a nanoscale gold band that can be functionalized with alkanethiols to be hydrophobic. We describe nanoscale electrical measurements with these post-electrodes on cells and demonstrate Giga-ohm seal formation at the electrode-membrane interface. Moreover, we use coarse-grained molecular dynamics simulations to elucidate the mechanism and structures of electrode-membrane interface formation.

**3463-Pos**

**Characterizing the Structure and Dynamics of Nanodisc Lipid Bilayers of Different Compositions**

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One of the key roles of the cellular membrane is the regulation and activation of membrane-anchoring proteins. The lipid composition of the membrane and the ionic content of the immediate solution significantly modify structural properties of the bilayer surface. Nanodiscs are lipoprotein particles of precisely controlled size and composition that proved to be valuable in experimental studies of protein-membrane interactions, for example in studying membrane binding and activation of blood coagulation factors. The enzymatic activity of several coagulation factors is regulated by their binding to anioic regions of the cellular membrane. Nanodiscs consist of a patch of lipid bilayer encircled in a belt-like fashion by a pair of amphipathic helical membrane scaffold proteins (MSP) and can serve as a membrane model. The structure and dynamics of lipid molecules in Nanodiscs are highly relevant to their physicochemical properties, and to the mode of interaction between Nanodiscs and membrane-anchoring proteins and peptides. We employ molecular dynamics simulations to investigate these aspects in solvated Nanodiscs. Extended simulations (on the order of 10s of nanoseconds) with Nanodiscs including anionic phosphatidylserine (POPS), zwitterionic phospatidylcholine (POPC), or POPS/POPC binary mixtures provide for detailed analysis of structural changes that occur due to lipid-lipid and lipid-ion interactions. The methodology supplies us with the atomic level description sufficient to investigate whether MSP influences the boundary lipids. Simulations revealed stable particles of consistent geometry. Presence of divalent cations Ca2+ shows its coordination with lipid head groups and modulates their orientation in the membrane bilayer, thus, preparing the stage for interaction with proteins.

**3464-Pos**

**Inter-Membrane Adhesion Mediated by Mobile Linkers: Effect of Receptor Shortage**

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Cell adhesion is a complex process essential for life. It is mediated by specific binding between cell surface proteins that eventually cluster and form supramolecular structures. However, the initial steps of cell adhesion, where physical forces can be expected to dominate over active processes, are barely understood.

Aiming at a rigorous analysis of the physical effects induced by membrane adhesion we developed a simplified passive model system. It consisted of a giant unilamellar vesicle (GUV) adhering via specific biotin-neutravidin interactions to a supported lipid bilayer (SLB). Receptors and ligands diffused freely within the plane of the respective membrane. A new microscopy set-up was developed enabling simultaneous imaging by reflection interference contrast microscopy (RICM) and fluorescence microscopy as well as determination of molecular diffusion by continuous photobleaching.

At high receptor concentrations we found GUV adhesion changed SLB fluidity as well as receptor mobility and distribution. The adhering membrane caused homogeneous accumulation and immobilization of the initially mobile receptors. Due to the introduction of these obstacles in the SLB its fluidity decreased significantly as well. Friction to the tightly bound GUV membrane furthermore enhanced the reduction of SLB fluidity.

At low receptor concentrations a characteristic ring-like accumulation pattern emerged. Due to the low efficiency of receptor diffusion at large distances receptors were accumulated only at the edge of the adhering GUV. In addition, GUV adhesion was found to be incomplete. The balance between the loss of translational entropy of the receptors and the gain in Gibbs'free enthalpy by receptor-ligand binding determined the final adhesion state.

The present results suggest that in addition to employing different receptor/ligand pairs, cells may regulate cell-cell adhesion by careful control of the receptor surface concentration.

