structural models using multi-body docking implemented in HADDOCK. Our models revealed novel modes of DNA binding for the ATP-free MR complex and provide the first depiction of a single-stranded DNA-bound complex. We tested our models through biochemical activity assays to determine the effect of disruption of key interactions, via mutation, in our models.

**87-Pos**

**Studying the Interaction Mechanism of Azurin-Derived Peptide P28 and Tumor Suppressor P53**

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p53 is a pro-apoptotic tumor suppressor. As a transcription factor, p53 directly regulates a variety of cellular processes, including apoptosis, cell cycle, cell senescence, metabolism, and antioxidation. Azurin is a copper-containing redox protein secreted by Pseudomonas aeruginosa. It consists of 128 amino acids. Azurin has attracted much attention because it preferentially enters a variety of cancer cells and produces cytotoxicity. p28 is a segment of azurin, corresponding to residues 50 to 77. So far, several different mechanisms have been proposed to explain the anticancer activity of azurin and p28, where azurin/p28 stabilizing p53 by forming a binary complex is the most important and widely studied one. However, except the predictive structures given by molecular simulation and molecular docking, no information about the structure of p28-p53 or azurin-p53 complex is available. Consequently, it is impossible to rationally modify the amino acid sequence of p28 to further enhance the interaction between p28 and p53 and to improve the anticancer activity of p28. Through the comprehensive use of biophysical and structural biology characterization techniques and computer simulation, we systematically explored the interactions between p28 and the DNA binding domain (DBD) of p53. Through alanine mutagenesis, GST pull-down and surface plasmon resonance experiments, it was found that the L68A mutation had the greatest effect on the p28/p53 DBD binding affinity. Through fluorescence polarization experiments, the p28 binding site on p53 DBD was found to be overlapped with the DNA binding site. The p28-interacting residues on p53 DBD were further identified by nuclear magnetic resonance, including Y236, S240, V274, V173, R267, S215, I255, G245, L145, K305, M133, Y103, A161, R156, C277, and T211. Finally, the best binding model consistent with available experimental information was obtained through molecular docking.

## Posters: Protein Structure, Prediction, and Design

**88-Pos**

**The Trade-off between Thermostability and Function in Designed DNA-Binding Proteins**

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Protein designers are ever improving their ability to design proteins that fold stably and perform a function, yet meeting both of these goals at once has been a challenge. Here we investigate whether there is a trade-off between thermostability and function by designing chimeras of two three-helix bundle proteins. The Engrailed homeodomain is a DNA-binding protein that serves as a transcription factor in *D. melanogaster* development and has a melting temperature of 52°C. Based on its backbone structure, a variant, UVF, was previously designed and found to be thermostable ($T_m > 99°C$) but was not functional. Molecular dynamics and coarse-grained simulations of chimeras of the two proteins suggest that UVF's fully hydrophobic core contributed entropically to its stability, and increased salt bridges on its surface contributed enthalpically. Here, we used equilibrium unfolding to test these thermodynamic predictions. Further, we used molecular dynamics simulations of the proteins bound to DNA to design and assess mutations to the chimeras to improve upon their DNA binding function. DNA-binding was then tested experimentally using electrophoretic mobility shift assays.

**89-Pos**

**Prediction of Amphipathic Helix - Membrane Interactions with Rosetta**

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Amphipathic helices have hydrophobic and hydrophilic/charged residues situated on opposite faces of the helix. They can anchor peripheral membrane proteins to the membrane, be attached to integral membrane proteins, or exist as independent peptides. Despite the widespread presence of membrane-interacting amphipathic helices, there is no computational tool within Rosetta to model their interactions with membranes. in order to address this need, we developed the AmphiScan protocol with PyRosetta, which runs a grid search to find the most favorable position of an amphipathic helix with respect to the membrane. The performance of the algorithm was tested in benchmarks with the RosettaMembrane, *ref2015_memb*, and *franklin2019* score functions on six engineered and 44 naturally occurring amphipathic helices using membrane coordinates from the OPM and PDBTM databases, OREMPRO server, and MD simulations as reference. The AmphiScan protocol predicted the coordinates of amphipathic helices within less than 3Å of the reference structures and identified membrane-embedded residues with a Matthews Correlation Constant (MCC) up to 0.61. Overall, AmphiScan stands as fast, accurate, and highly-customizable protocol that can be pipelined with other Rosetta and Python applications.

