2698-Plat Different Lipid Anchors Recruit Proteins into Distinct Membrane Domains: A FCCS Study on Activated T Cells

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While the heterogeneity of the plasma membrane of eukaryotic cells is by now a well-established fact, the precise architecture of this important cellular structure is still seriously debated. Here, we focus on the role of specific lipid anchor motifs in the organization of T-cell plasma membranes into distinct domains of particular composition. To that end we generated a combinatorial library of protein constructs by fusing different lipid-modification sites of lipid anchored proteins with one of two fluorescent proteins. Two of these constructs that encode for either myrestilation, palmytilation, geranylation or glycosylphosphatidylinositol (GPI) elaboration and are labeled with either enhanced green fluorescent protein (EGFP) or monomeric red fluorescence protein 1 (mRFP1) were co-expressed in each cell. We used dual color fluorescence cross-correlation spectroscopy (FCCS) to exploit co-movement of the same or different lipid anchors as a signature of spatial cluster formation, thereby circumventing the limitations of direct imaging of nanometer sized membrane structures. Our FCCS studies on activated T-cell membrane sheets and membranes of whole T-cells show that most anchors only co-localize with themselves, while different anchors move independently from each other. These findings not only suggest that the plasma membrane is composed of a variety of different domains (each with specific protein content), but also that the lipid anchor structure plays a key role in the specific recruitment of proteins into their target domains.

Protein Folding Stability - I

2699-Pos An All-Atom Structure-Based Model for Protein Folding: Interplay Between Geometry and Energetics

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Board B1

The funneled energy landscape is dominated by a protein's native state and gives a theoretical foundation for minimally frustrated structure-based models. For simulation, the protein is typically reduced to a one bead per residue representation. These simulations reproduce a folding mechanism in agreement with experiments. In order to get a more detailed description of a protein, one can add layers of complexity. One possibility is including all atoms of a protein in structure-based simulations to better capture the process of side-chain packing. We introduce an all-atom, structure-based model to explore the interplay between geometry and energy during folding. The model is applied to several proteins. It is shown that the folding mechanism stays stable over a wide range of parameters, while the energetic properties like folding temperature and folding barrier vary. Further, several definitions for the native contacts are evaluated and a simple contact map definition is proposed.

2700-Pos Fold Specific Knowledge-based Potential Functions for Protein Structure Prediction

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Board B2

In protein structure prediction, potential functions are used to guide the conformational search or to select the native structure from a set of candidate structures. Therefore, an accurate and efficient potential function is crucial in structure prediction. Quasi-chemical knowledge-based potential functions derived from known protein structure databases have been proven to be quite effective in structure selection. However, it has also been showed that knowledge-based potential functions might be training database dependent due to some unrealistic assumptions, such as the use of sequence independent reference state. In this study, we exploit the dependence of knowledge-based potentials on training databases. A set of *fold-specific* knowledge-based potential functions instead of a universal one is derived. We trained these potential functions at the fold level of SCOP classification and found that such fold-specific knowledge-based potentials are more sensitive and can discriminate almost all native structures from the decoys in both 4State reduced and LMDS decoy sets if the fold can be correctly predicted in advance. Even with a much simpler function than some sophisticated potential functions, the performance of our fold-specific potential functions is quite competitive. The results thus not only verify the dependence of the quasi-chemical knowledge-based potentials on the training databases, but also demonstrate that the sensitivity of the quasi-chemical knowledge-based potentials can be improved by applying a proper fold-specific potential, if the fold of the protein to be predicted is known. The conditional clause can be dropped by incorporating with a reliable fold prediction server, in which case the applicability of these fold-specific knowledge-based potential functions can be extended even to those proteins with unknown folds.

2701-Pos Calculations of Protein Thermodynamics via Distance Constraints: Refined Account of Hydration Effects

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Board B3

Ability to predict protein thermodynamic properties such as kinetic and thermodynamic stabilities, unfolding temperature, and free-energy landscapes is crucial for understanding protein function and for protein drug design. Even for small proteins however such analyses are still impractical by conventional approaches such as MD or Monte-Carlo simulations. We have developed a novel all-atom approach for modeling protein thermodynamics, termed a distance-constraint model (DCM). It is based on (*i*) a free-energy decomposition scheme and (*ii*) a distance constraint representation of the protein thermodynamic ensemble. The key advantages of our approach are the accurate account of the conformational entropy by utilizing rigidity theory algorithms, and the ability to analyze large proteins within a matter of minutes or hours. While the DCM was shown successful in predicting numerous thermodynamic properties [1,2], one of its drawbacks is the lack of transferability of three empirical parameters. In this work, our new upgrades to the DCM which include more accurate account of hydrogen bonding to solvent will be presented, and their impact on transferability of parameters and accuracy of thermodynamic predictions will be described.

