ , in the following manner

$$V = V_1 + (1 - \lambda) V_{\text{intr}} \quad (6)$$

where V_1 is the potential energy for the single peptide, and V_{intr} is the interaction between the two peptides. With this rescaling of the potential, ΔG for the reaction described in Eq. 5 can be computed by solving a one-dimensional integral of the form

$$\Delta G = - \int_0^1 \left\langle \frac{\partial V}{\partial \lambda} \right\rangle_\lambda d\lambda \quad (7)$$

where brackets denote the ensemble average at a specific λ value.

In order to circumvent the endpoint singularity problem usually encountered [33] in thermodynamic integration in the non-bonded interactions represented by L-J potentials, a linear mixing soft-core potential [34] was implemented. In this technique, the interactions are represented by a λ -dependent modified L-J potential, $V_{\text{non-bonded}}$, of the following form:

$$\frac{V_{\text{non-bonded}}}{4\epsilon} = (1 - \lambda) \sum_{i < j} \left\{ H_1 \left[\frac{1}{\alpha\lambda + \left(\frac{r_{ij}}{\sigma}\right)^6} \right]^2 - H_2 \left[\frac{1}{\alpha\lambda + \left(\frac{r_{ij}}{\sigma}\right)^6} \right] \right\} \quad (8)$$

where α is an adjustable constant set to 0.5. Previous studies have shown [34] that this soft-core potential effectively allows for proper thermodynamic integration even when using a relatively small number of integration points. The calculation of the change in Gibbs

free energy was reduced to the evaluation of a one-dimensional integral, which was solved numerically using six points in the Gauss-Legendre integration algorithm:

$$\int_0^1 \left\langle \frac{\partial V}{\partial \lambda} \right\rangle_{\lambda} d\lambda = \sum_{i=1}^6 W(\lambda_i) \left\langle \frac{\partial V}{\partial \lambda} \right\rangle_{\lambda_i} \quad (9)$$

where $W(\lambda)$ are the weights for a particular value of λ .

All canonical ensemble averages were computed using various Monte Carlo (MC) techniques. Each standard MC (SMC) move consisted of random particle displacements, which were performed with an acceptance probability, p , given by

$$p = \min = [1, \exp(-\beta \Delta U)] \quad (10)$$

where $\beta = 1/k_B T$, k_B is the Boltzmann constant, T is the absolute temperature, and ΔU is the change in internal energy between the configurations being sampled.

One of the main difficulties in characterizing conformational changes in biomolecular systems is the large fluctuation in the thermodynamic properties of the system. Hence, proper sampling of the configuration space is crucial for adequate convergence. To circumvent this problem, the replica-exchange algorithm was implemented [35–37]. This method allows the interchange between configurations that have different temperatures, β_2 and β_1 , with an acceptance probability given by

$$p = \min [1, \exp[(\beta_2 - \beta_1) \Delta U]]. \quad (11)$$

Tandem walkers in temperature were run using SMC steps. Every ten SMC steps, an interchange of configurations between replicas at different temperatures was attempted. The interchange was attempted between the actual configuration and one of its neighboring configurations. The temperature separation was chosen such that the acceptance ratio of interchanges was 20% or higher. A total of 10^8 warm-up steps were taken, followed by 10^8 steps where data was collected. The step size was adjusted in order to obtain 50% acceptance for the moves. Additional replica exchange moves were performed based on the hydrophobic aided replica exchange method (HAREM) [38]. In these moves, jumps between the various systems having different hydrophobicity were attempted. The acceptance probability in HAREM is given by Eq. 10, with the difference in energy being computed between two configurations of different primary sequences at the same temperature. For example, an exchange between configurations in systems S1 and S2 is possible with a probability of acceptance given by Eq. 10. This replica exchange procedure enhances the hydrophobicity of the systems, hence improving sampling in possible aggregated configurations. Each Monte Carlo move consisted of moving each coarse-grained residue once, with the residue chosen randomly. For every ten SMC steps, a replica-exchange MC step was randomly chosen, i.e., either the standard exchange of configurations at different temperatures or a HAREM move.

