hallmark of Parkinson’s disease (PD). The aggregation involves structural transitions from monomeric AS to oligomeric presumably neurotoxic and fibrill 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 hHc4Nc4 construct formed by 203 aminoacids spanning the C-linker and the CNB domain (from aa 521 to aa 733). The construct was deposited on (3-Mercaptocapto)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. In 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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HCN Channels are integral membrane proteins whose activation involves a conformational change of the cyclic nucleotide binding (CNB) domain that modulates channel gating. We have performed single molecule force spectroscopy experiments on the hHc4Nc4 construct formed by 203 aminoacids spanning the C-linker and the CNB domain (from aa 521 to aa 733). The construct was deposited on (3-Mercaptocapto)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. In 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.

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 FNIII1/Cys & FNIII2/Cys can have different unfolding/folding kinetics. In a previous study (JBC 286:26375-82), buried Cys in domains 2,3,11,12,16 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 A1, a Misfolding Disease Target
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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 fibrillogenesis. 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 ~1A), with various variations in chemical shift values in residues surrounding the mutation (i.e., in the complementarity determining regions). Despite the structural similarity, we found differences in their dynamical signatures. SEA and deuteron 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 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_mutation 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 LeuTαα. Currently, we focuss on the reconstitution of purified LeuTαα into liposomes and have our LRET measurements in a reconstituted system that allows to use more physiological ionic gradients.
in caldesmon and certain myosins. This novel structural motif contains a high proportion of charged E, R and K residues, which appear as alternating patches of like-charged residues throughout the sequence. The resulting salt bridge interactions between E and R/K sidechains are thought to stabilise and stiffen the straight helical structure, allowing the SAH to act as a spacer between two flanking functional domains. Here we use single molecule force spectroscopy and molecular simulation to investigate the mechanical unfolding behaviour of the ~100 residue SAH domain from myosin 10. Both methods indicate a globally non-cooperative unfolding process, with unfolding occurring below ~50 pN. Simulations suggest that the SAH domain does differ from a non-charged helix, not only in the stability of the helix but also in the unfolding characteristics under application of force. Enhanced local bonding interactions in SAH domains increases their resistance to force above the baseline level set by a non-charged helix.

1313-Pos  Board B43
Interactions of Urea with the Folded and Unfolded States of Proteins
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Urea is a strong protein denaturant, yet, the mechanism of action of urea on the protein unfolding process is still largely unknown. To tackle this problem, we determined the partial molar volumes, \( V^\alpha \), and adiabatic compressibilities, \( K_{\alpha} \), of a set of model proteins to examine their interactions with urea. Specifically, we measured the partial molar volume and adiabatic compressibility of ribonuclease A, \( \alpha \)-chymotrypsinogen A, lysozyme, and apocytochrome c in aqueous solutions of urea at concentrations between 0 and 8M. At pH 2 and pH3, ribonuclease A and \( \alpha \)-chymotrypsinogen A, respectively, exhibit a two-state transition of unfolding over the range of urea concentrations studied. Even in 8M urea, lysozyme retains its native conformation and apocytochrome c remains unfolded at pH 7. The fact that lysozyme and apocytochrome c do not undergo any conformational transitions in the presence of urea provides an opportunity to study the interactions of urea with proteins in the native and unfolded states within the entire range of experimentally accessible urea concentrations. We analyze our resulting volumetric data within the framework of a statistical thermodynamic model in which each instance of urea interaction with a protein is viewed as a binding reaction that is accompanied by release of two water molecules. From this analysis, we calculate the association constants, \( K \), as well as changes in volume, \( \Delta V^\alpha \), and adiabatic compressibility, \( \Delta K_{\alpha} \), accompanying each urea-protein association event in an ideal solution. By comparing these parameters with similar characteristics determined for low-molecular weight analogues of proteins, we quantify the extent of cooperative effects involved in interactions of urea with any of the proteins studied in this work.

