reuptake of dopamine (DA), a neurotransmitter responsible for pleasurable feelings. Work by Shan et. al. suggests that two DA molecules must bind before transport through DAT can occur, while other research disagrees. For this reason, the transport mechanism remains ambiguous. Using molecular dynamics simulations and a dual bilayer system with two DAT proteins, our study mimics physiological conditions at a synapse. One protein began in its occluded state, with DA and sodium bound in the S1 binding pocket. In this poster, we report the results from our simulation in which a single DA dissociated from the protein into the intracellular matrix without the need for a second bound DA. The sodium ions have yet to leave the binding pocket.

Protein Folding and Unfolding

1297-Pos Board B27

Sequence-Function Relationships in Allostery Mediated by Disorder-To-Order Transitions

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Allostery functions in many biological systems including signal transduction, transcription regulation, and metabolism. Although disorder-to-order transitions contribute to numerous allosteric processes, the relationship of the functional allosteric response to the sequences that undergo these transitions is not known. The Escherichia coli biotin repressor, BirA, is an allosteric transcription regulatory protein that undergoes ligand-induced loop folding upon corepressor, bio-5′-AMP, binding. Ligand binding and dimerization are coupled processes with bio-5′-AMP binding enhancing the dimerization free energy by −4.0 kcal/mole. Previous work has demonstrated that adenylate binding is coupled to folding of a loop in which any single alanine replacement disrupts effector binding, loop folding and functional coupling. In this work, coupling between residues that contribute to the disorder-to-order transition was investigated by studying variant proteins with several combinations of alanine substitutions. Combined kinetic and equilibrium measurements reveal non-additive effects of multiple amino acid substitutions for all BirA functions. The results also indicate that specific combinations of alanine substitutions lead to reversion of the allosteric response toward that observed for the wild type protein. In combination the results suggest that, analogous to protein folding, full function in disorder-to-order transitions requires appropriate packing of the relevant side chains.

1298-Pos Board B28

High Precision FRET Reveals Dynamic Structures in the Drosophila Scaffold Protein Complex Stardust-DPATJ-DLin-7 Mediated by L27 Domains

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Cellular differentiation is frequently regulated by multi-protein complexes (MPC). There is immense interest in isolating individual components of MPCs and how their interactions regulate cellular differentiation. The studied evolutionary conserved MPC is located in the subapical region of embryonic epithelia in Drosophila melanogaster. It plays a central role in the maintenance of the epithelia cell polarity and morphogenesis. The MPC is composed of the four proteins Crumbs (Crb), Stardust (Sdt), DLin-7 and DPATJ. The scaffold protein Sdt contains two subsequent L27 domains mediating the interaction between DPATJ and DLin-7. The trans-membrane protein Crb binds to the PDZ domain of Sdt via its cytoplasmic tail.
We used high precision Förster resonance energy transfer (hpFRET) with Multi-parameter fluorescence detection on the single-molecule level in order to understand quantitative parameters and spatial dynamics of the Sdt-DLin-7 complex. Single-molecule data, homology modelling and FPS (FRET-restrained positioning and screening) highlight that DLin-7 is present in various conformations: a folded- and an unfolded-state. In the folded-state a major- and the minor-conformation were identified. The Sdt-bound-state is comparable to the major-state. Conclusive a binding- and folding-equilibrium is postulated and confirmed experimentally.

1299-Pos Board B29

Wide Exploration of OPEP Protein Energy Landscapes using Advanced Monte Carlo Methods

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The OPEP coarse-grain protein force field has been shown to provide very good results for folding small soluble proteins, using MD and REMD [1]. However no systematic study of the energy landscapes generated by this force field has been done. Such a study could enable the identification of non-native low-lying energy minima, whose presence in the potential energy landscapes could lead to kinetic traps, significantly slowing down the folding process, or even for some systems leads to the detection of non-native states of lowest free energy minima. The energy terms of the force field can then be re-optimized to create a more energy landscape. Here, we investigate the properties of OPEP-based energy landscapes with GMIN [2], a basin-hopping technique [3] to locate global free energy minima.
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1300-Pos Board B30

Origin of the Architecture of Biological Macromolecules - A Mean-Field Perspective

