SERT a 12-helix presynaptic plasma membrane protein terminates synaptic transmission by Na+ symport of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) from the extracellular mileu of synapse into the cell.
Crystal structures of Leucine transporter (LeuT), the bacterial homologue of SERT, represent a substrate-bound occluded conformation in which the the intracellular part of the transporter is closed and packed.
We have constructed an experimentally-validated homology model of SERT based on the LeuT structure and cognate information about other transporters in this family and performed SMD simulations by pulling serotonin from the primary binding site towards the cytoplasm, to explore the alternative inward-facing conformation and reveal the cytoplasmic permeation pathway. The initial state of the SERT model has 5-HT both in the primary binding site and the newly discovered secondary site (L. Shi et al, Mol Cell, 2008). Local structural rearrangements associated with substrate movement reveal changes in water solvent accessibility that explain the energetic drive of the transport mechanism and provide data for validation against experimentally determined accessibilities measured with the substituted cysteine mutagenesis accessibility method (SCAM). Interactions of the moving substrate in SMD identify the residues of SERT which line the transport pathway and their role in stabilizing the inward-open or inward-closed state of SERT. These states involve large-scale helix movements triggered by changes in the binding sites of the substrate and Na ions, and enabled by solvent stabilized changes in the states of an ionic interaction network at the cytoplasmic end of SERT. While the detailed representation of the process provided by SMD simulations at atomic resolution offers specific new hypotheses for experimental probing of the proposed mechanism, it also reveals new insights and mechanistic aspects that are not achievable in other ways.

354-Pos Board B233
Solvent Effects On Protein Mechanical Stability: A Steered Molecular Dynamics Study
Georgi Z. Genchev, Hui Lu.
University of Illinois at Chicago, Chicago, IL, USA.
The solvent is an integral component in all cellular processes and solvent composition is actively regulated in the living cell. Changes in solvent environment constitute a chemical signal that is transduced into a mechanical response: a chemically induced change of mechanical properties of a protein system. In this study, steered molecular dynamics simulations are used to stretch mechanical proteins set in non-aqueous solvent environment to reveal details and mechanism of protein - solvent interactions. We explore the atomic level mechanism and measure the effect of solvent substitution on the mechanical properties of proteins. We investigate the distance to the transition state during force induced unfolding as a function of solvent molecule size and the atomistic detail and timescale of participation of solvent molecules in the transition state structure. Resulting constant velocity and constant force extension profiles showed increased stability and resistance to unfolding force by proteins solvated in deuterium oxide vs. proteins solvated in water. Solvent molecules were found present the transition state structure and involved in forming a stabilizing bridge between the force-bearing sheer topology elements. Features of the simulations were also matched with previously reported experimental results.

355-Pos Board B234
Fluorescence Resonance Energy Transfer Reveals Key Binding Domains of Neurotrophin Receptor-Interacting Melanoma-Associated Antigen Homolog in Bone Morphogenetic Protein-Mediated Apoptosis
Jennifer A. Rochira[1,2], Rebecca A. Cowling[2,3], Joshua S. Himmelfarb[2,4], Samuel T. Hess[5], Joseph M. Verdi[2].
[1]Functional Genomics Interdisciplinary Ph.D. Program, Department of Physics & Astronomy, University of Maine, Orono, ME, USA, [2]Maine Medical Center Research Institute, Scarborough, ME, USA, [3]Current Location: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [4]Current Location: Department of Psychology, Brown University, Providence, RI, USA, [5]Department of Physics & Astronomy, Institute for Molecular Biophysics, University of Maine, Orono, ME, USA.
Apoptosis, one form of programmed cell death, is used by tissues to develop normally and maintain homeostasis. Lack of apoptosis underlies many diseases such as cancer, while an excess can cause neurodegenerative disorders. Understanding the molecular events that initiate either condition is necessary for the development of treatments. Bone morphogenetic proteins (BMP) play profound roles in development, such as regulation of neural progenitor apoptosis and glial differentiation. Binding of ligand to the BMP receptors triggers the canonical and non-canonical pathways involving Smad and TAK1 activation respectively. In the non-canonical pathway, p38 mitogen activated kinase ($p38^{MAPK}$) is upregulated in P19 cells, a model line of neural progenitors, triggering a signal cascade leading to apoptosis. X-linked inhibitor of apoptosis protein (XIAP) functions as a positive mediator linking TAK1 to the BMP receptors through TAB1. Neurotrophin receptor-interacting MAGE (NRAGE) homolog binds with XIAP in the XIAP-TAB1-TAK1 complex and is necessary in the non-canonical pathway for apoptosis. We have measured fluorescence resonance energy transfer (FRET) between enhanced green fluorescent protein attached to NRAGE deletion mutants and DsRed-monomer attached to XIAP. Results show that the interaction is direct and is facilitated by a unique tryptophan-glutamine-X-proline-X-X (WQXPXX) repeat in NRAGE. We have continued using FRET with enhanced cyan and yellow fluorescent proteins (FPs) in designs with the FP fused to the amino or carboxy termini of NRAGE deletion mutants and XIAP to compare the FRET efficiencies and verify that the FP placement does not affect the NRAGE-XIAP interaction. Additionally, we compare the FRET of overexpressed NRAGE-XIAP with that of the endogenous interaction using antibodies conjugated to Alexa Fluors.