1314-Pos  Board B44
Volume Changes Upon Unfolding of Globular Proteins: Computational and Experimental Studies
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Hydrostatic pressure is an important environmental variable that plays an essential role in biological adaptation. Increasing pressure, much like increasing temperature, perturbs the thermodynamic equilibrium between folded state and unfolded state. In thermodynamic terms stability is defined essential role in biological adaptation. Increasing pressure, much like Hydrostatic pressure is an important environmental variable that plays an

1315-Pos  Board B45
Conformational Flexibility and Structure in High-Pressure Excited States of Amyloglucosidase Revealed by SDSL-EPR
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Proteins exist in an ensemble of conformations at equilibrium, and while higher energy ("excited") states may play important functional roles, under normal conditions they are too sparsely populated to detect. The application of high hydrostatic pressure may be used to populate excited states, but characterization of the high-pressure conformational ensemble is complicated by the presence of multiple conformations exchanging on the ms-ns time scale. The method of site-directed spin labeling (SDSL) in combination with electron paramagnetic resonance (EPR) is ideally suited to explore such excited states. The intrinsic timescale of EPR (ns) is fast compared to that of conformational exchange, so the EPR spectrum captures a snapshot of conformational equilibrium frozen in time and can reveal the existence of multiple conformational substates. For each resolved substate, the spectral lineshape encodes information on the local backbone dynamics and tertiary fold. In the present work, a set of spin-labeled mutants of both holo- and amyloglucosidase were studied in the pressure range 0 - 2 kbar. Many EPR spectra in the well-ordered holomycoglobin native state are essentially pressure independent, demonstrating that the internal motion of the spin label side chain is pressure insensitive. Thus, pressure-dependent spectral changes observed in more flexible systems can be directly interpreted in terms of protein compressibility rather than side chain effects. Pressurization of amyloglucosidase up to 2 kbar populates a low-lying excited state that has been designated a "high-pressure molten globule". Though the work presented here shows it to be distinct from the conventional low pH molten globule that is an intermediate along the folding pathway. Interestingly, the EPR spectra of amyloglucosidase at 2 kbar suggest the existence of a conformation with native-like dynamics within the ensemble of conformations that comprise the high-pressure molten globule.

1316-Pos  Board B46
Single Protein Complexes Isomerization and Conformational Dynamics Using Trapped Ion Mobility Spectrometry: From MS to Seconds
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In the present work, examples of protein and peptide complexes conformational dynamics, from the solvent state distribution to the gas-phase "de-solvated" state distribution, are characterized for traditionally considered "unstructured" complexes. Conformational motifs and isomerization/conformational dynamics are identified and isomerization kinetics in the ms to few seconds timescale are measured for single molecules using a trapped ion mobility spectrometer - mass spectrometer (TIMS-MS). Theoretical calculations are used to simulate the experimental "TIMS box" single molecule -neutral bath gas phase dynamics and candidate structures are proposed for each conformational state. It is found that, side chain and backbone structural changes are the main motifs governing the conformational inter-conversion processes in the ms-s time scale. Examples will be shown for the case of folded/unfolded protein complexes and DNA-binding proteins.

1317-Pos  Board B47
Minima and Barriers on the Pressure-Temperature Free Energy Landscape of Phosphoglycerate Kinase
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Proteins can exhibit strikingly different free energy landscapes depending on which thermodynamic variable is manipulated to study folding. We have investigated the relationship between temperature- and pressure-induced denaturation of yeast phosphoglycerate kinase (PGK). Thermodynamically, the pressure-temperature phase diagram of PGK seems standard: with a slight deviation from two-state behavior at low temperatures, a predicted elliptical shape of stability emerges. Kinetically, the behavior is much more complex: although a temperature perturbation of the energy

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Figure. Example of an isomerization/conformational dynamics pathway for the [M-Zn2]2+ -intracted form of the HAGK2 DNA-binding protein (ATIP) containing the third DNA-binding motif.