3 Results and discussion

The results obtained are shown in Figs. 1 through 8. Figure 1 shows the $\langle C_V \rangle$ as a function of temperature for the dimeric system with all residues being hydrophobic (system S1), and at various concentrations. In all cases, two peaks were identified in low- and high-temperature regions. Upon visual examination of sampled configurations within these

Fig. 1 Average constant volume heat capacity as a function of temperature for the dimeric polypeptide system with all residues being hydrophobic. All quantities are reported in reduced units

two regions, and the behavior of the computed distribution of distances, various ordered aggregated structures were identified at low temperatures. Figure 2a through f show the six most frequently observed configurations in this low-temperature region. These well-ordered

Fig. 2 The lowest energy equilibrium structures obtained for the dimeric polypeptide systems with all residues being hydrophobic

Fig. 3 **a** Representative amorphous configuration for the dimeric polypeptide system with all residues being hydrophobic; **b** shows the same as **a**, but for a configuration where the dipeptide has been dissociated into single peptides

structures have been previously identified as the smallest aggregates in the early stages of amyloid fibril formation using coarse-grained [10–14] and all-atom simulations [15–19]. At very low temperatures, two structures were primarily sampled for this dimeric system: parallel and parallel shifted structures, depicted in Fig. 2a and b, respectively. These configurations, which are the two lowest-energy equilibrium structures, maximized the number of hydrophobic interpeptide interactions. Detailed examination of the first transition region shows that there is an equilibrium between ordered and disordered structures (example of a disordered structure is depicted in Fig. 3a). However, in the temperature range where the second peak occurred, coexistence of amorphous aggregates and structures where the two peptides have separated (Fig. 3b) was observed. Thus, in the temperature range of the first peak, ordered and disordered aggregates were sampled, whereas in the range of the second peak, sampling of amorphous and dissociated aggregates occurred.

As stated above, numerical integration of each $\langle C_v \rangle$ vs. T curve yielded ΔU for each transition. From the above discussion, it can be concluded that values of ΔU obtained from

Fig. 4 Change in internal energy of formation of amyloid and amorphous aggregates as a function of concentration for the dimeric polypeptide system with all residues being hydrophobic

Fig. 5 Change in the entropy of formation of amyloid and amorphous aggregates as a function of concentration for the dimeric polypeptide system with all residues being hydrophobic

the first peak are associated with the *ordered* $\rightarrow$ *disordered* equilibrium. Hence, energetic stabilization for the ordered aggregates can be defined as $-\Delta U$ (the backward reaction). On the other hand, the second transition corresponds to the *disordered* $\rightarrow$ *dissociated* equilibrium, with $-\Delta U$ for this equilibrium giving the energetic stabilization of the amorphous aggregates. The values for ΔU and ΔS as a function of concentrations for both aggregates were plotted in Figs. 4 and 5, respectively. It can be observed from Fig. 4 that, at the high concentration, the ordered aggregates were more energetically stable than the amorphous aggregates by 8.7 reduced units. However, as the concentration of the solution decreased, the ordered systems became less energetically stable, whereas ΔU for the amorphous aggregates decreased. Furthermore, at concentrations below 0.002 reduced units, the amorphous aggregates became more energetically stable than the ordered system. Figure 5 shows the entropic contribution to the stability of the systems, and, as expected at all concentrations, the amorphous aggregates are entropically favored. Due to the energetic stabilization of the ordered aggregates at high concentrations, all the discussion presented below was limited to the highest concentration studied.

When other dipeptide systems with differing hydrophobicity were considered, the $\langle C_v \rangle$ vs. T curves obtained were similar to S1, but with variations in the computed stability of both the ordered and amorphous aggregates. Figure 6 shows ΔU as a function of hydrophobicity (fraction of hydrophobic bonds). Similarly to S1, the S2 ordered systems were

Fig. 6 Change in internal energy of formation of amylogenic and amorphous aggregates as a function of the hydrophobic bonds of the dimeric polypeptide systems

Fig. 7 Same as Fig. 1, but for system 8 (S8)

more energetically stable than the amorphous aggregates. However, when S2 is compared to S1, the ordered aggregates were less stable, and the amorphous aggregates were more stable. In contrast to S1 and S2, in systems S3, S4, S5, S6, and S7, the ordered aggregates were always less stable than the amorphous aggregates. Moreover, for system S8, which had no hydrophobic bonds, the formation of fibrils was not observed. Figure 7 shows the $\langle C_v \rangle$ as a function of temperature for S7. Only one peak is observed, which is associated with the stability of amorphous aggregates. When results for S3 and S7 (both have an equal number of hydrophobic residues but different primary structures) were compared, ordered aggregates in S3 were more stable than S7, and amorphous aggregates in S7 were more stable than S3. This result is due to the fact that all five hydrophobic residues of S3 are consecutive, with the hydrophilic interactions in the terminals decreasing hydrophobic contacts, and hence decreasing possible ordered packing. These results indicate a strong dependence between the number of hydrophobic residues and the stability of the aggregates. When the fraction of hydrophobic bonds was greater than 0.80, ordered aggregates were more stable than amorphous aggregates. Additionally, for systems with X_{HB} less than 0.15, the formation of the ordered configurations were almost negligible and independent of the used concentration. The variation in entropy as a function of hydrophobicity showed that the amorphous aggregates are favored for all systems with no particular tendency.

