sub-diffusive (t<20ps) and super-diffusive (0.1ns<t<1ns) regimes. Apparently the latter has not been reported before, and it appears to be correlated with the out-of-plane fluctuations of the lipid molecules. The anomalous super- and sub-diffusive regimes lead to a self-intermediate scattering function with compressed- and stretched exponential relaxation, respectively. In principle, these predictions should be testable using neutron scattering (NS) experiments. In practice, however, the NS signal will most likely be totally dominated by the contribution from the abundant ordinary bulk water.
Computer time was generously provided by the University of Missouri Bioinformatics Consortium.

2726-Pos Board B712
Nanosecond Megavolt-Per-Meter Pulsed Electric Field Effects on Biological Membranes
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Nanosecond, megavolt-per meter, pulsed electric field (nanoelectropulse) technology _ a low-energy, nondestructive means for transiently electropermeabilizing biological cell membranes _ is used in cancer therapy, genetic engineering, and cell biology. Nanoelectropulses can manipulate subcellular structures and permeabilize intracellular membranes without permanent damage to the plasma membrane. These intracellular effects open new applications in apoptosis induction, gene delivery to the nucleus, and alteration of other cell functions. However, the mechanisms of nanoelectropulse effects have not been systematically characterized. To study the responses of cells to nanoelectropulses, we apply 4 ns electric pulses to Jurkat T lymphoblasts in an electrode microchamber constructed on a microscope slide, with varying pulse counts and field amplitudes. Cellular responses are monitored with fluorescence microscopy and a sensitive EM-CCD camera. YO-PRO-1 and propidium are used to evaluate cell plasma membrane permeability. Also, we record changes in cell size after pulse exposure to measure nanoelectropulse-induced swelling resulting from increased membrane permeability. To assess mitochondrial membrane permeability, we use three different methods: cobalt-quenched calcein, and rhodamine 123 and TMRE fluorescence. To observe nanoelectropulse-induced intracellular phenomena on a very short time scale we constructed a system coupling a nanosecond pulse generator and a fast photomultiplier tube (PMT) to a fluorescence microscope. With this system, we are able to detect weak signals from excited fluorophores immediately. We report the effects of nanosecond, megavolt-per meter electric pulses on cell plasma membrane permeability and mitochondria membrane permeability, and we discuss the interpretation of data obtained from a fluorescence microscopy imaging system with a PMT detector.

2727-Pos Board B713
Hydration and Mobility of Oxidized Phospholipid Bilayer: Fluorescence Solvent Relaxation and Fluorescence Correlation Spectroscopy Study
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Oxidative stress often leads to truncation of the sn-2 chain of polyunsaturated phospholipids and introduction of aldehyde or carboxyl group at its end. Such oxidized phospholipids (OxPLs) change biophysical properties of a lipid bilayer, and are involved in pathogeneses of numerous diseases. Herein we investigate the influence of oxidation on the dynamics and hydration of model lipid membranes. Local mobility and hydration and lipid lateral diffusion were measured using fluorescence solvent relaxation and fluorescence correlation z-scan spectroscopy, respectively. Lipid mixtures of palmitoyloleoylphosphatidylcholine (POPC) and 10 mol% of one of four OxPL POPC analogues (with oleoyl chain truncated to: 5′-oxo-valeroyl, 9′-oxo-nonanoyl, glutaryl, or azelaoyl) were investigated. Atomic-scale molecular dynamics simulations were employed to give a rationale for the observed changes. Truncated tails of OxPLs were found to loop back toward the aqueous solution, which leads to increased membrane fluidity, i.e., both the local headgroup mobility and the lateral diffusion are increased. Moreover, these changes depend on the chemical nature of the oxidized chains and the salt present. Our results show that products of lipid oxidation may influence physiology not only by protein-mediated signaling, but also simply by altering physical properties of a lipid bilayer.

