non-enzymatic, post-translational protein modifications such as racemization and isomerization. While in many cases aged or non-enzymatically damaged proteins can be repaired or replaced, some changes can inhibit protein turn-over resulting in an accumulation of damaged protein over time. One of the modifications that very-long lived proteins have been shown to undergo is racemization of aspartic acid and asparagine residues to the non-natural D-form from the common L-form via succinimide intermediate. In this study we have employed quantitative label-free mass spectrometry to study isomerization/racemization reactions site specifically in the repeat-1 domain of Tau protein. We found that Asp-265 isomerizes into four different isoforms. We also found that the Tau peptide undergoes an auto-catalytic cleaveage without the use of a protease. A possible mechanism for the formation of the four isomers is proposed. This data may provide useful information on the *in vivo* rates of tau racemization, providing insights into the mechanisms and potentially opening a door to finding biomarkers for Alzheimer's disease.

2395-Pos Board B87
Coupling Evolutionary Information with Functional Dynamics to Reengineer the Oligosaccharide Specificity of Cvn with Bp-Dock
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Cyanovirin-N (CVN) is a cyanobacterial lectin with potent anti-HIV activity, mediated by binding to Man(1,2)Man with high affinity and specificity. These sugar-binding sites are located in two quasi-symmetric domains (A and B) of the CVN protein. We explore both the evolutionary and functional dynamics of CVN and reengineer CVN mutants with different binding affinity for the oligo-mannosides, through integrated computational and experimental strategies. We perform statistical analysis on CVN homologs to find correlated mutations between amino acids[1] and in parallel; we also analyze the positions whose correlated fluctuations are critical for function through a dynamic flexibility index (dfi) metric.[2] Integrating the evolutionary information with the dynamically coupled positions that are critical for function, we model various CVN mutants and obtain these variants in complex with di-mannose sugar with a fast and flexible docking method called BP-Dock.[3] We later characterize these mutants experimentally with NMR or ITC. Overall, this study helps us to explore the sugar binding properties of CVN mutants obtained from evolutionary and functional analysis and thus provide better insights about the mechanism of glycan recognition.
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2396-Pos Board B88
De Novo Design of Anti-Inflammatory Peptides for Treating Patients with Severe Sepsis
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Sepsis, blood poisoning, is a complex and severe infectious disease with high mortality (25~50%). Up to date, sepsis remains a therapeutic dilemma despite some advances in drug therapy and intensive care. In the case of Gram-negative bacteria, endotoxins (lipopolysaccharides, LPS) are major components and well known to be responsible for the strong inflammation reaction resulting in systemic infection. Other cytokines, such as TNF-α released from LPS-activated macrophages, IL-6, and IL-1β, also have much influence on the sepsis disease.
The purpose of this study was to de novo design the anti-inflammatory peptides for capturing the LPS, TNF-α, IL-6, and IL-1β in the blood of patients with severe sepsis to reduce their concentrations. The peptides determined from the cytokine-receptor complexes binding sites (from Protein Data Bank) were firstly used for docking to validate the selected peptide-cytokine binding. These peptides were then modified as de novo design to search for preferable binding affinity. The binding free energies of these peptide-cytokine complexes were calculated for comparison. The de novo designed peptides can be synthesized for further bio-assay and then applied to manufacture a cytokines adsorber for treating patients with severe sepsis in the future.

2397-Pos Board B89
Molecular Determinants of Substrate Specificity in Type VII Secretion Systems
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Type VII protein secretion (T7S) systems are found among numerous bacterial species, and have been implicated in a wide range of functions including growth, nutrient uptake, and virulence. The pathogenic bacterium *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis disease, contains five T7S systems, termed ESX-1 through ESX-5. Importantly, virulence is entirely dependent on ESX-1; while another system, ESX-3, is essential for growth and viability. While every T7S system contains a translocase, EccC, and a heterodimeric substrate, EsxAB, individual knockouts of proteins within a specific system in *M. tuberculosis* are not compensated for by the remaining four paralogs *in vivo*. Thus, at least some substrates, including EsxA and EsxB, are specific to individual ESX systems. How substrates are selected by T7S systems, and how these substrates are distinguished by different T7S systems within a single organism, is currently unknown and could be important for drug and vaccine development. We investigated the question of substrate selectivity using biochemical and genetic techniques. Importantly, we solved the first crystal structure of the EsxAB heterodimer that includes two regions important for substrate binding, including a hitherto-unseen C-terminal helix on EsxB. We further identified several residues that are absolutely necessary for binding of substrate to EccC, and have determined that seven of them together encode the information required for specificity.

2398-Pos Board B90
Structural Basis for Arsenate-Phosphate Discrimination
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In Nature arsenate can in principle replace phosphate in its role as a key biological molecule (e.g. as in ATP) but arsenate analogues are less stable with respect to hydrolysis and reduction. Yet bacterial adoption of arsenic has been described and although the precise nature of the arsenic species is undetermined, it is possible that arsenate is incorporated as direct substitution for phosphate in cellular macromolecules. Several phosphate-binding proteins are able to selectively bind phosphate over arsenate despite their chemical similarities. We take advantage of this chemical similarity to elucidate arsenate-phosphate discrimination and to probe our understanding of phosphate-binding mechanisms in general. Specifically we consider a highly flexible synthetic hexapeptide capable of irreversibly binding phosphate as well as a number of highly selective phosphate binding proteins, including those with the evolutionarily conserved sequence known as a P-loop. A major challenge in structural analysis is that automatic ligand search methods are deemed to fail in detecting phosphate-binding sites in small and disordered phosphate-binding peptides. We use the Generalized Amber Force Field (GAFF) and extend it to include arsenate. Our ability to explore and explain experimental results using a combination of DFT, MD, and MCMC techniques are demonstrated in their ability to distinguish between the chemically close phosphate and arsenate ligands. Using a number computational techniques, we explore the large number of potential interactions that may be exploited to explain and control the specificity and selectivity of binding.

2399-Pos Board B91
Variation in the Binding Pocket of an Inhibitor of the Bacterial Division Protein FtsZ Across Genotypes, Nucleotide States, and Species
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The recent increase in antibiotic resistance in disease-causing bacteria calls for new approaches to drug target selection and drug development. Targeting proteins involved in the bacterial cell division process provides a novel mechanism of action that bypasses problems associated with the increasingly ineffective variants of older antibiotics. One potentially powerful target for inhibition is the bacterial cytoskeletal protein FtsZ, a GTPase homolog of eukaryotic tubulin. This highly conserved and essential protein acts as a scaffold to recruit other cell division machinery and can exert mechanical forces that may be responsible for constriction of the division site. Recent work on an allosteric inhibitor (PC190723) has shown promising in vitro and in vivo antimicrobial activities on Staphylococcus aureus, however the