Additional calculations were performed to obtain information related to the stability of these aggregates. Specifically, the change in Gibbs free energy of formation was computed as a function of temperature for all the sequences considered here. Figure 8 shows ΔG as a function of temperature, for various values of X_{HB} with annotations in the temperature

Fig. 8 Change in the Gibbs free energy of formation for the various systems as a function of temperature computed using thermodynamic integration

scale, which identifies the regions where ordered and amorphous aggregates were stable. In all cases, ΔG decreased as temperature decreased, implying that the system became more stable at low temperatures. Additionally, as X_{HB} of the system increased, the value of ΔG decreased and the stability of the ordered aggregates increased. This is similar to the previously observed results based on $\langle C_v \rangle$ vs. T curves. Interestingly, in the case of the amorphous aggregates, the dependence upon X_{HB} was observed when $X_{HB} > 0.80$, i.e., systems S1, S2, S3. In such systems, the stability of the amorphous aggregates depends on the content of hydrophobic interactions only when the number of hydrophobic bonds is large. In all other cases, the stability was independent of hydrophobicity.

4 Conclusions

We have used a simple reduced dimensionality model to describe the interaction of two peptides that form ordered and amorphous aggregates. At low temperatures, various structural arrangements of peptides were obtained in accordance with previous studies focused on identifying the smallest precursors in the formation of amyloid fibrils. The stability of each system was determined by integration the $\langle C_v \rangle$ vs. T curves, and by implementing thermodynamic integration techniques to the equilibria associated with the formation of aggregates. It was found that relatively high concentrations of the peptides were needed for ordered peptides to be energetically more stable than the amorphous peptides. Also, ordered peptides were more energetically stable than amorphous aggregates if the hydrophobic character of the peptides was relatively large and the primary sequence had consecutive hydrophobic residues. These results suggest that high concentration and a large number of hydrophobic bonds make the peptide prone to form ordered materials, and, as such, increase the likelihood of forming prefibrillar material in the initial stages of aggregation. At present, the thermodynamic stability of larger aggregates composed of peptides is being considered. Also, inclusion of hydrogen bonding, which has a significant effect at later stages of fibril formation, is being considered.

Although our model is based on a relatively simple description of interparticle forces, it is interesting to note that experimental data confirms our results in a qualitative manner. For example, experimental procedures [39] depend on the use of high concentrations to form amyloid fibrils. Our results suggest that in order for fibrils to be more stable than amorphous aggregates, relatively high concentrations are needed. Our results also suggest that the number of hydrophobic bonds is an important parameter in the formation of ordered aggregates. Interestingly, this parameter has not been previously considered as a crucial quantity in protein aggregation, though an analysis of various proteins fragments that are known to be prone to form fibrils shows a surprising correlation. Table 2 shows various protein fragments that have been studied experimentally [40–43] and the percentage of hydrophobic bonds. The fragments of α -beta amyloid, prion, and tau have a large fraction of hydrophobic bonds, and thus, ordered aggregation is expected. In the case of beta-microglobulin, various fragments were considered based on the results reported in reference [43]. In this study, it was concluded that there were no structural characteristics of the different fragments considered that explained the aggregation process—the fragments E and E' were the only fragments forming fibrils. However, from the fraction of hydrophobic bonds shown in Table 2 for these systems, it can be seen that the fragments E and E' have a relatively large number of hydrophobic bonds. Moreover, the fragments that do not form fibrils have less than 20% of hydrophobic bonds. From this comparison, we conclude

that the fraction of hydrophobic bonds might be a significant factor in the structure of aggregates.

Acknowledgement The INBRE/NIH program supported this research.

