such as Crouzon syndrome and bladder cancer. This mutation has been shown to stabilize the isolated TM domain dimers in lipid membranes, but it is not known if it stabilizes the full length FGFR3 receptor dimers in the plasma membrane of mammalian cells.
To address the effect of the mutation in mammalian cells, we have determined free energies of dimerization for the wild type and mutant FGFR3 in mammalian (HEK293T and CHO) plasma membranes using the QI Förster resonance energy transfer (FRET) technique [Li et al.,2008]. The measured change in the dimerization free energy due to the Ala391Glu mutation is 1.2 kcal/mol, consistent with previous reports of hydrogen bond strength in proteins, as well as results for the isolated FGFR3 TM domains. Thus, we have shown that the mutation stabilizes the full length FGFR3 dimers in mammalian cells. We propose that this dimer stabilization is the major cause for FGFR3 overactivation and human pathologies.
Li E,Placone J,Merzlyakov M, Hristova K (2008) Quantitative measurements of protein interactions in a crowded cellular environment.Anal Chem 80:5976-5985.

1281-Pos
The Physical Basis Behind Achondroplasia, the Most Common Form of Human Dwarfism
Lijuan He, Kalina Hristova.
Johns Hopkins University, Baltimore, MD, USA.
Fibroblast growth factor receptor 3 (FGFR3) is a receptor tyrosine kinase which plays an important role in long bone development. The Gly380Arg mutation in FGFR3 transmembrane domain has been linked to achondroplasia, the most common form of human dwarfism. However, the exact mechanism underlying the pathology is under debate. One hypothesis is that the mutation stabilizes the active FGFR3 dimer in the plasma membrane. To test this hypothesis, here we measure the activation of wild type and mutant FGFR3 in mammalian cells, and analyze the activation using a physical-chemical model accounting for dimerization, ligand binding and phosphorylation probabilities. Our results demonstrate that the achondroplasia mutation does not increase the dimerization propensity of FGFR3. Instead, the data suggest that the mutation induces a structural change in the unliganded dimer. We propose that this structural change is a cause for pathogenesis in achondroplasia.

1282-Pos
Spectroscopic Design of Phospholamban Mutants to Treat Heart Failure
Simon J. Gruber[1], Suzanne Haydon[1], Kim N. Ha[1], Roger J. Hajjar[2], Gianluigi Veglia[1], David D. Thomas[1].
[1]University of Minnesota, Minneapolis, MN, USA, [2]Mt. Sinai Medical School, New York, NY, USA.
Ca^{2+} cycling through the SR in muscle cells is largely controlled by the Ca-pump (SERCA). SERCA transports Ca^{2+} into the SR and is inhibited by phospholamban (PLB) at submicromolar [Ca^{2+}], and this inhibition can be relieved by adrenergic stimulation. Contraction takes place when the Ca-release channel opens and the intracellular [Ca^{2+}] is high. One of the most common symptoms of heart failure (HF) is impaired calcium handling, frequently resulting from decreased SERCA activity. We are using EPR and NMR to study the relationships among structure, dynamics, and function of PLB, with the goal of designing LOF-PLB mutants (PLB_M) that can compete with WT-PLB and thus relieve SERCA inhibition. Several studies have shown that a pseudophosphorylated PLB (S16E-PLB) is effective for gene therapy in rodents and sheep, and we are using spectroscopic methods to refine this approach. We have developed a system for examining the function and interactions of SERCA and PLB in HEK cells. Active SERCA is expressed at a level high above basal ATPase activity, and cells are co-transfected with WT-PLB and/or PLB_M to measure SERCA inhibition in living cells. Unlike S16E-PLB, these mutants are able to respond to adrenergic stimulation. In addition to quantifying SERCA activity in the presence of PLB_M, we measure the ability of each mutant to compete with WT-PLB for binding to SERCA. This is done by measuring fluorescence resonance energy transfer (FRET) between labeled SERCA and WT-PLB. If PLB_M displaces WT-PLB, less energy is transferred between fluorophores and a decrease in FRET is observed. Based on these results, rAAV is used to test PLB_M in rodent and porcine models of HF for efficacy *in vivo* and ability to respond to adrenergic stimulation, with the goal of developing a novel, gene therapy based treatment for HF.

