has yet to receive serious attention. We analyzed folding routes predicted by a variational model in terms of a generalized formalism of the capillarity scaling theory that assumes the volume of the folding nucleus grows with chainlength as $V_f \sim N^v$. We found that the scaling exponent for 27 two-state proteins ranges from 0.2 to 0.45 with an average of 0.33. This value is close to the exponent value corresponding to packing of rigid objects, though generally the nucleus has a much smaller mean packing fraction than the native state. We also studied the growth of the folding nucleus and interface along the folding route in terms of the density or packing fraction. We found three types of growth of the nucleus depending on how the growth of the folded core is balanced by changes in density of the interface. Finally, we characterize the sharpness of transition state by volume of per particle in interface region.

2727-Pos When Model Systems Misbehave: The Folding Of A Three-Helix Bundle

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Board B30

Are there common themes in the folding of diverse protein types? To complement our previous studies on slow-folding α/β proteins (Krantz et al., 2002; Pandit et al., 2006), we have characterized the folding behavior of the B domain of protein A (BdpA) a fast-folding three-helix bundle. Although this protein has long been used for evaluating folding models, numerous studies have failed to produce a consensus view of the folding transition state (TS). Specifically, the extent of native-like structure and the roles of each of the helices (H1, H2, and H3) vary among studies. Using engineered metal binding sites ("ψ-analysis") and amide H/D kinetic isotope effects, we find that the TS of BdpA contains a high fraction of the native topology. A singular TS containing helix H2 and the adjoining portions of H1 and H3 ("$H1H2_{H3}$") forms a coarse version of the native topology. However, the TS has relaxed (energy minimized) to some degree. This nonnative behavior has confounded previous experimental and theoretical studies, e.g. mutational studies underreport the α-helical content. These results serve as a double warning for how one characterizes a TS and whether a particular "model" system should in fact be considered one. Nevertheless, with our new results from a fully helical protein, we are positioned to conclude that many proteins, irrespective of their type, will fold through a TS structure that forms ~80% of the native topology (relative contact order).

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Protein Folding Stability - II

2728-Pos Investigating the Folding Properties of Superoxide Dismutase

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Board B31

The purpose of this study was to prepare bovine erythrocyte Superoxide Dismutase (BESOD) for Fluorescence Resonance Energy Transfer (FRET) analysis to elucidate the intermediate steps in its folding pattern in the presence of a denaturant. The protein, itself, is a dimer consisting of two homologous domains, each containing a single Tyrosine (Tyr) residue. Native BESOD was stripped of its Cu and Zn prosthetic groups by dialysis. The next step in the process was to create a donor/acceptor protein consisting of an unmodified domain (donor), and a domain whose Tyr residue has been modified by nitration (acceptor). This is crucial for FRET analysis as the unmodified Tyr residue in the protein absorbs light and emits fluorescence at 280 nm, whereas the nitrated Tyr residue absorbs the emitted fluorescence from the neighboring Tyr. This significant characteristic of the donor/acceptor protein will be used to determine the position of the unmodified Tyr residues to the nitrated Tyr residue, and thus will determine the intermediate steps of the folding and unfolding of this protein as denaturants are introduced to it. Tetranitromethane was used as a nitrating agent and UV spectroscopy was used to determine the success of the nitration reaction.

2729-Pos Investigating the Folding Properties of Superoxide Dismutase: Theory

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Board B32

A new computational method, the Nearest Neighbor Algorithm, was developed to predict protein folding pathways based on a hierarchy of nearest neighbor contacts. This method provides a virtually instantaneous calculation of the order in which a protein's tertiary structure accumulates during folding. The method was tested on the beta-sheet protein superoxide dismutase (SOD) and was compared directly with the results of Go-model simulations of SOD. The Nearest Neighbor Algorithm's ability to reproduce the Go-model results succeeded in some protein regions but failed in others. This finding suggests that excluded body effects are relevant to protein folding.

2730-Pos Macromolecular Crowding Increases The Mechanical Stability Of Protein Molecules

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Board B33

Cells contain a variety of macromolecules in the cytoplasm, which can collectively occupy a large fraction of the cellular volume. This macromolecular crowding phenomenon has been shown to have various effects on the kinetics of biomolecular processes and the thermodynamic properties of macromolecules. We have used an atomic force microscope based single molecule method to measure the effects of macromolecular crowding on the mechanical stability of individual protein molecules. The results show that the forces that are required to unfold ubiquitin molecules are enhanced when high concentration of dextran molecules is added to the buffer solution. The average unfolding forces of ubiquitin are increased by 24 pN when the dextran, with an average molecular weight of 40 kDa, concentration changes from from zero to 300 grams per liter in the buffer solution. The physical origin of the observed enhancement of the protein's mechanical stability is provided using the scaled particle theory. The dependence of the molecular crowding effects on the size of the dextran molecules, used as crowding agents, is also investigated.

