238-Pos Board B7
Analysis of the Role of Aromatic Interactions in Amyloid Formation by Islet Amyloid Polypeptide
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Aromatic-aromatic and aromatic-hydrophobic interactions have been proposed to play a role in amyloid formation by a range of polypeptides including islet amyloid polypeptide (IAPP, Amylin). IAPP is responsible for amyloid formation during type-2 diabetes. The polypeptide is 37 residues in length and contains three aromatic residues Phe-15, Phe-23, and Tyr-37. The ability of all single aromatic to leucine mutants, all double aromatic to leucine mutants and the triple leucine mutant to form amyloid were examined here. Amyloid formation was almost twice as rapid for the F15L mutant relative to wild-type, but was almost three fold slower for the Y37L mutant and almost two fold slower for F23L mutant. Seeds formed from each of the single mutants were effective at seeding amyloid formation by wild-type IAPP, suggesting that the fiber structures are similar. The F15LF23L double mutant has a larger effect than the F15LY37L double mutant on the rate of amyloid formation even though a Y37L substitution has more drastic consequences in the wild-type background than does the F23L mutation, suggesting non-additive effects between the different sites. The triple leucine mutant and the F23LY37L double mutant are the slowest to form amyloid. F15 has been proposed to make important contacts early in the aggregation pathway, but the F15L mutant data indicates that they are not optimal. A set of variants containing natural and unnatural amino acids at position 15 which were designed to conserve hydrophobicity but alter alpha-helix and beta-sheet propensity were analyzed to determine the factors which control amyloid formation. There is no correlation between beta-sheet propensity at this position and the rate of amyloid formation, but there is a correlation with alpha-helical propensity.

239-Pos Board B8
Modulating Amyloid Aggregation by Incorporation of Fluorinated Phenylalanine Derivatives in the Central Hydrophobic Cluster of Aβ10-35
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Aromatic amino acids have been suspected to drive the aggregation mechanism in amyloidogenic peptides by virtue of their hydrophobicity and aromaticity. Alzheimer's disease in particular is associated with the aggregation of a 39-42 residue polypeptide (Amyloid β, Aβ) and its subsequent deposition into amyloid plaques. The aromatic phenylalanine residues (Phe19 and Phe20) in the central hydrophobic cluster (CHC) of the peptide have been suspected to play a significant role in such aggregation. In the present study the amyloid β 10-35 (Aβ10-35) fragment has been used as a model system, where the CHC has been perturbed through the introduction of non-natural amino acids. We have prepared variants where Phe19 and Phe20 have been systematically replaced by pentafluorophenylalanine (F5-Phe) and their affect on amyloid aggregation and kinetics has been studied by biophysical techniques including circular dichroism (CD), fluorescence, UV resonance Raman (UVRR) spectroscopy and chemometrics. While Phe→F5-Phe mutations have been found to enhance conformational stability in Aβ16-22 (Senguen et al. Mol. BioSyst., 2011, 7, p486) by virtue of enhanced hydrophobicity, unfavorable steric interactions brought about by such substitution can also lead to destabilization in some systems (Cornilescu et al., Prot.Sc., 2007, 16, p14). Our experimental studies show that aggregation in both the wild type (wT) Aβ10-35 and the Phe 19→F5-Phe mutant proceeds via a two step conformational transition pathway (wTAβ10-35: T1= 9.18 ±1.4 hrs, T2= 9.79 ±0.44 days, F5-Phe19: T1= 10.72 ± 2.1 hrs, T2= 7.6 ± 0.47 days). However the Phe 20→F5-Phe mutants fail to aggregate into β-sheets as confirmed by both CD and fluorescence studies. Deep UVRR studies have also been performed to probe the fibrillation process and monitor the structural changes brought about by the F5-Phe substitution and their effect on amyloid aggregation.

