# SYMPOSIUM EE

## Materials Science of Phospholipid Assemblies

November 29 – December 1, 1999

### Chairs

**Phillip Messersmith**
Biological Matls Dept
Northwestern Univ
Ward 10-019
Chicago, IL 60611
312-503-1789

**Joel Schnur**
Ctr for Biommolecular Sci & Eng
Naval Research Laboratory
MC 6900
Washington, DC 20375
202-404-6000

**David Thompson**
Dept of Chemistry
Purdue Univ
West Lafayette, IN 47907-1393
765-494-0386

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* Invited paper

SESSION EE1: LIPID MONOLAYERS, BILAYERS AND BIOMOLECULAR INTERACTIONS
Chair: David H. Thompson
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 and Molecular Biology, Medicine, and Physiology and Pharmacology, Oregon Health Sciences University, Portland, OR; V. Vogel, Department of Bioengineering, University of Washington, Seattle, WA.

Far 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 subfraction 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. At a surface pressure of 13 mN/m at room temperature, following completion of the liquid-expanded (LE) $\rightarrow$ liquid-condensed (LC) transition, the LC phase of dipalmitoyl phosphatidylcholine (DPPC) develops corrugations within a region that covers half the monolayer and surrounds flat, 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 nanoparticles through a budding process. Above 60 mN/m, the budding process accelerates. Atomic force microscopy (AFM) on samples transferred to mica confirms the presence of multibilayer discs of diameter 15 - 150 nm. The morphologies of the flat domains suggest that these topographical instabilities result from a heterogeneous distribution of packing defects locked into the monolayer during the LE$\rightarrow$LC transition.

**9:15 AM EE1.3**
DIRECT OBSERVATION OF THE INTERACTIONS OF AMYLOID-BETA PEPTIDES WITH LIPID MONOLAYERS. Canay 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 molecules with various headgroups. 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 membrane surface may act as seed for further peptide aggregation.

**9:30 AM EE1.4**
CHEMICAL TRAPPING: A NEW METHOD FOR ESTIMATING ANION BINDING BY MICELLES AND VESICLES. Iolanda M. Cuccovia and Hernan Chaimovich, Department of Biochemistry, University of Sao Paulo, Sao Paulo, BRAZIL; Mahendra Kumar Jain, Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; Laurence S. Romsted and Jihu Yao, Rutgers University, New Brunswick, NJ.

The balance of forces controlling aggregate structure and stability and the distribution of components between the water and interfacial regions in organized solutions are not well understood, in part because their interfacial compositions are difficult to measure over wide ranges of solution compositions. Aggregate bound arenediazonium ions react with weakly basic nucleophiles such as halide ions and water and many of the other nucleophilic components commonly found at the surfaces of biomembranes. Stable products are formed in proportion to the concentration of each nucleophile within-the-immediate-vicinity of the aggregate surface and their yields are used to estimate their concentrations in the interfacial region and component distributions over wide ranges of solution compositions. Here we show how chemical trapping is used to estimate the interfacial anion and water concentrations around cationic micelles of cetyltrialkylammonium (alkyl = methyl, ethyl, n-propyl and n-butyl) chlorides and bromides with variable head group sizes. The results show that interfacial concentrations increase continuously with added surfactant and counterion and that they undergo marked increases at the sphere-to-rod transition. The same approach is then used to estimate the interfacial counterion concentrations in zwitterionic micelles as a function of added salt with variable cation type. Experiments in phospholipid micelles and vesicles with added NaCl show marked uptake of chloride ion by their interfaces and that these increases correlate with NaCl induced increases in phospholipase A2 hydrolysis of these micelles and vesicles. Future applications include tagging of the amide bonds of aggregate bound peptides and proteins should provide new information on their binding, topology and orientations at aggregate interfaces.

**9:45 AM EE1.5**
ELECTRONEUTRAL TRANSPORT OF ELECTRONS ACROSS BILAYER MEMBRANES BY PYRYLIUM AND PYRIDINIUM PHOTOREDOX MEDIATORS. Rafail F. Khairutdinov, Lisa Lucchesi, James K. Hurst, Washington State University, Department of Chemistry, Pullman, WA.

One major objective in our efforts to build vesicle-based systems for solar photoproduction 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 permeabilites and avoid rate-retarding membrane polarization, these compounds must be electrically neutral in both oxidation states. One system under investigation utilizes hydrophobic pyrylium ions (e.g., triphenylpyrylium, $TPP^+$) or thiopyrylium analogs as as transmembrane redox mediators across dihexadecyl phosphate SUV. Photoexcitation of a sensitizer molecule in the bulk aqueous phase containing an electron donor (e.g., dithiothreitol) led to efficient reduction of an electron acceptor occluded within the aqueous core of the vesicle when the mediator was present, but not when it was absent. Transient spectrophotometry revealed that reaction was initiated by oxidative quenching of the excited photosensitizer by $TPP^+$, and that the $TPP^o$ radical formed traversed the bilayer with a permeability coefficient of $\sim 1 \times 10^{-4}$ cm $s^{-1}$. The amount of electron acceptor reduced exceeded the amount of $TPP^+$ present by up to $\sim$40-fold, implying that on average each $TPP^+$ underwent 40 cycles of electron transport from electron donors outside the vesicles to electron acceptors within the vesicles. The mechanism proposed involves inward transmembrane diffusion of $TPP^o$, reoxidation within the inner aqueous phase followed by hydrolytic opening of the pyrylium ring to form the neutral 1,5-diketone (TPPD), outward transmembrane diffusion of TPPD to the external aqueous environment, and reformation of the pyrylium ring, closing the cycle of $TPP^+$ transformations. In this process $TPP^+$ functions as an $e^-/OH^-$ antiporter. Acid-dependent $TPP^+$-TPPD equilibration within the membrane was demonstrated spectrophotometrically. Analogous electron carrier mechanisms were determined for thiopyrylium ions. The thiopyrylium ions could also serve as photosensitizers when excited with near-uv photons, obviating the need for an independent sensitizer ion. A series of N-alkylcarboxy-4-cyanopyridinium ions were also shown to be capable of cyclic photoredox mediation across bilayer membranes; in this case, the diffusing species are thought to be neutral pyrydinium radicals and internally ion-paired zwitterions, so that they function as $e^-/H^+$ cotransporters.

