domains can be created by the interactions between membrane- anchored multivalent proteins. Utilizing the binding pairs, SH3 (SRC homology 3) and PRM (proline-rich motif), which were recently reported to form phase-separated micro-droplets in solution [Li et al., 2012, Nature, 483, 336-340], with histidine tags allowing efficient binding to lipid membranes containing nitrilotriacetic acid (NTA) lipids, we demonstrated that macroscopic protein domains appeared in both giant unilamellar vesicles (GUVs) and Langmuir monolayers. In GUVs, these domains remained circular over a large range of temperatures and protein concentration ratios. In Langmuir monolayers, domains showed reversible transitions from circular shapes to fractal ones depending on surface pressures. Overall, we have demonstrated that the interplay between lipid- protein and protein- protein interactions can induce phase separation of proteins on model membranes.

1278-Pos Board B170

Inhalation Anesthetics Change the Domain Structure of Model Ternary Lipid Raft Membranes

Michael Weinrich[1,2], David L. Worcester[3,4].
[1]National Center for Medical Rehabilitation Research, NIH, Bethesda, MD, USA, [2]Center for Neutron Research, NIST, Gaithersburg, MD, USA, [3]Department of Physiology and Biophysics, University of California, Irvine, CA, USA, [4]Biology Division, University of Missouri, Coumbia, MO, USA.

The mechanism of action for volatile anesthetics remains obscure despite clinical use for over 150 years. No single ion channel or protein receptor appears necessary and sufficient to account for anesthetic action, and the physical effects of volatile anesthetics on homogeneous model membranes appear too small to produce anesthesia. We recently reported that halothane changes the domain structure of a binary lipid mixture[1], increasing the ratio of disordered phase to ordered phase.

We have now studied two ternary model lipid raft mixtures with X-ray diffraction: Dioleoylphosphatidylcholine (DOPC)/dipalmitylphosphatidylcholine (DPPC)/cholesterol, and Dioleoylphosphatidylcholine (DOPC)/sphingomyelin (porcine)/cholesterol. Multi-layers were prepared upon glass slides, hydrated overnight at 98% relative humidity, and maintained at 27.0 $\pm$ 0.1 C on a Peltier-controlled stage in a sealed X-ray chamber. Volatile anesthetics were introduced as solutions in hexadecane. For both raft mixtures, two series of lamellar diffraction peaks are observed, with d-spacings differing by about 10%. These correspond to the liquid ordered phase and the liquid disordered phase. The relative intensities of diffraction for these phases change with increasing temperature and anesthetic concentration, both favoring the liquid disordered phase. A variety of different volatile anesthetics–halothane, isoflurane, chloroform, and hexane_all produce significant increases in the ratios of liquid disordered to liquid ordered lipid phases in these mixtures. These shifts occur at clinically relevant concentrations and are reversible upon withdrawal of anesthetic. There were no consistent effects of the anesthetics on the d-spacings of the lipid layers.

These findings suggest that some effects of volatile anesthetics may be mediated through physical changes in membrane domain structures that interact with membrane proteins.

1. Weinrich, M., Nanda, H., et al., Halothane changes the domain structure of a binary lipid membrane. Langmuir, 2012. 28(10): p. 4723-8.

1279-Pos Board B171

The Effect of Photosensitization on the Physical Properties of a Biological Membrane

Shany Ytzhak, Benjamin Ehrenberg.
Bar-Ilan University, Ramat Gan, Israel.

Depolarization of the Nernst electric potential on cells' membranes has been observed in cellular photosensitization, but it was not established whether lipid oxidation is a relevant factor leading to abolishing the resting potential of cells' membranes and to their death. In this work, we studied the effect of liposomes' lipid composition on the kinetics of membrane electric depolarization that is induced by photosensitization. We have studied this effect by two methods: 1. measuring the dissipation of K^+-diffusion electric potential that was generated across the membranes by employing an electrochromic voltage-sensitive spectroscopic probe that possesses a high fluorescence signal response to the potential. 2. Measuring the permeation kinetics of large fluorescent dye molecules, which are known to exhibit self-quenching of their fluorescence at high concentration, through the membranes by observing the increase of the fluorescence as their permeability through the membrane increases. We found a correlation between the structure and degree of unsaturation of lipids and the leakage of the membrane, following photosensitization. As the extent of non-conjugated unsaturation of the lipids is increased from 1 to 6 double bonds, the kinetics of depolarization become faster. When liposomes are composed of a lipid mixture similar to that of natural membranes and photosensitization is being carried out under usual photodynamic therapy (PDT) conditions, photodamage to the lipids is not likely to cause enhanced permeability of ions through the membrane, which would have been a mechanism that leads to cell death.

