concluded that the possible presence of water may be excluded. Our novel scheme is currently the only way to perform long-time MD simulations involving cation-π interactions with reasonable computational cost and with high accuracy. Based on this analysis, we propose a novel structural and functional element indentified in the active site of T1 lipase.

2925-Pos Board B30
Protein Degradation Kinetics and Biochemical Oscillations
Lida Xu, Zhilin Qu.
Protein ubiquitination and degradation are very important posttranscriptional regulatory processes which are required for biological systems. It is well known that for biochemical oscillations to occur, besides other posttranscriptional regulation, such as phosphorylation and dephosphorylation, the rates of protein synthesis and degradation have to be properly matched. However, the properties of protein degradation kinetics and how they affect biochemical oscillations are not well understood. In this study, we first developed a biochemical reaction model of protein ubiquitination and degradation based on the up-to-date experimental information and calculated the degradation rate against the concentration of the free substrate. The model was tuned to a recent experimental dataset of protein ubiquitination. We show that when the substrate concentration (or synthesis rate) is low the degradation rate linearly increases with the substrate concentration, but as the substrate concentration increases further it becomes nonlinear and eventually saturates. We then studied analytically how linear and nonlinear degradation kinetics affect the instabilities that leads to oscillations using three generic biochemical oscillation models: pure negative feedback induced oscillations (the Goodwin model); pure positive feedback induced oscillations; and combined positive and negative feedback induced oscillations. In all three cases, nonlinear degradation kinetics promotes oscillations and results in much larger oscillation amplitude than the linear kinetics does. On the other hand, the time delay due to protein ubiquitination and deubiquitination generally suppresses oscillations. We finally used computer simulations of cell cycle and circadian rhythm models combined with the detailed protein ubiquitination and degradation model to demonstrate our theoretical predictions from the simplified models.

Protein Aggregates II

2926-Pos Board B31
Halting the Amyloid March: How a Novel Ca_{2+}-Binding Protein, NUCB1, Prevents the Formation of Amyloid Fibrils
Ruchi Gupta, Neeraj Kapoor, Daniel P. Raleigh, Thomas P. Sakmar.
The aggregation of hydrophobic peptides into amyloid fibrils is a characteristic pathological feature observed in Type 2 diabetes, Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease. Both amyloid fibrils and their pre-fibrillar aggregates exhibit toxicity and it is generally believed that prevention of aggregation of pathogenic amyloid peptides would prevent disease progression. In our recent studies, we have discovered a unique anti-amyloidogenic function of the soluble form of human Ca2+-binding protein, NUCB1, namely sNUCB1. We show that sNUCB1 can inhibit amyloid fibril formation of both Amylin and Aβ42 at sub-stoichiometry concentrations. Amylin and Aβ42 are amyloidogenic peptides implicated in the pathophysiology of adult-onset diabetes mellitus and Alzheimer's disease, respectively. sNUCB1 can inhibit amyloidogenesis even after nucleation and protofibrillar stages and the process is reversed at high concentrations of Ca2+. Once fibrils are formed, sNUCB1 can also disaggregate them in a concentration dependent manner but only in the absence of Ca2+. The mechanistic investigation shows that sNUCB1 stabilizes an oligomeric state of hIAPP aggregate. Ca2+-free sNUCB1 binds to and stabilizes a prefibrillar species of hIAPP by ''capping'' the ends. We further demonstrate that Ca2+-free sNUCB1 bound prefibrillar species are dead-end products incapable of seeding the aggregation reaction of hIAPP. Furthermore, sNUCB1 stabilized prefibrillar species of Aβ42 show no toxicity in cell viability assays.

2927-Pos Board B32
Decrease in Size of Hen Egg white Lysozyme Aggregates with Decrease in Monomer Concentration from Micro to Nanomolar in Alkaline pH
Vijay K. Ravi, Kapil D. Singh, Aditya Iyer, C.N. Reddy,
Rajaram Swaminathan.
Earlier work from our laboratory has demonstrated that hen egg white lysozyme (HEWL) is an excellent model protein for investigating protein aggregation at an alkaline pH of 12.2. Exposure of HEWL to this pH at monomer concentrations from 0.3 to 120 uM, yielded amyloid fibrils as shown previously. Here we present the following concentration dependent changes observed with HEWL aggregates subsequent to incubation at pH 12.2 at room temperature from 0 to 24 hours. A) a two-fold increase in exposure of trp residues in HEWL to water at 0.3 uM compared to 120 uM as revealed by fluorescence quenching experiments with iodide B) a several fold increase in ANS fluorescence intensity accompanied by significantly blue-shifted emission on binding to HEWL moving from 0.3 to 120 uM. C) a marginal increase in dansyl probe fluorescence intensity accompanied by significant blue-shifted dansyl emission in dansyl conjugated HEWL moving from 0.3 to 120 uM. D) a gradual increase in steady state fluorescence anisotropy of dansyl probe in dansyl conjugated HEWL moving from 3 to 120 uM, although the dansyl mean fluorescence lifetime remained concentration independent between 3 and 120 uM. E) presence of all cys in -SH form with absence of any change in free [-SH] between 20 & 120 uM, as revealed by DTNP assays. In addition, FCS measurements with rhodamine conjugated HEWL demonstrated facile aggregation of HEWL in the concentration range 25 nM to 120 uM. The above results imply that, the size of HEWL aggregates, are clearly dependent on the initial monomer concentration, with lower concentrations like 300 nM favoring small aggregates that possess solvent exposed trp, diminished binding towards ANS and shorter mean fluorescence lifetime for dansyl probe.

