conditions (e.g. stress). TA toxins have a bacteriostatic effect that can lead to cell death if sustained. Although the mechanisms of action for a few TA toxins have been uncovered, the intracellular targets of many others have not been identified. Our latest structural and functional data on such complexes will be presented.

1286-Pos

Overproduction, Purification and Structure Determination of Human Dual Specificity Phosphatase 14

George Lountos, Joseph Tropea, Scott Cherry, David Waugh.
National Cancer Institute-Frederick, Frederick, MD, USA.

Dual-specificity phosphatases (DUSPs) are enzymes that participate in the regulation of biological processes such as cell growth, differentiation, transcription and metabolism. A number of DUSPs are able to dephosphorylate phosphorylated serine, threonine and tyrosine residues on mitogen-activated protein kinases (MAPKs) and thus are also classified as MAPK phosphatases (MKPs). As an increasing number of DUSPs are being identified and characterized, there is a growing need to understand their biological activities at the molecular level. There is also significant interest in identifying DUSPs that could be potential targets for drugs that modulate MAPK-dependent signaling and immune responses, which have been implicated in a variety of maladies including cancer, infectious diseases and inflammatory disorders. Here, the overproduction, purification and crystal structure at 1.88 Angstroms resolution of human dual-specificity phosphatase 14, DUSP14 (MKP6), are reported. DUSP14 has been reported in the literature to play potentially important roles in T cell regulation and may also be involved in gastric cancer. The determination of the three-dimensional structure of DUSP14 should aide the study of DUSP14 at the molecular level and may also accelerate the discovery and development of novel therapeutic agents.

1287-Pos

Structural Studies on Mutants of HMG CoA Reductase from Pseuodomonas Mevalonii

Moumita Sen, Nicklaus C. Steussy, Chandra Duncan, Victor W. Rodwell, Cynthia V. Stauffacher.
Purdue University, West Lafayette, IN, USA.

HMG-CoA reductase catalyzes the four-electron reduction of HMG-CoA to free CoA and mevalonate. This is one of the few double oxidation/reduction reactions in intermediary metabolism that take place in a single active site. In addition to the unusual enzymology, this reaction is of interest because it is the committed step of the fundamental mevalonate isoprenoid pathway. In animals this pathway produces cholesterol, the steroid hormones and a variety of signaling molecules based on the isoprenoid building block (1). In bacteria the pathway is equally important, and has been shown to be essential to the virulence of *Staphylococcal* and *Streptococcal* bacteria (2). To better understand the nature of this reaction, our laboratory has undertaken a comprehensive structural study of the mechanism of HMG-CoA reductase in bacteria utilizing the enzyme from *Pseudomonas mevalonii*.

HMG-CoA reductase is an obligate dimer, with each monomer consisting of a large domain, a small domain, and a flap domain (2, 3) that is disordered in the apoenzyme structure. The flap domain is ordered in the crystal structure only in the presence of ligand and co-factors, where it closes over the active site, positioned by a network of hydrogen bonds that include the ligand and co-factor. Two residues proposed to be important in flap domain movement have been mutated. Mutant proteins have been crystallized, soaked with various combinations of ligands and co-factors, and their structures have been solved at 1.95-2.40Å. These structures, reinforced with kinetic analysis of the mutants, demonstrate the essentiality of this closure in the reaction and reveal how these residues are involved in flap domain movement.

1288-Pos

Structure of the E. Coli Gyrase DNA Binding and Cleavage Core Reveals A Unique Domain

Allyn Schoeffler, James Berger.
University of California, Berkeley, Berkeley, CA, USA.

DNA topoisomerases are essential enzymes that sustain chromosome supercoiling homeostasis in all forms of life. DNA gyrase, a heterotetrameric type IIA topoisomerase, has the unique ability to introduce negative supercoils into DNA, helping maintain bacterial genomes in a compact, underwound state. Though all gyrase orthologs use a set of homologous domains and a central ''two-gate'' mechanism for passing one DNA segment through another, they also exhibit critical family-specific differences. For example, the metal- and DNA-binding TOPRIM domain of gyrases found in many gamma- and beta-proteobacteria contains a 170-amino acid insertion of unknown function. We have solved the crystal structure of the *E.coli* gyrase DNA binding and cleavage core, visualizing this insertion for the first time. Biochemical analyses of a structure-guided deletion mutant lacking this region reveal that it may help coordinate the activities of gyrase's distal ATPase and DNA binding gates.

