In this presentation we illustrate in detail the folding mechanism of a prototypical beta-hairpin, namely the C-terminal fragment of protein GB1, by means of all-atom molecular dynamics (MD) simulations in explicit solvent, using metadynamics to accelerate the sampling of standard MD and reconstruct the free energy of the process.
Our results show clearly that the unfolded ensemble of this protein does not comply with the classical view of a collection of disordered coil conformations. Indeed we found out that a fully stretched configuration is unstable towards the formation of a turn in the central region of the peptide. This loop can assume two different conformations: a native-like one, which eventually leads to the 2:4 native structure, and a non-native turn which characterizes the ensemble of the unfolded states among which an ordered 3:5 misfolded structure is particularly stable.
Our results, corroborated by several experiments, support the growing idea that the unfolded ensemble of proteins and even of small polypeptides can be characterized by some form of non-native structure.

404-Pos Board B283
Beta-barrel Proteins that Reside in the E. coli Outer Membrane In Vivo Demonstrate Varied Folding Behavior In Vitro
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Little is known about the dynamic process of membrane protein folding, and few models exist to explore it. We have doubled the number of *Escherichia coli* outer membrane proteins (OMPs) for which folding into lipid bilayers has been systematically investigated. We cloned, expressed, and folded nine OMPs: outer membrane protein X (OmpX), OmpW, OmpA, the *crcA* gene product (PagP), OmpT, outer membrane phospholipase A (OmpLa), the *fadl* gene product (FadL), the *yaet* gene product (Omp85), and OmpF. These proteins share a transmembrane β-barrel motif, but vary in barrel size and primary sequence. We quantified their ability to fold into a matrix of bilayer environments by SDS-PAGE. Several trends emerged from these experiments: higher pH values, thinner bilayers, and increased bilayer curvature promote folding of all OMPs. Increasing the incubation temperature promoted folding of several OMPs but inhibited folding of others. We discovered that OMPs do not have the same ability to fold into any single bilayer environment. We show that while environmental factors are influential, OMP folding must also be modulated by intrinsic protein properties. To rationalize the differences in folding results between OMPs, we explored their kinetic profile and determined their resistance to thermal denaturation. Although these proteins share a common structural motif and have evolved to reside in the same bilayer environment *in vivo*, we found that these OMPs vary in their folding behavior.

405-Pos Board B284
Bistable Entropy Landscape of Sequences and Folds of Proteins
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The evolutionary capacity of a protein fold is defined as the number of sequences that match a particular structure. The logarithm of the evolutionary capacity is proportional to the sequence entropy. We computed the sequence entropies for a representative set of protein structures from the Protein Data Bank (1590 folds). For each of the structures we sample about ten million sequences acceptable to the target fold, and estimate the evolutionary capacity for a range of energies with telescoping ratios. The calculations are conducted with three empirical energy functions that were designed for different tasks in computational biology. The probability of observing a protein with a capacity normalized with respect to length is doubly peaked. The proteins at the peak of lower capacity are most likely to belong to the SCOP classes of all-alpha and the alpha/beta classes. The other peak of capacity includes structures from all-beta and the alpha+beta classes similarly to the overall distributions. Experimentally determined mutants of each protein in the set were collected using BLAST with E-value cutoff of 10^{-10}. The evolutionary capacity and experimentally determined number of mutants are positively correlated for proteins at the peaks. Among all the proteins within the two peaks, a HIV reverse transcriptase at the higher peak has the maximum number of mutants. HIV virus is known for its high evolution rate, which corresponds to high evolutionary capacity in our research. We also examine the network in which sequences flip between alternative folds. The network density and dynamics will be reported.

406-Pos Board B285
Amyloid β Proteins, Modified by a Lipid Oxidation Product, Are Nucleation Sites for Fibril Formation on Lipid Membranes
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Oxidatively damaged lipid membranes are known to promote the aggregation of amyloid β (Aβ) proteins and fibril formation. When lipid membranes contain ω-6 polyunsaturated fatty acyl chains, 4-hydroxy-2-nonenal (HNE) is produced during oxidative stress. We previously demonstrated that HNE modifies the three His residues in Aβ proteins by Michael addition, which increases the hydrophobicity and affinity of Aβ proteins for the membrane surface, and promotes the aggregation of unmodified Aβ proteins into fibrils. There are two different mechanisms by which the promotion of fibril formation may occur. HNE-modified Aβ proteins may act as catalytic templates that stabilize monomers in a fibrillar conformation, but do not ultimately become a part of the fibril (template mechanism). Alternatively, they may act as seeds that reduce a kinetic barrier to adopt a fibrillar conformation by becoming a part of the fibril (seed mechanism). In this report, the concentrations of HNE-modified Aβ proteins in fibrils, in solution, and in lipid vesicle membranes were monitored in order to distinguish between these two mechanisms.
Results indicate that HNE-modified Aβ proteins associate primarily with lipid membranes, suggesting that HNE promotes fibril formation by the template mechanism, and that relatively little HNE-modified Aβ is incorporated into fibrils. This result is pathophysiologically significant because such templates are not consumed by the process of fibril formation and may be long-lived. The formation of isolated templates may also account for the patchy distribution of amyloid fibril plaques in brain tissue afflicted with Alzheimer's disease.