References

1. Schnabel, J.: The dark side of proteins. *Nature* **464**, 828–829 (2010)
2. Luhrs, T., Zahn, R., Wuthrich, K.: Amyloid formation by recombinant full-length prion protein in phospholipid bicelle solutions. *J. Mol. Biol.* **357**, 833–841 (2006)
3. Fawzi, N.L., Yap, E.H., Okabe, Y., Kohlstedt, K., Brown, S., Head-Gordon, T.: Contrasting disease and non-disease protein aggregation by molecular simulations. *Acc. Chem. Res.* **41**, 1037–1047 (2008)
4. Rochet, J.-C., Lansbury, Jr. P.T.: Amyloid fibrillogenesis: themes and variations. *Curr. Opin. Struct. Biol.* **10**, 60–68 (2000)
5. Chiti, F., Webster, P., Taddei, N., Clark, A., Stefani, M., Ramponi, G., Dobson, C.M.: Designing conditions for in vitro formation of amyloid protofilaments and fibrils. *Proc. Natl. Acad. Sci. USA* **96**, 3590–3595 (1999)
6. Bouchard, M., Zurdo, J., Nettleton, E.J., Dobson, C.M.: *Protein Sci.* **9**, 1960–1967 (2000)
7. Nilsson, M.R., Dobson, C.M.: Chemical modification of insulin in amyloid fibrils. *Protein Sci.* **12**, 2637–2641 (2003)
8. Malisauskas, M., Zamolin, V., Jass, J., Noppe, W., Dobson, C.M., Morizova-Roche, L.A.: Amyloid protofilaments from the calcium-binding protein equine lysozyme: formation of ring and linear structures depends on pH and metal ion concentration. *J. Mol. Biol.* **330**, 879–890 (2003)
9. Huang, A., Stultz, C.M.: Finding order within disorder: elucidating the structure of proteins associated with neurodegenerative disease. *Future Med. Chem.* **1**(3), 467–482 (2009)
10. Singh, G., Brovchenko, I., Oleinikava, A., Winter, R.: Peptide aggregation in finite systems. *Biophys. J.* **95**, 3208–3221 (2008)
11. Bellesia, G., Shea, J.-E.: Effect of β -sheet propensity in peptide aggregation. *J. Chem. Phys.* **130**, 145103–145113 (2009)
12. Bellesia, G., Shea, J.-E.: Diversity of kinetic pathways in amyloid fibril formation. *J. Chem. Phys.* **131**, 111102–111105 (2009)
13. Wang, Y., Voth, G.A.: Molecular dynamics simulations of polyglutamine aggregation using solvent-free multiscale coarse-grained models. *J. Phys. Chem.* **114**, 8735–8743 (2010)
14. Yang, S., Cho, S.S., Levy, Y., Cheung, M.S., Levine, H., Wolynes, P.G., Onuchic, J.N.: Domain swapping is a consequence of minimal frustration. *Proc. Natl. Acad. Sci. USA* **101**, 13786–13791 (2004)
15. Gnanakaran, S., Nussinov, R., Garcia, A.E.: Atomic-level description of amyloid β -dimer formation. *J. Am. Chem. Soc.* **128**, 2158–2159 (2006)
16. Fawsi, N., Phillips, A.H., Ruscio, J.Z., Doucleff, M., Wemmer, D.E., Head-Gordon, T.: Structure and dynamics of the $A\beta_{21-30}$ peptide from the interplay of NMR experiments and molecular simulations. *J. Am. Chem. Soc.* **130**, 6145–6158 (2008)
17. Wei, G., Jewett, A.I., Shea, J.-E.: Structural diversity of dimers of the Alzheimer amyloid- β (25–35) peptide and polymorphism of the resulting fibrils. *Phys. Chem. Chem. Phys.* **12**, 3622–3629 (2010)
18. Sgourakis, N.G., Yan, Y., McCallum, S.A., Wang, C., Garcia, A.E.: The Alzheimer's peptides Abeta40 and 42 adopt distinct conformations in water a combined MD/NMR study. *J. Mol. Bio.* **368**, 1448–1457 (2007)
19. Vitalis, A., Wang, X., Pappu R.: Atomistic simulations of the effects of polyglutamine chain length and solvent quality on conformational equilibria and spontaneous homodimerization. *J. Mol. Biol.* **383**, 279–297 (2008)
20. Klimov, D.K., Thirumalai, D.: Dissecting the assembly of $A\beta_{16-22}$ amyloid peptides into antiparallel β sheets. *Structure* **11**, 295–307 (2003)
21. Straub, J.E., Thirumalai, D.: Principles governing oligomer formation in amyloidogenic peptides. *Curr. Opin. Struct. Biol.* **20**, 187–195 (2010)
22. Brown, S., Fawsi, N.L., Head-Gordon, T.: Coarse-grained sequences for protein folding and design. *Proc. Natl. Acad. Sci.* **100**, 10712–10717 (2003)
23. Sorenson, M., Head-Gordon, T.: Matching simulation and experiment: a new simplified model for simulating protein folding. *J. Comp. Biol.* **7**, 469–481 (2000)
24. Ma, B., Nussinov, R.: Molecular dynamics simulations of alanine-rich β -sheet oligomers: Insight into amyloid formation. *Protein Sci.* **11**, 2335–2350 (2002)

