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