

2606-Pos Board B298**Human Guanylate-Binding Protein 1 Tethers Giant Unilamellar Vesicles in a Nucleotide-Dependent Manner**Sergii Shydlovskiy¹, Annika Hohendahl², Gerrit J.K. Praefcke^{3,4}, Aurélien Roux², Christian Herrmann¹.¹Physical Chemistry I, Ruhr University Bochum, Bochum, Germany,²Biochemistry Department, University of Geneva, Geneva, Switzerland,³Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany,⁴Institute for Genetics, University of Cologne, Cologne, Germany.

Human guanylate-binding protein 1 (hGBP1) is the most studied protein within the family of guanylate-binding proteins (GBPs), which has seven isoforms in humans. GBPs belong to the dynamin superfamily of large GTPases and are thought to act as mechanoenzymes. All members of the GBPs family are expressed to high level after treatment of the cells with interferons, and hGBP1 in particular is mostly expressed by interferon γ , and, similar to the family of Mx proteins, is involved in antiviral response. However, the molecular mechanism of antiviral activity of hGBP1 is poorly understood. In the course of posttranslational modification the protein is coupled to a lipid anchor (isoprenoid), which might be crucial for performing its function within the cell. We address the question of the molecular function of hGBP1 by studies of its farnesylated form *in vitro* in the presence and absence of lipid systems. We can show nucleotide-dependent polymerization of the farnesylated form of hGBP1 and, moreover, we can show that the non-farnesylated form of hGBP1 disturbs the latter processes giving a hypothesis of possible regulation of the biological function of the protein by other isoforms from GBPs family, which cannot undergo lipid modification, through the heterointeraction. Previous studies of protein interaction with lipids show the binding of the protein to the liposomes only in the active state of the protein. In contrast, by using the lipid model of giant unilamellar vesicles (GUV), we can show that the protein, which carries the farnesyl anchor, binds to the vesicles directly after nucleotide binding and does not require GTP hydrolysis. Also we can show that it tethers vesicles in a nucleotide-dependent manner and we assume this to be related to the biological function of the protein.

2607-Pos Board B299**Transmembrane Domains of Bacterial Cell Division Proteins FtsB and FtsL Form a Stable High-Order Oligomer - A FRET Study**

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FtsB and FtsL are two essential integral membrane proteins of the bacterial division complex or "divisome", both characterized by a single transmembrane helix and a juxta-membrane coiled coil domain. The two domains are important for the association of FtsB and FtsL, a key event for their recruitment to the divisome, that in turn enables recruitment of the late divisomal components to the Z-ring and subsequent completion of the division process. Here we present a biophysical analysis performed *in vitro* that shows that the transmembrane domains of FtsB and FtsL associate strongly in isolation. Using FRET, we have measured the oligomerization of fluorophore-labeled transmembrane domains of FtsB and FtsL in both detergent and lipid. The data indicates that the transmembrane helices are likely a major contributor to the stability of the FtsB-FtsL complex. Our analyses show that FtsB and FtsL form a 1:1 higher-order oligomeric complex, possibly a tetramer. This finding suggests that the FtsB-FtsL complex is capable of multi-valent binding to FtsQ and other divisome components, a hypothesis that is consistent with the possibility that the FtsB-FtsL complex has a structural role in the stabilization of the Z-ring.

2608-Pos Board B300**General Anesthetics do not Alter Lipid Bilayer Properties at Clinically Relevant Concentrations**Karl F. Herold¹, William Lee¹, R. Lea Sanford², Olaf S. Andersen², Hugh C. Hemmings, Jr¹.¹Anesthesiology, Weill Cornell Medical College, New York, NY, USA,²Physiology, Biophysics and Systems Biology, Weill Cornell Medical College, New York, NY, USA.