240-Pos Board B9
Homogeneous Nucleation with Parallel Pathways
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An extension of the thermodynamically-based nucleation theory that has been used successfully to describe sickle cell hemloglobin polymerization is presented. (Ferrone, Hofrichter, Eaton, 1985, JMB 183:611-631) This extension accounts for the possible interference of parallel assembly pathways such as liquid-liquid demixing, or disordered cluster formation, in a mathematically simple and physically intuitive way. We illustrate this theory by application to the crystallization of lysozyme, which exhibits a well-documented liquid-liquid demixing process and thus fills the role of an alternate pathway to assembly. The theory correctly describes an observed acceleration of the nucleation rates upon intersection of the barriers for nucleation and demixing. An interesting corollary of the theory presented here is that it provides a novel explanation for assembly processes that appear to proceed via monomeric nuclei.

241-Pos Board B10
Dissecting the Energies that Stabilize Sickle Hemoglobin Polymers
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Hemoglobin is a tetrameric protein that exists in two quaternary conformations: a T structure which is found in deoxygenated Hb, and an R structure that is found in liganded Hb. At concentrations above a well defined solubility, sickle hemoglobin in the T structure forms long, multistranded polymers (which generate the pathophysiology of the disease). The molecules in these polymers make significant contacts along the polymer axis (axial), which do not involve the point mutation of the disease, as well as diagonally directed contacts (lateral) that involve the mutation site docking into a non-mutant receptor region. We have conducted light scattering measurements to probe initial steps of aggregation (below solubility), as a function of temperature, concentration, primary and quaternary structure. HbS in the T structure shows much higher overall aggregation, but lower enthalphy than R-structure HbS as well as R and T structure HbA, the latter showing aggregation properties very similar to one another. We conclude that at room temperature the axial contacts are significantly weaker than the lateral ones. The enthalpy for the reaction, however, is much greater for the axial contacts than lateral, and axial and lateral strengths will be much more commensurate at physiological temperatures. Unexpectedly, the data require the presence of substantial fractions of dimers in polymerization, or alternatively, of locally stable intermediates, which have stability that is greater than either their predecessors or successors in the reaction pathway.

242-Pos Board B11
Impact of Interfacial Chemical Modifications on the Assembly of an Allosteric Protein - Isothermal Calorimetry and Oxygenation Measurements
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Most allosteric proteins -if not all- present in living organisms tend to associate naturally to form macromolecular assemblies. The classical tenet of allostery invokes that structural changes at quaternary level are responsible for triggering functional changes in the assembly. That is to say, functional changes are not expected to occur in macromolecular systems that lack quaternary structure (with a few exceptions).
In the present work, we have investigated the role of the α1β1 interface in α2β2 tetrameric hemoglobin (Hb), long considered inert, and studied the effects of chemical modifications on this interface on the dissociation of tetramers into dimers, as well as oxygenation properties of tetrameric Hb.
Chemical modifications of sulfhydryl groups of α104Cys and β112Cys present in the α1β1 interface allow the study of intradimeric communication. Reactions of these Cys residues were carried out with dithiopyridine. Disulfide bonded thiopyridyl groups thus produced faced the central cavity of the tetramer.
Tetramers were reassembled and dimer association equilibrium constants and oxygenation properties were measured. We found that any modification on the α1β1 interface produced a pronounced decrease in oxygen affinity. Surprisingly, the dimerization of the ligated derivatives was enhanced rather than impaired. These results suggest that the chemically modified α1β1 interface of the dimer produced such a striking effect. In other words, the quaternary effect originated from the dimer rather than the tetramer.

