

distribution of PICK1. To investigate whether the altered cellular distribution results directly from a change in the lipid binding capacities of the AH, we employ a Single Liposome Curvature Sensing (SLiC) assay. We use quantitative fluorescence microscopy to evaluate the binding of the phospho-mimicking mutants to nanosized liposomes in terms of MC-sensing, lipid affinity and membrane deformation.

Intriguingly, we find that this single phospho-mimicking mutation in the AH is sufficient to change the lipid binding capacities of the entire protein, likely causing the altered cellular distribution of the phosphorylated protein seen in the cells. As MC-sensing has been shown to be dependent on the AH of N-BAR proteins in general, we speculate that the finding may apply generally to phospho-regulation of N-BAR proteins.

### 3065-Pos Board B220

#### Membrane Penetrating Ability of Ebola Matrix Protein, VP40

Smita P. Soni<sup>1</sup>, Emmanuel Adu-Gyamfi<sup>2</sup>, Robert V. Stahelin<sup>2,3</sup>, Christina Leblang<sup>1</sup>.

<sup>1</sup>IU- School of Medicine, South Bend, IN, USA, <sup>2</sup>University of Notre Dame, Notre Dame, IN, USA, <sup>3</sup>IU-School of Medicine, South Bend, IN, USA.

Ebola from the *filoviridae* family of viruses causes severe and mostly fatal hemorrhagic fevers in primates and has been listed as a category IV pathogen by the NIH. Viral Protein 40 (VP40), the major matrix protein of Ebola virus, regulates the assembly and budding of the virus and alone harbors the ability to form virus-like particles (VLPs) from human cells. We hypothesize that VP40 is a high affinity lipid binding and membrane curvature-inducing protein with specificity for plasma membrane (PM) lipids. This specificity leads to localization of VP40 to the PS-rich inner leaflet of the PM and formation of VLPs. Using fluorescence spectroscopy to investigate VP40 binding and insertion within lipid vesicles (POPC:POPS) containing brominated lipids. Because the fluorescence of the tryptophan is variably quenched depending on its distance from the bromine atoms on the lipid acyl chain, a tryptophan introduced into the membrane binding interface was utilized as a probe to detect the depth of membrane penetration of VP40. Results were indicative of VP40's high affinity and specificity for PS in a PS-concentration dependent manner demonstrating the robust ability of VP40 to penetrate membranes. Further analysis of VP40 membrane insertion revealed a depth of penetration more than halfway into one monolayer of the membrane. Data also confirmed that VP40 binds with nanomolar affinity to vesicles that recapitulated the PM in comparison to the nuclear membrane. In addition VP40-mutants, which inhibit membrane binding and penetration, obstruct VLP formation and release. Cellular assays confirmed the lipid-binding specificity of VP40 in the PM of different cell lines and also demonstrated that deep membrane penetration is essential for VLP. We predict that these results will elucidate the molecular basis of VP40 induced membrane curvature changes, a prerequisite to the PM deformation required for VLP production.

### 3066-Pos Board B221

#### Spatial and Temporal Regulation of the Nedd4 Family of E3 Ubiquitin Ligases through Phospholipid Binding

Jordan L. Scott<sup>1</sup>, Robert V. Stahelin<sup>2</sup>.

<sup>1</sup>University of Notre Dame, South Bend, IN, USA, <sup>2</sup>Indiana University School of Medicine, South Bend, IN, USA.

The Nedd4 family of HECT ubiquitin ligases are essential regulators of cellular polarity, ion channel activity, motility, and inflammatory signaling. HECT family Ubiquitin ligases employ a catalytic cysteine residue to target cellular substrates for mono or poly ubiquitination. The Nedd4 family has nine members each with an N-terminal canonical C2 domain, protein recognition WW domains, and a catalytic HECT domain. While several reports in the literature indicate which substrates these proteins target, little is known about how their cellular localization and catalytic activity are regulated by their C2 domains. These domains have previously been shown to bind phospholipids and to be required for the localization of some members to the plasma membrane. In our laboratory, we employ surface plasmon resonance (SPR) technology to measure the affinity of proteins for specific lipids in an *in vitro* environment with a lipid vesicle coated surface. Using SPR, we have investigated the specific lipid binding properties of the Nedd4 family C2 domains to vesicles of specific composition. We find that several Nedd4 family C2 domains bind to phosphoinositides with nanomolar affinity. In addition, we have used vesicular sedimentation assays and immunological lipid blots to confirm these binding results. We are employing confocal microscopy and fluorescent C2 constructs to determine their cellular localization and to quantitatively determine their diffusion coefficients, oligomerization state in the cytosol and at cellular membranes. Finally, we are using both confocal microscopy and immunoblotting to determine how lipid binding regulates the ubiquitination state of Nedd4 family members.

