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 cardiolin and initiate the intrinsic apoptotic pathway, this dimer structure could potentially be a structure particularly suited to function in oxidizing cardiolin. 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.

Heme Coordination Versatility in a Truncated Hemoglobin
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GibN, 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), Glu43 (E7) and Glu47 (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 GibN 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 GibN 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 GibN.

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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 shown to 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 peptide association site 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.

Structural Behavior of Cardiac Troponin C Variants Present in Cardiomyopathic Patients
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Cardiac Troponin C (TnC) is a Ca2+-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 5YH, M103I and 1148V related to DCM) using an ensemble of thermodynamic and structural approaches. Ca2+-titrations monitored by bis-ANS fluorescence revealed that D145E, A31S and all mutations related with DCM decreased the Ca2+- 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 ± 1.4°C, holo: 65.4 ± 1.6°C) but different values for WT (apo: 65 ± 1.9°C and holo: >90°C) and C84Y (apo: 43.8 ± 1.5°C, holo: 66.6 ± 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 YSH, A31S, M103I and 1148V 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.

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 oxidation 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 halting methionine oxidation reaction. To verify the effect, electron paramagnetic resonance (EPR) spectroscopy performed with CaM at sub-micromolar [Ca2+] with probes near the residues of interest revealed two populations in the oxidized sample and only one in the unoxidized sample,
agreement well with the simulation and indicating that oxidation is responsible for a stable conformational shift. This conformational change induced by the noncovalent interaction between oxidized methionine and tyrosine’s aromatic ring could be the mechanism by which CaM responds to changes in the redox environment in a reversible manner.

1071-Pos Board B22
Targeting Melanoma with Small Molecules: Inhibitors of the Calcium-Binding Protein S100B
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Long used as a prognostic indicator, the calcium binding protein, S100B, has been directly linked to Malignant Melanoma (MM). S100B binds the typically wild-type tumor suppressor in MM and promotes its degradation. In order to restore p53 levels within MM along with its tumor suppressor and apoptotic activities, we have implemented a program for the discovery and optimization of S100B inhibitors (often referred to as SBIxS). In this process, we have uncovered and probed the three persistent binding sites within S100B. Liganding within these sites was characterized using structural biology techniques (NMR and X-ray crystallography) and inhibitor efficacy was evaluated using Fluorescent Polarization Competition Assays and cellular assays. Efforts to discover/synthesize and/or to improve existing inhibitors of S100B to restore p53 activity in human malignant melanoma are ongoing and SBIxS occupying the persistent binding Sites 1, 2, and 3 simultaneously are desired. Such compounds can then be examined for in vivo efficacy. The compounds presented offer potential bridging scaffolds between Sites 1, 2, and 3; and will act as the basis for the design of improved SBIxS. SBIxS may also have therapeutic value for treating other cancers with elevated S100B and wt p53 such as astrocytoma, renal tumors, and some forms of leukemia.

1072-Pos Board B23
Crystallization Studies of Calmodulin Binding Targets
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In a recent scientific study, 70 new calmodulin (CaM)-binding targets have been identified in human brain (1). For this research project, peptides corresponding to the CaM binding portion of a number of these targets, including, LAT1, RPS2, STIM1, CaMkv, and Rab11b, have been synthesized and purified. In a couple of cases, including Rab 11b, the full length protein, has also been expressed and purified. The ultimate aim is to obtain an understanding of how CaM can regulate so many targets with such selectivity. Toward this goal we are collecting target binding data as well as structures of a range of peptides and proteins in complex with CaM using x-ray crystallography. The peptides described in this work have been synthesized using Fmoc solid peptide synthesis and the Rab11b protein was expressed in E. coli and purified using a series of column chromatography steps. Crystallization conditions have been optimized for a specific subset of these complexes. Diffraction data were obtained for CaM-LAT1 at the Max Lab synchrotron facility in Lund, Sweden. The data obtained were then analyzed at Colgate University, NY, employing the software, Reffmac5, to furnish a crystal structure with a resolution of 2.1 Å and an R-factor of 25%. We will also present preliminary binding data of the LAT1 sequence bound to CaM, determined to be in the μM range, and obtained by isothermal titration calorimetry (ITC).


