determined the phase behavior of total synaptosomal lipids by repeating the NMR protocol on extracted lipids. To our surprise, there was no detectable signal for liquid-ordered and liquid-disordered lipid phases at the body temperature of either species. However, there was a difference in the temperature of the onset of order as synaptosomes were cooled below body temperature: the phase state of mouse synaptosomal membranes changes drastically below 24 °C, whereas this change occurs below 8 °C for the squid synaptosomal membranes. We then measured the composition of synaptosomes in terms of total lipid heads and tails, and the main difference arises from high concentrations of omega-3 polyunsaturated fatty acids and cholesterol in the squid. Fluorescence microscopy images of squid synaptosomes stained with lipid dyes confirmed the formation of domains below, but not above the phase transition temperatures obtained from NMR measurements. Thus although the membranes of synaptosomes contain lipids that can phase-separate, these lipids remain in the liquid-disordered state at the usual physiological temperatures for squid and mouse.

1267-Pos Board B159

LIP-2 Domain on the Cytoplasmic Terminal Tail (CTT) of HIV-1 GP41 affects T-Cell but not HIV Virion Membranes

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Kalita et al.1 produced a mutant MX2 of LIP-2 where two highly conserved arginine residues (R3 and R21) were replaced with negatively charged glutamic acid. Although MX2 mutation showed no loss of Env expression, Env processing or infectivity of the HIV virion, both viral-initiated T-cell death and T-cell synaptocytom formation (cell-cell fusion) were greatly decreased. In the present work we investigated the interactions of five LIP-2 variants with lipid mimics of the HIV virion and the T-cell membranes. The LIP-2 peptides were designed to investigate the role of electrostatics, as well as the effect of a Crac, or cholesterol-binding motif, preceding LIP-2, and of a palmitoylated cysteine. We obtained synchrotron x-ray diffuse scattering of oriented, fully-hydrated peptide/lipid bilayers that provides both structural and bending flexibility information. We find that none of the LIP-2 peptides changed the structure or properties of the HIV mimic membrane, whereas they clearly altered the T-cell membrane mimetic, and, importantly, did so differently for the wild type LIP-2 and MX2 mutant. Our work provides a structural explanation for the mutation studies, namely, that LIP-2 has an effect on the HIV lifecycle only when it alters a membrane.

1Kalia et al., J Viral 77, 3634-3646 (2003). Research reported in this abstract was supported by the NIGMS of the NIH under award # R01GM44976. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. ALB was supported by Howard Hughes Medical Foundation. JDS and RCM were supported by AI087533. X-ray data were taken at Cornell High Energy Synchrotron Source, which is supported by the NSF and the NIH/NIGMS under NSF Award DMR-0225180.

1268-Pos Board B160

Neuroprotective Effect of 17β Estradiol on Membrane Fluidity in Ageing Rat Brain

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Oxidants and fluidity of membrane lipids are known to play a major role in ageing and age-related neurodegenerative diseases. In the present study, authors observed in the in vitro effect of Tachykinin neuropeptide NKB, Amyloid Beta (25-35) and combined NKB and Aβ (25-35) on membrane fluidity, oxidative modification and protein surface hydrophobicity of proteins on 17β estradiol (E2) treated ageing female rat brain synaptosomes of 3 months (young), 12 months (adult) and 24 months (old) age groups. Authors found that membrane fluidity of synaptosomes decreased in ageing rat brain. Similarly, Aβ (25-35) decreased the membrane fluidity in ageing rat. These effects of ageing and Aβ (25-35) on membrane fluidity were restored by NKB and combined NKB + Aβ (25-35) with estradiol. The tryptophan fluorescence was used as a sensitive marker of protein oxidation. Tryptophan fluorescence significantly decreased in estradiol treated synaptosomes of ageing rats. To evaluate the effect of oxidative stress on membrane and protein conformation, fluorescent probe ANS was used. A decrease in ANS fluorescence in estradiol treated synaptosomes of ageing rats indicated that estradiol is associated with significant conformational changes and surface hydrophobicity of membranes and proteins. In contrast these changes were not detected with NKB and Aβ in estradiol treated synaptosomes. The present findings suggest that treatment with NKB along with estradiol restores age- and Aβ-induced alterations and demonstrating a possible beneficial role of NKB and estradiol in preventing some of the age related changes in the brain.

Membrane Physical Chemistry II

1269-Pos Board B161

Exploring Artificial Intercellular Nanotube Networks

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Tunneling nanotube (TNT) as a new type of intercellular communication channel was discovered in 2004 [1]. Over the past years, these membranous channels had been proved to be used to mediate intercellular transport of endosome-related organelles, cellular components, and the coordination of signaling [2-3] etc. More recently, several studies have been reported that to naturally form long-distance TNT cell-to-cell connection by combining microfabrication technique [4]. Our experimental aim is to explore an artificial method to create intercellular tunneling nanotube (TNT) networks among living cells. Based on our experimental results, we can successfully connect target cells to be intercellular nanotube networks by applying micromanipulation techniques. For analysis transport function of the created intercellular networks, we do fluorescence intensity test by injecting Ca2+ into cells (preloading Fluo-3 AM) through the forming tunneling nanotubes and finally get the expected results.