**10:30 AM *EE1.6**
SELF LIMITING AGGREGATION BY CONTROLLED LIGAND RECEPTOR STOICHIOMETRY. Joseph A. Zasadzinski, Edward Kisak, Michael Kennedy, Dirk Trommeshauser, 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 biotin coupled to phospholipids and incorporated in a vesicle membrane) on the colloid surface to crosslinking agents (multifunctional receptors such as avidin or streptavidin) in solution. A distinct transition occurs between limited and complete aggregation as a function of the ligand to receptor ratio. The limited aggregates formed are compact with a fractal dimension of 2.9. The size of the aggregates depends on the overall concentration of surface accessible biotin-ligands, which can be

controlled either by the biotin-lipid fraction in the bilayer at fixed vesicle concentration, or by increasing the vesicle concentration at fixed biotin-lipid fraction. The compact shapes and concentration dependence are the result of the free diffusion of the ligands on the vesicle surfaces. A simple model of the process based on Smolukowski aggregation kinetics coupled with a Langmuir-type surface reaction is consistent with experiment. This process might be generalized to any system of colloids with surface reactive groups that can be coupled by a soluble crosslinking agent.

**11:00 AM EE1.7**
CONSTRUCTION AND PROPERTIES OF PHOSPHOLIPID/ PROTEIN ASSEMBLIES. Anthony W. Coleman Institut de Biologie et Chimie des Proteines, CNRS UPR 412, Lyon, FRANCE; Marie-Helene Paclet, Francoise Morel, CHU, Grenoble, FRANCE; Jean-Paul Rieu and Bernard Roux, UCBL1, Lyon, FRANCE.

The presentation will concern the assembly of two types of proteins in differnt phospholipid assemblies and their study by Atomic Force Microscopy, using both contact and non-contact mode observation. The first section concerns the real time assembly of Phosphatase Alkaline 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 Lucferin are discussed both in terms of protein structure and anchoring properties.In the second section the assembly of cytochrome b558 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 activated complex of cytochrome b558 in which a number of other proteins assemble. From Topographic 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 *EE1.8**
MEMBRANE PROTEIN CRYSTALLIZATION IN LIPID MESOPHASES. Hong Qiu, Martin Caffrey, The Ohio State University, Dept of Chemistry, Columbus, OH; Peter Nollert, Biocenter, 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 glucoside has been used in such applications and recently was included as a member of a multicomponent, monoolein (MO)-containing system in which 3-D crystals of bacteriorhodopsin (bR) were grown. A cubic mesophase figured prominently in bR crystal growth but its exact role in the process has not yet been established. Molecular geometry considerations suggest that the complementary 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 for 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 mesophase stability. Possible involvement of coexisting lamellar and cubic phases in protein crystal growth will be discussed. [Supported by The National Institutes of Health (GM56969)]

**11:45 AM EE1.9**
MEMBRANE ACTIVE CYSTEINE CONTAINING OLIGO-PEPTIDES: INTERACTIONS WITH PHOSPHOLIPID VESICLES AND CHONDROCYTES. Stavroula Sofou, James Louis Thomas, Columbia University, Dept of Chemical Engineering, New York, NY.

Intracellular delivery and release of anionic polymers (such as polynucleotides) may be facilitated by environmentally responsive peptides. The different reduction potentials of intra- and extracellular media suggest that disulfide bond formation may be used to control peptide association, and consequently to modify peptide affinities for membranes and polynucleotides. To explore the effects of disulfide formation on peptide behavior, the oligopeptide with sequence $NH_2$-(Lys-Leu)$_3$-Cys-$CONH_2$ was synthesized by solid phase peptide synthesis using the Fmoc strategy. We have shown that the peptide can be reversibly dimerized via cysteine sulfhydryl oxidation (in air) and reduction (with dithiothreitol). 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% either dioleoyl phosphatidylethanolamine or egg phosphatidylcholine. A solution of calcein, a self-quenching, membrane impermeant dye, was entrapped into the vesicles. Calcein 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 calcein release. To examine the peptide interactions with cellular membranes, permeabilization of cell membranes to calcein was studied. Chondrocytes were plated on glass bottom microwells and then treated with buffers containing calcein and oxidized or reduced forms of the peptide. After varying times, cells were washed with buffer to remove the peptide-calcein 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 appropriately treated glass surfaces and exhibit lateral fluidity over large distances due to a thin ( about 10 - 15 Å ) lubricating layer of water trapped between the bilayer and the surface. Supported bilayers can be partitioned and corralled 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 membrane-associated proteins can be manipulated using applied electric fields [Biophys. J. 69, 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 PLANAR SOLID SUPPORTS. Paul Cremer and Tinglu Yang, Texas A&M University, Department of Chemistry, College Station, TX.

The cellular membrane is the most sophisticated surface detection systems 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 into 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 liquid-solid 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**
SYNTHESIS AND PHASE BEHAVIOR OF MACROCYCLIC TETRAETHER BISPHOSPHOCHOLINES. Aniruddha Patwardhan, Jong-Mok Kim, David H. Thompson, Dept of Chemistry, Purdue University, West Lafayette, IN.

An efficient route towards the synthesis of unsaturated (bis-diacetylenic) and saturated 40- and 48-membered macrocyclic bisphosphocholines has been developed using 2-phenyl-5-hydroxy-1,3-dioxane as a common glycerol synthon. Ring closure was accomplished using either high dilution Glaser oxidation or [(Cy3P)2Ru=CHPh]Cl2-catalyzed olefin metathesis conditions. Aqueous dispersions of these bolaamphiphiles have been studied using

DSC, calcein leakage assay, 31P & 23Na 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 $\beta$ helix.