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2702-Pos Secondary Structure Formation of Short Peptides Studied by Molecular Dynamics Simulations

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Board B4

Secondary-structure formation is a key step of protein folding. Two secondary-structure elements are usually connected by a turn or a loop that locates near the surface of the protein molecule. A β-hairpin has such a turn and two β-strands. We investigated the role of the turn region of a β-hairpin peptide ("G-peptide") for folding by molecular dynamics simulations. The amino-acid sequence of G-peptide is GEWTYDDATKTFTVTE.

We performed a multicanonical replica-exchange molecular dynamics simulation of G-peptide with explicit water molecules. The initial peptide conformation for the simulation was an unfolded conformation that was prepared without any use of the information of the native conformation. The number of replica was 8, and the simulation length was 34.75 ns for each replica. We observed three folding events from unfolded conformations to the native conformation. The analysis of the simulation results has shown that (1) not only the hydrophobic inter-side-chain contacts between the aromatic residues (Y5 and F12) but also the native-like turn formation take place in an early stage of folding of G-peptide and that (2) the native-like turn formation prevents misfolding of G-peptide to non-native β-sheet conformations with the aromatic inter-side-chain contact formed. [1] These results are consistent with experimental studies on folding and stability of G-peptide.

The foldability of G-peptide in molecular simulations, however, depends on the force field used in the simulation. We considered that the force-field dependence might be due to the force-field dependence of the conformational preference of the turn sequence. We performed molecular dynamics simulations of a shorter peptide whose amino-acid sequence corresponds to the turn region. Similarity (RMSD) to the native structure has been shown being dependent on the force field.

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2703-Pos Free-energy Based All-atom Protein Folding Using Worldwide Distributed Computational Resources

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Board B5

Following Anfinsen's thermodynamic hypothesis we have implemented massively parallel stochastic optimization methods for all-atom de-novo protein folding using our free-energy forcefield PFF02[1]. We have implemented this approach (POEM) using a world-wide volunteer computation a grid to predictively and reproducibly folded several proteins with up to 57 amino assets, including the engrailed homeodomain and protein A, from completely unfolded conformations. POEM identifies the native conformation of the protein as the global minimum of the protein free-energy forcefield PFF02, which stabilized the native conformation of all 32 monomeric proteins (without cofactors) against all decoys in the Rosetta decoy set[2]. In addition we could fold a set of 13 proteins with helical, sheet and mixed secondary structure from completely unfolded conformations to near-native conformations, to an average 2.87 Å resolution[1–3].

In this investigation, we deployed a BOINC server implementing an evolutionary strategy[4], which explores the free-energy landscape in many parallel dynamical processes, which communicate with one another through a central server. The overall computational work is thus segmented into medium size work-units, which can be processed independently. The algorithm evolves a population of conformations towards the global optimum of the free-energy surface by balancing energy improvement with population diversity. POEM@HOME (http://boinc.fzk.de) thus implements a complementary approach to existing distributed computational proteomics initiatives, such as Folding@Home or Rosetta@Home, to help analyze structure and function of large, experimentally relevant proteins.

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2704-Pos Mechanical Single-Molecule Folding and Unfolding Transition State phi-value Analysis

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Board B6

Protein folding is described as an energetically driven diffusional search on a high-dimensional energy landscape. In general, during the folding process, the denatured unstructured polypeptide chain has to pass a saddle point - the so-called transition state ensemble - before finally reaching its fully folded native state. This transition state ensemble is described in theory by a number of interactions between several amino acids. Here, we use systematic phi-value analysis combined with single-molecule double-jump force spectroscopy experiments to characterize the transitions states of the three-state folding pathway of the beta-sandwich protein ddFLN4. We find that both transition states are nearly independent. First, amino acids from beta-strands C-G form the transition state located around a cluster of amino acids for the folding intermediate. In a second step, mainly amino acids from the beta-strands A and B interact with each other to form the second transition state ensemble. Point mutations clearly either affect the first transition or the second transition, leading to the conclusion that before folding and transition state formation no complete hydrophobic collapse is needed.