**3465-Pos**

**Collective Membrane Dynamics under Osmotic Stress**

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Phospholipid membranes are highly dynamic, ordered structures that involve molecular motions of phospholipids together with collective fluctuations of the bilayer [1]. Membrane structural dynamics on these length scales are sensitive to changes in properties such as temperature, pressure, and chemical potential. Structural deformation accompanying the removal of water from the membrane is well characterized, yet perturbation of membrane dynamics under osmotic stress conditions has not been studied. Here we show that membrane dynamics as revealed by $^2$H NMR relaxation measurements are sensitive to osmotic stress. Specifically, we measured the segmental order parameters ($S_{CD}$) and $^2$H spin-lattice relaxation rates ($R_{1Z}$) over a broad range of hydration levels. Empirical correlations of acyl chain $S_{CD}$ and $R_{1Z}$ profiles follow a theoretically predicted square-law functional dependence. However, for a given acyl position $R_{1Z}$ is essentially independent of $S_{CD}$ as the hydration water is varied. This is expected if the correlation length of the collective and segmental fluctuations remains unperturbed. The fast segmental fluctuations are decoupled from larger amplitude lipid motions within the osmotically stressed membrane. This result contrasts with studies involving cholesterol, where variations of $S_{CD}$ on the order of those observed in the osmotic stress experiment lead to significant reductions in $R_{1Z}$ rates [2]. In this case, interaction with cholesterol couples local segmental dynamics to collective viscoelastic modes. These results show that the relation of $S_{CD}$ to $R_{1Z}$ is a characteristic marker of lipid matrix composition and collective lipid interactions. Furthermore, our results highlight intrinsic differences in the sensitivity of membrane dynamics, as may be encountered for peripheral protein-membrane interactions and integral membrane-lipid interactions. [1] M.F. Brown and S.I. Chan, *Encyclopedia of Nuclear Magnetic Resonance*, Wiley, New York 1996, 871-885. [2] G.V. Martinez *et al.* (2004) *Langmuir* **20**,1043-1046.

**3466-Pos**

**Differential Effect of Isoflurane on the Anisotropy of Diphenylhexatriene and its Cationic Trimethylamine Analog**

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The mechanism of action of volatile general anesthetics has not yet been resolved. Recent developments in the understanding of lipid physics, including the discovery of microdomains and computing the lateral pressure profile in simulations, suggest a need to revisit possible indirect effects of lipids on ion channels during anesthesia. We present strong experimental evidence that volatile general anesthetics localize at the headgroup region of a lipid bilayer and therefore increase lateral membrane pressure near the bilayer surface. Theoretically, this increase in pressure in the head group region, in conjunction with a decrease in pressure in the tail group region, may induce conformational changes in ion channels to produce the characteristic effects of volatile general anesthetics. To examine this idea, the anisotropy of fluorophores localized in either the head (trimethylamino-diphenylhexatriene, TMA-DPH) or tail group regions (DPH) of small unilamellar vesicles of dipalmitoylphosphatidylcholine was assessed. These measurements were repeated at multiple temperatures between 20 and 55 °C in the presence or absence of various concentrations of the anesthetic isoflurane. In treated samples, the main phase transition (41.5 °C) was shifted down by 2 to 10 °C depending on the concentration of isoflurane (3.8-13.0 mM). Melting reduced anisotropy by 0.1 (TMA-DPH) or 0.2 (DPH) units. Interestingly, isoflurane caused opposite changes in anisotropy of the two probes in the liquid crystalline phase: DPH anisotropy decreased by ~0.02 units whereas TMA-DPH anisotropy increased by the same magnitude. This observation suggests that isoflurane partitions into the headgroup region of the bilayer where it increases lateral pressure, while reducing it in the tail region.

