the long fiber axis while the β-strands run perpendicular (cross-β) to the fiber axis. β-helices are a parallel β-sheet motif that have similar properties to amyloid fibers. In this work, we explore the utility of the β-helix as a building block to capture and test attributes of amyloid fibers relevant to the design of self-assembling nanostructures. We have isolated and characterized a five-run portion of the triple-stranded, homotrimeric β-helix, GPS, of the bacteriophage T4. In order to construct a self-assembling β-helix, we have developed a small library based on amyloid sequence analysis and applied it to the five-run helix. Specific changes targeting internal interactions of the helix have been made. The mutations in these positions have been selected to allow for greater control of self-assembly and hold the promise of breaking the homonomic assembly into a heteromeric construction. A split adenylate kinase selection assay has been developed to monitor self-assembly of these alternative structures.

2925-Pos Board B80
Simulations of Glutathione Synthetase Indicate Conformational Asymmetry that may Contribute to Negative Cooperativity
Sasi Kodathala, Brian W. Beck.
Texas Woman’s University, Denton, TX, USA.
Glutathione (GSH), a major anti-oxidant found in all eukaryotic cells is produced by a homodimeric enzyme, Glutathione Synthetase (GS). The GS dimer is an allosterically negatively cooperative enzyme that loses affinity for substrate after initial binding of substrate. In our previous work, energy minimization calculations on fully solvated monomeric and dimeric conformations of GS, each with and without substrate bound, demonstrated differences in energies and conformations between some of the sequence identical residues of monomers of dimeric GS, suggesting GS may occupy energetically asymmetric conformations. Here, we report the analysis of 1 ns Molecular Dynamic (MD) simulations on fully solvated dimeric conformations of GS, with and without substrate. The differences in root mean square deviation (RMSD) along with the different energies of interface residues over time indicate intrinsic asymmetry between monomer conformations. These energy differences and the positions of the residues relative to the active site and the dimer interface suggest the monomers may exist in distinct conformational ensembles whose relative frequencies are asymmetrically shifted to be favorable to initial substrate binding, thus potentially contributing to the observed negative cooperativity.

2926-Pos Board B81
Multibody Force Fields: Characterizing Intrinsic Correlation within the PDB
Jared J. Thompson.
Purdue, West Lafayette, IN, USA.
Although dynamics simulations are a powerful tool in research, the most commonly used methods have limitations with respect to the time scales that can be achieved, even on the most sophisticated hardware available. To mitigate this problem, there has been significant research in structural abstraction or “coarse-graining” simulations. However, the process of coarse graining necessarily excludes potentially relevant information from an atomic-level representation and may ignore terms that describe higher-order interactions. Limited attempts to reintroduce this information back into simulations have employed multibody terms to simulate secondary structures such as β-sheets, however more complex attempts at including multibody interactions have not yet been published. In this offering, we describe our findings with respect to characterizing multibody interactions found within the PDB and explorations of potentially employing this work in the framework of a forcefield.

2927-Pos Board B82
Conservation of Functionally Important Global Motions in an Enzyme Superfamily across Varying Quaternary Structures
Emily K. Luebbering.
University of Missouri, Columbia, MO, USA.
The α-2-phosphohexomutase superfamily comprises enzymes involved in carbohydrate metabolism that are found in all kingdoms of life. Recent biophysical studies have shown for the first time that several of these enzymes exist as dimers in solution, prompting an examination of the oligomeric state of all proteins of known structure in the superfamily (11 different proteins; 31 crystal structures) via computational and experimental analyses. We find that these proteins range in quaternary structure from monomers to tetramers, with six of the 11 known structures being likely oligomers. The oligomeric state of these proteins is associated in some cases with enzyme sub-group (i.e. substrate specificity), but also appears to depend on domain of life, with the two archaeal proteins existing as higher order oligomers. with the oligomers, three distinct interfaces are observed, one of which is found in both archaeal and bacterial proteins. Normal mode analysis shows that the topological arrangement of the oligomers permits domain 4 of each protomer to move independently as required for catalysis. Our analysis suggests that the advantages associated with protein flexibility in this enzyme family are of sufficient importance to be maintained during the evolution of multiple independent oligomers. This study is one of the first showing that global motions are conserved not only within protein families, but across members of a superfamily with varying oligomeric structures.

