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**

**Hui Wang**, Michael Farchild, Dennis R. Livesay, Donald J. Jacobs.
University of North Carolina at Charlotte, Charlotte, NC, USA.

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**

**Jessica Lin**, Didem Vardar-Ulu.
Wellesley College, Wellesley, MA, USA.

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**

**Travis A. Hoppe**, Jian-Min Yuan.
Drexel University, Philadelphia, PA, USA.

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.

**413-Pos Board B292**

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

**Sanbo Qin**, Huan-Xiang Zhou.
Florida State University, Tallahassee, FL, USA.

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 $\mu$ 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**

Gregg Lois, Jerzy Blawzdziewicz, **Corey OHern**.
Yale University, New Haven, CT, USA.

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