2705-Pos Heat Capacities Of Protein Calculated By Using Extended Scaled Particle Theory

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Board B7

Calorimetry experiments often assume that heat capacity changes of protein denaturation are dominated by hydration effect of protein. Heat capacities of bovine pancreatic trypsin inhibitor (BPTI) are calculated by using extended scaled particle theory (XSPT). In this study, temperature dependence of hydration free energy is used to calculate the heat capacities and entropies of the protein. Calculated values of heat capacity changes, Delta Cp, show that the protein has constant values in the range of 273–373K. In XSPT, the expression of hydration free energy has the term named C2, which has the same dimension as the square of the mean radius of the solute molecule. Temperature dependence of hydration free energy is dominated by the C2 term. Thus Cp values are directly affected by the geometry of the protein conformation. We have applied two different approximations to the expression of hydration free energy which is a functional of excluded volume through the concept of hypothetical scaling. One of the approximations is the conventional one and the other is new and intended to treat interior cavities explicitly. Differences of Delta Cp values between calculated and experimentally measured ones reduce 30% in the case of explicit treatment than before. In addition, the C2 term leads us to make a simple explanation of the existence of the convergence temperature of entropy changes (Delta S) by borrowing the concept of bilinear model proposed by B. Lee.

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2706-Pos Intuitive Analysis Of Conformational Dynamics In Biomolecules

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Board B8

Molecular dynamics (MD) simulation generates large quantities of data which must be interpretable in an intuitive way to understand the function of the system studied. In previous work we presented an approach for the meaningful physical analysis of this data. We partitioned the space into configurational microstates before identifying few long-lived, i.e. dynamically metastable, states based on kinetic proximity. In this continuative study we introduce energy model based graph clustering as a beneficial supplement or full alternative to partitions. As a supplement one can better interpret the relationships between metastable states obtained from partitioning in view of their structural and physical properties. As an alternative to partitions dynamically metastable states are identified using energy model based graph clustering on the basis of configurational microstates whereas a given microstate is not necessarily assigned to a definite metastable. In both cases the result can be represented as drawings which facilitate the intuitive exploration of the data as a combination of configurational and kinetic information, microstates, metastable states and connecting transition states. Here we present this complete solution of data preparation, visualization and interpretation for the MD simulation of several polypeptide systems.

2707-Pos Effect of the Amino Acid Composition of Surface Residues on the Structure and Stability of a Protein

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Board B9

Using the information from the genome projects, recent comparative studies of thermostable proteins have revealed a certain trend of amino acid composition in which polar residues are scarce and charged residues are rich on the protein surface. To clarify experimentally the effect of the amino acid composition of surface residues on the structure and stability of *Escherichia coli* Ribonuclease HI (RNase HI), we constructed six variant proteins in which five to eleven polar residues were replaced by charged residues (5C, 7Ca, 7Cb, 9Ca, 9Cb, and 11C). The thermal denaturation experiments indicate that all of the variant proteins are 3.2° to 10.1°C in T_m less stable than the wild-type protein. The crystal structures of resultant protein variants 7Ca, 7Cb, 9Ca, and 11C closely resemble that of the wild-type protein in their global fold, and several different hydrogen bonding and ion-pair interactions are formed by the substitutions. Comparison of the crystal structures of these variant proteins with that of the wild-type protein reveals that thermal destabilization is apparently related to electrostatic repulsion of the charged residues with neighbors. This result suggests that charged residues of natural thermostable proteins are strictly posted on the surface with optimal interactions and without repulsive interactions. This means that protein thermostabilization requires a fine-tuned placement of surface charged residues.

2708-Pos A Novel Method for Predicting Native Structure of Proteins Based on Liquid State Thermodynamics

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Board B10

Understanding the thermodynamic basis for protein folding is of fundamental importance in the field of biophysics. The native structure of a protein is uniquely determined by its amino acid sequence. This structure is thought to be most stable in terms of the free energy of the protein in water. Although this principle is well established and thermodynamic data concerning the protein folding/unfolding have been accumulated since the late 1980s, the prediction of the protein structure from its sequence is still an unsolved problem. Consequently, there must be an important term missing in past thermodynamic analysis. Recently we have shown that the gain in the translational entropy (TE) of the solvent is a dominant factor upon protein folding and that the native structure of protein G (2GB1) has the maximum value for the TE of water among an ensemble of structures created by molecular simulation.

In this study, we present the idea that the native structure is designed to maximize the TE of water and balance the dehydration penalty in the protein interior. As energy function, we employ the TE of water for provided protein structures and the energy loss of dehydration for those atoms that could form a hydrogen bond in the protein interior without doing so. In molecular simulation, force field parameters are tested by calculating the total energy of structures of 'decoy' sets to check whether one can distinguish the native structure from other non-native structures. In order to check the robustness of our concept, we tested one of the decoy sets. By using our energy function, we succeeded in discriminating the native structure from all decoy structures in the sets.

