energy transfer measurements these studies are revealing significant differences in the oligomeric state of the N-terminal transmembrane domain of Fukutin-I in response to bilayer composition with implications for the regulation of protein trafficking.

3447-Pos Board B552
The Intermembrane Ceramide Transport Catalyzed by CERT is Sensitive to the Lipid Environment
Peter Mattjus, Jessica Tauf, Matti Kjellberg, Julian G. Molotkovsky, Kentaro Hanada.
The in vitro activity of the ceramide transporter, CERT, has been studied using a fluorescence assay. CERT is responsible for the in vivo non-vesicular trafficking of ceramide between the endoplasmic reticulum and Golgi. In this study we have examined how the membrane environment surrounding the ceramide substrate, the membrane packing density and the membrane charge, are affecting the ceramide transfer activity. To examine this we have used an anthrylvinyl-labeled ceramide analogue. We found that if ceramide is in a tightly packed environment such as in sphingomyelin or dipalmitoylphosphatidylcholine containing membranes, the CERT transfer activity is markedly reduced. Ceramide in fluid membranes on the other hand are available for CERT mediated transfer. CERT also favors membranes that contain phosphatidylinositol 4-monophosphate, due to its binding capacity of the pleckstrin homology domain towards phosphatidylinositol 4-monophos-
phatidylcholines with various hydrocarbon chain lengths. A reduction in membrane thickness led to a positive shift in the operating point of the membrane potential at which charge transfer associated with motile response takes place. The shift observed was up to 150 mV. An increase in membrane thickness had the opposite effect. Shifts were about 56 mV for 1% reduction in thickness. This result was interpreted as an indication that conformational changes of preinhibitory form is a domain towards phosphatidylinositol 4-monopho-

3448-Pos Board B555
Membrane Thickness Dependence of Non-Mammalian Prestins
Chisako Izumi, Jonathan E. Bird, Kuni H. Iwasa.
Prestin is significant for voltage-dependent somatic motility of outer hair cells in the cochlea, which is important for mammalian hearing. This membrane protein undergoes conformational changes in response to changes in the membrane potential in a manner similar to piezoelectricity. Previously we have shown that mammalian prestin is sensitive to membrane thickness by changing membrane thickness by perfusion of gamma-cycloextrin loaded with phosphatidylcholines with various hydrocarbon chain lengths. A reduction in membrane thickness led to a positive shift in the operating point of the membrane potential at which charge transfer associated with motile response takes place. The shift observed was up to 150 mV. An increase in membrane thickness had the opposite effect. Shifts were about 56 mV for 1% reduction in thickness. This result was interpreted as an indication that conformational changes of prestin, namely the conformation with larger membrane area has thinner hydrophobic area that interfaces lipid bilayer. In the present study, we examined whether or not non-mammalian prestins are also sensitive to membrane thickness. We found that the membrane thickness dependence of platytpus prestin was quantitatively similar to that of mammalian prestin. In contrast, chicken prestin did not show a systematic membrane thickness de-

3449-Pos Board B554
A Systematic Approach Towards Elucidation of the Mode of Action of a Bacterial Thermosensor
Joost Ballering, Larisa E. Cybulski, Diego de Mendoza, J. Antoinette Killian.
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 trans-

3450-Pos Board B555
Thermosensor Desk Measures Membrane Thickness
Larisa E. Cybulski, Mariana Martin, Diego de Mendoza.
The bacterium Bacillus subtilis adjusts the composition of membrane lipids to cope with temperature variations. The histidine kinase DesK is a five-pass transmembrane thermosensor suited to remodel membrane fluidity in B. subtilis according to temperature. To understand the mechanism of sens-
ing, individual transmembrane segments (TMS) were fused to the cytoplasmic catalytic domain of DesK (DesKC) and the ability to respond to temperature analyzed. Surprisingly, a hybrid TMS composed of 17 amino-
coids of the first TMS and the C-terminal 14-residue portion of TMS fused to DesKC, fully retains in vivo and in vitro the sensing properties of full-

3451-Pos Board B556
Modulation of the Activity of an Integral Membrane Protein by Phospho-
lipids in Mixed Micelles
Although the importance of lipid-protein interactions in determining the bio-

