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