243-Pos Board B12
A New Non-Canonical Control Mechanism in an Allosteric Protein -An Inert Interface Comes to Life
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The classical tenet states that allosteric proteins exist in at least two different conformations, each one exhibiting distinct functional characteristics that interconvert each other. Basically, a conformational change leads to a functional change. This is accomplished by proteins that almost in all cases (with very few exceptions) are oligomeric, i.e., are formed by two or more subunits. This implies that, as it is in the minimal case of a dimer, at least one protein interface is present.
In the case of the hemoglobin, according to the Monod-Wyman-Changeux, Two-State Concerted Model, the archetypal allosteric protein exists theoretically in either a tense (''T'') or relaxed (''R'') conformation. X-ray crystallographic studies have revealed that these two conformations correspond to

two different structures. 1H-NMR studies have shown that either structure is characterized by specific sets of salt bridges formed between residues on both αβ dimers, the so-called α1β2 (or α2β1) interdimeric interactions. The accepted consensus states that the structural change consists of a ~15°rotation of one dimer over the other when converting from ''T'' to ''R'', and vice versa, whereas the αβ dimers themselves do not experience any conformational change, i.e., the α1β1 (or α2β2) interface remains unaltered. Functionally, ''T'' and ''R'' are characterized by low and high affinity for the ligand, respectively.
In the present work, we have altered chemically this allegedly inert intradimeric α1β1 interface and found striking functional changes that cannot be explained in terms of the canonical allosteric model, yet ''T'' and ''R'' structural traits were unequivocally present. This finding exemplifies the functional versatility that a protein can attained, exceeding the limits of what is called ''of physiological significance''. Experimental data that support this finding will be presented.

244-Pos Board B13
The Effect of Small Regulatory RNA on Globular Protein Aggregation
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Small regulatory RNA segments, such as microRNA (miRNA) or silencing RNA (siRNA) binding to proteins could effect the association mechanisms of protein aggregation. Extended protein aggregation, known as fibrillization, has links to degenerative diseases like Alzheimers or prion disease. The formation of fibrils is thought to be a non-specific property of proteins, and can be demonstrated with well-known model globular proteins like serum albumin, lysozyme, beta-lactoglobulin and the like. Much research has been devoted to this field, but with the recent discovery of micro/silencing RNA, small regulatory RNA generally less than 70 base pairs, the question arises on the effect of these nucleic acids on the aggregation process behind fibril formation. Therefore, this study attempted for the first time to probe two effects in the fibrillization process: first, the binding affinity of the selected microRNA MIR106A to the model proteins lysozyme and bovine serum albumin in fibril forming conditions, and second, the long-term effect of the protein-nucleic acid complex on the fibril formation process. Fluorescence spectroscopy, to include time-resolved anisotropy decay of fluorescein or dansyl-labeled complexes, and sizing techniques like atomic force microscopy to track aggregation patterns were incorporated.

245-Pos Board B14
Clarifying Alpha Crystallin Chaperone Function by using an Insulin B-Chain Aggregation Model
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James A. Hebda, **Patricia B. O'Hara**.
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The crystallin family of proteins exist in high concentrations in the lens of the eye by are also found throughout the body. In the lens, alpha-crystallin isoforms A and B exist in a ratio of 2 to 3. Their role in the eye is to provide a structural matrix and prevent protein misfolding. The inducible aggregation of the insulin-B chain serves as a model to the protein aggregation that occurs in the human lens over time. This protein aggregation contributes to lens opacity, which is the beginning of cataract formation. In this study, we explore the kinetic and thermodynamics of such system in order to understanding the mechanism of alpha-crystallin chaperone function in insulin aggregation. Insulin aggregation can be measured through light scattering. Our preliminary result suggest a mechanism for aggregation in which a B-chain insulin dimer formation precedes aggregation and that alpha crystallin and insulin monomer form a 2 to 1 complex that inhibits this aggregation. Thermodynamic and kinetic constants will be presented for the purified alpha chrystallins as well as mixtures and bulk protein.

