proton channels from influenza A and B viruses by solution NMR spectroscopy. The channel structures reveal pore features that are important for proton gating and proton relay. Structural details of the anti-influenza drug, rimantadine, bound to the channel suggests an unexpected allosteric mechanism of drug inhibition and drug resistance, which has been verified by thorough functional and mutagenesis experiments.
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1034-Symp
Proton Transport Through Channels: Insights and Surprises from Molecular Simulation
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The behavior of hydrated excess protons passing into and through transmembrane proton channels will be presented, based on the results of a novel multi-scale computer simulation methodology. The unique electrostatics related to the dynamic delocalization of the excess proton charge defect will be elaborated, as well as its effect on the channel proton transport and selectivity properties. The often opposing and asymptotic viewpoints related to electrostatics on one hand and Grotthuss proton shuttling on the other will be reconciled and unified into a single conceptual framework. Specific simulation results will be given for various channel systems, including the M2 channel of influenza A, proton selective mutant aquaporin-1 channels, the ClC Cl^-/H^+ antiporter, and models of the Hv1 voltage gated proton channel. Comparison to experimental results will be discussed where possible.

Platform L: Protein Folding Pathways

1035-Plat
Protein Folding: Independent Unrelated Pathways or Predetermined Pathway with Optional Errors
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There is a fundamental conflict between two different views of how proteins fold. Kinetic experiments and theoretical calculations are often interpreted in terms of different population fractions folding through different intermediates in independent unrelated pathways (IUP model). However, detailed structural information indicates that all of the protein population folds through a sequence of intermediates predetermined by the foldon substructure of the target protein and a sequential stabilization principle. These contrary views can be resolved by a predetermined pathway_optional error (PPOE) hypothesis. The hypothesis is that any pathway intermediate can incorporate a chance misfolding error that blocks folding and must be reversed for productive folding to continue. Different fractions of the protein population will then block at different steps, populate different intermediates, and fold at different rates, giving the appearance of multiple unrelated pathways. A test of the hypothesis matches the two models against kinetic folding results for two proteins, hen lysozyme and staphylococcal nuclease, which have been interpreted previously in terms of independent parallel pathways. Folding kinetics of both proteins fit equally well to the two models, indicating that the measured experimental data does not require alternative parallel pathways. The fitted PPOE reaction scheme leads to known folding behavior, whereas the IUP properties are contradicted by experiment. The appearance of a conflict with multipath theoretical models seems to be due to their different focus, namely on multitrack microscopic behavior versus cooperative macroscopic behavior. The integration of three welldocumented principles in the PPOE model (cooperative foldons, sequential stabilization, optional errors) provides a unifying explanation for how proteins fold and why they fold in that way.

1036-Plat
Multiple Routes and Milestones in the Folding of HIV-1 Protease Monomer
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Evolution has lead proteins to display funneled energy landscapes with small degrees of ruggedness. However, a funneled landscape does not preclude the presence of multiple kinetically relevant folding routes. Here we show that for an extremely relevant biological case, the monomer of HIV type 1 protease (HIV-1-PR), multiple pathways and milestones can coexist along the folding process.
We provide a comprehensive picture of the folding mechanism of HIV-1-PR monomer using a variety of theoretical and computational techniques. These include all-atom molecular dynamics simulations in explicit solvent, an analysis of the network of structure clusters found in multiple high-temperature unfolding simulations and a complete characterization of the free energy surface carried out using an all-atom structure based potential and a combination of metadynamics and parallel tempering.
Our results confirm that the monomer in solution is stable and show unambiguously that at least two (un)folding pathways exist. Moreover, we demonstrate how the formation of a hydrophobic core can be considered a milestone in the folding process which must occur along all the routes that lead towards the protein's native state. These results also provide a theoretical framework that is able to rationalize both the experimental evidences and the evolutionary data for HIV-1-PR monomer. Finally, our characterization of the ensemble of possible folding routes substantiates a rational drug design strategy based on inhibiting the folding of each of the subunits that build the HIV-1 protease homo-dimer.