1283-Pos
The Activity of a Low-Affinity L-Arginine Transporter Quenches Peroxynitrite-Induced Fluorescence in Ventricular Cardiomyocytes
Jayalakshmi Ramachandran, **R. Daniel Peluffo**.
UMDNJ-New Jersey Medical School, Newark, NJ, USA.
We discovered a low-affinity, high-capacity L-arginine (L-Arg) transport process in rat cardiomyocytes consistent with the activity of the CAT-2A member of the y(+) family of cationic amino acid transporters (Peluffo, *J Physiol*, **580:**925-936, 2007), set to function in parallel with the previously described high-affinity, low-capacity CAT-1 (Lu et al., *Biosci Rep*, **29:**271-281, 2009). In assessing the role of a low-affinity transporter in this setting, we propose that CAT-2A protects cardiac muscle cells by ensuring the availability of proper L-Arg levels for the synthesis of nitric oxide (NO) *via* NO synthase (NOS). To test this hypothesis, acutely-isolated cardiomyocytes were loaded with the dye coelenterazine that greatly increases its fluorescence quantum yield in the presence of peroxynitrite (ONOO) and superoxide radicals. Cells were then exposed to 20 or 100 μM ONOO and changes in fluorescence were followed with a spectrofluorometer. Addition of extracellular L-Arg reduced ONOO-induced fluorescence in a concentration-dependent manner, an effect that was not mimicked by D-arginine or L-lysine and was fully blocked by the NOS inhibitor L-NAME. L-Arg reduced fluorescence with K_i values of 0.84 ± 0.12 and 1.26 ± 0.16 mM at 20 and 100 μM ONOO , respectively. L-Arg ''zero effect'' on ONOO-induced fluorescence was also dependent on ONOO concentration, with values of 145 and 363 μM for 20 and 100 μM ONOO , respectively. Below these values, decreasing concentrations of L-Arg progressively increased ONOO-induced fluorescence, an effect that was also blocked by L-NAME. All these effects can be explained by NOS-mediated NO synthesis, which may turn to ONOO production at limiting L-Arg. Since ONOO has detrimental effects on cardiac contractility, these results suggest a cardioprotective role for the low-affinity L-Arg transporter, ensuring proper supply of NOS substrate under a variety of physiological and pathological conditions.

1284-Pos
Studies on the Structure and Function of the Intracellular Region of the Plexin-B1 Transmembrane Receptor
Prasanta K. Hota[1], Yufeng Tong[2], Junia Y. Penachioni[3], Luca Tamagnone[3], Hee-Won Park[2], Matthias Buck[1].
[1]School of Medicine, Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, USA, [2]Structural Genomics Consortium, Department of Pharmacology, University of Toronto, Toronto, ON, Canada, [3]Institute for Cancer Research and Treatment, University of Torino, Candiolo, Italy.
Plexin family are unique transmembrane receptors protein known to regulate several cellular processes including axonal guidance in the developing nervous system. Upon activation, plexin initiates signaling processes, which involve several small GTPases of the Ras and Rho families (R-Ras, Rac1, Rnd1, and RhoD) that regulates cytoskeletal dynamics and cell adhesion. Plexins are unique amongst transmembrane receptors because its several cytoplasmic regions interact directly with small GTPases. Specifically, plexins possess a domain with homology to GTPase activating proteins (GAPs). As part of their activation, plexin family shows GAP activity toward R-Ras. However, the mechanism of activation is not known till date because of lack of information about the structure and function of these receptor proteins. In this context, we have studied the structure, function of intracellular region of PlexinB1 and their binding interaction with small GTPase. The structure is monomeric and binds to Rac1, Rnd1 as well as Rras, but not H-Ras. These findings suggest that the monomeric form of the intracellular region is primed for GAP activity and extend a model for plexin activation.
[1] Tong, Y., et al. (2008) Structure 16, 246-258.[2] Bouguet-Bonnet, S. & Buck, M (2008) J.Mol.Biol. 377,1474-87.[3] Tong, Y, et al. (2007) J.Biol. Chem. 282, 37215-37224.[4] Hota, P. & Buck, M. (2009) Protein Science, 18(5):1060-71.[5] Tong, Y., et al. (2009) J. Biol. Chem. Submitted.

Protein Structure I

1285-Pos
Structural and Functional Studies of Bacterial Toxin-Antitoxin Systems
Gregory Verdon[1], Lauren DeStefano[2], Chi Wang[3], Gregory Boel[3], Guy Montelione[4], Nancy Woychik[5], John F. Hunt[3].
[1]Weill Medical College of Cornell University, New York, NY, USA, [2]UMDNJ-RWJMS Department of Molecular Genetics and Microbiology & Immunology, Piscataway, NJ, USA, [3]Columbia University, Dept. of Biological Sciences, New York, NY, USA, [4]Rutgers - State University of New Jersey, New Brunswick, NY, USA, [5]UMDNJ-RWJMS Department of Molecular Genetics and Microbiology & Immunology, PIscataway, NJ, USA.
TA systems have recently been linked to medically important processes such as biofilm formation, bacterial persistence after exposure to antibiotics, and bacterial pathogenesis. Toxin-Antitoxin (TA) systems are stable protein complexes consisting of a toxin, whose action is mechanistically distinct from exotoxins (e.g. botulinum, anthrax or cholera toxins), in complex with an antitoxin, its specific inhibitor. The release of TA toxin occurs under specific

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