2731-Pos Macromolecular Crowding Effects on the Structure and Dynamics of VlsE

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Board B34

The intracellular environment is crowded due to the presence of high concentrations of macromolecules, including proteins, nucleic acids, ribosomes, and carbohydrates. This means that a significant fraction of the intracellular space is not available to other macromolecular species. It has been estimated that the concentration of macromolecules in the cytoplasm is in the range of 80–400 mg/ml, corresponding to a volume occupancy of up to 40 %. Due to excluded volume effects, macromolecular crowding will stimulate any reaction that responds to changes in the available volume. It is proposed that the major result of macromolecular crowding is a stabilizing effect on the folded protein that originates in lowered entropy of the unfolded state because of polypeptide compaction. Experimental and theoretical work has demonstrated large effects of crowding on biological processes, including protein binding, folding and aggregation.

Here we investigated the native- and unfolded-state structures and the folding dynamics of a model alpha-helical protein, *Borrelia burgdorferi* VlsE, as a function of macromolecular crowding *in vitro*. Crowded conditions were created experimentally with inert synthetic polymers termed crowding agents. Spectroscopic and rapid mixing methods were used to monitor changes in the protein structure at equilibrium and in kinetic folding pathways with increases macromolecular crowder concentration. Ficoll 70 (*i.e.,* a highly branched copolymer of sucrose and epichlorohydrin) and dextran 70 (*i.e.,* a flexible long-chain poly(D-glucose) with sparse and short branches) were used as crowding agents. VlsE was found to unexpectedly increase secondary structure from the native state and slow down the rate of unfolding under crowded conditions. The results indicating this unique behavior are discussed.

2732-Pos A Comparative Study on the Structure and Thermal Stability of Camel and Bovine Alpha-lactalbumin

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Board B35

Alpha-lactalbumin, as a small two domains globular protein containing 123 amino acids, is the specifier component of lactose synthase complex in the lactating mammary gland of all mammals. Sequence alignment of bovine and camel alpha-lactalbumin (BLA and CLA, respectively) shows that the sequence similarity and identity between the proteins of these two species are 82.9 and 69.1%, respectively. Thermal unfolding of CLA and BLA in 20 mM Tris, $CaCl_2$ 2 mM and pH 7.5 determined by differential scanning calorimetry (DSC), fluorescence spectroscopy and circular dichroism. Thermal denaturation of both CLA and BLA was found to be reversible and transition temperatures (T_m) of CLA and BLA were found to be 72.1 and 64.4 °C, respectively. Far-UV circular dichroism (CD) also showed that CLA is more stable against thermal denaturantion and its secondary structure does not change until 60 °C, and then slight decrease in the content of helix is accompanied by an apparent concurrent increase in the content of beta structures. The structural change starts at lower temperatures and is more pronounced for BLA than CLA. However, CLA secondary structure contains more beta structure and less random coil and helix than BLA at room temperature. Decrease of the intrinsic fluorescence intensity and the red shift of the emission λ_{max} indicate Trp exposure to solvent and also a cooperative unfolding as temperature increases for both species. The extrinsic fluorescence emission, 1-anilino-8-naphthalene sulfonate (ANS), results revealed that the accessible hydrophobic surface of CLA is more than BLA. In CLA, there are more hydrophobic residues in B helix (23–34) within alpha domain hydrophobic core. The observed difference in thermal stability is presumably in part related to this difference in distribution of hydrophobic residues.

2733-Pos Thermodynamics of the Coil-to-β-Sheet Transition in a Membrane Environment

