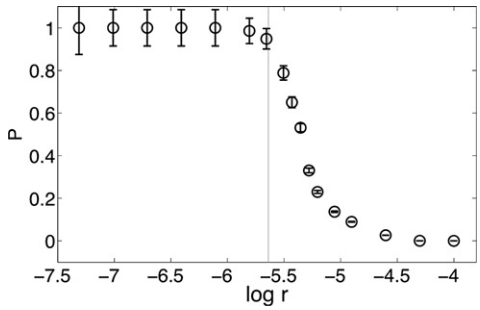
always occur on funneled energy landscapes. We introduce a theoretical framework to understand folding in the presence of metastable and intermediate states by considering the statistics of protein conformational dynamics on rugged energy landscapes. Our analysis reveals that, even for the most frustrated proteins, reliable folding can occur on rugged energy landscapes and is sensitive to the rate that external parameters are adjusted to induce folding. When folding is reliable, there is always a well-defined reaction path leading to the native state.

We test the predictions of our statistical analysis using simulations of a model protein. In the accompanying figure we plot the probability P to inhabit the native state after inducing folding by reducing the temperature at rate r. Reliable folding only occurs below a limiting rate that is correctly predicted by theory (red line).



3038-Pos Board B85

2*D*-string Theory of Biomolecular Bundle Space

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The *Biomolecular Bundle Space* is presented as a topological space with a *finite group* structure of size 36, DO_{GU}. The helical patterns of 2*D*-strings replace vibrational patterns of 1*D*-strings. Possible configurations of these helical patterns form 9 distinct *polyhedra*, from tetrahedron to icosahedron, and the *fundamental element* is a pair of equilateral triangles forming the *diamond simplex*. These polyhedra are distributed over the 3*D*-branes reflecting the DO_{GU} group structure. The two-sheet *3D-branes* are a *torus* for the sheet having the *symmetric* diamond simplex, and a *Klein bottle* for that having the *asymmetric* diamond simplex. The torus brane represents the backbone structure of the *nucleic acids, DNA, RNAs* and the Klein bottle brane that of *proteins*. We present the *fundamental elements* of the bundle space *B* and its *projection p* to the corresponding *base space X*. The base space has, as a translational symmetry, congruence (*mod* 6). The *transcription code* (Genetic Tableau) is directed by the *Transcription Shuttle* tetrahedron, the *translation code* (*tRNA+rRNA*) by the *Translation Key* truncated tetrahedron, and the *René Thom Cobordism code* (Protein Space) by the entire set of 9 distinct polyhedra of the biomolecular bundle space. The relative rotational energies of the polyhedral elements of the DO_{GU} group are calculated by the organizing centers of the *René Thom's catastrophes*. This classification provides a unified approach to analyze the relationships within the bundle space.

3039-Pos Board B86

Microsecond Explicit Solvent Molecular Dynamics Simulations of Protein Folding

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Explicit solvent molecular dynamics (MD) simulations of protein folding offer information on the protein folding process with tremendous temporal and spatial resolution. Long timescale protein folding trajectories can aid in interpretation of experimentally observed protein folding kinetics, and in the design of new fast-folding mutants. At the same time, protein folding simulations offer a demanding test for MD force fields to assess their accuracy in describing long-timescale conformational transitions in proteins. Until recently, however, little overlap existed between the timescales accessible to simulations and the time required for small proteins to fold. Recent experimental advances have lead to the discovery and characterization of a variety of proteins which fold on the 1-10 microsecond timescale; at the same time, increased computational power has made multiple microsecond timescales accessible for explicit solvent MD simulations.

We performed multiple-microsecond folding simulations of two well-characterized fast-folding proteins, namely the villin headpiece subdomain and Pin1 WW domain. The villin headpiece folds to a native state after ~6 microseconds of molecular dynamics simulation. Furthermore, a common folding mechanism is observed in multiple simulations from different starting conditions, where all secondary structure elements form over 1-2 microseconds after an initial hydrophobic collapse, but the native structure is only obtained after a complete dissociation and rearrangement of the secondary structure elements relative to each other. In the case of the WW domain, the protein misfolds in all simulated folding attempts. Conformational free energy calculations indicate that the WW domain's native state, a three-stranded beta sheet, is significantly higher (~ 9 kcal/mol) in free energy for the force field (CHARMM22/CMAP) used than several misfolded helical structures obtained from folding simulations. Our results agree with several other recent studies in suggesting a bias toward helical secondary structure in modern MD force fields.

3040-Pos Board B87

Ab Initio Determination of Tryptophan Fluorescence Quenching by Histidine Cation in HP35-N27H, Barnase, and T4Lysozyme

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The fast folding, 35-residue villin headpiece, HP35, has been at the center of numerous protein folding rate simulations. Eaton et al. have experimentally followed the folding with the N27H mutant, plausibly because the protonated His27 quenches the fluorescence from Trp23 in the folded form by electron transfer, but not when unfolded. Because of this, at least in some simulations of the folding, a major criterion for the folded form is close proximity of His27 to Trp 23. Protonated His is indeed a potent quencher of Trp fluorescence in solution and in some, but not all, proteins. For quenching to occur the energy of the Trp-to-His charge transfer(CT) state must be low enough to be in resonance with the excited state of the Trp. This resonance is dictated by the electric potential difference between the Trp and His due to local protein environment. In some proteins the electric field enables quenching, and in others it does not. We have carried out QM-MM simulations of quenching by His cation for Trp23 in in villin, Trp94 in barnase and Trp138 in Q105H T4 lysozyme using ab initio electronic coupling [Callis et al J. Phys. Chem. B; 2007; 111(35); 10335-10339] Preliminary results indicate that for villin and T4 lysozyme the lowest CT state is that of the amide backbone of Trp. In contrast, the lowest CT state for barnase has His cation as the electron acceptor. We have also computed electronic coupling matrix elements between 3-methylindole and imidazole cation for all three proteins. The average coupling is 58, 329, and 2 cm-1 for the T4 lysozyme, barnase, and villin cases, respectively, suggesting that the change of Trp fluorescence upon folding in villin is not because of quenching by His27.

3041-Pos Board B88

Cu Involvement In Prion Oligopeptide Stability: Experiments And Numerical Simulations

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The enormous sociological impact of neurodegenerative diseases (like Alzheimer disease, Transmissible Spongiform Encephalopathies, Parkinson disease, etc.) has pushed the attention of researchers towards the study of the rôle played by metals in the misfolding process, as they are regarded as a possible concurrent cause of protein aggregation and plaque formation.

Metals are, in fact, essential players in many of the fundamental activities of cells. Storing, metabolism and trafficking of metals through the cellular membrane and within the cytoplasm is mediated by many proteins via well tuned mechanisms because of the toxicity of free ions.

With a combination of X-ray Absorption Spectroscopy (XAS) technique and numerical ab initio simulations we have investigated the physico-chemical basis of the aggregation phenomenon, which is suspected to be at the basis of the development of the amyloidosis.

In this presentation we will summarize the results of the our experimental and numerical investigations aimed at understanding the possible rôle of Cu in stabilizing the Prion protein

structure and in the formation of protein polymers.

3042-Pos Board B89

Folding Pathway And Free Energy Landscape Of Villin Headpiece Subdomain HP35 Studied by String Method

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The string method with swarm of trajectories is applied here to find the most probable folding pathways of the 35-residue villin headpiece subdomain (HP35). The converged pathway, represented by 61 discrete images, fully

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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