SYMPOSIUM EE
Materials Science of Phospholipid Assemblies

November 29 – December 1, 1999

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Symposium Support
†Avanti Polar Lipids, Inc.
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*Invited paper
SESSION EE1: LIPID MONOLAYERS, BILAYERS AND BIOMEMBRANES INTERACTIONS
Chair: David M. Pickup
Monday Morning, November 29, 1999
Salon A/B [M]

8:30 AM *EE1.1
TRANSITIONS OF PHOSPHOLIPID MONOLAYERS INTO THE THIRD DIMENSION. W.R. Schief, Department of Physics, University of Washington, Seattle, WA; S.B. Hall, Departments of Biochemistry, Biophysics, and Chemistry, Oregon Health Sciences University, Portland, OR; V. Vogel, Department of Bioengineering, University of Washington, Seattle, WA.

For below the monolayer collapse pressure, light scattering microscopy now reveals that complex transitions into the third dimension occur for pure phospholipid monolayers at the air/water interface. Occurrence of these topographic transitions remained unnoticed in the past, since the instabilities are on the nanoscale and involve only a small fraction of the surface material. DPPC monolayers, for example, undergo a budding transition above the surface pressure of 20 mN/m at room temperature. While the bud size for DPPC monolayers remains on the nanoscale, they can outgrow into microscopic discs or tubules in mixtures of phospholipids with cholesterol. An overview will be given summarizing the phenomena observed for various phospholipids as pure monolayers or in mixtures with cholesterol using light scattering microscopy. Brewster angle microscopy, fluorescence microscopy and atomic force microscopy.

9:00 AM *EE1.2
NANOSCALE INSTABILITIES OF A DPPC MONOLAYER IN THE LC PHASE. W.R. Schief, Department of Physics, University of Washington, Seattle, WA; L. Touryan, Department of Bioengineering, University of Washington, Seattle, WA; S.B. Hall, Departments of Biochemistry and Molecular Biology, Medicine, and Physiology and Pharmacology, Oregon Health Sciences University, Portland, OR; V. Vogel, Department of Bioengineering, University of Washington, Seattle, WA.

Light scattering microscopy reveals previously undetected topographical complexity in phospholipid monolayers at the air/water interface. As a surface pressure of 13 mN/m or room temperature following completion of the liquid-expanded (LE) - liquid-condensed (LC) transition, the LC phase of dipalmitoyl phosphatidylcholine (DPPC) develops corrugations within a region that covers half the monolayer and surrounds fine, chiral-shaped domains. The topographical structure of the domains and the surrounding region are revealed by analysis of scattered intensities in light of capillary wave theory. With compression above a pressure of 20 mN/m, the corrugated region becomes decorated with nanotopes through a budding process. Above 60 mN/m, the budding process accelerates. Atomic force microscopy (AFM) on samples transferred to silica confirms the presence of millihitlly discs of diameter 15 - 150 nm. The morphologies of the first domains suggest that these topographical instabilities result from a heterogeneous distribution of packing defects locked into the monolayer during the LE-LC transition.

9:15 AM EE1.3
DIRECT OBSERVATION OF THE INTERACTIONS OF AMYLOID-BETA PEPTIDES WITH LIPID MONOLAYERS. Casey Ege, Ka Yee C. Lee, The University of Chicago, Dept of Chemistry, Chicago, IL.

Interaction of amyloid-beta peptides with lipid membranes is central to the neurotoxic activity of the peptide, and is one of the leading mechanisms of pathogenesis. Using fluorescence microscopy and monolayer techniques, we have studied the interactions between two versions of amyloid-beta peptides (differing in the number of residues, 40 and 42) and lipid bilayers with cholesterol and phosphatidylserine. We found that the peptide interact specifically with some lipid molecules, inserting itself into the monolayer and disrupting the packing of the lipids. The anchoring of the peptide at the bilayer surface may act as seed for further peptide aggregation.

9:30 AM EE1.4
CHEMICAL TRAPPING: A NEW METHOD FOR ESTIMATING AMLIONE BINDING MICELLES. Jolanda M. Cuccia and Herrn Chaimovich, Department of Biochemistry, University of Sao Paulo, Sao Paulo, BRAZIL; Mhendire Kumar Jain, Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; Lucienne S. Romani and Jhiu Yao, Rutgers University, New Brunswick, NJ.

The balance of forces controlling aggregate structure and stability and the distribution of aggregates between aqueous and micellar phases in organized solutions are not well understood, in part because of the complexity of the system. Aggregates can be obtained self-limiting by controlling the ratio of reactive groups (ligands such as bivalent to phospholipids and incorporated in a vesicle membrane) on the colloid surface to crosslinking agents (multifunctional receptors such as milkin or streptakinin) in solution. A distinct transition occurs between limited and complete aggregate as a function of the ligand to receptor ratio. The limited aggregates formed are compact with a fractal dimension of 2.9, while the aggregates of the overall concentration of surface accessible biotin-ligands, which can be

9:45 AM EE1.5
ELECTROTRANSPORT OF ELECTROACTIVE COMPOUNDS ACROSS BILAYER MEMBRANES BY PYRHYLUM AND PYRHYLUM PHOTOREDOX MEDIATORS. Rashed F. Khatunmad, Lisa Lubchens, James K. Hurst, Washington State University, Department of Chemistry, Pullman, WA.

One major objective in our efforts to develop vesicle-based systems for photoreduction of dihydrogen is to develop compounds that can cycle as redox mediators between photosensitizers and phase-separated electron acceptors, thereby effecting long-lived charge separation of photogenerated oxidants and reductants. To possess high intrinsic permeabilities and avoid rate-limiting membrane polarization, these compounds must be electrically neutral in both oxidation states. One system under investigation utilizes hydrophilic pyrhyllum ions (e.g., triphenylpyrhyllum, TPP+) or pyrhyllum analogs (e.g., transmembrane redox mediators across bilayer) to enhance photostability. Photosensitization of a sensitizer molecule in the bilayer aqueous phase containing an electron donor (e.g., diiodobenzol) lead to efficient reduction of an electron acceptor localized within the aqueous core of the vesicle when the mediator was present, but not when it was absent. Transient spectrophotometry revealed that reaction was initated by oxidative quenching of the excited photosensitizer by TPP+, and that the TPP+ radical formed traversed the bilayer with a permeability coefficient of 1.0 x 10^-7 cm s⁻¹. The amount of electron acceptor reduced exceeded the amount of TPP+ present by up to 0.5 mole, implying that on average each TPP+ underwent 40 cycles of electron transfer from electron donors outside the vesicles to electron acceptors within the vesicles. The mechanism proposed involves inward transmembrane diffusion of TPP+, reoxidation within the inner aqueous phase, followed by hydrolysis opening of the pyrhyllum ring to form the neutral 1,5-diketone (TPPD), outward transmembrane diffusion of TPPD to the external aqueous environment, and reformation of the pyrhyllum ring, closing the cycle of TPPD reoxidation.

10:30 AM *EE1.6
SELF LIMITING AGGREGATION BY CONTROLLED LIGAND RECEPTOR STOICHIOMETRY. Joseph A. Zawadzki, Edward Kisiel, Stephen L. Kennedy, Dirk Truemmerhauser, University of California, Dept. of Chemical Engineering, Santa Barbara, CA.

Colloidal aggregation can be made self-limiting by controlling the ratio of reactive groups (ligands such as bivalent to phospholipids and incorporated in a vesicle membrane) on the colloid surface to crosslinking agents (multifunctional receptors such as milkin or streptakinin) in solution. A distinct transition occurs between limited and complete aggregate as a function of the ligand to receptor ratio. The limited aggregates formed are compact with a fractal dimension of 2.9, while the aggregates of the overall concentration of surface accessible biotin-ligands, which can be...
11:00 AM EEI.7
CONSTRUCTION AND PROPERTIES OF PHOSPHOLIPID PROTEIN ASSEMBLIES. Anthony W. Coleman Institut de Biologie et Chimie des Proteines, CNRS CPR 312 Lyon, FRANCE.
Marc-Helene Paul, Institut Curie, Montreal, Canada
Jean-Paul Rueu and Bernard Roux, UCBL Lyon, FRANCE.

The presentation will concern the assembly of two types of proteins in different phospholipid assemblies and (here) microscopy, using both contact and non-contact mode observation. The first section concerns the real time assembly of Phosphatase Alkylamide into Langmuir Blodgett bilayers of DPPS. The protein contains a GPI anchor which may be inserted into the phospholipid bilayer as an anchor. Both the protein containing the anchor and also the protein in which the anchor is absent have been studied. Both Topographical and Lateral Force imaging, under an aqueous subphase show that the assembly process proceeds in different manners, depending on the presence or absence of the GPI anchor. Comparison of these assemblies with those proteins, such as Luciferase, are discussed both in terms of protein structure and anchoring properties. In the second section, assembly of cytochrome b568 into DPPC liposomes has been studied, using the natural and de-glycosylated protein. The effect of glycosylation is to change the size and rigidity of the liposome-protein complex. The study has been extended to include the study of the extended complex of cytochrome b558 in which a number of other proteins assemble. From Topographical measurements, the height of the assembly has been derived and also the implications of the orientation of the protein sub-units on the activation of the assembly have been studied. The interaction of cytochrome b558 with various antibodies has been studied using force-distance measurement techniques.

