Conotoxins are short peptides that are isolated from predatory marine cone snail venom. They are unique from other peptides in that they have the ability to differentiate between various types of ion channels and this makes them ideal diagnostic tools in the characterization of neuronal pathways and in drug development. The subject of our study, α- conotoxins, are 13 to 15-amino acid peptides containing 4 cysteine residues. Thus, three possible regioisomers can form via disulfide bond formation upon synthesis of these compounds. We report a method to model the conformational folding of α-conotoxins and the factors that affect the synthesis of specific regioisomers. We use a combination of molecular dynamics methods to determine the geometric factors (S-S distance, for instance) and *ab initio* methods to determine the conformational energy and molecular orbital information. Experimental work by the Hargittai group has determined that the nature of the amino acid at the 9 position strongly directs the formation of a specific regioisomer. Our model agrees with experimental observations that identify the role of the amino acid proline in directing the proper folding of α-conotoxins. We have observed that the presence of proline (1) directs the folding towards the proper (native) sulfur-sulfur pairs, and (2) allows less flexibility in the folding. The molecular orbital methodology has also provided insight into the directionality and energetics of the disulfide bond formation.

3049-Pos Board B96

All Atom Protein Folding Simulations With Distributed Computational Resources

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On the basis of Anfinsen's thermodynamic hypothesis we have developed a free-energy forcefield PFF02 for all-atom de-novo protein simulation, which we recently implemented in a massively parallel computational environment. POEM (Protein Optimization using Energy Methods), which implements PFF02 along with several simulation protocols, identifies the native conformation of the protein as the global minimum of the protein free-energy forcefield. PFF02 was validated by stabilizing the native conformation of all 32 monomeric proteins in the Rosetta decoy sets (without cofactors) against the corresponding decoys. In addition we could fold a set of 24 proteins with helical, sheet and mixed secondary structure from completely unfolded conformations to near-native conformations to an average 2.87 Å resolution using traditional computational networks.

The simulations we report here were performed on the POEM@HOME (http://boinc.fzk.de) volunteer computing architecture using a multiple population evolutionary strategy, which explores the free-energy surface in many parallel Monte-Carlo random walks. Various distinct temperature populations are evolved to the global free-energy minimum by balancing energy improvement and population diversity. This massively parallel algorithm enables the exploration of the free-energy landscape of relatively large proteins.

Here we report simulations of three proteins that fold from completely extended conformations to native structural ensembles within experimental resolution. The largest of the three is the 43-amino acid fructose-repressor DNA-binding domain 1UXD. Analyzing the trajectory we find concomitant hydrophobic collapse and secondary structure formation. We observe intermediate collapsed states with native secondary structure content and few native tertiary contacts, which subsequently arrange into the native structure.

Membrane Protein Function II

3050-Pos Board B97

Colicin Ia Uses Cir Protein Both As Its Primary Receptor and As Its Translocon

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Colicin Ia is a bactericidal protein that kills *E. coli* by making a voltage-dependent ion channel in their inner membrane and de-energizing them. To do this, the colicin or its pore-forming domain must cross the outer bacterial membrane. Like all colicins, Ia first binds to an outer membrane receptor. The crystal structure of colicin Ia bound to its outer membrane receptor, the 22-strand plugged β-barrel protein, Cir, suggests the plug doesn't move upon Ia binding. Therefore, another pathway is needed for the colicin to cross the outer membrane. Group A colicins, like E3, use the periplasmic and inner membrane proteins TolA,B,Q,R,Pal in translocation; a second outer membrane protein, such as the porin OmpF or TolC, has been shown to serve as a ''second receptor'' and be involved in passage of the colicin across the outer membrane.

