We compared uptake kinetics and aggregation kinetics in buffer and in cell culture medium at different Abeta 42 concentrations to test whether aggregation precedes uptake or vice versa. To compare the uptake of different Abeta species, pre-aggregated Abeta oligomers or small fibrils were added to the cells. We found that pre-aggregation accelerated the formation of intracellular aggregates, which suggests that Abeta oligomers and / or small fibrils may be taken up more rapidly than Abeta monomer.
In the future the form and location of intracellular Abeta will be monitored by high resolution fluorescence nanoscopy combined with atomic force microscopy.

2247-Pos Board B17
Biophysical Studies on Protein Aggregation and Amyloid Fibril Formation
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Protein misfolding leading to aggregation and amyloid fibril formation has been implicated in a number of debilitating human disorders. The primary causative agent is commonly identified to be an aberrant misfolded-form of a protein that self-assembles into oligomers which eventually lead to the formation of ordered cross-β-rich amyloid fibrils. The transiently-populated oligomeric intermediates enroute to amyloid assembly have drawn considerable attention owing to their higher cytotoxicity compared to that of mature amyloid fibrils. Our efforts are directed towards unraveling the mechanisms of amyloid fibrillation using a diverse array of biophysical tools involving steady-state and time-resolved fluorescence, circular dichroism, Raman spectroscopy, dynamic light scattering, electron microscopy and atomic force microscopy [1-3]. Our recent findings on aggregation of an all α-helical protein namely serum albumin revealed that low pH-induced partially-unfolded, molten-globule-like conformers associate to form obligatory oligomeric intermediates that serve as precursors to amyloid fibrils. Comparison of the kinetics of protein conformational- and size changes using multiple structural probes in-tandem indicated that oligomerization followed by conformational conversion leads to the formation of β-rich fibrils. Recently, we have extended our biophysical studies to other proteins such as ovalbumin. I will also discuss our recent results on chain collapse and oligomerization of intrinsically disordered proteins that are capable of forming amyloid fibrils.
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**Equal contribution.*

2248-Pos Board B18
Single Molecule Fluorescence Studies of Amyloid-Beta 1-42 Aggregation
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The proteolytic cleavage of the transmembrane amyloid precursor protein (APP) produces amyloid-β peptides (Aβ) that vary from 38 to 43 amino acids in length. Two of these peptides, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, are the major components of the extracellular amyloid plaques characteristic of Alzheimer's disease (AD). Within these plaques, the Aβ is found aggregated into long polymeric assemblies rich in β-sheet structure that are known as amyloid fibrils. Although the correlation between plaque load and disease severity is poor there is strong evidence that small soluble oligomers of Aβ formed during the early stages of the aggregation process are the agents of AD-associated neurotoxicity (1). Single molecule fluorescence techniques have the potential to resolve the size and structural heterogeneity of these oligomers, which are often difficult to discern by ensemble methods. Most importantly, they allow the characterisation of small oligomeric species at the nucleation stage of the aggregation as the structures of amyloid seeds remain ambiguous (2). Equimolar mixtures of $A\beta_{1\text{-}42}$ singly labelled with either HiLyteFluor-488 or HiLyteFluor-647 were studied using single molecule fluorescence confocal microscopy and FRET, allowing the characterisation of oligomers present during aggregation of monomers and disaggregation of fibrils. Additionally, we have extended our single-molecule studies to examine the species formed during the co-aggregation of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ to understand the interaction at physiological concentrations and ratios. The thorough detection and characterisation of these potentially toxic oligomeric species provides a basis with which to screen therapeutic agents and other modulators of aggregation *in vitro* which could inform *in vivo* studies in the future.
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2249-Pos Board B19
Replica Exchange Statistical Temperature Molecular Dynamics Simulations of Peptide Dimerization
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We present a new computer simulation algorithm called Replica Exchange Statistical Temperature Molecular Dynamics that combines the multicanonical sampling technique of Statistical Temperature Molecular Dynamics with temperature replica exchange. This algorithm is related to Wang-Landau sampling but uses a dynamical update of the density of states to achieve flat energy sampling within a replica-dependent temperature range. This algorithm is used to investigate the thermodynamics of dimerization of two polypeptide chains. Three two-peptide systems are investigated: two hydrophobic peptides, two hydrophilic peptides, and one of each. Each monomer is modeled using a coarse-grained peptide model that has an α-helix as the lowest energy configuration. For each dimer system, interesting folding behavior is observed. It is found that at low temperatures, both peptides are helical and the lowest-energy configuration maximizes inter-peptide contact; at high temperatures, both peptides are random coils; and at intermediate temperatures, one peptide is folded and the other unfolded. Formation of the peptide dimer causes one peptide to fold at a *higher* temperature than an isolated monomer and the other to fold at a *lower* temperature than an isolated monomer. Dimerization causes one peptide to become more stable and the other peptide to become less stable. It is also shown that at intermediate temperatures, neither peptide adopts a random coil configuration: the helical peptide induces a conformational change in the unfolded peptide. The Potential Energy Surface (PES) is also determined for each dimer and the effect of hydrophobic/hydrophilic nature of the peptide on the PES is discussed.

2250-Pos Board B20
Protein Structure in Amorphous Solid Phase
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Protein-based medicines often require freezing or stabilization in carbohydrate glasses for storage prior to use. The structural stability of these proteins is of the great importance in the conditions required for pharmaceutical purposes. Problems involving aggregation and stability of the protein in freeze-dried formulations are of the challenges for the pharmaceutical industry.
Small-angle neutron scattering (SANS) is uniquely qualified to study the structure of proteins in the liquid and solid phases that are biotechnologically relevant for proteins. The structural and conformational changes of a model protein, lysozyme, during the destabilizations in water- ice and carbohydrates systems were studied using SANS and MD simulations. X-ray diffraction measurements were performed to verify existence of cubic and hexagonal ice structures in protein-ice system. Measurements and modelling efforts to understand protein structural changes will be discussed and the interaction distances measured by SANS and proposed model protein structures in different carbohydrate glasses will be compared.

2251-Pos Board B21
Osmolyte Effect on Aggregation of ß-Lactoglobulin Amyloid-Prone Peptides by Explicit Molecular Dynamics Simulation
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Whereas the toxicity of pathogenic amyloids relies on protein misfolding, other nonpathogenic or even functional amyloid structures can regulate physiological activity in a number of domains. Understanding the structural transition within this class of amyloids will provide insights into the general mechanism for ordered to agregation-dependent transitions. We have performed explicit molecular dynamics simulations using GROMACS with GROMOS53A6 force field and SPC water model. We have investigated 6 and 12 peptides of sequence: Ac-y^{146}HIRLSFN^{152}NH_2, from bovine ß-lactoglobulin; theses peptides are known to display high aggregation propensity under specific experimental conditions (5 M urea). We have shown by MD that the peptides form in water and in 5 M urea, in less than 100 ns, a structural aggregate displaying antiparallel ß-sheets, with an hydrophobic core protected from water. Furthermore, we have examined the effect of two different osmolytes (2.5 and 5 M Urea and 1.5 M Trehalose) on the nature of the interactions favoring the ß-structure of