all-atom molecular dynamics simulations we provide extensive insight into microsecond dynamics of a villin solution at different concentrations, as a simple model of cellular environment. Since inaccuracies of current force fields are known to result in exaggerated protein aggregation we increase protein-water interactions to better reproduce available experimental results. The simulations show that protein rotational diffusion slows down more significantly than translational diffusion with increasing concentration, while protein internal dynamics remain largely unaltered. These findings correlate with observed formation of sub-microsecond persisting protein clusters which size distribution shifts toward larger clusters with increasing protein concentration. We show that diffusion coefficients estimated for simulation-derived cluster structures weighted by the cluster size distribution mostly reproduce the observed overall diffusion. It indicates the clusters formation as a primary determinant of diffusion slow-down upon crowding. Finally, we shed light on protein diffusion in a heterogeneous protein solution, near a lipid membrane and in the presence of metabolites.

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Hidden Native State Ensembles of NFκB Dimers Provide Insights into their Different DNA-Binding Affinities

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The most abundant NFκB family member, p65 (also called RelA), exerts its transcriptional regulatory function as a homo- or hetero-dimer with p50. The two dimers have different but overlapping DNA binding specificities, considered to be the key to fine control of gene regulation. Past crystallographic studies on NFκB-DNA complexes showed high structural homology among different NFκB dimers and left a puzzle: how do NFκB dimers bind to DNA with different affinities? In this study, we discovered drastically different conformational dynamics between two free NFκB dimers: p65p50 and p65 homodimer. Hydrogen-deuterium exchange mass spectrometry (HDXMS) revealed different exchange levels in the p65 DNA-binding domains (DBDs) of the homodimer as compared to the heterodimer, suggesting a sub-global conformational difference between p65 bound to p50 and p65 bound to another p65. Furthermore, molecular dynamics (MD) simulations elucidated the conformational ensembles that give rise to different HDX patterns. We found that when not bound to DNA, the two DBDs of the NFκB dimer can twist around the linker between the dimerization domain and the DBD with a large amplitude (~30 Å). Through MD simulations, we observed very distinct conformational relaxation processes from DNA-bound to free states for p65p50 and p65 homodimer. While the two DBDs in p65p50 move apart from each other, those in p65 homodimer come together and form new contacts. The new protein interface captured by MD in the p65 homodimer explains the different HDX behaviors between NFκB dimers. Moreover, we suggest that the differences in DNA-binding affinities are a consequence of distinct conformational dynamics displayed by the free NFκB dimers. By combining HDX-MS and MD simulations, we here provide an example of understanding how signaling proteins function from the standpoint of conformational dynamics.

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Correlated Motions in Several Variants of the DHFR-NADPH Complex

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Correlated motions are thought to be related to the catalytic step for dihydrofolate reductase (DHFR). We performed several molecular dynamics studies of the DHFR-NADPH complex, the analog of an apo structure existing prior to the binding of dihydrofolate. We compare correlated and anti-correlated motions and their timescale to motions previously observed in the catalytically active state. This is done both via cross correlation matrices and via wavelet analysis. We also compare correlated motions across several DHFR mutants, and at several pH levels.

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Dissecting the Structural Mechanism of a Naturally Occuring Variant of the Prion Protein in Preventing Prion Disease

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Prion diseases are a group of fatal neurodegenerative disorders associated with the misfolding and aggregation of mostly alpha-helical cellular prion protein (PrP^C) into the beta-rich infectious (scrapie) form of the prion protein (PrP^{Sc}). A recent experimental study reported that the naturally occurring G127V variant of human PrP^C is intrinsically resistant to prion conversion and completely prevents prion disease. However, the structural basis of the protective effect of V127 variant remains mostly unknown. Herein we performed multiple microsecond-scale molecular dynamics simulations on both wildtype and V127 variant of human prion protein at neutral pH with the aim to understand why V127 variant can prevent prion conversion. Our simulations show that the G127V mutation not only increases the rigidity of the loop between strand-2 (S2) and helix-2 (H2) but also enhances the stability of H2 through the formation of hydrophobic interactions between V127 and P165 and that of the salt bridge between R164 and D178. The increased rigidity of S2-H2 loop and the enhanced H2 stability in V127 variant may inhibit the prion conversion of human PrP^C, thus prevents prion disease. Our hypothesis is supported by the fact that prions are poorly transmissible to animals with their PrP^C carrying a rigid loop and a recent finding showing that stabilization of H2 of prion protein prevents its misfolding and oligomerization. In addition, our MD simulations at acidic pH demonstrate that G127V mutation facilitates the helix-to-sheet transition of the C-terminal region of H2 that is critical to the misfolding of wildtype PrP^C. Our findings offer structural basis for understanding the role of human 127V variant in preventing prion conversion and may provide key mechanistic insights into prion propagation and the development of rational therapeutics.

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Drivers of Conformational Variability in Transthyretin Monomers under Amyloidogenic Conditions

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The unfolding, misfolding and subsequent oligomerization of the protein transthyretin (TTR) are associated with two amyloid diseases: senile systemic amyloidosis and familial amyloid neuropathy. Dissociation of the native tetramer is the rate-limiting step in TTR amyloid formation; afterwards, monomers partially unfold and reassemble into toxic oligomeric species that eventually form mature amyloid fibrils. Recent studies have used both solution and solid state NMR to identify conformational changes associated with the misfolding of TTR monomers; however, the specific interactions that give rise to these conformational changes remain unclear. To investigate the early events in the unfolding of TTR monomers, we have performed multiple molecular dynamics simulations of wild type and mutant TTR under amyloidogenic conditions. By analyzing over 10 μs of simulation data, we find that in the absence of stabilizing interactions present in the native state, interactions with solvent molecules reshape the conformational landscape of TTR and give rise to a heterogeneous ensemble of partially folded structures. Specifically, we find that β-sheet secondary structure, β-sheet tertiary structure, and the packing of solvent-exposed side chains are destabilized when a natively buried β-sheet is exposed to solvent. These findings highlight how non-native solvent-protein interactions can drive amyloid formation and may aid in the development of therapeutic strategies to mitigate oligomer toxicity.

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Quantifying Peptide Binding Affinities from Non-equilibrium Work

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Peptides have great potential as novel medicinal agents. Computational design of peptides binding to a target protein has received great interest of researchers in the recent years. Structural prediction of relative binding affinities of peptides to proteins is an important step in the design of potentially therapeutic peptides, yet both peptide-protein structure prediction and methods for quantitative affinity prediction are far from being accurate. In the past, methods based on single static structures as well as Molecular Dynamics simulations have been used for peptide binding affinity prediction. End-point methods such as MM-PBSA/GBSA are usually preferred for this purpose, however their accuracy for peptide ligands is usually limited. We investigate the applicability of steered MD (sMD) simulations as an alternative structural Free Energy estimation method for peptide binding affinity prediction. The method is based on pulling the peptide away from the binding region of protein and recording the pull force along the pulling trajectory. The work performed during this nonequilibrium process is then taken as an indicator of binding affinity. We applied this method to predict the ranking of different peptide ligands of a Major Histocompatibility Complex Class I protein in terms of experimentally-determined binding affinities. The peptide-MHC complexes had either crystal structures available or were modeled by docking or side-chain conformation prediction based on a template peptide ligand. Several pulling velocities were tested. Our results indicate that, if the pulling velocity is sufficiently low and the peptide contacts are correctly modeled, good correlation between experimental and predicted binding affinities can be obtained by the sMD method.