Unlike the Tol-dependent colicins, no ''second receptor'' has ever been identified for the group B, or TonB-dependent colicins, such as colicin Ia. We show here that colicin Ia uses one copy of Cir as its receptor, for the initial binding step, and then searches for a second copy of Cir for translocation across the outer membrane. We constructed a chimeric colicin with the C-terminal channel-forming domain and N-terminal translocation domain (T-domain) of colicin Ia and the receptor-binding domain of colicin E3. Although this colicin now requires the E3 receptor protein, BtuB, presumably for binding, it still requires both the Cir and TonB proteins for killing. Furthermore, we show that the purified 225-residue colicin Ia T-domain can compete with this hybrid colicin and protect *E. coli* from killing, *in vivo*. These results imply that the T-domain binds to and opens a channel in Cir through which the colicin reaches the periplasm.

3051-Pos Board B98

A Relay Mechanism in Rhomboid Intramembrane Protease

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Intramembrane proteases are a family of highly conserved membrane proteins that cleave other transmembrane (TM) helical segments within the plane of the lipid membrane. GlpG rhomboid protease, the best characterized of these intramembrane proteases, has six helical segments and a unique loop lying in the plane of the membrane. TM5 acts as the lateral gate that opens to allow docking of the incoming substrate; the role of the L1 loop, which extends away from the active site, is uncertain. Site-directed mutagenesis experiments have identified a triple serine L1 mutant (Y138S/F139S/L143S) with a significantly reduced catalytic activity, and a triple valine mutant of the TM5 gate (L229V/F232V/W236V) with enhanced activity relative to the wild-type protease (Baker et al, Proc. Natl. Acad. Sci. USA 104, 8257-8262, 2007). To dissect the roles of TM5 and L1, we performed all-atom molecular dynamics simulations of the L1 and TM5 mutants in hydrated bilayers of 1-palmytoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine (POPE). The results reveal a relay mechanism that transmits structural and dynamical perturbations between the remote TM5/L1 structural elements of the protease. Perturbation of L1 is transmitted to the active site and TM5 via intra-protein hydrogen bonds to which conserved amino acid residues contribute. Likewise, perturbation of TM5 leads to changes in protein dynamics and local structural rearrangements of the remote L1 loop. In the (inactive) L1 triple serine mutant, but not in the (highly active) TM5 triple valine mutant, several intra-protein interactions become locked in a new geometry. The communication between L1 and the TM5 helical gate TM5 suggests a regulatory role for loop L1.

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3052-Pos Board B99

Structural Basis of Lipid Effects on G-Protein-Coupled Receptor (GPCR) Activation

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Several new crystal structures published in the past year give insight into the activation mechanism of G-protein-coupled receptors (GPCRs). The inactive receptor is stabilized by interactions of TM-3 with TM-7 in the ligand binding pocket on the extracellular side and with TM-6 on the cytoplasmic side. Both interactions are weakened upon activation. The helix movement model of receptor activation suggests that conformational changes in the ligand-binding pocket are transmitted to the cytoplasmic surface. The model is consistent with structural changes from the inverse agonist-bound receptor ground state (rhodopsin) to the G-protein-interacting conformation ('activated' opsin). We demonstrate analogous changes in the TM-3/TM-7 interaction from long molecular dynamics simulations (>600 ns) of β_2-adrenergic receptor (AR) in two forms, bound to carazolol (inverse agonist) and to adrenaline/epinephrine (agonist), respectively.[1] The activated opsin structure exhibits movement (tilt and rotation) of TM-6, which generates the G-protein-binding site and disrupts stabilizing (''ionic lock'') interactions of E247(6:30) with the (D/E)RY motif on TM-3. Movement of TM-6 is independent of a broken ionic lock as seen in inverse agonist-bound $\beta_{1/2}$-AR. On the other hand, movement of TM-6 appears to be the structural basis for several lipid effects on receptor activation. We have shown that in bilayer membranes receptor activation is facilitated by non-lamellar phase-promoting (phospho-) lipids with small head-groups and/or bulky acyl chains. Moreover, mismatch of bilayer hydrophobic thickness with the receptor results in oligomerization and/or local molecular

crowding, which in turn inhibits receptor activation. [1] T. Huber, S. Menon, and T.P. Sakmar. 2008. Current Topics. Structural Basis for Ligand Binding and Specificity in Adrenergic Receptors: Implications for GPCR-targeted Drug Discovery. Biochemistry **47**, *in press*.