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

Domain patterns were fabricated from pre-polymerized n-octadecyltrichlorosilane (OTS) monolayers by Langmuir-phase assisted self-assembly on SiO2/Si substrates. Pattern structures were examined by atomic force microscope (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: ring shaped domains formed from LE phase precursor; whereas round-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 out-of-ring 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 BILAYER MEMBRANES USING NEUTRON REFLECTIVITY. 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. Plant, Biotechnology Division, NIST, Gaithersburg, MD.

Neutron reflectivity measurement techniques are being developed to characterize the structure of novel synthetic alkanethiol and phospholipid biomimetic systems, or hybrid bilayer membranes (HBMs), 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 octadecanethiol and a monolayer of $d_{54}$-DMPC, and on THEO-HBMs with a monolayer of thiahexaethyleneoxide octadecane, which contains an ethyleneoxide moiety at the gold surface, in place of the octadecanethiol layer. Data were obtained in $D_2O$ solution at 28°C, where the DMPC layer is in the fluid phase, both in the absence and in the presence of the membrane-active peptide, melittin, in the solution. To help determine if melittin penetrates into the alkanethiol layer, measurements were also made on a THEO-HBM with a deuterated alkanethiol layer and a non-deuterated DMPC layer in the presence of melittin. Neutron scattering length density (SLD) profiles of the lipid structure 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 Leckband, Tammy 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 between membranes 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 possibility, we used direct force measurements, turbidity, and fluorescence microscopy i) to quantify the impact of glycolipids on the interactions between cell membranes, ii) to quantify the relevant forces that mediate those interactions, and iii) to estimate the carbohydrate densities on cell surfaces that could support cell adhesion. Despite the increased range of th steric repulsion between the lipid bilayers that these bulky headgroups confer, membranes displaying neutral carbohydrates did adhere. Furthermore, while bilayers bearing negatively charged sugars repelled each other, both in the absence and in the presence of calcium, in the presence of calcium, neutral glycolipids appeared to attract some negatively charged sugars. The possible forces responsible for these interactions, as well as their implications for cell-cell adhesion, will be discussed.

**4:00 PM EE2.7**
PHOSPHOINOSITIDES AS PROBES FOR PROTEIN KINASES. Jan W. Thuring, Gavin 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)-phosphatidyl inositols [(D)-PtdIns] constitute an important class of phospholipids that play key roles in signal transduction. These phospholipids are derived from PtdIns through the action of distinct phosphoinositide-3-kinases (e.g. PDK1 which is an activator of PKB/Akt which is known to mediate several signal transduction pathways). We have prepared and immobilized (D)-inositol phospholipids on a solid support via the fatty acid side chains. The resulting affinity matrices have been used to isolate and characterize various (D)-PtdIns binding proteins. This paper will discuss the advances that modern solid state materials can provide in helping to identify the structures of new proteins which regulate PtdIns binding.

**4:15 PM EE2.8**
VISIBLE LIGHT INITIATED POLYMERIZATION OF ACRYLATE FUNCTIONALIZED PHOSPHATIDYLCHOLINE MONOLAYERS. Janine Orban, Elliot L. Chaikof, Laboratory of Biomolecular Materials Research, Department of Surgery, Emory University, Atlanta, GA.

Cytomimetic biomaterials derived from an understanding of membrane localized cellular processes provides a rational strategy for the development of biosensors, biofunctional surface coatings, and tissue engineered constructs with improved performance characteristics. In prior investigations, we have stabilized self-assembled acrylate-functionalized phosphatidylcholine (acrylate-PC) monolayers on hydrophobic surfaces by in situ heat initiated 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 1-vinyl 2-pyrrolidone, as an accelerator. The effect of vesicle fusion time, irradiation time, as well as alkylated 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 $H_2O_2$ SENSING FORMED BY THIN FILM ELECTRO-POLYMERIZATION OF THE AMPHIPHILIC DECYL ESTER OF D-TYROSINE. DangDuc Long, Tiean 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 0.20 mM in a pH 6.5 buffered phosphate solution as measured by electrochemical techniques. We demonstrate that electrochemical polymerization of DEDT, via cyclic potential scanning between -0.20 and 1.00 V versus Ag/AgCl in pH 6.5 solution, forms stable films on Pt electrodes. Enzymes such as horseradish peroxidase (HRP) can be either surface adsorbed or physically entrapped within the polymer during electropolymerization. The ability of these thin film covered Pt electrodes to measure varying [$H_2O_2$] concentrations directly, or via enzymatic intermediates, was examined at two different V values. The first is +0.85 V, the potential for direct oxidation of $H_2O_2$. The second is -0.05 V, the potential for reduction of $H_2O_2$. Formation of a DEDT electrode film with stable chronoamperometric response properties for $H_2O_2$ detection is enhanced by carrying out the electropolymerization below the c.m.c. of DEDT. Formation of a DEDT electrode film at [DEDT] above the c.m.c., results in an electrode with a low and time dependent $H_2O_2$ sensitivity level. The -0.05 V potential for $H_2O_2$ sensing is advantageous, since its use avoids electrochemical interferences from compounds such as ascorbate and urate in certain clinical situations. These results demonstrate that stable $H_2O_2$ 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 greatfully acknowledged.)

SESSION EE3: PEG-CONTAINING MATERIALS – MOLECULAR AND BIOLOGICAL PROPERTIES
Chair: Marcel B. Bally
Tuesday Morning, November 30, 1999
Salon A/B (M)

**9:00 AM *EE3.1**
KINETIC VS THERMODYNAMIC CONTROL OF PROTEIN ADSORPTION BY GRAFTED POLYMER LAYERS. Javier Satulovsky, Igal Szleifer, 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 amount of lysozyme and fibrinogen adsorption 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 suggests 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 parameters that determine the 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**
COILING OF CYLINDRICAL MEMBRANE STACKS WITH ANCHORED POLYMERS. Daniel Kandel, Ilan Tsafrir, Joel Stavans, Weizmann Inst of Science, Dept of Physics of Complex Systems, Rehovot, ISRAEL.

