Dual effect of crowders on fibrillation kinetics of polypeptide chains revealed by lattice models

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Dual effect of crowders on fibrillation kinetics of polypeptide chains revealed by lattice models

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We have developed the lattice model for describing polypeptide chains in the presence of crowders. The influence of crowding confinement on the fibrillation kinetics of polypeptide chains is studied using this model. We observed the non-trivial behavior of the fibril formation time τ_{fib} that it decreases with the concentration of crowders if crowder sizes are large enough, but the growth is observed for crowders of small sizes. This allows us to explain the recent experimental observation on the dual effect of crowding particles on fibril growth of proteins that for a fixed crowder concentration the fibrillation kinetics is fastest at intermediate values of total surface of crowders. It becomes slow at either small or large coverages of cosolutes. It is shown that due to competition between the energetics and entropic effects, the dependence of τ_{fib} on the size of confined space is described by a parabolic function. © *2013 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4804299]

I. INTRODUCTION

Neurodegenerative pathologies such as Huntington's, Alzheimer's, Parkinson's, and prion diseases are associated with formation of amyloid oligomers and fibrils that have cross β-sheet structure.[1] Understanding mechanisms governing fibrillation kinetics of peptides and proteins plays a key role in finding out the way for their effective treatment. So far most of investigation has been focused on exploring kinetics of oligomerization in ideal homogeneous milieu. However, all living processes take place in crowded environment which comprises DNA, protein, lipid, and sugar occupying 20%–30% volume of the typical cell cytoplasm.[2] Therefore this factor should be taken into account.

Recently some progress has been made in apprehending the effects of macromolecular crowding on folding, conformation, and function of proteins.[3–7] The aggregation of proteins is a more complex process because it depends not only on the monomer sequence but also on intermolecular interactions, nucleation rates, diffusive properties, etc.[8–10] Despite this apparent complexity, one can delineate general features for fibril growth in crowded environment showing that the propensity to self-assembly may not depend on some details of studied systems. For example, having used a quartz crystal microbalance (QCM) assay with a high level of accuracy in measuring fibril growth rates, Dobson and co-workers showed that cosolutes accelerate the fibril elongation.[11] This finding is supported by numerical simulations[12,13] and in line with the depletion theory.[3,14] The similar dependence of fibrillation rates on crowder concentration has been found by Linse *et al.*[15] who showed that copolymeric nanoparticles accelerate β_2-microglobulin. This was explained by assuming accumulation of β_2-microglobulin at the nanoparticle surface favoring fast formation of critical nuclei for fibril growth. The opposing effect has been observed for amyloid β peptides fibril growth of which is retarded by nanoparticles.[16] It has been interpreted as a result of depletion of solution concentration leading to block of the growing ends. The most striking experimental observation has been made by Cabaleiro-Lago *et al.* who reported on the dual effect of amino modified polystyrene nanoparticles on Aβ_{40} and Aβ_{42} peptide fibrillation.[17] At a constant peptide concentration, the fibril formation is accelerated by crowders at low crowder concentration, while at high crowder concentration this process is slowed down. Thus, contrary to other groups which have seen either acceleration[15,18] or retardation,[16,19] they have observed both effects by one type of nanoparticle. This interesting dual effect is presumably due to a competition between two different mechanisms of interaction between protein and crowders which is tuned by the total crowder surface area.[17] However theoretical understanding of such phenomena is still missing.

The main goal of this paper is to explain the dual effect using the lattice model.[20,21] We have shown that there exists a critical crowder size below which the fibril formation time $\tau_{\rm fib}$ does not decrease as observed in the previous theoretical works[12,13] but increases with the concentration of crowders. More importantly, in agreement with the experiment,[17] the dependence of $\tau_{\rm fib}$ on the coverage of crowders has the U-shape character which is a typical for the dual effect.

