

In many protein ion channels found in biological membranes, a central cavity is formed between the membrane-spanning subunits of the protein, though which ions flow selectively. There is no general consensus regarding whether nonpolar cavities inside proteins are water filled or empty, or what conditions are necessary and sufficient for these two cases. The potassium channel from the soil bacteria *Streptomyces lividans* named KcsA, is the first of the potassium-selective channel proteins whose structure has been solved crystallographically (Doyle, D.A. et al., *Science* **280**, 1998). KcsA has become the principal model for the pore domain in voltage-gated K<sup>+</sup>, Na<sup>+</sup>, and Ca<sup>2+</sup> channels due to highly conserved structural motifs in the pore domain characteristic for these channels. The cavity dimensions for KcsA inferred from crystallographic structures allow less than 20 water molecules to be accommodated. Crystallographic maps, however, do not typically reveal the true conformation of the channel in a fluid lipid environment, nor can they reveal water in cavities due to its high thermal disorder and low scattering density. In this report, we present neutron diffraction data on KcsA pore domain incorporated in lipid liquid crystalline phases. Hydrogen to Deuterium substitution is used to determine the amount of water and its distribution across the pore domain of the channel.

### 331-Pos Board B100

#### In Situ Visualization of Mouse Skeletal Muscle Ryanodine Receptor by Cryo-Electron Tomography

**Cahyo Budiman**<sup>1,2</sup>, Hirotohi Furusho<sup>2</sup>, Yumiko Mishima<sup>2</sup>, Lars-Göran Öfverstedt<sup>2</sup>, Ulf Skoglund<sup>2</sup>, Ichiro Maruyama<sup>1</sup>.

<sup>1</sup>Information Processing Biology Unit, Okinawa Institute of Science and Technology (OIST), Onna son, Japan, <sup>2</sup>Structural Cellular Biology Unit, Okinawa Institute of Science and Technology (OIST), Onna son, Japan. Among three isoforms of mouse ryanodine receptors (RyRs), which share 70% similarities with their amino acid sequences, the type 1 skeletal muscle ryanodine receptor (RyR1) is the most extensively studied and known. Structurally, RyR1 is made up of four identical subunits. Each subunit has a molecular mass of approximately 500-600 kDa. This isoform contains at least two functional domains: a carboxyl-terminal hydrophobic domain or trans-membrane domain that contains the Ca<sup>2+</sup> release channel pore, and a large amino-terminal domain or cytoplasmic domain that contains sites responsible for the channel regulation. Physiologically, RyR1 functions as a channel for calcium-induced calcium release (CICR) during excitation-contraction coupling (EC-coupling), in which Ca<sup>2+</sup> release from the sarcoplasmic reticulum is followed by the subsequent muscle contraction. In central core disease, mutations in RyR1 may promote disruption of EC-coupling resulting in skeletal muscle weakness and lower limb deformities. The important role for RyR1 in this physiological function drives the need for high resolution and comprehensive structural information. Despite many structural studies on RyR1, however, the mechanism underlying this phenomenon remains enigmatic. Advances in techniques and approaches to observe a structure of RyR1 is important to address this question. In this study, we used immunogold for in situ visualization of mice RyR1 by electron tomography. To our knowledge, this is the first report on the application of electron tomography for in situ visualization of RyR1.

### 332-Pos Board B101

#### Force Dependent Changes in Non-Erythroid Spectrin and Ankyrins

**Eleni K. Degaga**<sup>1</sup>, Martin B. Forstner<sup>1,2</sup>.

<sup>1</sup>Physic Department, Syracuse University, Syracuse, NY, USA, <sup>2</sup>Syracuse Biomaterials Institute, Syracuse University, Syracuse, NY, USA. Mechanotransduction in cells describes the process by which external physical stimuli are converted into biochemical activity and plays an important role in many biological functions on both the cell and tissue level. However, the specific mechanisms by which mechanical forces lead to particular molecular and cellular responses are much less understood. We investigate the changes in non-erythroid spectrin and ankyrins as a result of equi-biaxial strain application to cells in culture. Specifically, we focus on the spectrins' role in the ubiquitination process - a vital process in the regulation of protein degradation- of spectrin and ankyrins. We utilize immune-fluorescence staining in combination with quantitative fluorescence imaging as well as biochemical methods to measure changes in cell's spectrin and ankyrin content. Protein expression levels and localization between cells exposed to mechanical stimuli of different temporal and spatial profiles are compared.

### 333-Pos Board B102

#### Electrical Response of Bilayers to the Bee Venom Toxin Melittin

**Gregory Wiedman**<sup>1</sup>, Kate Herman<sup>1</sup>, Peter Searson<sup>1</sup>, William C. Wimley<sup>2</sup>, Kalina Hristova<sup>1</sup>.