356-Pos Board B235
Conformational Dynamics of Antithrombin III With its Allosteric Activator Heparin
Lauren Boucher[1], Richard de la Cruz[2], Susan Bock[2], Patrick L. Wintrode[1].
[1]Case Western Reserve University, Cleveland, OH, USA, [2]University of Utah, Salt Lake City, UT, USA.
Antithrombin III (ATIII) is a serpin that is involved in the regulation of blood coagulation through the inhibition of blood clotting enzymes. Heparin is an allosteric activator of ATIII that binds to helix-D and causes a conformational change in the reactive center loop (RCL), expelling it from its position partially inserted into beta-sheet A. RCL expulsion in turn increases ATIII activity toward fXa several hundred-fold. Hydrogen/deuterium exchange and mass spectrometry were used to probe the dynamics of ATIII in the presence and absence of a synthetic heparin pentasaccharide (Fondaparinux). Results of our initial hydrogen/deuterium exchange mass spectrometry experiments provide direct, solution phase evidence that heparin cofactor binding alters conformational dynamics in four specific regions of the antithrombin molecule. (1) Helix D – Heparin binding reduced H/D, consistent with hD extension upon cofactor binding. (2) Breach region – Beta strands 3A and 5A, flanking the site for RCL insertion into sheet A, showed reduced H/D exchange, consistent with increased rigidity of the breach region and stabilization of a loop-expelled form. (3) Proximal RCL and hinge region – H/D exchange for residues 376-387, which includes the N-terminal hinge region of the RCL, increased in the presence of heparin, indicating greater solvent exposure and expulsion from beta sheet A. (4) Distal RCL – Deuterium exchange for 388-402, which includes s1C and the distal side of the RCL, decreased significantly in the presence of heparin, suggesting that s1C extension-mediated stabilization on the C-terminal side of RCL contributes to exposure of the proximal its end upon cofactor binding. Thus, dynamic H/D exchange studies of free and heparin-bound antithrombin molecules in solution will be useful for validating and refining models of ATIII heparin activation inferred from crystal structures.

357-Pos Board B236
Structural Evaluation of the Effects of Disulfide Bond Eliminations on Scorpion Toxin κ-Hefutoxin1 from *Heterometrus Fulvipes*
Mehriar Amininasab, Maryam Ghobeh.
University of Tehran, Tehran, Iran, Islamic Republic of.
κ-Hefutoxin1, a novel weak potassium-ion-channel toxin present in the venom of the scorpion *Heterometrus fulvipes*, is a 22-residue peptide which has a unique spatial fold consisting of two parallel helices linked by two disulfide bridges without any β-sheets. In order to evaluate the structural contribution of the disulfide bonds, wild and three mutant forms of κ-Hefutoxin1 were cloned, expressed, purified and structurally analyzed. To do so, synthetic genes encoding wild and three mutant forms of k-Hefutoxin1 were designed using appropriate codons and synthesized using overlapping primers. In the mutant forms, alternative pairs of cystein residues, which participate in the formation of disulfide bonds, were replaced by serine (Mut1: C4S, C22S; Mut2: C8S, C18S; Mut3: C4S, C22S, C8S and C18S). To facilitate cloning into expression vector, EcoRI and BamHI restriction sites were inserted to the flanking ends of the genes. Moreover, Tev cleavage site was added to the N-terminal part of the genes. The amplified k-Hefutoxin1 genes of wild and mutant forms were cloned into pET32a vector, followed by transformation into *E.coli* host strain DH5α. The positive colonies with recombinant plasmid were first screened by PCR analysis and finally confirmed by sequencing. Then, the correct recombinant plasmid was transformed into *E.coli* host strain BL21 in which protein expression was induced by IPTG. After assuring protein expression by polyacrylamide gel electrophoresis, the cells containing wild and mutant forms of