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