2709-Pos

Board B12

WITHDRAWN

2710-Pos Few Native Non-local Interactions are Formed Prior to the Rate Limiting Step in the Refolding of *E. Coli* Adenylate Kinase

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Board B13

Do non-local or local interactions (long loops formation versus secondary structure elements formation) dominate the critical initial phase of the folding transitions of globular proteins? An answer to this questions was sought by determination of 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 two secondary structure elements (helix, residues 169–188 and strand, residues 188–203) and four very long chain sections whose ends are in juxtaposition in the native structure were determined in the denatured state, in the initial transient state, at 5–10 ms after initiation of refolding and in the native state.

The double mixing stopped flow experiments showed that the ends of the chain segment between residues 28 and 71, which were widely separated in the denatured state formed native like proximity within the dead time of the instrument (~5 ms). 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 are compatible with 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.

2711-Pos Single-molecule Fluorescence Studies Of Protein Folding

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Board B14

The heterogeneous nature of unfolded protein ensembles makes protein folding an ideal arena for single-molecule experiments. We have been developing single-molecule fluorescence tools that allow us to study folding/unfolding trajectories, as well as to characterize denatured states of proteins, especially under conditions where they coexist with folded states. A novel and delicate method was devised for immobilizing protein molecules for the purpose of recording their time-dependent folding trajectories, based on their encapsulation in surface-tethered lipid vesicles. A new single-molecule spectrometer allows us to automatically scan the surface, identify vesicles containing proteins and collect fluorescence from these vesicles. Using FRET as our signal, we study the dynamics of folding of individual molecules of barstar and adenylate kinase and contrast their behavior. In particular, the very heterogeneous nature of adenylate kinase folding trajectories is dissected.

We also use single-molecule fluorescence methods to study denatured protein molecules diffusing freely in solution, and characterize the equilibrium collapse preceding folding. This collapse is akin to the coil-globule transition in homopolymers, and we have probed it in particular in the denatured state of the small protein L. A new theory recently developed in our lab allows us now to analyze our data in the context of all literature results on denatured-state collapse. The analysis exposes a universal behavior: The average energy of amino acid residues in the denatured state is independent of the specific protein and depends only on denaturant concentration. This universality can be rationalized by Tanford's transfer model.

2712-Pos Membrane Catalyzed HIAPP Folding Followed With 2DIR Spectroscopy

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Board B15

The human islet amyloid polypeptide (hIAPP) creates fibers in the pancrease of Type 2 diabetics. We are studying the structures and folding kinetics of this peptide in the presence of vesicles using 2DIR spectroscopy with the aim of detecting and characterizing intermediates in fiber formation. We will report kinetics of hIAPP secondary structure changes during the fibrillogenesis pathway as the peptide converts from a random coil into beta-sheet fibers. Until now, the only structural information of the folding process comes from circular dichroism spectroscopy. With 2D IR spectroscopy, a much more explicit and accurate picture of the folding process is being gained.

2713-Pos Deep Ultraviolet Resonance Raman Spectroscopy is a Powerful Tool for Protein Structural Characterization at All Stages of Fibrillation

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Board B16

Understanding the biochemical mechanism of amyloid fibrillation is one of the most intriguing and pressing problems in modern biology and medicine. Amyloid fibrils are non-crystalline and insoluble, and thus are not amenable to conventional X-ray crystallography and solution NMR. Several specialized techniques with less general capabilities have been developed and utilized for probing fibrillar structure. We report here on the application of deep UV resonance Raman (DUVRR) spectroscopy for protein structural characterization at all stages of fibrillation. In particular, DUVRR spectroscopy combined with advanced statistical analysis including 2D-correlation spectroscopy was utilized for studying nucleus formation during the fibrillation of hen egg white lysozyme (1). Combining ESI-MS, Raman and fluorescence spectroscopies with advanced statistical analysis allowed us for concluding that the partial unfolding of lysozyme, the first step of in vitro fibrillation, is a two-state transition (2). Finally, the evolution of protein secondary and tertiary structure as well as the structure of the cross-β core of lysozyme fibrils were established based on DUVRR spectroscopic studies (3).