General anesthetics are a widely used class of drugs but, despite their clinical use for >160 years, their exact molecular mechanism(s) remain to be elucidated. A mechanism proposed early on was direct interaction with the lipid bilayer, in some unspecified manner to alter cellular function, which lead to the unitary lipid-based hypothesis of anesthetic action. More recent studies show that general anesthetics interact specifically with various proteins, in particular membrane-embedded ion channels. For example, the inhibition of voltage-gated sodium channels by volatile anesthetics leads to reduced neurotransmitter release in excitable cells. But, though a number of anesthetic targets have been identified, it remains unclear whether the bilayer

per se may be involved as well. We therefore examined whether various general anesthetics (isoflurane, sevoflurane, halothane, desflurane, chloroform, diethyl ether, F3, cyclopropane, ketamine and etomidate) and related nonanesthetics (F6 and flurothyl) alter lipid bilayer properties at clinically relevant concentrations. The effects on lipid bilayer properties were tested using the gramicidin-based fluorescence assay (GBFA). The results show that none of the anesthetics or nonanesthetics tested altered lipid bilayer properties at the clinical concentration of 1 MAC (minimal alveolar concentration) with a membrane mole-fraction ranging from 1×10^{-3} (for F6) to 0.1 (for diethyl ether and sevoflurane). Even at two- to four-fold higher concentrations only minimal effects on the bilayer were observed; at much higher (supratherapeutic) concentrations, however, certain anesthetic agents did alter lipid bilayer properties. These results suggest that general anesthetics do not alter ion channel function by altering lipid bilayer properties in a manner that is sensed by a bilayer-spanning channel at clinically relevant concentrations.

2609-Pos Board B301**Arf1 Induced Membrane Remodeling and Morphological Changes Studied by Cryo-Em, Confocal Microscopy and Langmuir Film Balance**Sebastian Daum¹, Annette Meister², Kirsten Bacia¹.¹ZIK HALOmem, Martin-Luther-University Halle-Wittenberg, Halle, Germany,²Martin-Luther-University Halle-Wittenberg, Halle, Germany.

The small GTPase Arf1 of the RAS superfamily plays an important role in vesicular trafficking. On the Golgi membrane, the formation and fission of coat protein I (COPI) transport vesicles proceeds via local deformation of the lipid bilayer by a curvature generating COPI protein coat. The assembly of this complex is initiated by the GTPase Arf1 in a nucleotide-dependent manner. After GDP/GTP exchange, soluble Arf1 becomes membrane bound by insertion of its myristoylated N-terminal amphipathic helix (myrAH) into the proximal leaflet of the Golgi membrane. The subsequent liberation of transport vesicles requires the full COPI complex and has been observed *in vivo* and *in vitro*.

As the role of Arf1 in the process of curvature induction has not been fully elucidated, we have studied binding and incorporation of recombinant S. cerevisiae Arf1p into lipid mono- and bilayers using binding assays with a Langmuir film balance setup and artificial, unilamellar liposomes. We observe myristoylation-dependent binding to membranes and an increase in membrane surface area upon addition of Arf1p. Confocal laser scanning microscopy and cryo electron microscopy reveal highly curved membrane structures upon incorporation of myristoylated Arf1p. Our results support a mechanism of curvature induction based on the bilayer couple theory.

2610-Pos Board B302**The Dynamics of P-Rex 2 Membrane and Protein Interactions**Anne-Marie Bryant¹, Rakesh Kumar Harishchandra¹, Alonzo H. Ross², Arne Gericke¹.¹Worcester Polytechnic Institute, Worcester, MA, USA, ²University of Massachusetts Medical School, Worcester, MA, USA.

P-Rex 2 (phosphatidylinositol (3,4,5)-trisphosphate Rac exchanger 2) protein is a Rac guanine nucleotide exchange factor (GEF) whose activity is tightly regulated through activation by PI(3,4,5)P3 (phosphatidylinositol 3,4,5-trisphosphate) and G-protein $\beta\gamma$ subunits along with phosphorylation and domain-domain interactions. A previous study has shown that P-Rex 2 DH-PH domains interacts with PTEN (phosphate and tensin homologue deleted on chromosome 10), which is a frequently mutated tumor suppressor in human cancer that dephosphorylates PI(3,4,5)P3 and antagonizes PI3K signaling. P-Rex 2 binds to PTEN through its DH-PH domain, inhibits PTEN's lipid phosphatase activity and activates the PI3K pathway. This suggests that P-Rex 2 may function as a regulator of PTEN and activator of the PI3K pathway, thereby, contributing to a variety of pathological and physiological processes, such as tumorigenesis, diabetes, and aging. We are investigating the role of P-Rex 2 DH-PH domains in lipid binding and PTEN protein binding using steady-state fluorescence, stopped flow photometry, and ITC (isothermal titration calorimetry). This study aims to delineate P-Rex 2 membrane and protein interactions at the molecular level.