Experiments show that polymers with hydrophobic anchors grafted along their hydrophilic 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 CO-POLYMERS ON LIPOSOME STRUCTURE AND STABILITY. Katarina Edwards, Nill Bergstrand, Markus Johnsson, 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 recognition 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 tri-block copolymers, have begun to be explored in the search for alternative strategies. Inclusion of PEG carrying molecules in the liposomal membrane may, however, induce undesirable 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, important bilayer 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? Barry R. Lentz, Department of Biochemistry and Biophysics and Program in Molecular & Cellular Biophysics, University of North Carolina, Chapel Hill, NC.

The sequence of events involved in poly(ethylene glycol)-mediated fusion of 450 *Å* small unilamell-ar ves-icles (SUVs) has been studied. Fusion events were monitored using light scattering for vesicle aggrega-tion; fluorescence lifetime of membrane probe lipids (DPHpPC and NBD-PS) for mem-brane mixing; aqueous fluorescent marker ($Tb^{3+}$/DPA and $H^{+}$/HPTS) for contents 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 mem-branes to near molecular contact. Manipu-lations that reduce packing density in the backbone regions of both contacting membrane leaflets 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 accompa-nied 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-committed 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 continued to a fusion pore in the presence or absence of PEG. The sequence of events and Arrhenius activation energies for this are analogous to those observed for protein-mediated cell mem-brane fusion events, suggesting a commonality between these two processes. Supported by USPHS grant GM32707 to BRL.

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

Various micropipet methods that have been specifically developed since 1980 to study 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 essential materials property data that are required for the successful design and deployment of lipid vesicle capsules in applications such as drug delivery. Here, the strength and compliance of the membrane, its interfacial interactions, its transition properties, and its exchange with drugs and other molecules are of particular interest. Strength, cohesion, and low 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 resist exchange of 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 macromolecules with the lipid surface, but this time by a steric rather than cohesive mechanism. Drug uptake and solubilization by lipid bilayers and micelles have also been shown to be dependent on interface compliance, with softer interfaces binding a greater amount of a drug like paclitaxel. Once retained in the liposomal carrier, it becomes important to release the drug from the carrier for it to act on its target cell, and so temperature, pH or mechanical transitions that result in repartitioning or leakage of the drug is a key feature of more sophisticated systems.

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

One popular method of producing lipid vesicles involves pushing or extruding a lipid suspension through the cylindrical pores of polycarbonate membranes.[1]. By characterizing vesicles produced under different extrusion conditions and from different lipids,[2] we have found that vesicles are only produced above a certain threshold extrusion pressure. This minimum extrusion pressure is related to the lysis, or rupture, tension of the membrane. We have used this fact to develop a method of measuring the lysis tension of vesicles composed of various lipids and lipid mixtures.

[1]M.J. Hope, M.B. Bally, G. Webb, and P.R. Cullis, *Biochim. Biophys. Acta.* **812**, 55-65 (1985).
[2]D.G. Hunter and B.J. Frisken, *Biophys. J.* **74**, 2996-3002 (1998).

SESSION EE4: DRUG AND GENE DELIVERY
Chair: David H. Thompson
Tuesday Afternoon, November 30, 1999
Vermont (M)

**1:30 PM EE4.1**
PHOSPHOLIPID NANOTUBULES: FORMATION AND APPLICATIONS IN TOPICAL DELIVERY. Vitthal Kulkarni, Duncan Aust, James Wilmott, James Hayward, The Collaborative Group, Ltd., East Setauket, NY.

The formation of tubular microstructures from diacetylenic phospholipids or from certain sphingolipids is well documented. The driving force for formation of tubular microstructures develops from the anisotropic packing interactions arising from molecular chirality, strong in-plane intermolecular interactions and tilted orientation of the acyl chains. The tubular microstructures of diacetylenic phospholiipds 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, no applications of tubular microstructures have been recognized in the delivery of health care products. We envision nanotubules to have potential application for the delivery of active ingredients through topical application. With this view, we are investigating formation of the tubular microstructures from mixed systems of phospholipds and naturally occurring sphingolipids. We have successfully formed tubular microstructures from mixed lipid systems consisting of phosphatidylcholine and ceramides. The tubules ranged from 40-60 nm in diameter and 1-2 $\mu$ in length. We are further characterizing these nanotubulear microstructures for various properties including capture volume capacity, stability, toxicity, and skin penetration. We plan to discuss the details of our findings during the presentation.

**1:45 PM EE4.2**
IMPROVING BIOAVAILABLILITY USING PHOSPHOLIPID- AND POLAR LIPID-DRUG CONJUGATES. Milton B. Yatvin, 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 membranes. This selective association provides a possible avenue of drug targeting. A novel aspect of our inventions 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 lipid trafficking of the lipid prodrugs inside the cells 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 lipid 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 POLYELECTROLYTES COMPLEXED WITH CATIONIC LIPID: HIGHER ORDER SELF ASSEMBLY & APPLICATIONS. C.R. Safinya, G.C.L. Wong, A. Lin, N. Slack, Y. Li, A. Martin & T. Pfohl, Materials and Physics Depts and Biochemistry and Molecular Biology Program, University of California, Santa Barbara, CA.

It has been known 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 arises, in part, because they mimic viruses as chemical carriers of therapeutic molecules (functional DNA, peptides) into cells for delivery application [1,2]. The materials also have potential uses as templates for mesoscale micro-machine elements and molecular sieves [3]. We will present synchrotron x-ray diffraction and optical microscopy which has revealed a variety of novel self-assembled phases. Supported by NSF DMR 9972246 and NIH 1R01 GM59288-01.
[1] A.D. Miller, "Cationic Liposomes for Gene Therapy", Angewandte Chemic (International Edition), *Reviews* 37, 1768-1785 (1998).
[2] A. Lin, N. Slack, A. Ahmad, C. George, C. Samuel, C.R. Safinya (submitted); J.O. Raedler, I. Koltover, T. Salditt, C.R. Safinya, *Science* 275, 810 (1997); T. Salditt, I. Koltover, J.O. Raedler, C.R. Safinya, *Phys. Rev. Lett.* 79, 2582 (1997); I. Koltover, T. Salditt, J.O. Raedler, C.R. Safinya, *Science* 281, 78-81 (1998); C.R. Sarifinya, I. Koltover, J.O. Raedler, Curr. Opin. Coll. & Interf. Sci. 3, 69 (1998).
[3] G.C.L. Wong, Y. Li, I. Koltover, C.R. Safinya, Z. Cai, W. Yun, Appl. Phys. Lett. 73 (14), 2042 (1998).

