DNA recombination and DNA repair. Buffer and salt conditions influence the aggregation and activity of RecA. In low salt conditions, RecA is a DNA-dependent ATPase. However, prior research demonstrated that high salt concentrations allow RecA to hydrolyze ATP in the absence of DNA and at levels comparable to those obtained in the presence of DNA [Pugh, B. F. and Cox, M. M. (1988) *Journal of Biological Chemistry 263*, 76-83]. We have used circular dichroism (CD) and fluorescence spectroscopies to better understand the salt-induced effects on RecA structure and function. CD and fluorescence studies were performed in order to monitor the thermally induced unfolding of RecA in the presence of a variety of salts. We found that different salts had unique effects on RecA unfolding transitions and stability. Unfolding studies performed under salt conditions known to activate RecA's ATPase activity showed unique, thermally stable RecA structures. A comparison of the influences of different ions on RecA unfolding will be presented. These studies may help to elucidate how different ions influence RecA activity, structure, aggregation, and stability.

**421-Pos Board B300**

**Folding/Unfolding of Glycolipid Transfer Protein: Molten Globule-Like Intermediates?**

Ravi Kanth Kamlekar[1], Roopa Kenoth[1], Helen M. Pike[1], Franklyn G. Prendergast[2], Sergei Yu. Venyaminov[2], **Rhoderick E. Brown[1]**.
[1]University of Minnesota, Austin, MN, USA, [2]Mayo Clinic College of Medicine, Rochester, MN, USA.

Glycolipid transfer proteins (GLTPs) are small, soluble, single-chain proteins (~24 kDa) that selectively accelerate the intermembrane transfer of glycolipids in vitro. The GLTP-fold is unique among lipid-binding proteins. However, little is known about GLTP stability and folding/-unfolding. During isolation of heterologously expressed GLTP, FPLC size exclusion chromatography showed peaks corresponding to monomer, multimer, and a third peak of intermediate elution volume. Unexpectedly, native gel electrophoresis showed that the intermediate protein peak migrated as monomer rather than dimer, raising the possibility of a molten globule-like state. Intrinsic GLTP tryptophan fluorescence showed a blue-shifted (~2nm) emission wavelength maximum (λmax), indicating an altered tryptophan environment compared to monomer. ANS binding resulted in a large blue shift (~20nm) in λmax and dramatically enhanced emission intensity (~120%). Far-UV-CD showed retention of ordered secondary structure (>95%), but substantially reduced cooperativity during thermally-induced melting. Near-UV-CD analysis of induced optical activity of GLTP Trp/Tyr residues was insufficient to establish tertiary folding changes. To further evaluate GLTP unfolding intermediates, the effect of urea was studied. Trp emission changes suggested a two-step unfolding pathway involving intermediate formation at 4M urea and characterized by blue-shifted Trp emission. Additional urea induced further unfolding marked by red-shifted Trp emission. Far-UV-CD analyses of the 4M urea-induced intermediate indicated reduced ordered secondary structure and cooperative melting at lower temperature compared to native GLTP, but the near-UV-CD signal did not provide definitive insights into tertiary folding status. ANS binding showed 1nm blue shift and 60% increase in fluorescence intensity compared to untreated GLTP. Conditions are identified under which GLTP may exist in molten globule-like and other partially unfolded states. Studies on the significance of these intermediates with respect to function are underway. [Support: NIH/NIGMS GM45928 & GM34847, NIH/NCI CA121493, The Hormel & Mayo Foundations]

**422-Pos Board B301**

**Elucidating The Specificity Determinants Responsible For ClpX-Adaptor Interaction**

**Tahmeena Chowdhury**, Peter Chien, Robert T. Sauer, Tania A. Baker.
MIT, Cambridge, MA, USA.

The ClpXP proteolytic machinery in bacteria consists of the AAA+ ATPase protein ClpX and the serine peptidase ClpP. ClpX recognizes, unfolds, and translocates substrates into the protease chamber where hydrolysis of the polypeptide occurs. To control substrate-specificity, the cell uses adaptor proteins, such as SspB, to modulate substrate selection. The N-terminal domain of ClpX is the adaptor-docking site and also directly recognizes some substrates. However, very little is known about the sequence specificity of this important ClpX N-domain protein-binding site.

