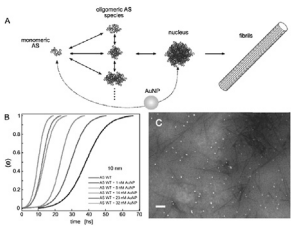
hallmark of Parkinson's disease (PD). The aggregation involves structural transitions from monomeric AS to oligomeric presumably neurotoxic and fibril formation. In spite of its importance for the understanding PD pathobiology and devising rational, targeted therapeutic strategies, details on the aggregation 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