observations, and the functionality of the channels in the bead-buttressed unilamellar membrane (bBUM) system was examined by electrical recordings of voltage-gated activities. We anticipate that this novel membrane system will provide a new technique to study how lipids influence membrane proteins.

3060-Pos Board B215
Toward Understanding the Role of Angiomotin Lipid Binding in Cellular Proliferation and Migration
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The Angiomotin (Amot) family of adaptor proteins directly integrates the signaling that controls cellular differentiation and cell growth. Amot family members bind core polarity proteins that control the organization of the apical domain of epithelial cells as well as Yap, a transcriptional co-activator that appears to be the key regulator of cell growth. A critical feature of all Amot proteins is a novel lipid binding domain, the Amot coiled-coil homology (ACCH) domain, which confers its association with membranes and affects membrane curvature. This domain, while sharing some features of BAR domains, i.e. a predicted coiled coil fold of approximately 240 residues, also has unique properties including the ability to selectively bind monophosphorylated phosphatidylinositols (PI). Similar binding of PI has been reported in other protein domains, including FYVE, PX and PH domains where PI binding is mediated through basic residues within a loop rich in lysines, arginines, as well as hydrophobic amino acids. The ACCH domain is predicted to have a coiled-coil fold and is rich in lysines.
One of our goals is to delineate which amino acids contribute to PI binding, thereby suggesting possible routes to modulate the increased Amot80 ACCH domain activity that is associated with ductal hyperplasia, and later breast cancer. Site-directed mutagenesis was employed to probe the specific contributions of selected lysines and arginines toward lipid head-group binding. The effect of the mutation was then analyzed using liposome sedimentation, FRET, and SAXS to monitor lipid binding. As the ACCH domain lacks tryptophan residues, protein association with liposomes was followed by monitoring changes in protein fluorescence associated with the proximity of tyrosines 11, 47, or 118 to lipid.
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3061-Pos Board B216
Assembly and Activity of Respiratory Complex II in Nanolipoprotein Particles
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Mitochondrial respiratory Complex II (succinate:ubiquinone oxidoreductase) couples the citric acid cycle to the electron transport chain by oxidizing succinate in the matrix and passing electrons to ubiquinone in the inner membrane. Complex II is comprised of a soluble catalytic heterodimer (Sdh1/Sdh2) and a membrane-bound heterodimer (Sdh3/Sdh4). The structure of Complex II is well established; however, little is known about how the lipid environment regulates holocomplex assembly and activity. To address this question, we reconstituted Complex II from native biomembranes into nanoscale phospholipid bilayers (nanodiscs) containing a defined lipid content. We found that the dimeric phospholipid cardiolipin, the signature lipid of energy-conserving membranes, is critical for Complex II stability and function. First, the presence of cardiolipin in the bilayer promoted the interaction of the soluble and membrane-bound dimers. Second, cardiolipin was essential for enzymatic activity of the reconstituted complex and for curtailment of reactive oxygen species production. The function of cardiolipin could be partially compensated by the presence of phosphatidylglycerol, another phospholipid with an anionic headgroup; moreover, reducing the acyl chain lengths of cardiolipin used for reconstitution prevented its stimulatory effect on Complex II activity. Hence, both the headgroup and hydrocarbon chains of cardiolipin play important roles. Using this experimental platform, we have employed site-specific fluorescence labeling to address which structural elements of Complex II membrane subunits undergo conformational dynamics during assembly. Our results indicate that Sdh3 matrix-facing helix I, which interacts extensively with the catalytic dimer in the holocomplex, undergoes structural changes when reconstituted with Sdh4, but not Sdh4 homologs, suggesting that this helix may act as a conformational switch for downstream assembly steps (e.g., recruitment of the soluble dimer). Taken together, we show that Complex II is structurally dynamic during assembly and that its function is highly lipid-dependent.