**3467-Pos**

**Deformation of Vesicles Controlled by Local Spontaneous Curvatures of Membrane**

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The metabolic pathways are fundamental processes to maintain the life, which is supported by the cell membrane deformations such as, membrane adhesion, fusion and pore formation. In the present living organisms, the membrane

deformations in the metabolic pathways are conducted by the related proteins. However, we believe that such membrane deformations can be caused by controlling the physical properties of the membrane without the aid of proteins. In this context we demonstrate the deformations of vesicle by controlling the spontaneous curvature of lipids.
First, we focused on the lipids with negative spontaneous curvature (NL: DPPE and DPhPC). The binary vesicles composed of NL and lipids with zero spontaneous curvature (ZL: DOPC and DPPC) show a phase separation between NL rich domains and ZL rich matrix under the immiscibility temperature. When the two phase separating vesicles are contacted using micropipettes, they adhere each other through the domain rich in NL.
Second, we have investigated the effects of the lipids with positive spontaneous curvature (PL: DHPC) on the shape of vesicles. The binary vesicles composed of PL and ZL form spherical shape in one-phase region. By decreasing the temperature, the binary vesicles show a burst and then form a single pore on the vesicle. There are three types of pores, simple circular, rolled-rim and wrinkle-rim, depending on the ratio of PL to ZL. The pore closes with increase the temperature and finally vesicles return to the spherical shape again. We discuss this shape deformation of vesicles by calculating time development equation of the membrane free energy.
We believe that the control of the spontaneous curvature of lipids is a key to realize the model metabolic pathways without proteins.

**3468-Pos**
**A Biomolecular Photodiode for Imaging of Cell Membrane Potential**
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Despite the recognized importance of electrical signals in certain biological systems, there has been limited success in the creation of a reliable voltage sensor for imaging of such activity. Using standard molecular biology techniques, we have created a biomolecular photodiode consisting of a membrane-bound cytochrome c protein fused with a GFP (green fluorescent protein) variant. A similar photodiode assembly has been shown to produce unidirectional photocurrent in vitro with the cytochrome acting as an acceptor of excited state electrons from the FP donor upon excitation with visible light. Electron transfer between the cytochrome and the FP is a highly voltage dependent process. By embedding this assembly in the plasma membrane of living cells, it is subjected to the same electric potential as the membrane. As the membrane potential of the cell changes, as in an action potential, the extent of electron transfer is expected to vary significantly, resulting in a change in fluorescence intensity of the FP donor. As this is a very fast process with high sensitivity to changes in electric potential, the biophotodiode has potential to form a robust sensor of electrical activity in cells. The feasibility of the sensor is investigated in several ways, including modeling, electrophysiology, and direct application of current to purified membrane fragments.

**3469-Pos**
**Sub-Diffusion and Super-Diffusion of Hydration Water Molecules at Biological Interfaces**
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The structure and dynamics of hydration water at the surface of biomolecules (e.g., proteins and lipids in biological membranes) are fundamental for their stability and functioning. Due to the interactions at the surface of a solvated biological membrane, the dynamics of the hydration waters and that of the membrane molecules are to some degree correlated. In spite of previous efforts, little is known about the time and length scale of these correlations. Here, we report on a 0.1 microsecond all-atom molecular dynamics simulation study aimed at investigating the dynamics of the hydration water at the interface of a dimyristoylphosphatidylcholine (DMPC) lipid bilayer. We find that, mainly due to hydrogen bonding with the lipid bilayer, the mean-square displacement of the interfacial water has four well defined dynamical regimes, with characteristic power law ($t^n$) time (t) dependence: (1) ballistic, for $t < 10$ fs, with $n=2$ ; (2) sub-diffusive, for 0.2 ps $< t <$ 20 ps, with $n<1$; (3) super-diffusive, for 0.1 ns $< t <$ 1 ns, with $1<n<2$; and (4) Fickian diffusion, for $t >$ 10 ns, with $n=1$. The super-diffusive regime (characterized by a self intermediate scattering function with compressed exponential relaxation) of the hydration water molecules has not been observed before, and possibly determines the length and time scales of the correlation between the dynamics of water and lipid membrane. Furthermore, the water-lipid interactions give rise to an average liquid-like structure of the interfacial water molecules on a length scale corresponding to the average lipid-lipid separation, and the relaxation time of this structure is an order of magnitude larger than what is expected from the self motion of the water.
Computer time was generously provided by the University of Missouri Bioinformatics Consortium.

