Unlocking the Atomic-Level Details of Amyloid Fibril Growth through Advanced Biomolecular Simulations

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Molecular fibrils formed by aggregated amyloid peptides of various lengths and sequences are common pathological fingerprints of a large range of amyloid-related diseases, from type 2 diabetes to Parkinson’s, Huntington’s, or Alzheimer’s disease. Despite their tremendous biomedical importance, both the structural properties of peptide amyloid fibrils and the molecular mechanisms involved in their formation and propagation (i.e., the formation of nucleated seeds and fibril segments) remain notoriously difficult to investigate either experimentally or computationally (1–4).

Experimental structural studies have been primarily hindered by the characteristic insoluble yet noncrystalline nature of amyloid aggregates, and required novel and often nonconventional approaches for prying into the atomic-level details of amyloid fibrils and microcrystals (4,5). Kinetic measurements of amyloid fibril growth are equally demanding though, fortunately, several pioneering studies shed light on the detailed growth mechanisms occurring in a variety of amyloid fibril-forming peptides (6–8). Most notably, these studies proposed and evidenced a dock-and-lock type of mechanism of monomer addition at the ends of preformed amyloid fibrils of both wild-type and synthetic Alzheimer’s amyloid beta (Aβ) peptides. Interestingly, the locking step seems to occur generally at a much slower rate than the rather weak binding events that govern the initial docking stage (8). The existence of a significantly slower and strongly sequence-controlled subsequent locking step suggests possible methods for therapeutic intervention that could target specifically the kinetic imbalance between the locking and docking timescales (2,8).

Computationally, molecular simulations of structural, thermodynamic, or kinetic properties of amyloid fibrils are equally challenging. This is due to the typically large sizes of these molecular systems, to the slow timescales involved in the fibril association, and, especially, to the dissociation processes occurring either in solution or upon interaction with lipids (1,3,9–13). While modern advances in both hardware and software for molecular simulations have enabled substantial classical molecular dynamics (MD) studies of fibrils formed by many types of amyloid peptides, it is increasingly clear that the field would benefit much from novel algorithmic and methodological approaches that could enable the thorough study of the rare events involved in their formation. Significant insight into the amyloid formation mechanism has been gained by using simplified (i.e., coarse-grained and implicit solvent) simulations of the peptide aggregates (14–16). However, the atomistic-level (i.e., without using coarse-grained or implicit solvent representations) investigation of slow processes, such as the amyloid peptide dynamics at fibril ends during docking, requires enabling technologies that go far beyond faster processor speeds and the enhanced parallel processing on high-performance computing hardware.

In Biophysical Journal, Schor et al. (2) present a pioneering computational study that is aimed exactly at developing methods that address the challenging issues mentioned above: the study of the atomically detailed dock-and-lock molecular elongation mechanism of amyloid fibrils. To enable this study, the authors perform a tour de force in developing and using state-of-the-art advanced molecular simulation methods that extend the applicability of classical MD simulations well beyond their traditional usage. First, the authors have identified a seven residue-long peptide, \textit{LVEALYL}^{11LVEALYL17}, as the ideal candidate for their study. This amyloidogenic heptapeptide—a part of the insulin B-chain shown to be responsible for fibril formation—is one of the smallest amyloid fibril-forming molecules, making it a computationally appealing target. At the same time, it has the advantage that its high-resolution structure in fibril-like microcrystals has recently been solved (5), which decreases even further the computational resources required for minimizing typical uncertainties related to the choice of initial conditions in any MD study.

At the heart of the authors’ approach stays, however, the implementation of their transition path sampling (TPS) method (17) that, unlike alternative approaches for rare event sampling, can be used in conjunction with unbiased MD simulations, at the temperature of interest, to find an ensemble of most probable reactive transition paths that connect a sequence of metastable states. The knowledge of this ensemble is essential for the proper evaluation of the transition molecular mechanism and of the statistical ensemble of transition states located along reaction pathways, which are particularly difficult to characterize experimentally (18).

An inherent problem in analyzing statistical transitions in high-dimensional kinetic systems is finding an optimal, ideally lowest-dimensional reaction coordinate (RC) that can best capture the physical mechanism behind the transition process, with its true free energy barriers (19,20).
authors overcome this challenge by using a maximum likelihood method which allows the screening of many potentially relevant low-dimensional order parameters (OPs) proposed as RC candidates, as well as of all their possible linear combinations (20). Interestingly, the authors find that, for amyloid fibril growth, as few as two OPs (which quantify the integrity of fibril-stabilizing hydrogen bonds) can be sufficient for obtaining a close-to-optimal (i.e., in a maximum likelihood sense) low-dimensional RC. Adding a third OP such as the radius of gyration of the locking peptide does not increase the likelihood significantly, since this information is already captured by the other two OPs (2). This result raises the hope that it is generally possible to identify in a systematic way low-dimensional RCs in other complex biomolecular systems as well.

Finally, another essential and novel ingredient in this TPS implementation is to bootstrap the multidimensional TPS search by using a realistic initial candidate for the transition path that is generated by steered molecular dynamics (SMD) with a low-velocity pulling protocol rather than by using a (more typical) high-temperature MD simulation. In this approach, the authors thoroughly use multiple SMD simulations to carefully pull one LVEALYL peptide away from the fibril by increasing their center-of-mass distance along the fibril axis. The SMD runs are then analyzed using Jarzynski’s relation, and the best initial candidate for the subsequent TPS simulations is selected (2).

Remarkably, this study succeeds to unlock significant atomistic-level details on the molecular mechanisms responsible for the growth of LVEALYL amyloid fibrils. As suggested by previous experimental and computational studies of amyloid formation (14,21), these new calculations reveal the existence of multiple competing pathways contributing to the slow conformational search during the locking step. The authors describe two main routes on which the formation of backbone hydrogen bonds characteristic of β-sheet-rich amyloids can occur: either before or after the proper packing and reorientation of important side chains (e.g., Glu) at the interface between adjacent β-sheets. Interestingly, while this study can pinpoint with atomistic detail the molecular interactions that are characteristic of the LVEALYL system, it also reaches more general inferences such as the importance of docked states as transition intermediates that play a central role in achieving the proper alignment of attaching peptides to the fibril template. In agreement with previous simulations of amyloid peptides of similar sizes but different sequences (10,11,15,16), this study reveals the important role of hydrogen-bonding in the locking stage, and suggests a generic character of the lock mechanism in the formation and growth of amyloid peptide aggregates.

As importantly, besides the biomedical relevance of the study by Schor et al. (2) to shedding new light on the molecular mechanisms of amyloid fibril formation-dissociation, this innovative work opens up new pathways toward the development of novel statistical mechanics-based computational algorithms that can significantly advance the way molecular simulations are performed and analyzed. In particular, maximum likelihood-based methods appear to play an increasingly important role in both optimizing the amount of information that can be extracted from trajectory data, and in designing new, more efficient molecular simulation methods (2,20,22,23). Modern approaches—such as presented by Schor et al. (2)—using the TPS-based method in conjunction with RC optimization may “hold the key” to overcoming the perpetual limitations imposed on computational molecular biophysics by large system sizes, slow transition times, and the need for detail and accuracy required to make connection with modern experiments.

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REFERENCES


