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1150-Pos Board B60
Diffusion of Unfolded Acyl-Coenzyme A-Binding Protein Over a Complete Range of Denaturant
Yujie Chen, Vincent A. Voelz, Olgica Bakajin, Vijay S. Pande, Lisa J. Lapidus.
The unfolded states of acyl-coenzyme A-binding protein (ACBP) were studied from the point of view of diffusion dynamics. Using the method of Trp/Cys contact quenching, we monitored the intramolecular diffusion of the unfolded chain over a wide range of denaturant in both equilibrium and using a novel microfluidic mixer to capture dynamics before folding. Theoretically, both a worm-like chain model and molecular dynamics (MD) have been used to generate loop terminal distance distributions required for data analysis. MD simulation also provides a direct comparison to measured rates through mean squared displacement over time. We observed a deep compaction of protein conformation from 6M GuHCL to 0.2M GuHCL resulting in a 100-fold decrease of intramolecular diffusion coefficient. This protein, however, is still shown to be more diffusive than protein L in physiological conditions, suggesting a strong sequence dependence of intramolecular diffusion.

1151-Pos Board B61
Early-Aggregation Studies of Diabetic Amyloid in Solution and Membranes
Sadanand Singh, Chi-Cheng Chiu, Kyle Q. Hoffmann, Allam S. Reddy, M. Zanni, J.L. Skinner, Juan J. de Pablo.
Islet amyloid polypeptide (IAPP, also known as amylin) fibrils, as well as intermediates in their assembly, have been associated with type II diabetes. The factors that trigger islet amyloid deposition *in vivo* are not well understood. To better understand the mechanism and cause of such aggregation, molecular simulations with explicit solvent models have been used to compare monomer structure and the early aggregation mechanisms in different environments, including water, 35%(wt) aqueous trehalose, and charged membranes.
Using free-energy maps generated through a variety of novel, enhanced sampling free-energy calculation techniques, we have found that, in water, the peptide adopts three major structures. One has a small α-helix at N-terminus and a small β-hairpin at other end. The second is a complete β-hairpin, and the third is a random coil structure, with, the β-hairpin state being the most stable. From studies of the dimerization of monomers in water, we have found that the early aggregation proceeds by conversion of all α-helical configurations to β-hairpins, and by two β-hairpins coming together to form a parallel β-sheet. From studies in the presence of a negatively charged bilayer membrane consisting of a 3:1 mixture of POPC-POPG we have found that, in water, the monomer inserts itself into the membrane through the N-terminus α-helical state. It can also be adsorbed onto the membrane surface as a β-hairpin. From detailed free energy maps we have further found that the N-terminal α-helical inserted configuration is most stable.
Together, our results have revealed a hitherto unavailable detailed mechanism for IAPP early stage-aggregation, and for the role of crowding and bilayer membranes in that aggregation. Several aspects of the proposed mechanism have been verified by concerted 2D IR experimental measurements, thereby adding credence to the validity of our predictions.

1152-Pos Board B62
Early Closure of Loops in the Refolding of Adenylate Kinase: A Possible Key Role for Non Local Interactions in the Initial Folding Steps
Eldad Ben-Ishai, tomer Orevi, Gil Rahamim, Sivan Gershanov, Gershon Hazan, Dan Amir, **Elisha Haas**.
We hypothesize that non-local interactions (long loops formation prior to secondary structure elements formation) dominate the critical initial phase of the folding transitions of globular proteins. In order to test this hypothesis we study the kinetics of refolding of specifically labeled chain segments in a model protein, *E. Coli* adenylate kinase (AK), using time resolved fluorescence resonance energy transfer (FRET) detected stopped flow experiments (the ''double kinetics" approach). The AK molecule consists of a single chain of 214 residues folded in three domains. The changes of the mean and the width of the distributions of the end-to-end distance of three secondary structure elements (helix, residues 169-188 and strands, residues 188-203 and 79-86) and five loop sections whose ends are in juxtaposition in the native structure were determined in the denatured state, in the initial transient state, at 5 ms after initiation of refolding, during the refolding and in the native state. The ends of the two N terminal loops (residues 1-24 and 28-71) attained native end to end distance distribution within the deadtime of the mixing device. The other three labeled chain sections attained the native proximity only during the rate limiting main transition, in parallel with the formation of short secondary structure elements. These results support the hypothesis that few very effective non-local interactions can be essential factor in stabilization of the early transient structures of folding of globular proteins and determine the direction of the folding pathway.