407-Pos Board B286
Rescuing Functional Protein from Amyloid-Like Structure
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Formation of amyloid deposits is the molecular background of several diseases. Protein oligomers and aggregates formed in the process are connected to the observed pathogenesis. Amyloids represent the end stage of a multi-step aggregation cascade. Although some recovery of the enzyme activity from amyloid deposits has been reported in the case of the lysozyme (Booth II et al., Nature 385 (1997) 787-93), the recovery of the active structure from amyloid or amyloid-like deposits has not been studied. Here we show that phosphoglycerate kinase can be refolded into the biologically active structure from amyloid-like fibrils. First, amyloid-like fibrils were grown from phosphoglycerate kinase. The conversion of the protein structure was confirmed by electron microscopy, enzyme activity assays, as well as by Congo red and Thioflavin T binding measurements. Next, the protein was refolded into its native structure. Biological equivalence of the reference and recovered enzyme was confirmed by enzyme activity and differential scanning calorimetry measurements. We found that stabilizing the native fold is not enough for the efficient recovery of the native enzyme. The aggregates have to be destabilized before the formation of the native structure is initiated.

408-Pos Board B287
Dissecting the N-terminal Helical Domain of Apolipoprotein B
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High levels of low density lipoprotein (LDL, the bad cholesterol) are associated with cardiovascular disease, the leading cause of death in western countries. Very low density lipoprotein (VLDL, the precursor to LDL) secretion requires proper folding of the N-terminal domains of apolipoprotein B (ApoB). The N-terminal domain of ApoB is homologous to lipovitellin, whose structure is known. It is composed of a β-barrel, followed by a helical domain and two β-sheet domains. There is little direct structural information on any part of ApoB. Structural studies of the N-terminal domain of ApoB are complicated by aggregation in the absence of lipids. Our initial goal was to isolate an independent folding domain suitable for study by NMR. Two constructs encoding ApoB6.4-8 and ApoB6.4-9 were found to be cooperatively folded and show helical CD spectra. Single point mutations (e.g. L343V) within this region of ApoB have been shown to cause Familial Hypobetalipoproteinemia (FHBL). FHBL is characterized by low levels of plasma ApoB-containing lipoproteins. These mutations result in retention and degradation of ApoB in the ER. Despite the conservative mutation, our hypothesis is that the mutations cause a structural defect in this helical domain which disrupts efficient secretion of VLDL.

409-Pos Board B288
Peptide Structure Stabilization: A Study Of Aromatic-aromatic Interaction And pH Effect On A β-hairpin Stability
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Aromatic side chains in proteins are often involved in aromatic pairs, most of which form interacting networks of three or more aromatic side chains. (Petsko

et al., Science, 1985, 229, 23-28) Analysis of neighboring aromatic groups can lead to improved understanding of protein folding mechanisms and stability. We have used the β-hairpin forming peptide Trpzip2 (Cochran et al., PNAS, 2001, 98, 5578-5583) as a template to study the effect of aromatic-aromatic interaction on peptide stability. Optical spectra (ECD, FTIR) and NMR of the original TZ2 peptide and its Tyr and Val-substituted mutants were measured to characterize their conformation and thermal stability. Compared to the strongly interacting Trp-Trp edge-to-face geometry, Tyr-Tyr shows much weaker aromatic-aromatic interaction. These aromatic-aromatic interactions were also compared to simple hydrophobic interaction by substituting Val for two interacting Trp residues in this peptide. Aromatic interaction showed a stronger effect than hydrophobic interaction for stabilization of this peptide. The thermal unfolding process of three Val mutants was studied under both neutral and acidic conditions by IR and ECD. We see more random coil content at acidic pH than those at neutral pH from analysis of the IR amide I' band, which reflects peptide secondary structure, and the transition temperatures obtained using both IR and CD are lower at acidic pH than at neutral pH, which suggests that this peptide is less stable at acidic pH.

410-Pos Board B289

Molecular Partition Functions For Amino Acids And Beyond

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The Distance Constraint Model (DCM) provides a novel paradigm for describing protein thermodynamics [1] based on a Free-Energy Decomposition (FED), which assigns free energy contributions to specific molecular interactions. Unique to the DCM, non-additivity in entropy components is explicitly calculated using constraint theory as the total free energy of a system is reconstituted from its molecular parts. In prior work, a minimal DCM (mDCM) involving three empirical fitting parameters has been used to successfully predict protein flexibility and stability in proteins [1-4]. Although the mDCM captures essential physics, it is based on an oversimplified FED involving effective energy and entropy parameters universally applied to all residues. Employing, in part, principal component analysis, we constructed a residue-specific energy and entropy spectrum that depends on secondary backbone conformation states. The resulting molecular partition function predicts surprisingly rich temperature-dependent energy and entropy contributions. This method is general, and works for any type of chemical group and other types of molecular interactions, such as hydrogen bonds. Our new FED is self-consistent, complete and computationally simple. It defines the first all-atom DCM parameterization that is analogous to a molecular mechanics force field. This work is supported by NIH R01 GM073082, and we gratefully acknowledge partial support for H. Wang from the Charlotte Research Institute through a Duke Postdoctoral Fellowship.