2611-Pos Board B303**The Effect of Hydrophobic Matching between Lipids and Transmembrane Peptides on Sterol Bilayer Affinity**

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Lipid self-organization is believed to be essential for shaping the lateral structure of membranes, but it is becoming increasingly clear that also membrane proteins can be involved in the maintenance of membrane architecture. Cholesterol is thought to be important for the lateral organization of eukaryotic cell membranes and has also been implicated to take part in the sorting of cellular

transmembrane proteins. It is therefore of interest to study the influence of lipid-protein interactions on membrane trafficking to find out how transmembrane proteins influence the lateral sorting of cholesterol in phospholipid bilayers. We have measured the equilibrium partitioning of the fluorescent cholesterol analog cholestatrienol between large unilamellar vesicles and methyl- β -cyclodextrin to determine the effect of hydrophobic matching on the affinity of sterols for phospholipid bilayers. The sterol partitioning was measured in 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers with and without the peptides WALP19, WALP23 or WALP27. The results showed that hydrophobic matching will affect the affinity of the sterol for the bilayers. Stronger sterol binding to the bilayers was achieved by an increase in positive hydrophobic mismatch (except in extreme situations), and a large negative hydrophobic mismatch decreased the affinity of the sterol for the bilayer. Peptide insertion into the phospholipid bilayers was also observed to depend on hydrophobic matching. In conclusion, the results showed that hydrophobic matching can affect lipid-protein interactions in a way that may facilitate the formation of lateral domains in cell membranes. This may well be of importance in membrane trafficking.

2612-Pos Board B304

Functional and Structural Characterization of Pulmonary Surfactant Protein SP-C in Nanodiscs: A Nanotechnological Approach

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Because membrane proteins need to be taken out of their natural environment and reconstituted into artificial milieus to perform structure-function studies, the search for mimetic membranes that retain their native structure and functional activity constitutes a challenge.

Nanodiscs emerge as a powerful tool for achieving this goal. These discoidal particles are constituted by a phospholipid bilayer encircled by a membrane scaffold protein, which determines the size of the nanodiscs. We are focused on the study of pulmonary surfactant protein C (SP-C), a key component of the pulmonary surfactant lipoprotein complex that reduces the surface tension in the pulmonary alveoli preventing them from collapse during respiration. This transmembrane protein is a 35 amino acid peptide dually palmitoylated in its N-terminal segment. SP-C is one of the most hydrophobic proteins that are known and its low stability and the high tendency to form aggregated beta-sheets in aqueous solvents difficult its structural and functional characterization even in the presence of detergents.

In the present work, nanodiscs containing both native and a non-palmitoylated recombinant version of SP-C have been obtained using POPC as a lipid membrane model. The incorporation of SP-C into nanodiscs provides a novel approach for structural and functional studies of this membrane protein in a bilayer mimetic system.

2613-Pos Board B305

Solid-State NMR and FTIR Study of a Neuronal Calcium Sensor (NCS) Protein, Recoverin

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Recoverin is a peripheral protein present in retinal photoreceptors. This protein is one of the fourteen members of the neuronal calcium sensor protein (NCS) family. These proteins have different functions such as phototransduction in the case of recoverin. The conformation of these proteins varies as a function of calcium concentration. At low Ca^{2+} concentration, recoverin is in a cytosolic form with its myristoyl group sequestered into a hydrophobic cavity and the binding of 2 Ca^{2+} results in the extrusion of the myristoyl group. This is the so-called *Calcium Myristoyl Switch*. The study of the structure and membrane binding of recoverin in the absence and presence of calcium is therefore of great interest. On the other hand, the membrane composition of the rod outer segments is more than 60% of polyunsaturated lipids. This parameter could therefore modulate the membrane interaction and binding of recoverin. We have investigated in the present study the conformation of recoverin in solution with and without calcium and myristoyl. We have also investigated the interaction of recoverin with phospholipids with different polar headgroups and acyl chains using a combination of solid-state NMR and FTIR spectroscopies as well as the effect of cholesterol at a 10% molar ratio in membranes. Our results demonstrate that protein aggregation is favored in the absence of calcium and for the non myristoylated form of the protein. In addition, ³¹P solid-state MAS NMR results suggest an important role of the myristoyl group and of the hydro-

