fusion peptide of HIV-1 is a crucial player in viral entry into a target cell. Emerging evidence of the presence of cholesterol-enriched microdomains in a plasma membrane makes it important to understand the role of cholesterol in fusion peptide - membrane interactions. In this work we investigate the effect of cholesterol concentration on the capability of HIV-1 gp41 peptide to insert into lipid monolayers using insertion assays, epifluorescence microscopy, and synchrotron X-ray reflectivity (XR) and grazing incident-angle X-ray diffraction (GIXD) techniques. Lipid monolayer formed at the air-liquid interface models an approximate environment of the outer leaflet of a target cell membrane where viral fusion occurs. A target membrane was mimicked with 1,2-Dipalmitoyl-sn-glycero-3-phophocholine (DPPC), Cholesterol, and binary mixtures of Cholesterol:DPPC (with molar ratio between 0.15 and 0.85). In the binary mixture cholesterol has been found to be shielded from aqueous environment with DPPC headgroups at every cholesterol concentration used, in agreement with the *umbrella* model. The fusion peptide was found to insert readily into all lipid monolayers without destroying the morphology of a membrane. Binary mixture monolayers with low content of cholesterol were found to be the most vulnerable to the peptide insertion among all examined membrane mimics. XR results indicate that decrease in thickness of a membrane induced by gp41 fusion peptide insertion is more pronounced for monolayers with higher cholesterol content suggesting an important role of cholesterol in fusion pore formation. FP was found to penetrate the pure cholesterol and DPPC monolayers fully, while in mixed cholesterol:DPPC monolayers the fusion peptide inserts only partially, interacting strongly with cholesterol benzene rings.

1146-Pos New Tools For Biophysical Studies Of Hemagglutinin-mediated Membrane Fusion

Frederic EGHIAIAN, Paul LeTissier, John E. Corrie, John J. Skehel, Claudia Veigel

Medical Research Council, NIMR, Mill Hill, United Kingdom.

Board B121

The influenza Hemagglutinin (or flu HA) is responsible for immobilising influenza virus particles on the surface of the host cell and for fusion between the host cell and the viral membranes. Following endocytosis, HA-mediated membrane fusion occurs during intracellular transport, when viral surface proteins are being exposed to low pH in the endosomes. Low pH triggers a conformational change upon which the functional segment of HA named "fusion peptide" becomes solvent exposed and contacts the host's membrane. The mechanisms by which the flu HA facilitates subsequent merging of lipid membranes and opening of a fusion pore in the merged membrane are unclear: in particular it is not known how many HA molecules are needed for membrane fusion or whether structural intermediates of HA contribute to different stages of fusion. In order to obtain a more detailed understanding of the basic mechanisms underlying HA-mediated membrane fusion we use a combination of molecular biology and single molecule biophysical techniques. We are expressing mutant HA for single molecule imaging of the fusion process. We also use a photoactivatable caged proton to allow for a fast pH jump, in order to study the fusogenic conformational change of single HA molecules at high time resolution. The caged proton did not affect HA-induced membrane fusion. Also our biochemical studies indicate that the known low-pH refolding of HA2 correlates with a structural rearrangement of HA2 C-terminal region.

(Supported by MRC and The Royal Society)

1147-Pos Possible Mechanism Of Endosomal Escape Of Tat: Membrane Fusion Of Intraluminal Vesicles With Late Endosomes

Sung-Tae Yang, Leonid V. Chernomordik, Kamran Melikov

NICHD, Bethesda, MD, USA.

Board B122

Cellular uptake of the HIV Tat-derived peptide enables to deliver a variety of the bioactive molecules including proteins and nucleic acids. It has recently been recognized that endocytosis/pinocytosis plays a major role in the mechanism of Tat-mediated internalization. Following the endocytosis, however, the mechanism of escape of Tat from endosomes remains elusive. Here we suggest a model for its endosomal escape. We demonstrate that Tat induces both fusion and leakage of the liposomes containing lysobisphosphatidic acid (LBPA), which is a characteristic lipid of intraluminal vesicles of multivesicular body. In contrast, liposomes in which LBPA was replaced by its stereoisomer phosphatidylglycerol were unaffected by Tat. Both Tat-induced fusion and leakage of LBPA containing liposomes were promoted by acidic pH and elevated Ca^{2+} concentration. In addition, Tat efficiently induced membrane fusion of LBPA with the liposomes mimicking the late endosomal lipid composition. Based on these results, we propose that the electrostatic trans-interaction of anionic LBPA with cationic Tat initiates the membrane fusion of intraluminal vesicles with late endosome and enables the escape of Tat from the endosome.

Membrane Structure - I

1148-Pos Electroelastic Coupling between Membrane-Embedded Charges and Membrane Fluctuations: Continuum Multi-Dielectric Treatment

Michael B. Partensky, Gennady V. Miloshevsky, Peter C. Jordan

Brandeis University, Waltham, MA, USA.

Board B123

There is growing interest in understanding effects due to electrostatically promoted membrane-water fluctuations, which may influence voltage gating in proteins. Membrane geometry is significantly influenced by electrostatic interactions with membrane-

embedded charges. We use a modified continuum model to study the interactions of charges with surface fluctuations for arbitrarily shaped membrane-water interfaces in multi-dielectric environments. We surround a point charge by a low dielectric constant sphere and solve the linear Poisson-Boltzmann equation, directly calculating the potential due to the reaction field via a method that eliminates self-energy contributions. This approach permits analysis of a charge in a mixed lipid-water environment, e.g. a charge crossing the fluctuating membrane-water interface or interacting with a water plume penetrating the hydrophobic core of the membrane. We determine the energetics and optimized shapes of such aqueous deformations interacting electroelastically with charges located at various positions in the membrane.

1149-Pos Theory of the Phase Behavior of a Model Bilayer Membrane with Coupled Leaves

Gregory G. Putzel, Michael Schick

University of Washington, Seattle, WA, USA.

Board B124

We discuss the thermodynamic behavior of a bilayer composed of two coupled leaves and derive the Gibbs Phase Rule for this system. A simple phenomenological model of such a bilayer is considered in which the state of the bilayer is specified by only two quantities: the relative number of ordering lipids in the outer leaf, and in the inner leaf. Two cases are treated. In the first, both inner and outer leaves could undergo phase separation when uncoupled from one another. This is a situation often encountered in model membranes. The bilayer can exist in four different phases, and can exhibit three-phase coexistence. In the second case, an outer layer which can undergo phase separation by itself is coupled to an inner leaf which can not. This is the case which is most probably of relevance to the plasma membrane. We find that when the coupling is weak, the bilayer can exist in only two phases, one in which the outer layer is rich in ordering lipids and the inner leaf is somewhat richer in them than when uncoupled, and another in which the outer layer is poor in ordering lipids and the inner leaf is poorer in them than when uncoupled. Increasing the coupling increases the sensitivity of the inner leaf composition to small changes in that of the outer leaf. For sufficiently large coupling, a phase transition occurs and the bilayer exhibits four phases as in the first case considered. Our results are in accord with several observations made recently.

1150-Pos Flexible-to-semiflexible Chain Crossover On The Pressure-area Isotherm Of Lipid Bilayer

Sergei I. Mukhin, Igor N. Krivonos

Moscow Institute for Steel and Alloys, Moscow, Russian Federation.

Board B125

We found theoretically [1] that competition between $\sim K_f q^4$ and $\sim Qq^2$ terms in the Fourier transformed conformational energy of a single lipid chain, in combination with inter-chain entropic repulsion in the hydrophobic part of the lipid (bi)layer, may cause a crossover on the bilayer pressure-area isotherm $P(A) \sim (A\text{-}A_0)^{-\alpha}$. The crossover manifests itself in the transition from $\alpha=5/3$ to $\alpha=3$. Our microscopic model represents a single lipid molecule as a worm-like chain with finite irreducible cross-section area A_0, chain flexural rigidity K_f and chain stretching modulus Q in a parabolic potential with self-consistent curvature B(A) formed by entropic interactions between hydrocarbon chains in the lipid layer. The crossover area A* obeys relation $Q/\mathrm{Sqrt}\{K_f B(A^*)\} \sim 1$. We predict a peculiar possibility to deduce effective elastic moduli K_f and Q of the individual hydrocarbon chain from the analysis of the isotherm possessing such crossover. Also calculated is crossover-related behavior of the area compressibility modulus K_A, equilibrium area per lipid A_t, and chain order parameter $S(\Theta)$.

