1066-Pos Board B17
Identification and Characterization of a Yeast Iso-1-Cytochrome C C-Terminal Domain Swapped Dimer
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Domain swapped protein dimers consist of a swapped domain linked by a hinge loop. They have been proposed as a means of achieving larger assemblies potentially contributing to biological cellular activity or conferring disease. Here we present a crystal structure of a C-terminal domain swapped dimer in yeast iso-1-cytochrome c. In this structure the C-terminal alpha helix from one monomer positions itself in the native position of the opposite monomer and vice versa. The highly dynamic heme crevice loop, the most highly conserved portion of the cytochrome c sequence, spans the gap acting as the hinge loop. Interestingly, conversion of the heme crevice loop to the hinge loop results in a loss of the native Met80-heme ligation. This produces an open heme coordination site on each subunit of the dimer. As cytochrome c requires an open heme coordination site to act as a peroxidase, to oxidize cardiolipin and initiate the intrinsic apoptotic pathway, this dimer structure could potentially be a structure particularly suited to function in oxidizing cardiolipin. In fact, a recently reported C-terminal domain swapped dimer of horse cytochrome c demonstrates increased peroxidase activity relative to the monomer. Although the yeast and horse dimer are similar, the hinge loop orientations differ. The hinge loop is two residues longer in the yeast dimer resulting in an increased distance between the heme groups and an altered angle of the hinge loops relative to the horse cytochrome c dimer. Even though they contain similar structure and sequence, the domain swapped yeast iso-1-cytochrome c dimer demonstrates decreased stability compared to the horse cytochrome c dimer.

1067-Pos Board B18
Heme Coordination Versatility in a Truncated Hemoglobin
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GlbN, the ''truncated'' hemoglobin from *Synechocystis* sp. PCC 6803, coordinates the heme iron with His46 (E10, distal) and His70 (F8, proximal). Displacement of His E10 by an exogenous ligand (e.g., cyanide in the ferric state) drives a significant conformational change allowing Tyr22 (B10), Gln43 (E7) and Gln47 (E11) to establish a hydrogen bond network stabilizing the distal ligand.
THB1, a closely related hemoglobin from *Chlamydomonas reinhardtii*, also binds cyanide and forms the same network of interactions. However, in the absence of an exogenous ligand, the neutral amino group of Lys53 (E10) coordinates the heme iron on the distal side [1]. Because of the importance of the coordination scheme in controlling the reactivity of heme proteins, the flexibility of the truncated hemoglobin fold, and the rarity of lysine coordination, we explored the ability of GlbN to use lysine at position E10 as an axial ligand. The His46Lys replacement yielded a protein with UV-Vis and NMR spectra similar to those of His46Leu and comparable pH response, suggesting that GlbN does not accommodate lysine coordination at position E10. However, the spectra were incompatible with water coordination and suggested that in the ferric state these variants were low-spin endogenously hexacoordinate complexes. We performed amino acid replacements within the distal H-bond network to characterize the perturbed heme environment and ligand sets. A combination of pH titrations and NMR experiments illustrates the delicate balance of interactions governing the heme pocket conformation of GlbN.
[1] Johnson et al. (2014) *Biochemistry* 53:4573
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1068-Pos Board B19
Modeling the Calcium and Integrin Binding Protein 2
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The inhibition of the first calcium and integrin binding protein, CIB1 has been observed to interfere with signaling in breast-cancer cells by inducing non-apoptotic cancer cell death without harming healthy cells. This has led to a push for creating targeted therapy drugs which inhibit the CIB1 protein. While inhibiting CIB1 kills cancer cells, inhibiting the second protein, CIB2, could lead to hearing loss or deafness. Since CIB2 and CIB1 share similar sequences, I wanted to determine whether CIB2 could be inhibited by the potential targeted therapy drugs used to inhibit CIB1. Since no structure for CIB2 exists, I first created a homology model for the protein by threading the sequence for CIB2 onto the 2LM5 PDB structure of CIB1. This model was relaxed using Discrete Molecular Dynamics and analyzed for stability. Maestro and Peptide Docking simulations were then used to examine the model for druggable sites and association sites. The druggable sites were used for virtual screening with MedusaDock, where the results for CIB2 were to be compared to a previous virtual screening done for CIB1. Any drugs that were found to bind to both proteins would be excluded as targeted therapy candidates. By analyzing the model I was able to determine that CIB2 has one bipartite druggable site and two peptide association sites. Since the peptide association sites and druggable site on CIB2 were different in location and composition to the peptide and druggable site on CIB1, I concluded that the targeted therapy drugs created for CIB1 will have no effect on the functioning of CIB2. In the future, my lab intends to determine which association site on CIB2 corresponds to the actual peptide-binding site, as well as analyze the virtual screening results and experimentally validate the data from our simulations.