1280-Pos Board B172

Impact of Oxidized Phospholipids on Membrane Organization

Martin Wallgren[1], Quoc Dat Pham[2], Martin Lidman[1], Paavo K.J. Kinnunen[3], Martin Hof[4], **Gerhard Gröbner**[1].
[1]Chemistry, Umeå University, Umeå, Sweden, [2]Physical Chemistry, Lund University, Lund, Sweden, [3]Helsinki Biophysics and Biomembrane Group, Aalto University, Helsinki, Finland, [4]J. Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Lipids have often been seen as basic structural membrane subunits with proteins doing the actual work. This view has changed in recent years where it has been shown that lipids are also directly involved in numerous physiological processes and are often required for specific membrane protein functions. However, how a membrane and its function becomes modified under intracellular oxidative conditions, which e.g. trigger mitochondria-mediated apoptotic cell death, is still not really known. Oxidative stress can generate oxidized phospholipids (OxPls), which have a great impact on mitochondrial membrane integrity. Therefore, we studied the impact of OxPls on DMPC based bilayers membranes by differential scanning calorimetry (DSC) and solid state nuclear magnetic resonance (NMR) spectroscopy. Incorporation of OxPls with functional groups (carboxyl or aldehyde) at their truncated sn-2-chain ends generated information about the effect which OxPl species exert on the basic structural and physico-chemical properties of DMPC bilayers. DSC experiments revealed significant changes in the thermotropic phase behavior of these vesicles in the presence of OxPls as a function of their concentration. In addition, solid state 31P NMR provided molecular information of the behavior of the DMPC headgroups when OxPls were present. In addition changes could also be monitored during temperature induced phase transitions, where OxPls induced a very complex phase behavior. Between 293 K (onset of Lα-phase) and 298 K two overlapping NMR subspectra occured which indicated the co-existence of two liquid-crystalline lamellar phases. Most likely one phase reflected an OxPls poor domain and the other an OxPl-rich domain. In summary, the presence of OxPls seems to alter the mitochondrial membrane organization, which has serious implication for the role of this membrane and its Bcl-2 proteins involved in mitochondrial apoptosis.

1281-Pos Board B173

The Location of Vitamin E in Model Membranes and its Effect on Oxidation

Drew Marquardt[1], Norbert Kučerka[2], Justin A. Williams[3], Jeffrey Atkinson[4], Stephen R. Wassall[5], John Katsaras[6,7], Thad A. Harroun[1].
[1]Department of Physics, Brock University, St Catharines, ON, Canada, [2]Neutron Beam Centre, National Research Council, Chalk River, ON, Canada, [3]Department of Physics, IUPUI, Indianapolis, IN, USA, [4]Department of Chemistry, Brock University, St Catharines, ON, Canada, [5]Department of Physics, IUPUI, Indianapolis, IN, USA, [6]Canadian Neutron Beam Centre, National Research Council, Chalk River, ON, Canada, [7]Biology and Soft Matter, Neutron Scattering Science Division, Oak Ridge National Laboratories, Oak Ridge, TN, USA.

There are no proven health benefits to supplementing with Vitamin E, so why do we require it for healthy living? The whole notion that vitamin E is an in-vivo antioxidant is now being seriously questioned. We believe the debate in literature is due to much of the existing data being collected using techniques which require the presences of non-biological and invasive probes, and often in the wrong model systems. Using neutron diffraction, supported by solid state 2H NMR, we have correlated vitamin E's location in model membranes with its antioxidant activity. Experiments were conducted using phosphatidylcholine (PC) bilayers whose fatty acid chains varied in their degree of unsaturation. PC bilayers made up of mixed acyl chains (i.e., saturated and unsaturated) and different headgroup moieties were also studied. UV/Vis spectroscopy studies were conducted to examine vitamin E's oxidation at its various locations within the different model membranes. Both water soluble and lipid soluble initiators were used to start the oxidation process.