Recently, a homolog of *Escherichia coli* SspB was identified in *Caulobacter crescentus*. Despite structural and functional homology, there is limited sequence similarity between the two SspB adaptors. To learn more about ClpX-adaptor interaction specificity, we carried out a systemic mutational analysis of *C.crescentus* SspB to identify the residues responsible for its interaction with ClpX. Functional assays monitoring adaptor-stimulated degradation of a model fluorescent substrate (GFP with a degradation signal) were used to assess SspB variants. In addition, monitoring direct-binding between the ClpX N-domain and variant fluoresceinated-SspB peptides by fluorescence anisotropy provided a quantitative assay for the interaction. Results reveal that a minimal C-terminal region (residues: $^{152}$KIVSLDQFRKK$^{162}$) of SspB is responsible for docking with ClpX, and at least 5 specific residues ($I^{153}$, $V^{154}$, $L^{156}$, $R^{160}$, $K^{162}$) within this region play key roles in the interaction. Additional residues ($F^{159}$, $K^{161}$) may also be contributing albeit more subtly. This binding region is much longer and shares little sequence homology with the *E.coli* SspB ClpX-binding region ($^{161}$LRVVK$^{165}$). However, *C.crescentus* SspB binds *E. coli* ClpX and can clearly deliver substrates to it. This cross-species interaction demonstrates the sequence-versatility of the ClpX N-domain interactions in a functionally relevant manner and highlights the challenge in identifying ''consensus sequences'' for AAA+ protease interaction signals.

**423-Pos Board B302**

**The Effect of Salts and Co-Solvents on the Cytochrome c Folding Pathway within a Sol-gel Glass**

**Eric S. Peterson**, Michael A. Erbele, Brock B. Jas.
Concordia College, Moorhead, MN, USA.

The folding reaction of ferric cytochrome c (cyt c) was examined in the presence of several salts and co-solvents in both solution and within porous silica sol-gel glasses to characterize the conformational changes that cyt c can undergo if constrained to remain in a compact state. The sol-gel pores restrict protein motions to a volume slightly larger than the native state. Unfolding was induced by changing pH and addition of guanidine. The populations of four species characterized by their heme ligation were determined using UV/VIS absorption spectroscopy: the native HM state (His18/Met80), the partially folded HW (His18/water) and HH (His18/His33) intermediates, and the 5C (water) unfolded state. In solution, the native HM state unfolds primarily into the HH species, while in the gel the HW species is formed preferentially. This indicates that the steric constraints within the gel pores hinder some backbone motions. We and others have previously shown that the water solvating the protein within the gel pores is more ordered than bulk water, leading to a decreased hydrophobic effect. Here we present the effects of several salts and co-solvents on the folding pathway of cyt c. We find that addition of some, but not all, salts and co-solvents can alter both the folding kinetics and which conformations are accessed by the protein. The results are discussed in the context of molten globule folding models and the Hofmeister ranking of chaotropic agents.

**424-Pos Board B303**

**Prosegment Catalyzes Pepsin Folding to a Kinetically Trapped Native State**

**Derek R. Dee**, Rickey Y. Yada.
University of Guelph, Guelph, ON, Canada.

Pepsin, an archetypical aspartic peptidase, initially contains an N-terminal, 44 residue prosegment (PS) that, upon folding, is removed to yield native pepsin (Np). Np is irreversibly denatured above pH 6, the basis for which was not understood. Our previous studies comparing Np and a denatured and subsequently refolded state (Rp) indicated that Rp was thermodynamically more stable than Np, having a higher $T_m$ and being reversibly unfolded (Dee, D., et al., 2006, *Biochemistry 45*, 13982). Thus, it was suspected that Np is blocked from refolding by a large energy barrier, which is reduced by the action of the PS.

It was found that the exogenous addition of a synthetic 44 residue PS peptide catalyzes folding of Rp to the Np state. The PS displays foldase activity that follows Michaelis-Menten kinetics ($K_m = 3.1 \pm 2.0$ μM, $k_{cat} = 0.011 \pm 0.002$ sec$^{-1}$), binds with higher affinity to the product (Np, $K_i = 41 \pm 6$ nM) of the folding reaction than to the substrate (Rp, $K_d = 2.0 \pm 0.2$ μM), and increases the rate of folding by a factor of $10^5$ compared to uncatalyzed folding. By comparing the rates of Np unfolding ($t_{1/2} = 9 \pm 1$ days) and uncatalyzed refolding of Rp to Np ($t_{1/2} = 64 \pm 6$ days), it is shown that Np is thermodynamically metastable relative to Rp, in agreement with previous calorimetry data (Dee, D. et al.).

The data support a model whereby the PS catalyzes folding before being removed, resulting in a kinetically trapped Np state that is stabilized by a large barrier to unfolding rather than by a lower free energy. Considering the high structural and functional similarities among the aspartic peptidases, PS-catalyzed folding and kinetic stabilization mechanisms may be quite common.