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Board B36

Biologically important peptides such as the Alzheimer peptide Aβ (1–40) display a reversible random coil-to-β-structure transition at anionic membrane surfaces. In contrast to the well-studied random coil-to-α-helix transition of amphipathic peptides, there is a dearth on information on the thermodynamic and kinetic parameters of the random coil-to-β-structure transition. We present a new method to quantitatively analyze the thermodynamic parameters of the membrane-induced β-structure formation. We have used the model peptide $(KIGAKI)_3$ and eight analogues in which two adjacent amino acids were substituted by their D-enantiomers. The positions of the d,d pairs were shifted systematically along the three identical segments of the peptide chain. The β-structure content of the peptides was measured in solution and when bound to anionic lipid membranes with circular dichroism spectroscopy and isothermal titration calorimetry. The thermodynamic parameters were found to be linearly correlated with the extent of β-structure formation. β-Structure formation at the membrane surface is characterized by an enthalpy change of $\Delta H\beta = -0.23$ kcal/mol per residue, an entropy change of $\Delta S\beta = -0.24$ cal/molK per residue and a free energy change of $\Delta G\beta = -0.15$ kcal/mol residue. We have further synthesized peptides containing the KIGAKI module with a chain length varying between n = 6 to 30. The above parameters are valid for peptides with a chain length of $n > 12$. For short chains with n = 12 β-structure formation becomes unfavorable with $\Delta G\beta = 0.08$ kcal/mol per residue, $\Delta H\beta = -0.23$ kcal/mol and $\Delta S\beta = -0.24$ cal/molK. The residual free energy of membrane-induced β-structure formation for long peptides is close to that of membrane-induced α-helix formation.

2734-Pos Stability and Folding of a TRX Fragment

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Board B37

Molecular recognition between C-terminal helix 5 and fragment TRX1-93 of *E. coli* thioredoxin (TRX) embodies reciprocal structure selection where both partners gain order. Packing of helix 5 against the remainder of the protein stabilizes the TRX fold (Santos et al. 2007 *Biochemistry* ***46***, 5148–5159), pointing to the potential role on specific stabilization played by a row of four L residues (**L**SKGQ**L**KEF**L**DAN**L**A) clustered along one side of the helix.

The significance of contacts involving L99, E101, F102, L103 and L107 to stabilize secondary structure was assessed by alanine mutagenesis. The helix-forming trend of peptide variants was measured by the strength of TFE-induced far-UV CD bands. Binding of peptide variants of TRX94-108 to the reduced form of fragment TRX1-93 was explored by near-UV CD, MS and MD simulations.

The last five residues of helix 5 in *M. tuberculosis* TRX insert into a groove adjacent to the active site (Hall et al. 2006 *Acta Crystallogr D Biol Crystallogr* ***62***, 1453–1457). Here, the C-terminal helix is somewhat relaxed and the particular crystal packing might help stabilize this unusual conformation. Thus, native TRX conformation would persist even if helix 5 becomes disorganized. In addition, it is a fact that oxidized full-length *E. coli* TRX is stabilized by ~3.4 kcal/mol as compared to the reduced form.

In this vein, we demonstrated that -upon C32-C35 disulfide bridge formation- >50% of fragment TRX1-93 acquires a native-like state, as evidenced by near-UV CD and MS. In the view of energy landscapes, reduced TRX1-93 would map a rather flat funnel where the molten globule conformation is populated. By contrast, the energy surface would deepen (or become smoother) when a specific peptide recognition event or an 'allosteric' covalent backbone stiffening interaction contribute to the consolidation of the native form.

2735-Pos Hierarchical Investigation Of The Structural Vs. Mechanical Response Of Erythrocyte Membrane

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Board B38

We report here an investigation of the correlation between the nonlinear mechanical response of the erythrocyte membrane and its molecular structure using a hierarchical modeling paradigm that includes a molecular based model and a complete cell double-layer FEM model. The molecular based model describes the dynamics of a small cluster of junctional complexes (the basic units in the protein skeleton) coupled with the lipid bilayer. This model incorporates a state-of-the-art understanding of the three-dimensional molecular architecture of the junctional complex, the skeleton-bilayer interaction, fluid-structure interaction, and stress-induced protein unfolding. This model is thus capable of simulating relations between the mechanical properties of individual components and their interconnectivity with the mechanics of the membrane itself, and predicting consequences of mutations and diseases that cause alternations in the molecular architecture. The FEM model describes the membrane as a structure including two continuous layers, the outer layer corresponding to the lipid bilayer and the inner layer representing the protein skeleton. The mechanical properties of the inner layer are provided via the molecular-based model. Both vertical interaction (i.e. the decoupling force between the two layers) and horizontal interaction (i.e. the inner layer sliding as allowed by the lateral mobility of transmembrane proteins of the skeleton) are modeled. This multiscale model has been applied to simulate overall deformations and local response of normal and mutant cells (e.g. cells with Southeast Asian Ovalocytosis). Comparisons with experiments are discussed.

