1532-Pos Board B302
Quantitative Scheme for Full-Field Polarization Rotating Fluorescence Microscopy (PROM) using a Liquid Crystal Variable Retarder
John F. Lesoine.
National Institute of Standards and Technology, Gaithersburg, MD, USA.
We present a quantitative scheme for full-field polarization rotating fluorescence microscopy (PROM). A quarter-wave plate, in combination with a liquid crystal variable retarder (LCVR), provides a tunable method to rotate polarization states of light prior to its being coupled into a fluorescence microscope. A calibration of the polarization properties of the incident light is performed in order to correct for elliptical polarization states. This calibration allows the response of the sample to linear polarization states of light to be recovered. Three known polarization states of light can be used to determine the average fluorescent dipole orientations in the presence of a spatially varying DC offset or background polarization-invariant fluorescence signal. To demonstrate the capabilities of this device we measured a series of full-field fluorescence polarization images from fluorescent analogs incorporated in the lipid membrane of Burkitts lymphoma CA46 cells. The fluorescent lipid-like analogs used in this study are molecules that are labeled by either a DiI (1,1'-Dioctadecyl 3,3,3',3'-Tetramethylindocarbocyanine) fluorophore in its head group or a BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) molecule in its acyl chain. A spatially-varying contrast in the normalized amplitude was observed on the cell surface, where the orientation of the DiI molecules is tangential to the cell membrane. The internally labeled cellular structures showed zero response to changes in linear polarization, and the net linear polarization amplitude for these regions was zero. The method is then applied to study acyl chain structural phase transitions in model vesicles in addition to the probing of acyl chain structural order during cellular chemotaxis. This instrument provides a low cost calibrated method that may be coupled to existing fluorescence microscopes to perform investigations of cellular processes that involve a change in molecular orientations.

1533-Pos Board B303
Dynamics of PIP_2-Ca^{2+} Structures in Lipid Bilayers
Ian Mc Cabe.
Syracuse University, Syracuse, NY, USA.
Changes of intercellular Ca^{2+} concentrations are one of the most ubiquitous signaling events that accompany or precede large scale cellular responses. These fluctuations control such responses as: T-cell activation, cell motility, neuronal signaling, and muscular contractions. One particular Ca^{2+} pathway includes Phospholipase-C (PLC) acting on phosphatidylinositol 4,5-bisphosphate (PIP_2) in the presence of calcium ions. We are in particular interested in the direct modulation of PIP_2 organization in the membrane in such events due to Ca^{+2}. At physiological conditions, PIP_2's headgroup is multiply negatively charged (>3 effective charges) and interacts with the cationic Ca^{2+}. By coordinating several PIP_2 head-groups, calcium ions can induce condensation and aggregation of PIP_2. A series of experiments were conducted on supported lipid bilayers containing physiological quantities of PIP_2. Fluorescence correlation spectroscopy (FCS) was used to study the response of the PIP_2 to changes in the concentration of Ca^{2+} ions in the surrounding buffer solution. As Ca^{2+} concentration increases, the FCS indicates that PIP_2 goes from a freely diffusing single species to a multiple species system. The diffusion rates of the additional species decrease with increasing $[Ca^{2+}]$, thus indicating increasing aggregate sizes with increasing, but physiological relevant Ca^{2+} concentrations.

1534-Pos Board B304
Curcumin Alters Membrane Phase-Separation, Particle Size Distribution, Permeability, and Anisotropy Differently in Unsaturated or Saturated Small Unilamellar Vesicles
William D. Ehringer, Andrea S. Gobin, Patricia M. Soucy,
Patrick G. Hoblitzell, Martin G. O-Toole, Brigette H. Totten,
Robert S. Keynton.
University of Louisville, Louisville, KY, USA.
Curcumin is an extract of turmeric, and is an effective reactive oxygen species scavenger, with potential anti-cancer benefits. In this study, we examined the effects of curcumin on membrane bilayer permeability, fusion, anisotropy, hydrodynamic radius and negative staining EM using two lipid vesicle delivery systems: 1) 99% Soy phosphatidylcholine (Soy PC) and 2) DMPC/DPPG/Cholesterol (1:1:0.7 mol/mol/mol) (MPC). Curcumin was added to these two vesicle populations at 10:1, 50:1, and 100:1 (lipid mol/curc mol). The structure of curcumin suggests it would most likely intercalate into the head group region of the membrane bilayer, and thus our hypothesis was that the unsaturated fatty acids of Soy PC vesicles would accommodate curcumin with less membrane perturbation than the saturated MPC vesicles. Curcumin increased membrane permeability to carboxyfluorescein leakage (CF) in both Soy PC and MPC small unilamellar vesicles in a concentration-dependent manner; however, Soy PC vesicles were 35% more permeable to CF than MPC vesicles. The fusion of the Soy PC and MPC was examined by vesicle mixing using the Tb/DPA-assay. Both Soy PC and MPC vesicles fused within seconds of addition, and the rate of fusion was dependent on buffer calcium concentration. Curcumin had membrane concentration-dependent effects on the fluorescence anisotropy of DPH-PC and TMA-DPH Soy PC and MPC vesicles. Lipid vesicle hydrodynamic radius and TEM negative stained vesicles indicate a unimodal distribution for Soy PC vesicles (r = 100-120 nm), while MPC vesicles had a bimodal distribution (r = 100-130 nm and 3000-4000 nm). Thus curcumin can more readily intercalate into more unsaturated lipid vesicle bilayers, and we conclude that for maximal delivery of curcumin to cells using a liposomal therapy, a more unsaturated vesicle system is suggested.