Protein folding in confined space has been studied in detail,[22–24] but the effects of confinement on fibril formation has not been investigated yet. Therefore, our second goal is to study the fibrillation kinetics of polypeptide chains in cavity. This also helps us in exploring its analogy with aggregation in the presence of cosolutes leading to better understanding the mechanism of the dual effect.

II. MATERIALS AND METHODS

A. Polypeptide chain

We will extend the toy lattice model developed for studying the oligomerization kinetics[20] to the case when crowders coexist with polypeptide chains. In this model each chain consists of M connected beads that are confined to the vertices of a cube. The simulations are done using N identical chains and $M = 8$. The sequence of a chain is +HHPPHH−, where + and − are charged beads. The inter- and intra-chain potentials include excluded volume and contact (nearest neighbor) interactions. Excluded volume is imposed by the condition that a lattice site can be occupied by only one bead. The energy of N chains is

$$E = \sum_{l=1}^{N} \sum_{i<j}^{M} e_{sl(i)sl(j)} \delta(r_{ij} - a) + \sum_{m<l}^{N} \sum_{i,j}^{M} e_{sl(i)sm(j)} \delta(r_{ij} - a), \quad (1)$$

where r_{ij} is the distance between residues i and j, a is a lattice spacing, $sm(i)$ indicates the type of residue i from mth peptide, and $\delta(0) = 1$ and zero, otherwise. The first and second terms in Eq. (1) represent intrapeptide and interpeptide interactions, respectively. The contact energies between H beads e_{HH} is −1 (in the units of the hydrogen bond energy ϵ_H). The propensity of polar (including charged) residues to be "solvated" is mimicked using $e_{P\alpha} = -0.2$, where $\alpha = $ P, +, or −. "Salt-bridge" formation between oppositely charged beads is accounted for by a favorable contact energy $e_{+-} = -1.4$. All other contact interactions are repulsive. The generic value for repulsion $e_{\alpha\beta}$ is 0.2. For a pair of like-charged beads the repulsion is stronger, i.e., $e_{++} = e_{--} = 0.7$. Our toy force field roughly mimics the interactions between amino acids. The chains were confined to the vertices of the three-dimensional simple cubic lattice. The concentration of chains was kept ≈57 μM (roughly, the cubic sizes are 135, 165, and 217a for $N = 6$, 10, and 24 monomers and in what follows we set the lattice space $a = 1$) for all systems. This concentration has the same order of magnitude as that used in typical experiments.

The 8-bead sequence monomer in our model folds to the compact native state,[20] while the conformation of ground or fibril state of multi-sequence systems has the fibril structure where monomers are antiparallel. The fibril structure for $N = 6$ is shown in Fig. 1(a), while structures for $N = 10$, 16, and 24 are shown in Fig. S1 of the supplementary material.[30] The folding temperature of monomer $T_F = 0.55$ which is identified as room temperature in our model.[20] Further calculations will be performed at a bit higher temperatures (see below), where the fibril formation is fast to reduce CPU time.

B. Crowders

Crowders are modeled as inpenetrable cubics (Fig. 1(b)) and their size is denoted by R_c. They do not interact either with each other or with polypeptide chains. However the excluded volume is imposed in such a way that any site of the lattice can be occupied either by a polypeptide chain or crowder, but not both.