References

[1]. I. N. Krivonos, S. I. Mukhin "Flexible-to-semiflexible chain crossover on the pressure-area isotherm of lipid bilayer", Journal of Experimental and Theoretical Physics, vol. *132*, No. 5, in press (2007).

1151-Pos Kinetic Methods in Determining Ion Binding to a Simulated Zwitterionic Bilayer

Sagar A. Pandit[1], David L. Bostick[2], Max L. Berkowitz[3]

[1] *University of South Florida, Tampa, FL, USA*
[2] *The Scripps Research Institute, La Jolla, CA, USA*
[3] *The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.*

Board B126

Half-microsecond molecular dynamics simulations are performed on hydrated dipalmitoylphosphatidylcholine bilayer systems in NaCl solution. Using the ionic hydration number as a progress variable, binding of Na+ and Cl− ions to the membrane is observed. In this study we obtain estimates on the association and dissociation time scale for the ions by modeling ion binding with simple kinetic equations. The effect of these time scales on the apparent surface potential is discussed.

1152-Pos Molecular Organization In A Phospholipid Membrane Containing *Trans* Unsaturation

Smita P. Soni[1], Jennifer Runyan[1], Garrison Birch[1], Jesse A. Ward[2], Stephanie E. Sen[1], Scott E. Feller[2], Stephen R. Wassall[1]

[1] *IUPUI, Indianapolis, IN, USA*
[2] *Wabash College, Crawfordsville, IN, USA.*

Board B127

We synthesized deuterated analogs of 1-elaidoyl-2-stearoylphosphatidylcholine (*t*18:1-18:0PC) and 1-oleoyl-2-stearoylphosphatidylcholine (*c*18:1-18:0PC) and employed solid state ^{2}H NMR,

complemented by MD simulations, to compare molecular organization in a model membrane containing a single "manmade" *trans* or "natural" *cis* double bond. Moment analysis of ^{2}H NMR spectra recorded as a function of temperature showed that the chain melting temperature T_m for *t*18:1-[$^2H_{35}$]18:0PC (32 °C) lies well above *c*18:1-[$^2H_{35}$]18:0PC (7 °C). The smaller depression in transition temperature relative to 18:0-[$^2H_{35}$]18:0PC (52 °C) is consistent with the *trans* unsaturated chain adopting a more linear conformation and causing less disruption of chain packing in the gel phase than the *cis* unsaturated chain. Order parameters S_{CD} evaluated, and reproduced in MD simulations, for the [$^2H_{35}$]18:0 *sn*-2 chain of *t*18:1-[$^2H_{35}$]18:0PC and *c*18:1-[$^2H_{35}$]18:0PC in the liquid crystalline phase coincide within <5%. They are 20% below 18:0-[$^2H_{35}$]18:0PC, which is attributed to the greater flexibility conferred upon a monounsaturated chain by the reduced energy barrier to rotation about the single C-C bonds next to a *trans* or *cis* double C=C bond. Now we synthesize [$^2H_{33}$]*t*18:1-18:0PC and [$^2H_{33}$]*c*18:1-18:0PC to directly probe *t*18:1 vs. *c*18:1 *sn*-1 chains. MD simulations predict that while the average conformation of the *trans* unsaturated chain resembles a saturated chain, the amplitude of the angular fluctuations undergone approaches that of a *cis* unsaturated chain.

(Supported by ACS, PRF 43281-AC7.)

1153-Pos Simulation Studies Of The Properties Of Hydrated POPC Lipid Bilayers Interacting With A Nanoporous Solid Silica Substrate

Scott Feller[1], Matthew Roark[1], Paul Tumanen[2], H. Larry Scott[2]

[1] *Wabash Col, Crawfordsville, IN, USA*
[2] *Illinois Institute of Technology, Chicago, IL, USA.*

Board B128

One of the goals of the National Center for the Design of Biomimetic Nanoconductors (NCDBN) is the design of protocells, consisting of nanoporous solids or gels, loaded with electrolyte of desired composition, coated with membrane with desired transport and recognition properties. In support of experimental investigations of Center members we have carried out a series of molecular dynamics (MD) simulations of POPC bilayers interacting with nanoporous silica substrates that are used by Brinker and co-workers (Doshi et al, Science 290, 1107 (2000)) in experimental NCDBN work. Results from two sets of simulations will be reported. For both sets the nanopore consists of an amorphous silica solid surface with lateral dimension 51 × 56 nm and thickness 27 nm, containing a central cylindrical pore of diameter ∼1 nm. All surface bonds are hydroxylated. In the simulations, a POPC bilayer is placed at varying distances above the pore, and the system is fully hydrated. We have simulated this system using two MD protocols: an all-atom POPC using CHARMM, and a united atom POPC using GROMACS. In this poster we report results of the simulations, including structural alterations in the POPC bilayer due to intreractions with the pore, and we compare the two simulation methodologies.

Supported by NIH Roadmap for Medical Research Grant to the NCDBN (PN2 EY016570)

1154-Pos Interleaflet Interactions in Lipid Bilayers

Marcus D. Collins, Sarah L. Keller

University of Washington, Seattle, WA, USA.

Board B129

We recently showed that the leaflets of a lipid bilayer interact sufficiently strongly that one leaflet can induce substantial phase separation in the other, or suppress it. We do not observe incomplete overlap of domains; that is, the boundary edge of a liquid domain in one leaflet is always exactly aligned with the boundary of a similar domain in the opposite leaflet. This implies a strong interaction which takes the form of a surface tension between the two leaflets. However, it remains unclear what gives rise to this interaction. Suggestions have included interdigitation of hydrocarbon chains at the bilayer midplane, and rapid translocation of cholesterol from one leaflet to the other. Some models can be ruled out based on the expected form of the interleaflet interaction. Here we examine the effects of various additive molecules expected to increase or decrease the strength of the interactions between the two leaflets.

1155-Pos Coupling between local Curvature and Composition in Lipid Bilayers: atomistic Simulation of Tail Length Effects

Hao Wang[1], Jason de Joannis[2], Jeffrey Gaulding[3], Birgit Albrecht[4], Yong Jiang[5], Kunal Khanna[1], James T. Kindt[1]

[1] *Emory University, Atlanta, GA, USA*
[2] *Accelrys Software Inc., San Diego, CA, USA*
[3] *GlaxoSmithKline, Research Triangle Park, NC, USA*
[4] *Loyola College, Baltimore, MD, USA*
[5] *Scripps Research Institute, La Jolla, CA, USA.*

Board B130

We investigate lipid partitioning between different curvature environments driven by differences in tail length through mixed molecular dynamics-Monte Carlo simulation. Specifically, we simulate bilayers composed of dimyristoyl-phosphatidylcholine (DMPC) mixed with didecanoyl- phosphatidylcholine (DDPC) or with an asymmetric-tail lipid such as myristoyl-hexanoyl phosphatidylcholine (MHPC). Environments include the edge of a ribbon and regions of positive and negative curvature in a strongly buckled bilayer. Our conclusions are:

1. no apparent segregations of lipids of different tail length are observed in a strongly curved but unbroken environment; instead, lipids tend to adjust the splay between their two tails to achieve efficient packing in curved environments.
2. the buckling surface pressure increases monotonically with the proportion of the longer-tail DMPC lipids.
3. the shorter-tail lipid is enriched at the edges of a bilayer ribbon, and

4. enrichment at the edges is more pronounced for asymmetric-tail lipids than for symmetric-tail lipids with the same average tail length.

1156-Pos Modeling the Smooth A-B+ LPS Membrane of Pseudomonas aeruginosa

Roberto D. Lins, Thereza A. Soares, Tjerk P. Straatsma

Pacific Northwest National Laboratory, Richland, WA, USA.