### 3067-Pos Board B222

#### Mode of Action of the Bacterial Thermosensor DesK Involved in Regulating Membrane Fluidity

Joost Ballering<sup>1</sup>, Larisa E. Cybulski<sup>2</sup>, Martijn C. Koorengel<sup>1</sup>, Martin Ulmschneider<sup>3</sup>, Diego de Mendoza<sup>2</sup>, J. Antoinette Killian<sup>1</sup>.

<sup>1</sup>Membrane Biochemistry & Biophysics, Bijvoetcenter for Biomolecular Research, Utrecht University, Utrecht, Netherlands, <sup>2</sup>Instituto de Biología Celular y Molecular de Rosario-CONICET, Universidad Nacional de Rosario, Rosario, Argentina, <sup>3</sup>Institute of Structural and Molecular Biology Department of Biological Sciences Birkbeck University of London, London, United Kingdom.

The *Bacillus subtilis* membrane harbors the temperature sensing and signaling protein DesK. At low temperatures it triggers expression of a desaturase, which introduces double bonds into pre-existing phospholipids, thereby regulating membrane fluidity. Recently it was discovered [1] that both sensing and transmission of DesK, which has five transmembrane segments, can be captured into one single chimerical transmembrane segment, the so-called 'minimal sensor' (DesK-MS). This protein can be functionally reconstituted in lipid bilayers, thus providing an excellent model system to study the molecular details of a biologically important signaling mechanism.

Here we used synthetic peptides corresponding to functional and non-functional mutants of the minimal sensor in artificial membranes of phosphatidylcholines as convenient model systems. We studied the conformational properties, tilt, and exposure at the lipid/water interface at different bilayer thickness and upon varying temperature by using circular dichroism and fluorescence studies. Based on these results and on mutational studies, we propose a model for the mode of action of DesK-MS, in which an N-terminal "sunken buoy" motif and a C-terminal hydrophilic motif are crucial for DesK-MS functioning. Finally, we explored the possibility of isolating and characterizing DesK-MS in its native membrane in the form of "native nanodiscs" by using copolymers of styrene and maleic acid (SMA). Results of these studies will be presented.

[1] Cybulski LE, Martin M, Mansilla MC, Fernandez A, de Mendoza D. Membrane thickness cue for cold sensing in a bacterium. *Curr Biol.* 2010 20(17):1539-44

### 3068-Pos Board B223

#### Separation of Timescales for Endophilin Dimerization and Membrane Binding

Benjamin R. Capraro, Ken Chang, Zheng Shi, Tingting Wu, Chih-Jung Hsu, Tobias Baumgart.

University of Pennsylvania, Philadelphia, PA, USA.

The membrane association of endophilin is a pivotal step in clathrin-mediated endocytosis. In this process, the basis for the functional role of endophilin is believed to involve the promotion of membrane curvature, which in turn depends on membrane shaping by the dimeric structure of endophilin. Thus, the dynamic nature of endophilin-membrane association and dimerization are functionally important. However, little is known about the timescales and factors determining the kinetics of the interactions involved. To illuminate these aspects, we study the kinetics and equilibria of endophilin N-BAR dimerization and membrane binding. We determine the dimerization equilibrium constant by using subunit exchange FRET. We characterize N-BAR membrane association, under conditions where the dimeric species predominates, by stopped-flow, observing prominent electrostatic sensitivity. Our results suggest that membrane insertion of amphipathic helices rapidly follows association, in a non-rate-limiting manner. Relative to membrane binding, we find that dimerization is governed by far slower kinetics. Thus, monomer-dimer exchange does not contribute to the kinetic mechanism of membrane binding. These results underscore a separation of timescales for endophilin dimerization and membrane binding, which may facilitate temporal regulation of functional membrane processes.

### 3069-Pos Board B224

#### Structural Changes of Alpha 1-Antitrypsin Under Osmotic Pressure and in the Presence of Lipid Membranes

Luis A. Palacio<sup>1</sup>, Christopher B. Stanley<sup>2</sup>, Andrew K. Fraser<sup>1</sup>, Merrell A. Johnson<sup>1</sup>, Horia I. Petrache<sup>1</sup>.

<sup>1</sup>Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, <sup>2</sup>Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Alpha 1-Antitrypsin (A1AT) is a glycoprotein that has been shown to have protective roles of lung cells against emphysema, a disease characterized by lung tissue destruction [1]. Most known glycoproteins have been shown to play a role in cellular interactions but the exact role of the glycan chains is still under investigation. Previous electrophysiological measurements show that A1AT has a strong affinity to lipid bilayers perturbing the function of ion channels