25. Bains, B., Rouleau, G.A., Bouchard, J.P., Farde, M., Tome, F.M.: Oculopharyngeal muscular dystrophy. *Semin. Neurol.* **19**, 59–66 (1999)
26. Lee, J.K., Barker, J.A., Abraham, F.F.: Theory and Monte Carlo simulation of physical clusters in the imperfect vapor. *J. Chem. Phys.* **58**, 3166–3180 (1973)
27. Frenkel, D., Smit, B.: *Understanding Molecular Simulations: from Algorithms to Applications*, 2nd edn. Academic Press, New York (2001)
28. Ramirez, E., López, G.E.: Isotopic effect in the solid-liquid line of quantum clusters. *Mol. Phys.* **105**, 2399–2406 (2007)
29. López, G.E.: Quantum effects in the solid-liquid phase diagram of Ne_{13} and $(\text{para-H}_2)_{13}$. *J. Chem. Phys.* **117**, 2225–2232 (2002)
30. Bruylants, G., Wouters, J., Michaux, C.: Differential scanning calorimetry in life science: thermodynamics, stability, molecular recognition and application in drug design. *Curr. Med. Chem.* **12**, 2011–2020 (2005)
31. Cooper, A., Johnson, C.M.: Differential scanning calorimetry. *Methods Mol. Biol.* **22**, 125–136 (1994)
32. Grochola, G.: Constrained fluid λ -integration: Constructing a reversible thermodynamic path between the solid and liquid state. *J. Chem. Phys.* **120**, 2122–2127 (2004)
33. Valleau, J.P., Torrie, G.: *Modern Theoretical Chemistry*, vol. 5. In: Berne, B. (ed.) Plenum Press, New York (1997)
34. Steinbrecher, T., Mobley, D.L., Case, D.A.: Non-linear scaling schemes for Lennard-Jones interactions in free energy calculations. *J. Chem. Phys.* **127**, 214108 (2007)
35. Strozak, M.A., López, G.E., Freeman, D.L.: Gibbs free energies for formation of argon clusters adsorbed on graphite. *J. Chem. Phys.* **97**, 4445 (1992)
36. Quintana, I.M., Ortiz, W., López, G.E.: Determination of the structure and stability of water clusters using temperature dependent techniques. *Chem. Phys. Lett.* **282**, 429 (1998)
37. Hukushima, K., Takayama, H., Nemoto, K.: Exchange Monte Carlo method and application to spin glass simulation. *J. Phys. Soc. Jpn.* **65**, 1604–1616 (1996)
38. Liu, P., Huang, X., Zhou, R., Berne, B.J.: Hydrophobic aided replica exchange: An efficient algorithm for protein folding in explicit solvent. *J. Chem. Phys.* **110**, 19018–19022 (2006)
39. Hirota-Nakaoka, N., Hasegawa, K., Naiki, H., Goto, Y.: Dissolution of β_2 -microglobulin amyloid fibrils by dimethylsulfoxide. *J. Biochem.* **134**, 159–164 (2003)
40. Petkova, A.T., Yau, W.M., Tycko, R.: Experimental constraints on quaternary structure in Alzheimer's beta-amyloid fibrils. *Biochemistry* **45**, 498–512 (2006)
41. Prusiner, S.B., McKinley, M.P., Bowman, K.A., Bolton, D.C., Bendheim, P.E., Groth, D.F., Glenner, G.G.: Scrapie prions aggregates to form amyloid-like birefringent rods. *Cell* **35**, 349–358 (1983)
42. von Bergen, M., Friedhoff, P., Biernat, J., Heberle, J., Mandelkow, E.M., Mandelkow, E.: Assembly of τ protein into Alzheimer paired helical filaments depends on a local sequence motif ($^{306}\text{VQIVYK}^{311}$) forming beta structure. *Proc. Natl. Acad. Sci. USA* **97**, 5129–5134 (2000)
43. Jones, S., Manning, J., Kad, N.M., Radford, S.E.: Amyloid-forming peptides from β_2 -microglobulin: insights into the mechanism of fibril formation in vitro. *J. Mol. Biol.* **325**, 249–257 (2003)