Board B131

Lipopolysaccharides (LPSs) form the major constituent of the outer membrane of Gram-negative bacteria, and are believed to play a key role in processes that govern microbial metal binding, surface adhesion, and microbe-mediated oxidation/reduction reactions. It is also a major causative agent of nosocomial illness, eliciting both chronic and acute infections in burn, immunocompromised, and cystic fibrosis. Phenotypic variation in the relative expression of A- and B-band in the LPS of *P. aeruginosa* seems to alter its overall surface characteristics influencing adhesion and favoring survival. Classical molecular dynamics simulations of the rough and smooth A-B+ LPS membranes of Pseudomonas aeruginosa have been carried out and the influence of the B-band O-antigen chain on the structure and electrostatic surface potential is characterized. Our results indicate that the B-band presents a remarkable flexibility remaining fully solvated and does not interact with the sugar units from the LPS core surface residues, in agreement atomic force microscopy experiments. The presence of the O antigen promotes a slight membrane expansion and significantly alters the electrostatic surface potential. Based on these findings and on recent experimental observations that the absence of the B-band is associated with a planktonic life style in *P. aeruginosa* (*i.e.*, acute infective capable bacteria), we postulate that electrostatics is probably the major factor behind LPS remodeling towards cell adhesion and biofilm formation.

1157-Pos Molecular Organization at the Ion-Water-Lipid Interfaces of DOPS Bilayers

See-Wing Chiu[1], Sagar A. Pandit[2], Eric Jakobsson[1], H. Larry Scott[1], Ananth Grama[3]

[1] *University of Illinois, Urbana, IL, USA*
[2] *University of South Florida, Tempa, FL, USA*
[3] *Purdue University, West Lafayette, IN, USA.*

Board B132

Anionic unsaturated lipid bilayers such as dioleylphosphatidylserine (DOPS) are suitable model systems that mimic real cell membranes. Understanding of detailed molecular organization of ion-water-lipid interfaces may provide important insights into the mechanisms of protein binding to membranes. In this poster we describe multiple Molecular Dymanics (MD) simulations of hydrated DOPS lipid bilayers. The simulations consist of bilayers containing 128 DOPS molecules and 128 counter soidum ions. Force fields for the simulations were parameterized by our group (Biophys. J. Annu. Meeting 2007, 2611-Plat, Molecular Dynamics Simulation of a Dioleylphosphatidylserine Lipid Bilayer). Simulations were run at levels of hydration that vary from 9 waters per lipid to 66 waters per lipid. We calculate the effects of hydration level on structural properties of DOPS including area per molecule, order parameters, dipole potential, elecron density profiles and form factors. We also study the distribution of water and counterions at the membrane interface using radial distribution functions.

1158-Pos Probing Membrane Undualtions: Molecular Dynamics Simulation of GM1 in a large POPC bilayer

Sagar A. Pandit[1], Larry Scott[2]

[1] *University of South FLorida, Tampa, FL, USA*
[2] *Illinois Institute of Technology, Chicago, IL, USA.*

Board B133

Gangliosides are a class of glycosphingolipids that play important roles in brain membrane function and pathologies, including HIV, Alzheimers and Prion diseases. Gangliosides are associated with sphingolipid and cholesterol rich domains found in lipid rafts. The biological importance of gangliosides is clear, but their role in modulating membrane structure is not. Given that experimental studies suggest that Gmq is found near caveolae, one likely role for this lipid is in the induction and control of localized membrane curvature. In this poster we describe very large scale simulations of bilayers consisting of 1000 POPC molecules in one leaflet and 1990 POPC plus 10 GM1 molecules in the other leaflet. The goal here is to examine the role of Gangliosides in the undulation and bending properties of POPC lipid bilayers. We describe the calculation of the required forcefield parameters, and then the results of the large scale MD simulations of the ganglioside GM1 in the POPC membrane. The scale of the simulations, and the initial placement of theGM1 molecules in close proximity in one leaflet, enable the simulation of GM1-induced undulation fluctuations in the POPC bilayer. Structural properties of the bilayer that result from the presence of the GM1 will be described.

1159-Pos Correspondence Between The Lateral Stress Profile And Spontaneous Curvature Of Membranes

Horia I. Petrache[1], Michael F. Brown[2]

[1] *Department of Physics, Indiana University Purdue University Indianapolis, Indianapolis, IN, USA*
[2] *Department of Chemistry, University of Arizona, Tucson, AZ, USA.*

Board B134

Membrane mechanics are determined by a balance of attractive and repulsive forces involving lipid head groups and acyl chains that is manifested in biological functions such as mechanosensitivity and organelle shape regulation. The lateral pressure profile is an appealing theoretical description of these membrane forces relevant to lipid-protein interactions and membrane functions [1]. However, while easily calculated from molecular dynamics simulations, the lateral stress profile is not directly accessible experimentally. Here we combine X-ray scattering and solid-state ^{2}H NMR spectroscopy to relate the lateral pressure profiles to experimental observables:

(i) the *spontaneous curvature* values as obtained from SAXS and
(ii) the lateral *mean-torque profiles* as obtained from ^{2}H NMR [2].

Lipid acyl chain order parameters measured by solid-state ^{2}H NMR carry information on the lateral pressure profile. Similarly, the membrane spontaneous curvature is related to the (bending) moments of the lateral stress profile. The mean-torque profile from ^{2}H NMR together with the spontaneous curvature and bending energies measured by SAXS explain mechanical features of biomembranes ranging from mechanosensitivity to cellular shape regulation. Mean-torque profiles are calculated by mapping the measured order parameters into the corresponding potential of mean-torque felt by individual carbon segments. This approach reveals that a universal chain packing profile exists for saturated acyl chains. We have shown that measured shifts in the chain packing profiles correspond to shifts in the balance of forces between headgroups and acyl chains. This shift in the force balance gives rise to the observable membrane mechanics and provides a framework for relating lipid composition to membrane function.

References

[1]. M. F. Brown (1994) *Chem. Phys. Lipids* ***73***, 159.
[2]. H. I. Petrache *et al.* (2000) *Biophys. J.* ***79***, 3172.
[3]. H. I. Petrache *et al.* (2001) *J. Am. Chem. Soc.* ***123***, 12611.

1160-Pos Lipid Area Refinement Based On A Simultaneous Analysis Of Neutron And X-ray Scattering Data And All-atom Molecular Dynamics Simulations

Norbert Kučerka[1], John Nagle[2], Jonathan Sachs[3], Scott Feller[4], Jeremy Pencer[5], Andrew Jackson[6], John Katsaras[1]

[1] *National Research Council, Chalk River, ON, Canada*
[2] *Carnegie Mellon University, Pittsburgh, PA, USA*
[3] *University of Minnesota, Minneapolis, MN, USA*
[4] *Wabash College, Crawfordville, IN, USA*
[5] *Atomic Energy of Canada Ltd., Chalk River, ON, Canada*
[6] *NIST Center for Neutron Research, Gaithersburg, MD, USA.*

Board B135

Quantitative structures were obtained for fully hydrated, fluid phase dioleoyl-phosphatidylcholine (DOPC at 30 °C) and dipalmitoyl-phosphatidylcholine (DPPC at 50 °C) bilayers. The structures were evaluated by the simultaneous analysis of x-ray and neutron scattering data using a new structural model for the bilayer scattering density profile (SDP). The model's design was guided by results from molecular dynamics simulations, and addresses features found in both electron and neutron scattering length density profiles of a lipid bilayer. After developing and testing the SDP model, using simulated data, it was applied to evaluating small-angle x-ray and neutron scattering data of DOPC collected under two different external contrast conditions. In the case of DPPC bilayers, additional contrast variation data were obtained through the use of specifically deuterated DPPC analogues (i.e. DPPC_d62, DPPC_d13 and DPPC_d9). Analysis of the data yielded the lateral areas for liquid crystalline DPPC and DOPC bilayers. Although, the obtained area/DPPC molecule was not so different from previously published reports, this was not the case for DOPC bilayers whose area/lipid was found to be $\sim$ 10 % smaller. This newly developed area refinement method will reconcile long-standing inconsistencies in lipid areas.

