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Thermal Adaptation Strategies used by TBP
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The TATA binding protein (TBP) is a monomeric transcription factor present in archaea and eukarya, in organisms adapted to temperatures spread over a 100°C range. Thermal adaptation implies a balance between stabilization and function, which entails keeping ligand selectivity and also the amplitude of conformational fluctuations within physiologically reasonable bounds. To explore relevant contributions to thermal adaptation of the TBP fold, we perturbed the native structures of six TBP variants coming from organisms with optimal growth temperatures between 10°C and 105°C, with molecular dynamics simulations at 273, 298, 323, 348 and 373K, and 1 atm pressure, with the CHARMM27 forcefield in NAMD, for 11 ns each. We characterized the temperature response of free energy components obtained using the MM-PBSA approach. Hyperthermophilic TBPs have a less steep response of their free energy to temperature than the mesophiles or the psycrophile, suggesting that TBP alters the curvature of its free energy surface as a global stabilization strategy; this coincides qualitatively with a measure of resiliency derived from the fluctuations of H atoms over 100 ps intervals. The van der Waals energy becomes less attractive with increasing temperature, and this correlates with a decrease in protein density and an increase in the number of sampled side-chain rotamers, but the steepness of the response is not correlated to the thermal stability of TBP. The electrostatic energy decreases upon temperature increase, and opposes all other internal energy components. This electrostatic stabilization with increasing temperature is due to the decrease in dielectric properties of water, and is reflected in an increase in the number of salt bridges at the expense of TBP-water interactions; this is particularly effective in hyperthermophiles.
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Interactions Of fluorescent Labeled Beta-amyloid Peptides With Self-assembled Nanospheres And Their Impacts On Fibrillation
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Aggregation of amyloid β-peptide (Aβ) into fibrils plays a vital role in the pathogenesis of Alzheimer's disease. In this work, we have studied the interactions of two model peptides which include the Aβ (1-42) peptide, and the short segment Aβ (13-21) with self-assembled nanospheres. The Aβ (13-21) segment was examined as this region is believed to play a crucial role in the beta-sheet formation and fibrillation. The nanospheres were prepared by self-assembly of a newly synthesized bolaamphiphile, bis(N-α-amido-methionine)1,5 pentane dicarboxylate. We observed that the Aβ peptides showed significant binding to the nanospheres, which resulted in inhibition of beta-amyloid fibril formation. The binding of amyloid β peptides to the nanospheres was confirmed by fluorescence spectroscopy, zeta-potential analysis, FTIR, circular dichroism analysis and TEM analysis. We found that the binding of the amyloid β-peptides to the nanospheres was pH, temperature and concentration dependent as well as depended upon the hydrophobicity/ hydrophilicity of the solvent systems. Upon interacting with the nanospheres, a significant transition from β-sheet structure to alpha-helix or random coil formation is observed under acidic to neutral conditions, while relatively less interactions are observed under basic conditions. This indicates that the electrical charge density of the nanospheres play a significant role in the binding interactions with the peptides. Significant fluorescence quenching was observed when the fluorescent labeled beta-amyloid peptides were bound to the nanospheres under certain conditions. The apparent binding constants calculated were in the order of $K = 20$ to 200 M^{-1} depending on the experimental conditions. It appears that the self-assembled nanospheres may mimic the vesicles formed from certain biological bilayer membranes and suggest that the nanospheres may potentially serve as a new family of bioactive agents for inhibiting amyloidogenesis.

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An Investigation Of Gastric-like Aspartic Proteinase Molecular Chimeras
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Proplasmepsin II (zPMII) represents a structurally unique member of the aspartic proteinase family, with a prosegment-enzyme interaction that has never been reported. It has been a generally accepted assertion that the prosegment in pepsin-like aspartic proteinases is critical to aspartic proteinase folding, and to investigate this further two chimeric proteins were generated, one with the pepsinogen prosegment fused to the mature region of plasmepsin II (PMII) (peppro PMII) and a second with the prosegment of PMII fused to pepsin (PMIIpropep). Both chimeras were expressed from *E. coli,* however, PMIIpropep was extremely unstable and was rapidly degraded by trypsin, suggesting protein misfolding. Since a stable enzyme could not be generated PMIIpropep was not further studied. Alternatively, peppro PMII was capable of both autoactivation and synthetic substrate cleavage. In addition, both the zymogen and mature form of the enzyme had the same predicted secondary structures, suggesting that altering the PMII prosegment did not affect this level of protein conformation although the prosegment may play a role in enzyme stability. DSC and CD measurements indicated that peppro PMII had reduced thermal stability as compared to zPMII. It is proposed that this reduction of temperature stability resulted from the loss of the ability of the prosegment in PMII to stabilize the C-terminal domain of the enzyme. The ability of PMII to fold in the presence of a completely non-homologous prosegment suggests that the prosegment in gastric-like aspartic proteinases is not always critical to enzyme folding but likely plays a role in protein stabilization.

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Reduced Cell Surface Stability Of Rescued Herg Trafficking Mutants
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Long QT syndrome (LQTS) is a cardiac disorder that is characterized by a prolonged QT interval on the electrocardiogram, syncope and in severe cases sudden death. LQTS type 2 is associated with loss-of-function mutations in the human-ether-a-go-go-related gene (hERG1) that encodes the α-subunit of the cardiac delayed rectifier repolarization current, IKr. hERG mutations have been most commonly associated with defects in protein trafficking that lead to channel retention in the ER. This impaired biosynthesis can be partially rescued by low temperature and/or pharmacological chaperones. We know little about the metabolic fate of rescued mutant hERG channels and whether functional recovery may be limited by altered endocytic membrane trafficking of these channels. We undertook this study to test the hypothesis that rescued mutant hERG channels are less stable in the cell membrane and are processed differently than wt channels. Initial experiments involving immunoblotting following cyclohexamide treatment indicated that mutant hERG proteins have reduced stability compared to their wt counterpart in post-Golgi compartments. To further assess the cell surface stability and rate of internalization of hERG we conducted cell surface antibody binding assays. The results of long term binding assays confirm that mutant hERG channels are less stable at the plasma membrane than wt hERG. The results of short term binding assays suggest that this may be due in part to the accelerated internalization of mutant hERG. Using fluorescent ratio-image analysis of FITC-labeled channels revealed that mutant hERG channels are targeted preferentially to lysosomes, while wt channels are recycled. These results suggest that besides the biosynthetic processing defect, a complex alteration of peripheral membrane trafficking may contribute to the LQTS phenotypic manifestation. Furthermore, the results suggest the involvement of a post-Golgi quality control mechanism responsible for the elimination of defective hERG channels.

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Structural and Thermodynamic Characterization of T4 Lysozyme Mutants and the Contribution of Internal Cavities to Pressure Denaturation
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Protein pressure denaturation cannot be explained by the liquid hydrocarbon transfer model of protein denaturation derived from thermal denaturation studies. Recent studies imply that the mechanism of pressure denaturation is the penetration of water into the protein rather than the transfer of hydrophobic residues into water. To investigate water penetration and the volume change associated with pressure denaturation, we studied the solution behavior of four T4 lysozyme mutants having different cavity volumes at low and neutral pH up to a pressure of 400 MPa using small-angle X-ray scattering and fluorescence spectroscopy. At low pH, L99A T4 lysozyme expanded from a compact, folded state to an extended but partially unfolded state. The denaturation volume change correlated positively with the total cavity volume, indicating that all of the molecule's major cavities are hydrated with pressure. As a direct