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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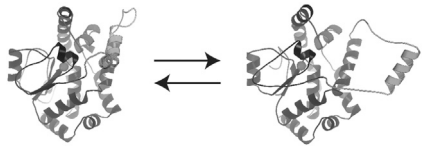
Mycobacterium tuberculosis protein tyrosine phosphatase B (PtpB), which plays a key role in tuberculosis virulence, protects its active site from oxidation through a large-amplitude conformational change. The proposed regulatory mechanism for this protective motion involves a local unfolding event. We 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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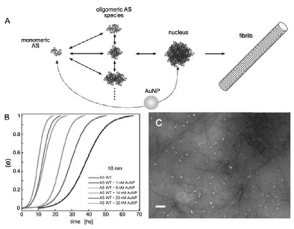
Alpha-synuclein (AS) is a presynaptic protein lacking a unique secondary structure in solution. AS amyloid aggregates in dopaminergic neurons are the

hallmark of Parkinson's disease (PD). The aggregation involves structural transitions from monomeric AS to oligomeric presumably neurotoxic and fibril formation. In spite of its importance for the understanding PD pathobiology and devising rational, targeted therapeutic strategies, details on the aggregation process remain largely unknown. Methodologies and reagents capable of controlling aggregation kinetics are essential tools for the investigation of the molecular amyloid mechanisms. In this work we investigated the influence of citrate capped gold nanoparticles on the aggregation kinetics of AS using a fluorescent probe (MFC) sensitive to the polarity of the molecular microenvironment via an excited state intramolecular proton transfer (ESIPT). The particular effects on the half time, nucleation time and growth rate were ascertained. Gold nanoparticles produced a strong acceleration, with an influence on the nucleation and growth phases of the mechanism. The effects were dependent on the size and concentration of the nanoparticles, being strongest for nanoparticles 10 nm in diameter, with a 3-fold increase in the overall aggregation rate at low concentrations as 20 nM.



1308-Pos Board B38

Unfolding Dynamics of the Cyclic Nucleotide Binding Domain and C-Linker of HCN Channels

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The cyclic nucleotide binding (CNB) domain of a variety of proteins is composed by a binding pocket formed by several alpha helices and beta sheets. In HCN channels, the CNB domain is connected to the C-linker, forming the cytoplasmic domain that modulates channel gating. We have performed single molecule force spectroscopy experiments on the hHCN4 construct formed by 203 aminoacids spanning the C-linker and the CNB domain (from aa 521 to aa 723). The construct was deposited on (3-Mercaptopropyl)trimethoxysilane (MPTs) functionalized mica surfaces and we collected Force-distance (F-d) traces in the absence and in the presence of 2mM cAMP. In the presence of cAMP force peaks at contour length (Lc) of 33.4 ± 1.5, 43 ± 1.5 and 52.5 ± 2nm were detected with forces between 45 and 64pN, and at longer values of Lc corresponding to 63.8 ± 2.5 and 84.5 ± 1.5 nm with forces between 86 and 107pN. In the absence of cAMP force peaks at Lc of 34 ± 1.5, 59.5 ± 2 and 65 ± 1.5 are detected with forces between 46 and 52pN, and at Lc of 74 ± 1.5 and 86 ± 2.5 with forces between 80 and 106pN. As alpha helices unfold at lower forces than beta sheets, these results suggest that F-d traces up to a Lc of about 55nm are associated to the unfolding of alpha helices and from 55 to 90nm to the unfolding of beta sheets. The force peak with a value of Lc around 43nm observed in the presence of cAMP is not seen in the absence of cAMP suggesting that several alpha helices are not properly folded in the absence of cAMP, to a greater extent than previously thought (Taraska et al. 2009). These results suggest also that the beta sheet has a different folding than that in the absence of cAMP.