**90-Pos**

**High Resolution Cryo-Em Structure of the EspA Filament from EPEC**

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Antimicrobial resistance is a growing concern for the global population, predominantly in animal husbandry, hospitals, and developing countries. Several pathogens are of concern, including *Escherichia coli*. The rise in resistance has contributed to the decline in development of novel antibiotics, thus pushing us closer to a pre-antibiotic era. Virulence mechanisms that bacteria use are often not required for their survival and therefore are promising targets for the development of anti-virulence compounds. The T3SS is a proteinaceous channel that spans the inner and outer membranes of the bacterial cell, projecting into the extracellular medium where it interacts with the host cell membrane to deliver virulence factors. The main components of the T3SS are: (1) the basal body, spanning the inner and outer membranes of the bacterial cell; (2) the needle that projects from the basal body, and (3) the tip and pore-forming translocon that completes the channel from bacteria to host. Entero-pathogenic and -hemorrhagic *E. coli* (EPEC/EHEC) possess a distinct T3SS from that of other species; EPEC/EHEC possess a needle extension, or filament, in place of the 'tip' component, called EspA. The function of EspA is required for colonization of EPEC within the gut and *espA* EPEC knockouts are associated with decreased virulence. Here we describe the cryo-EM structure of natively-sheared EspA filaments from EPEC, determined to 3.6 Å resolution. Within the filament lumen, a pattern of positively charged residues adjacent to a hydrophobic groove lines the lumen of the filament in a spiral manner, suggesting a mechanism of translocation through the channel mediated via electrostatics. Using structure-guided mutagenesis of these residues, in vivo studies corroborate the role of these residues in secretion function. The high-resolution structure of the EspA filament will aid in structure-guided drug design of novel anti-virulence therapeutics.

**91-Pos**

**Completing the Partially Resolved Complex Crystal Structure of Aurora Kinase A-/-N-MYC by Molecular Modeling**

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A recent crystallization study resolving the complex between the Aurora A kinase (AurA) and the N-Myc provides invaluable insights into indirect targeting of the N-Myc oncogene which is otherwise regarded as undruggable. Although the N-Myc construct in the crystallization experiment covers two Myc Box (MB 0-1) motifs, only MB1 motif was validated in the crystal structure (PDBID: 5G1X). in this study, we unraveled the roles of the MB motifs of N-Myc in the AurA interaction by all-atom molecular dynamics (MD) simulations and free energy calculations. Overall, we have performed MD simulations of 3 different AurA complexes with different N-Myc constructs for 500 ns. Repeated simulations (n=5) of the 5G1X crystal structure consistently showed that the N-Myc without the MB0 motif is either partially or completely dissociated from the kinase. Free energy calculations by the MM-PBSA method similarly showed that the affinity of N-Myc was markedly increased when the MB0 motif is constructed in the 5G1X structure. Furthermore, we tested different AurA complexes harboring another protein partner that binds to the same Aura surface with the MB0 motif. Altogether our results suggested (i) the necessity of the MB0 motif of N-Myc for a stable AurA interaction, and

(ii) the N-terminal lobe of AurA as the primary protein-protein interaction surface rather than its activation loop residing between the two lobes. The models presented by this study are particularly insightful for the studies aiming to target the N-Myc oncogene, reflecting the promise of molecular modeling techniques as complementary tools to experiments.

**92-Pos**

**Evolutionary Models of Fold-Switching Proteins**

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It has recently been argued that fold-switching proteins, which can adopt multiple distinct folds, may be much more abundant than was previously thought. We aim to test this notion using statistical models built on evolutionary relationships between protein families. Specifically, we infer fitness landscapes from patterns of homolog sequence variation within and between different families. Using Monte Carlo simulations to sample sequence space, we then assess the extent to which such landscapes can accurately discern sets of contacts associated with distinct folds. We compare results for synthetic proteins, known to switch between all-alpha and alpha/beta structures, to the naturally-occurring RfaH family, which contains fold-switching transcription factors. information gleaned from these simulations allows us to propose novel sequences likely to exhibit fold-switching.

**93-Pos**

**Bioinformatics Analysis of Proteins Involved in Bacterial Curli Assembly Indicate Conserved Amino Acids that May Play a Role in Structure/ Function**

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Curli fibers are a cell surface filament found on gram-negative bacteria that are essential for host cell colonization. The formation of Curli requires 6 different proteins: CsgA, CsgB, CsgC, CsgE, CsgF, and CsgG. CsgC, CsgE and CsgF are thought to be chaperone proteins that prevent the premature intracellular aggregation of CsgA and CsgB, the two structural proteins of Curli. CsgC, CsgE, and CsgF are also thought to be involved in the intracellular to extracellular transport of CsgA and CsgB through the outer-membrane channel protein CsgG. Although the 3D structures of CsgC, CsgE, CsgF, and CsgG have been determined details of the structure-function relationship are still under investigation. We conducted protein bioinformatics analysis and found regions with significant conservation that may be important for the structure and/or function of CsgC, CsgE, CsgF and CsgG. We will use this information to guide our *in-vitro* and *in-vivo* experimental work aimed at elucidating the mechanistic details of Curli fiber assembly.