**2:30 PM EE4.4**
MONOLAYER FORMATION AFTER ADDITION OF PLASMID 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 cationic lipids and DNA (Lasic and Templeton, 1996). It is also known that two dimensional columnar invertebrate hexagonal lattices ($H^c_{II}$) are generated when 1,2dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE)-composed cationic liposomes interact with DNA. This configuration is more efficient in terms of transfection activity compared to the lamellar phase (L$\alpha$c) formed when 1,2 dioleoyl-sn-glycero-3-phosphocholine (DOPC) is used as the helper lipid (Koltover, et al 1998). However, it is not clear whether bound lipids are organized in a bi-layer structure or in hydrophobic complex with DNA, surrounded by lipid monolayer. In an effort to clarify the macromolecular 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 contained both lipids in a (1:1 molar ratio) as 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 supports our intention that destabilization of liposomes can generate lipids in a micellar or monomer 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 argued 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**
POLYMORPHIC STRUCTURE OF CATIONIC LIPOSOME-DNA COMPLEXES. Brigitte Sternberg-Papahadjopoulos, Keehung Hong, Weiwen Zhong, Demetrios Papahadjopoulos, California Pacific Medical Center, Research Institute, San Francisco, CA.

Complexes formed during interaction of cationic lipsomes with polynucleotides such as DNA (CLDC) belong to the family of liquid-based non-viral vectors. They self-assemble into a variety of polymorphic structures, including non-bilayer, *honeycomb*-type structure ($H_{11}$), and bilayer structures such as *spaghetti/meatball*-type structures, spherical and invaginated particles, oligolamellar structures, and *map-pin* structures.
We have chosen mainly freeze-fracture electron microscopy but also cryo-electron microscopy for recording the polymorphic structures, and for studying factors and conditions triggering the formation and stabilization of specific structure types. Furthermore, we took microscopically snapshots of the interaction 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 vivo* and *in vitro* of CLDC, 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 CLDC and cultured cells, showed frequently endocytosis events after incubation times of 2-4 hours. However, after short incubation times of 10-30 minutes, fibrillar *spaghetti*-like structures were frequently observed intact and inside the cells. Comparison of *in vitro* transfection activity of CLDC measured on SK-BR-3 cells with their *in vivo* transfection activity expressed in mouse 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, serum-stable complexes coexisting 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 polyelectrolyte reaction 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 isothiuronium headgroups. The detergents have alkyl chains of C8 - C12 and contain hydrophilic isothiuronium headgroups that give relatively high critical micelle concentrations (CMC) to the detergents (> 10 mM). The isothiuronium group also masks a sulfhydryl group on the detergent. However, the isothiuronium 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 isothiuronium group, the CMC of the surfactants, 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 ~ -13 mV zeta 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 cleavable 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 PM EE4.7**
DRUG AND GENE DELIVERY APPLICATIONS OF SYNTHETIC ACID-LABILE DIPLASMENYL LIPIDS. Jeremy A. Boomer, Marquita M. Qualls, Junhwa Shin, David H. Thompson, Dept. of Chemistry, Purdue University, West Lafeyette, 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 L$\alpha$ to HII phase transitions within these acidic sites. Previously, we have demonstrated that plasmenylcholine-type lipids have excellent acid hydrolysis and contents release kinetics (Gerasimov et al., Biochim. Biophis. Acta. 1997, 1324, 200-214; Rui et al., J. Am. Chem. Soc. 1998, 120, 11213-11218). We now report the synthesis and bioactivity of three new acid sensitive lipids, based on a chiral 1,2-di-O-(1Z*/*, 9Z*/*-octadecadienyl)-sn-glycerol platform, that employ phosphocholine, poly(ethyleneoxide), and O-carbamoyl-N-diethylenetriamine headgroups. The release kinetics and transfection activity of these materials will be described.

**4:15 PM EE4.8**
DISRUPTION OF MIXED SURFACTANT LIPOSOMES BY CATIONIC POLYAMIDOAMINE DENDRIMERS. Kerill Titiyevskiy, Michael Grey, James L. Thomas, Department of Chemical Engineering and Applied Chemistry, Columbia University, 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 spectroscopies have been used to characterize dendrimer-membrane interactions. Using a self-quenching fluorescent dye, we find that membranes containing a non-lamellar, anionic lipid (either stearic or oleic acid), stabilized with phosphatidylcholine (PC) or phosphatidylethanolamine, are transiently permeabilized by higher generation dendrimers, while pure PC liposomes are unperturbed. EPR measurements on spin-labelled lipids in vesicles have been used to characterize the membrane reorganization induced by dendrimers. In addition, measurement of the binding of dendrimers to unilamellar vesicles, using a centrifugation assay, show tight complexation of the polycationic polymer and polyanionic vesicles.

**4:30 PM *EE4.9**
STABILIZED PLASMID-LIPID PARTICLES FOR SYSTEMIC GENE DELIVERY. Pieter R. Cullis, Biochemistry Department, University of British Columbia, Vancouver, CANADA; Inex Pharmaceuticals, Burnaby, CANADA.

Gene therapies for systemic diseases such as cancer or inflammatory disorders clearly require systemic vectors. However, currently available gene delivery systems have limited utility for systemic applications. Viral systems are rapidly cleared from the circulation following intravenous (i.v.) injection, limiting potential transfection sites to first pass organs such as the lung, 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 small, long -circulating liposomes results in preferential accumulation at tumour sites. It therefore follows that encapsulation of plasmid in small liposomal systems should result in enhanced delivery to tumour sites. A method for encapsulating plasmid in small (diameter 70 nm) stabilized plasmid-lipid particles (SPLP) employing a detergent dialysis procedure will be described. These SPLP contain one plasmid per particle and are stabilized in aqueous media by the presence of a poly(ethyleneglycol) (PEG) coating. It will also be demonstrated that SPLP exhibit extended circulation lifetimes following i.v. injection and promote delivery of intact plasmid to a distal tumour site, with concomitant reporter gene expression. Initial studies utilizing SPLP for suicide gene therapy applications employing thymidine kinase gene delivery in combination with ganciclovir will also be reported.