1161-Pos Temperature Dependence of Structure, Bending Rigidity and Bilayer Interactions of Dioleoylphosphatidylcholine Bilayers

Jianjun Pan[1], Stephanie Tristram-Nagle[1], Norbert Kučerka[2], John F. Nagle[1]

[1] *Carnegie Mellon University, Pittsburgh, PA, USA*
[2] *Canadian Neutron Beam Center, NRC, Chalk River, ON, Canada.*

Board B136

X-ray diffuse scattering was measured from oriented stacks and unilamellar vesicles of dioleoylphosphatidylcholine (DOPC) lipid bilayers to obtain the temperature dependence of the structure and of the material properties. The area per molecule A was 75.5Å^2 (45°C), 72.4Å^2 (30°C) and 69.1Å^2 (15°C), which gives the area expansivity α_A =0.0029/deg at 30°C, and we show that this value is in excellent agreement with the polymer brush theory. The bilayer becomes thinner with increasing temperature; the contractivity of the hydrocarbon portion was α_{Dc} = 0.0019/deg; the difference between α_A and α_{Dc} is consistent with the previously measured volume expansivity α_{Vc} = 0.0010/deg. The bending modulus K_C decreased as exp (455/T) with increasing T in Kelvins. Our area compressibility modulus K_A decreased with increasing temperature by 5%, the same as the surface tension of dodecane/water, in agreement again with the polymer brush theory. Regarding interactions between bilayers, the compression modulus B as a function of interbilayer water spacing D_W' was found to be nearly independent of temperature. The repulsive fluctuation pressure calculated from B and K_C increased with temperature and the Hamaker parameter for the van der Waals interaction was nearly independent of temperature; this explains why the fully hydrated water spacing D_W' that we obtain from our structural results increases with temperature.

1162-Pos Microbeam X-Ray Diffraction on Single Phospholipid Liposomes Fixed by Optical Tweezers

Peter Laggner[1], Heinz Amenitsch[1], Michael Rappolt[1], Barbara Sartori[1], D. Cojoc[2], Christian Riekel[3]

[1] *Austrian Academy of Sciences, Institute of Biophysics and Nanosystems Research, Austria*
[2] *CNR-INFM, Lab TASC, Basovizza, Italy*
[3] *ESRF, Grenoble, France.*

Board B137

The present knowledge about structure and interactions of liposome systems is largely limited to bulk aqueous dispersions of phospholipids which generally are heterogeneous ensembles of particle sizes and thermodynamic activities. In order to overcome this limitation and to manipulate, characterize and measure the micro-diffraction of individual phospholipid liposomes, an optical tweezers setup combined with an imaging microscope has been developed and installed at the microfocus beamline ID13 at ESRF, Grenoble. Clusters of about 50 multi-lamellar liposomes (< 10 μm diameter) were trapped in a liquid-filled capillary and investigated by a scanning microbeam X-ray diffraction with (1 μm beam) to explore the fixing capabilities and to study the size and internal structure of the liposome cluster. The results demonstrate the feasibility to investigate structure and interactions of single liposomes, e.g. during fusion experiments or during phase transitions.

1163-Pos Analysis of Membrane Tether Lifetime: a Magnetic Tweezers Study

Marius C. Staiculescu, Mingzhai Sun, Gabor Forgacs

University of Missouri Columbia, Columbia, MO, USA.

Board B138

Membrane tethers are nanotubular structures extracted from lipid bilayers through the use of a force transducer. Due to their role in cell-cell adhesion, migration and signaling they have been the subject of intense studies employing various techniques, such as micropipette aspiration, atomic force microscopy, optical tweezers and magnetic tweezers. Our study employed a magnetic tweezers to generate constant force and super-paramagnetic beads as the force transducer to investigate the viscoelastic properties and the lifetime of membrane tethers extracted from HB cells (a malignant tumor cell line). The beads were attached to the membrane tethers through non-specific bonds. They were exposed to a magnetic field and their displacement was monitored through video microscopy and plotted as a function of time. The analysis of the displacement vs. time revealed multiple jumps in the bead velocity. The jumps were interpreted as ruptures of individual tethers or the simultaneous detachment of multiple tethers. The number of tethers rupturing at each jump in bead velocity and the total number of tethers initially extracted was calculated by fitting the bead's trajectory with a viscoelastic model. The time interval between two distinct subsequent rupture events was considered as the lifetime corresponding to a collection of N tethers. This lifetime is determined by the nature of the chemical bonds formed between the tethers and the bead. From the experimental data we determined the average lifetime as function of N. By fitting the result with a Bell model a characteristic dissociation rate and reactive compliance under a specific force and bead surface could be calculated. Our method provides a way to extract molecular parameters from biophysical data collected at the cell level.

Supported by NSF-056854

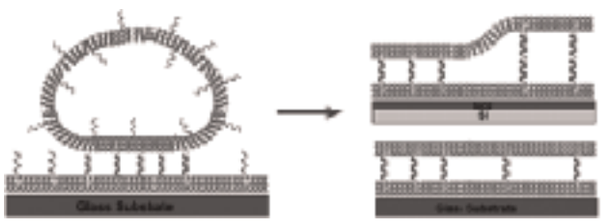
1164-Pos Dna-tethered Lipid Membranes Formed By Giant Vesicle Rupture

Minsub Chung, Mark Yee-Hung Chan, Steven G. Boxer

Stanford University, Stanford, CA, USA.

Board B139

Various approaches have been used to separate supported lipid membranes from the influence of the solid substrate by using polymer cushions or covalently bonded spacers. Following upon applications of DNA-tethered vesicles*, we have developed a DNA-tethered lipid membrane system which employs DNA as spacers between a supporting membrane and tethered "2nd-story" membrane. Giant vesicles presenting DNA attach to the supported lipid membrane which presents the complementary strand via hybridization. Tethered giant vesicles are observed to flatten, then rupture to form a tethered membrane patch. Changing the length of the DNA tethers allows control over the spacing between the two membranes, which can be analyzed by fluorescence interference contrast microscopy. The tethered membrane patches tend to be unstable due to lipid mixing with the underlying supporting membrane, typically within 30–60 minutes. Pre-treating the supporting membranes with water-soluble Texas Red labeled lipids back-fills the supported membranes and relieves the instability. Measurements on these stabilized patches, patches positioned by micromanipulation on patterned surfaces, and mixtures of different DNA tether lengths will be reported. This architecture is ideally suited for experiments on integral membrane proteins.



1165-Pos Characterizaton of the Formation of Giant Liposomes from Hydrogel Films

Kim Horger, Michael Mayer

University of Michigan, Ann Arbor, MI, USA.

*Yoshina-Ishii *et al., JACS*, ***125,*** 3696 (2003); ***127***, 1356 (2005)

Board B140

Giant liposomes are cell-sized spheroidal shells of lipid bilayers that are useful models for studying the properties of cell membranes, such as membrane fusion or lipid raft formation. Other studies of biological membranes involve integral or peripheral membrane proteins. The function of these proteins can be affected by the ionic strength and pH of the surrounding solution. Therefore, preparation of giant liposomes in physiological solutions is desirable, but the yield of giant liposomes using traditional methods, such as gentle hydration and electroformation, is often low in ionic solutions.

We previously introduced a technique of obtaining giant liposomes in physiological solutions by hydrating lipid films from films of agarose. Here we present the results from forming giant liposomes in various conditions including various lipid compositions, concentrations and types of salt contents in the hydrating solution, pH of the hydrating solution, and types of hydrogels that we used to support the lipid films. We assessed the effect of an electric field during formation, the penetration of lipids into the hydrogel film prior to hydration of the lipid film, the effect of agarose on the diffusivity of the membranes, and the lamellarity of the liposomes. We found that forming giant liposomes on films of hydrogels greatly increased the range of conditions under which giant liposomes form spontaneously. The flexibility of this technique may prove to be useful for creating membranes that incorporate membrane proteins.

1166-Pos High Resolution Structure of Solid-Supported, Single Bilayer Bio-Membranes

Chad E. Miller[1], Jarek Majewski[1], Erik B. Watkins[1], Tonya L. Kuhl[2]

[1] *Los Alamos National Laboratory, Los Alamos, NM, USA*
[2] *University of California, Davis, Davis, CA, USA.*

Board B141

We report the first grazing incidence x-ray diffraction (GIXD) measurements of single phospholipid membranes, composed of two lipid monolayers, at the solid-liquid interface. These measurements are complementary to neutron reflectivity studies where deuteration was used to address particular details of the bilayer. Previously, GIXD of lipid membranes was confined to the study of amphiphilic monolayers at the liquid-air interface and multilayer membrane stacks in humid environments. Our results reveal that lipid composition has a significant effect on whether a membrane has domains that are coupled between lipid leaflets. Other structural details of single bilayers such as intermolecular spacing of lipid molecules, membrane thickness, and comparisons to related monolayer studies are reported. We believe that the method presented in this paper can be easily implemented to study more complicated and biologically relevant systems and can become a standard method in probing bio-membrane structure and its interaction with proteins.