**94-Pos**

**Developing Web Sites that Integrate Data and Diverse Approaches**

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BioMakie.jl is a Julia package that facilitates visual investigation of biological data. It aims to provide extra tools to view and control data of such things as protein structures and sequences. BioMakie utilizes other packages in the BioJulia ecosystem like BioSequences and BioStructures.jl, plus outside packages like MIToS.jl to perform comparisons, load data, and to do most of the computation/numerical analysis. Makie defines objects that can contain data and be tracked. Any changes or updates to the data inside these objects can be ''heard'' by other objects and this can drive event chains that update many interdependent values and their graphical representations immediately. This enables a unique data-driven visual approach to studying bioinformatics in pure Julia. We are presently using this platform to integrate protein sequences, structures, and phylogenies.

**95-Pos**

**Refinement of Protein Homology Model by Enhanced Directional Sampling Guided by Bioinformatics**

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An accurate protein structure is a prerequisite for computational drug discovery and mechanistic analysis of protein's interactions. Homology modelling is a widely used method to predict atomic-level structure of protein whose structure is experimentally undetermined. It predicts structure accurately even with moderate sequence identity (∼50 percent) template, however, its accuracy decreases when template has low sequence identity (<50 percent). We developed a homology model refinement method to obtain an accurate structure of those proteins for which high sequence identity template is not available but enough data on the history of protein's evolution is available. This tool performs sequential directional sampling of protein conformations guided by a native state residue-residue contact map obtained from statistical coupling analysis of protein's evolutionary history. Statistical coupling analysis of a protein's multiple sequence alignment (MSA) can provide information on the degree of energetic coupling between residues. This energetic coupling imposes an evolutionary pressure on protein residues and extraction of co-evolutionary information of protein's residues from MSA can be used to obtain residue-residue spatial contact map of few functional residues. We have used this low-resolution SCA-predicted contact map of functional residues to guide the conformational sampling towards native state. A modified version of recent enhanced sampling method, molecular dynamics with excited normal modes (MDeNM), which excites protein velocity along the slow normal modes is developed for enhanced directional sampling. The modification in MDeNM is introduced to selectively excite velocity of protein atoms towards the native conformation. Using this method, a less accurate homology model of HIF-2α PASB domain (110 residues) generated with a 28 percent sequence identity template is refined to obtain the accurate structure matching the experimental structure.

**96-Pos**

**Disulphide Lock-Probing Fibrillogenesis Trigger in Aciniform Spider Silk**

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Proteinaceous solutions produced within the abdominal spinnerets of spiders undergo significant structural transformations to form silk fibres employed in constructing various architectures to support their biological functions like nesting, anchorage, reproduction, predation etc. A female orb-weaver spider can produce seven types of silk, each distinct in residue content, stickiness, morphology, and mechanical properties, hence, giving a family of highly desirable biomaterials. Aciniform (or wrapping) silk is used in both prey-wrapping and egg-casing. Wrapping silk from *Argiope trifasciata* (AcSp1) comprises a core repetitive domain (≥14) of identical 200 amino acid-long units ('W units') flanked by non-repetitive terminal domains. Unlike most silk fibres, significant α-helical character is observed in the fibres of AcSp1. Previous studies suggest a compact *'beads-on-a-string'* structure rich in α-helical character for the soluble W unit. Notably, one of the α-helices (H5) was found to be relatively less stable. This observation led us to hypothesize -that localized unfolding of helix H5 is key for fibrillogenesis of AcSp1. Namely, this would initiate lengthening of the ''string-like'' linker region and decrease protein compactness of the W units, favouring protein entanglement and increase in intermolecular interactions, which would in turn promote β-sheet formation during fibrillogenesis. To test this hypothesis, two serine-to-cysteine mutations were engineered within the W unit(s), one in H5 and another in the globular core, to generate a disulphide staple linking H5 to the core. Compellingly, the oxidized mutant cannot form fibres, while the reduced form is fully functional. Through atomic-level structural and dynamics analysis of the oxidized vs. the reduced, and native forms of the soluble W unit(s), we are testing the hypothesis that denaturation and decompaction of H5 is crucial in initiating fibrillogenesis in aciniform silk. This, in turn, will facilitate engineering of W units with modified mechanical behaviour.

**97-Pos**

**Exploring the Specificity of 3-Ketosteroid-Δ1-Dehydrogenase by use of Site-Directed Mutagenesis**

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Dehydrogenization of steroids at C1,2-positions can significantly enhance their pharmaceutical efficacy. Compared with chemical dehydrogenization, the enzymatic modification of steroids by 3-ketosteroid-Δ1-dehydrogenase (KstD) has shown tremendous advantages that occur in the mild and environment friendly conditions. However, the substrate specificity of KstD has not been thoroughly explored so far. Recently we identified a novel KstD (i.e., KstD211) from Mycobacterium neoaurum HGMS2 that can