2736-Pos Effects of Triethylene Glycol on the Folding and Stability of Some Polypeptides

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Board B39

Previously we have investigated the effects of osmolytes/cosolvents on the stability and hydration of several single-domain proteins. We have found out that triethylene glycol (TEG) induced the unfolding of myoglobin and cytochrome c at low to moderate concentrations followed by the folding at higher concentrations. The helicity of the proteins in the presence of high TEG concentrations was higher than that of the proteins in their native state. Consequently, we have investigated the effects of TEG on the stability apocytochrome c (a natively unfolded protein) and poly-L-lysine in the random coil form. In the presence of high TEG concentrations, these unfolded/disordered polypeptides were found to adopt a helical shape as witnessed by the circular dichroism (CD) spectra in the far UV range. The degree of TEG-induced folding (of poly-L-lysine and apocytochrome c) was similar to that of trifluoroethanol (TFE), which is a potent helix-inducer.

2737-Pos Contributions of charged residues to the folding stability Of FKBP12

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Board B40

Charged residues in proteins may have significant effects on the folding and binding stability. We have developed computational models for predicting electrostatic contributions to protein folding and binding stability [1, 2]. To rigorously test and further refine these models, we carried out experimental studies on the effects of charge mutations on the folding stability of FKBP12. FKBP12 has a close homologue, FKBP12.6, differing in 18 of the 107 residues. Of the 18 substitutions, 8 involve charged residues. The changes in the unfolding free energy for these 8 substitutions on FKBP12 were found to vary from −0.34 to 0.65 kcal/mol. Double and triple mutations were introduced to accumulate the stabilization effects of individual substitutions, resulting an increase in stability of 0.84 kcal/mol. On the other hand, neutralizing one or both partners of a conserved salt bridge reduced the stability by as much as 0.64 kcal/mol. Additional charge mutations were modeled after a thermophilic FKBP, MtFKBP17, which aligns with FKBP12 in 89 of the latter's 107 positions with 28 identical residues. Eleven charge mutations were studied, with changes in the unfolding free energy varying from −3.61 to 0.15 kcal/mol. Comparison between mutations modeled after FKBP12.6 and those after MtFKBP17 demonstrates that point mutations grafted from more distant proteins tend to be more destabilizing. The experimental results provide valuable insight into the molecular basis of protein stability and will serve as an important benchmark for refining computational models for predicting protein stability.

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2738-Pos Metal Ion Selectivity and Affinity of the LIN-12/Notch-Repeat

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Board B41

Notch receptors are transmembrane glycoproteins of a highly conserved signaling pathway that regulate cell growth, differentiation, and death in multicellular organisms. Notch activation requires two successive ligand-induced proteolytic cleavages that enable the intracellular Notch to translocate to the nucleus and regulate gene transcription. Notch proteins exhibit a highly conserved modular architecture, which includes three tandem LIN-12/Notch-Repeats (LNRs) responsible for maintaining the receptor in its resting conformation prior to ligand binding. These highly conserved modules contain a characteristic arrangement of three disulfide bonds and a group of aspartate/asparagine residues that coordinate a Ca^{2+} ion, essential for the correct folding of an LNR. In this work, we used the first repeat from human Notch1 (hN1LNRA) as a model system to investigate the binding specificity and affinity of different metals to an LNR via isothermal titration calorimetry (ITC). We expressed hN1LNRA in *Escherichia coli,* purified, and refolded it *in vitro* in the presence of Ca^{2+}. To obtain the apo-hN1LNRA for the ITC experiments, we repurified the folded protein under acidic conditions before dialyzing it against experimental buffer conditions. We tested several divalent atoms, including calcium and zinc, which are essential components of biological systems, as well as rare earth metals, such as terbium, which are similar to calcium in size. We also compared the binding affinities of these metals for the wild type hN1LNRA to a mutant form of hN1LNRA, where the serine in position 19 is replaced by an aspartate that is a part of the Ca^{2+} coordination in most other LNRs. This work represents an important step in elucidating the basis for metal ion selectivity by the LNRs and unrevaling the details of the regulation of the Notch signaling pathway by the LNRs, which rely on metal binding for their structural integrity.

2739-Pos A Comparison Of The Counteracting Effects Of Osmolytes Trimethylamine N-oxide And Betaine On The Interactions Of Urea With Zwitterionic Glycine Peptides