1167-Pos

Board B142

WITHDRAWN

1168-Pos Interaction of an Ionic Liquid with a Supported Phospholipid Bilayer is Lipid Dependent

Kervin O. Evans

NCAUR-ARS, USDA, Peoria, IL, USA.

Board B143

Liquid salts, ionic liquids as they are commonly called, have found use as solvents in which to conduct transformation of vegetable oils into new products. These reactions are often catalyzed via immobilized enzymes. However, some enzymes were found to loose activity and are in need of some protection. Phospholipid bilayers may serve as this protective agent. Unfortunately, there is little to no literature that explores interaction of ionic liquids with phospholipid bilayers. The presented work forces on characterizing how ionic liquids interact with supported phospholipid bilayers using atomic force microscopy. Data show that the interaction between a liquid salt and a supported phospholipid bilayer depends on the lipid composition of the supported phospholipid bilayer. When phosphatidylcholine (PC) is the only lipid present, the bilayer became "roughened". When there were negative charged lipids (phosphatidic acid - PA or phosphatidylglycerol - PG) present, round protrusions formed with frequency of protrusions found as PA>PG. However, when the lipid phosphatidylethanolamine (PE) was present, distinct patches formed. In all cases, the bilayer surrounding the protrusions or patches was unperturbed by the ionic liquids.

1169-Pos Reorganization of Membrane by Activated IgE Receptors Observed with SEM

Ethan N. Chiang, Dwaipayan Sil, Christopher Ober, Barbara Baird

Cornell University, Ithaca, NY, USA.

Board B144

Antigen-mediated crosslinking of immunoglobulin E (IgE) bound to its high affinity receptor FcεRI on mast cell initiates a transmembrane signaling cascade that results in cell activation and release of chemical mediators involved in allergic response. As part of this transmembrane signaling process, lipids and proteins undergo orchestrated rearrangements and redistributions including microdomain formation. To understand the functional role of these microdomains the ability to resolve their size, composition and structure is required. While fluorescence microscopy has been widely employed to study the spatial and temporal organization of fluorescently labeled biomolecules in cellular systems, its resolution is still ultimately diffraction limited. High resolution scanning electron microscopy (SEM) allows us to overcome this resolution barrier and directly visualize sub-micron membrane domains. By utilizing a combination of secondary and backscattered electron detection, both membrane topography and the distribution of gold labeled proteins and lipids can be analyzed on the surface of intact cells. Using a variation of Ripley's K-Function as well as autocorrelation analysis, we have observed a change in the nano-scale

membrane redistribution of IgE before and after stimulation with multivalent ligands. To address the question of how antigen structure influences membrane compartmentalization following receptor engagement, we employed rigid, y-shaped DNA ligands functionalized with specific groups to control the distance between antigen binding sites and thereby IgE-bound receptors complexes. We observe a dependence on ligand structure of the nano-scale organization of membrane domains. With SEM, we are mapping out the distribution of selected membrane components and thereby examining the spatial organization of the membrane involved in IgE mediated cell signaling.

1170-Pos Effect of Annexin A5 Binding on Lipid Diffusion: A Fluorescence Correlation Spectroscopy Study

Kanika Vats[1], Kristofer Knutson[2], Anil Bhatta[3], Anne Hinderliter[2], Erin D. Sheets[1]

[1] *Penn State University, State college, PA, USA*
[2] *University of Minnesota, Duluth, MN, USA*
[3] *Minnesota State University, Moorhead, MN, USA.*

Board B145

Annexins are a extrinsic membrane protein family that characteristically binds to negatively charged phospholipids in a Ca^{2+}-dependent manner. To understand the mechanisms controlling annexin A5 (Anxa5) interactions with the cell surface, we use supported lipid bilayers as a cell membrane model. In this study, we measure the lateral diffusion of both annexin A5 (Anxa5) and the underlying lipids in the presence and absence of bound Anxa5 using fluorescence correlation spectroscopy (FCS). Fluorescently labeled Anxa5 is incubated with planar bilayers composed of POPC ± 40 mol% POPS in the presence of Ca^{2+}, resulting in protein clustering on the bilayer surface. The lateral diffusion of the bound protein was measured using FCS and the data was fit to two-component simple Brownian diffusion. To assess the effect of Anxa5 binding on lipid bilayer diffusion with FCS, we use both head-group (TexasRed-DHPE) and acyl-chain labeled fluorescent lipid analogs (NBD-PS and NBD-PC). We find that in the presence of bound Anxa5, TexasRed-DHPE exhibits two-component simple Brownian diffusion, in contrast to the one-component diffusion exhibited by the bilayer in the absence of Anxa5. When NBD-PC is used as the fluorescent lipid analog, Anxa5 binding reduces the diffusion by an order of magnitude, as compared to the protein-free bilayer. Similarly, when Anxa5 is present NBD-PS undergoes two-component diffusion, in which both components are slower than in the absence of Anxa5. Upon comparing Anxa5 with NBD-PS diffusion in presence of Ca^{2+} and Anxa5, rate of diffusion of the slower of the two component (i.e. bound fraction) seems comparable ($D \sim 10^{-10} cm^2/s$). These results suggest that the structure of the membrane is affected by the binding of an extrinsic protein to its surface, which has been suggested by theoretical studies.

1171-Pos Entering The Solid-order Phase By Lowering Temperature Makes *Staphylococcus aureus* Membranes Resistant To PLA_2-IIA

Hector J. Ocampo, Nicolas Afanador, Martha Vives, Juan C. Moreno, Chad Leidy

Universidad de los Andes, Bogota, Colombia.

Board B146

Secretory human phospholipase A_2 type IIA (s*PLA_2-IIA*) catalyzes the hydrolysis of the sn-2 ester bond in glycerolipids to produce fatty acids and lysolipids. One of its primary functions appears to be the targeting of bacterial membranes during bacterial infection, in particular, due its preference for anionic lipid membranes, and its high levels of expression during the inflammatory response. In previous work we have shown that sPLA_2-IIA has significantly higher activity in the liquid-disordered (*ld*) phase, compared to the solid-ordered (so) phase in model membranes composed of DMPG. Since bacterial membranes present *ld*/*so* phase transitions close to physiological temperature, we explore whether bacterial membranes also become resistant to PLA_2-IIA hydrolysis below their melting temperatures. We determine by FTIR the *ld*/*so* phase transition of *Staphylococcus aureus* (*S. aureus*) to be centered at 15.3°C. We find that the activity of the enzyme drops sharply below this temperature. We also study restructuring of the bacterial membrane during hydrolysis. Above the melting temperature the FTIR thermograms show the emergence of a higher temperature cooperative event after PLA_2-IIA activity, which is likely to result from accumulation of fatty acids due to the hydrolysis process. We also observe a general rigidification of the bacterial membranes when exposed to the enzyme at temperature above the melting temperature. The activity of the enzyme is also evaluated in terms of its effects on the bacterial viability. In general we find that bacteria are protected from PLA_2-IIA hydrolysis by entering into the solid-ordered phase regime.

1172-Pos Angiotensin II Interaction with Model Membranes

Julia Preu[1,2], Thomas Gutberlet[1], Thomas Heimburg[2]

[1] *Paul Scherrer Institute, Villigen, Switzerland*
[2] *University Copenhagen, Copenhagen, Denmark.*

Board B147

The renin-angiotensin system plays a critical role in circulatory homeostasis. Part of this system is the peptide hormon Angiotensin II (Ang II), a potent vasoconstrictor that aids in blood pressure regulation, as well as in body fluid balance maintenance. On the heart, acting in both endocrine and paracrine fashions Ang II regulates contractility, remodelling, growth, apoptosis, and reduces cell coupling and conduction velocity in cardiac muscles. Apart from the interaction with protein receptors Ang II interacts with the cellular membrane. Ang II also adopts a well-defined structure in a phospholipid environment, as studied by Carpenter et al. using NMR [1].

Our interest focuses on the concentration-dependent interaction of Ang II on different lipid dispersions, in particular charged phospholipids DMPG, and the structural changes induced by the insertion at different temperatures and ionic strengths. By means of small angle neutron and X-ray scattering, calorimetry and light scattering we provide an inside in the interaction and induced structural changes of Ang II with model membranes compared to studies of pure DMPG model membranes.

