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Posters

Protein Structure and Conformation IV

2571-Pos Board B1

Biochemical State of the Aryl Carrier Protein Directs Sequential Domain-Domain Interactions in the Yersiniabactin Synthetase System

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Nonribosomal peptide synthetases (NRPSs) are modular enzymatic systems responsible for the production of complex secondary metabolites in bacteria and fungi. Each module is comprised of (at least) three core domains whose combined action leads to the selection, activation, and incorporation of a single small molecule into a growing peptide. Central to each module is the carrier protein (CP), which is first primed via attachment of a 4′-phosphopantetheine moiety (ppant arm) to a conserved serine to generate the active holo form. The ppant arm then covalently harbors activated monomers and growing peptides and shuttles them between the active sites of catalytic domains in both the same and adjacent modules. During CP priming and peptide elongation, a CP thus exists in multiple different post-translational states and must interact with multiple catalytic domains. Understanding how NRPSs are able to efficiently orchestrate this series of sequential protein-protein interactions between a CP and its partner catalytic domains is key to understanding the molecular mechanism of NRP synthesis. In functionally analogous fatty acid synthases (FAS) and polyketide synthases (PKS), the post-translational state of a CP (holo vs. substrate loaded) has been implicated in directing the sequence of interactions in these systems. However, the role these modifications play in modulating protein-protein interactions in a NRPS has not previously been explored. Here, we provide evidence that the biochemical state of a CP (apo vs. holo vs. monomer loaded) alters the affinity of the CP for its partner catalytic domains in a NRPS system. Our results demonstrate that each partner catalytic domain preferentially binds to a different biochemical state of the CP and suggests a means by which directionality in protein-protein interactions is achieved in NRPSs.

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Systematic Perturbation of Protein:Protein Interfaces may Aid in Functional Classification

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Protein:protein interactions play vital roles in many biological reactions. In a previous study we constructed a database of protein:protein interfaces (FLIPdb) and have shown that calculations of the computational alanine scanning (CAS) energy of residues along the interface can distinguish functional categories of proteins.

Here, to further understand the underlying principles of protein interactions, we examine the effects of systematically translating one interfacial subunit over a grid in relation to the other stationary subunit for the structures in FLIPdb. A three-dimensional potential energy surface was generated from the change in spatial coordinates and energy of alanine substitution that accompanies each shift in conformation. Specific characteristics of the potential energy surfaces, including the volume within the surface, maximum depth, width at half depth, and energy per residue were found to discriminate between different functional classes of protein interfaces with an accuracy of approximately seventy-six percent. These results may suggest that Functionally-Linked Interfaces of Proteins (FLIPs) are more sensitive to perturbation and are thus more specific than Functionally uncorrelated Contacts (FunCs).

2573-Pos Board B3

Structural Analysis of Lipocalin-Type Prostaglandin D Synthase Complexed with Prostaglandin J_2

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Lipocalin-type prostaglandin (PG) D synthase (L-PGDS), is a member of the lipocalin superfamily, is found and exists in the brain. L-PGDS catalyzes the isomerization of PGH_2, a common precursor molecule of various prostanoids, to produce PGD_2, a potent endogenous somnogen. L-PGDS-produced PGD_2 is sleep-inducing and to accumulates in the brain during prolonged periods of wakefulness. In addition, We have recently found that the enzyme activity of L-PGDS was inhibited by PGJ_2, a metabolite of PGD_2 metabolite. Therefore, the structural information regarding the of L-PGDS/PGJ_2 complex should provide potential information for the drug development of drugs for treating sleep disorders. In this study, we investigated the three-dimensional structure of recombinant mouse L-PGDS complexed with PGJ_2 by means of NMR and evaluated the binding mechanism from the deduced molecular structure.

The Chemical shift assignments for the of backbone and side-chain protons of the L-PGDS/PGJ_2 complex was made carried out using two-dimensional ^1H-^{15}N HSQC, three-dimensional HNCACB, CBCA(CO)NH, HBHA(CO)NH, CCH-TOCSY, and HCCH-TOCSY. NOEs were collected from three-dimensional ^{13}C-edited NOESY data. In addition, to investigate the binding site of PGJ_2, we compared the HSQC of L-PGDS/PGJ_2 complex with that of apo-L-PGDS. Significant changes in the chemical shifts of the catalytic Cys65 and other residues which are locatedexist in the bottom area of the binding pocket of L-PGDS were observed upon PGJ_2 binding, indicating that PGJ_2 competitively binds to the substrate binding site.

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The Origin of CDR H3 Structural Diversity

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Antibody CDR H3 loops are critical for adaptive immunological functions. Although the other five CDR loops adopt predictable canonical structures, H3 conformations have proven unclassifiable, other than an unusual C-terminal ''kink'' present in most antibodies. To determine why the majority of H3 loops are kinked and to learn whether non-antibody proteins have loop structures similar to H3, we searched a set of 15,679 high-quality non-antibody structures for regions geometrically similar to the residues immediately surrounding the loop. By incorporating the kink into our search, we identified 1,030 H3-like loops from 632 protein families. Some protein families, including PDZ domains, appear to use the identified region for recognition and binding. Our results suggest the kink is conserved in the immunoglobulin heavy chain fold because it disrupts the β-strand pairing at the base of the loop. Thus, the kink is a critical driver of the observed structural diversity in CDR H3.

2575-Pos Board B5

Biophysical, Biochemical and Functional Studies of a Novel Fungal Tec1 Paralog

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Tec1 is a member of TEA/ATTS transcription factors family and a key regulator of cell development and adhesion in fungi. In *Candida albicans*, Tec1 is required for yeast to filamentation switch and virulence. It also plays a role in biofilm formation. Here, we report the identification of a novel Tec1 paralog, Tec2, which has been implicated in white/opaque switching. First, using the NMR and X-ray crystallographic structures of the DNA-binding TEA domain, solved in the Veeraraghavan laboratory, we constructed three-dimensional structural models of the Tec1 and Tec2 proteins to gain insights into their activities. We then investigated the DNA binding activity of Tec2 and compare it with that of Tec1. Finally, we report on the potential biological role for Tec2, determined using knockout and overexpression studies in *C. albicans*. We are currently investigating the three-dimensional structure of Tec2.

2576-Pos Board B6

Structure and Molecular Dynamics of the Ig58/58 Domains of Obscurin

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Obscurin (800-900 kDa) is a giant muscle protein vital to muscle cell maintenance and organization. It is the only known connection between the contractile apparatus and the sarcoplasmic reticulum and also binds to specific cytoskeletal, signaling, or membrane-associated proteins. Obscurin domains Ig58/59 bind to titin ZIg9/10, which is hypothesized to stabilize the sarcomeric cytoskeleton. Mutations in this obscurin region lead to malformed muscle architecture and, eventually, to hypertrophic cardiomyopathy (HCM). For obscurin/titin binding to occur, all four of these domains must be present. In order to fully characterize this physiologically important region of obscurin, and by extension determine the molecular factors that drive HCM, here we present the Ig58 and Ig59 structure using both X-ray crystallography and heteronuclear