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The structures of the key classes of biological macromolecules: proteins, nucleic acids and polysaccharides can be dissected into very regular motifs, which are alpha-, beta, and double helices and sheets. In this communication we demonstrate that these regular patterns arise as a result of dipole-dipole interactions of the polar groups (peptide, nucleic-acid-base or sugar-ring groups) and the coupling of these interactions with backbone-local interactions, described at the mean-field level; the averaging is carried out by rotating the dipole of a polar unit about its virtual-bond axis. This principle is behind the coarse-grained UNRES force field for large-scale protein simulations developed in our laboratory, which has performed well in the blind protein-structure-prediction CASP exercises, and our recently developed NARES-2P and SUGRES-1P force fields for nucleic acids and polysaccharides, respectively. We demonstrate how changing the orientation of the dipole with respect to the rotation axis of the polar unit and changing the pattern of local interactions result in the formation of different regular helical and sheet patterns. Moreover, we demonstrate that the structure of regular patterns and loop linkers can be obtained in a semi-analytical manner as solutions of the Discrete Nonlinear Schroedinger Equation (DNLSE) with a Hamiltonian constructed from the mean-field terms. These developments open avenues both to large-scale physics-based simulations of the structure and dynamics of biological macromolecules and to qualitative understanding of the relationship between the pattern of interactions and structure, which can contribute to our understanding of processes that originate in major conformational changes upon mutation or alteration of environment, which are behind, e.g., cancer and conformational diseases. Examples of ab initio simulations with the UNRES, NARES-2P, and SUGRES-1P are presented.

1301-Pos Board B31

Protein Flexibility and Stability: Thermophiles Know Best

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Understanding the relation between protein flexibility, stability and function remains one of the most challenging, open questions in biophysical chemistry. For example, proteins need to be flexible to facilitate substrate binding but locally rigid to sustain substrate specificity. Exemplary cases are thermophilic enzymes from archaea and bacteria. These proteins are stable and functional at elevated temperatures but generally lack activity at ambient conditions. Therefore, their thermal stability has been correlated to enhanced mechanical rigidity through the ‘‘corresponding states’’ paradigm [1]. There are, however, a number of studies on thermophilic proteins that have questioned this view [2].
In this work we present a comprehensive computational study, that questions the ‘‘rigidity paradigm’’, at least in its universal character. We compare a pair of homologous G-domain proteins, with their melting temperatures differing by 40 K. Our study points to a clear result: at ambient condition the hyperthermophilic protein has comparable or even enhanced flexibility with

respect to the less stable mesophile. When focusing on different time- and length- scales specific behaviors arise. At an atomistic scale, it is found that in the hyperthermophile a more regular alternation of rigid and flexible regions stabilizes a key part of the protein where the unfolding of the mesophile begins. We furthermore find that the conformational landscape of the hyperthermophile is characterized by a higher number of substates, or otherwise an enhanced conformational flexibility that is suggested to broaden its stability curve and raise the melting temperature. We finally compare, for the two proteins, the unfolding paths upon increasing temperature, the kinetic barrier along the early steps of unfolding and the temperature dependency of the stability.

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1302-Pos Board B32

Exploring the Relation between Unfolded Protein Ensembles, Transformations between Structures, and Refolding Kinetics

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We develop a method for generating a diverse conformational ensemble, to characterize properties of the unfolded states of intrinsically disordered or intrinsically folded proteins, with or without disulfide bonds. We can thus examine physical properties of the unfolded ensembles for various proteins, including chemical shifts, residual dipolar couplings, clustering properties, and scaling exponents for the radius of gyration with polymer length. We apply our generated ensembles to the problem of folding kinetics, by examining whether the ensembles of some proteins are closer geometrically to their folded structures than others. We find that for a randomly selected dataset of 15 non-homologous 2- and 3-state proteins, quantities such as the average root mean squared deviation between the folded structure and unfolded ensemble correlate with folding rates as strongly as absolute contact order. We introduce a new order parameter that measures the distance travelled per residue, which naturally partitions into a smooth "laminar" and subsequent "turbulent" part of the trajectory. This latter conceptually simple measure with no fitting parameters predicts refolding rates with remarkable accuracy ($r = -0.95$, $p = 1e\text{-}7$). The high correlation between folding times and sterically modulated, reconfigurational motion supports the rapid collapse of proteins prior to the transition state as a generic feature in the folding of both two-state and multi-state proteins. This method for generating unfolded ensembles provides a powerful approach to address various questions in protein evolution, misfolding and aggregation, transient structures, and molten globule and disordered protein phases.