2928-Pos Board B33
Does Thioflavin-T Detect Oligomers Formed During Amyloid Fibril Assembly
Christopher Persichilli, Shannon E. Hill, Jason Mast, **Martin Muschol**.
Deposits of insoluble protein fibrils with cross β-sheet structure are the hallmark of numerous human disorders, including Alzheimer's disease and type II diabetes.Recent results have indicated that oligomeric intermediates, emerging during amyloid fibril assembly, represent the main molecular species responsible for toxicity to cells and tissues. Hence, detection of oligomers is critical for studying amyloid toxicity, for discerning the details of amyloid fibril self-assembly, and for developing inhibitors of oligomer formation. Thioflavin-T (ThT) is among the most commonly used indicator dye for the detection of mature amyloid fibrils *in vitro*. However, it is not clear which of the various intermediates of fibril growth (oligomers, protofibrils and protofilaments) are detected by ThT fluorescence.
To investigate this question, we used ThT for monitoring amyloid fibril formation of hen egg white lysozyme under partially denaturing conditions. We correlated changes of ThT fluorescence with dynamic light scattering (DLS) and atomic force microscopy (AFM) on the same samples. We have previously shown that the combination of DLS and AFM reliably detects all intermediates formed during amyloid fibril growth [1]. Furthermore, we could study the ability of ThT to discern among two distinct pathways of lysozyme fibril formation: oligomer-free assembly *vs.* an oligomeric assembly pathway.
We found that ThT fluorescence did not detect oligomer growth or the nucleation of oligomeric filaments (protofibrils) during lysozyme fibril growth. However, ThT fluorescence increases did coincide with the formation of monomeric filaments (protofilaments) in the oligomer-free assembly pathway. These observations imply that ThT fluorescence is not a generally suitable tool for the detection of oligomeric intermediates during amyloid fibril growth. The selectivity of ThT for protofilaments over oligomers also suggests that the internal structure of oligomeric *vs.* monomeric filaments is distinctly different.
[1] Hill et al., Biophys. J. (2009), 96:3781-3790.

2929-Pos Board B34
Photo-Induced Fibrillar Formation of Chicken Egg White Lysozyme Under Native Conditions
Jin-Bing Xie, Meng Qin, Zhi-Qiang Yan, Yi Cao, **Wei Wang**.
Proteins are generally quite stable under native condition. However, recent findings showed that many proteins can form amyloid-like fibrils under native conditions by introducing destabilizing mutations or inserting amyloidogenic sequences to proteins at the gene level. Can posttranslational modifications at protein level also induce the fibrillar formation? Here we show that alternation of disulfide bonds, as one kind of such modifications, can initialize fibril formation under native conditions. We used UV-illumination to control the breakage and formation of disulfide bonds in chicken egg white lysozyme (CEWL). Through a cassette of tryptophan based photochemistry, the two terminal disulfide bonds in CEWL can be selectively ruptured. Such alternation mildly disrupts the local structure and results in structural flexibility of the C-terminal fragment, which allows the C-terminal fragment hooping between ''open'' and ''close'' states by thermal fluctuation. The ''open'' state can serve as the precursor for fibrillar aggregation. We found that in these fibrils the CEWL molecules are still native-like and connected through intermolecular disulfide bonds, different from those in amyloid-like fibrils with beta-sheet structures. Based on our experimental evidences and all-atom molecular dynamics simulation, we proposed an ''runaway domain-swapping'' model for the structure of CEWL fibrils, in which each CEWL molecule swaps its C-terminal fragment into the complementary position of the adjacent molecule along the fibrils via intermolecular disulfide bonds. Furthermore, we also found that the fibrils