References:

1270-Pos Board B162

Nucleobases and Ribose Bind to and Stabilize Aggregates of a Prebiotic Amphiphile

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One scenario for the origin of life postulates that catalytic RNA can be synthesized and encapsulated in a membranous compartment to form primitive cells. Here we examine physical interactions between fatty acid aggregates, especially vesicles, and the bases and sugar composing RNA. Our goal is to employ an array of fundamental physical techniques to establish the plausibility of a scenario in which aggregates of amphiphiles preceded RNA and facilitated its synthesis by binding and concentrating the bases and sugars of which it is composed. We find that fatty acid membranes do indeed bind nucleobases and ribose more effectively than they bind several related bases and sugars. Moreover, we find that nucleobases and ribose also inhibit flocculation of fatty acids by salt, and do so more effectively than several other bases and sugars. This result is important because it addresses how prebiotic membranes could have been stabilized in early oceans with high salt concentrations. Taken together, our results yield mutually reinforcing mechanisms of adsorption, concentration and stabilization that could have driven the emergence of a population of stable vesicles enriched in the components of RNA.

1271-Pos Board B163

Characterization of Archaea Tetraether Hybrid Liposomal Doxorubicin

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We have previously shown that liposomes made of the polar lipid fraction E (PLFE) isolated from the thermoacidophilic archaean Sulfolobus acidocaldarius exhibit enhanced vesicle stability in comparison to conventional dieter or diether lipids (reviewed in Chong et al (2012) Archea, doi:10.1155/2012/1271-(9)). In the present study, we have characterized several physical properties of tetraether hybrid liposomes composed of PLFE and non-archaeal diether lipids such as dipalmitoylphosphatidylcholine or porcine brain sphingomyelins/cholesterol. Particle size, zeta-potential and membrane packing of the hybrid liposomes are examined using dynamic light scattering, laser Doppler electrophoresis, Laurdan’s generalized polarization, respectively. In addition,
entrapment efficiency and spontaneous release of anti-cancer drug doxorubicin is studied by using absorption spectroscopy and fluorescence de-quenching method. These physical measurements are performed on liposomes with varying PLFE molar ratios at different temperatures. The obtained results may help to optimize and design liposomal drugs with greater stability and higher therapeutic efficacy. (supported by NSF DMR1105277)

1272-Pos Board B164
PLFE Lipids Stabilize Liposomal CA4P
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Liposomal encapsulation using bipolar tetraethyrlipids such as the polar lipid fraction E (PLFE) isolated from the archaeae Sulfolobus acidocaldarius offers many advantages over conventional phospholipid mediated or free drug delivery. PLFE provide increased stability to lipid vesicles. Liposome mediated drug delivery can reduce the off target effects caused by anti-cancer drugs. Therefore, we hypothesize that PLFE archaeosomes, offer the anti-cancer drug brentastatin A4 disodium phosphate (CA4P) a higher therapeutic efficacy by increasing the drug’s stability, circulation time, and targeting. In this study, the fluorescent properties of CA4P have been utilized for drug leakage assays with different compositions of PLFE/POPC in unilamellar vesicles of varying sizes. We have found that PLFE lipids stabilize liposomes and decrease rate of CA4P leakage. We also have shown that this effect is manifested in the cytotoxicity assay against human MCF-7 breast cancer cells. (supported by NSF DMR1105277)

1273-Pos Board B165
Hydrodynamic Co-Localization of Molecules in Supported Lipid Bilayers Detected by Secondary Ion Mass Spectrometry
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Here we report on the use of secondary ion mass spectrometry (SIMS) to study the hydrodynamic co-localization of membrane components in supported lipid bilayers formed by the fusion of multi-component giant unilamellar vesicles to oxidized silicon substrates. In these experiments, hydrodynamic drag forces arising from flow above the supported lipid bilayer (SLB) results in the directed motion of molecules protruding from the SLB. In this particular case, protrusion of the cholera toxin B into the aqueous layer serves as a handle for the directed motion of its natural ligand, ganglioside GM1, and any other molecule (i.e. cholesterol) strongly associated with it. Orthogonal isotopic labeling or fluorination of every lipid bilayer component allowed generation of molecule-specific images, using a nanoSIMS, that map the lateral re-distribution of molecules in a lipid bilayer as a result of hydrodynamic flow. Furthermore, simultaneous detection of up to seven different ion species, including secondary electrons, allowed generation of ion ratio images whose signal intensity values could be correlated to composition through the use of calibration curves from standard samples.