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Board B42

To compare the counteracting effects of methylamines trimethylamine N-oxide (TMAO) and betaine on the actions of urea, we have determined the apparent transfer free energies ($\Delta G'_{tr}$) of amino acids of zwitterionic glycine peptides: glycine (Gly), diglycine (Gly_2), triglycine (Gly_3), and tetraglycine (Gly_4) from water to TMAO, or betaine and urea, and also the blends of osmolyte (TMAO or betaine) and urea at a 1:2 ratio as well as various urea concentrations in the presence of 1 M osmolyte, through the solubility measurements, at 25°C under atmospheric pressure. The protecting osmolytes rapidly raise the positive $\Delta G'_{tr}$ values, indicating that osmolyte can stabilize the glycine peptides. Surprisingly, the denaturant urea also slightly increases the positive values of $\Delta G'_{tr}$ in the simple amino acids (AA) of Gly and Gly_2, whereas urea lowers the negative free energies of Gly_3 and Gly_4, favoring the unfolded state of higher glycines. Additionally, the $\Delta G'_{tr}$ values of the blends of osmolyte with urea revealed that the natural osmolytes TMAO or betaine strongly counteracted the urea actions on glycine peptides. However, the protecting osmolytes partially counteracted the deleterious effects of urea on the Gly_4 in the case higher urea concentrations (4–8 M) in the presence of 1 M osmolyte. Our results indicate that TMAO counteracts the urea effects on glycine peptide molecules more effectively than betaine.

2740-Pos Structural Thermodynamics of Protein Preferential Solvation

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Board B43

Protein stability and solubility is known to depend strongly on the presence of osmolytes, because of a preference of the protein to be solvated by either water or osmolyte. It has traditionally been assumed that only this relative preference can be measured, and that the individual solvation contributions of water and osmolyte are inaccessible. However, it is quite possible to determine hydration and osmolyte solvation separately using Kirkwood-Buff theory, and this fact has recently been utilized by several researchers. Here, we provide a thermodynamic assessment of how each surface group on proteins contributes to the overall hydration and osmolyte solvation, thus providing a space-resolved solvation pattern. Our analysis is based on experiments with model-compounds that were previously demonstrated to allow for a very successful prediction of osmolyte-dependent protein stability. We find the protein hydration to be largely independent of the specific patch on the protein surface, and of the type of osmolyte used. The major solvation effects originate from the osmolytes. Interestingly, the presence of saccharides in solution leads to simultaneous accumulation of both sugar and water in the vicinity of peptide groups. Thus, the overall "preferential exclusion" of sucrose around proteins results in part from an accumulation that is less than the accumulation of water - resulting in a "deficit" of sucrose relative to water.

2741-Pos Destabilizing Aggregation Conditions of Aß Proteins: Alzheimer's disease Passive Immunotherapy

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Board B44

Amyloid beta (Aß) protein deposits in the brain are an important hallmark of Alzheimer's disease (AD). Antibody-based therapy (passive immunization) can assist in the dissolution and clearance of these deposits. However, judicious evaluation and optimization *in vitro* is needed in order to understand the molecular effects of this type of therapy. Atomic force microscopy (AFM) and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy have been mainly used to directly evaluate and monitor the protein aggregates physical and chemical changes during antibody-based assays. We have studied the effects of adding antibodies during Aß protein growing-phase (i.e. fibrils) and determined the conformational changes suffered by the protein. One of the effects observed is the dissolution of Aß protein aggregates as function of antibody specificity, which can be either to $Aß_{1–40}$ or $Aß_{1–42}$ or to both proteins. We have determined that antibody to Aß molar ratios ranging from 1:10 to 0.6:10 equally enhanced the dissolution of Aß aggregates. But, when smaller molar ratios are used, we observed that the expected dissolution is minimal. In fact, the opposite effect has been recorded. That is, the aggregation of Aß protein increases. With our analysis, it is possible to connect the physical and chemical changes during the process of dissolution triggered by antibody addition. It is expected that this study contribute to understand and optimize antibody-based treatments for Alzheimer's disease.

2742-Pos Tocopherol Reduces Kinetics of Rhodopsin Photoactivation and Thermal Denauration

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Board B45

Tocopherol is found in human rod outer segments. Its function is typically ascribed to protection from oxidation, however recent reports describe roles for tocopherol in cellular regulation at the enzymatic and genetic levels. To investigate the non-antioxidant roles of tocopherol in visual signaling, we reconstituted bovine rhodopsin into tocopherol-containing 1-stearoyl-2-docosohexanoyl-phosphatidyl choline (SDPC) bilayers. The effect of 10 and

