amyloid form of $PAP_{248-286}$ had little effect on either vesicle aggregation or fusion. To further investigate this effect we have solved the structure of $PAP_{248-286}$ in SDS micelles. A largely α-helical conformation of $PAP_{248-286}$, lying parallel to the membrane surface, is implicated in promoting bridging interactions between membranes by the screening of the electrostatic repulsion that occurs when two membranes are brought into close contact. This suggests non-specific binding of small oligomeric forms of SEVI in an α-helical conformation to lipid membranes may be an additional mechanism by which SEVI enhances the infectivity of the HIV virus.

Lipids and Signaling on Membrane Surface

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The Preferential Reconstitution Of Ampa Receptor Proteins Into Model Lipid Domains With Cholesterol Studied By Atomic Force Microscopy - an Imaging And Force Spectroscopy Study

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In our research we have conducted an atomic force microscopy (AFM) study of trafficking-like behaviour of neural receptor proteins into lipid raft-like domains. In our initial research we formed artificial rafts by varying a mixture of four phospholipids found in the synapse in order to mimic a synaptic membrane. The most commonly occurring receptor protein in the central nervous system, the AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), was then reconstituted into these mixtures. The results show a preferential reconstitution of these membrane proteins into lipid rafts of a certain height. AMPA receptors are implicated in long term potentiation, a process thought to underlie learning and memory, with up-regulation of AMPAR numbers in the post-synaptic membrane possibly being a key component of this process.

In order to come closer to the mixtures naturally occurring in the synapse we furthered these studies to incorporate cholesterol. The results were a preferential reconstitution of AMPAR proteins but this time into the low domain when cholesterol is present. These surprising results were better understood when we treated this system as a ternary mixture with gel phase lipids, liquid phase lipids and cholesterol acting as an impurity. We studied the phases in terms of the domain heights as well as their mechanical properties. When cholesterol was present, the protein-deficient high domains were stiffer and more viscous.

The lateral extent of the lipid domains is typically ~100nm, so they have structural similarities with the lipid rafts observed to occur in synaptic membranes, albeit with much simpler composition. Dynamic AFM measurements reveal information about the mobility of receptors within and between domains which may shed light on this process.

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Piezoelectricity of phospholipids: Are cell membranes also piezoelectric?

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Recently it was found[i] that mechanical deformation of films of L-α-phosphatidylcholine in the L_a phase induces an electric polarization. It was suggested that this effect is due to the chiral smectic A (SmA*) type liquid crystal structure of the bilayers, which under molecular tilt becomes a ferroelectric (SmC*) phase, where the electric polarization is normal to the tilt plane. However no control measurement on the racemic material has been presented to prove this suggestion. Here we demonstrate that indeed the chirality of phospholipids makes fluid lipid bilayers piezoelectric. By periodically shearing and compressing nonaqueous lamellar phases of synthetic right enantiomer 2,3-Dihexadecanoyl-sn-glycero-1-phosphocholine (D-DPPC) the synthetic left enantiomer 1,2-Dihexadecanoyl-sn-glycero-3-phosphocholine (L-DPPC) lipids and their racemic mixture (DL-DPPC) ,we induced a tilt of the molecules with respect to the bilayer normal and produced electric current perpendicular to the tilt plane, with the chiral lipids only. Because most of the living cell membranes contain chiral lipids, we hypothesize that piezoelectricity may have a role in the function of cell membranes. For example, this coupling allows for a wide variety of sensory possibilities of cell membranes such as mechano-reception, magneto-sensitivity, and proton membrane transport. Preliminary results on electromechanical couplings in *Saccharomyces cerevisiae* (Baker's yeast) and their protoplasts will be also reported and discussed.

Endnotes
[i] A. Jákli, J. Harden, C. Notz and C. Bailey, *Liquid Crystals,* 35 (4), 395-400 (2008).