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411-Pos Board B290

Lin-12/Notch Repeat B: The Effects Of Disulfide Bonding And Hydrophobic Residues On Its Autonomous Folding

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Notch proteins are transmembrane proteins involved in controlling cell differentiation, cell growth, and cell death. An extracellular negative regulatory region (NRR) contains three contiguous LIN-12/Notch Repeats (LNRs) - LNRA, LNRB, and LNRC. Previous work from our lab showed that the total number of residues N-terminal to the first cysteine residue from human Notch1 LNRB (hN1LNRB) was critical for correct disulfide bond formation. Because of its more central location in relation to the rest of the protein, hN1LNRB participates in extensive interactions via its hydrophobic residues in the context of the full Notch protein. When expressed and folded in isolation, some of these residues are expected to be exposed to solvent and possibly contribute to the requirement of the additional N-terminal residues for autonomous *in vitro* folding with formation of the correct disulfide bonds. To test this hypothesis we mutated W52 to A52 and compared its folding pattern to that of the wild-type hN1LNRB. The effect of the total number of disulfide bonds on the autonomous folding of hN1LNRB was also investigated. In this study, the first of three pairs of disulfide bonds in hN1LNRB was eliminated by mutating C45 and C69 to A. Two mutant forms with two pairs of disulfide bonds were folded *in vitro* under the same conditions as the wild-type and the folding patterns were compared. The data and comparative analysis we present in this work demonstrate the importance of specific hydrophobic interactions and the total number of disulfide bonds as key determinants for the correct folding of an LNR module in addition to the total number of amino acids and Ca^{2+} ion coordination within the module.

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Lattice Model Studies of Designability and Alpha-helix to Beta-sheet Transitions of Short Peptide Chains

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The folding kinetics of lattice peptide chains are studied using a directed Markov-chain model. The small-world characteristics of the conformation space has been noted in recent publications, encouraging further study in the move set topology. We extend this idea to study the entropic effects that result from different move sets and energy functions. Markov-chain models allow for an efficient exploration of the steady-state probabilities, parametrized by temperature. This is an improvement over Monte-Carlo simulations, where an efficient exploration of the entire state-space requires some form of adaptive temperatures scheme. Interesting results are shown at intermediate temperatures, where higher energy states are favored over their lower counter-parts due to their connectivity in the move set. We also provide evidence for rudimentary alpha-helix to beta-sheet transitions for specific peptide configurations and the designability of such structures. Given a transition we can elucidate the kinetics of the pathways between them using first passage times.

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Atomistic Modeling of Macromolecular Crowding Predicts Modest Increases in Protein Folding and Binding Stability

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Theoretical models predict that macromolecular crowding can increase protein folding stability, but depending on details of the models (e.g., how the unfolded state is represented), the level of stabilization predicted can be very different [1, 2]. In this study we represented the folded and unfolded states realistically, with conformations sampled from explicit-solvent molecular dynamics simulations at room temperature and high temperature, respectively. We then designed an efficient algorithm to calculate the allowed fraction, f, when the protein molecule is placed inside a box of crowders. The allowed fraction corresponds to an increase of $\mu = -k_B T \ln f$ in chemical potential. The difference in μ between the folded and unfolded states predicts the effect of crowding on the folding free energy. Even when the crowders occupied 35% of volume, the stabilization reached only 1.5 kcal/mol for cytochrome b_{562}, a 106-residue four-helix bundle protein. This prediction of modest stabilization is consistent with experimental data [3, 4]. Interestingly, we found that a mixture of different sized crowders exerts a greater effect than the sum of the individual species of crowders, in agreement with an earlier model [5]. The stabilization of crowding on the binding stability of barnase and barstar, based on atomistic modeling of the proteins, is similarly modest. The atomistically detailed calculation results can be fitted to a fundamental measure theory, affording the theory predictive power.

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414-Pos Board B293

Forced Unfolding of CTPR proteins

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We perform Brownian dynamics simulations of coarse-grained models for CTPR proteins to study the dynamics of unfolding while the proteins are subjected to stretching forces. We use a variant of an HP model with cooperative attractive interactions that give rise to a cascade of bond breaking events during unfolding. In the attached figure, we show a force versus extension curve for CTPR2 in which a single cooperative unfolding event occurs near an extension of 5 nm and the 'worm-like-chain' behavior for pulling out thermal fluctuations of the unfolded chain occurs near 30 nm. The inset shows the structure of CTPR2 during the unfolding process; note that initially the superhelices must twist so that CTPR2 can stretch. Our