15mol% tocopherol on rhodopsin function was determined spectroscopically by measuring the equilibrium amount of the signaling-competent conformation metarhodopsin II (MetaII) following photoactivation. The rate of MetaII formation was measured by flash photolysis. Effects of tocopherol on rhodopsin thermal stability were determined by differential scanning calorimetry (DSC). Phospholipid acyl chain packing was determined via DPH time-resolved fluorescence polarization anisotropy. Addition of 10 and 15mol% tocopherol reduced the amount of MetaII formation by 12 and 28%, respectively, and the incorporation of tocopherol slows the rate of MetaII formation. DSC thermograms demonstrate that tocopherol increases the thermostability of rhodopsin by up to 0.8°C. Model-dependent analysis of the scan rate-induced shift in Tm shows that the kinetics of rhodopsin denaturation are decreased by tocopherol, with a reduction in denaturation activation energy of up to 20%. Lipid acyl chain packing order is increased in proportion to the amount of tocopherol in the membrane, while DPH fluorescence lifetimes are decreased. The reduction of DPH fluorescence lifetime by tocopherol indicates increased water penetration, which suggests increased packing defects in the lipid headgroup region. The combination of more constrained packing in the acyl chain region and looser packing in the headgroup region suggests that tocopherol produces a change in bilayer curvature stress. The results demonstrate that high levels of tocopherol inhibit the photoactivating conformation change of rhodopsin and increase its thermal stability.

2743-Pos Kinetic And Thermodynamic Characterization Of BsSCO's Interactions With Copper

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Board B46

SCO proteins are molecules that are involved in the assembly of the respiratory enzyme complex cytochrome *c* oxidase. Evidence from studies with *Bacillus subtilis* support a specific role for BsSCO in assembly of the dinuclear Cu_A center of cytochrome *c* oxidase. BsSCO binds one equivalent of Cu(II) with high affinity and we have estimated the K_D of this interaction by assessing the relative stability of BsSCO-Cu(II) by differential scanning calorimetry (K_D<3.5 pM). In contrast BsSCO binds copper (I) with relatively low affinity (K_D= 10 uM). Dissociation of BsSCO-Cu(II) is exceedingly slow at neutral pH and low ionic strength ($t_{1/2}$>5 days), and is promoted at high ionic strength in the presence of one extra equivalent of Cu(II) ($t_{1/2}$ = 100 s). Kinetic studies of Cu(II) binding reveal a two-step process leading to the final BsSCO-Cu(II) complex. The initial BsSCO/copper complex forms in a bimolecular reaction with a rate of $3 \times 10^4\ M^{-1}\ s^{-1}$ with an absorption maximum shifted to 380 nm compared to the A_{max} at 352 nm in the final complex. The transition from the initial complex to the final form is a unimolecular process and occurs at a rate of $1.5\ s^{-1}$. Denaturation of BsSCO in guanidine-HCl shows a relatively small effect of copper binding on the unfolding kinetics of the overall protein structure, but the release of copper is slow following unfolding. These data suggest that BsSCO strongly prefers Cu(II) over Cu(I) and this affinity is largely determined by slow dissociation kinetics. If BsSCO mediates Cu(II) delivery to Cu_A then the dissociation rate of BsSCO-Cu(II) must be promoted.

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2744-Pos Role of Water in Conformational Behavior and Assembly of Collagen Molecules

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Board B47

Supramolecular assembly of collagen, the most abundant protein in the human body, determines the local mechanical environment that is vital for tissuehomeostasis. Misfolding of collagen or derangement in collagen turnover have been implicated in a number of diseases. However, factors that determine the conformational stability of collagen triple helices and their assemblies remain poorly understood. We used molecular dynamics simulations to study the stability and behavior of a collagen-mimetic peptide (PDB ID: 1BKV) in an explicit-water environment. We developed a method to monitor the torsional motion of the triple helix, which enabled detailed analysis of its temperature-dependent local unwinding. In particular, unwinding initiates in the region devoid of imino acids at the glycine-isoleucine bond, which is known to be a common cleavage site.

We found that water plays a stabilizing role by forming bridges through hydrogen bonding with backbone atoms and preventing propagation of unwinding. Although water bridges were highly dynamic and had lifetimes on the order of only a few picoseconds, their presence was enough to keep the molecule stable at 273 K, while unwinding was observed above 300 K, consistent with the fact that isolated collagen molecules are unstable at body temperature. While individual water bridges and hydrogen bonds become short-lived at higher temperatures, the overall organization of water around the collagen was found to be stable, with a clearly defined boundary for the first hydration shell. The diameter of the hydrated collagen including the first hydration shell is about 14 Angstroms, which agrees well with the experimentally measured inter-collagen distance. These results suggest that water not only controls the unwinding behavior of collagens, but it forms a lubrication layer that mediates collagen self-assembly.