11:15 AM EEI.8
MEMBRANE PROTEIN CRYSTALLIZATION IN LIPID MIMOPHASES. Hong Qiu, Martin Cafrey, The Ohio State University, Dept. of Chemistry, Columbus, OH; Peter Nallert, Bicecenter, Basel, SWITZERLAND.

The medium chain length alkyl glycosides are high solubility, non-ionic detergents. Because of their mild nature, they have found extensive use in solubilizing membrane proteins for subsequent structure characterization, reconstitution and crystallization studies. Octyl glycoside has been used in such applications and recently was included as a component of a mixed micelle containing system in which 3D cryo axis of bacteriorhodopsin (bR) were grown. A cubic mesophase formed prominently in bR crystal growth but its exact role in the process has not yet been established. Molecular geometry considerations suggest that the compositional molecular shapes of OG and MO should lead to a destabilization of the highly curved cubic phase of hydrated MO in favor of a lamellar type structure in the presence of OG. We have tested this hypothesis by constructing the temperature composition phase diagram of the MO/OG/water system and by characterizing structurally the corresponding mesophases using x-ray diffraction. The data support the hypothesis. The effect of OG on water activity must also be considered in the context of membrane stability. Possible involvement of existing lamellar and cubic phases in protein crystal growth will be discussed. [Supported by The National Institutes of Health (GM59691)]

11:45 AM EEI.9
MEMBRANE ACTIVE CYSTEINE CONTAINING OLIGopeptides. INTERACTIONS WITH PHOSPHOLIPID VESICLES AND CATIONIC PHOSPHOLIPIDS. Stuart Schreiber, Howard Hughes Medical Institute, University of California, Los Angeles, CA.

Membrane active cysteine containing oligopeptides such as phospholipids, are synthesized by a combination of recombinant and radiolabeled synthetic routes. The different reduction potential of intra and extracellular media suggest that disulfide bond formation may be used to control peptide association, and consequently to modify peptide affinities for membrane structure. To explore the effects of reduced formation on peptide behavior, the oligopeptide with sequence NHH, Cys-Lys-Leu-Cys-CONH, was synthesized by solid phase peptide synthesis using the Fmoc strategy. We have shown that the peptide can be reversibly halo-cysteinylated via cysteine (in air) and reduction (with diithiothreitol). To examine the interactions of the peptides with model lipid membranes, small sonicated vesicles were prepared consisting of 30 mol% oleic acid and 70 mol% dilauroyl phosphatidylcholine and a solution of calcin, a self-quenching, membrane impermeant dye, was entrapped into the vesicles. Calcine release, as a result of peptide-membrane interactions, was monitored using steady state fluorimetry for different peptide concentrations. The dimeric peptide is significantly more effective in causing calcine release. To examine the peptide interactions with cellular membranes, permeabilization of cell membranes to calcine was studied. Cholesterol was placed on glass bottom microslides and then treated with buffer containing calcium and oxidized or reduced forms of the peptide. After varying times, cells were washed with buffer to remove the peptide-calcine solution. Cellular internalization was examined using fluorescence microscopy.

SESSION EE2: SUPPORTED MEMBRANES
Chair: David H. Thompson, Monday Afternoon, November 29, 1999
Salon A/B (M)

1:30 PM EE2.1
ELECTRICAL MANIPULATION OF FLUID SUPPORTED BILAYERS. Steven G. Boxer, Alexander van Oudenaarden, Stanford University, Department of Chemistry, Stanford, CA.

Supported lipid bilayers offer an important alternative to vesicles for studying biological membranes, and they have been shown to possess many properties similar to native cell membranes. Supported bilayers can be assembled on a large number of substrates and exhibit lateral fluidity over large distances due to a thin (~10-15 Å) lubricating layer of water trapped between the bilayer and the surface. Supported bilayers can be partitioned and controlled by scratching the membrane on the surface or by assembly on surfaces with patterned barriers created by photolithographic processing [Science, 275, 651 (1997)] or by electron beam lithography. Charged components such as lipids and membranes associated proteins can be manipulated using applied electric fields [Biophys. J. 68, 1972 (1995); 71, 2716 (1996)]. A novel combination of diffusion barriers and electrical manipulation is the fabrication of a geometrical Brownian ratchet (A. van O. and SGB, in press). Charged, fluorescently labeled phospholipids were driven in one direction by an electric field through a two-dimensional periodic array of asymmetric barriers to lateral motion. Diffusion spreads the phospholipid molecules in the orthogonal direction, and the asymmetric barriers rectify the Brownian motion causing a directional transport of molecules. The geometrical ratchet can be used as a continuous molecular sieve to separate mixtures of membrane-associated molecules that differ in electrophoretic mobility and diffusion coefficient.

2:00 PM EE2.2
CREATING PHOSPHOLIPID MEMBRANE BASED SENSORS FROM SPATIALLY ADDRESSED BILAYER ARRAYS ON PATTERNED SOLID SUPPORTS. 6.0. Creager, Texas A&M University, Department of Chemistry, College Station, TX.

The cellular membrane is the most sophisticated surface detection device ever designed. The cell interacts with its environment by presenting a host of carbohydrate, peptide, and protein moieties in a continuously mixing environment on the external leaflet of its plasma membrane. These receptors recognize a wide variety of foreign objects with which the cell comes in contact. These include small molecules, proteins and even other cells. One attractive option for mimicking this process is the use of supported phospholipid bilayers. These biomimetic membranes can be deposited at the solid-liquid interface to form supported planar membranes that maintain the two-dimensional fluidity that is vital to their function. We have designed arrays of chemically distinct phospholipid membranes with unique chemical components at each address. These systems represent the ideal geometry for sensor design, because the fluid membranes remain confined on the support allowing viruses, proteins, and peptides of interest to be passed over them in a standard flow cell geometry.

2:15 PM EE2.3
SYNTHESES AND PHASE BEHAVIOR OF MACROCYCLIC TETRAETHER BISPHOSPHOCHOLINES. Anindita Patwardhan, Jong-Mok Kim, David H. Thompson, Dept of Chemistry, Purdue University, West Lafayette, IN.

An efficient route towards the synthesis of unsaturated (bicyclic) tetraether bisphosphochoelines has been developed using 2-propenyl 5-hydroxy-1,3-dioxole as a common glycol synthon. Ring closure was accomplished using either high dilution Glaser oxidation or [(CuPd)]2+ = CHPdCH3 or chlorin mediated conditions. Aqueous dispersions of these homolipidazines have been studied using
DSC, calcein leakage assay, 31P & 32P NMR, and CD spectroscopy. Our results suggest that membrane vesicles formed from these lipids are impermeable to small ions, surprisingly robust, and capable of incorporating gramicidin in its native β-helix.

2:30 PM EE2.4 CONTROL THE FORMATION OF ORGANIC PATTERNS TOWARDS BIOLOGICAL APPLICATIONS. Bong Wang, Atul N. Parikh, Jaime D. Beers, Andrew P. Shreve, Basil T. Swanson, Los Alamos National Laboratory, Chemical Science & Technology Division, Los Alamos, NM.

Domain patterns were fabricated from pre-polymerized n-octadecyalkylcholine (OTS) monolayers by Langmuir-phase assisted self-organization with GOX/substrate. Pattern structures were examined by atomic force microscopy (AFM). It was found that the types of pattern structures can be controlled by simply varying compression pressure, and hence the initial phase, of the precursor Langmuir film formed from LE phase precursor, whereas rounded-shaped domains formed from LE/LC mixed phase precursor; and uniform solid films were derived from LC phase precursor. Moreover, when fluid phospholipid vesicles were allowed to spread onto the domain patterns, it was observed that the ring-shaped domains arrested the vesicles, forming hybrid bilayers restricted by the domain boundaries due to the high contrast in hydrophobicity between the encircled area (containing relatively high density of OTS molecules) and the outer background. The domain patterns were further applied to arrest and immobilize the GM1 receptors by flowing a mixture of GM1 and vesicles onto the films. Specific binding and non-specific binding of cholera to GM1 will also be discussed.

2:45 PM EE2.5 STRUCTURAL CHARACTERIZATION OF BIOMIMETIC BILAYERS USING INFRARED REFLECTIVITY SPECTROSCOPY. S. Krueger, N.F. Berk, J.A. Dura and C.F. Majkrzak, NIST Center for Neutron Research, NIST, Gaithersburg, MD, C.W. Meuse and A.L. Platt, Biotechnology Division, NIST, Gaithersburg, MD.

