New and Notable

Milestoning Simulation Reveals Mechanism of Helix-Breaking

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In recent years, significant interest has developed in simulating the kinetics of slow processes, especially as applied to biological systems. Typically, these processes lie outside the range of direct atomistic molecular dynamics (MD) simulations and require specialized techniques, such as accelerated MD (1), transition path sampling (2), transition interface sampling (3), Markov state modeling (4), or Milestoning (5,6). Milestoning provides a very promising approach to description of long-term dynamics, because it is based on coarse-graining of atomistic simulations. The initial method involved partitioning a reaction coordinate into fragments and running multiple MD trajectories over the fragments, allowing the buildup of a kinetic matrix for the overall process (5). The more recent version of milestoning does not require a reaction coordinate, but relies on a partitioning of conformational space into cells and generating transitions between cells (6). By computing multiple short trajectories covering fragments of conformational space, milestoning achieves accelerated sampling and improved statistics with a high efficiency, for both diffusive-type and activated-type processes. Additionally, the calculations are trivially parallelizable (5). The method has been applied to a number of interesting biological processes, including modeling the recovery stroke in myosin (7) and the initial stages of unfolding of an α-helix (8).

In this issue, milestoning is applied to the early unfolding events in a coiled-coil under mechanical load (9). These kinds of studies are gaining in interest due to the recognition of the role of mechanical forces in biological processes and increasing availability of experimental data (8). The earlier study of the isolated helix found break initiation times of 4 ns without load, 5 ns at 10 pN, and 2 ns at 100 pN. This unexpected nonmonotonic behavior was described as a catch-bond. Analysis of the kinetic network showed that changing load influenced both timescales and mechanisms of the unfolding. Thus, the narrower 3_{10}-helix form was found to be the intermediate at high loads, while the wider π-helix form was significantly populated for local unfolding under moderate loads, though not as an intermediate (8). This work extends the simulations to a coiled-coil, finding marked differences between this system and an isolated helix (9). For the coiled-coil, the single-residue cracks tended to heal before propagating further at loads <100 pN, due to helix-helix interactions. Propagation of cracks within and between helices occurred systematically only at the highest loads used, 200 pN. Interestingly, unfolding events tended to be localized at a specific site, residue E929. This charged residue located at a hydrophobic position in the heptad repeat appears to form a weak link in the highly stable coiled-coil structure. The average time for first appearance of a crack was 280 ns at 50 pN and 20 ns at 200 pN, ~20 times longer than for a single helix (9).

Overall, milestoning was able to provide highly interesting new insights into the early events of helix unfolding, showing that this process is quite complex and worth further investigation. The milestoning approach for treating slow complex processes has a number of advantages. The basic calculations involve atomistic MD simulations, and thus provide an accurate picture of the system motions. The calculations are very efficient, enabling simulations for reactions of arbitrarily slow rate by generating an appropriate subdivision of states. The results include both kinetic and thermodynamic description of the system. Most importantly, meaningful mechanistic insights are obtained by analysis of the coarse-grained kinetic network. Wider application of this exciting method should be encouraged.

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