## SESSION EE5: POSTER SESSION
Chairs: Phillip B. Messersmith and Joel M. Schnur
Tuesday Evening, November 30, 1999
8:00 P.M.
Exhibition Hall D (H)

**EE5.1**
SPATIALLY-RESOLVED ELECTRON ENERGY-LOSS MAPPING OF LIPIDS IN A MULTI-COMPONENT LIPID/PROTEIN SYSTEM. A. Aitouchen, S. Shi*, M. Libera and M. Misra* Stevens Institute of Technology, Hoboken, NJ. *Unilever Research, Edgewater, NJ.

Because of their amphiphilic 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 on length scales 2-100 nm, and morphological studies of such structures 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 currently are developing alternate approaches to generate image contrast based on spatially resolved electron energy-loss spectroscopy. This 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 these lipid species have characteristic spectroscopic fingerprints due to both pi 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 bovine serum albumin (BSA). Multiple-least squares fitting of characteristic reference spectra from each of these three materials is used to deconvolute the spatial distribution of ceramide, cholesterol, and BSA 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. Hayat Onyuksel, Manisha Patel, Syed Akhter, Israel Rubinstein, Depts 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 amphiphilic peptides/proteins. The purpose of this study was to determine the changes in molecular conformation and bioactivity of a 28 amino acid neuropeptide, vasoactive intestinal peptide (VIP), after self-association with phospholipid micelles. Phospholipid micelles, about 17 nm in size, were prepared by dispersing distearoyl-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 and absence of micelles was determined by circular dichroism. The vasodilatant activity of VIP as aqueous and micellar 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 helixicity increased with temperature. The bioactivity of VIP significantly increased in a dose dependent fashion in the presence of micelles (3±1% versus 10±1% with 0.01 nmol VIP and 9±2% versus 26±2% with 0.1nmol VIP, n=4, $p<0.05$). In conclusion, phospholipid micelles can modify the conformation of an amphiphilic 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.

**EE5.3**
INFLUENCE OF DIACETYLENIC FUNCTIONALITY ON THE SELF-ASSEMBLING PROPERTIES OF PHOSPHOLIPIDS.
Alok Singh, Center for Bio/Molecular Science & Engineering, Naval Research Laboratory, Washington, DC.

Considerable efforts have been devoted to understand transformation of microscopic morphologies in bilayers membranes derived from phospholipids. Diacetylenic moiety incorporated in the acyl chains of a phospholipid is known to exert such an influence by transforming multilamellar vesicles into tubules. Two types of phospholipids have been studied for their ability to modulate microstructure morphologies. In the first example, a charge neutral phospholipid, 1,2 bis(heptacosa-8,10-diynoyl)-sn-glycero-3-phosphocholine has been used in the preparation of giant unilamellar vesicles (GUV) by applying electric field to its aqueous dispersion. GUVs underwent efficient photo-polymerization process. Unpolymerized GUVs showed structural stability over a large temperature range. In a second example, morphologies from a diacetylenic phospholipid equipped with charged headgroup consisting of iminodiacetic acid functionality has been examined. Iminidiacetic moiety is connected to phosphate group by a linker consisting of 1-3 ethylenoxy units. Thus, in the presence of metal ion phospholipid with one ethylenoxy linker produced bilayer strands, with diethylenoxy linker produced ribbons, and the one with triethyelenoxy linker produced helices and helically wrapped tubules. These results are more interesting due to fact that the racemic phospholipids were used in the study.

**EE5.4**
CATIONIC SINGLE-CHAIN BOLAAMPHIPHILES AS NEW MATERIALS FOR APPLICATIONS IN MEDICINE AND BIOTECHNOLOGY. Volkmar Weissig and Vladimir P. Torchilin, Department of Pharmaceutical Sciences, Northeastern University, Boston, MA.

Liposome 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 new properties is ever increasing. A vast body of literature exists about the structure, dynamics, and phase behavior of amphiphilic aggregates composed of either single-chain, two-chain, or four-chain polar lipids. Also, two-chain bolaamphiphiles, 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 dequalinium, which we call DQAsomes [1]. Dequalinium is a quinolinium derivative in which two positive charge centers are connected by a hydrocarbon chain consisting of ten CH2 groups. This molecule, therefore, represents a complete new class of amphiphiles, which we name single-chain bolaamphiphiles. We have analyzed the self-assembly behavior of single-chain bolaamphiphiles using Monte Carlo simulations [2] and in a structure-activity relationship study testing a series of dequalinium derivatives (synthesized by C. R. Ganellin, London). These studies have revealed essential str uctural requirements for stable vesicle formation [3]. Based on the intrinsic properties of dequalinium, such as the in vivo selectivity for carcinoma cells and selective accumulation in mitochondria, we propose DQAsomes as a novel and unique drug and gene delivery system.
References [1] V. Weissig et al (1998) Pharmaceutical Research 15, 334-337;
[2] V. Weissig et al (1998) Proceed. Intl. Symp. Control. Rel. Bioact. Mater. 25, 312;
[3] V. Weissig, et al (1999) Proceed. Intl. Symp. Control. Rel. Bioact. Mater. 26, in press.

**EE5.5**
LIPOCORES: NOVEL DRUG-RICH NANOPARTICLES.
Walter R. Perkins, Eric Mayhew, Andrew S. Janoff, The Liposome Company, Princeton, NJ.