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Gastrin-Releasing Peptide Adopts An Orientation Parallel To The Membrane Plane As A Preferred Orientation In DMPC Bilayers: Multiple Molecular Dynamics Simulations

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Gastrin-releasing peptide (GRP) binds to GRP-receptor (GRPR), a member of GPCR family. GRP is one of the bombesin peptides and they are implicated in obesity and cancer. Understanding the mechanism of GRP-GRPR interactions at molecular level is extremely significant and requires the knowledge of the structure of peptide-receptor complex. Since the complex structure is not available, the structures of ligand and the free receptor could be used to model the complex. GRP is flexible in aqueous medium but it is likely to adopt a stable structure when it binds to membrane according to ''Membrane Compartments Theory'' [*Biopolymers* **37**, 5-16 (1995)].

The C-terminal decapeptide of GRP is biologically active and is modeled as a helix using a related peptide structure determined in SDS micelles [*FEBS Lett.* **460**, 263-269 (1999)]. Its amino acid sequence is GNHWAVGHLM. We carried out multiple independent simulations of GRP peptide in explicit DMPC bilayers which differed in the orientation of GRP inside the bilayers and force-field. At the end of 10 to 20 ns production runs, five out of six simulations resulted in the peptide orientation that is nearly parallel to the membrane plane. This indicates that this orientation is a preferred one and is independent of CHARMM or GROMOS force-fields. In the sixth simulation, the peptide was deeply inserted inside the bilayer. We analyzed the stability of helix, interaction of individual residues with different lipid components and water penetration in both layers. The helix structure is stable in majority of the simulations. Our results indicate that the residues Gly-7 and His-8 are important in maintaining the helical structure and orienting the peptide.

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Lipid Composition Modulates the Stability of DNA Acting as Model Membrane-bound Receptors

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Many important signaling processes occur in the interactions between lipid organelles: a multitude of ligands and receptors are localized to the surface of lipid structures and vary in many ways, including their length and the strength of their interactions. DNA strands with hydrophobic modifications anchor to the surface of lipid membranes. These membrane-anchored DNA diffuse within the lipid matrix and can bind specifically to their complement: minimal properties of real membrane receptors. The properties of these DNA ''receptors'' can be varied systematically to explore the physical advantages of variables such as receptor length, binding strength and repeated sequences in the binding domain.

We show that the binding equilibrium between DNA-functionalized vesicles is dependent upon lipid composition. We develop a model as a framework to understand this phenomenon by extension of the Bell model to the non-constant force-fields between lipid membranes. We find that the inter-membrane interactions can either suppress or favor receptor binding and discuss the possible implications for biological receptor-mediated signaling processes.

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Phosphatidylinositol-(4,5)-bisphosphate Acting As A Ligand Of PKCalpha Modulates The Membrane Localization Of This Enzyme In Living Cells

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Rapamycin-triggered heterodimerization strategy is becoming an excellent tool for rapidly modifying phosphatidylinositol(4,5)-bisphosphate [PtdIns(4,5)P2] levels at the plasma membrane and for studying their influence indifferent processes. In this work, we studied the effect of modulation of the PtdIns(4,5)P2 concentration on protein kinase C (PKC)α membrane localization in intact living cells. We showed that an increase in the PtdIns(4,5)P2 concentration enlarges the permanence of PKCα in the plasma membrane when PC12 cells are stimulated with ATP, independently of the diacylglycerol generated. The depletion of this phosphoinositide decreases both the percentage of protein able to translocate to the plasma membrane and its permanence there. Our results demonstrate that the polybasic cluster located in the C2 domain of PKCα is responsible for this phosphoinositide-protein interaction. Furthermore, the C2 domain acts as a dominant interfering module in the neural differentiation process of PC12 cells, a fact that was also supported by the inhibitory effect

obtained by knocking down PKCα with small interfering RNA duplexes. Taken together, these data demonstrate that PtdIns(4,5)P2 itself targets PKCα to the plasma membrane through the polybasic cluster located in the C2 domain, with this interaction being critical in the signaling network involved in neural differentiation.