3053-Pos Board B100

Changes in the Secondary and Tertiary Structures of Secreted Phospholipase A_2 upon Activation

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Activation of human pancreatic phospholipase A_2 (PLA_2) in the presence of DPPC/DPPG (7:3) vesicles was induced by a temperature shift from 4 to 38 ^oC. PLA_2 activity was monitored by changes in fluorescence of bis-Pyrene-PC (2.5 mol % in the membranes), while simultaneous far- and near-UV circular dichroism (CD) spectra identified changes in the secondary and tertiary structures of the protein in real time. The 4-to-38 ^oC temperature shift caused dramatic changes in both bis-Pyrene-PC fluorescence and the protein CD spectra. The monomer fluorescence signal of bis-Pyrene-PC rapidly increased and the excimer signal decreased, demonstrating PLA_2 activation. Drastic weakening in the α-helical CD signal of the protein, i.e., a 20% decrease in the n-π* transition intensity at 222 nm, was detected upon enzyme activation. The α-helical signal exhibited a significantly smaller change upon a similar temperature shift under non-catalytic conditions (1 mM EGTA), while little changes were detected in the absence of lipid. Strong changes in the tertiary structure during PLA_2 activation were also identified. Initially, at 4 ^oC, the near-UV CD spectra showed a weak negative band around 280 nm. Upon a shift to 38 ^oC, strong positive CD bands rapidly developed around 250 and 280 nm, implying significant changes in the conformation and/or the microenvironment of Tyr and Trp side chains of PLA_2, possibly accompanied with a global tertiary structure perturbation associated with deformation of the abundant disulfide bonds in the protein. These experiments provide new information on the structure-function relationship of PLA_2 by near-simultaneous measurements of PLA_2 activity and its secondary and tertiary structures.

3054-Pos Board B101

Global Fitting and Kinetic Modeling of the Drug Transport Cycle of Human P-glycoprotein

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Human P-glycoprotein (ABCB1) is important in pharmacokinetics, tissue distribution, oral bioavailability and disposition of therapeutic compounds. Overexpression of P-glycoprotein can lead to adverse clinical effects such as the failure of cancer chemotherapy by the induction of multidrug resistance. Thus a detailed understanding of P-glycoprotein's drug transport cycle is a prerequisite to modulating its various activities. Here we present a detailed kinetic and mechanistic model of drug transport by P-glycoprotein. This was achieved by global fitting of time-based progress curves of the transport of spin-labeled verapamil as a function of ATP concentrations and measurements of the force-flux relationships observed during drug transport. By measuring the ATPase activity simultaneously with the transport velocity in proteoliposomes containing purified P-glycoprotein we established a limiting stoichiometry of one ATP hydrolyzed per spin-labeled verapamil molecule transported. Next, using standard chemical kinetic rate laws, we compared different proposed models of drug transport by trying to globally fit available experimental data numerically to coupled differential equations generated by each competing model. Our original seed values and constraints were generated from measurements of K_M, *Vmax*, and K_i values for all reacting ligands during the steady state transport cycle together with knowledge of the overall thermodynamics of the transport cycle. We achieved a unique global fit of the progress curves of spin-labeled verapamil transport as a function of ATP concentration. A single internally consistent set of rate constants was shown to account for the data. Additionally, these same rate constants also accounted for the experimentally observed force-flux relationships when utilized in our ''reaction power stroke'' model. In contrast we were not able to fit the data as uniquely and as effectively with other competing drug transport models. Supported by NIH grant GM52502.