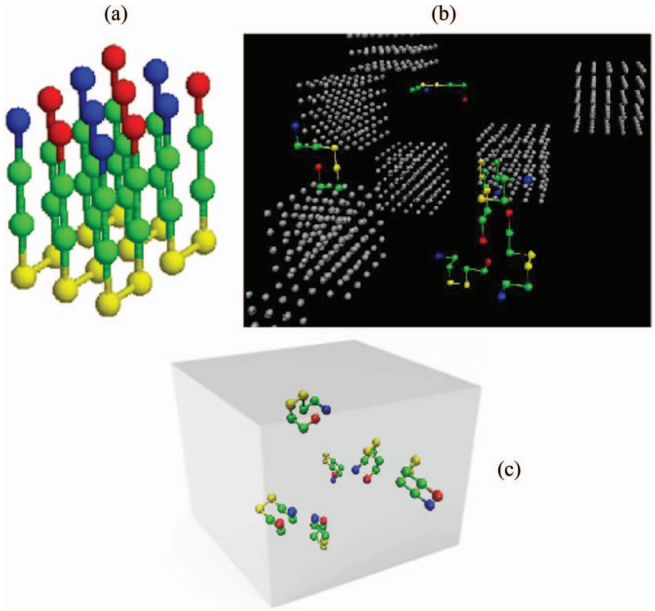

FIG. 1. (a) Fibril conformation in the lattice model for $N = 6$ chains. (b) Snapshot of crowders (grey cubes) and polypeptide chains (color). (c) Random configuration of chains in a confined box.

C. Modeling of confining box

In order to study the confinement effect, we allow monomers to move within a box with different sizes (Fig. 1(c)). Instead of periodic boundary conditions, the free boundary conditions are applied for Monte Carlo (MC) simulations.

D. Monte Carlo moves

We use MC algorithm to study the kinetics of fibril formation of polypeptide chains. MC moves include global and local ones. A local move[25,26] corresponds to tail rotation, corner flip, and crankshaft rotation. Global moves, which are rigid-body moves, correspond to either translation of a peptide by a in a randomly chosen direction or rotation by 90° around one of the randomly chosen coordinate axes. The acceptance probabilities of global and local moves are 0.1 and 0.9, respectively (see Ref. 20 for more details). We measure time in units of Monte Carlo steps (MCS). The combination of nine local and one global moves constitutes one MCS.

Every 5 MC moves for chains, cubics which represent crowders are allowed to translate as a whole randomly in one of the six possible directions. Simulations were performed by enclosing N chains and crowders with concentration Φ_c, defined as the volume fraction of crowders, in a box with periodic boundary conditions.

E. Fibril formation time τ_{fib}

The simulation is started from random configurations of chains (confinement) or of chains and crowders (crowding

case) (Figs. 1(b) and 1(c)) and the fibril formation time is defined as the first passage time to the fibril state.[20,21] To get the robust result for each set of parameters, 50–200 MC trajectories are generated and τ_{fib} is computed as the average of all first passage times.

III. RESULTS AND DISCUSSION

A. Crowder effect on the fibrillation kinetics

1. Aggregation times depend on crowder sizes

It has been shown that secondary structures of peptides strongly depend on size and shape of crowder.[13] However the effect of these factors on τ_{fib} has not been explored.[12,13] Using the lattice model, we calculated the fibril formation times for different crowder sizes and concentrations (Fig. 2). For smallest cubic crowders of size $R_c = 1$, the fibril formation becomes very large, we, therefore, make a separate plot in the supplementary material (Fig. S2 of the supplementary material[30]). Clearly, one has two scenarios for the dependence of τ_{fib} on the crowder concentration Φ_c (Fig. 2). For cubic crowders of sizes $R_c > 2$ the fibril formation time decays with Φ_c. This scenario has been also observed in the experiments[11,15,18] as well as in simulations using off-lattice models[12] and may be explained using general theoretical arguments based on the depletion theory.[3,13,14] The depletion effect which results in nonspecific entropy-induced attraction between polymers in the presence of nonabsorbing cosolutes, arises from the volume excluded to the crowders by aggregating polymers. This attraction should promote fibril formation of polypeptide chains as shown in Fig. 2.