**3470-Pos**
**Two-Dimensional Continuum Percolation Threshold as a Function of the Radius of the Diffusing Particles**
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Lateral diffusion in the plasma membrane is obstructed by proteins bound to the cytoskeleton. The most important parameter describing diffusion in the presence of immobile obstacles is the percolation threshold, where long-range conducting paths disappear and the long-range diffusion coefficient therefore goes to zero. The thresholds are well-known for point diffusing particles on various lattices or the continuum. But for diffusing particles of nonzero radius, the threshold depends on the excluded area, not just the obstacle concentration. For the triangular lattice, the threshold is known to be highly sensitive to the size of the diffusing particle [Saxton, Biophys J 64 (1993) 1053], but lattice calculations give very low resolution. Here high-resolution results are obtained for circular obstacles on the continuum. Random obstacle configurations are generated by Brownian dynamics or Monte Carlo methods, and tested for percolation by examining bond percolation on the Voronoi diagram of the obstacles. The percolation threshold is expressed as the diameter of the largest diffusing particle that can cross a set of obstacles at a prescribed number density. For the simplest case, random overlapping obstacles, the analytical solution is known and the Monte Carlo results agree with it quantitatively. When the obstacles are disks with a $1/r^{12}$ repulsion, the percolating diameter is around 10% lower than for overlapping obstacles. Disks with a $1/r^{6}$ or $1/r^{18}$ repulsion behave similarly. The results are used to find the thresholds for lipids, and for proteins of various diameters. (Supported by NIH grant GM038133)

**3471-Pos**
**Comparison of Lipid Monolayers and Bilayers by Comparative Molecular Dynamics Simulations of a Lipid-Like Dye Molecule**
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Using p-polarized internal reflection fluorescence microscopy in monolayer membrane systems, Livanec and Dunn have demonstrated that the order of dipalmitoylphosphatidylcholine (DPPC) lipid molecules decrease as the surface pressure increases (Livanec and Dunn (2008) Langmuir 24 (24), 14066-14073). Although monolayer experiments provide more convenient control of membrane properties such as surface pressure, temperature, and lipid compositions, bilayers can offer a more dependable representation of a true biological membrane. This raises the question whether monolayer membranes can be used to study bilayer membranes. In order to explore the correspondence between monolayer and bilayer membranes, molecular dynamics (MD) simulations with lipid reporter dye molecule BODIPY-PC in a DPPC explicit membrane were performed. The correspondence was measured in different surface pressures: high pressure (40 mN/m), normal pressure (25 mN/m), and low pressure (3 mN/m). Each pressure system consisted of 5 bilayer and 5 monolayer systems, totaling up to 30 different simulations. Each simulation ran for 3 ns of equilibration and 47 ns production. Using trajectories, BODIPY-PC molecule's orientation was characterized in terms of tilt, azimuthal, and rotation angles. The calculations were compared between bilayer and monolayer systems to examine structural similarities. In addition, non-bonded energies (or the enthalpic contribution) of the BODIPY-PC molecule in different pressure systems were compared in order to address the driving force governing tilting of the dye molecule in different pressures. The calculated tilt, azimuthal, and rotation angles suggest that monolayer and bilayer systems yield quite similar results. The energy calculations demonstrate that entropy dominates the tilting of a dye molecule in low and normal pressure whereas enthalpy plays a higher role in tilting of a dye molecule in high pressure.

**3472-Pos**
**Membrane Interactions in Ionic Solutions**
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Because of complicated molecular dynamics, calculations of membrane interactions require more complicated models than straightforward electrostatics. The net intermembrane forces measured experimentally by x-ray scattering and osmostic stress must first be decomposed into various components including pure electrostatics, van der Waals, and a less understood hydration force. In addition, in most situations, an entropically generated force is required to account for the repulsive effect of membrane fluctuations (Helfrich force). Another challenge is to corroborate the experimental findings with already calculated results for particular setups. Situations in which ions are excluded from the vicinity of the membrane introduce new complications in the model. We theoretically explore to what extent we can explain these results by calculations of electrostatic interactions in ionic solutions using a Poisson-Boltzmann approach.