3452-Pos Board B557
Small-Angle Neutron Scattering Reveals Colicin N Inserts into Clefts on the Outside of the OmpF Trimer
We wish to understand the mechanism by which colicins translocate across the outer-membrane of competing bacteria to mediate cell death. Pore-forming colicin N hijacks E. coli outer-membrane protein OmpF and exploits it as both a receptor and translocator to cross the outer-membrane [1]. It is currently
a matter of debate if the translocation route taken by colicin N is through the OmpF internal pore or via the external protein-lipid interface. Recent electron microscopy data from our laboratory suggests the latter route for translocation [2]. In order to re-address this question we undertook small-angle neutron scattering experiments. By using a combination of deuterated OmpF and hydrogenated colicin N we were able to define the three dimensional structure of the colicin N-OmpF complex. This revealed that colicin N inserts into clefts on the outside of the OmpF trimer, supporting the case for translocation via the protein-lipid interface.


Fig1. The ab initio SANS model of OmpF (light grey) and colicin N (dark grey) complex.

3453-Pos Board B558
Analysis of Glycoprotein Interactions with Multichannel Membranes
Luis A. Palacio, Pat DeMoss, Horia I. Petrache
We investigate the interaction of glycopolymers (including ovalbumin and alpha 1 antitrypsin) with lipid membranes by using a number of physical methods including light spectroscopy and ion current measurements. Glycoproteins are a class of proteins that are decorated with sugar (glycan) chains. Most known glycopolymers have been shown to play a role in intercellular interactions but the exact role of the glycan chains is still under investigation. The ion-current measurements involve the channel forming peptide gramicidin A (gA) which is used as a reporter. Alteration of gA activity indicates an interaction of the added glycoprotein with the membrane. For the analysis of such events, single channel recordings are preferable because the calculation of channel on and off times is straightforward. Obtaining single channel events however depends critically not only on the amount of gA present in the sample but also on the amount of the added glycoprotein. Therefore, the majority of the recordings exhibit multichannel events. We show how the multichannel events can be used (rather than discarded) to calculate single channel parameters. This analysis allows us to characterize the interaction of glycopolymers with lipid bilayers which can help elucidate the role of glycosylation in cell signaling.

3454-Pos Board B559
Do Sphingolipids Control Viral Membrane Protein Activity?
John M. Sanderson, James A. Freeth, Helen K. McPhee, Harriette E. Foster
The membrane-behaviour of the matrix (M) protein from respiratory syncytial virus, a prototypic enveloped virus, has been characterised by surface techniques (tensiometry, Brewster angle microscopy and atomic force microscopy) and experiments using lipid bilayers (FRET, light microscopy). Experiments have been conducted using both model lipid mixtures and reconstituted natural membranes.

The protein is able to penetrate lipid monolayers and bilayers composed of phosphocholine/cholesterol or phosphocholine/phosphoethanolamine, inserting between lipid molecules. In contrast, in the presence of sphingomyelin, the protein exhibits a different behaviour, with a simple partitioning (i.e. penetration) at low concentrations, replaced by peripheral association at higher protein concentrations. A picture emerges of specific interactions between the protein and sphingomyelin influencing membrane behaviour. These findings are discussed in respect of the structure of the protein (PNAS, 2009, 106, 4441-4446), the formation of viral filaments during key stages of the infection cycle and the isolation of the protein from detergent-resistant membrane fractions.

3455-Pos Board B560
On the Treatment of Dynamics During Combined $^3$H GALA and $^{15}$N/$^1$H PISEMA Analysis of Transmembrane Peptide Tilt using Solid-State NMR Data
Vitaly V. Vostrikov, Christopher V. Grant, Stanley J. Opella, Roger E. Koeppel II.
Knowledge of transmembrane alpha-helix dynamics is important for interpretation of solid-state NMR observables. While precession of tilted peptides about the bilayer normal is commonly observed, additional dynamic features, such as anisotropic contributions from distributions of helix tilt or helix rotation, have the potential to influence the analysis. Previously, we compared independent analysis of $^3$H-ala nine (“GALA”) and $^{15}$N/$^1$H-backbone data sets (“PISEMA”) as constraints for determining helix tilt. Here we report combined analysis of $^3$H quadrupolar splittings together with $^{15}$N/$^1$H dipolar couplings, using two methods to treat the dynamics, for the systematic evaluation of several membrane-spanning peptides based on the GWALP sequence (acet-yl-GGALW(LA)6LWLAGA-amide), which tilt by 2°-30° in lipid bilayer membranes.