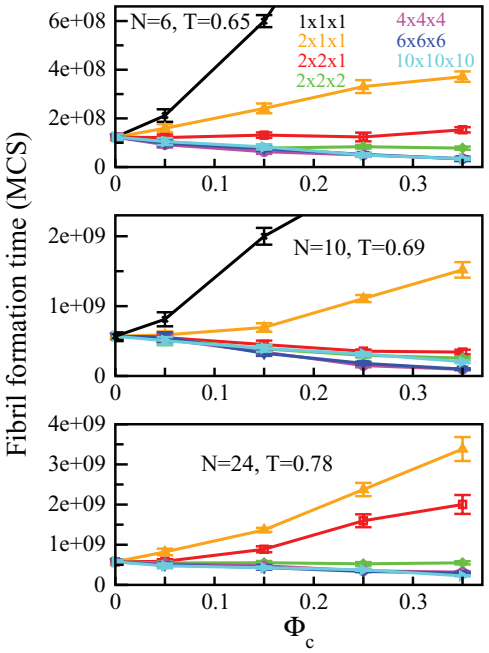

FIG. 2. Dependence of τ_{fib} on the crowder concentration Φ_c for various sizes of crowders. Orange and red refer to non-cubic (rectangular parallelepiped) crowders with dimensions $2 \times 1 \times 1$ and $2 \times 2 \times 1$. Other crowder sizes are also shown. The number of polypeptide chains $N = 6$, 10, and 24.

The experiments of Cabaleiro-Lago *et al.*[17] showed that in the presence of 120 nm amine-modified polystyrene nanoparticles the fibrillation kinetics of Aβ_{40} is faster at small concentrations of crowders but it becomes slower at high concentrations. One may think that this contradicts our results shown in Fig. 2. However, this is not so because at high concentrations, the free volume becomes small and the fibril formation should slow down for any systems. This is clearly shown in Fig. S3 of the supplementary material[30] where the dependence of τ_{fib} of 10 chains in the presence of $2 \times 2 \times 2$ crowders on Φ_c is presented up to $\Phi_c = 0.55$. Thus our results shown in Fig. 2 are restricted to those concentrations of large-size crowders where the increase of τ_{fib} does not occur yet and both acceleration and deceleration are captured by our model.

The second scenario for crowding effect on aggregation (Fig. 2) occurs when cosolute sizes are small. In this case, for a constant crowder concentration, the number of crowders becomes large and their random movement wins over the depletion effect leading to interference with aggregation. Another possible explanation is that for small crowders their total surface area becomes large (Fig. S4 of the supplementary material[30]) shrinking the free space for conformational changes of proteins. Therefore, the fibril formation rates are considerably reduced, particularly in the case when crowders are cubes of the smallest size $R_c = 1$ (Fig. S2 of the supplementary material[30]). Such a reduction has not been obtained in the previous simulations[12] presumably because the crowder size has been chosen not small enough. In the present lattice model, where specific interactions between crowding particles and polypeptide chains are not taken into account, the small size of particles is the only reason for the increase of τ_{fib}. However, this effect may be caused by other factors that would be captured by more sophisticated models. Clarifying this issue requires further investigation.

2. Dual effect

To make direct comparison with the experimental results,[17] we plot the fibril formation time as a function of the total surface area of crowders which is defined as $A_{\text{total}} = \Phi_c V A_c / V_c$ (Fig. 3). Here V and V_c are the box volume and volume of individual crowder, while A_c is the surface area of a crowder. We obtain the good agreement with the experiment result on Aβ fibril formation in the presence of modified polystyrene nanoparticle[17] that τ_{fib} first decreases and then increases with the coverage of nanoparticles (Fig. 3). As explained above, at small crowder coverages, the entropy of aggregating agents is largely resulting in slow self-assembly. This situation is similar to slow protein folding at high temperatures.[27,28] The retardation at large surface areas (Fig. 3) comes from the reduction of entropy of proteins in very crowded environment. Note that this case corresponds to crowders of small sizes (Fig. S4 of the supplementary material[30]). The fast fibril formation occurs in the region where the energetics and entropic contributions compromise.