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2714-Pos Spectroscopic And Enzymatic Studies Of Human Cytoplasmic Aconitase Reveal A Complex Unfolding Process

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Board B17

Human cytoplasmic aconitase is a large (98 kDa), multi-domain protein that functions as either a metalloenzyme or an RNA-binding protein, depending on intracellular iron levels. As the metalloenzyme, cytoplasmic aconitase possesses a [4Fe–4S] cluster and

catalyzes the isomerization of citrate to isocitrate. We have used intrinsic tryptophan fluorescence and circular dichroism spectroscopy to monitor tertiary and secondary structural changes as a recombinant form of cytoplasmic aconitase was subjected to thermal and chemical denaturation. These methods were complemented by EPR spectroscopic measurements of the iron-sulfur cluster and enzyme activity assays under native and denaturing conditions. The urea-induced equilibrium unfolding process was complex and could not be described by a two-state model, but thermal denaturation did appear to be two-state. Both thermal and urea-induced denaturation processes were irreversible. The endpoints of the thermal melting curve and the urea titration produced protein species with distinct spectroscopic properties, implying that there are separate unfolded states depending on the method of denaturation. Enzyme activity was enhanced by the presence of urea, up to concentrations of 1M, and measurable activity persisted up to 2 M urea at 37°C. In the absence of urea, activity reaches a maximum at about 50 °C, a value that was slightly higher than the apparent melting temperature of ~ 45 °C in the absence of ligand, suggesting that the presence of substrate enhances protein stability. Biochemical and biophysical data have been combined to produce descriptive models for the urea and thermally induced unfolding of cytoplasmic aconitase.

2715-Pos FT-IR Analysis Of Thermal Stability Of Calmodulin

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Board B18

Ca^{2+}/saturated calmodulin (Ca^{2+}/CaM) has highly thermal stability but not in the Ca^{2+}-free form (apo-CaM). However, the structural mechanism of such heat stability is poorly understood yet. Recently, a fourier-transform infrared spectroscopy (FT-IR), Confocheck$^{Â®}$ System that enables measuring the secondary structure of protein in physiological solution has been developed. In this study, we investigated the temperature-dependent change in the secondary structure of CaM in the physiological solution. A novel Ca^{2+} binding specific band appeared at 1628cm $^{-1}$ derived from vibration of beta-sheet stretching by transmittance measurement using FT-IR. By measuring of transmittance and attenuated total reflection (ATR) of thermal stability of CaM, the area around 1628cm^{-1} of apo-CaM was dramatically increased dependent on the temperature but not in Ca^{2+}/CaM. The integration of the second derivative spectrum value at 1628cm^{-1} indicates that Ca^{2+}/CaM was more stable to heat than apo-CaM. CaM unfolded beyond 55C in the presence and the absence of Ca^{2+}measuring by dynamic light scattering (DLS). The apo-CaM was more strongly aggregated than Ca^{2+}/CaM. Taken together, beta-sheet structure around the Ca^{2+} ion should be important for the thermal stable of CaM.

2716-Pos Rational Design of a Switchable Calcium and Lanthanide Binding Protein

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Board B19

Ca(II) as a messenger of signal transduction regulates numerous target molecules via Ca(II)-induced conformational changes. Investigating the determinants for Ca(II)-induced conformational change is often impeded by multiple cooperative metal binding or protein oligomerization in natural occurring proteins. Here we report a single-site Ca(II)-binding protein (termed CD2.trigger) designed by altering the charged residues at sensitive locations of the host protein rat Cluster of Differentiation 2 (CD2) to dissect the relative contribution of key determinants for Ca(II)-dependent conformational changes. The CD2.trigger binds to Tb(III), La(III) and Ca(II) with dissociate constants (K_d) of 0.3 ± 0.1, 0.7 ± 0.1, and 90 ± 25 μM, respectively. This protein is largely unfolded in the absence of metal ions at physiological pH while Ca(II) or Ln(III) binding results in folding to the native conformation. Removing the charged ligand residues by either mutation or protonation facilitates the folding and thermal stability of the protein. Like nature trigger proteins, Ca(II)-induced protein folding process follows an enthalpy-entropy compensation relationship. These results provide insight into the role of charge repulsion in Ca(II)-induced conformational changes and facilitate the design of Ca(II) modulated triggers to control protein function.