1535-Pos Board B305
Anomalous Diffusion of Proteins Coupled to Membrane Thickness, Height, and Lipid Order
Brian Camley, Frank L.H. Brown.
University of California, Santa Barbara, Goleta, CA, USA.
Diffusion of proteins in lipid membranes has traditionally been described by a hydrodynamic model that treats the membrane as a two-dimensional fluid surrounded by a less viscous outer fluid. As a result, the diffusion coefficient of proteins in a membrane is predicted to have a weak (logarithmic) dependence on the protein size. This result has been experimentally confirmed for micron-scale objects in membranes and related systems such as thin smectic-A films, but the evidence for the size-dependence of protein diffusion coefficients is mixed, with some groups observing a much stronger, 1/R dependence on protein radius. This has led to speculation that the primary source of drag on a protein is not viscous, but comes from coupling to other fields, such as lipid ordering. We discuss simple continuum models of the dynamics of proteins with hydrophobic mismatch, spontaneous curvature, active protein-membrane interactions, and coupling to lipid chain order. We show that these coupling mechanisms will generically create diffusion that is anomalous at short times, which may lead to the observation of differing diffusion coefficients by different techniques.

1536-Pos Board B306
Organization and Dynamics of the Serotonin-1A Receptor in Live Cells
Amitabha Chattopadhyay.
Centre for Cellular and Molecular Biology, Hyderabad, India.
It is important to understand the dynamic organization of membrane-bound molecules in order to arrive at a comprehensive view of cellular signaling mediated by membrane-bound receptors.[1] We addressed the organization and dynamics of the human serotonin$_{1A}$ receptor fused to enhanced yellow fluorescent protein expressed in CHO cells. Serotonin$_{1A}$ receptors are prototypical members of the G-protein coupled receptor superfamily and represent a prime target for therapeutic actions of several anxiolytic and antidepressant drugs.[2] Our recent work using z-scan fluorescence correlation spectroscopy (zFCS) provides novel insight on the effects of cholesterol depletion and actin cytoskeleton destabilization on receptor confinement.[3] Interestingly, results from FRAP measurements performed under conditions of mild cytoskeletal destabilization suggest that receptor signaling is correlated with receptor mobility.[4] We recently proposed, utilizing homo-FRET in live cells, that the serotonin$_{1A}$ receptor is present as constitutive oligomers and implicated the presence of higher-order oligomers.[5,6] Taken together, these results on the cellular organization and dynamics of the serotonin$_{1A}$ receptor provide useful insight in understanding the function of the receptor in healthy and diseased states.
1. Saxena, R., and Chattopadhyay, A. (2011) *J. Neurochem.* 116: 726-733.
2. Pucadyil, T.J., Kalipatnapu, S., and Chattopadhyay, A. (2005) *Cell. Mol. Neurobiol.* 25: 553-580.
3. Ganguly, S., and Chattopadhyay, A. (2010) *Biophys. J.* 99: 1397-1407.
4. Ganguly, S., Pucadyil, T.J., and Chattopadhyay, A. (2008) *Biophys. J.* 95: 451-463.
5. Ganguly, S., Clayton, A.H., and Chattopadhyay, A. (2011) *Biophys. J.* 100: 361-368.
6. Paila, Y.D., Kombrabail, M., Krishnamoorthy, G., and Chattopadhyay, A. (2011) *J. Phys. Chem. B* (in press).