For the same value of A_{total} the larger Φ_c is, the faster is fibril formation (Fig. 3). This is because the larger

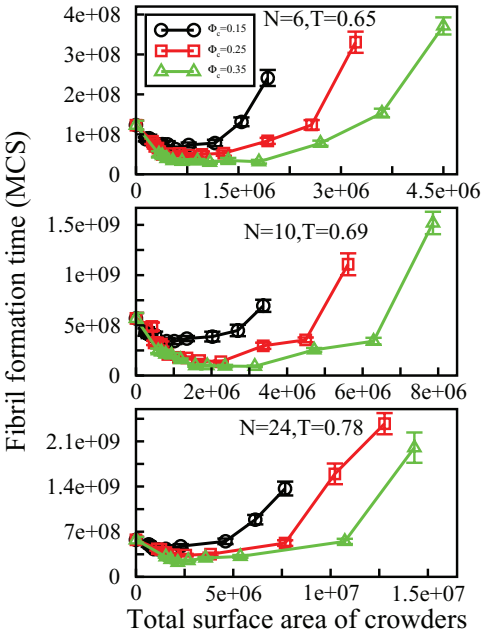

FIG. 3. Dependence of τ_{fib} on the total surface area of crowders at three values of crowder concentration Φ_c. $T = 0.65$, 0.69, and 0.78 for the number of chains $N = 6$, 10, and 24, respectively. The results are averaged over 50–200 MD trajectories for each set of parameters.

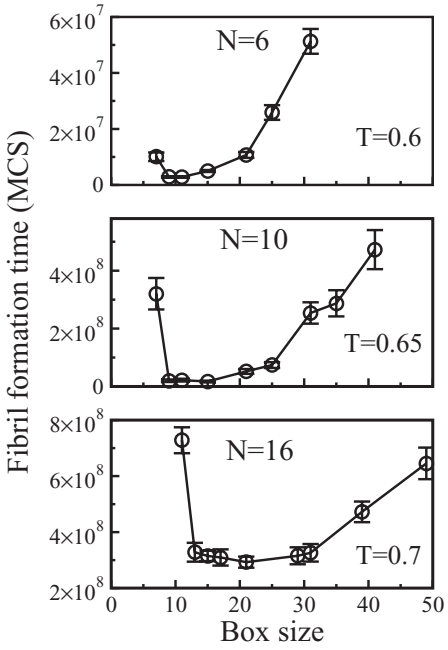

FIG. 4. The U-shape dependence of τ_{fib} on the sizes of confining cubic box. The results obtained from 50 to 200 MC trajectories for each box size. Error bars come from averaging over MC runs.

crowder concentration corresponds to crowders of larger sizes (for crowders of cubic shape $A_{\text{total}} = \frac{\Phi_c V}{R_c}$) which promote aggregation.

The dual effect may be also seen plotting τ_{fib} against sizes of crowding agents (Fig. S5 in the supplementary material[30]). Here again one has the U-shape dependence reflecting the effect of crowders on competition between energetics and entropic contributions of aggregating particles.

Since fibril assembly potentially involves spontaneous aggregation and subsequent fibril elongation, we also consider the effect of crowders on the aggregation. The aggregation time τ_{agg} is defined as the first passage time to reach the state where any chain has at least one contact with the remaining chains. The dependence of τ_{agg} on the total surface area of crowders is shown in Fig. S6 of the supplementary material[30] for $N = 6$ (similar results were obtained for $N = 10$ and 24, but not shown). The weak dual effect is observed for $\Phi_c = 0.35$ implying that the influence of crowders on aggregation of polypeptide chains is less pronounced than on fibril growth. This is presumably because the first process is considerably faster than the second one (Fig. 3 and Fig. S6 of the supplementary material[30]).