Neutron reflectivity measurement techniques are being developed to characterize the structure of novel synthetic anionophores and phospholipid biomimetic systems, or hybrid bilayer membranes (HBM\(\text{S}\)), which are formed on gold-coated single crystal silicon substrates, and which are in contact with aqueous solution. Parameters of interest include thickness of the bilayer and its individual components, hydration of the head groups, depth of penetration of peptides into the bilayer and structural changes in the bilayer due to the presence of peptides. Measurements have been made on HBMs consisting of a monolayer of octadecylthiol and a monolayer of d\(_4\)-DMPC, and on THEO-HBM\(\text{S}\) with a monolayer of thioninehexadecyloctadecane, which contains an ethylenecidoxymo at the gold surface, in place of the octadecylthiol layer. Data were obtained in \(\text{D}_2\text{O}\) solution at 28°C, where the DMPC layer is in the fluid phase, both in the absence and in the presence of the membrane perturbation, melittin, in solution. To determine if melittin penetrates into the alkaneethanol layer, measurements were also made on a THEO-HBM\(\text{S}\) with a deuterated alkaneethanol layer and a non-deuterated DMPC layer in the presence of melittin. Neutron scattering from small, low-density fractions (SLD) produced by lipid, strongly perpendicular to the plane of the bilayer have been obtained from the reflectivity measurements. The reflectivity data and resultant SLD profiles will be discussed in terms of structural models for the HBMs and the location of melittin in the bilayer.

3:30 PM EE2.6 DIRECT MEASUREMENTS OF FORCES BETWEEN GLYCO- LIPIDS. Deborah Lackland, Timothy Calvert, Zhi Wu Yu, University of Illinois, Dept of Chemical Engineering, Urbana, IL.

In this study, we measured directly the molecular forces that mediate the interactions of small membrane containing lipids with neutral and with charged carbohydrate headgroups. These molecules are abundant on cell surfaces, and some investigations have suggested that binding between carbohydrates may mediate weak cell-cell adhesion. To explore this hypothesis, we used a surface force apparatus to measure the forces (in nN) between glycophospholipids and synthetic polymer layers. The strength of the interactions should be proportional to the negative charge density of the polyanion. The magnitude of the interaction will be a function of such factors as the extent of electrostatic screening, changes in the hydration layer, and changes in the hydration layer. The results suggest that these interactions may be important in cell-cell adhesion.

4:00 PM EE2.7 PHOSPHONOMETHIDES AS PROBES FOR PROTEIN KINASES. John W. Thirion, Gein F. Painter, Ze-Yi Lim, Andrew B. Holmes, Melville Laboratory, Department of Chemistry, Cambridge, UNITED KINGDOM; Phillip T. Hawkins, Leonard R. Stephens, Babraham Institute, Babraham, Cambridge, UNITED KINGDOM.

3-Phosphorylated (D)-phosphonyl methyl esters (D-PHMs) constitute an important class of phospholipids that play key roles in signal transduction. These phospholipids are derived from PHMs through the action of distinct phospholipomethyltransferases (e.g., PKB which is an activator of PKB/Akt which is known to mediate several signal transduction pathways). We have prepared and immobilized (D)-methyl phospholipid on a solid support via the fatty acid side chains. The resulting affinity matrices have been used to isolate and characterize various (D)-PHMs binding proteins. This paper will discuss the advances that modern solid state materials can provide in helping to identify the structures of new proteins that regulate PHMs binding.

4:15 PM EE2.8 VISIBLE LIGHT INITIATED POLYMERIZATION OF ACRYLIC FUNCTIONALIZED PHOSPHATIDYLCHOLINE MONOLAYERS. Jamie Orban, Elliot L. Chikof, Laboratory of Bioceramics Research, Department of Surgery, Emory University, Atlanta, GA.

Cytometric biomimetics derived from an understanding of membrane localized cellular processes provide a rational strategy for the development of bioensors, biofunctional surface coatings, and tissue engineered constructs with improved performance characteristics. In prior investigations, we have stabilized self-assembled acrylate functionalized phosphatidylcholine (acylcholesterins) in hydrophobic surfaces by in situ heat induced free radical polymerization. In this report, we will describe an alternative approach based on visible light induced polymerization using Eosin Y and triethanolamine, as photosensitizer and photoinitiator respectively, and 1vinylpyrrolidone, as an acrylate monomer. The effect of wave fusion time, irradiation time, as well as alkylamine substrate type on monolayer formation and stability have been determined using a variety of surface sensitive techniques. These systems exhibit robust stability under a variety of conditions.

4:30 PM EE2.9 ELECTRODES FOR \(\text{H}_2\text{O}_2\) SENSING FORMED BY THIN FILM ELECTRO-POLYMERIZATION OF THE AMPHIPHILIC DECYL ESTER OF D-TYROSINE. Dong-Dac Long, Tian Zhou and Kenneth A. Marx, Center for Intelligent Biomaterials, Department of Chemistry, University of Massachusetts, Lowell, MA.

Amphiphilic decyl ester derivatives of D-Tyrosine (DEDT) self-assemble into long rod like or tubular aggregate structures in aqueous phosphate buffered solution visualized by SEM or light microscopy. These possess n.m.c.m. value of 0.20 m\(\text{m}\) in pH 6.5 buffered solution. Their polymerization has been achieved using a novel approach based on reduction of the borohydride derivative, DEDT, with sodium borohydride in aqueous solution. The resulting polymer is a highly porous material exhibiting very low bulk resistance. In electrochemical studies using three electrode systems, the DEDT polymer exhibits a well defined redox transition at -0.7 V vs. Ag/AgCl in pH 6.5 solution, forming stable films on Pt electrodes. Enzymes such as horseradish peroxidase (HRP) can be adsorbed or physically entrapped within the polymer during electropolymerization. The ability of these thin film covered Pt electrodes to electrochemically detect \(\text{H}_2\text{O}_2\) electrochemically varied directly or in enzymatic intermediates, was examined at two different values. The first is \(-0.85 V\), the potential for direct oxidation of \(\text{H}_2\text{O}_2\). The second is \(-0.05 V\), the potential for reduction of \(\text{H}_2\text{O}_2\). Formation of a DEDT electrode film with stable chronoamperometric response properties for \(\text{H}_2\text{O}_2\) detection is enhanced by cutting out the polymerization below the c.m.c. of DEDT. Formation of a DEDT electrode film at \(\text{D}_2\text{O}\) above the c.m.c., results in an electrode with a low and time dependent \(\text{H}_2\text{O}_2\) sensitivity level. The \(-0.05 V\) potential for \(\text{H}_2\text{O}_2\) detection is in agreement with the results from previous work, which avoids electrochemical interference from compounds such as ascorbate and urate in certain clinical situations. These results demonstrate that stable \(\text{H}_2\text{O}_2\) sensing electrodes may be formed electrochemically from amphiphilic decyl esters of D-Tyrosine. In certain sensing situations, the DEDT film electrode may be advantageous due to selective permeabilities associated with the hydrophobic derivatization. (Support from a TURI Grant from UML is gratefully acknowledged.)

SESSION EE3: PEG-CONTAINING MATERIALS – MOLECULAR AND BIOLOGICAL PROPERTIES

Chair: Marcel B. Bally
Tuesday Morning, November 30, 1999
Sesion A/B (M)
9:00 AM *EE3.1
KINETIC CYTOMEDICINE: CONTROL OF PROTEIN ABSORPTION BY GRAFTED POLYMER LAYERS.
Javier Satulovsky, Igal Selaifer, Department of Chemistry, Purdue University, West Lafayette, IN.

The ability of grafted polymer layers to the surface of a liposome to reject proteins from solution has been studied with a molecular theory. The predictions of the theory are in very good agreement with experimental observations for the ability of short and long PEG chains to reduce the loss of liposomes by surface contamination on hydrophobic surfaces. The calculated isotherms are assuming that the adsorption has reached thermodynamic equilibrium. The extension of the theory to study the kinetic behavior of the adsorption suggest that the equilibrium assumption in comparing with experimental systems is adequate. Further, the kinetic studies strongly suggest that the ability of lipid-PEG to increase longevity of liposomes in the blood stream is due to the kinetic slow down of protein adsorption. The molecular basis of the determination of equilibrium adsorption will be compared to those that dictate the kinetic properties. The relationship between the ability of the polymer layer to prevent protein adsorption and the structure of the grafted chains will be discussed in detail.

9:30 AM EE3.2
COATING OF CYLINDRICAL MEMBRANE STACKS WITH ANCHORED POLYMERS.
Daniel Kindel, Ian Tanfiri, Joel Swanson, Weizmann Inst of Science, Dept of Physics of Complex Systems, Rehovot, ISRAEL.