2745-Pos Arrhenius-like Activated Kinetics of Protein Folding

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Board B48

The activated kinetics of the folding mechanism of membrane proteins was studied following Arrhenius Theory using a high-

resolution Modulation Calorimetry (MC) technique. Different CopA membrane proteins, e.g. mesophilic and thermophilic, were studied with and without ligands. Heating scans were performed at different ramp rates from near equilibrium to 60 K/hr using the MC technique. A sudden increase in the activation energy of the folding process of thermophilic membrane protein (Archaeoglobus Fulgidus: AfCopA) was revealed with ligands (e.g. ATP). Significant changes were also observed in the activation energy for mesophilic protein folding. This kinetics appears to follow an Arrhenius behavior as the folding mechanism shifts towards lower temperature as the ramp rate increases. These observations will be discussed in terms of folding and the stability mechanism of proteins in the presence of ligands.

2746-Pos Determination of Pair Potentials for Protein Folding: A Coarse-grained Representation

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Board B49

We present a method to find out the pairwise potentials driving protein folding. In this method the main factor of folding is the attractive energy between nonbonded residues that are in contact in native state. A dynamic optimization program with a Go-type potential is used to minimize the distance between the native contact pairs for the whole trajectory. The coarse-grained model of Chicken villin headpiece, a 36 residue protein, has been prepared by representing each amino acid as a bead. The dynamics of the protein is based on the Newton's equations of motion. The excluded volumes and bond lengths are implemented as constraints. A force is stated between each bead pair and it is determined by the optimization program. As a result of this optimization, the optimum pathway and the pairwise forces operating along this pathway are obtained. The results of the optimization are analyzed using two different approaches. First, the pairwise distances are calculated from the trajectory data and the potential of mean force for each residue pair is obtained using this distance data. This provides the distance dependent potential between pairs. Second, pairwise forces are fitted to the pairwise distance data. Results are averaged over several optimizations that start from different initial conformations

2747-Pos A new search principle for protein structure prediction:Zipping and Assembly Method using FRODA (ZAMF)

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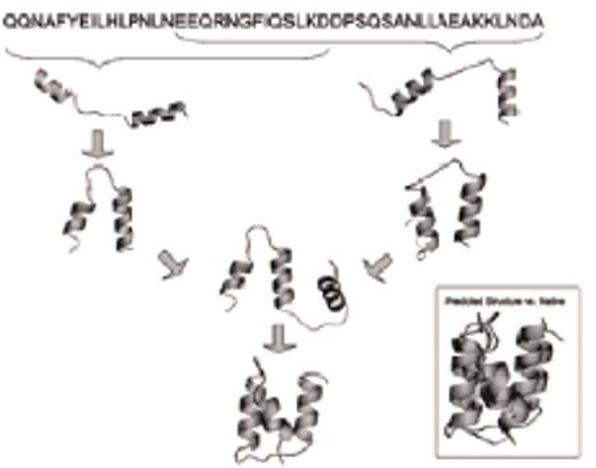
Board B50

'Protein folding problem' stemming from Levinthal's paradox (i.e. how proteins fold fast even though they have vast conformational space) has been answered by the zipping and assembly mechanism (ZA). According to ZA mechanism, an unfolded chain first explores locally favorable structures at multiple independent positions along the chain. Then, these local structures engage neighboring amino acids in the chain sequence to form additional contacts, growing individual local structures by zipping or assembling. Using ZA principle, an all-atom structure prediction method has been developed, called zipping and assembly method (ZAM). ZAM has successfully predicted protein structures of small single domain proteins. It uses replica exchange molecular dynamics for conformational sampling which creates a bottleneck in the assembly stage.

We modify ZAM assembly stage by introducing FRODA which is a Monte Carlo based geometric simulation. Since FRODA can explore the large-amplitude motions of larger systems so much faster than molecular dynamics, we can speed up the assembly stage and generate the complete enumeration of all topologies quickly. The results show that the native structure of proteins can be sampled during the FRODA-assembly stage within a RMSD <3 Å.



2748-Pos Plant Type 1 Metallothioneins: Candidates for Intrinsically Unstructured Proteins

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Board B51

Type 1 plant metallothioneins (MTs) are low molecular weight (7–8 kDa), sulphydryl-rich, aromatic residue lacking, metal-binding proteins with two terminal metal-binding clusters separated by a long hinge region of about 50 amino acids. The length and conserved sequences in the hinge region distinguish type 1 MTs from their