1274-Pos Board B166
Interaction of 1,4-Naphtoquinone with Cell Membranes Models Studied with Tensiometry and Vibrational Spectroscopy
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Antiepileptic drugs are natural or synthetic compounds that act against the development of cancer cells, whose chemical interactions with cell membranes have a mechanism of action not sufficiently known so far. For this reason, it is imperative the understanding at the molecular level of drug-cell interactions, and using models for cell membranes is a suitable strategy for that. In this study, we employed Langmuir monolayers of lipids as cell membrane models, and 1,4-Naphthoquinone, which is a potent inhibitor of human cancer cell growth and angiogenesis, was investigated. The drug was incorporated in monolayers of zwitterionic lipids such as DPPC (dipalmitoyl phosphatidyl choline), and negative ones, such as DPPS (dipalmitoyl phosphatidyl choline). Surface pressure-area isotherms showed that the drug induces to a condensation of the monolayer, influences the first-order transition of the lipid from liquid-expanded to liquid-condensed phases, and alters the visco-elastic properties of the monolayers. Also, Polarization Modulation Infrared Absorption-Reflection Spectroscopy (PM-IRRAS) indicated that the drug acts in a first moment in the polar heads of the phospholipids, which causes further distortion of the alkyl chains of the phospholipids. These results are important not only because brings information on drug-membrane interactions at the molecular level, but also because envisage the enhancement of the use of antiepileptic drugs in cancer treatment.

1275-Pos Board B167
Lipid Membrane Phase Dynamics
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We study lipid phase behavior using giant unilamellar vesicles to model cell membrane dynamics. In our system, we investigate the effects of cross-linking in the head groups position via biotinylated lipids, avidin, and its analogues. Cross-linking is the linking of two molecules (biotinylated lipids) via a linking agent (avidin). Vesicles allow us to isolate the lipid rearrangement due to cross-linking, a common activity on cell surfaces. By comparing specific binding strength of the coupling and self-association, we study the role that cross-linking plays in membrane behavior. Using anti-avidin we attempt to induce aggregation of the membrane bound protein, producing micron size phase domains from initial one-phase vesicles. Confocal microscopy enables us to image this change in the membrane dynamics. Using phase specific dyes, we probe phase segregation on the nanometer scale from the addition of a cross-linker to the system. Förster Resonance Energy Transfer (FRET) enables us to detect clustering on the submicron (1-10 nm) scale, beyond the limits of conventional microscopy. Both techniques allow us to quantify the phase behavior due presence of the cross-linking agent. Using FRET we detect lipid rearrangement associated with the transition from one-phase vesicles to two-phase vesicles using two different fluorescent dyes, a donor and acceptor. From judicious choice of donor and acceptor dyes, we detect the changes in fluorescence acceptor signal as a function of clustering. We are pursuing life-time studies to complement our current FRET analyses. From this simple cross-linking system, we model membrane responses to protein complex formation and oligomerization.

1276-Pos Board B168
Meta-Cresol Affects Lipid Raft Organization in Membrane-Model Systems and Increases Membrane Leakage in Neural Cells
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m-cresol is an excipient stabilizer used in numerous pharmaceutical formulations, including injectable insulin and vaccines. Therefore, we studied the effects of m-cresol in a range of concentrations from 10nM to 3mM on membrane model systems mimicking lipid-rafts and living neural-cells. First, the intrinsic fluorescence of m-cresol was studied. Both its fluorescence lifetime and anisotropy increased in the presence of liposomes, indicating a decreased mobility of the molecule. This interaction was dependent on membrane lipid composition. To elucidate this process, liposomes were labeled with several membrane probes spanning a range of in-depth locations and with preference for distinct lipid domains. For the probes located in the bilayer core (DPH and trans-paraninic acid), no effect was detected even for an m-cresol concentration of 300M, whereas for the more superficial NBD-DOPC and NBD-Dim, 30M m-cresol induced a significant fluorescence lifetime decrease. Atomic force microscopy experiments were performed on ternary supported lipid bilayers containing raft-like liquid ordered domains (Lo). Indeed, it was observed that upon addition of m-cresol in the M range, a reduction of the Lo occurs without changing their thickness. For higher m-cresol concentrations, raft-like domains are not detected at all.
Whole-cell voltage-clamp recordings from pyramidal-neurons isolated from the CA1 region of rat hippocampus (p21-p29) and from N1E-115 neuroblastoma cells were also performed. m-Cresol was applied during constant superfusion and the following parameters were monitored: series-resistance, whole-cell capacitance, holding-current (Vm=-70 mV), and another read-out for the leak-current. Results show that only the leak current was altered by m-cresol (>100 M). As a whole, we show that m-cresol interacts with the membrane, affecting lipid raft organization, with functional implications on neural-cell integrity. We thank F.C.T. Portugal for financial support (Ciência2007, SFRH/BD/64442/2009, Pest-OE/QUI/UI0612/2011).

1277-Pos Board B169
Two-Dimensional Macroscopic Protein Domains Induced by the Interplay between Lipid- Protein and Protein- Protein Interactions
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It has been suggested that lipids and proteins are not homogeneously distributed in cell membranes; they can segregate into dynamic micro/ nanodomains, serving as centers for signal transduction, membrane trafficking, and cytoskeletal organization. Here we ask the question whether two- dimensional protein