246-Pos Board B15
Structural Metal Mediated Self Assembly of Collagen Mimetic A:B:C Heterotrimer Peptides into Higher Oder Structures
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Collagen is the most abundant protein in the human body with many favorable properties making it an attractive target for biomaterials. Currently, animal derived collagens are utilized in biomedical, cosmetics and for other applications. But, they are costly to purify and risk of prior contamination. Synthetic collagen can overcome some of these issues and potentially allow greater control of material properties. Recently, collagen like homotrimer peptides has been utilized to form metal trigger higher order structures. In this study we present the case of a higher order structure formed by heterospecific A:B:C collagen like peptides using a structural metal. Here using electrostatic interactions in conjunction with the metal binding site to drive the formation of higher order assembly of heterotrimer collagen like peptide. The assembled structures range from particle to fibers to disks. We hypothesize that using these heterotrimer peptides will allow control over the higher order structures and chemical functionality of biomaterials. Also, the insight gain from this study will help to improve the molecular design of biomimetic materials.

247-Pos Board B16
Aggregation of Trp>Glu Mutants of the Human Gamma-D Crystallin: A Model for Hereditary or UV-Induced Cataract
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Cataract is a protein misfolding disorder resulting from formation of light-scattering aggregates of proteins from the crystallin family. These long-lived proteins account for 90% of all protein in the human eye lens. The γD crystallin consists of two symmetrical domains, each of which has a duplicated Greek key fold. Maintaining this topologically complex native fold of the γD crystallin is essential for lens transparency. A number of point mutants in the γD crystallin gene cause early-onset cataract. UV-B exposure accelerates cataract onset, and UV irradiation of purified wild-type γD results in aggregation in vitro. A distinctive feature of the Greek key fold is the presence of highly conserved buried tryptophans. Replacements of tryptophan by charged glutamate groups may represent a model of UV-induced photodamage – introduction of a charged group into the hydrophobic core generating ''denaturation from within.'' We show that such Trp>Glu mutants can display vastly increased aggregation propensity under physiological conditions *in vitro*. Furthermore, a striking property of these mutants is their ability to drive the wild-type protein into the aggregated state. Domain swapping mechanisms can account for this aggregation behavior.

248-Pos Board B17
Artificial Honeybee Silk: A Recombinant Protein as a Biomimetic Structural Material
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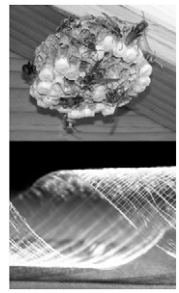
Honeybee larvae produce silken cocoons that provide mechanical stability to the hive. The silk proteins are small and non-repetitive and therefore can be produced at large scale by fermentation in *E. coli*. Recombinant silk proteins which have a coiled coil structure can be fabricated into a range of forms including sponges and fibers. The resultant material is soluble in water and requires a post-production stabilizing treatment. Aqueous methanol treatment induces the formation of a stabilizing ß-sheet structure, with the amount of ß-sheet being controlled by time or methanol concentration. Dry heat treatment at 190°C also produces a water insoluble material but without significant secondary structural changes. Honeybee silk proteins are particularly high in lysine, serine, threonine, glutamic acid and aspartic acid. The stability of the heat treated material is attributed to the generation of covalent cross-links including lysinoalanine and isopeptide groups. The unique ability to stabilize material by controlling secondary structure rearrangement and covalent cross-linking allows us to design recombinant silk materials with a wide range of properties and potential applications.



249-Pos Board B18
Self-Replicating Amyloid-Beta Oligomers Open Doors to New Molecular Mechanisms in Alzheimer Disease
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Aggregates of amyloid-β (Aβ) peptides have been implicated in the etiology of Alzheimer disease (AD). Among the different forms of Aβ aggregates, low molecular weight species ranging between 2- and 50-mers, also called ''soluble oligomers,'' have emerged as the species responsible for early synaptic dysfunction and neuronal loss. Emerging evidence suggests that the neurotoxic oligomers need not be formed along the obligatory nucleation-dependant fibril formation pathway. In our earlier work, we reported the isolation of one such ''off-pathway'' 12–18-mer species of Aβ42 generated from fatty acids called large fatty acid-derived oligomers (LFAOs) (Kumar, A., Bullard, R. L., Patel, P., Paslay, L. C., Singh, D., Bienkiewicz, E. A., Morgan, S. E., and Rangachari,