2728-Pos Board B714
Energies and Dynamics of Ca^{2+}/MG^{2+}-Mediated Vesicle Docking, Measured using Single Particle Tracking
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Ca^{2+} plays an essential role in several biomolecular pathways, including signal transduction, vesicle fusion, and muscle contraction. Many of these processes involve biological membranes, and while Ca^{2+} has been found to mediate the association of membrane-bound structures, little is known about the energies and dynamics of interaction.
A principal case is neuronal exocytosis, where a synaptic vesicle encounters several membrane interfaces prior to fusion. First, it has to navigate through a dense pool of other synaptic vesicles, until it reaches the plasma membrane and undergoes docking. Upon stimulation, an influx of Ca^{2+} adjacent to the plasma membrane initiates vesicle exocytosis by membrane fusion, thus progressively bringing the apposing membranes into contact. While the proteins responsible for both docking and fusion has been intensively studied, less attention has been devoted to the study of docking between pure lipid membranes in the presence of intracellularly available divalent cations, e.g. Ca^{2+} and Mg^{2+}. We applied a model system, previously developed in our laboratory[1-3], comprising supported lipid bilayers and single small unilamellar vesicles (SUV) to study docking mediated by Ca^{2+}/Mg^{2+}. The lateral diffusion of single SUVs along supported membranes was imaged via TIRF microscopy to selectively acquire signal at the surface-solution interface, thus resolving SUV positions spatiotemporally. From tracking data we derived interaction rates and energies, in addition to dynamics information like mobility patterns and diffusion coefficients. With these assays we found that divalent cations, at physiologically relevant concentrations, were sufficient to mediate vesicle docking as well as affect the diffusive dynamics of SUVs in a concentration-dependent manner. We speculate that this behavior may be of biological significance for e.g. neuronal exocytosis.
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2729-Pos Board B715
Three-Dimensional Construction of Bilayer Networks using Shape Encoded Hydrogel
K. Tanuj Sapra, Hagan Bayley.
The newly invented system - droplet interface bilayer (DIB) - is beginning to emerge as a versatile platform not only for studying the basic properties of membrane proteins but also to construct novel nano-devices. Small volumes of aqueous buffer when injected into hexadecane take the form of droplets. These droplets when loaded with lipids assemble a lipid monolayer at the water/oil interface. By carefully touching two such monolayer encased aqueous droplets a bilayer can be formed which is conducive to the insertion of membrane proteins.
Here we demonstrate that contacting two hydrogel surfaces coated with lipid monolayers can form a lipid bilayer, forming a so-called hydrogel interface bilayer (HIB). We use two of the most commonly available hydrogels, a natural polymer agarose and a synthetic polymer polyethylene glycol diacrylate (PEG-DA), to form HIBs. The lipid bilayers formed using both the polymers are conducive to the insertion of membrane proteins as shown by the insertion of α-hemolysin pore.
Thus far, solid-supported lipid bilayers for reconstituting membrane proteins have been assembled in a 2-D plane. We present a simple approach to form lipid monolayers on a manipulable 3-D system based on hydrogels. Millimeter sized particles were formed by using low melt agarose or PEG-diacrylate. Agarose and PEG-DA based hydrogels can be molded in different shapes and coated with a lipid monolayer, which on bringing together gives a lipid bilayer as monitored by an increase in the capacitance. Polymers molded in different shapes have been used in a number of applications ranging from reaction coding in enzyme catalysis to providing a 3-D environment for cell growth to drug delivery. We propose the use of such solid particles incorporating membrane pores for the use of constructing communicating electrical devices.

2730-Pos Board B716
Probing the Membrane Potential of Liposomes with Impedance Spectroscopy
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Various numerical and some experimental studies have shown that the low frequency dispersions in the dielectric spectrum of a cell suspension depend on the membrane potential of the individual cells. Here we report the dielectric properties of phosphatidylcholine liposomes and its changes with the variation of membrane potential. Liposomes have been prepared to have a higher concentration of potassium ions (K^+) inside the membrane compared to external medium. Under valinomycin (K^+ ionophores) these liposomes will generate a negative membrane potential which is verified by the fluorescent measurements. Impedance measurements are carried out from 100 mHz to 1 MHz with different membrane potentials. Both the dielectric and the conductivity spectrums display low frequency dispersions that are dependent on membrane potential. Possible future applications include noninvasive sensors to monitor membrane potential and other physical properties of living cells.