surfactant, and DPPC/POPG/Palmitic Acid (68:22:9) (lipidTA), a system widely used as a basis for clinical surfactants. We found similar equilibrium surface tensions after 5 min of adsorption of all the samples, regardless the protein and lipid system, although SP-B exhibited somehow slower initial adsorption in lipidTA. Significant differences were found in SP-B activity under quasi static compression-expansion cycling for the two lipid systems tested. In lipidTA, SP-B allowed reaching tensions near 2mN/m, whereas in lipidS surface tension did not fall below 20mN/m. However, SP-B-containing samples produced similarly low tensions, within the two lipid compositions, once cycled dynamically at physiologically relevant compression-expansion rates. Analysis of film stability under mechanical perturbations showed that SP-B introduces a significant resistance of the films to relaxation, which is particularly remarkable in lipidS samples. This stability was maximal in the simultaneous presence of SP-B and SP-C.

**297-Pos**

**The NHERF2 Dependent Dynamic Ca2+/LPA Regulation of NHE3 Mobility and Interaction At the Epithelial Brush Border**

**Boyoung Cha**, Xinjun C. Zhu, Nicholas C. Zachos, Rafiquel Sarker, Molee Chakraborty, Olga Kovbasnjuk, Mark Donowitz.
The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Na+/H+ exchange 3 (NHE3) plays an essential role in NaCl absorption intestine and kidney. NHE3 rapidly cycles between the plasma membrane and recycling endosomal compartment under basal conditions. Those regulations require PDZ domain containing NHERF proteins. Especially NHERF2 is required both in Ca2+ inhibitory and LPA stimulatory regulations in NHE3 activity. In this study, using the FRET technique, the dynamic binding between NHERF2 and NHE3 at the brush border was investigated in the presence of Ca2+ or LPA. Zeiss 510 Meta confocal microscopy was used to perform FRET (acceptor photobleaching) between NHE3-YFP and CFP-NHERF2 on the apical brush border in polarized epithelial kidney OK cells.
We observed that NHERF2 and NHE3 exhibited 10-20% FRET signaling at the microvilli and not at the juxtanuclear region under basal conditions. As a negative control, there was no FRET signaling between CFP-NHERF2 and YFP-GPI. With treatment of Ca2+ ionophore, A23187(0.5μM) or LPA(100μM), FRET signaling was transiently abolished within one minute for A23187 and within 30min for LPA and recovered at 1 hr later.
The dynamic interactions between NHE3 and NHERF2 by LPA and Ca2+ in OK cell microvilli were quantified by FRET. We conclude that the dissociation of NHEFR2 from NHE3 at the microvillus leads to NHE3 activity inhibition by A23187 by increasing the NHE3 endocytosis and leads to stimulation of NHE3 activity by LPA by increasing NHE3 translocation to the brush border.

## Protein Assemblies

**298-Pos**

**Structural Survey of Large Protein Complexes in Desulfovibrio Vulgaris**

**Bong-Gyoon Han**, Ming Dong, Mark D. Biggin, Robert M. Glaeser.
Lawrence Berkeley National Laboratory, University of California, Berkeley, Berkeley, CA, USA.

Large protein complexes purified by a tagless strategy have been analyzed for an unbiased survey of the stable, most abundant multi-protein complexes in Desulfovibrio vulgaris Hildenborough (DvH) that are larger than Mr ~400 k. The quaternary structures were determined for 8 out of 16 complexes by single-particle reconstruction of negatively stained specimens. The success rate of getting structure was about 10 times greater than that of previous ''proteomic'' screens. For the remaining complexes, the subunit compositions and stoichiometries were analyzed by biochemical methods. Our results show that the structures of large protein complexes vary to a great extent from one microorganism to another. None of the complexes except for GroEL and the ribosome could not be modeled from the previously known homologous structures due to organism dependent variation of quaternary structures. This result indicates that the interaction interfaces within large, macromolecular complexes are much more variable than has generally been appreciated. As a consequence, the quaternary structures for homologous proteins may not be sufficient to understand their role in another cell of interest. The diversity of subunit stoichiometries and quaternary structures of multiprotein complexes that has been observed in our experiments with DvH is relevant to understanding how different bacteria optimize the kinetics and performance of their respective biochemical networks. It is further anticipated that imaging the spatial locations of such complexes, through the analysis of tomographic reconstructions may also be important for accurate computational modeling of such networks. While templates for some multi-protein complexes such as the ribosome or GroEL could be derived from previously determined structures, it is quite clear that single-particle electron microscopy should be used to establish the sizes and shapes of the actual complexes that exist in a new organism of interest to prepare valid templates.