**425-Pos Board B304**

**Osmolytes Control Peptide Folding and Aggregation**

**Regina Politi**, Shahar Sukenik, Daniel Harries.
Hebrew University, Jerusalem, Israel.

Nature has developed many strategies to ensure that protein folding occurs in vivo with efficiency and fidelity. Among the most widely employed

strategies is the use of small solute molecules called osmolytes that most often confer stability to folded proteins by preferential exclusion from macromolecular surfaces. Recent evidences indicate that modest changes in environmental conditions set by osmolytes and other cosolutes can have profound effects on protein and peptide conformation and aggregation. Such aggregation processes constitute a hallmark of neurodegenerative pathologies, including Alzheimer's, Huntington's, and Parkinson's diseases. This study examines the effect of natural osmolyte on a model peptide that can fold from a ''random coil'' to β-hairpin, or aggregate into fibrils. We use Fluorescence and Circular Dichroism measurements as well as perform Molecular Dynamic simulations to determine the mechanism by which osmolytes control the structure and thermodynamic stability of the peptide, and to follow changes in peptide aggregation kinetics. We find that excluded osmolytes such as sugars and polyols cause peptides to favor a more compact (folded) structure relative to more extended (unfolded) conformations, and that this stabilization sensitively depends on the osmolyte used. Water structuring in close proximity to peptide surfaces crucially affects this process. Understanding the role of osmolytes in regulation will not only allow to predict the action of osmolytes on macromolecular interactions in stressed and crowded environments typical of cellular conditions, but will also provide insights on how osmolytes may be involved in pathologies or in their prevention.

**426-Pos Board B305**

**The Effects of Reduction Potential and Number of Disulfide Bonds on the Correct Folding of Lin-12/Notch Repeats (LNRs) Using Human Notch 1 LNRA as a Model System**

**Lauren Choi**, Didem Vardar Ulu.
Wellesley College, Wellesley, MA, USA.

Notch receptors are multi-domain trans-membrane proteins that are important for cell-cell communication and development. Deregulated Notch signaling has been linked to many human diseases such as sclerosis, artereopathy and leukemia. The extra-cellular domain of the Notch Receptor contains the Ligand Binding Domain and the Negative Regulatory Region (NRR), which includes three Lin-12/Notch Repeats (LNR), small disulfide-rich sequences of 35 residues. It has been previously shown that the first LNR from human Notch1, hN1LNRA, requires Ca2+ and a certain reduction potential that ensures the correct formation of three specific disulfide bonds believed to be critical for LNR structure and function. However, the first LNR in human Notch 4 and some of the LNRs found in PAPP (pregnancy-associated plasma protein-A), only possess four cysteines thereby can only form two disulfide bonds.

In this work we present our findings on the effect of various reduction potentials as well as the elimination of the first disulfide bond in the in vitro folding of hN1 LNRA through a comparative analysis. The kinetics of the folding process for both the wild-type and the four-cysteine mutant form of hN1LNRA is studied by trapping various folding intermediates in a time-course manner, which is possible due to the slow rate of disulfide bond formation. Our results indicate that even though the wild-type hN1LNRA is very tolerant to variations in the specific redox potential in obtaining its ultimate correct folding, the its folding kinetics is significantly impacted. This is in contrast to the mutant form, which does not fold into a single species under identical refolding conditions.

**427-Pos Board B306**

**Helical Flexibility Governed by the Placement of Alanine Residues in a Series of Aib-Rich Model Peptides**

Matthew Cocchiola, Valentine Sackmann, **Adrienne P. Loh**.
University of Wisconsin - La Crosse, La Crosse, WI, USA.