1289-Pos

Structure of Crohn'S Disease-Related Proteins and their Binding to Class II MHC

Lihui Liu, Guo Yi, Hongmin Li.
Wadsworth Center, Albany, NY, USA.

T cell response to enteric bacteria is important in inflammatory bowel disease. pfiT is a T-cell superantigen associated with human Crohn's disease. The bacterial superantigens are a class of protein toxins that share the capacity to induce massive activation of the human immune system. These molecules simultaneously bind to major histocompatibility complex class II molecules on the surface of antigen-presenting cells and T-cell receptors (TCRs) on T cells to stimulate large numbers of T cells. The aim of this study is to analyze the molecular mechanism of superantigen recognition by host receptors. Here, we report the crystal structure of pfiT. This protein was overexpressed in *Escherichia coli* and purified though GST-affinity and size exclusion chromatography. The protein is selenomethionine labeled and single wavelength anomalous dispersion method was used for determination of the crystal structure. The superantigen crystallizes in the monoclinic space group $P2_1$, with two molecules in asymmetric unit cell. The structure was determined to 2.5Å resolution. In addition, we performed radiolabeled competitive binding assays between three superantigens: pfiT, *Mycoplasma arthritidis*-derived mitogen (MAM), PA2885, a novel open reading frame (ORF) in the *Pseudomonas aeruginosa* genome. Analyses showed that both the microbial homologuepfit and PA2885, just as potent superantigen MAM, are capable of binding to target mammalian cells. Moreover, we labeled these superantigens with FITC and analyzed them by FACS in PBMC. The statistic results show that antibody against HLA-DR has strong effect to block these SAg' binding ability with PBMC, and antibodies against HLA-DQ and DP can also compete binding site in a much weaker manner. These findings support the concept that pfiT, PA2885, MAM are superantigens and can bind to class II MHC molecule .

Research is funded by the Crohn' & Colitis Foundation of America.

1290-Pos

Structural and Metal-Binding Characterization of the C-terminal Metallochaperone Domain of the Membrane Fusion Protein SilB from *Cupriavidus Metallidurans* CH34

Beate Bersch[1], Kheiro-Mouna Derfoufi[2], Fabien De Angelis[2], Elisabeth Ngonlong Ekendé[2], Max Mergeay[3], Jean-Marie Ruysschaert[2], **Guy Vandenbussche[2]**.
[1]Institut de Biologie Structurale, Grenoble, France, [2]Université Libre de Bruxelles, Brussels, Belgium, [3]Belgian Nuclear Research Centre, Mol, Belgium.

The β-proteobacterium *Cupriavidus metallidurans* CH34 has an outstanding ability to grow on harsh environments such as heavy-metal contaminated sites. The regulated transport of heavy metal ions out of the cell via tripartite efflux systems is one of the mechanisms used by the bacteria for detoxification. These protein complexes span the entire bacterial cell envelope, and are composed of an inner membrane transporter belonging to the resistance nodulation cell division (RND) family, an outer membrane protein member of the Outer Membrane Factor (OMF) family, and a periplasmic adaptor protein, member of the Membrane Fusion Protein (MFP) family. SilABC is one of the 12 putative efflux systems detected in *C. metallidurans* CH34 genome and is most probably involved in silver and copper trafficking. We report here on the characterization of the C-terminal domain of the periplasmic adaptor protein SilB. This C-terminal extension exists only in SilB homologs and is not present in other MFPs. A potential Ag(I)/Cu(I) coordination site was detected on the basis of the amino acid sequence and the metal-binding specificity was confirmed by mass spectrometry. NMR solution structure of the apo-form showed that SilB C-terminal domain adopts a β-barrel structure. Comparison of chemical shift data between the apo-and metallated-form demonstrated the implication of two methionine, one histidine and one tryptophan residues in the metal coordination site. Fluorescence quenching and UV-visible data are consistent with a cation-tryptophan π-interaction. With respect to its three-dimensional structure and metal-binding specificity, the SilB C-terminal domain closely resembles CusF, a small periplasmic protein belonging to the CusCFBA efflux system involved in silver and copper resistance in *E. coli*. Our study suggests

that SilB C-terminal domain could function as a metallochaperone to the SilABC system.