B. Confinement effect on the kinetics of fibrillation of polypeptide chains

In order to explore the analogy between crowder and confinement effects on aggregation kinetics, we have calculated the fibril formation times for 6, 10, and 16 chains at $T = 0.6$, 0.65, and 0.7, respectively. At these temperatures the fibril growth in bulk (without confinement) is fastest.[20] The minimal box size is $L_c^{\min} = 7, 7$, and 10 for $N = 6$, 10, and 16, respectively. $L_c^{\min} = 7$ is equal to the end-to-end distance of a 8-bead chain in the fully extended state. Below minimal sizes, the free space becomes so small that the fibril elongation is topologically almost impossible. The U-shape dependence of fibril formation times on size of confining boxes (Fig. 4) may be interpreted based on the entropy argument. At large L_c, the movement of chains becomes more random due to large conformational entropy leading to the increase of τ_{fib}. For small cavities, the fibril formation time increases again as the transition to the fibril state requires partial disassociation of chains which is difficult in a tight confining space. The fibril growth becomes fast at $L_c \sim 11$ for $N = 6$ and 10, and $L_c \sim 20$ for $N = 16$ (Fig. 4). One can show that for the same concentration of polypeptide chains τ_{fib} in confined space is larger than in bulk.

Comparing Fig. 4 and Fig. S5 of the supplementary material,[30] one can see the similarity between crowding and confinement that crowders of large sizes can effectively serve as walls of confining cavity leading to increase of τ_{fib}. For crowding particles of small sizes there is no analogy between these two phenomena but the slowing down of aggregation is largely caused by entropy reduction in narrow free space.

The U-shape behavior is also observed for folding times of β-hairpin in encapsuled space.[24] The confinement effects on the kinetics of protein dimerization has been considered by Wang *et al.*[29] who also reported a similar dependence for dimerization times. Thus the U-shape dependence that comes from the competition between energetics and entropy is universal for protein folding, oligomerization, and fibril formation times.

IV. CONCLUSION

For the first time we have theoretically explained the dual effect of crowders on the fibril growth kinetics using

our own lattice model. The non-monotonic dependence of τ_{fib} on the total surface area A_{total} of crowding particles comes from the competition of the energetics and entropic contributions. The decrease of the fibril formation times with the crowder concentration may be explained by the depletion theory. Our study reveals that this behavior appears in the case when crowder sizes are not too small compared to aggregating agents. The increase of τ_{fib} with Φ_c in our model is associated with small sizes of cosolutes, i.e., purely of topological nature. The question of whether such a dependence occurs in systems with crowders of large enough sizes but with realistic interactions between molecules remains open. Its answer requires development of either new theory or simulations with more realistic models. We have demonstrated that the crowding effect is similar to the confinement provided sizes of aggregating particles become large. Our study calls for development of the theory for aggregation in the presence of small crowders. This situation is probably important for crowding nanoparticles.

It should be noted that the dependence of folding[27] and fibril formation[21] times on the temperature also has the U-shape suggesting that temperature and sizes of confining cavity and crowders have the similar effect on folding and aggregation kinetics. This is because they have the same nature associated with the interplay between the energetics and entropic factors of the system although phenomena are different. This universal behavior may be captured even by the simple lattice model.