Experiments show that polymers with hydrophobic anchors grafted along their hydrophobic backbone induce a coiling instability in cylindrical multilamellar stacks of phospholipid membranes. We propose a theoretical model for this phenomenon. According to our interpretation, polymer molecules tend to diffuse to regions of high membrane curvature. This coupling between polymer concentration and the curvature leads to the prediction of a threshold polymer density. Below threshold membrane tubes are straight on the average, but above the threshold concentration the tubes form maximally tight coils. These predictions are consistent with experimental results. Our system is unique in that coils form in the absence of twist.

9:45 AM EE3.3
EFFECTS OF PEG-LIPIDS AND PEG CONTAINING COPOLYMERS ON LIPOSOME STRUCTURE AND STABILITY.
Kajsa Edvardsson, Nils Bergstrand, Markus Johansson, Mats Silvander, Uppsala Univ, Dept of Physical Chemistry, Uppsala, SWEDEN.

Liposomes have during the last few years come into widespread use as vehicles for systemic delivery of various drugs. For many applications, including those requiring sustained release or accumulation in a particular organ or tissue, long circulation times are a prerequisite for effective treatment. Increased circulation times may be achieved by the attachment of long flexible polymer chains, such as polyethylene glycol (PEG), to the surface of the liposomes. The presence of the polymers effectively hinders close approach of destabilising blood components like phospholipases and lipoproteins and, furthermore, restricts the recycling and removal of the liposomes by the host immune system. The most common way of achieving PEG-stabilisation is by conjugation of the polymer to a proportion of the lipids in the liposome membrane. Lately, non-lipid molecules, such as PEG containing copolymers, have begun to be explored in the search for alternative strategies. Inclusion of PEG carrying molecules in the liposomal membrane may, however, induce undesirale alterations in aggregate structure and other biophysical properties. We have carried out systematic studies, based primarily on cryo-transmission electron microscopy and different photophysical techniques, which show that major structural rearrangements may occur at comparably low PEG-lipid, or copolymer, concentrations. Furthermore, changes in the aggregate properties, such as packing order and permeability, may be affected at concentrations well below those where alterations in aggregate structure are detected.

10:30 AM EE3.4
PEG-MEDIATED LIPID BILAYER FUSION: A MECHANISM IN COMMON WITH SECRETORY AND VIRAL FUSION.
Harry H. Linsen, Department of Biochemistry and Biophysics and Program in Molecular Cellular Biophysics, University of North Carolina, Chapel Hill, NC.

The sequence of events involved in polyethylene glycol-mediated fusion of 450 Å small unilamellar vesicles (SUVs) has been studied. Fusion events were monitored using light scattering for vesicle aggregation; fluorescence lifetime of membrane probe lipids (DiTP, PC and NBD-PS) for membrane mixing; aqueous fluorescent marker (TMR, H2DPA and H2TPPS) for core mixing; and quasi-elastic light scattering for the change in size of vesicles Poly(ethylene glycol) is a highly hydrated polymer that can bring vesicle membrane-interfaces to near molecular contact. Manipulations that reduce vesicle packing density in both outer and inner leaflets of the membrane bilayers are necessary to achieve fusion between contacting vesicles. Curvature stress appears to be essential. Once this condition is achieved, the sequence of events involved in vesicle fusion is shown here to be: 1) outer leaflet mixing accompanied by 2) transient pore formation, both occurring on a time-scale of ~10 seconds and leading to an initial, reversible intermediate; followed by 3) a 1-3 minute delay leading to formation of a fusion-competent second intermediate; followed by 4) inner leaflet mixing on a time-scale of ca. 150 seconds; and 5) contents mixing on a time-scale of 150-300 seconds. Inner leaflet mixing begins simultaneously with, but is completed before, contents mixing. Fusion products, which seem to be large vesicles, are estimated to be formed from 4-6 SUVs. Two fusion intermediates are identified. Using quasi-elastic light scattering, the initial intermediate was shown to revert to SUVs upon removal of PEG, while the second intermediate irreversibly contributed to fusion pore opening or absence of PEG. The sequence of events and Arnesen activation energies for this are analogous to those observed for protein-mediated cell-membrane fusion events, suggesting a commonality between these two processes. Supported by USPHS grant GM52575 to BRL.

11:00 AM EE3.5
THE MECHANOCHEMISTRY OF LIPID VESICLES: IMPLICATIONS FOR DRUG CARRIER DESIGN.
David Needleman, Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC.

Various micropipet methods that have been specifically developed since 1980 have studied the mechanochemical features of lipid bilayer vesicles. The information gained from such studies not only characterizes the membrane and its inter membrane interactions from a fundamental materials science perspective, it also provides vital materials property data that are required for the successful design and deployment of lipid vesicles in applications such as drug delivery. Here, we discuss the strength and compliance of the membrane, its interfacial interactions, its transition properties, and its mechanical contact with drugs and other molecules are of particular interest. Strength, cohesion, and permeability are obviously required in order to retain a particular compound in the aqueous interior. A cohesive interface (e.g., cholesterol-rich) has also been shown to exchange surfactant micelles and pH-sensitive polymers with the bilayer. Because of this resistance to molecular-induced instability, cholesterol-rich liposomes have been shown to circulate longer in the blood stream, directly correlated with the membrane elastic modulus. Interfacially, PEG-grafted lipids have also extended the circulation time in the blood, again by limiting interactions of micromolecules with the lipid surface, but this time by a steric rather than a cohesive mechanism. Drug uptake and solubilisation by lipid bilayers and micelles have also been shown to depend on interface compliance, with softer interfaces binding a greater amount of a drug like paclitaxel. Once retained in the lipidic carrier, it becomes important to release the drug from the vesicles without affecting its target cell, and so, temperature, pH or mechanical transitions that result in reorganization or leakage of the drug is a key feature of the next generation systems.

11:30 AM EE3.6
LYSIS TENSION OF LIPID VESICLES. Barbara J. Frenken and Philip Patsy, Dept of Physics, Simon Fraser Univ, Burnaby, BC, CANADA.

One popular method of producing lipid vesicles involves pumping or extruding a lipid suspension through the cylindrical pores of polycarbonate membranes. By characterizing vesicles produced under different extrusion conditions and from different lipids, we have found that vesicles are only produced above a certain threshold extrusion pressure. This minimum extrusion pressure is reduced to the lyso, or rupture, tension of the membrane. We have used this fact to develop a method of measuring the lysin tension of vesicles composed of various lipids and lipid mixtures.

1:30 PM EE4.1
PHOSPHOLIPID NANOBULES: FORMATION AND APPLICATIONS IN TOPICAL DELIVERY.

SESSION EE4: DRUG AND GENE DELIVERY
Chair: David H. Thompon
Tuesday Afternoon, November 30, 1999
Vermont (M)
The formation of tubular microstructures from diacyl-phospholipids or from certain sphingolipids is well documented. The driving force for formation of tubular microstructures develops from the unidirectional packing interactions arising from molecular chirality, strong in-plane intermolecular interactions and tilted orientation of the acyl chains. Microstructures of diacyl-phospholipids have been used in applications ranging from making inorganic composites to controlled release of antimicrobial agents. These applications are mostly in material engineering area.

Interestingly, applications of tubular microstructures has more recently been recognized in the delivery of health care products. We envision nanotubes to have potential application for the delivery of active ingredients through topical application. With this view, we have been investigating formation of the tubular microstructures from mixed systems of phospholipids and naturally occurring sphingolipids. We have successfully formed tubular microstructures from mixed lipid systems using phosphoethanolamides and ceramide. These tubes ranged from 40-60 nm in diameter and 1-2 μm in length. We are further characterizing these nanotubular microstructures for various properties including capture volume capacity, stability, toxicity, and skin penetration.

We plan to detail the discussion of our findings during the presentation.

1:45 PM EE4.2
IMPROVING BIOAVAILABILITY USING PHOSPHOLIPID- AND POLAR LIPID-DRUG CONJUGATES. Mikon B. Yasin, Michael J. Meredith, Oregon Health Sciences University, Portland, OR; Walter A. Shaw, Stephen W. Burgess, Avanti Polar Lipids, Alabaster, AL.

