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
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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
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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
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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
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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
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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