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[1]F. Chiti and C. M. Dobson, Annu. Rev. Biochem. **75**, 333 (2006).
[2]R. J. Ellis, Trends Biochem. Sci. **26**, 597 (2001).
[3]H. X. Zhou, G. N. Rivas, and A. P. Minton, Annu. Rev. Biophys. **37**, 375 (2008).
[4]M. S. Cheung, D. K. Klimov, and D. Thirumalai, Proc. Natl. Acad. Sci. U.S.A. **102**, 4753 (2005).
[5]S. S. Cho, G. Reddy, J. E. Straub, and D. Thirumalai, J. Phys. Chem. B **115**, 13401 (2011).
[6]B. van den Berg, R. Wain, C. M. Dobson, and R. J. Ellis, EMBO J. **19**, 3870 (2000).
[7]D. Homouz, M. Perham, A. Samiotakis, M. S. Cheung, and S. P. Wittung, Proc. Natl. Acad. Sci. U.S.A. **105**, 11754 (2008).
[8]J. Kuriyan and D. Eisenberg, Nature (London) **450**, 983 (2007).
[9]R. Krishnan and S. L. Lindquist, Nature (London) **435**, 765 (2005).
[10]R. J. Ellis and A. P. Minton, Biol. Chem. **387**, 485 (2006).
[11]D. A. White, A. K. Buell, T. P. J. Knowles, M. E. Welland, and C. M. Dobson, J. Am. Chem. Soc. **132**, 5170 (2010).
[12]A. Magno, A. Caflisch, and R. Pellarin, J. Phys. Chem. Lett. **1**, 3027 (2010).
[13]E. P. O'Brien, J. E. Straub, B. R. Brooks, and D. Thirumalai, J. Phys. Chem. Lett. **2**, 1171 (2011).
[14]S. Asakura and F. Oosawa, J. Chem. Phys. **22**, 1255 (1954).
[15]S. Linse, C. Cabaleiro-Lago, W. F. Xue, I. Lynch, S. Lindman, E. Thulin, S. E. Radford, and K. A. Dawson, Proc. Natl. Acad. Sci. U.S.A. **104**, 8691 (2007).
[16]C. Cabaleiro-Lago, F. Quinlan-Pluck, I. Lynch, S. Lindman, A. M. Minogue, E. Thulin, D. M. Walsh, K. A. Dawson, and S. Linse, J. Am. Chem. Soc. **130**, 15437 (2008).
[17]C. Cabaleiro-Lago, F. Quinlan-Pluck, I. Lynch, K. A. Dawson, and S. Linse, ACS Chem. Neurosci. **1**, 279 (2010).
[18]W. H. Wu, X. Sun, Y. P. Yu, J. Hu, L. Zhao, Q. Liu, Y. F. Zhao, and Y. M. Li, Biochem. Biophys. Res. Commun. **373**, 315 (2008).
[19]B. Klajnert, M. Cortijo-Arellano, M. Bryszewska, and J. Cladera, Biochem. Biophys. Res. Commun. **339**, 577 (2006).
[20]M. S. Li, D. K. Klimov, J. E. Straub, and D. Thirumalai, J. Chem. Phys. **129**, 175101 (2008).
[21]M. S. Li, N. T. Co, C. K. Hu, J. E. Straub, and D. Thirumalai, Phys. Rev. Lett. **105**, 218101 (2010).
[22]A. Brinker, G. Pfeifer, M. J. Kerner, D. J. Naylor, F. U. Hartl, and M. Hayer-Hartl, Cell **107**, 223 (2001).
[23]R. J. Ellis, Nature (London) **442**, 360 (2006).
[24]D. K. Klimov, D. Newfield, and D. Thirumalai, Proc. Natl. Acad. Sci. U.S.A. **99**, 8019 (2002).
[25]H. J. Hilhorst and J. M. Deutch, J. Chem. Phys. **63**, 5153 (1975).
[26]M. S. Li, D. K. Klimov, and D. Thirumalai, J. Phys. Chem. B **106**, 8302 (2002).
[27]M. Cieplak, T. X. Hoang, and M. S. Li, Phys. Rev. Lett. **83**, 1684 (1999).
[28]M. S. Li and M. Cieplak, Phys. Rev. E **59**, 970 (1999).
[29]W. Wang, W. X. Xu, Y. Levy, E. Trizac, and P. G. Wolynes, Proc. Natl. Acad. Sci. U.S.A. **106**, 5517 (2009).
[30]See supplementary material at http://dx.doi.org/10.1063/1.4804299 for figures on the dependence of fibril formation times on the crowder concentration for cubic crowder of size 1 and on the crowder sizes, and dependence of the total coverage of crowding particles on their sizes.