2928-Pos Board B83
A Web Site for Detecting Protein Structural Domain Neighbours
Jean Garner1, Franck Samson1, Richard Shrager2, Jean-François Girbat1, Chin-Hsein Tai3, Victhret Sam3, Peter J. Munson2.
1INRA, Jouy en Josas, France, 2NIH, Bethesda, MD, USA.
A query protein structure is compared with the VAST program to a database of target structures from the PDB (PDB40, list of protein structures having less than 40% of identical residues; 19 500 structures version 2011). The threshold of the VAST program is lowered in order to find the largest possible number of structures having a local similarity with the query protein. The purpose of the web site is to define structural domains in the query protein using the recurrence of these locally similar substructures. (http://genome-jouy.inra.fr/domire/). The list of matches is subsequently sorted according to two criteria: the number of aligned residues by VAST is at least 40% of the number of residues of the target, and 80% of the target length is aligned including gaps of non aligned residues if less than 40. Besides this list, a residue-residue alignment of the structural neighbour on the amino acid sequence of the query protein is provided together with a 3D view of their superposition. The object of this sorting is to help in detecting remote homologues and isolated protein structures matching the domain structures of a protein.

2929-Pos Board B84
Development of Semi-Synthesis Methods to Incorporate Fluorophore/ Thiouamide Pairs into Proteins
Rebecca F. Wisnser, Solongo Batjargal, E. James Petersson.
University of Pennsylvania, Philadelphia, PA, USA.
A thioamide, a single atom substitution on the peptide backbone, can be used as a minimally perturbing probe for various applications. Previously, the incorporation of thioamides has been limited to synthetic small peptides. Here, we demonstrate that thioamides in both C-terminal thiosters and N-terminal Cys fragments are compatible with native chemical ligation conditions. Recently, we have shown that p-cyano phenylalanine is quenched by a backbone thioamide in a distance-dependent fashion. Unnatural amino acid mutagenesis and native chemical ligation can be used together to site-specifically incorporate this minimally perturbing spectroscopic pair into a protein of interest. As a proof of principle, we have generated thioamide labeled versions of the amyloid protein α-synuclein and monitored its conformational changes during aggregation by fluorescence quenching.

2930-Pos Board B85
NIST/UMD Biomolecular Labeling Laboratory (B12)
Zvi Kelman.
National Institute for Standards and Technology, Gaithersburg, MD, USA.
The NIST/UMD Biomolecular Labeling Laboratory (BL2) is a joint facility of the National Institute of Standards and Technology (NIST) and the University of Maryland (UMD). The mission of the facility is to provide users the environment, equipment, and expertise necessary to produce stable isotope labeled biomolecular samples so they can be used for structural and biophysical methods including, but not limited to, neutron scattering, NMR and mass spectroscopy. The facility intends to support research performed at NIST, UMD and other universities and institutions from the US and other countries.

Molecular Chaperones

2931-Pos Board B86
Acid-Induced Activation of the Periplasmic Chaperone HdeA
Linda Foit1, Bin Zhang2, Jenny George3, Lucia Brunetti4, Charles Brooks III2, James Bardwell5.
1University of Michigan/HHMI, Ann Arbor, MI, USA, 2University of Michigan, Ann Arbor, MI, USA.
Enteric bacterial pathogens like Escherichia coli are able to survive passage through extremely acidic environments like the human stomach. This acid-resistance strongly depends on the activity of the small periplasmic chaperone HdeA. Inactive as a dimer at neutral pH, HdeA elegantly uses its own acid-induced unfolding and monomerization to become rapidly activated upon shift to low pH. In its active and intrinsically disordered monomeric state, HdeA tightly binds to acid-unfolded client proteins. Upon pH neutralization, HdeA slowly releases its client proteins, allowing them to refold.