phobic pocket of recoverin in its interaction with lipids. The investigation of the membrane binding of recoverin with a deuterated myristoyl group by ²H solid-state NMR at 900 MHz clearly demonstrates a *Calcium Myristoyl Switch* for recoverin for specific lipids.

2614-Pos Board B306

Probing S100A12 Interactions with Model Membranes

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Proteins belonging to the S100 family regulate many cellular functions, including cell growth, differentiation, motility, contraction and intracellular signal transduction. S100A12 is a small calcium-binding protein highly abundant in the cytosol of granulocytes, where it has a possible role in signal modulation of inflammatory processes.

In this study, the binding of porcine S100A12 (Calgranulin C) to lipid bilayers and the effect of ions (calcium and zinc) in modulating its interaction with liposomes was investigated using synchrotron radiation circular dichroism (SRCD), fluorescence emission and surface plasmon resonance spectroscopies. The binding of S100A12 to phospholipid vesicles occurred both in its apo- and holo- forms, however the protein was bound more tightly to negatively-charged liposomes. Moreover, the presence of the ions facilitated its interaction with liposomes, producing distinct conformational changes and severely reducing its thermal stability.

These S100A12-lipid interactions may be a way of translating physiological changes in calcium/zinc levels into specific cellular responses. In addition S100A12 may exist in a dynamic exchange between cytosolic and membrane-associated states, regulated by specific cellular signals.

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2615-Pos Board B307

Lipid Selectivity in Lipid Efflux Induced by Proteins and Peptides

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Some proteins and peptides have the ability to extract lipids from membranes. In some systems, such as some toxins, it leads to cell death. In other systems, this process is vital. For example, bovine seminal plasma contains phosphocholine-binding proteins that associate with sperm membranes upon ejaculation and cause lipid efflux; this effect modifies the membrane lipid composition, an essential step in the maturation process of fertile sperm. In general, the affinity of these peptides/proteins for membranes and their impact on membrane permeability are well characterized. However the induced lipid efflux and particularly its lipid specificity are much less investigated. Using model membranes with different compositions, we identified the lipid selectivity of the membrane lipid efflux of two systems. First, we characterized the lipid efflux induced by BSP1, the most abundant Binder-of-Sperm protein in bull seminal plasma. The protein binding displays a distinct affinity for phosphatidylcholine. However, even though the protein anchoring on membranes is PC-specific, we demonstrated that the lipid extraction is practically without any lipid specificity; BSP1 molecules solubilise a lipid patch to form small complexes with a stoichiometry of 10-15 lipids per protein. Second, we characterized the lipid specificity of lipid efflux induced by melittin, a helical peptide with a secondary amphipathic character. Phosphatidylethanolamine, and cholesterol, two molecular species ordering bilayers, reduce the membrane affinity of melittin. We determined that, in parallel, the lipid fraction extracted by melittin is depleted of these lipids. It is proposed that the peptide accumulates in regions of bilayers where the bilayers are less ordered and, when its concentration is sufficient, detaches this patch from the membrane. These findings illustrate the need to investigate globally lipid efflux processes, from the peptide/protein binding to the mixed lipid-peptide/protein self-assembly formation.

2616-Pos Board B308

Elucidating Lipid Domains Function by Combinatorial Screening of Protein-Lipids Interactions

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Cell membranes composed of thousands of lipid species, differing in their alkyl chains, headgroups and degree of saturation. Changes in lipid composition or even the absence of a single lipid have shown to lead to severe pathologies and death. The leading hypothesis which explains the role of lipids in membrane functionality is that the lipids segregate into distinct domains. These lipid domains can with high specificity incorporate or exclude proteins, hence inhibit or accelerate biological processes at the membrane surface. Knowledge of