2717-Pos A Comparison of the Site-Specific Thermal Unfolding Mechanism of Two Helix-Turn-Helix Proteins

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Board B20

We investigated the thermal unfolding of two helix-turn-helix proteins using circular dichroism (CD) and Fourier Transform Infrared Spectroscopy (FTIR) with site-specific ^{13}C isotopic labeling. Helix-turn-helix is the simplest α-helical structural motif that combines both secondary and tertiary structural elements. As models, we used a naturally occurring 40-residue sub-domain of the bacteriophage P22 scaffolding protein and the *de novo* designed 38-residue αtα protein. Both of the proteins as well as several ^{13}C isotopically labeled variants were synthesized using FMOC solid phase peptide synthesis. To specifically monitor unfolding of the particular structural elements, we synthesized numerous isotopically labeled variants with ^{13}C substituted amino acids (on C=O) in specific regions throughout the two α-helices and turn which comprise both the *de novo* designed αtα and the P22 subdomain. The temperature-dependent CD and FTIR spectra were first ana-

lyzed by a singular value decomposition (SVD). For both of the unlabeled model proteins, the thermal denaturation monitored by CD and FTIR can be described by a three-state process. However, the site-specific ^{13}C signals (monitored by FTIR) for both proteins reveal the presence of multiple partially-unfolded structures. That these individual regions either unfold or remain partially folded indicates that tertiary inter-helical interactions within the motif are stabilizing the overall structure. These regions also possess unique stabilities, indicating that the folding mechanism is characteristic for each protein: while P22 appears to unfold from its N-terminus and has a reasonably stable core near the turn, the *de novo* designed αtα starts unfolding from the turn and terminal sides and its stable region is located towards the center of both helices which is consistent with three-dimensional structures. Therefore, the use of ^{13}C isotopically-edited infrared spectroscopy makes it possible to attain a site-specific view of how proteins unfold.

2718-Pos Screened Non-bonded Interactions On Weighted Residue Networks To Design Fast-folding Proteins

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Board B21

It has been observed that the topology of the native state is an important determinant of protein folding kinetics and there is a significant correlation between folding rates and relative contact order (RCO) in two-state small single domain proteins (1). However, as a purely geometric property, RCO does not take into account residue interactions, which also play an important role in folding kinetics. We introduce weights into the residue network of contacts (2), according to the cumulative distribution of the inter-residue statistical contact potentials (3), and define a weighted RCO. Using the weighted RCO, we can capture not only gross structural properties, but also the effect of specific residues and redundant features of sequences. We perform an analysis on the ultrafast folding proteins and mutants of CI2, protein A and L. Our results indicate that:

1. the weighted RCO has a significant correlation with the folding rate like RCO;
2. the weighted RCO computed for the subset of contacts, (i.e., the set of contacts that are screened using a cutoff on the statistical potential) has a better correlation with the folding rate than that of the original residue network; and
3. the weighted RCO works for mutants and could potentially help identify or design fast-folding proteins.

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2719-Pos Dynamics of Kinetically and Thermodynamically Stabilized States of Pepsin

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Board B22

Structure, function, stability and flexibility are interrelated factors in describing proteins, and must be studied as a whole. Many proteins are thermodynamically stabilized (TS) and under native conditions exist at the free energy minimum of the folding landscape. However, recently enzymes which are kinetically stabilized (KS) in the native state have been characterized, namely α-lytic protease and *Streptomyces griseus* protease B (Jaswal, S.S., et al., 2005, *J. Mol. Biol. 347*, 355–366). Thus, proteins have adapted various methods of stabilization. There is currently a lack of information regarding the structure and dynamics of KS proteins, which is critical to understanding the folding of proteins.

Our recent studies on different folded states of pepsin, a classical aspartic peptidase, indicated that native pepsin (Np) is KS while an inactive, denatured state (Rp) is TS (Dee, D., et al., 2006, *Biochemistry 45*, 13982–13992). Np is irreversibly denatured (by pH $>$ 6, chemicals or heat), and when placed under refolding conditions Rp is formed. In addition to having a higher Tm, the unfolding of Rp is reversible. The internal dynamics of Np and Rp have been compared using quasielastic neutron scattering (QENS), in order to compare the flexibility of a KS and a TS protein fold. The data (timescale ~ 70–10 ps, length scale ~ 20–4 Å) indicated that the TS Rp conformation was more rigid than the KS Np conformation. In contrast, Jaswal et al. (2005, *J. Mol. Biol. 347*, 355–366) proposed that KS proteins are more rigid than their TS homologues. However, our result that Rp was less flexible than Np is consistent with the observation that flexibility is inversely related to thermal stability. Further analysis of Rp and Np should yield insight into the role of dynamics in shaping the protein folding landscape.