1309-Pos Board B39

Unfolding the Structure of LeuT Employing Luminescence Resonance Energy Transfer

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Background: Neurotransmitter sodium symporters (NSS) are located in the brain and retrieve neurotransmitters from the synaptic cleft to end synaptic transmission. Solute carier class six proteins (SCLC6) are of great pharmacological importance in terms of their localization and function. The crystal structures obtained from a bacterial homolog, the leucine transporter LeuTAa, in open to outward, occluded and open to inward conformations are present in frozen state with high resolution. Due to its close kinship with SLC6 proteins, LeuTAa serves as a paradigm for these transporters.

Methods: In order to address the dynamicity of the substrate transport cycle in LeuTAa, we use the Lanthanide based resonance energy transfer (LRET) technique. This method is a spin-off of the fluorescence resonance energy transfer method according to Förster employing the introduction of the genetically encoded lanthanide binding tags (LBT) as donor elements. Exogenous cysteine residues labelled with cysteine specific fluorophores are used as acceptor elements. This technique is an alternative to address the movement of helices, with great resolution and has been employed successfully to examine potassium channels.

Results: We screened for the functional LBT_mutants using the scintillation proximity assay. The LeuT_A335-LBT-G336 mutant displayed function in terms of its binding activity. Within this background, we generated cysteine mutants. To date, we have successfully measured the intramolecular distances in different LBT_LeuT_Cys mutants. Furthermore, we observed intramolecular distance changes from these purified proteins in detergent micelles.

Conclusion: Our LRET measurements will help us to understand the transport cycle and help to complete the missing steps in substrate transport cycle of LeuTAa. Currently, we focus on the reconstitution of purified LeuTAa into liposomes and have our LRET measurements in a reconstituted system that allows to use more physiological ionic gradients.

1310-Pos Board B40

Determining the Rate of Unfolding and Refolding of FNIII Domains by Labeling Buried Cysteine

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We used thiol reactive DTNB to measure the kinetics of labeling of buried Cys in ten FNIII domains from fibronectin. This gave a comprehensive analysis of unfolding/folding kinetics of each domain, equivalent to analysis by H-D exchange (Table 1). Domains with similar stabilities like FNIII3Cys & FNIII12Cys can have very different folding/unfolding kinetics. In a previous study (JBC 286:26375-82), buried Cys in domains 2,3,11,12,6 labeled with maleimide in FN matrix fibrils. In the present study these are not obviously distinguished from 7, which did not label.

1311-Pos Board B41

Looks can be Deceiving: a Single Mutation on an Ig Domain Alters Dynamics while Conserving Structure. Implications for Ai, a Misfolding Disease

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Light chain amyloidosis (AL) is a misfolding disease characterized by the extracellular deposition of immunoglobulin light chains (LCs) as insoluble aggregates [1]. The lambda 6a germline protein (6aJL2) and its point mutants are models to study AL fibrilllogenesis. R24G is a point mutant with 30% loss in stability and seven times faster fibril formation [2,3] than the germline. To look into the structural and dynamical differences in the native state of these proteins, we carried out MD simulations and NMR experiments at room temperature. Representative solution structures of both proteins are very similar to each other (RMSD heavy atoms ~1Å), with variations in chemical shift values in residues surrounding the mutation in the complementarity determining regions. Despite the structural similarity, we found differences in their dynamical signatures. SEA and deuterium exchange experiments show more protected N-H groups in 6aJL2. Accordingly, backbone solvent exposed area is shifted to lower values, and N-H groups are involved in intramolecular hydrogen bonds more often, in 6aJL2. A flexible and more conformationally diverse native state for R24G is proposed, as side chain entropy and alpha-carbon RMSF values are incremented in almost all residues of R24G, compared to 6aJL2. Also, order parameters at 30°C indicate higher mobility for R24G. This flexibility may allow R24G mutants to reach aggregation-competent states more efficiently than the germline protein.

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1312-Pos Board B42

Super-Long, Single Alpha Helices: A Mechanical Unfolding Study

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Most α-helices in proteins are short and found in positions where stabilising interactions exist with neighbouring secondary structure elements. However, a number of very long, isolated, single α-helical (SAH) domains have been discovered. For example, >100-residue unbroken helices have been observed