It has been established that peptides composed primarily of the amino acid Aib (α-aminoisobutyric acid) fold into $3_{10}$-helices. Aib is structurally similar to alanine but with an additional methyl group at the α-carbon. The α,α-dialkylation creates significant steric hindrance, which is responsible for the helical preference of Aib. We are studying the effects of steric hindrance on the flexibility of Aib-rich helices. $^1$H NMR spectra of peptides dissolved in a deuterated solvent ($CD_3OD$) are obtained as a function of time and temperature. Rate constants for amide proton/solvent deuteron exchange are found using a pseudo first order model. Activation energies are obtained using the Arrhenius equation. Larger activation energies suggest stronger intramolecular H-bonds and a more rigid helix. Preliminary results on an Aib octamer (known to form a regular $3_{10}$-helix) show similar activation energies for all but the first two solvent-exposed amides, suggesting that the helix is fairly rigid in solution. When alanines are substituted at the fourth and fifth positions (4,5-AA), the exchange rates at Ala4 and Aib6 *decrease* relative to the other hydrogen-bonded amides, while that at Ala5 *increases*. Thus, the reduction in steric hinderance at Ala4 and Ala5 creates a local compression in the helix, opening one face of the helix and pinching the other. FTIR spectra of 4,5-AA shows a broader distribution of helical conformations than observed for the Aib octamer. Placement of the two alanines instead at positions three and six (3,6-AA) results in a narrow conformational distribution by FTIR similar to that of the Aib octamer. NMR data also suggest a more regular $3_{10}$-helical conformation for 3,6-AA than for 4,5-AA. Thus the positioning of the less hindered Ala residues is a significant driving force in determining the helix flexibility.

## Molecular Recognition in Silico

**428-Pos Board B307**

**Free Energy Calculations of Sparsomycin Analogs Binding to the Ribosome with Molecular Dynamics Simulations**

**Xiaoxia Ge**[1,2], Benoit Roux[2].
[1]Weill Medical College of Cornell University, New York, NY, USA, [2]The University of Chicago, Chicago, IL, USA.

The accurate calculation of absolute binding free energy is one of the holy grails of computer-aided drug design. The emerging successes reported in computing the binding free energy of small ligands to proteins using molecular dynamics (MD) simulations indicated that such physics-based approaches hold the promise of expediting the rational drug discovery process. Among numerous receptor-ligand systems, ribosome-antibiotic binding provides an important paradigm for studying the molecular recognition of RNAs by small molecules. The interactions of the 50S bacteria ribosomal subunit with antibiotic sparsomycin and its derivatives have been studied through the calculation of the binding free energy and the characterization of conformational dynamics. The standard binding free energies of the complexes were calculated using free energy perturbation (FEP) method. Restraining potentials affecting the orientational, translational and conformational freedom of the ligand and receptor were applied and then removed during the simulations to enhance the sampling and the convergence. The loss of ligand conformational entropy upon binding was estimated with Umbrella Sampling method by calculating the Potential of Mean Force as a function of the RMSD relative to the reference conformation of the ligand. Due to the large size of the ribosome, the Generalized Solvent Boundary Potential method was used to reduce the computational cost of MD/FEP calculations. For a deeply buried binding pocket in the ribosome, the fluctuation of solvent occupancy during the alchemical free energy calculation was also characterized by combining the MD with Grand Canonical Monte Carlo simulation. This computational study further revealed the mechanism of ribosome-antibiotics interactions and shed light on the design of ribosomal drugs. With the above stated developments, the evaluation of the binding free energies has become computationally more appealing for large systems.

**429-Pos Board B308**

**Computational Discovery Of The Electronegative Channel In RNA Loop-loop Interactions**

Andrey Semichaevsky, Abhishek Singh, **Yaroslava G. Yingling**.
North Carolina State University, Raleigh, NC, USA.

The most common motifs found in nature and used in bionanotechnology are hairpin loops which consist of a helical part and a loop with unpaired residues. The unpaired residues in these elements can lead to further super-assembly of RNA structures via formation of the loop-loop interactions. These loop-loop interactions regulate biological functions in both prokaryotic and eukaryotic organisms such as gene expression in different viruses and are also actively used in bionanotechnology for self-assembly of RNA building blocks into novel nanostructures. It has been observed that the super-assembly of RNA directly depends on the presence and specific concentration of ions. In order to understand the role of ions in loop-loop formation and stability, we conducted a series of explicit solvent atomistic molecular dynamics simulations of distinct kissing loops elements taken from various organisms. In our simulations we varied the concentration of different ions (such as Na+, K+, Mg2+, and Cl−) from zero to 1M solution and examined known destabilizing mutations. We discovered that in most organisms the loop-loop assembly process depends on the presence of electronegative and hydration channel. The properties of this channel are independent of the concentration and the type of ions. The size of this channel and RNA sequence determines the stability. We also examined the formation of the channel during self-assembly and discovered the critical threshold for the channel formation.

**430-Pos Board B309**

**Docking of a Linker Histone to The Nucleosome With Flexible Linker DNAs**

**Georgi V. Pachov**, Rebecca C. Wade.
EML Research gGmbH, Heidelberg, Germany.

In the cell nucleus, DNA wraps around histone proteins, forming nucleosome particles, and packs into a highly negatively charged structure, the chromatin.