References

[1]. Carpenter, K. A. et al., Eur. J. Biochem. *251* (1998) 448–453

1173-Pos Structure and thermotropic behavior of the Staphylococcus aureus lipid lysyl-dipalmitoylphosphatidylglycerol

Sabine Danner, Georg Pabst, Karl Lohner, Andrea Hickel

Austrian Academy of Sciences, Graz, Austria.

Board B148

Staphylococcus aureus is a Gram-positive bacterium that colonizes the skin of about 30% of healthy people. Usually, *S. aureus* is considered a harmless colonizer, but it is, on the other hand, also able to cause a wide spectrum of clinical manifestations. Treatment of *S. aureus* infections becomes increasingly difficult because of its resistance against methicillin and all other beta-lactam antibiotics (methicillin-resistant *Staphylococcus aureus*, MRSA). Antimicrobial peptides are natural candidates in the search for new effective antimicrobial agents to fight *S. aureus*. However, *S. aureus* is capable to change the charge distribution of the outer leaflet of its cytoplasmic membrane by increasing the content of positively charged lysyl-phosphatidylglyerol, leading to a repulsion of cationic antimicrobial peptides. This prompted us to characterize the structural and thermotropic properties of lysyl-dipalmitoylphosphatidylglycerol (lysyl-DPPG) applying differential scanning calorimetry and small- and wide-angle x-ray scattering. Microcalorimetry revealed that the main transition of lysyl-DPPG is pH independent. However, the thermotropic behavior is affected by the presence of degradation products such as lysine, free fatty acids and DPPG, whose formation is in turn dependent on lipid storage time, hydration and pH. X-ray experiments yielded first information on chain packing and morphology of lysyl-DPPG. We found that lysyl-DPPG forms unilamellar vesicles and that the hydrocarbon chains are interdigitated below the chain melting transition. This can be explained by the large headgroup area of lysyl-DPPG due its charged lysine group especially, if the headgroup is arranged parallel to the bilayer plane.

1174-Pos Characterization of Lipopolysaccharides From the Predatory Bacterium Bdellovibrio bacteriovorus and its E. coli Prey

Catherine B. Volle[1], Stephanie Azan[1], Eileen Spain[2], Megan E. Nunez[1]

[1] *Mount Holyoke College, South Hadley, MA, USA*
[2] *Occidental College, Los Angeles, CA, USA.*

Board B149

Bdellovibrio bacteriovorus is a small, Gram-negative bacterium that preys upon other Gram-negative bacteria. Predation involves a specific adhesion process between the bdellovibrio cell and the outer membrane of the prey. It is unclear what is being recognized, but it is likely that lipopolysaccharides (LPS) are involved. Our goal is to carefully examine the similarities and differences between prey cells and predator cells using chemical and physical techniques. By comparing the LPS from *E. coli* prey strains to the predator LPS we hope to identify the salient feature that identifies prey cells as edible. As a model for the outer membranes, we characterize monolayers from prey and predator LPS on the surface of a Langmuir-Blodgett trough. We then transfer the monolayers to a solid substrate, and examine them by Atomic Force Microscopy (AFM). The physical properties of the prey and predator membranes can thus be compared in terms of their domain size and shape. We also recently discovered a probable genetic basis for a chemical difference in the predator's LPS composition and we are using mass spectrometry to confirm this divergence.

1175-Pos Coping With Stress - Small Amounts Of Type II Lipids Can Affect Biological Processes

Annela Seddon[1], Christina Turner[2], Jessica Knott[2], Emma Clay[2], Piers Gaffney[2], Oscar Ces[2], Paula Booth[3], Richard Templer[2]

[1] *University of Chicago, Chicago, IL, USA*
[2] *Imperial College, London, United Kingdom*
[3] *University of Bristol, Bristol, United Kingdom.*

Board B150

Work during the last two decades has shown that stored curvature elastic stress (SCES) within lipid membranes affects the proteins which reside within the membrane. The effects of SCES on the activity, binding or insertion and folding steps of the protein can be elucidated by the judicious choice of lipids. This stress can be controlled by the addition of lipids which have a requirement to curve towards (Type I), or away from (Type II), the membrane - water interface; however, as they are constrained into a flat bilayer, stress occurs within the membrane as the lipids fight against their natural tendency for curvature. The effects of SCES have been demonstrated in systems ranging from the activation energy of insertion of a single transmembrane helix, to the activity of the complex G protein coupled receptor, Rhodopsin. Increased curva-

ture stress has also been shown to increase the conductance of the voltage-gated ion channel Alamethicin and plays a role in the conformational stability and activity of Gramicidin A. Manipulation of SCES also affects the folding of Bacteriorhodopsin. Whilst studying SCES in biological systems, we have found that at very small amounts of DOPE in DOPC (typically <5 mole %), unexpectedly large increases in protein folding rate and membrane fusion occur that might be expected at a much higher mole fraction. Examining these lipid mixtures by small angle x-ray scattering (SAXS) and ^{31}P NMR shows phase behaviour which deviates from expected. These results suggest that small patches of high stress which may occur in the membrane, allowing the global membrane stress to be lowered. These patches appear to promote protein folding and membrane fusion and may give insight into the mechanism by which stress controls key biological functions in membranes.

1176-Pos Effects of Short-Chain Alcohol Isomers on the Phase Behavior of Phospholipid Bilayers

Adam K. Schawel[1], Megan D. Reeves[1], Chih-Yin Cheng[2], Phoebe K. Dea[1], Weidong Wang[1]

[1] *Occidental College, Los Angeles, CA, USA*
[2] *U.C. Irvine, Irvine, CA, USA.*

Board B151

Differential scanning calorimetry and ^{31}P-NMR were used to study the effects of butanol isomers on the thermotropic phase behavior of dipalmitoylphosphatidylcholine (DPPC) bilayers. The threshold concentration for the onset of interdigitation for each isomer was determined by the disappearance of the pretransition and the onset of a large hysteresis between the heating and cooling scans of the gel-to-liquid main transition. Higher threshold concentrations and greater biphasic behavior were found to be correlated with increased solubility of the isomers in the aqueous phase. Systems were studied up to solution concentrations approaching solubility limits and, in the case of *tert*-butanol, through concentrations >5.0 M. As the solution concentration of *tert*-butanol increased, there was an abrupt shrinking of the hysteresis, initially with well-resolved shoulder peaks indicating mixed phases. The eventual disappearance of the shoulder peaks was correlated with a breakdown of the multilamellar structure identified using ^{31}P-NMR. Additional calorimetry studies characterized the phase behavior of several pentanol/DPPC systems. The threshold concentrations and biphasic behavior were correlated to solubility as in the butanol systems although the threshold concentrations were lower in systems with pentanol.

1177-Pos Is α-Tocopherol the "Glue" that Holds PUFA Non-Raft Domains Together?

William Stillwell[1], Daniel S. LoCascio[1], Sevgi Turker Gorgulu[2], Stephen R. Wassall[1]

[1] *IUPUI, Indianapolis, IN, USA*
[2] *Middle Eastern Technical University, Ankara, Turkey.*

Board B152

Recently membrane structural studies have focused on "lipid rafts". These liquid ordered microdomains, enriched in saturated sphingolipids and cholesterol, house characteristic cell signaling proteins. By definition, rafts float in a sea of liquid disordered lipids that must include ("non-raft") domains that to date have received very little attention. Highly disordered domains rich in polyunsaturated fatty acid (PUFA)-containing phospholipids, in particular, are the antithesis of rafts. The experiments reported here represent a first attempt to define a stabilizing mechanism for these "non-raft" domains. Cold temperature extraction in the detergent Triton X-100, differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC) are used to compare the relative affinities of cholesterol and the anti-oxidant α-tocopherol for "raft" and "non-raft" lipids in model membranes. We show that cholesterol associates primarily with the "raft" lipid sphingomyelin (SM) while α-tocopherol prefers "non-raft" docosahexaenoic acid (DHA)-containing phospholipids. From these studies we propose that α-tocopherol may be the molecular "glue" that stabilizes PUFA-rich "non-raft" domains together in a manner similar to that assigned to cholesterol in holding "rafts" together.

1178-Pos Infrared Spectroscopic Characterization Of Phosphoinositide Monolayers At The Air/water Interface

Yasmin Blaih, Arne Gericke

Kent State University, Kent, OH, USA.

