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 Channels
Andrea Pedroni, Anna Moroni, Andrea Alfieri, Loredana Casalis, Paolo Fabris, Vincent Torre
SISSA, Trieste, Italy, Università degli Studi di Milano, Milano, Italy, Eletra Sincrotrone, Trieste, Italy.

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 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 and we collected Force-distance (F-d) traces in the absence and in the presence of 2mM cAMP. In the presence of cAMP traces up to a distance of 43nm observed in the presence of cAMP is not seen in the absence of cAMP (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±1.5 and 52.5±2nm were detected with forces between 45 and 64pN, and at longer values of Lc corresponding to 63.8±0.5, 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±2.5 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. R24G is a point mutant with 30% loss in stability and seven times faster fibril formation than the wild type. 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 variance 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 resonances are incremented in almost all residues of R24G, compared to 6aJL2. Also, backbone solvent exposed area is shifted to lower values, and N-H resonances are incremented in almost all residues of R24G, compared to 6aJL2.

1309-Pos Board B39
Unfolding the Structure of LeuT Employing Luminescence Resonance Energy Transfer
Azmat Sohail, Markus Daerr, Terrence G. Oas, Harold P. Erickson.
Duke Univ Med Cntr, Durham, NC, USA.

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 FNIII3/Cys & FNIII12/Cys 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.

1310-Pos Board B40
Determining the Rate of Unfolding and Refolding of FNIII Domains by Labeling Buried Cysteine
Riddhi S. Shah, Terrence G. Oas, Harold P. Erickson.
Duke Univ Med Cntr, Durham, NC, USA.

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 FNIII3/Cys & FNIII12/Cys 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.