We describe here the physical characteristics of drug-rich stable spherical particles (lipocores) and the process by which they are made. Unlike conventional lipid-based particles (i.e., liposomes, emulsions, micelles), lipocores are comprised solely of a core of a poorly water soluble drug surrounded by PEG-lipid (or a negatively charged lipid). Consequently, the drug/lipid ratio of these particles is high (typically 9:1 and up to 49:1, mol/mol). Lipocores have been made with DSPE-PEG(2000) combined with the antibiotic hamycin or with acylated derivatives of paclitaxel or vinblastine. Bromine was included at the a-carbon of the acyl chain for paclitaxel derivatives (hence, BrC16-T, where C16 is the acyl chain length). For BrC16-T lipocores, the particles were stable for many months and exhibited anti-cancer activity concomitant with reduced toxicity in mouse models. Lipocores of 30 - 100 nm mean diameter were produced by an ethanol injection process and from turbidimetry it appeared that particle formation was complete within approximately 10 sec. For paclitaxel derivatives, lipocore 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 particles occurred regardless of the type of PEG-phospholipid used (i.e., acyl chain length, chain saturation, and polymer length) and could also be formed using the negatively charged lipid N-glutaryl-DOPE. Large aggregates resulted when PC was combined with BrC16-T, indicating that a steric or electrostatic barrier is critical for particle stability. From cryo- and freeze-fracture EM, lipocores appeared to be solid spheres without internal lamellae. BrC16-T/DSPE-PEG(2000) particles had no measurable aqueous captured volume and estimation of the surface area subtended per lipid suggested that each particle was coated with a single lipid monolayer. H-NMR, fluorescence anisotropy (DPH) and order parameter (10-doxyl-nonadecane) measurements revealed that motions within the particles were highly restricted.

**EE5.6**
PREPARATION, PHYSICAL AND BIOLOGICAL PROPERTIES OF LIPOSOMES PREPARED FROM CANCEROSTATIC ACTIVE ALKYLPHOSPHOLIPIDS. Reiner Zeisig, Dieter Arndt and Iduna Fichtner, Max-Delbruck-Center for Molecular Medicine, Berlin, GERMANY.

Alkylphospholipids (APL) are synthetic etherlipid like compounds with high anticancer activity against hormone independent human mammary carcinomas. 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 LUVET with an amount of about 50 mol% CH. The stability correlates with the incorporation of APL liposomes into a target model monolayer consisting of PC, PE, PS and CH, and with the induction of marker release from model liposomes of same composition. Resonance energy transfer experiments demonstrated not a clear 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 $\mu$M. 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. Sterical stabilization of APL liposomes significantly reduces liposome uptake by macrophages as a result of an increase of the fixed aqueous layer thickness (FALT) of liposomes from 0.83 nm to 3.57 nm and inhibit thus activation effects. Human mammary carcinoma xenotransplanted 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 cancerostatic formulations in investigated models.

**EE5.7**
LIPOMASC$^{TM}$ PROTOTYPES: LIPOSOMAL VEHICLES FOR ENHANCED TUMOUR TARGETING AND DELIVERY.
Ajay K. Agrawal, John Brew, Cristina Delgado, Derek Fisher, Robert G. Buckley and Gillian E. Francis, Poly MASC Pharmaceuticals Plc, London, UNITED KINGDOM.

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 extrusion into the tumour, tumour to blood cocentration ratios are reduced well below 1 [1]. This effect can be avoided by using various special formulations of PEG-liposome (modulating both the PEG and lipid composition) which impact on other factors, not merely ci rculation time. PEGylated liposomes were prepared using external PEGylation of preformed vesicles and tested for tumour localisation in colon carcinoma xenografts (LS147T). At 24 hours, tumour:blood concetration ratios were 2.22±0.19 mean± SEM, n=22 for all l i p o MASC $^{TM}$ preparations tested and no values were below 1. In comparison, a ratio of 0.3 was reported for Stealth $^{TM}$ liposomes [2]. In addition, the % injected dose per g of tumour at 24 h post-injection for the l i p o MASC $^{TM}$ prototype E was 4.51±0.39 (n=17) well above the 2.67 % injected dose p g of tumour reported for Doxil $^{TM}$[3]. In part, the improved tumour uptake was due to PEG with 144%±23% (n=14) of unPEGylated control values. In contrast to Stealth $^{TM}$ liposomes where the the addition of PEG decreased 24h tumour:blood ratios from 0.5 to 0.3 in l i p o MASC $^{TM}$ prototypes B to G had tumour:blood concentration ratios 128%±12% (n=14) higher than unPEGylated controls, indicating a fundamental difference in at least some factors impacting on tumour localisation. With respect to mormal tissues, PEG also improved tumour:muscle concentration ratios (163%±33%, n=14) and tumour:colon concentration ratios (126%±19%, n=14). l i p o MASC $^{TM}$ prototype E has been succesfully loaded with a weak base using remote loading by proton gradient and has therefore potential for enhanced delivery

of cytotoxics to tumour tissue with reduced toxicity.
[1] Francis et al. J. 1996 Drug Targeting 3:321-340).
[2] Martin et al 1991 WO/91 05546.
[3] Vaage et al 1994, Cancer 73:1478-1484.

**EE5.8**
MOLECULAR DESIGN OF NOVEL PHOSPHOLIPID MUTANTS AND CHARACTERIZATION OF THEIR AGGREGATES UPON SELF-ASSEMBLY. Santanu Bhattacharya, Saubhik Haldar, Indian Institute of Science, Dept of Organic Chemistry, Bangalore, INDIA.

To decipher the roles of the hydrocarbon chain-lipid backbone linkage functionalities on the properties of the phosphatidylcholine (PC) lipids toward the formation of bilayer membranes, a series of mutants of dipalmitoyl phosphatidylcholine have been synthesized. In these phospholipids either one or both (O-C=O) linkages at the hydrocarbon-glycerol backbone of the phospholipids have been replaced with $CH_2CH_2$-units keeping the chain lengths of these lipids identical with the corresponding 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 entrapment studies. The biophysical characterizations of the membranes formed from these mutant lipids reveal that the thermotropic transition parameters such as $T_m$, $\Delta H$, $C.U.$ and membrane permeability all depend significantly with chain-linkages. Results of these studies will be presented.