3055-Pos Board B102

Agonist and Antagonist Interactions in Beta Adrenergic Receptors

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G-protein coupled receptors (GPCRs) are a large family of integral membrane proteins involved in signal transduction pathways, making them appealing drug targets for a wide spectrum of diseases. The recently solved X-ray structures of beta1 (B1AR) and beta2 (B2AR) adrenergic receptors bound to inverse agonists/antagonists open up a large field of potential investigations to understand the binding modes and mechanisms of activation of GPCRs.

To investigate their structural and dynamic properties under pseudo in vivo conditions, we performed extensive molecular dynamics simulations (in an explicit membrane) of adrenergic receptors in complex with partial inverse agonists and agonists as well as in their apoform. To this end, we applied MD-based enhanced sampling techniques (steered MD, metadynamics, …) to describe ligand binding and to elucidate the process of ligand entrance and release.

In this contribution, we rationalize the differences in binding mode between B1AR and B2AR for both agonists and antagonists (focusing on a limited set of key residues surrounding the binding pocket that are different between B1AR and B2AR). We also discuss main structural changes upon agonist/antagonist binding also in comparison with the most thoroughly studied GPCR, rhodopsin.

3056-Pos Board B103

Expression and functional characterization of Metabotropic Glutamate Receptor Type 6 (mGluR6) in detergent micelles

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Metabotropic glutamate receptors (mGluRs) are G protein coupled receptors which are implicated in different brain functions and dysfunctions including learning, memory, pain perception, neurodegeneration, schizophrenia and addiction. Metabotropic glutamate receptor type 6 (mGluR6) is a subtype of mGluRs which is exclusively expressed in ON-bipolar cells and is involved in night vision. Recent genome-wide association studies have discovered involvement of mGluR6 in heroin addiction. mGluR6 is emerging as a new drug target but structure-function relationships of this receptor and in general of mGluRs are poorly understood. These receptors have very low expression levels in their native cells and are only active in a membrane environment. The major problem in studying these receptors with biophysical approaches is in obtaining sufficient quantities of functional protein. To overcome this, we have constructed a tetracycline inducible mammalian stable cell line expressing full length human mGluR6. We optimized the detergent and buffer conditions required for mGluR6 purification. We are developing a reliable, quantitative *in vitro* assay for verifying the function of purified mGluR6 in different detergents. We have been successful in developing a medium scale expression and purification system of mGluR6. Preliminary data suggests that this receptor responds to a number of ligands relevant to its implicated role in vision and addiction with changes in activity and structure as evidenced by G protein binding and cysteine accessibility measurements, respectively.

3057-Pos Board B104

Calcium Enhances The Proteolytic Activity Of BACE: An In Vitro Biophysical And Biochemical Characterization Of The BACE-Calcium Interaction

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BACE is a novel type I transmembrane aspartyl protease that has been implicated in the pathogenesis of Alzheimer's disease. Cleavage of the amyloid precursor protein by the beta-secretase, BACE, is the first step in the production of the amyloid-beta peptide and is a prime target for therapeutic intervention. Using circular dichroism, we provide evidence that show differences in stability between active (pH 4.8) and inactive (pH 7.2) BACE. Active BACE (T_m ~51°C) is comparably much less stable than the inactive form (T_m ~84°C). In this study we have also examined Ca^{2+} binding to BACE, the effect of this binding on the secondary and tertiary structural characteristics of BACE, and the influence of this binding on the specific activity of the purified protein. Initially, we used isothermal titration calorimetry to characterize the Ca^{2+}-BACE interaction. Our results suggest that there is a high affinity of binding ($K = 2.0 \times 10^5\ M^{-1}$) between Ca^{2+} and BACE and that the binding process was exothermic (-3.5 kcal/mol). Circular dichroism and endogenous tryptophan fluorescence measurements demonstrated that the secondary and tertiary structure, respectively, is sensitive to increasing concentrations of Ca^{2+}. We also could demonstrate that low concentrations of Ca^{2+} (μM) significantly increased the proteolytic activity of BACE. Collectively, these results define a role for Ca^{2+} in both modulating the structure and proteolytic activity of BACE and suggest