The low bioavailability of many drugs is a limiting factor in their use. In an attempt to overcome these problems we have linked drugs to phospholipids to create prodrugs that have selective affinities for specific biological target sites. This selective association provides a possible avenue of drug targeting. A novel aspect of our invention is that they provide for both intracellular organelle and organ targeting of the biologically active compounds. Of particular interest is the ability of some of the prodrugs to cross the blood brain barrier and attain very high concentrations in the brain and in brain tumors relative to free drug. Intracellular targeting is predicated upon the exploitation of active transport of lipid prodrugs into the target cells followed by intracellular trafficking of this selectivity association pro-drug to the appropriate intracellular target. To this end, the site within the polar lipid molecule of covalent linkage between the polar lipid and the organic spacer or biologically active compound, or both, is in the hydrophobic tail. It has been well established that the cellular enzymes responsible for intracellular lipid trafficking recognize different polar lipids by their chemically distinct head groups. Thus, productive intracellular trafficking readily occurs using polar lipid conjugated drugs that are covalently linked through the hydrophobic tail of the lipid. Whereas, exploitation of these cellular systems essentially precludes conjugation of biologically active compounds to the head group.

2:00 PM EE4.3
 BIOLOGICAL POLY ELECTROLYTES COMPLEXED WITH CATIONIC LIPIDS AS AN ALTERNATIVE TO THE CHEMICAL ORDNA COMPLEXES. C.R. Safinya, G.C.L. Wong, A. Lin, N. Slack, Y. Li, M. Martin & T. Pethil, Materials and Physics Dept and Biochemistry and Molecular Biology Program, University of California, Santa Barbara, CA.

It has been shown that the complexation of DNA with oppositely charged multivalent ions leads to a number of interesting condensed phases some aspects of which may relate to DNA condensation and de-condensation in vivo required for different biological functions. Currently, there is a large surge in interest in unraveling the structures of complexes consisting of biological polyelectrolytes (e.g. DNA, polypeptides) complexed with oppositely charged cationic lipids. This interest has, in part, been encouraged by the use of these molecular and ionic carriers of therapeutic molecules (functional DNA, peptides) into cells for delivery application [1]. The DNA plus cationic lipids have also potential uses as templates for mesoscale micro-machine elements and molecular sieves [2]. We will present synchrotron small angle diffraction and optical microscopy which has revealed a variety of novel self-assembled phases. Supported by NSF DMR 9922946 and NIH IR11 GM56986-0.1. [1] A.D. Miller, "Cationic Liposomes for Gene Therapy", Angeracheimische Chemie (International Ed.), 39, 1784-1785 (1997).

2:30 PM EE4.4
Metastable Formation After Addition of Plasmodial DNA to Cationic Liposomes. Mechanism Leading to Generation of Hydrophobic Lipid DNA Complex. Pierrot Harvie, Lawrence Mayer and Marcel B. Bally, Division of Medical Oncology, Department of Advanced Therapeutics, British Columbia Cancer Agency, Vancouver, CANADA.

It is well established that formation of lipid-based DNA delivery systems is a consequence of a self-assembly process triggered by electrostatic interactions between DNA and environment (Loizos and Templeton, 1996). It is also known that two dimensional columnar invertebrate hexagonal lattices (H_{2}) are generated when 1,2-dioleoyl-3-glyceryl-3-phosphoethanolamine (DOPE)-complexed cationic liposomes interact with DNA. This configuration is more efficient in terms of transfection activity compared to the lamellar phase (L_{c}) formed when 1,2-dioleoyl-3-glycerol-3-phosphocholine (DOPC) is used as the helper lipid (Kyle et al. 1998). However, it is unclear whether bound lipids are organized in a bilayer-like structure or in hydrophobic complex with DNA, surrounded by lipid monolayer. In an effort to clarify the micromolecular structure generate by interaction of liposomes with DNA, we performed this self-assembly reaction in a Langmuir trough where we could monitor the surface pressure at the air-water interface arising from lipid monolayer formation. A lipid-DNA charge ratio of 2:1 the monolayer formed upon addition of DNA to DODAC containing liposomes displayed a surface tension of 16 mN/m for liposomes composed of DODAC:DOPE (1:1 molar ratio) and 27 mN/m for DOPC:DODAC 1:1 molar ratio liposomes. Monolayer formation was reduced when experiments were performed at 4°C. Interestingly, the lipid monolayers in 1:1 molar ratio was not determined using radioactive labeled lipids collected with hydrophobic paper. The formation of a monolayer at the air-water interface after mixing DNA with cationic liposomes suggests that formation of these complexes of liposomes can generate lipids in a monolayer or multilayer form which can bind DNA. The method used to generated lipid-DNA complex plays an important role in the structures adopted. Based on this information, it is found that formulation technology relying directly on the use of hydrophobic lipid-DNA complex intermediates may be better suited in comparison to use of cationic liposomes for preparation of well defined lipid-based gene transfer systems.

2:45 PM EE4.5
Polymeric Structure of Cationic Liposome DNA Complexes. Brigette Sterenberg-Papahadjopoulos, Keeling Hong, Weiren Zhang, Genomics Papahadjopoulos, California Pacific Medical Center, Research Institute, San Francisco, CA.

Complexes formed during interaction of cationic liposomes with polynucleotides such as DNA (CLDC) belong to the family of lipid-based non-viral vectors. They self-assemble into a variety of polymeric structures, including non-bilayer, hexagonl-type structure (H_{1}), and bilayer structures such as spaghetti/ meatball-type structures, spherical vesicles, particles, oligomer structures, and map-pin structures. We have chosen mainly freeze-fracture electron microscopy but also cryo-electron microscopy for recording the polymeric structures, and for studying factors and conditions that affect the formation and stabilization of specific structure types. Furthermore, we have used micrographically snaps of the self-assembly of specific structure types with cultured cells. In order to find out the "active" structure in terms of transfection, we investigated the transfection activity both in vitro and in vitro of CDLC, and studied in parallel their morphology in serum as well as in cell medium.

Conditions favoring the formation of an individual structure type include charge neutralization, valency of the cationic component, type and ratio of the helper lipid, type and degree of condensation of the nucleotide component, as well as ionic strength of the aqueous medium. Studies on the interaction between CDLC and cultured cells, showed that frequently endocytosis after incubation times of 24 hours. However, after short incubation times of 1-30 minutes, fibrillar spaghetti-like structures were frequently observed intact and inside the cells. Comparison of in vitro transfection activity of CDLC measured on SK-HEL-1 cells with their 1:10 ratio to in vivo transfection activity expressed in mice lung following i. v. injection revealed a fundamental difference: For in vitro activity, high transfection rates are associated with hexagonal lipid precipitates, whereas in vivo activity is associated with small, semistable complexes consisting with map-pin-structures.

3:30 PM EE4.6
Self-Assembly of DNA DELIVERY SYSTEMS. Francis Szoka, Ming Ouyang, Jeff Sperinde, University of California, San Francisco, CA.

The assembly of DNA into small particles at high concentrations has been a challenging problem for those working in the area of gene
delivery. Viruses accomplish this using a sequential ordered process while most attempts in the gene delivery field have assembled complexes in an unordered process using polyethylene glycol (PEG) addition conditions. Recently, template directed methods have used the DNA backbone to assemble the complex and small particles containing one DNA molecule can be produced. We have developed two processes to control the assembly of complexes. The first uses a series of novel cationic detergents that contain chemically cleavable hydrophilic isothiouronium headgroups. The detergents have alkyl chains of C12 or C2 and contain hydrophilic isothiouronium headgroups that give relatively high CMC concentrations (CMC) to the detergents (> 10 mM). The isothiouronium group also masks a sulfhydryl group on the detergent. However, the isothiouronium group can be cleaved in a controlled manner under basic conditions to generate a reactive thiol group. The thiol group can undergo a further reaction after the detergents have accumulated on a DNA template to form a disulfide linked lipid containing two alkyl chains. The pH dependent kinetics of cleavage of the isothiouronium group, the CMC of the surfactant, the formation of the complexes, and the transfection efficiency of the DNA complexes have been investigated. Using the C12 detergent, a ~6 KB plasmid DNA was compacted into a small particle with an average diameter of around 40 nm with a ~15 mV net potential at high DNA concentration (up to 0.3 mg/mL). Under appropriate conditions, the small particle retained transfection activity. The second method uses enzymatically degradable monomers to control the process of complex assembly. We will describe the molecules, process and gene delivery results using these template directed methods in gene therapy.

4:00 P.M. EE4.7

**DRUG AND GENE DELIVERY APPLICATIONS OF SYNTHETIC ACID-LABILE DIPLASMIDYL LIPIDS.** Jeremy A. Boomer, Marquita M. Qualis, Junhwa Shin, David H. Thompson, Dept. of Chemistry, Purdue University, West Lafayette, IN.

The low pH environments characteristic of endosomal compartments and ischemic tissues provide an intrinsic pathway for triggering site-specific contents release from appropriately designed delivery vehicles. Accordingly, research in our group has focused on the design, synthesis and application of novel acid-sensitive lipids that will undergo facile 

**EE5: SPATIALLY-RESOLVED ELECTRON ENERGY-LOSS MAPPING OF LIPIDS IN A MULTICOMPONENT LIPID/PROTEIN SYSTEM**

**A. Aitzoucho, S. Shi, M. Libern and M. Marzl**

Svens Institute of Technology, Hoboken, N. J. *Univerkie Research, Edgewater, NJ.

