

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.

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

Michael Weinrich^{1,2}, David L. Worcester^{3,4}.

¹National Center for Medical Rehabilitation Research, NIH, Bethesda, MD, USA, ²Center for Neutron Research, NIST, Gaithersburg, MD, USA,

³Department of Physiology and Biophysics, University of California, Irvine, CA, USA, ⁴Biology Division, University of Missouri, Columbia, 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 mixture¹, 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)/dipalmitoylphosphatidylcholine (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 ± 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.

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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.

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Impact of Oxidized Phospholipids on Membrane Organization

Martin Wallgren¹, Quoc Dat Pham², Martin Lidman¹, Paavo K.J. Kinnunen³, Martin Hof⁴, Gerhard Gröbner¹.

¹Chemistry, Umeå University, Umeå, Sweden, ²Physical Chemistry, Lund University, Lund, Sweden, ³Helsinki Biophysics and Biomembrane Group, Aalto University, Helsinki, Finland, ⁴J. 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 L α -phase) and 298 K two overlapping NMR subspectra occurred 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.

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

Drew Marquardt¹, Norbert Kučerka², Justin A. Williams³, Jeffrey Atkinson⁴, Stephen R. Wassall⁵, John Katsaras^{6,7}, Thad A. Harroun¹.

¹Department of Physics, Brock University, St Catharines, ON, Canada,

²Neutron Beam Centre, National Research Council, Chalk River, ON,

Canada, ³Department of Physics, IUPUI, Indianapolis, IN, USA,

⁴Department of Chemistry, Brock University, St Catharines, ON, Canada,

⁵Department of Physics, IUPUI, Indianapolis, IN, USA, ⁶Canadian Neutron Beam Centre, National Research Council, Chalk River, ON, Canada,

⁷Biology 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