2720-Pos Go-in' Big: A Folding Simulation of GFP

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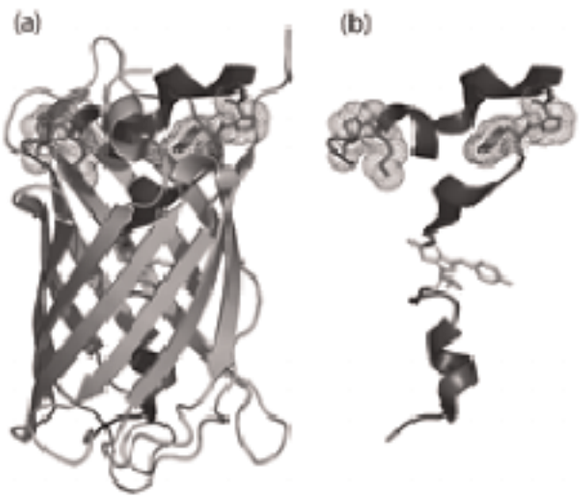
Board B23

Folding simulations with G[[Unable to Display Character: ō]]-type potentials model a smooth energy well, and probe topological effects on folding. However, computational needs limit the proteins which can be simulated. The structure of green fluorescent protein (GFP) has the highest contact order observed in natural proteins, and recent experimental studies have shown that GFP containing a mature chromophore has an extremely rough

energy landscape. Given the rough landscape observed in experiment for GFP, will G[[Unable to Display Character: ō]]-model simulation predict this frustration? Here, we use a modified multicanonical method to enhance sampling for the 228 residue GFP. Kinetic simulations show multiple intermediates, characteristic of a rough landscape. Thermodynamic simulations show two transitions, and an intermediate similar to a kinetic intermediate. Structural analysis of both kinetic and equilibrium intermediates show areas of low contact probability which correlate to residues which have previously been mutated to smooth the energy landscape in experiment (see figure). Also, application of the modified multicanonical method still picks up intermediates, even after reweighting the energy well to increase sampling. Finally, application of a G[[Unable to Display Character: ō]]-type potential to a protein with extremely high contact order shows that this technique can be applied to more complex systems.



2721-Pos The Search for Dimerization of Procaspase-3

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Board B24

We have examined the assembly of the procaspase-3 subunits into the native dimer. Earlier studies showed that the monomer was sufficiently stable to be populated upon dimer dissociation. The equilibrium data were best described to a four-state equilibrium model in which the native dimer undergoes an isomerization to a dimeric intermediate, and the dimeric intermediate dissociates to a monomeric intermediate, which then unfolds. Stopped-flow circular dichroism and fluorescence emission studies point to a complicated burst phase. These results suggest that more than one species is formed very rapidly. By using a catalytically active, yet uncleavable, mutant of procaspase-3, we show that a late folding reaction correlates to formation of the active site. The protein is fully folded after about 60 minutes. Due to the small change in circular dichroism and fluorescence signals upon dimer formation, current studies focus on determining the rate of dimerization using anisotropy and fluorescence quenching techniques.

2722-Pos Determinants of Unique Repressor Function

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Board B25

Sequence comparisons of protein families can identify conserved residues that dictate common function. However, this information cannot predict the distinct function of each homologue, such as altered ligand affinity, specificity, or allosteric regulation. Unique functions are derived from sequence changes at nonconserved positions ("specificity deter[[Unsupported Character - Codename -]]mi[[Unsupported Character - Codename -]]nants"). Specificity determinants are confirmed in the ligand binding sites of many proteins. In addition, "long-range" specificity determinants exist in other protein regions, including domain-domain interfaces.

We are using the LacI/GalR transcription regulators to bridge bioinformatics predictions and experimental understanding of specificity determinants. Using engineered homologues, we identified several specificity determinants in the interface between the DNA-binding and regulatory domains. One engineered repressor (LLhP) is a hybrid of the LacI DNA-binding domain and the PurR regulatory domain. *In vivo* characterization of LLhP with varied amino acids at linker specificity determinants suggests:

1. Exchanging the regulatory domains between two wild-type proteins alters the function of the DNA-binding domain.
2. Individual specificity determinants contribute to different aspects of DNA-binding function (affinity *vs* specificity *vs* allosteric response).

This work presents *in vitro* characterization of purified repressors that test these hypotheses. Experiments include thermodynamic DNA-binding assays with a variety of DNA ligands. Variants at sites 48 and 55 appear to affect DNA-binding affinity. Variants at site 58 abolish all detectable binding. Variants at site 61 appear to affect specificity for recognized DNA ligands and may enhance the magnitude of allosteric response. Comparison of *in vitro* and *in vivo* function suggests that some LLhP variants may acquire specificity for DNA binding sites in the *E. coli* genome that are not recognized by wild-type LacI. Notably, the residues in LacI that directly contact DNA are identical in LLhP. Therefore, other positions in LLhP must contribute indirectly to DNA specificity.