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Probing Phosphoinositide Kinetics With A Voltage-sensitive Phosphatase

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Voltage-sensitive phosphatases (VSPs) have a voltage sensor linked to a phosphoinositide (PI) 5-phosphatase, which hydrolyzes plasma membrane PI-(4,5)-bisphosphate (PIP2) to PI(4)P (Iwasaki, PNAS 105, 7970). We used PIP2 hydrolysis by VSP from Ciona intestinalis (ci-VSP) and zebrafish (dr-VSP) to better understand PIP2 binding and resynthesis. PIP2 was monitored using the PIP2-sensitive M-current (KCNQ2/3) and FRET between a pair of PIP2-binding probes (PH-PLCδ1-CFP & PH-PLCδ1-YFP). Depolarizations to +100 mV lasting >50 ms reduced M-current and PH-probe FRET. PIP2 depletion was saturated by depolarizations lasting 500-1000 ms. Evidently PH-probe FRET and M-current respond quickly to changes in plasma membrane PIP2.

After repolarization, PH-probe FRET and M-current relaxed to baseline values with time constants of ~10 s in a wortmannin-insensitive manner. This reflects endogenous PIP 5-kinase converting PI(4)P back to PI(4,5)P2. Overexpression of PIP 5-kinase increased the length of depolarization required to deplete PIP2, and speeded PIP2 recovery after repolarization.

Recovery of PIP2 after VSP activation is ~10x faster than after PLC activation. However, it only requires PIP 5-kinase, whereas recovery after PLC activation requires PI 4-kinase and PIP 5-kinase in series. Thus PI 4-kinase must be the slower enzyme. To estimate the rate of PI 4-kinase, we compared translocation of a fluorescent probe that reports plasma membrane PI(4)P (PH-OSH2, T.Balla) to translocation of the PIP2-binding probe in confocal time-lapse imaging. Upon PLC activation through M1 receptors, the plasma membrane PI(4)P signal decreased 20 s later than the PIP2 signal. Both probes recovered with similar time courses. FRET photometry between either PIP2-probes or PI(4)P-probes showed comparable results. The simultaneous recovery of both probes is consistent with the hypothesis that recovery of PIP2 is governed by rate-limiting synthesis of PI(4)P by the PI 4-kinase, followed by rapid conversion of PI(4)P into PI(4,5)P2 by the PIP 5-kinase.

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Interaction Of $PTEN_{1-21}$ Peptide With Phosphatidylinositol-4,5-Bisphosphate: A ^{31}P NMR Relaxation Study.

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Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) is by far the most abundant of all phosphoinositides (about 1% of all membrane phospholipids) and is found primarily at the cytoplasmic leaflet of the plasma membrane. $PI(4,5)P_2$ can be converted by class I PI 3-kinases to $PI(3,4,5)P_3$, which is a second messenger molecule affecting processes like cell survival and proliferation. PTEN is an important tumor suppressor protein that converts $PI(3,4,5)P_3$ back to $PI(4,5)P_2$, thereby keeping the basal levels of $PI(3,4,5)P_3$ low. We have demonstrated recently that PTEN is allosterically activated by $PI(4,5)P_2$, which interacts with the N-terminal end of PTEN. Here we study the interaction of the $PI(4,5)P_2$ binding domain of PTEN (PTEN1-21) with model membranes containing $PI(4,5)P_2$ using ^{31}P-NMR. Using both magic angle spinning (MAS) and static solid state NMR we probe the interaction of PTEN1-21 with lipid phosphates. We determined the intrinsic T_1 and T_2 relaxation times of the phosphomonoester groups of $PI(4,5)P_2$ and the phosphodiester group of dioleoylphosphatidylcholine, which form the lipid matrix into which $PI(4,5)P_2$ was dispersed. PTEN1-21 is highly basic, containing several Lys and Arg residues which are thought to give rise to a largely electrostatic PTEN/$PI(4,5)P_2$ interaction. We show here that the binding of PTEN1-21 to $PI(4,5)P_2$ bilayers dramatically affects the membrane structure, indicating that the PTEN1-21/$PI(4,5)P_2$ interaction is likely more than a simple electrostatic interaction. This is in accordance with our recent findings that PTEN/phosphoinositide interaction is specific for $PI(4,5)P_2$. Lys13 is crucial for this specific interaction and this study explores the interaction of the Lys13 of PTEN with the phosphomonoester groups of $PI(4,5)P_2$.