1069-Pos Board B20
Structural Behavior of Cardiac Troponin C Variants Present in Cardiomyopathic Patients
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Cardiac Troponin C (TnC) is a Ca^{2+}-binding protein and plays an important role in regulation of muscle contraction. Mutations in cTnC are implicated in phenotypic characteristics known as hypertrophic and dilated cardiomyopathy (HCM and DCM, respectively). However, the structural mechanisms underlying cardiac dysfunction are unknown. The main goal of this work is to investigate changes in stability and dynamics of seven cTnC variants (A8V, D145E, C84Y and A31S related to HCM; and Y5H, M103I and I148V related to DCM) using an ensemble of thermodynamic and structural approaches. Ca^{2+}-titrations monitored by bis-ANS fluorescence revealed that D145E, A31S and all mutations related with DCM decreased the Ca^{2+}-induced hydrophobic exposure, while C84Y substantially enhance it by the N-domain exposure compared to WT. Thermostability monitored by circular dichroism revealed similar melting temperatures between apo and holo states for D145E (apo: 66.4 $\pm$ 1.4°C, holo: 65.4 $\pm$ 1.6°C) but different values for WT (apo: 65 $\pm$ 1.9°C and holo: >90°C) and C84Y (apo: 43.8 $\pm$ 1.5°C, holo: 66.6 $\pm$ 0.8°C). Shape restorations from small angle X-ray scattering were used to evaluate conformational changes induced by the mutations. In the apo state, an increase in the radius of gyration values upon increasing concentrations of urea was observed for the Y5H, A31S, M103I and I148V mutants compared to WT. Furthermore, the D145E displayed the most affected shape compared to WT and perturbed residues were located at the C-domain as confirmed by chemical shift perturbation analysis. In addition, the D145E secondary structure was not significantly altered by dihedral angles prediction from the NMR assignment data. These observations open up new avenues for the comprehension of the complex behavior of HCM and DCM mutations in cTnC that has heretofore been not evaluated at structural level.

1070-Pos Board B21
Methionine-Aromatic Interactions in Calmodulin: A Replica Exchange MD and EPR Spectroscopy Study
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Methionine oxidation introduces methionine-aromatic interactions that play a significant role in secondary structure conformation and stabilization. Calmodulin (CaM) contains nine methionine residues that function as targets of reversible oxidation, serving as a mechanism through which the cell senses and responds to oxidative stress. Replica exchange molecular dynamics (REMD) simulations illustrated that methionine oxidatin of the N-terminal helix of CaM introduces two configurations that involve oxidized methionines at positions 144 and 145 interacting with tyrosine 138. As these configurations do not occur in the unoxidized helix, we propose the conformational change is induced by the stabilizing methionine-aromatic interaction. To verify the effect, electron paramagnetic resonance (EPR) spectroscopy performed with CaM at submicromolar [Ca^{2+}] with probes near the residues of interest revealed two populations in the oxidized sample and only one in the unoxidized sample,