Board B153

Phosphoinositides have been shown to mediate a large variety of important physiological processes by affecting the activity and the localization of membrane associated proteins. Phosphoinositide properties are largely determined by the characteristics of their headgroup, which at physiological pH is highly charged but also capable of forming hydrogen bonds. The spatiotemporal control of phosphoinositide mediated signaling events requires the local enrichment of phosphoinositides, which depend on the interchange between attractive and repulsive forces. Factors expected to affect mutual phosphoinositide interaction are pH, cations, or positively charged proteins. We have characterized the structural properties of dipalmitoyl phosphatidylinositol mono-, bis- and trisphosphate monolayer films at the air/water interface by infrared reflection-absorption spectroscopy (IRRAS) in the presence of low and high salt concentrations and monovalent (Li^+ and Na^+) and bivalent (Ca^{2+}) cations. While on pure water subphases the Φ/A isotherms for all phosphoinositide derivatives were characteristic for a condensed monolayer, a monolayer expansion was found for medium (10 mM) and high salt concentrations (150 mM), which was associated with a larger molecular tilt angle and reduced acyl chain order. Alternately, the interaction with Ca^{2+} or Mg^{2+} resulted in largely untilted phosphoinositide molecules. The analyses of the infrared bands associated with the phosphomonoester groups as well as the observed molecular tilt angle changes suggest that the hydrogen bond pattern and the headgroup orientation is cation dependent. Furthermore, the results highlight that attractive forces

due to intermolecular hydrogen bond formation balance the repulsive electrostatic forces due to the negative charge of the phosphoinositide headgroup.

1179-Pos Molecular Structure of Amyloid-like Fibers Induced by Membranes

Ana Coutinho[1,2], Luís Loura[3,4], Manuel Prieto[1]

[1] *Centro Química-Física Molecular , IST, Lisbon, Portugal*
[2] *Dep. Quím. Bioq., UL, Lisboa, Portugal*
[3] *Fac. Farmácia, UC, Coimbra, Portugal*
[4] *Centro Química, UE, Évora, Portugal.*

Board B154

The formation of millimeter-length fibers by proteins, upon interaction with negatively charged membranes has been reported (e.g., [1]), and these fibers are considered to display amyloid-like behaviour, such as characteristic dye staining and lipid-induced β-sheet formation.

As a model system for this type of molecular aggregate, we studied the interaction of lysozyme with large unilamellar vesicles (LUV) of POPC:POPS (80:20). The formation of large aggregates was detected from light scattering data, and at saturation each lysozyme binds $\sim$ 14 negatively charged PS molecules; since the mean charge of lysozyme is +8, steric restrictions, and not strict neutralization of charge, seem to be controlling maximal adsorption of lysozyme to the vesicles.

Lipids derivatized with suitable probes (BODIPY-PC, donor, and Rhodamine-PE, acceptor) were used in Förster resonance energy transfer (FRET) experiments. In addition, the protein interaction with the vesicles, was also studied from FRET data of two protein-donor/membrane probe-acceptor pairs (wild-type protein Trp, donor/DPH, acceptor and Alexa 488, donor/Rhodamine-PE, acceptor) to the lipid probe incorporated in the membrane. From the fitting of models to time-resolved data assuming different bilayer geometries [2], it is concluded that lysozyme induces vesicle aggregation in a "pinched lamellar" structure, with reduced interbilayer distance in the regions where there is bound protein, and increased interbilayer distance (stabilized by hydration repulsion) outside these areas.

The data obtained will be discussed on the framework of reported evidence from microscopy and AFM [1], and allow to postulate a detailed geometry for this type of amyloid-like aggregates.

Acknowledgement

Financial support from FCT (Portugal) is acknowledged.

References

[1]. J.-M. Alakoskela, et al., *Biochemistry,* **2006,** *45,* 13447–13453
[2]. L. M. S. Loura, et al., *J. Phys. Chem.* **2006,** *110*, 8130–8141

1180-Pos The Transitions Between Lamellar and Bicontinuous Inverted Cubic Phases Are Quasi Second-Order Phase Transitions

David P. Siegel

Givaudan Inc., Cincinnati, OH, USA.

Board B155

A model for the curvature free energy of bicontinuous inverted cubic (Q_{II}) phases with respect to the lamellar (L_α) phase in excess water ($\mu_Q{}^c$) was developed [1]. It accurately describes the observed temperature dependence of the Q_{II} phase unit cell constant, c_{eq}, in a one-component phospholipid system [2]. Here it is shown that if $\mu_Q{}^c$ is the only component of the free energy difference between these phases, then the transitions between them in excess water are second-order phase transitions: there is no discontinuous change in molar entropy at the transition temperature T_K. When the L_α phase is heated to T_K, theoretically, the c_{eq} of nascent Q_{II} phase is initially infinite, but decreases rapidly with increasing temperature above T_K [1]. In reality, other components of the free energy difference between the phases, like the difference in interbilayer interactions within them, delay the appearance of Q_{II} phase until a higher temperature, $T_{Q,}$ where c_{eq} is finite [3]. In general, the other components produce a small difference in the molar entropies at T_Q: the transition is no longer strictly second-order. The behavior of c_{eq} at temperatures near T_Q partially explains the relatively slow kinetics of L_α/Q_{II} transitions [2,3] and the facile supercooling of Q_{II} phases to temperatures $< T_K$ [3,4]. The unit cell constant of supercooled Q_{II} phases ($T < T_K$), c_{sup}, is strongly affected by the temperature history of the sample. The method for measuring the Gaussian curvature elastic modulus via measurements of $c_{eq}(T)$ [1] only works at temperatures $> T_K$, and the measurements of $c_{eq}(T)$ must always be done in the heating direction.

References

[1]. Biophys. J.*91*:608 [2006].
[2]. J. Membr. Biol. *195*:165 [2003].
[3]. Siegel & Tenchov, Biophys. J. (in press).
[4]. Biophys. J. *75*:853 [1998].

1181-Pos High Speed Imaging Of Purple Membrane Reveals A Differential Leaflet-by-leaflet Melting Mechanism

Sonia Antoranz Contera[1], Kislon Voitchovsky[1], Joanna Sobek[1], Hayato Yamashita[2], Takayuki Uchihashi[2], Toshio Ando[2], J.F. Ryan[1]

[1] *University of Oxford, Oxford, United Kingdom*
[2] *Kanazawa University, Kanazawa, Japan.*

Board B156

Purple membrane is composed of bacteriorhodopsin (bR) and lipids (1:10), assembled in a 2-dimensional hexagonal lattice. bR is a proton pump and a model system for membrane protein function.

PM is one of the best models for elucidating the interactions that are responsible for the assembly and stability of integral membrane protein complexes. During the melting of PM two very fundamental problems can be investigated: The role of leaflet specific bR-lipid and bR-bR interactions; and a long-standing question, the physics of two-dimensional melting.

Using high-speed atomic force microscopy (HS-AFM) we have imaged the dynamics of the lattice melting at T~60C (<1nm resolution, ~40 frames/s) identifying 2 regimes:

– -Edge melting: bR trimers diffuse in and out of edges and the crystal disassembles into trimeric units. At interfaces between islands of different lattice orientation the crystal melts from the edges and the trimers remain stable.
– -Bulk melting: the melting starts by nucleation of holes in the cytoplasmic leaflet corresponding to phospholipids leaving the intertrimer space between 2 individual monomers.(a 3-7 nm lipid vesicles are observed next to defects).

The lipid defect provokes the collapse of the adjacent cytoplasmic part of bR. This acts as a nucleation site for more lipid holes and collapsed monomers to appear along the hexagonal lattice. Eventually the cytoplasmic leaflet appears as a disordered 2-D fluid of half-collapsed bR proteins. Finally the lattice disassembles in the extracellular side and the crystal melts.

Concomitantly the membrane elasticity modulus is measured using AFM indentation and compared with models of 2-D melting (KTHNY theory). These results complement our previous experiments which showed that the cytoplasmic leaflet bR-mobile phospolipid interactions are electrostatic while the stiffer extracellular leaflet is stabilised by specific steric bR- static glycolipid interactions.