1037-Plat
Characterizing Energy Landscapes of Proteins and Identifying Shape-Determining Factors
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Protein folding is a long standing problem in biology, whose mechanism is still not completely understood. Funnel-shape energy landscape has been proposed as a plausible folding mechanism. However, the factors that determine the funnel-shape energy landscapes is largely unknown. In this study, we use hydrophobic-hydrophilic (HP) model to investigate the factors that affect the funnel-shape of protein energy landscapes. We designed a clustering method based on graph theory to analyze the conformations sampled using a recently developed Monte Carlo method, FRESS. We found that the way conformations move from one to another defined by a particular sampling method (move set) has a significant effect on the shape of protein energy landscapes. To our surprise, both protein-like sequences and random sequences with around 50% hydrophobic residues have a stable state represented by a single dominant cluster, consisting of a large number of similar conformations. The energy landscapes resemble a funnel, where there are many paths to minimum energy conformations in the dominant cluster from conformations of higher energies. We also found that sequences with hydrophobic residues above or below the optimal range around 50% do not have a single stable state. In stead, there are many much smaller clusters, representing multiple local energy minima. Our finding is consistent with the compositions of hydrophobic and polar residues in globular proteins (fold to unique structures) and intrinsically disordered proteins (IDPs). Our study suggests that in computational simulations, move sets affect significantly the shape of protein energy landscapes; hydrophobic interaction is likely a major force leading to the funnel-shape energy landscape of proteins; and the composition of hydrophobic and polar residues is an important sequence feature for the formation of funnel-shape of protein energy landscapes.

1038-Plat
Common Folding Mechanism of a Peptide Revealed by Multiple MD Simulations
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Here, we report on the folding of a 15 residue beta-hairpin peptide (Peptide 1) using multiple unbiased, atomistic molecular dynamics (MD) simulations. Fifteen independent MD trajectories, each 2.5 microseconds-long for a total of 37.5 microseconds are performed in explicit solvent, at room temperature and without the use of enhanced sampling techniques. The computed folding time of 1-1.5 microseconds obtained from the simulations is in good agreement with experiment. A common folding

mechanism is observed, in which the turn is always found to be the major determinant in initiating the folding process, followed by cooperative formation of the inter-strand hydrogen bonds and the side chain packing. Furthermore, direct transition to the folded state from fully unstructured conformations does not take place. Instead, the native hairpin is always observed to form from partially structured conformations involving a non-native (ESYI) turn from which the native (NPDG) turn forms, triggering the folding to the beta-hairpin.

1039-Plat
Residue Specific Analysis of Frustration in Folding Landscape of Repeat Alpha/Beta Protein Apoflavodoxin
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Topological frustrated proteins can give rise to complex folding pathways and transition state profiles. To better understand such systems, we combine experimental and computational methods to study *Desulfovibrio desulfuricans* apoflavodoxin by producing several point mutation variants. By equilibrium unfolding experiments, we first revealed how different secondary-structure elements contribute to overall protein resistance towards heat and urea. Next using stopped-flow mixing coupled to far-UV circular dichroism (CD), we probed how individual residues affect the amount of structure formed in the experimentally-detected burst-phase intermediate. Together with *in silico* folding route analysis of the same point-mutated variants and computation of the growth in nucleation size during early folding, computer simulations suggested the presence of two competing folding nuclei at opposite sides of the central β-strand 3 (i.e. at β-strands 1 and 4), which cause early topological frustration (*i.e.,* misfolding) in the folding landscape. Particularly, the extent of heterogeneity in the folding nuclei-growth correlates with the *in vitro* burst phase CD amplitude. In addition, Φ-value analysis (*in vitro* and *in silico*) of the overall folding barrier to apoflavodoxin's native state revealed that native-like interactions in most of the β-strands must form in transition state. Our study reveals that an imbalanced competition between the two sides of apoflavodoxin's central β-sheet directs initial misfolding while proper alignment on both sides of β-strand 3 is necessary for productive folding.