We observe vitamin E up-right in all lipids examined, with its overall height in the bilayer lipid dependant. Interestingly we observe vitamin E's hydroxyl in the headgroup region of the bilayer for both the fully saturated and poly unsaturated lipids. Vitamin E was most effective at intercepting water borne oxidants

than radical initiated within the bilayer core. However for lipids where vitamin E resides slightly lower(glycerol backbone) we observe comparable antioxidant activity against both water borne and hydrocarbon borne oxidants. Thus showing lipid species can modulate the location of vitamin E's activity.

1282-Pos Board B174

Exploring the Sequence Determinants of Spontaneous Membrane-Translocating Peptides

Jing He[1], Taylor Fuselier[1], Kalina Hristova[2], William C. Wimley[1].
[1]Tulane University, New Orleans, LA, USA, [2]The Johns Hopkins University, Baltimore, MD, USA.

The development of cell penetrating peptides (CPPs) has long promised to enable the delivery of a wide variety of polar compounds into cells. Yet the lack of tools for engineering and designing such peptides creates a bottleneck in the discovery pipeline. In addition, a consensus raised from mounting studies favors a mechanism that requires one or more types of energy-dependent endocytotic pathways for cellular uptake of most CPPs, which significantly limits their applications. The goal of this work was to learn to engineer spontaneous membrane translocating peptides (SMTPs) that move across lipid bilayers and cellular membranes in an energy-independent manner. A previous orthogonal screen of a synthetic peptide library (N=10,368) for SMTPs revealed a conserved 9-residue motif (PLI[L/Y]LRLLR) in a family of 12-residue SMTPs that translocate rapidly without causing any bilayer destabilization. In this work, we used one of these SMTPs, PLIYLRLLRGQF, as the template for rational iterations of SMTPs designed to reveal the sequence determinants of spontaneous translocation. Translocation of the variants was measured in large unilamellar vesicles (LUVs) containing entrapped protease. We also measured vesicle permeabilization caused by each of these analogs. For each variant, the rate of peptide translocation and its effect on membrane permeability is discussed. Our results shed light on the determinants of spontaneous translocation, which may allow for the discovery and engineering of potent SMTPs to overcome the membrane barrier for drugs delivery.

1283-Pos Board B175

Exploring Amphotericin B-Membrane Interactions: Free Energy Simulations

Anna Neumann, Maciej Baginski, Jacek Czub.
Gdansk University of Technology, Gdansk, Poland.

Amphotericin B (AmB) is a membrane-active polyene antibiotic used to treat serious fungal infections. Biological action of AmB is due to the formation of transmembrane channels. Membrane sterols are known to be crucial for the AmB antifungal activity, i.e. AmB is more active against fungal cell membranes containing ergosterol than against the mammalian membranes with cholesterol.

We explored molecular determinants of AmB selectivity for ergosterol-containing membranes using computational methods. By means of molecular dynamics simulations we studied various aspects of the interactions between AmB and lipid bilayers of different composition (containing or not 30% of ergosterol or cholesterol). More precisely, we examined (1) AmB insertion into a membrane, (2) changing tilt angle between AmB and the bilayer plane (for AmB embedded in a membrane) as well as (3) AmB dimerization in a membrane. To provide a thermodynamic description of these processes, we calculated the free energy profiles describing each of them in the three different membrane systems. The results indicate that at low, chemotherapeutically relevant concentrations of AmB at which the antibiotic expresses its channel-forming activity, AmB is mostly monomeric in ergosterol-containing membranes and it exists predominantly as a dimer in cholesterol-containing (and sterol-free) ones. We also show that compared to the other two studied membranes, it is the most favorable for AmB to insert the ergosterol-containing bilayer. The differences in the behavior patterns of AmB in bilayers of different composition are mainly of energetic origin. From the free energy profiles for (2) and (3) we also determine the most preferred location and orientation of AmB within the studied bilayers.

1284-Pos Board B176

Conductance of Ideally Cation-Selective Ion Channel Depends on Anion Type

Torri C. Roark[1], Philip A. Gurnev[2], Sergey M. Bezrukov[2], Horia I. Petrache[1].
[1]Indiana University Purdue University Indianapolis, Indianapolis, IN, USA, [2]National Institutes of Health, Bethesda, MD, USA.