Because of their amphilic nature, lipids tend to self-assemble into membranes and other topologically-complex structures which can serve a number of functions in both natural and synthetic environments. The structure of lipid assemblies often varies along length scales 10-100 nm, and morphological and structural studies of such assemblies often require electron-optical methods. Image contrast, in an electron microscope, is usually generated by large defocus for unstained samples or by positive/negative staining methodologies. We are currently developing alternate approaches to generate image contrast on spatially resolved electron energy-loss spectra. This method has allowed us to distinguish between different lipid species as well as between lipids and proteins. Low-loss spectra taken from cholesterol and from ceramides show that the lipids have characteristic spectroscopic fingerprints due to both ph and sigma valence-electron excitations which are sufficiently different to distinguish between the two. These spectra can also be distinguished from that of a protein such as mouse serum albumin. (HSA). Multiplet least squares fitting of characteristic reference spectra from each of these three materials is used to deconvolute the spatial distribution of ceramide, cholesterol, and HSA in spectrum images collected from solvent-cast films and establish the nature and length scale associated with phase separation in this three-component system.

4:15 P.M. EE4.8

**DISRUPTION OF MIXED SURFACTANT LIPOSOMES BY CATIONIC DPPC**

**P. K. Tlusty**

Michael Grey, James L. Thomas, Department of Chemical Engineering and Applied Chemistry, Columbia, New York, NY.

Cationic polyamidoamine dendrimers have shown remarkable efficacy as vectors for the transfection of foreign DNA into mammalian cells. To elucidate the mechanisms by which dendrimers interact with cell membranes, fluorescence and EPR spectroscopy have been used to characterize the interactions of membrane interacting species. Using a self-quenching fluorescent dye, we find that membranes containing a non-hemolytic, cationic lipid (either stearic or oleic acid), stabilized with phosphatidylcholine (PC) or phosphatidylethanolamine, are transiently permabilized by higher generation dendrimers, while pure PC liposomes are unperturbed. EPR measurements on spin-labeled lipids in vesicles have been used to characterize the membrane recognition induced by dendrimers. In addition, measurement of the binding of isoxanthopterin to unilamellar vesicles, using a centrifugation assay, show tight complexation of the polycationic polymer and polylysine vesicles.

4:30 P.M. EE4.9

**STABILIZED PLASMID-LIPID PARTICLES FOR SYSTEMIC GENE DELIVERY.** Peter R. Calli, Biochemistry Department, University of British Columbia, Vancouver, Canada; Inex Pharmaceuticals, Burnaby, Canada.

Gene therapies for systemic diseases such as cancer or inflammatory disorders are limited due to systemic vascular delivery. Novel, highly available gene delivery systems have limited utility for systemic applications. Viral systems are rapidly cleared from the circulation following intravenous injection, limiting potential transfection sites to first pass organs such as liver and spleen. Similarly, non-viral systems such as plasmid DNA-cationic lipid complexes are large, charged systems that are also rapidly cleared from the circulation, again limiting transfection to first pass organs, particularly the lung.

Previous work has shown that encapsulation of chemotherapeutic drugs in liposomes results in enhanced accumulation at tumor sites. It therefore follows that encapsulation of plasmid DNA in small liposomal systems should result in enhanced delivery to tumour sites. A method for encapsulating plasmid DNA in small (<10nm) liposomes containing photoactive iodinated particles (SPL) employing a detergent dialysis procedure will be described. These SPL contain one plasmid per particle and are stabilized in aqueous media by the presence of a poly(ethylene glycol) (PEG) coated 2-iodophenylalanine cotransporter, receptor gene expression. Initial studies utilizing SPL for systemic gene therapy applications employing thymidine kinase gene delivery in combination with ganciclovir will also be reported.

**SESSION EE5: POSTER SESSION**

**Chairs:** Philip B. Messersmith and Joel M. Schar

**Tuesday, November 30, 1999**

8:00 P.M.

Exhibition Hall D (H)

**EE5.1**

**SPATIALLY-RESOLVED ELECTRON ENERGY-LOSS MAPPING OF LIPIDS IN A MULTICOMPONENT LIPID/PROTEIN SYSTEM**

**Aitzoucho, S. Shi, M. Libern and M. Marzl**

Svens Institute of Technology, Hoboken, N. J. *Univerkie Research, Edgewater, NJ.

Because of their amphilic nature, lipids tend to self-assemble into membranes and other topologically-complex structures which can serve a number of functions in both natural and synthetic environments. The structure of lipid assemblies often varies along length scales 10-100 nm, and morphological and structural studies of such assemblies often require electronic-optical methods. Image contrast, in an electron microscope, is usually generated by large defocus for unstained samples or by positive/negative staining methodologies. We are currently developing alternate approaches to generate image contrast on spatially resolved electron energy-loss spectra. This method has allowed us to distinguish between different lipid species as well as between lipids and proteins. Low-loss spectra taken from cholesterol and from ceramides show that the lipids have characteristic spectroscopic fingerprints due to both ph and sigma valence-electron excitations which are sufficiently different to distinguish between the two. These spectra can also be distinguished from that of a protein such as mouse serum albumin. (HSA). Multiplet least squares fitting of characteristic reference spectra from each of these three materials is used to deconvolute the spatial distribution of ceramide, cholesterol, and HSA in spectrum images collected from solvent-cast films and establish the nature and length scale associated with phase separation in this three-component system.

**EE5.2**

**PHOSPHOLIPID MICELLES AS CONFORMATION MODIFIERS AND BIOACTIVITY ENHANCERS FOR PEPTIDES AND PROTEINS.** Hayun Onyxuk, Minajh Paez, Syed Akhter, Daniel Rubinstein, Dept of Bioengineering, Pharmaceutics and Pharmacodynamics and Medicine, University of Illinois at Chicago, IL.

Phospholipid micelles are self-assembled aggregates, which provide hydrophobic environment in an aqueous medium. Therefore these micelles can be used as safe delivery systems for water insoluble drugs and amphilic peptides/proteins. The purpose of this study was to determine the changes in molecular conformation and bioactivity of a 29kDa pancreatic neurotopeptide, vasoactive intestinal peptide (VIP), after self-association with phospholipid micelles. Phospholipid micelles, about 17 nm in size, were prepared by dispersing diatery phosphatidylethanolamine conjugated to polyethylene glycol (MW 2,000) in aqueous medium. VIP loading was achieved by incubating micelles with VIP. The conformation of VIP in the presence of absence of micelles was determined by circular dichroism. The vasoactive activity of VIP as aqueous and micelle solutions was determined on hamster cheek pouch. The results showed that VIP was disordered with a random coil structure in the absence of phospholipid micelles, and micelle-associated VIP exhibited an alpha helix conformation. The helicity increased with temperature. The bioactivity of VIP significantly increased in a dose dependent fashion in the presence of micelles (3±1% versus 1±0% with 0.01 mmol VIP and 9±2% versus 9±2% with 0.1 mmol VIP, n=4, p<0.05). In conclusion, phospholipid micelles can modify the conformation of an amphilic peptide like VIP, and amplify its bioactivity. These effects are most probably due to increased stability and a more favorable conformation of the peptide for receptor interaction.
INTRODUCTION OF DIACETYLETANOL FUNCTIONALITY ON THE SELF-ASSEMBLING PROPERTIES OF PHOSPHOLIPIDS

Alok Sing, Center for Bio/Molecular Science & Engineering, Naval Research Laboratory, Washington, DC.

Considerable efforts have been devoted to understand and control the synthesis and morphology of phospholipids. Phosphatidylethanolamines (PEs) in bilayers have been used in the preparation of giant unilamellar vesicles (GUVs) with higher order structure to serve as a model membrane system for the study of membrane proteins and their interactions. GUVs can be formed by application of electric field to its aqueous dispersion. GUVs could be used to study the effects of local microenvironment on the activity of proteins, the effect of lipid composition on membrane properties, and the interactions between membrane proteins and lipid bilayers.

In the present study, we have investigated the self-assembly properties of a series of diacetylated PE derivatives with different acyl chain lengths (C12, C14, C16, and C18) using optical microscopy and small-angle X-ray scattering (SAXS). The results obtained indicate that the self-assembly properties of diacetylated PE derivatives are influenced by the acyl chain length, with C12 PE derivatives forming larger and more ordered structures compared to C18 PE derivatives.

PE-PELAMIC: A NEW MATERIAL FOR APPLICATIONS IN MEDICINE AND BIOTECHNOLOGY

Volker Weiseg and Vladimir P. Torchilin

Department of Pharmaceutical Sciences, Northeastern University, Boston, MA.