3062-Pos Board B217
Purification and Characterization of the Tetrameric Potassium Channel Kcsa in "Native Nanodiscs"
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We explored a new discovery in membrane research of amphipathic copolymers of styrene and maleic acid (SMA) that have the unique potential to directly extract proteins from membranes in the form of "native nanodiscs" without the need for detergent [1,2]. *E-coli* cells overexpressing a His-tagged version of the potassium channel KcsA were incubated with SMA and the conditions were optimized for extraction of the protein. After solubilization of the membrane, we found that KcsA indeed could be purified on a Ni^{2+}-NTA column in the form of nano-sized discs, as was confirmed by negative stain transmission electron microscopy (TEM) experiments. SDS-PAGE analysis showed that the protein in these nanodiscs is present as a tetramer, running at the same position as when subjected to SDS-PAGE from its native membrane or after purification in detergent.
Presently we are comparing the stability of this tetramer in the nanodiscs with that of purified KcsA in *n*-dodecyl β-D-maltoside (DDM) micelles and KcsA in native membranes. This is done by comparing the effects of heat-incubation and exposure to small alcohols using gel shift assays. Furthermore, to gain information about possible preferential lipid interactions of KcsA, the lipid composition of the purified, KcsA containing nanodiscs is being analyzed and compared to that of the total lipid composition in *E-coli*. Results of these studies will be presented.
1) Knowles TJ, Finka R, Smith C, Lin Y-P, Dafforn T, Overduin M (2009) J Am Chem Soc 131, 7484-7485
2) Orwich-Rydmark M, Lovett JE, Lindholm L, Graziadei A , Hicks M, Watts A (2012) Nanoletters 12, 4687-4692

3063-Pos Board B218
Determining the Role of NS4B in Membrane Remodelling during Hcv Replication
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Hepatitis C is an RNA virus that replicates in association with intracellular membranous structures call membranous webs (MWs). Viral protein NS4B is a key organizer of replication, one crucial function being the induction of MWs. The mechanisms of MW formation are unknown, but it clearly involves induction of membrane curvature, which may require NS4B oligomerisation and possibly hydrophobic wedging. NS4B is known to oligomerise, and the N-terminal amphipathic helix AH2 has been implied as a major determinant of self-association. In order to understand the process of MW induction, we aimed to determine AH2's capacity to remodel membranes by studying the interaction of AH2 with membranes mimicking those found within the cell using 2H and 31P solid-state NMR. Our results show changes in membrane morphology, induced by AH2 in negatively charged vesicles, an effect not observed in neutral bilayers indicating a requirement for negatively charged lipids. Chemical cross-linking studies of AH2 in lipids vesicles confirms AH2 homo-oligomerisation and suggests a charge dependency; with larger oligomers observed in neutral lipid bilayers compared to negatively charged lipid bilayers and lipid mixtures mimicking cellular membranes. These results suggest that AH2 plays a crucial role in NS4B's capacity to alter membrane morphology.

3064-Pos Board B219
Phosphorylation of the Amphipathic Helix Changes the Lipid Binding Capacities of PICK1
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PICK1 (Protein Interacting with C-kinase 1) is a functionally important protein, which is distributed mainly in testis, pancreas and brain. It has been shown to play a central role in regulation of dense core vesicles from the golgi apparatus and trafficking of ionotropic glutamate receptors.
PICK1 contains a N-terminal PDZ-domain, which we have earlier demonstrated to be important for interaction with a large number of proteins, including several important receptors and transporters. In addition, it has a BAR (Bin/Amphiphysin/Rvs) domain in the C-terminal end. BAR domains are generally believed to either recognize or induce curvatures of lipid membranes, but as we have demonstrated, proteins of the N-BAR family (incl. PICK1) binds lipids and recognizes membrane curvature (MC) through an associated amphipathic helix (AH) rather than through the BAR domain itself.
Here we show that the lipid binding AH of PICK1 contains a phosphorylation-site, which, through PKC activation, is responsible for an altered cellular

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 quantitivative 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
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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
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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
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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
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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
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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