1153-Pos Board B63
Equilibrium Studies of Protein Denaturation by Urea - Examining Backbone and Sidechain Contributions
Deepak R. Canchi, Angel E. Garcia.
Urea is a commonly used denaturant in protein folding studies, and it is of great interest to determine its interaction with various protein groups to elucidate the molecular basis of its effect on protein stability. Using the Trp-cage miniprotein as a model system, we report changes in preferential interaction coefficient of the protein upon urea denaturation and examine the contribution from the backbone and sidechain groups. The preferential interaction was obtained from reversible folding/unfolding Replica Exchange MD simulations of Trp-cage in presence of urea, over a broad range of urea concentration. The simulations were carried out using two different forcefields. AMBER94 and AMBER99sb, in conjunction with TIP3P water and KBFF urea models. In a given ensemble, folded or unfolded, we find the sidechains have a larger preferential interaction than that of the backbone. The increase in preferential interaction upon unfolding can be accounted entirely by sidechain contributions in AMBER94 simulations. In AMBER99sb, we find that both backbone and sidechain contribute to the increase in preferential interaction, with the sidechain contribution being slightly larger. The differences between two forcefields can be attributed to the unfolded ensemble sampled, with AMBER99sb favoring conformations with larger surface area and lower helical content. Both forcefields show that the preferential binding is dominated by binding of urea to sidechain atoms. This work has been supported by the National Science Foundation (Grant MCB-0543769).

1154-Pos Board B64
Exploration of the Conformational Landscape of an Amyloidogenic Ig Domain
Nina Pastor, César Millán-Pacheco, D. Alejandro Fernández-Velasco.
On their way from the denatured state to their native conformation, proteins can jump into aggregation funnels. This is the underlying cause of a large group of misfolding diseases, such as light chain amyloidosis. In this particular case, variable light chain immunoglobulin domains are expressed in excess, due to a B-cell dyscrasia, and form fibers that deposit primarily in the kidneys and heart. We explore the conformational dynamics of lambda 6 VL domains, both in native and in denaturing conditions, through extensive molecular dynamics simulations in explicit solvent. Denaturation pathways are characterized following the loss of native contacts for selected residues, and the increase in solvent accessibility for the most amyloid prone regions of the protein, as determined by ZipperDB. We have identified putative unfolding intermediates, which have lost many of the antiaggregation moieties of Ig domains, and could thus constitute seeds for fiber nuclei. Comparison of amyloidogenic versus non-amyloidogenic Ig domains reveals that the former have more regions with higher propensities for forming fibers, and these are located in more structurally labile zones of the folded protein. Furthermore, the structures generated in simulations at 398K of non-amyloidogenic proteins resemble those populated by amyloid prone variants, at room temperature. This is in line with the thermodynamic hypothesis, which states that unstable proteins are more likely to form amyloid fibers.
Funded by CONACyT (102182). The simulations were carried out in Centro Nacional de Supercómputo (San Luis Potosí), Sputnik II (Instituto de Biotecnología, UNAM), Orion (PROMEP-UAEM), and Entalpia (Facultad de Medicina, UNAM)

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Trp Cage Folding in Confinement
Kristen A. Marino, Peter G. Bolhuis.
While protein-folding studies are typically performed in bulk solution, the effects of confinement and crowding are poorly understood and in fact may alter the thermodynamics and kinetics of protein folding in a cellular environment. Here we have used all-atom simulations to examine the model Trp-cage miniprotein adsorbed to a purely hydrophobic surface and confined between hydrophobic walls. Replica exchange molecular dynamics simulations were performed to construct the folding free-energy landscape from which meta-stable states not seen in the bulk were indentified. In both cases, these states consist of different conformations of the protein adsorbed to the wall. The intermediates likely affect the free energy barriers to folding. Adsorption of the protein to a hydrophobic wall raises the energy barrier to folding, while lowering the barrier to folding of the confined protein. Although the confined protein is adsorbed to the wall, it appears that confinement stabilizes the α-helix, driving the protein toward the native state. Further, the presence of additional states likely changes the folding mechanism of the protein. Therefore we use multiple state transition path sampling to elucidate the differences between folding of Trp-cage in the bulk and confined between hydrophobic walls. Our simulation results can provide a realistic starting point for experiments on the effect of confinement on the folding behavior in Trp-cage and other small model proteins.