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Plasma Membrane Order In T Cell Signalling

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Plasma membrane nanodomains, referred to as lipid rafts, more ordered than the bulk membrane play an important role in T cell signalling by forming signalling platforms in activated T cells. However, the existence of lipid rafts in resting T cells is contentious. Using laurdan, a membrane probe whose peak emission wavelength depends on the lipid environment, evidence is presented for the existence of ordered nanodomains in resting T cells.

T cell signalling can be initiated by stimulating the T cell receptor (TCR), crosslinking the lipid raft markers GM1 (sphingolipid) or glycosylphosphatidylinositol (GPI) anchored proteins. The aggregation of lipid raft components induces the same response in Jurkat T cells as the ligation of an antigen to the TCR. Changes in membrane order linked with reorganization of the plasma membrane upon Jurkat T cell activation were followed at 37°C. Fluorescent images were analyzed for generalised polarisation values - a measure of the relative abundance of liquid ordered and liquid disordered domains. TCR patching does not increase the overall membrane order suggesting that membrane domains of high order are brought together in the patches. This supports the existence of small ordered membrane domains in resting T cells that aggregate upon activation. Patching of GM1, the GPI-anchored protein CD59 and the non lipid raft marker CD45 significantly increases the overall membrane order. So does general crosslinking of membrane components with Concanavalin A. Remodelling of the actin cytoskeleton is an integral part of TCR signaling and T cell activation. Disrupting actin polymerization using latrunculin B decreases membrane order and stabilizing actin filaments with jasplakinolide increases membrane order. An increase in membrane order appears to be a general effect of plasma membrane component patching and is likely due to a global induction of actin polymerization at the plasma membrane.

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Adsorption Of Bar-domain Proteins To Charged Lipid Membranes Causes Deformations And Lipid Demixing

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Many proteins participating in cellular processes contain BAR domains that have been implicated in membrane shaping and deformation. These BAR domains can either induce significant membrane curvatures or sense high-curvature regions on cell membranes, but the mechanism for this action is still not well understood. One suggestion is that BAR domains work collectively and achieve significant bilayer deformations only through a suitable organization at membrane interfaces. In contrast, evidence from some atomistic simulations suggests that a single BAR can substantially deform a lipid membrane locally. Here we present results from a self-consistent mean-field model of BAR domain association on membranes, suggesting that a single Amphyphisin BAR is capable of producing a steady state, where the initially near-planar membrane curves significantly. However, using our approach we predict that such deformation will occur only for membrane patches that have the propensity to attain high spontaneous curvature, and that such favorable preconditioning may be the result of either local lipid demixing, or of a preceding insertion of the BAR domain's amphyphatic N-helix. Both events have been predicted to bring about asymmetry in the two membrane monolayers. To contrast, our simulations also show that local segregation of charged lipids under the influence of the adsorbing BAR domain alone cannot produce high enough asymmetry between bilayer leaflets, and that in the absence of additional energetic sources that favor membrane asymmetry, the membrane will remain near-flat within fluctuations upon BAR adsorption. Thus, we conclude that N-helix insertions may have a critical mechanistic role in the function of the BAR domain, and that the electrostatic interactions between BAR and membrane are essential for sensing and stabilization of bilayer curvature.

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Inducing and Reversing Anesthesia with Temperature Variation - Experiments on an Excised Frog Sciatic Nerve

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The Meyer-Overton Rule and other more recent experimental observations suggest that the fluidity of the lipid membrane is involved in nerve propagation and in mechanisms behind anesthesia. In other words, Hodgkin-Huxley may not be