By comparing individual and combined analyses of specifically $^3$H or $^{15}$N labeled peptides incorporated in mechanically or magnetically aligned lipid bilayers of differing thickness, we investigated the influence of data set size/identity, and of explicitly modeled dynamics, on the average apparent orientations of the peptides. We conclude that the peptides with small (less than 10°) apparent tilt values can be fitted by extensive collections of solutions, which can be narrowed by incorporating additional $^{13}$N as well as $^3$H restraints. Conversely, peptides that exhibit larger tilt angles have a narrower range of distributions of tilt and rotation that are consistent with the experimental data. The resulting smaller range can then be fitted using smaller sets of experimental constraints, or even with $^3$H or $^{15}$N data alone. Importantly, for the peptides that tilt significantly more than 10° from the bilayer normal, the contribution from rigid body dynamics can be approximated by a simple scaling factor (principal order parameter), and the concomitant apparent peptide orientation remains reasonably accurate.

3456-Pos Board B561
Characterization of Chain Order of an Acylated-Lactoferricin Peptide by Solid-State NMR Spectroscopy and All-Atom Molecular Dynamics Simulations
Denise V. Greathouse, Tod D. Romo, Alan Grossfield
The hexapeptide, Li6B (RRWQWR-NH2), derived from a cationic antimicrobial 25-residue fragment of bovine lactoferrin, retains broad spectrum activity. The two tryptophans and three arginines are thought to promote membrane interaction. Attachment of a short fatty acid to the N-terminus (C6-Li6B) further enhances membrane binding (Greathouse, et al. 2008. J. Pept. Sci. 14:1103). The mechanism by which antimicrobial peptides interact with bacterial cell membranes is proposed to depend on lipid composition. Mammalian membranes are comprised primarily of neutral lipids, whereas bacterial membranes contain a significant fraction of negatively charged lipids. We have shown using all-atom molecular dynamics simulations that association of C6-Li6B with negatively charged membranes (3:1 POPE:POPG) begins with the arginines, followed by the tryptophans, with the C6 ‘tails’ inserting last into the membrane (Grossfield, et al. 2010. Biophys J. 98:92A). By contrast, the association with a zwitterionic membrane (POPC) is led by the C6 tail, followed by the tryptophans and arginines. Solid-state $^3$H NMR experimental results confirmed that the lipid order parameters are not significantly changed when C6-Li6B is bound to negatively-charged membranes, whereas a slight decrease in order is observed with zwitterionic membranes. We now present the order parameters for a deuterated 6-carbon acyl chain (C6-di1) attached to either Li6B or a single glycine control. Solid-state $^3$H NMR results show an increase in acyl chain order for C6-Li6B in POPE:POPG compared to POPC, whereas the chain order for C6-Gly is the same in POPE:POPG and POPC. Additionally, the quadrupolar splittings of individual methylene deuterons are resolved at acyl chain carbons 2-4 in C6-Li6B but not in C6-Gly, suggesting restricted motion for the membrane-embedded acyl-peptide. Results from all-atom molecular dynamics simulations will be compared with experimental data from solid-state NMR.

3457-Pos Board B562
How Transmembrane Model Peptides Affect Lipid Head Group Orientation: An Application of 14N NMR

Studying interactions in lipid bilayers is not an easy task. It usually requires complex labeling strategies to answer specific questions, but sometimes such labels dramatically affect the subtle balance of interactions involved, especially at the membrane interface. One of the facile routes to study lipids in model membranes has been proposed to be 14N NMR.

In this study we used fully hydrated lipid vesicles of phosphatidylocholine, mixed with positively charged (DMPA) and negatively charged (DMPG) amphiphiles, to monitor the effect of surface charge on the orientation of the phosphatidylopholine head group with 14N wide line NMR. The results showed

[Image 176x156 to 284x309]