<sup>1</sup>Johns Hopkins University, Baltimore, MD, USA, <sup>2</sup>Tulane University Medical School, New Orleans, LA, USA.

Melittin is a 26-residue amphipathic alpha-helix that causes membrane destabilization via a mechanism that is still disputed. While a transmembrane pore model has been a central part of the mechanistic dialogue for decades, there is growing evidence that a transmembrane pore is not required for melittin activity. In part, the controversy is due to limited experimental tools to probe the bilayer's response to melittin. Electrochemical impedance spectroscopy (EIS) is a technique that can reveal details of molecular mechanism of peptide activity, as it yields direct measurements of membrane resistance and capacitance of supported bilayers. In the work presented here, this technique was used to study the response of surface-supported bilayers of different lipid compositions to melittin. The EIS results directly demonstrate that the response of a bilayer to melittin cannot be explained by a simple transmembrane pore model.

### 334-Pos Board B103

#### High-Yield Expression and NMR Structural Studies of Antimicrobial Peptides, LPcin Analogs

**Yongae Kim**, Ji-Ho Jung.

Hankuk University of Foreign Studies, Yong-In, Korea, Republic of.

Lactophorin (LPcin), a cationic amphipathic peptide consists of 23-mer peptide, corresponds to the carboxy terminal 113-135 region of component-3 of proteose-peptone. LPcin is a good candidate as a peptide antibiotic because it has an antibacterial activity but no hemolytic activity. Three different analogs of LPcin, LPcin-yk2 which has mutant amino acids, LPcin-yk1 and LPcin-yk3 that has shorter mutant amino acids are recently developed by using peptide engineering in our lab. These three LPcin analogs show better antibiotic activities than LPcin and no toxicity at all.

In order to understand the structural correlation between LPcin structure and antimicrobial activity under the membrane environments, we tried to express and purify as large as amounts of LPcin and three different LPcin analogs. We finally optimized and succeed to overexpress in the form of fusion protein in *Escherichia coli* and purified with biophysical techniques like Ni-affinity chromatography, dialysis, centrifuge, chemical cleavage, and reversed-phase semiprep HPLC. In order to identify the purified peptides, we performed Mass, CD and NMR spectrometry for their characteristic structure in membrane environments. In here, we will present the optimizing processes for high-yield expression and purification and solution NMR spectra and solid state NMR spectra for antimicrobial mechanisms.

### 335-Pos Board B104

#### New Insights into CFTR as GSH Transporter

**Guangyu Wang**.

University of Nevada, Reno, NV, USA.

The cystic fibrosis transmembrane regulator (CFTR) chloride channel is an important member of the ATP-binding cassette superfamily because dysfunction or low expression of CFTR was found in patients with human cystic fibrosis. Further studies show low mitochondrial GSH levels in the CFTR-knockout mouse lung. Because impaired mitochondrial oxidative metabolism plays a critical role in lung diseases, CFTR has been proposed to be expressed on the mitochondria to promote GSH transportation across mitochondria to protect the lung from oxidative damage. However, it has not been well-established that CFTR functions as GSH transporter under the physiological conditions. Here, patch clamp studies demonstrate that only outward GSH currents across inside-out membrane patches were found with WT CFTR while both inward and outward GSH currents were observed with the constitutively active K190C/K978C CFTR construct with a high open probability. Thus, asymmetric GSH permeation may be state-dependent. Further studies show that the constitutively active CFTR mutant T338C/K190C/K978C was completely inhibited by extracellular Cu<sup>2+</sup> and inhibition was fractionally reversed by 10mM intracellular membrane-impermeant GSH and completely by intracellular membrane-permeant TPEN. In contrast, extracellular Cu<sup>2+</sup> failed to suppress the activity of the CFTR mutant K190C/K978C. The application of 10mM GSH or TPEN to the intracellular side of this construct did not increase the channel activity, either. These results suggest that native CFTR may function as GSH transport under the physiological condition and designed transition metal binding sites at the channel pore can be used to define its GSH permeability.

### 336-Pos Board B105

#### Molecular Mechanism of Membrane Targeting by Endosomal Adaptor Proteins

**Daniel G.S. Capelluto**<sup>1</sup>, Shuyan Xiao<sup>1</sup>, Sharmistha Mitra<sup>1</sup>, C. Alicia Traugher<sup>1</sup>, Stephanie Gomez<sup>1</sup>, Mary K. Brannon<sup>1</sup>, Carla V. Finkielstein<sup>2</sup>.