Gramicidin A (gA) is a cation selective ion channel that has been used in many biophysical studies of lipid bilayers, in particular for investigations of lipid-protein interactions [1, 2] and membrane electrostatics [3]. In addition, it was found that ionic interactions with neutral lipid membranes also affect the kinetics of gA channels [4]. Here we report measurements of gA ion-channels for a series of sodium and potassium salts that show an *anion*-dependence of gA conductance. We find that gA conductance varies significantly with the anion type with ClO_4 and SCN producing distinctly larger conductance values than Cl, F, and H_2PO_4. These results can provide new insights into ion-lipid membrane interactions and ion channel functions in general. [1] Andersen et al., Annu. Rev. Biophys. Biomol. Struct., 1996, [2] Lunbaek et al., PNAS 2010, [3] Rostovtseva et al. Biophys. J. 1998, [4] Rostovtseva et al. Biophys. J. 2008.

1285-Pos Board B177

Nanoscopic Cell Membrane and Pore Profiles Combining Molecular Dynamics and a 3D Electromagnetic Tool

Sophie Kohler[1], Ming-Chak Ho[2], Zachary A. Levine[2], Philippe Leveque[1], Delia Arnaud-Cormos[1], **P. Thomas Vernier**[2].
[1]XLIM, CNRS, University of Limoges, Limoges, France, [2]University of Southern California, Marina del Rey, CA, USA.

Nanosecond, megavolt-per-meter electric pulses applied to biological cells can target subcellular structures with minimal loss of plasma membrane integrity, opening up new perspectives for intracellular manipulations. Experimentally observed effects of intense nanopulses include intracellular calcium release, externalization of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, and non-thermal cell death by apoptosis. Molecular dynamics (MD) simulations have shown that PS re-distribution occurs after the electric-field-driven formation of nanometer-sized pores in the plasma membrane and is facilitated by electrophoresis of PS along the pore walls. Nanopulse-induced pore creation occurs on a nanosecond time scale, but the underlying molecular mechanisms are not yet clear. Experimental observations of the process of pore formation are challenging because of the time and spatial scales required.

In this study, we combine MD simulations and a quasi-static approach using a custom implementation of 3D finite-difference analysis to investigate the physical mechanisms of electropore creation. First, MD simulations of pore formation in phospholipid bilayers in external electric fields are performed at nanoscopic scale. From these simulations we extract the charge densities across the electroporated bilayer. Second, the charge densities are injected into a new, custom, quasi-static algorithm based on the Poisson equation. The software computes 3D nanoscopic profiles of the transmembrane potential, electric field, and electric field gradient. The goal of the two-step simulation is to establish whether and how electric field gradients, water and phospholipid head group dipole moments, and the site of initial water intrusion in pore initiation are correlated.

1286-Pos Board B178

Biophysical and Biological Behaviour of Ciprofloxacin and Ciprofloxacin Derivatives: A Route to Counteract Bacteria Resistance?

Paula Gameiro, Mariana Ferreira.
Requimte, Departamento de Química e Bioquimica, Faculdade de Ciências, Universidade do Porto, PORTO, Portugal.

Studies of bacterial membranes are fundamental in the understanding and counteracting of bacterial resistance. Direct attack on bacterial membranes is thought to be a way to counteract bacterial resistance mechanisms, which are mostly based on intracellular adaptations. Therefore, a current trend in antibiotic research is specifically targeting bacterial membranes, leading to permeation of the membrane, inducing the formation of membrane domains and ultimately leading to cell death. The fact that such effects are not observed in the interaction with mammalian cells illustrates the capacity that such antibiotics have to discriminate between bacterial and mammalian cells, most likely due to the differences in the lipid composition of the two types of cell membranes.

In this work we present fluorescence spectroscopy studies of the interaction of ciprofloxacin and phenanthroline/copper/ciprofloxacin complex with several lipidic mimetic systems. POPE/POPG (0.75:0.25), POPE/POPG/Cardiolipin (0.67:0.23:0.10), E. coli total lipid extract and DMPC liposomes were used and the results obtained point out to a very different behavior between ciprofloxacin and its copper complex. Preliminary results of the interaction of these compounds with OmpF proteoliposomes will also be presented. These results, together with biological data, suggest that the ciprofloxacin complex can be an alternative to counteract bacterial resistance to these antibiotics.

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