1291-Pos

Crystal Structure and Metal-Binding Specificity of ZneB, the Periplasmic Adaptor Protein of A Heavy Metal Resistance System from Cupriavidus Metallidurans CH34

Fabien De Angelis[1], John K. Lee[2], Joseph D. O'Connell III,[2], Larry J.W. Miercke[2], Koen H. Verschueren[3], Vasundara Srinivasan[3], Rebecca A. Robbins[2], Cédric Govaerts[1], Jean-Marie Ruysschaert[1], Robert M. Stroud[2], Guy Vandenbussche[1].
[1]Université Libre de Bruxelles, Brussels, Belgium, [2]University of California in San Francisco, San Francisco, CA, USA, [3]Vrije Universiteit Brussel, VIB, Brussels, Belgium.

In Gram-negative bacteria, tripartite efflux systems are involved in the transport of a broad range of toxic compounds such as drugs or heavy metals out of the cell. These protein complexes span the entire bacterial cell envelope, and are composed of an inner membrane transporter belonging to the resistance nodulation cell division (RND) family, an outer membrane protein member of the Outer Membrane Factor (OMF) family, and a periplasmic adaptor protein, member of the Membrane Fusion Protein (MFP) family. The periplasmic adaptor plays an important role in the recruitment of the two integral membrane partners and for the assembly of a functional transport complex. It has been proposed that this component could also contribute to the binding of the substrate. We report here on the characterization of ZneB, the MFP of a Heavy Metal Efflux-RND system from Cupriavidus metallidurans CH34. Using mass spectrometry, we have demonstrated that ZneB has a high specificity for zinc binding with a metal stoichiometry of 1:1 to the protein. The protein was crystallized in the presence of zinc and the apo- and metallated-forms were detected in the same asymmetric unit. The involvement of two histidine and a glutamate residues in the metal ion coordination site was confirmed by site-directed mutagenesis. The comparison of apo- and Zn-bound conformations based on the crystal structures and on data obtained in solution reveals important conformational changes upon zinc binding, suggesting an active role of the MFP in the efflux mechanism. The characterization at the molecular level of the efflux system proteins and the comparison with their counterparts in homologous RND-type transport systems involved in multidrug resistance will allow a better understanding of the resistance mechanisms.

1292-Pos

Crystal Structure of the N-terminal Region of Brain Spectrin Reveals A Helical Junction Region and A Stable Structural Domain

Marta A. Witek[1], Shahila Mehboob[2], Yuanli Song[1], Fei Long[1], Bernard D. Santarsiero[2], M.E. Johnson[2], L.W.-M. Fung[1].
[1]University of Illinois at Chicago, Chicago, IL, USA, [2]Center for Pharmaceutical Biotechnology, Chicago, IL, USA.

The crystal structure of a recombinant protein consisting of the first 147 residues of brain α spectrin was solved to 2.3 Å. The N-terminal region consists of the partial domain (Helix C') and the anti-parallel, triple helical coiled-coil first structural domain (helices A1, B1, and C1). The data revealed that each asymmetric unit contained two crystallographically independent structures (1 and 2). The crystal structure of the first structural domain resembled that of the first structural domain of erythroid α-spectrin, determined before by solution NMR studies, with some specific differences, especially at the N-terminal region, including Helix C' and the region connecting Helix C' with the first structural domain (the junction region). The first ten residues are in a disordered conformation, followed by Helix C' with an apparent, flexible bend. The junction region exhibits a helical conformation in contrast with an unstructured junction region in erythroid α spectrin. A special feature that has not been reported in other spectrin domains is the long and flexible A1B1 loop of 13 residues. This loop is likely the recognition site for interaction with other proteins. Hydrogen bonds and hydrogen bond networks were identified in the first structural domain and compared with those in erythroid α-spectrin. We suggest that these hydrogen bonds might contribute toward the stability of brain and erythroid spectrin.

1293-Pos

An Overlapping Kinase and Phosphatase Docking Site Regulates Activity of the Retinoblastoma Protein

Alexander Hirschi[1], Matthew Cecchini[2], Rachel Steinhardt[1], Frederick A. Dick[2], Seth M. Rubin[1].
[1]UC Santa Cruz, Santa Cruz, CA, USA, [2]University of Western Ontario, London, ON, Canada.