mammalian counterparts, suggesting additional roles other than metal binding and detoxification. Homology modeling of a Cd-binding durum wheat MT (dMT) indicates mammalian-like folds for the metal-binding domains, and *ab initio* calculations yield a DNA-binding like structural motif for the hinge region. Here, we study the structural features of full-length dMT in metal-free (apo) and metal-bound (holo) states using various biophysical and biochemical techniques. Cd-bound dMT is expressed in *Escherichia coli* as a GST fusion protein and cleaved from this tag for further analyses. Small angle X-ray solution scattering measurements and gel-filtration chromatography revealed holo-dMT as a dimer whereas apo-dMT is monomeric as shown by native state mass spectrometry experiments. Far-UV circular dichroism (CD) measurements revealed apo- and holo-dMT as a random coiled protein. Near-UV CD measurements showed two maxima at 246.6 and 266 nm for holo-dMT. The amount of bound Cd was determined as 5±1 M equivalent by inductively coupled plasma optical emission spectroscopy. When Cd metals were stripped, the two CD maxima disappeared and this form of dMT showed CD spectrum similar to that of the acid unfolded dMT, suggestive of disordered structure for the apo form. Interestingly, Kratky plot of the SAXS data lacks the characteristic maximum observed for globular proteins, indicating that Cd-bound dMT is in a molten globule like conformation. Taken together, our results put forward the possibility of dMT as a candidate for an intrinsically unstructured protein.

2749-Pos Thermal Stability Of The TATA Box Binding Protein

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Protein function implies a compromise between stability and flexibility. Proteins from organisms adapted to high temperatures appear to compensate larger thermal fluctuations with increased stability. We probe the structural, energetic and fluctuation amplitude responses of TBP with 5 ns molecular dynamics simulations at temperatures ranging from 0 to 100°C, carried out in the NPT ensemble at 1 atm. We simulated TBPs from *Pyrococcus*, *Sulfolobus*, *Saccharomyces* and *Arabidopsis*, with unfolding Tms of 101, 85, 60 and 60°C, respectively; *Pyrococcus* TBP has a disulfide bond, and its effect was studied simulating a reduced version of the protein (unfolding Tm of 97°C). The fluctuation amplitudes and free energy of the proteins (estimated within the MM-PBSA formalism) increase with temperature, but not at the same rate for all the systems. Resiliency measures[1] suggest that TBP has used entropic, rather than enthalpic stabilization as a strategy for thermal adaptation; accordingly, the number of intramolecular H-bonds does not change with temperature. The disulfide bond contributes enthalpically to the stability of *Pyrococcus* TBP. The rate of increase of fluctuation amplitudes with temperature at each residue revealed heating-resistant zones, and others that are highly responsive. The first lie at the interface between the β-sheet and helices 2 and 2′, as suggested by [2]; the extent of these zones decreases with the Tm. The latter map to the N- and C-termini, the stirrups and the loop connecting the N- and C-subdomains. The disulfide bond increases the stability of the connections between helix 1 and the β-sheet, and also the loop between strands 3 and 4.

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2750-Pos Biological Thermodynamics: The Thermal Set Point and the Origins of Life

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Board B53

Application of the Planck-Benzinger thermal work function to biological systems has demonstrated a basic pattern for life processes, in that there is a lower cutoff point, $<T_h>$, where entropy is favorable but enthalpy is unfavorable, i.e. $\Delta H^o(T_h)(+) = T\Delta S^o(T_h)(+)$, and upper cutoff, $<T_m>$, above which enthalpy is favorable but entropy unfavorable, i.e. $\Delta H^o(T_m)(-) = T\Delta S^o(T_m)(-)$. Only between these two limits, where $\Delta G^o(T) = 0$, is the net chemical driving force favorable for interacting biological processes. In the case of water vapor condensation the compensatory temperatures, $<T_h>$ and $<T_m>$, are 30 K and 380 K. Each living system is made up, in some part, of water. Hence we suggest that the single point at which the system is its most stable, defined as the thermal set point, $<T_S>$, must fall between the limits of 30 K and 380 K. We find that each biological system will exhibit a negative minimum of Gibbs free energy change at this well-defined temperature, $<T_S>$. Each system will have its own unique value of $<T_S>$, where the bound unavailable energy $T\Delta S^o = 0$. At this point, $\Delta H^o(T_S)(-) = \Delta G^o(T_S)(-)_{minimum}$, the maximum work can be accomplished. For water vapor condensation, thermal set point falls at 260 K and $<T_{Cp}>$ at $\Delta Cp^o(T) = 0$ is 130 K. In examining interacting protein systems, it would appear that the heat capacity change of reaction of water within the system determines the behavior of the other thermodynamic functions. It is apparent from the application the Chun approach to studies of numerous biological interactions that the origins of life in any system are inevitably linked to a single, unique thermal set point.

Protein Structure

2751-Pos Structure and Interactions of Mini-B, a Fragment of Surfactant Protein B

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