1182-Pos Understanding The Process Of Membrane Photolithography

Hillary Smith[1], Jaroslaw Majewski[1], Atul Parikh[2], Alan Szmodis[3]

[1] *Manuel Lujan Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, NM, USA*
[2] *Department of Applied Science, University of California, Davis, CA, USA*
[3] *Biophysics Graduate Group, University of California, Davis, CA, USA.*

Board B157

Deep-ultraviolet radiation is useful in providing highly localized photodecomposition of phospholipids. Illumination of a phospholipid bilayer with deep-UV light through a simple mask results in the patterning of fluid phospholipids bilayers on unpatterned substrates, including non-planar substrates. The mechanism by which photodecomposition of the membrane occurs is poorly understood. This process was investigated through a study of decomposition as a function of exposure time for two types of lipids, using neutron reflectometry. Neutron reflectometry is a surface sensitive technique that can probe the chemical density of thin layers deposited at buried, solid-liquid interfaces with near-molecular resolution.

1183-Pos Adhesion Of Lipid Vesicles On Patterned Substrates

Sovan Lal Das, Jian Zhang, Qiang Du

Pennsylvania State University, University Park, PA, USA.

Board B158

Adhesion is a key mechanism for survival of many cells and organisms. It is also known that stem cells differentiate into various cell types including neurons, myoblasts, and osteoblasts depending on the stiffness of the substrate that they adhere to. The cytoskeletal network within the cell provides the key mechanism for the cell membrane adhesion. However, studies of adhesion and cell mechanics using techniques such as atomic force microscopy showed that the mechanical properties of the cell membrane also play an important role. To elucidate the influence of the mechanical properties of membrane on adhesion,we study the adhesion of lipid bilayer vesicles. Lipid bilayer vesicles are the closed membranes that form spontaneously in aqueous environment under suitable conditions and represent a simplest model for the cell membrane without the complexity of membrane bound proteins.

We investigate the adhesion of vesicles, under the influence of a constant contact potential, to substrates with various geometry. In the axisymmetric configurations, we find that the transitions from a free vesicle to a bound state depend significantly on the substrate shape. In general, the values of the contact potential at which these transitions take place are lower for a concave-shaped substrate than that for a flat shaped substrate investigated in earlier studies. Furthermore, we observe that the transitions happen at higher values of the contact potential when the substrate is convex. These values of the contact potential at the transition also depend on the curvature of the substrate. We extend our study to discuss an adhesion potential that varies with distance. This potential incorporates the influence of the distance between the membrane surface and the substrate to their interaction.

Subsequently, we focus on the adhesion to a substrate with nonaxisymmetric patterns using a phase field model.

1184-Pos Domain Formation and Lateral Lipid Diffusion in Nanoporous Substrate Supported Lipid Bilayers

EMEL I. GOKSU[1], BARBARA A. NELLIS[1,2], WAN-CHEN LIN[1,3], SUBHASH H. RISBUD[1], MARJORIE L. LONGO[1]

[1] *Chemical Engineering and Materials Science, University of California, Davis, DAVIS, CA, USA*
[2] *Chemistry, Materials, and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA*
[3] *Department of Chemistry, University of California, Berkeley, Berkeley, CA, USA.*

Board B159

Supported planar lipid bilayers (SPB) are excellent biomimetic analogues for cell membranes and can be well-characterized by surface sensitive techniques such as Atomic Force Microscopy (AFM). SPBs assembled on glass, mica, or other solid supports

suffer from two significant difficulties: integral membrane proteins inserted in the lipid bilayer encounter non-physiological interactions with the support and the substrate-proximal leaflet is not accessible to modifications. Nanoporous substrates provide a good method of overcoming these problems. In this study, nanoporous xerogel structures were used to support two-phase lipid bilayers made of 1,2-Distearoyl-Glycero-3-Phosphocholine (DSPC) and 1,2-Dioleoyl-Glycero-3-Phosphocholine (DOPC) in 1:2 mole ratio. Domain formation on xerogel surfaces was achieved. The surfaces of the xerogels and lipid bilayers supported by xerogels were characterized in terms of roughness by using AFM. The domain sizes were measured by using the epifluorescence microscopy images. The lateral mobility of the lipids was confirmed by Fluorescence Recovery After Photobleaching (FRAP) method and the diffusion coefficients were calculated by using Fluorescence Correlation Spectroscopy (FCS). The results were compared to mica supported lipid bilayers. We observed that the lipid bilayer followed the surface topography of the xerogel under contact mode imaging conditions. Both the average size of the domains and the standard deviation on xerogel surfaces were found to be larger compared to mica surfaces. Future work will explore several materials chemistries to make xerogel supports for lipid layer assembly.

1185-Pos Photo-induced phases in the Lipid Bilayer

linda S. Hirst, Jing Yuan

Florida State University, Tallahassee, FL, USA.

Board B160

The self-assembly of biological amphiphiles has proved a fascinating topic in recent years, the hollow cylindrical lipid tubule morphology being of particular interest due to its potential relevance to intercellular transporting channels and applicability to controlled-release systems, chemical micro-reactors and nano-conduits. Co-existence of the liquid-ordered and liquid-disordered phases in the lipid bilayer has recently been observed in biologically-relevant three-component giant unilamellar vesicles. We have generated stable, photo-induced micron-scale phase separation in lipid tubules formed from ternary lipid mixtures, inducing a new bilayer disc structure. This investigation not only aids in our understanding of lipid sorting phenomena in cell membranes (suggesting a mechanism for bilayer disc formation in retinal rod-cells), but is also a fascinating route to the generation of new, functional structures.

Membrane Structure - II

1186-Pos Condensing And Fluidizing Effects Of Ganglioside G_{M1} On Phospholipid Films

Shelli L. Frey, Eva Y. Chi[1], Cristobal Arratia[2], Jaroslaw Majewski[3], Kristian Kjaer[4], Ka Yee C. Lee[1]

[1] *Department of Chemistry, Institute for Biophysical Dynamics, and the James Franck Institute, University of Chicago, Chicago, IL, USA*
[2] *Department of Physics, University of Chile, Santiago, Chile*
[3] *Manuel Lujan Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, NM, USA*
[4] *Max-Planck Institute of Colloids and Interfaces, Potsdam, Germany.*

Board B162

In model membrane mixtures that mimic lipid raft compositions, the ganglioside, G_{M1}, which contains four neutral sugars and a negatively charged sialic acid, is enriched in the more ordered domains. In an effort to understand the organization and partitioning of G_{M1} in cell membranes, we have modeled the outer leaflet of the cell membrane using Langmuir monolayers of 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC) and added varying concentrations of G_{M1}. At low biologically relevant concentrations, G_{M1} has a condensing effect on the DPPC monolayer while at higher concentrations, it acts to fluidize, with a switch-over point between the two behaviors at a ratio of 3:1 DPPC:G_{M1}. To examine phase morphology and organization, the monolayers were transferred onto solid substrates and imaged with atomic force microscopy. At concentrations below the switch-over point, G_{M1} is located in nanoscale clusters within the condensed DPPC domains, as shown by a height difference attributed to the bulky sugar groups. The total surface area of these nanosize domains is larger than that attributable to G_{M1} molecules alone, suggesting the higher regions are due to G_{M1} and DPPC packing preferentially in condensed geometric complexes. At higher concentrations, G_{M1} is also located with DPPC in the more fluid phase. X-ray grazing incidence diffraction and reflectivity measurements characterized the molecular packing and structure of pure and mixed monolayers, showing quantitatively how the presence of G_{M1} both condenses and alters the molecular ordering of the phospholipid film. To pinpoint the structural portion of G_{M1} that gives rise to the condensing effect, parallel experiments have been performed with structurally related gangliosides, ceramide, and a PEGylated lipid series. Our results indicate that the bulky sugar headgroup of G_{M1} is responsible for the significant effects on phase behavior and organization of the surrounding lipid molecules.

1187-Pos Perfluorocarbon Compounds Are Chemically Inert But Able To Induce Unusual Vesicle Aggregation And Alter Membrane Packing

Berenice Venegas, Marla R. Wolfson, Parkson L.-G. Chong

Temple University School of Medicine, Philadelphia, PA, USA.

Board B163

Because of their unique physicochemical characteristics, perfluorocarbons (PFCs) have multiple biomedical applications, especially as oxygen transporting agents. Despite the high clinical interest in the development of PFC applications, there is a lack of a fundamental understanding about the interactions of PFCs with cell membranes. Here, large unilamellar vesicles made of POPC and cholesterol (molar ratio: 7:3) were used in conjunction with spectroscopic