Liposomes technology and its application in medicine has advanced significantly during the last 10 years as visible by the approval of lipid-based formulations for human use. However, the demand for advanced liposomes and related carriers with novel properties is ever increasing. A vast body of literature exists about the structure, dynamics, and phase behavior of amphiphilic aggregates composed of single-chain, two-chain, or four-chain polar lipids. Also, two-chain headgroup bilayers, lipids in which both polar head groups are connected by two parallel hydrocarbon chains, have been extensively studied. We have recently described the formation of liposomes made from dequainlin, which we call DQosomes [1]. Dequainlin is a quinquilinum derivative in which two positive charge centers are connected by a hydrocarbon chain consisting of ten CH2 groups. This molecule, therefore, represents a novel new class of amphiphiles, which we name single-chain holamphipides. We have analyzed the self-assembly behavior of single-chain holamphipides using Monte Carlo simulations [2] and in a structure-activity relationship study testing a series of dequainlin derivatives [3].


LIPOCORES: NOVEL DRUGRFC NANOPARTICLES


We describe here the physical characteristics of drug-rich stable spherical liposomes and the process by which they are made. Unlike conventional lipid-based particles (i.e., liposomes, emulsions, micelles), liposomes comprised of a drug-rich layer of a cholesterol-soluble drug surrounded by a liquid crystalline layer of PEG-lipid (or a negatively charged lipid). Consequently, the drug/lipid ratio of these particles is high (typically 3:1 or 4:1, mol/mol). Liposomes have been made with PEG-PEG (2000) combined with the antibiotic lysozyme or with acylated derivatives of paclitaxel or vinblastine. B Nicotinamide was included as the nitrogen of the dipalmitoyl lecithin base. For BrC16, liposomes, the particle sizes can be controlled for many months and exhibit reduced anti-cancer activity activity in suspension, with the drug leaching into the surrounding aqueous phase after 5-7 min. For paclitaxel derivatives, liposome formation was dependent upon the length of the attached acyl chain; for chain lengths less than C12, crystals were co-produced. For BrC16+T, formation of liposomes occurred regardless of the type of PEG-phospholipid linkage used, no significant increase in particle size, saturation, and polymer length could be achieved. BrC16+T, indicating that a steric or electronic barrier is critical for particle stability. From cryo-electron microscopy, the diameter of the liposomes was found to be less than 100 nm. BrC16+T liposomes appeared to be solid spheres without internal lamellae. BrC16+T/DSPC-PEG (2000) particles had no measurable aqueous uptake and the size of the surface area and volume remained constant after 72 h. BrC16+T was co-delivered with a single liposome monolayer. 1H-NMR, fluorescence anisotropy (DPh) and order parameter (1H-deuteronoracine) measurements revealed the motions within the particles were highly restricted.

PREPARATION, PHYSICAL AND BIOLOGICAL PROPERTIES OF LIPOSOMES PREPARED FROM COMBINATION OF BR flC16, LIPOSOMAL CACONUT, AND ALKYLPHOSPHOLIPIDS

Reiner Zeha, Dieter Arndt and Edna Fichtner, Max-Delbrück-Center for Molecular Medicine, Berlin, GERMANY.

Alkylphospholipids (APLs) are synthetic ether lipid-like compounds with high anti-cancer activity against hormone-independent human mammary carcinoma. Their amphiphilic character makes it possible to prepare different types of liposomes from APL if cholesterol (50-100 mol%, based on APL) and a charged component (20 mol%) is added. APL liposomes are remarkable stable in buffer and in plasma in a composition dependent way. The most stable vesicles are negatively charged LUVs with an amount of about 50 mol% CH. The stability correlates with the incorporation of APL liposomes in a target membrane model consisting of PC, PE, PS and CH, with the induction of marker release from model liposomes of some composition. Resonance energy transfer experiments demonstrated no correlation of composition and lipid mixing between APL liposomes and model vesicles. Cytotoxicity of APL liposomes in vitro correlates with the stability and could be increased by decreasing the CH amount to an inhibition concentration of 20–50 µM. APL liposomes are able to activate different macrophage cells to induce the release of tumoricidal factors like TNF and NO. The strongest release was caused by a synergistic action of liposomal APC and lipopolysaccharide. Sterically stabilized APL liposomes significantly reduced uptake by macrophages as a result of an increase of the fixed aqueous layer thickness (FLAT) from liposomes of 0.83 nm to 3.57 nm and inhibit thus activation effects. Human mammary carcinoma xenografted to nude mice were used to demonstrate the therapeutic effect in vivo. The use of liposomes reduced clearly hemolysis, the most serious side effect of APL. Sterically stabilized APL liposomes with a reduced amount of CH were the most effective cancer ras inhibitors in investigated models.

LIPOMAS™ PROTOTYPE VEHICLES FOR ENHANCED TUMOUR TARGETING AND DELIVERY


PEGylated liposomes can be used to exploit leaky tumour vasculature and achieve tumour targeting (the so-called enhanced permeability and retention - EPR - effect). If, however, one focuses on prolongation of the circulation time to achieve maximum extravasation into the tumour, tumour to blood concentration ratios are reduced well below 1 [1]. This effect can be modeled by using various special formulations of PEG-liposome (modulating both the PEG and lipid composition) which impact on other factors, not merely circulation time. PEGylated liposomes were prepared using external PEGylation of preformed vesicles and tested for tumour localisation in colon carcinoma xenografts (LS147). At 24 hours, tumour-blood concentration ratios were 2.52±0.19 mean± SEM, n=29 for all lipoparticles tested (i.e., none of the formulations tested). A ratio of 0.3 was reported for Stealth liposomes [2]. In addition, the % injected dose per g of tumour at 24 h post injection was 3% (± 2%) for the 1 p.p. MACS™ prototype and 4.51±0.39 (n=17) well above the 2.67% injected dose p g of tumour reported for Dose liposomes [3]. In part, the improved uptake was due to PEG with 144%±23% (n=14) of unPEGylated controls. In contrast to Stealth liposomes where the addition of PEG decreased the tumour-blood ratio, from 0.5 to 2.5, MACS™ prototype B to G had tumour-blood concentration ratios 198%±12% (n=14) higher than unPEGylated controls, indicating a fundamental difference in at least some factors impacting on tumour localisation. With respect to normal tissues, PEGylated tumour vascular concentration ratios (100%±30%, n=14) and tumour:colon tumour concentration ratios (120%±10%, n=14). 1 p.p. MACS™ prototype E has been successfully loaded with a weak base using remote loading by proton gradient and has therefore potential for enhanced delivery.
of cytotoxicity to tumour tissue with reduced toxicity.

E5.8 MOLECULAR DESIGN OF NOVEL PHOSPHOLIPID MUTANTS AND CHARACTERIZATION OF THEIR AGGREGATES UPON SELF-ASSEMBLY. Santhu Bhattacharya, Shubhik Hakkar, Indian Institute of Science, Dept of Organic Chemistry, Bengaluru, INDA.

To decipher the roles of the hydrocarbon chain-lipid backbone linkage functionalities on the properties of the phospholipid (PC) lipids toward the formation of bilayer membranes, series of mutants of dipalmitoyl phosphatidylcholine have been synthesized. In these phospholipids either one or both (C6-C6) linkages at the hydrocarbon-glycerol backbone of the phospholipids have been replaced with CH2=CH2-units keeping the chain lengths of these lipids identical with those found naturally occurring phospholipids with diester linkages. The aqueous suspensions of the mutant lipids afforded vesicular aggregates as confirmed by dynamic light scattering, transmission electron microscopy and water-soluble dye entrance of the obtained calcium phosphate varied completely from the thin layer precipitated on the organic monolayer to the confined particles formed inside the lipid vesicles to the three-dimensional mesomorphs organized in the precursor solgel.

E5.9 COMPARISON ON PRECIPITATION OF CALCIUM PHOSPHATE BY ORGANIC MONOLAYER, UNILAMELLAR PHOSPHOLIPID VESICLES AND HYDROTHERMAL SELF-ASSEMBLY. Y. Zeng, Z.S. Cai, Y. Zhang, Z.S. Cai, Department of Materials Science and Engineering, Tsinghua University, Beijing, PR CHINA.

Biominalization centers around the idea that organisms control the nucleation growth and form of inorganic. As ubiquitous biological phospholipid, phospholipid has been extensively utilized as a model to study biominalization. The present studies use phospholipid and other organisms to investigate the effects of their assemblies on the precipitation of inorganic. It shows that the morphology of these templating organisms has essential effects on both the phase and the constructs of inorganic. Organic monolayer templated calcium phosphate, unilamellar phospholipid vesicles templated calcium phosphate, and minimally self-assembled calcium phosphate were investigated. The results revealed that hydroxyapatite minerals could be consistently formed in all three cases, regardless of the great difference in the precursor or intermediate phase. However, the constructs of the obtained calcium phosphate varied completely from the thin layer precipitated on the organic monolayer to the confined particles formed inside the lipid vesicles to the three-dimensional mesomorphs organized in the precursor solgel.