1073-Pos Board B24
Tuning of Structure-Function Relationships by Macromolecular Crowding
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The cellular interior is crowded with high concentrations of macromolecules and small organic solutes and strict maintenance of this environment, where ~30% of the total volume is occupied, is essential to cellular function. The volume occupied by these molecules confines the 3D space in which any particular protein can reside and is thought to modulate protein-protein interactions by affecting diffusional encounter and reaction rates. In this work, we investigate the mechanistic consequences of crowded environments on the structure-function relationship of the ubiquitous protein calmodulin (CaM). CaM is highly flexible and dynamic, making it particularly susceptible to crowded environments, and its conformational plasticity is essential for accommodating binding to its 300 identified targets. We determined that crowded environments, created with purified polymer systems and sucrose, stabilize compact conformers of CaM, reduce translational and rotational diffusion, modulate association and dissociation kinetics with a calmodulin-binding target, and control rates of conformational transition. Most notably, these effects were determined by the size and concentration of the crowding reagent indicating that the composition of the environment differentially tunes CaM structure, dynamics, and target association kinetics. Our results are shown in a general kinetic framework where there are consequences for target selectivity and cellular signaling.

1074-Pos Board B25
Understanding the Structural Changes of the Calcium Binding S100A1 Protein with Molecular Dynamics Simulations
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The S100A1 protein is ubiquitous in the human body and is particularly localized to the heart and brain tissue, within which it may influence the onset of diseases such as cardiomyopathy and Alzheimer’s disease. S100A1 is a homodimer that binds four calcium ions to drive a conformational change, whereby two helices separate to expose target protein binding sites. However, the physiochemical drivers of this conformational transition between the apo (no bound ions) to the holo (bound ions) states remain unclear. To understand the atomistic basis of conformational changes in S100A1, we performed nanosecond-scale molecular dynamics simulations (MD) of the apo and holo states in explicit solvent. These MD studies reveal a variety of conformational trends including helical orientations, hydrogen bond contacts, and backbone fluctuations of the calcium binding regions. In response to previous studies demonstrating that the addition of polar functional groups to cysteine residue 85 (Cys85) increases the calcium ions’ binding affinity, we furthermore examined the influence of glutamic acid and glutamine mutations at site 85 (Cys85Glu/Cys85Gln) on the aforementioned conformational behavior. We finally relate structural differences between the apo and holo states for the wild-type and Cys85 mutant cases to alterations on S100A1’s experimentally observed alterations in calcium handling. We speculate that similar trends may emerge in similar calcium-binding proteins.

1075-Pos Board B26
Single-Molecule FRET Studies of the ER Calcium Sensor STIM1
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The ER membrane (EM) protein STIM1 is the ER Ca2+- sensor that initiates store-operated Ca2+ entry through Orai channels in lymphocytes and many other cells. Communication of ER Ca2+ store depletion to Orai channels in the plasma membrane involves a transition of STIM1 dimers from an inactive to an active state, but the conformational changes underlying this transition remain poorly characterized. We have developed an in-vitro assay to study the conformation and dynamics of STIM1 at the single-molecule level. Purified STIM1 cytosolic fragments were labeled at single sites with Alexa555 and Alexa647 by site-directed mutagenesis and cysteine-nucleimide chemistry. These served as donor and acceptor dyes for high-resolution intradimer distance measurements using single-molecule FRET. Labeled STIM1 fragments were encapsulated in 100nm diameter, surface-immobilized liposomes and imaged with a prism-based TIRF microscope. We identified three broad regions within the STIM1 fragments, defined by their characteristic FRET signatures. Within the ER-proximal N-terminal region containing the predicted calcium-binding domain (aa74-339), we observed several transiently stable conformational states, ranging from closely apposed (FRET ~0.8) to widely separated (FRET ~0). The different FRET states occurred with similar probability, and transitions between them were frequent. In the region 363-449, encompassing the CRAC activation domain (CAD), stable intermediate to high FRET values (0.6-0.9) were dominant, indicating close apposition of paired sites in the dimer. Interestingly, low-FRET states (0.1-0.4) were also observed at several sites, suggesting the existence of an open conformation with relatively low probability. Finally, C-terminal to the CAD (aa512-660) we found stable, broad low-FRET signals, consistent with the predicted unstructured nature of this region. FRET ratios progressively declined from ~0.2 to 0 towards the C-terminal end, indicating increasing separation. Further development of this approach will enable direct, high-resolution probing of STIM1 activation under controlled conditions.