2723-Pos Structure-based Protein Engineering for Alpha-amylase Inhibitory Activity of Plant Defensin

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Board B26

The structure of a novel plant defensin isolated from the seeds of the mung bean, *Vigna radiate*, has been determined by ^{1}H nuclear magnetic resonance spectroscopy. The three-dimensional structure of VrD2, the *V. radiate* plant defensin 2 protein, comprises an α-helix and one triple-stranded anti-parallel β-sheet stabilized by four disulfide bonds. This protein exhibits neither insecticidal activity nor α-amylase inhibitory activity in spite of showing a similar global fold to that of VrD1, an insecticidal plant defensin that has been suggested to function by inhibiting insect α-amylase. Our previous study proposed that loop L3 of plant defensins is important for this inhibition. Structural analyses and surface charge comparisons of VrD1 and VrD2 revealed that the charged residues of L3 correlate with the observed difference in inhibitory activities of these proteins. A VrD2 chimera that was produced by transferring the proposed functional loop of VrD1 onto the structurally equivalent loop of VrD2 supported this hypothesis. The VrD2 chimera, which differs by only five residues compared with VrD2, showed obvious activity against Tenebrio molitor α-amylase. These results clarify the mode of α-amylase inhibition of plant defensins and also represent a possible approach for engineering novel α-amylase inhibitors. Plant defensins are important constituents of the innate immune system of plants, and thus the application of protein engineering to this protein family may provide an efficient method for protecting against crop losses.

2724-Pos 3D-phase Diagram For Apomyoglobin

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Board B27

To compare conformational states of apomyoglobin in the wide range of conditions, 3D-phase diagram was constructed in coordinates of pH-, temperature-, and urea concentration. Coexistence of the native, intermediate and unfolded states, their stability and conformational transitions between them were investigated by tryptophan fluorescence and circular dichroism in far UV region. The maximum stability of the native state was observed at pH 6.2 and around 310 K in the absence of urea. The native state showed a similar heat melting above pH 5.0, however its stability with respect to urea unfolding increased with pH. Below pH 5.0, both heat and cold denaturation of the native apomyoglobin can occur. At heat denaturation the native-intermediate state transition is observed, while during cold denaturation the native state of the protein undergoes a transition to the unfolded state via small population of the intermediate state. Decreasing pH leads to decrease of heat melting temperature, but for cold one - to enhancing it. Intermediate states, observed during denaturation by different agents, have some differences in their far UV CD spectra bearing gradual character. It is shown that all intermediate states, originated at any denaturation, lie in a single integrated range of the phase diagram.

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2725-Pos Purification and Characterization of Recombinant *durum* Metallothionein Domain Constructs

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Board B28

Metallothioneins (MTs) are small proteins with high cysteine content and high binding capacity for metals like Zn, Cu and Cd. MTs exist in a wide range of organisms and are classified in one super- family according to the distribution of cysteine motifs in their sequences. Type 1 plant MTs, similar to their mammalian counterparts, have the cysteine motifs clustered in the N-and C-termini constituting the β- and α-domains respectively. The two domains are connected by an unusually long (about 42 amino acids) hinge region whose structural and functional properties are unclear. Recent studies indicate that, despite the classification into a single family, all MTs do not have a single unifying function, and while some MTs help metal homeostasis others play a role in detoxification of heavy metals (e.g. Cd and As).

We identified an *mt* gene in Cd resistant durum wheat coding for a type 1 MT (dMT) and the recombinant protein (dMT) was over-expressed in *E. coli* as GST fusion (GSTdMT). For detailed structural and functional investigations GST-fusion constructs of βhinge-, αhinge- and the hinge-domains of dMT were over-expressed in *E. coli*. Proteins were purified and results of characterization by size exclusion chromatography, SDS- and native-PAGE, UV-VIS absorption spectroscopy, atomic absorption spectroscopy, dynamic light scattering, and small-angle solution X-ray scattering will be presented. Studies on the isolated domains indicate distinct metal-binding properties and structural features for these regions. GST fusion of the hinge appears to be stable reflecting an intrinsic structural organization for this domain. These findings will be discussed in terms of relevance for the native structure of dMT.

2726-Pos Quantifying the capillarity picture Of protein folding

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Board B29

Quantifying the capillarity picture of protein folding A full description of the structure of the transition state ensemble of protein folding includes the specificity of the structured residues composing the folding nucleus as well as the spatial density. To our knowledge, the density of the folding nucleus and interface of specific proteins

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.