E5.11 DEVELOPMENT OF METAL ENCRUSTED PHOSPHONIC ACID MICELLES AS POSSIBLE DRUG DELIVERY VEHICLES. Marcus R. Helfrich, Lauren M. Huffman, Meng OuYang and Catherine J. Page, University of Oregon, Department of Chemistry, Eugene, OR.

Micelles and vesicles have been recognized as possible drug delivery agents for several decades. These systems are promising in that the exterior of the aggregates can be functionalized to provide a means of targeted delivery for a drug trapped within the hydrophobic core. Our current research is focused on the development of a unique vesicle delivery system in which the exterior of the drug containing aggregate has been encrusted with a metal. This metal crust provides a route to which other molecules can be conjugated to target drug delivery as well as it stabilizes the underlying vesicle. The characterization of one such system composed of n-dodecanephosphonic acid and water with a variety of iron(III) salts will be presented as an in transmission electron microscopy (TEM), fluorescence spectroscopy, and static light scattering (SLS) study.

SESSION E6: TUBES, TEMPLATES AND POLYMORPHISM

Chairs: Philip B. Messersmith and Joel M. Schum
Wednesday Morning, December 1, 1999
Vermon (M)

8:30 AM E6.1 PHOSPHOLIPID TEMPLATES FOR FABRICATION OF MICRO- AND NANO-METER-SCALE POLYMERIC FILAMENTS. Jeffrey Linhardt, Kevin Higgen, David Tisdall, California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, CA.

Microminiurculation of phospholipid vesicle membranes can be used to produce micro- and nanometer-scale tubes that serve as effective templates for photopolymerization of water-soluble monomers and polymers. The resulting polymeric fibers are tough and elastic, and can be configured in a manner that provides controlled, point-to-point connections between attachment sites arranged in two or three dimensions. This lecture will examine the chemistry of attachment and photopolymerization as well as the materials properties of the resulting filaments.

9:00 AM E6.2 METALIZED DECAYETYLIC LIPID TUBES, AND SOME OF THEIR APPLICATIONS. Paul E. Schoen, Dom Tzatavitis, Ron R. Price, Naval Research Lab, Washington DC.

Decanoyl PC lipids form micro-dimension hollow cylinders with relatively high aspect ratios, a shape which suggests the possibility of applications as microencapsulation and RF dielectrics. However, tubes are electrically insulating and are easily destroyed by heat, solvents and mechanical stress. We have developed tubule metallization techniques which allow them to be used as electrical and magnetic properties, make them strong and impermeable, and to a limited extent, allow them to be more easily manipulated. Some utilized tubule applications will be discussed, especially their use in dielectric composites.

9:30 AM E6.3 CALORIMETRIC, SPECTROSCOPIC, AND ELECTRON MICROSCOPIC CHARACTERIZATION OF NANOSCALE LIPID ASSEMBLIES FORMED FROM MIXTURES OF POLYMERIZABLE AND NONPOLYMERIZABLE PHOSPHOLIPIDS. Matthew Stone, Phillip Messersmith, Northwestern University, Division of Biological Materials, Chicago, IL.

We recently reported that equimolar mixtures of 1,2-dimyristoyl-1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DCS,4P) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DNPC) form micelles of diameter 55±10 nm and length between 10 to greater than 100 micrometers. These nanotubes were found to be stable when stored at 4°C, but transformed into an interconnected helical ribbon phase at or above room temperature. In an effort to fully characterize these phases, we have synthesized isotopically labeled DNPC (deuterated-DNPC). Mixtures of deuterated-DNPC with DCS,4P were characterized by electron microscopy, scanning...
Self-Assembly of Micellar Tubules of the Amphiphilic Decyl Ether of D-Tyrosine and their Enzymatic Polymerization Measured at the Quartz Crystal Microbalance (QCM) Surface. Tien Zhou and Kenneth A. Marx, Center for Intelligent Biomaterials, Department of Chemistry, University of Massachusetts, Lowell, MA.

Amphiphilic decyl ester derivatives of D-Tyrosine (DEDT) self-assemble into long rod-like or tubular aggregate structures in aqueous phosphate buffered solution as visualized by SEM or light microscopy. They possess a c.m.c. value of 0.17 mM in a pH 6.0 solution, as measured by light scattering. We demonstrate that the QCM, through measurement of the quartz crystal frequency, is capable of detecting the formation of rod-like aggregates as a function of increasing pH values from 3 to 7. Using the Smoluchowski equation to estimate the rigid QCM bound surface mass, we estimate a 
\[ M_{\text{agg}} \] of 8.3 for the equilibrium involving both the deprotonation of the carboxylic group and the subsequent aggregation of DEDT. Once formed and bound to the QCM surface, we initiated enzymatic polymerization of the self-assembled DEDT monomers through addition of horseradish peroxidase (HRP) and then hydrogen peroxide. By monitoring changes in the quartz crystal frequency (f) and motional resistance (R), we demonstrated that the viscoelastic properties of the polymerized aggregates change relative to the unpolymerized aggregates. A final state is achieved in which the altered physical properties of the polymerized aggregates make the solution above the QCM surface behave as a Newtonian fluid, producing a nearly pure viscosity-density energy dissipative effect on the measured f and R values. These results demonstrate that the self-assembled QCM technique has valuable applications to characterizing changes in turbid micellar systems that interact with the QCM surface or alter the motional resistance in the solution immediately above the surface-solution interface. (Acknowledgement Seed Money Grant from Res. Fdn. at UMASS to Center for Intelligent Biomaterials)

Polymerization of Liquid Crystalline Assemblies. David P. O'Brien, University of Arizona, Department of Chemistry, Tucson, AZ.

The self-assembly of amphiphiles in water yields various lamellar and nonlamellar phases depending on concentration, temperature, and pressure. The organized nature of these materials offers several attractive features for applications in both biological and materials sciences. In many cases the potential utility requires a means to make the self-assembled systems more robust. In some cases the desired properties can be attained through surface charge or the association of polymers at the assembly surface, whereas in other instances polymerization of the assembly is more aggregate. The lecture will focus on the latter case with particular emphasis on a strategy that relies on the formation of self-assembled monolayers from reactive amphiphiles, followed by polymerization of the amphiphiles within the assembly.

Cytoaffinic Strategies for Bio-Material Design. Elliott L. Chalof, Emory University, Departments of Surgery and Bioengineering, and Georgia Institute of Technology, School of Chemical Engineering.

A number of examples of biomimetic design will be described with a particular emphasis on the biological membrane as a starting point for engineering biofunctional surface coatings for blood contacting applications and tissue engineered constructs. Membrane-based mechanisms for the control of molecular recognition and interfacial transport will likely prove to be powerful strategies for improving the clinical performance characteristics of a variety of artificial organ systems.

Structure of Polymerized Liposomes as Mucosal Delivery Vehicles for Complex Moleculare Drugs and Vaccines. Likan Liang, Elyan Bolotin, Kyle Barcher, Fan Ma, Denise Barche, Kathy Kech, David Fast, Dan Markovic, Hansi Dean, Robert Guest, Endorex Corporation, Lake Forest, IL.

Liposomes composed of a variety of phospholipids and other lipids have been used extensively as drug delivery vehicles. Several lipid-complex or true liposomal drugs are now marketed. However, the utility of this class of liposomes is limited to parenteral delivery because of their susceptibility to disruption in the GI tract or at other mucosal surfaces. To overcome instability, liposomes were constructed using the polymerizable phospholipid 1,2-dipalmitoyl-3-phosphatidyl choline (DODPC). Liposomes composed of DODPC formed typical bilayer membranes that could be cross-linked by polymer formation. Polymer content and extent of cross-linking could be experimentally varied depending on reaction conditions. Polymerized liposomes were stable through a range of Triton X-100, depending on polymer content. Liposomes used for animal experiments were 150-200 nm ultrafine vesicles (>90%) and could be effectively charged with hydrophilic complex molecules ranging in molecular weight from 5K - 150K. Correlation of liposome structure based on polymer content to biological outcome was assessed for several protein drugs and vaccines. Oral administration of liposomes in mice resulted in serum biodistribution and bioactivity of human growth hormone or insulin. Likewise, oral or intranasal administration of a single dose of liposomal tetanus toxoid vaccine resulted in brisk serum and local antibody responses boosted by two successive immunizations. The intranasal response was larger than oral and comparable to subcutaneously administered vaccine. Analysis of liposome structure indicated that membranes with high polymer content (>80% cross-linking) were less effective biologically than liposomes with 40-50% polymer content. These results indicate that polymerized liposomes can be used to deliver complex molecules mucosally, and that extremely stable membranes (>90% polymer content) may fail to release drugs systemically.