1040-Plat
Folding Kinetics of IkB: Excursions Through the Energy Landscape
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The IkB proteins, inhibitors of the transcription factor NF-kB, are comprised of tandem repeats of a conserved primary structure. The tandem repeats are stabilized as a repeated tertiary structural motif, at least when complexed with NF-kB and DNA. Structural fluctuations in the native state deviate substantially from a simple repeating pattern, as reported by site-resolved hydrogen exchange rates, NMR chemical shifts and relaxation parameters, and energetically frustrated intraprotein contacts.
Here we have studied the folding of the first four ankyrin repeats of human IkB-alpha and -beta, using coarse-grained structural models to extensively simulate dynamics under structurally-parameterized Hamiltonians. The folding reaction coordinate was sampled with biasing potentials and the free energy as a function of the reaction coordinate was calculated with weighted histogram analysis. The trajectories were used to assign the thermodynamic changes between substates in the folding mechanism. Kinetic models connecting the folding substates were used to predict experimentally known (un)folding rates.

1041-Plat
Protein Folding Landscapes for Alpha- and Beta-Miniproteins Using All-Atom Simulations with an Optimized Force-Field
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With recent advances in simulation methodology and computer hardware, it has become possible to access physical phenomena occurring on the microsecond timescale with molecular dynamics simulations. Even though most naturally occurring proteins fold on a much slower timescale (milliseconds to seconds), the design of miniproteins (e.g., trpcage, trpzip, beta-hairpin) and proteins (e.g., Villin) which fold close to the protein folding-speed limit (~ 1 μs) have made feasible direct, high resolution, simulations of protein folding. At the same time, the shortcomings in the current energy functions for proteins are becoming increasingly evident with clear biases in secondary structure preference of different force fields being reflected in which proteins they are able to fold. To be able to compare and complement experimental observations, an ideal force-field would be able to fold different types of secondary structure without additional modifications or inputs.
We will present results from extensive replica exchange molecular dynamics simulations for folding of GB1 beta-hairpin and trpcage (representing beta and alpha structures respectively) with a force field based on AMBER ff03 and optimized only to reproduce the helix-coil transition. We obtain converged equilibrium distributions for runs starting from a completely unfolded state and from the native state, with folded populations at room temperature in quantitative agreement with experiment. The folded structures for both the proteins (starting from a completely unfolded structure) have average backbone dRMS from the experimental structures of less than 1 Å. Finally, we will present results for the 35-residue protein Villin. Although convergence of equilibrium distributions in this case is not computationally feasible, we nonetheless obtain a folded structure within 1 Å dRMS of the native structure, starting from a completely unfolded coil-like conformation.

1042-Plat
Amyloid Fiber Precursors in Native and Denaturing MD Simulations of an IG Light Chain Domain
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The native structure of proteins is generally necessary for function. Mutations and changes in the environment can lead to altered conformations that are prone to aggregation, leading to the formation of amorphous or fibrillar aggregates, which are associated with diseases such as systemic amyloidoses. Precursors for these aggregates can come from any point in the folding route for the protein, and there is a direct correlation between poor protein stability and its potential to form fibers. Immunoglobulin variable light chain domains are prominent in primary systemic amyloidoses; in particular, class 6a appears in ~40% of clinical cases, despite being expressed only in ~2% of B-cells under normal conditions. 6aJL2 is a prototype for these protein domains, containing the germline sequence of the VIa gene fused to the lambda J2 segment, built to explore the properties of this particular class. An allotypic variant, containing the R24G mutation, is even more susceptible to fibril formation, and is also less stable. Both forms are two-state folders. Starting from the crystal structure of 6aJL2, we carry out MD simulations of the original 6aJL2 protein and the R24G variant under native conditions (298K) and under strong denaturing conditions (573K), to explore the native basin and the unfolding pathway of these proteins, in order to identify possible precursors for the formation of fibers. We compare the results of the simulations to available circular dichroism, fluorescence and NMR data. Preliminary data provide a rationale for the lower stability of R24G, due to the loss of both stacking and H-bond interactions. Analysis of both native and denaturing simulations suggest that unfolding for both variants starts at a proline-rich loop and makes the N-terminus of the protein available for seeding the amyloid.