characterizes the mechanism of HP35 folding. The three alpha helices in HP35 exhibit distinct patterns of formation, and each forms at a different stage in the folding process. The one-dimensional free energy profile is computed by integrating the mean force along the folding pathway and roughly two major energy barriers are observed. The biggest folding energy barrier is estimated to be 4.1 kcal/mol and the second one about 3.3 kcal/mol. The two major free energy barriers divide the whole folding process into three metastable states, namely the unfolded, native and a partially folded state in between, which is characterized by an aromatic core including residue Phe6, Phe10 and Phe17. This three-state picture is consistent with the biphasic kinetics inferred from previous laser temperature jump experiments and a recent computational study using temperature replica exchange MD simulations. Markov states model (MSM) is then built to estimate the rate of folding. We find that folding time scale implied from MSM is much faster than experimental folding time, although good Markovian behavior is observed in present model. Possbile reasons for this difference and alternative ways to improve the MSM are discussed.

3043-Pos Board B90

Protein Coarse-Grain Potentials for Folding Simulations

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Protein coarse-grained models are simplified representation of proteins that in principle can be used to perform long time scale simulations for the study of their folding dynamics, thermodynamics, and native structures. The main challenge in realizing these models is to find a physically accurate energy parameterization. Here two approaches are considered for this purpose. The first is the popular knowledge based potential approach, where the energies are extracted from the sequence and structure of known proteins. The advantages and limitations of this approach are examined from the perspective of minimal lattice models. It is concluded that this approach is less accurate in the determination of non-bonded interactions. The other approach involves the straightforward coarse-graining of individual residue pairs by performing molecular dynamics simulations. This approach does not suffer from the approximations involved in knowledge-based potentials and have the advantage that their quality can be controlled. The final energy model is built from a balanced combination of knowledge based potentials and coarse-grained interactions from molecular dynamics. Applications of this model to protein structure prediction are presented.

3044-Pos Board B91

Pressure-induced Structural Changes Of Amyloid-β Peptide:a Md Simulations Study

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Major constituents of the amyloid plaques found in the brain of Alzheimer's patients are the 39-43 residue amyloid-β(Aβ)peptide. Extensive in vitro as well as in vivo biochemical studies have shown that 40- and 42-residue peptides play major roles in the neurodegenerative pathology of Alzheimer's disease (AD). It is known that Aβ40 and Aβ42 have obvious different conformation in its native state, even though they differ in only two (IA) amino acid residues at the C-terminal end. In this study, to characterize the pressure-induced structural changes in both Aβ40 and Aβ42 peptide monomers, we perform 6 independent long-time molecular dynamics (MD) simulations at variable pressure of 0.1Mpa, 200Mpa and 1000Mpa for total of 360ns. In aqueous solution, α-helix to β-sheet conformational transition for Aβ40 under the pressure of 200Mpa was observed, and higher pressures such as 1000Mpa could retain the unfolding rate of α-helix. However, the pressure-induced structural change of Aβ42 was different from Aβ40, under 200Mpa pressure, the β-sheet in Aβ40 of propensity increases, and the high pressure can restrain the Aβ42 to from β-sheet. The results of MD simulations are beneficial to understanding the mechanism of amyloid formation and designing the compounds for inhibiting the aggregation of Aβ and amyloid fibril formation.

Keywords: Amyloide-β peptide, Molecular dynamics simulation, conformational transition, Pressure

3045-Pos Board B92

Atomistic and Coarse-grained MD study on mutated alpha-Synuclein in Water Box

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Alpha-synuclein (αS) belongs to a natively unstructured protein family. The misfolded αS is recognized as a possible causative agent in the pathogenesis of Parkinson's disease (PD). Genetic studies have identified two dominant mutations A30P and A53T, which are associated with the early onset of PD. We show here that these two mutants observably affect the folding process of αS in a water box. Based on the NMR minimized average structure (PDB ID = 1XQ8) of the wild type (WT) αS, three mutated αS models are created: αS with mutation A30P; αS with mutation A53T; αS with mutation A30P and A53T. The WT αS model is also used for comparison. For each simulated system, which contains a monomeric protein and a water box, the temperature and pressure were set as constant: 300K and 1atm. Atomistic simulations were performed for 30 ns each, using CHARMM22/CMAP force field (MacKerell et al, 2004). Then, using the MARTINI force field v2.1 (Marrink et al, 2008), coarse-grained simulations were performed for 400ns each to simulate the conformation changes of αS over a longer time scale. The coarse-grained simulations demonstrate similar equilibrium structures for both mutated and WT αS systems. In addition, the atomistic simulations indicate that the two mutations significantly increase the rate of denaturation in the N terminus.

3046-Pos Board B93

Deciphering Protein Mechanical Stability By Comparing Different Folds

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Cellular functionality is in large dependent on the ability to respond properly to external stimuli thorough signaling networks. Traditionally, this concept has been described by means of chemical reaction pathways, however, lately it has become apparent that mechanical force also plays a crucial role in many physiological processes. Signal transduction is achieved by regulating the reversible folding and binding of single proteins. The combination of single molecule force measurement and computer modeling has been used successfully in studying such force induced protein signaling events. Steered molecular dynamics (SMD) is one of the most popular simulation methods used in such modeling.

In this work, we systematically use SMD to investigate the mechanical properties of a number of proteins involved in mechanical signaling events. Specifically, SMD illustrates the atomic level protein conformational changes induced by mechanical forces. These conformational changes have been used to propose means by which mechanical and chemical signals are interconnected to achieve regulatory ends. Additionally, we are able to compare the relative mechanical stability of different folds, thereby eluding to how certain folds are specifically tailored to withstand mechanical stress.

3047-Pos Board B94

Super-proteins From Fitness-threshold Selection Statistics

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It is in principle possible to assign a fitness value (organism reproductive rate) to every sequence of any given protein. The resulting fitness landscape can be regarded as the convolution of two mappings: one from genotype to phenotype with another mapping from phenotype to fitness. Despite this intrinsic complexity, we may expect organism fitness to depend on protein properties bearing upon catalysis, interactions with other molecules, stability, et cetera. However, the specific relation between fitness, sequence space, evolution and protein molecular properties is not understood, a fact which hampers efforts to tap the enormous potential for protein and organism engineering contained in the exponentially-growing sequence databases. Here we show that a simple evolutionary hypothesis on the statistics of purifying natural selection over a fitness threshold is operational and leads to protein multi-feature optimization. We thus obtain variants of E. coli thioredoxin showing simultaneous, large-scale optimizing modulations in stability, folding/unfolding kinetics, bulk-solvent oxidoreductase activity and the two chemically and evolutionary different mechanisms of enzymatic catalysis revealed by single-molecule force clamp spectroscopy. Furthermore, preliminary experiments suggest that these variants may induce in-vivo resistance to thermal and oxidative stresses. We anticipate, therefore, applications in fields that involve organism engineering (microbial biotechonology, synthetic biology).

3048-Pos Board B95

Molecular Modeling of Folding and Preferred Regioisomer Formation in α-Conotoxins

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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