1303-Pos Board B33

Coupling between Protein Conformation and Local Unfolding Highlights the Role of Disorder in Protein Function and Suggests a New Target for Tuberculosis Treatment

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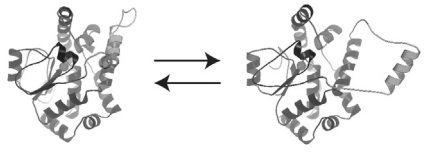
Mycobacterium tuberculosis protein tyrosine phosphatase B (PtpB), which plays a key role in tuberculosis virulence, protects its active site from oxidation through a large-amplitude conformational change. The proposed regulatory mechanism for this protective motion involves a local unfolding event. We quantitatively show that local unfolding transitions can be coupled to other conformational changes in proteins. Molecular dynamics simulations were used to determine the conformations of a disordered protein region and the energy landscape for local unfolding. The accuracy of this energy landscape was tested and confirmed experimentally, providing rigorous support for our simulation approaches. Our work demonstrates that local unfolding can depend on protein conformation by changes in the stability of the disordered region. These results provide a mechanism for the internal regulation of protein conformational changes, which highlights new targets for the development of tuberculosis therapeutics.



1304-Pos Board B34

Computational Methods for Measuring the Free Energy of Folding in the Ribosomal Exit Tunnel

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As a protein is synthesized in the ribosome, the nascent peptide chain, starting from the peptidyl transferase center (PTC), elongates along the ribosomal exit tunnel, which is ~10-20Å in diameter and ~100Å long. It has been shown that proteins can partially fold inside the exit tunnel and that the ribosome can stabilize the native secondary structure of proteins. However, the mechanism for this stabilization is not yet known at the atomic scale. To determine this mechanism, one can contrast the free energy of α-helix formation in water and in the ribosomal exit tunnel using molecular dynamics (MD) simulations. To determine the free-energy landscapes in water, we employed two computational methods - umbrella sampling (US) and adaptive biasing forces (ABF) - on various polyalanine-containing peptides, using the end-to-end distance of the polyalanine sequence as our reaction coordinate. Since this reaction coordinate does not produce a 1-to-1 correspondence to helical content, successive constraints were added to the simulations, and the changes in the free energy upon addition of each set of constraints were examined. We also applied extended ABF using the helical content of the polyalanine sequence as a reaction and compared the results with the end-to-end distance coordinate. Finally, we used these computational methods to calculate the free-energy landscape along the entire ribosomal exit tunnel with polyalanine-containing sequences placed at different locations.

1305-Pos Board B35

Structure and Dynamics of Intermediate Protein States by NMR and Simulations

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The detailed characterization of the structure and dynamics of proteins and peptides in solution is crucial for a comprehensive understanding of complex biophysical mechanisms. Backbone dynamics from nanoseconds to seconds allow proteins to explore high-energy conformational states playing key roles in many biological processes. Using novel interdisciplinary approaches combining NMR experiments and simulations we have been able to effectively study protein dynamics and structures, including those "invisible" high-energy states that cannot be characterized by classical approaches of structural biology. These methods have proved to be highly effective in characterizing elusive states such as transition states in enzymatic processing and intermediates along the pathways of amyloid formation.

1306-Pos Board B36

Elucidating the Structural Basis of α-Synuclein Fibrillation using Small Camelid Nanobodies

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α-Synuclein has been widely accepted, since its discovery, as an intrinsically disordered protein that plays a central role in Parkinson's disease, as well as other neurodegenerative disorders associated with protein aggregation. Extensive recent data substantiate the pathogenicity of the early aggregates of α-synuclein, rather than the characteristic amyloid fibrils observed in the late stages of the aggregation process. Therefore, understanding the molecular steps and the mechanisms by which this natively unfolded protein aggregates is crucial for the purpose of identifying novel diagnostic and therapeutic strategies for the treatment of synucleinopathies. A powerful therapeutic approach is to target the initial events in the reaction process, in order to promote the solubility of the monomeric form of α-synuclein and prevent the formation of potentially harmful assemblies. Thus, in our study, we aim at understanding the structural properties of the monomer that determines its aggregation propensity, using nanobodies, the antigen-binding domains derived from camel heavy chain antibodies. These molecules are valuable probes for elucidating whether conformational changes in the monomeric protein cause the aggregation, as result of their exquisite specificity, high affinity and small size (14 KDa). Our strategy is based on the study of the interactions between α-synuclein and two specific nanobodies that bind to its C-terminus and modulate its fibrillation. The structure and dynamics of α-synuclein in its free and bound states are characterized via a combination of NMR spectroscopy and in silico tools. More specifically, chemical shifts measurements, RDCs and restrained Molecular Dynamic simulations are applied to provide a comprehensive energy sampling and description of the conformational ensemble populated by α-synuclein, and thus help gain detailed insight into the mechanism by which nanobodies modulate the aggregation process of α-synuclein.

1307-Pos Board B37

Influence of Gold Nanoparticles on the Kinetics of Alpha-Synuclein Aggregation

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Alpha-synuclein (AS) is a presynaptic protein lacking a unique secondary structure in solution. AS amyloid aggregates in dopaminergic neurons are the