Insights into the molecular mechanisms that regulate the phosphorylation state and corresponding activity of the retinoblastoma tumor suppressor protein (Rb) are fundamental to understanding the control of cell proliferation. While much focus has been placed upon regulation of Cyclin-dependent kinase (Cdk) activity towards Rb, less is known about Rb dephosphorylation catalyzed by the major Rb phosphatase, protein phosphatase-1 (PP1). Using x-ray crystallography, we have determined the crystal structure of a PP1:Rb peptide complex to 3.2Å that reveals an overlapping kinase and phosphatase docking site. Kinetic assays show that Cdk and PP1 docking to Rb are mutually exclusive and that this docking site is required for efficient dephosphorylation, as well as phosphorylation of Rb. Cell cycle arrest assays demonstrate that the ability of PP1 to compete with Cdks is sufficient to retain Rb activity and block cell cycle advancement. These results establish a novel mechanism for the regulation of Rb phosphorylation state in which kinase and phosphatase compete for substrate docking.

1294-Pos

Crystal Structures Of Yeast Mitochondrial ATPase with Uncoupling Mutations

Diana Arsenieva, Yamin Wang, Jindrich Symersky, Luke Vistain, David M. Mueller.
Rosalind Franklin University, North Chicago, IL, USA.

Yeast mitochondrial ATP synthase is a transmembrane protein responsible for synthesis of more than 90% of ATP under aerobic conditions. The water soluble portion of ATP synthase, F1, is composed of five subunits with stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ and has a combined molecular weight of 360 kDa. The three active sites of ATPase are formed at the interfaces between alternating α- and β-subunits. ATPase is capable of efficient ATP hydrolysis, accompanied by rotation of central stalk subunits, $\gamma\delta\epsilon$, within the $\alpha_3\beta_3$ core and is capable of ATP synthesis if central subunits are forced to rotate in the opposite direction. A number of mutations in ATP synthase have been identified that result in the uncoupling of catalytic function and proton flow across the mitochondrial membrane. These uncoupling mutations cluster at the interface between γ-subunit and $\alpha_3\beta_3$ catalytic core of ATPase. In this work, four X-ray crystal structures of ATPase with single amino acid substitutions αN67I, αF405S, βV279F, and γI270T were solved at resolutions ranging from 3.2 Å to 2.74 Å. This study will present a structural comparison of the mutant structures with the wild type structures to understand the mechanism of coupling. However, the crystal structures likely represent the ground state of catalytic reaction cycle while the mutations may result in notable distortions of enzyme structure during other stages of catalytic cycle. Additionally, the uncoupling of the ATPase may be caused by changes in the energy of interaction between the portions that are rotating in the molecular machine thereby altering transition to the higher energy states. Structural based hypotheses are presented to explain the role of these residues in the coupling of the enzyme. Supported by NIH R01GM0662223

1295-Pos

Molecular Mechanism of the Peptidoglycan Hydrolysis by FlgJ, A Putative Flagellar Rod Cap Protein From Salmonella

Yuki Kikuchi[1,2], Hideyuki Matsunami[2,3], Midori Yamane[1], Katsumi Imada[1,2], Keiichi Namba[1,2].
[1]Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka, Japan, [2]Dynamic NanoMachine Project, ICORP, JST, Suita, Osaka, Japan, [3]Okinawa Institute of Science and Technology, Urume, Okinawa, Japan.

The axial structure of the bacterial flagellum consists of three parts: the filament as a helical propeller; the hook as a universal joint; and the rod as a drive shaft connecting the hook and the rotor. The assembly of the axial structure, which occurs at its distal end, requires cap complexes attached to the growing end. FlgD and FliD are cap proteins necessary for hook and filament growth, respectively. For efficient penetration of the growing rod through the peptidoglycan (PG) layer, it is likely that the rod cap locally degrades PG. FlgJ is a putative rod cap protein with a PG-hydrolyzing activity (muramidase). Previous studies have shown that the N-terminal region of FlgJ interacts with the rod proteins and that the C-terminal region shows a sequence similarity to muramidase family, such as autolysin and AcmA. To understand the mechanisms of rod formation, we determined the atomic structure of a C-terminal fragment of FlgJ at 1.7 Å.

The crystal structure revealed the entire muramidase domain of FlgJ. In spite of no significant sequence similarity, the putative active site of FlgJ closely resembles that of hen egg white lysozyme (HEWL), which is a well-studied muramidase. A glutamic acid residue at position 184 in FlgJ is invariant among FlgJ family and the E184Q mutant of FlgJ shows no muramidase activity, indicating