**299-Pos**

**Ranolazine Preserves the Integrity of Mitochondrial Supercomplexes**

**Ashish K. Gadicherla**, Meiying Yang, Amadou K.S. Camara, Mohammed Aldakkak, Age D. Boelens, Bassam Wakim, David F. Stowe.
Medical College of Wisconsin, Milwaukee, WI, USA.

Mitochondrial respiratory complexes are known to exist in multi-complex assemblies (respirasomes). These respirasomes and their constituents are known to be damaged during ischemia reperfusion (IR) injury. In the present study we examined if ranolazine, a late sodium current blocker, and also a partial fatty acid oxidation inhibitor, preserves these assemblies after cardiac IR injury. Guinea pig hearts (n=6) were isolated and perfused with Krebs Ringer buffer (KR) and exposed to one of the following three protocols: 1) KR perfusion for 30 min, (time control), 2) 30 min global ischemia, or 3) ranolazine (10 μM) perfusion for 10 min just before 30 min global ischemia. Mitochondria were isolated by differential centrifugation and then subjected to blue native Polyacrylamide Gel Electrophoresis (BN-PAGE) to examine for damage to the respirasomes. We observed that there is a loss of protein bands after electrophoresis at 720 kDa and at 250 kDa in the untreated ischemic group. These bands were restored in the ranolazine treated group. These proteins will be subject to identification. Our results indicate that cardiac ischemia causes a loss of integrity of respiratory complexes, which is restored partially by ranolazine. A candidate for ranolazine's protective effect is cardiolipin, which stabilizes the respiratory chain supercomplexes, and which may be less oxidized after ranolazine treatment.

**300-Pos**

**A Biophysical Investigation of the Non-Classical Release Complex of Fibroblast Growth Factor-1**

**Katie Hamblin**[1], Anna E. Daily[2], T.K.S. Kumar[2].
[1]University of Arkansas, Eureka Springs, AR, USA, [2]University of Arkansas, Fayetteville, AR, USA.

Fibroblast Growth Factor-1 (FGF-1) is a potent angiogenic agent that is released via the non-classical protein secretion pathway. Angiogenesis, the process of formation of new blood vessels, is vital to the formation of tumors, and is also responsible for tumor metastasis, as cancer cells travel from one part of the body to another through the newly formed vessels. Export of FGF-1 is based on the Cu2+ -dependent structure of multi-protein complexes, which involves the S100A13, a Ca2+ binding protein belonging to the family of S100 protein. The goal of this study is to characterize the structure of the FGF-1/S100A13 Data will be presented analyzing the interaction between FGF-1 and an S100A13 peptide that has been designed to mimic the binding region of FGF-1 on S100A13. The binding interaction was characterized using various biophysical techniques including ITC, DSC, proteolytic digestion, and multi-dimensional NMR spectroscopy. Characterization of the binding FGF-1/S100A13 interface is expected to shed light on the molecular mechanism(s) underlying the non-classical secretion of FGF-1.

**301-Pos**

**The Monomerization of a Dimeric, Calcium-Binding Protein Involved in the Non-Classical Export of Fibroblast Growth Factor 1**

**Emily M. Erstine**, Anna E. Daily, T.K.S. Kumar, T.K.S. Kumar.
University of Arkansas, Fayetteville, AR, USA.

The non-classical secretion of fibroblast growth factor 1 (FGF1) is a poorly understood process. FGF1 is known to interact with the calcium-binding protein S100A13, which escorts FGF1 to the cytoplasmic surface of the cell membrane. The dimeric, highly alpha-helical structure of S100A13 has been well characterized. In addition to binding to $Ca^{2+}$, S100A13 has been shown to bind to $Cu^{2+}$. Binding of $Cu^{2+}$ to S100A13 is believed to be crucial for the formation of the FGF1 release complex. In order to gain a better understanding of the structural forces involved in the organization of the multi-protein FGF1 release pathway, we have embarked on the determination of the 3D structure of the FGF1 release complex in solution using multi-dimensional NMR spectroscopy techniques. As a first step toward achieving this objective, we have designed an S100A13 monomer through site-specific mutations at the S100A13 dimeric interface. Results on the characterization of the S100A13 monomer using ITC, DSC, CD spectroscopy, and multi-dimensional NMR spectroscopy will be presented.

**302-Pos**

**Reconstructing the Neisseria Type IV Pilus System in E.coli**

**Lorraine S. Meyer**, Katrina T. Forest.
University of Wisconsin - Madison, Madison, WI, USA.

Type IV Pili, long, thread-like structures found on the surface of many species of bacteria, are important virulence factors involved in motility, DNA/phage uptake, biofilm formation, and adhesion. Energy for the system is supplied by a set of cytoplasmic, hexameric ATPases which interact with proteins within the bacterial membrane to traffic pilin monomers to and from the pilus. The