**EE5.9**
COMPARISON ON PRECIPITATION OF CALCIUM PHOSPHATE BY ORGANIC MONOLAYER, UNILAMELLAR PHOSPHOLIPID VESICLES AND HYDROTHERMAL SELF-ASSEMBLY. F.Z. Cui, Y. Zhang, Z.S. Luo and Q. Cai, Department of Materials Science and Engineering, Tsinghua University, Beijing, PR CHINA.

Biomineralization centers around the idea that organics control the nucleation, growth and form of inorganics. As ubiquitous biological amphiphiles, phospholipid has been extensively utilized as a model to study biomineralization. The present studies use phospholipid and other organics to investigate the effects of their assemblies on the precipitation of inorganics. It shows that the morphology of templating organics has essential effects on both the phase and the constructs of inorganics. Organic monolayer templated calcium phosphate, unilamellar phospholipid vesicles templated calcium phosphate and hydrothermally self-assembled calcium phosphate were investigated. The results revealed that hydroxyapatite minerals could be consistently formed in all the 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 mesolamellae self-organized in the precursor sol-gel.

**EE5.10**
PEPTIDE-BASED BIOMOLECULAR MATERIALS: TAPES, FIBRILS AND FIBRES. A. Aggeli, M. Bell, N. Boden, L. Carrick, R. Harding, I.A. Nyrkova and A.N. Semenov, Centre for Self-Organising Molecular Systems, University of Leeds, Leeds, UNITED KINGDOM.

Our aim is to harness biomolecular self-assembly, and in particular protein-like self-assembly, to engineer novel, multifunctional biomaterials. We have rationally designed [1], [2] *de novo* peptides (7-25 residues in length), which self-assemble in water or in polar organic solvents, into micrometer long, semi-flexible, $\beta$-sheet, tape-like polymers with scission energies (ie peptide-peptide association energies) comparable to the strength of covalent bonds. At volume fraction of 0.002 the tapes form a three-dimensional network of topological entanglements which converts the solution into transparent self-supporting gel with mesh size of the order of 10 100nms depending on peptide concentration. The mechanical properties of these materials can be controlled by external chemical (eg pH) and physical (eg shear) triggers. We have found that for some peptides that the tapes aggregate into finite stacks (fibrils) with a periodic left-handed twist about their long axis. These fibrils can, in turn, wrap around each other to form rope-like fibres. The fibrils and fibres are far more rigid than the individual tapes and form nematic fluid solutions at volume fractions of 0.001, and nematic gels at volume fraction of 0.01. The fibrils are stabilised by competition between the gain of energy from tape-tape attraction and the loss of energy from the elastic distortion during incorporation of intrinsically twisted $\beta$-sheet tapes into growing stacks. Similarly, the formation of rope-like fibres is driven by attraction between the edges of $\beta$-sheets in neighbouring fibrils. We believe this is a generic model for the behaviour of chiral, tape-like polymers, and that it is also relevant to the formation and extraordinary stability of pathological amyloid fibrils in vivo, such as the ones diagnostic of Alzheimers disease.
[1] Responsive gels formed by the spontaneous self-assembly of peptides into polymeric $\beta$-sheet tapes. A. Aggeli, M. Bell, N. Boden, J.N. Keen, P.F. Knowles, T.C.B. McLeish, M. Pitkeathly and S.E. Radford, **Nature**, 386, 259-262, 1997.
[2] Engineering of peptide-based *-sheet nanotapes, Aggeli, A., Bell, M., Boden, N., Keen, J.N., McLeish, T.C.B., Nyrkova, I., Radford, S.E., & Semenov, A., **Journal of Materials Chemistry**, *Special Issue on Molecular Assemblies and Nanochemistry*, 7, 1135-1145, 1997.

**EE5.11**
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 base to which a tether molecule can be attached to the aggregate to target drug delivery as well as it stablizes the underlying vesicle. The characterization of one such system composed of n-dodecanephosphonic acid with and without a variety of iron(III) salts will be presented as evaluated by transmission electron microscopy (TEM), fluoresence spectroscopy, and static light scattering

## SESSION EE6: TUBULES, TEMPLATES AND POLYMERIZATION

Chairs: Phillip B. Messersmith and Joel M. Schnur
Wednesday Morning, December 1, 1999
Vermont (M)

**8:30 AM *EE6.1**
PHOSPHOLIPID TEMPLATES FOR FABRICATION OF MICRON- AND NANOMETER-SCALE POLYMERIC FILAMENTS. Jeffrey Linhardt, Kevin Thigpen, David Tirrell, California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, CA.

Micromanipulation of phospholipid vesicle membranes can be used to produce micron- and nanometer-scale tubes that serve as effective templates for photopolymerization of water-soluble monomers and prepolymers. The resulting polymeric filaments are tough and elastic, and can be configured in a manner that provides controlled, point-to-point connections between attachment sites arrayed 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 *EE6.2**
METALLIZED DIACETYLENIC LIPID TUBULES, AND SOME OF THEIR APPLICATIONS. Paul E. Schoen, Dan Zabetakis, Ron R. Price, Naval Research Lab, Washington DC.

Diacetylenic PC lipids form micron dimension hollow cylinders with relatively high aspect ratios, a shape which suggests the possibility of applications such as micro encapsulation and RF dielectrics. However, tubules are electrical insulators and are easily destroyed by heat, solvents and mechanical stress. We have developed tubule metallization techniques which endow them with useful electrical and magnetic properties, make them strong and impermeable, and to a limited extent, allow them to be more easily manipulated. Some metallized tubule applications will be discussed, especially their use in dielectric composites.

**9:30 AM EE6.3**
CALORIMETRIC, SPECTROSCOPIC, AND ELECTRON MICROSCOPIC CHARACTERIZATION OF NANOSCALE LIPID ASSEMBLIES FORMED FROM MIXTURES OF POLYMERIZABLE AND NONPOLYMERIZABLE PHOSPHOLIPIDS. Matthew Strege, Phillip Messersmith, Northwestern University, Division of Biological Materials, Chicago, IL.

We recently reported that equimolar mixtures of 1,2-bis(tricosa-10,12-diynoyl)-sn-glycero-3-phosphocholine (DC8,9PC) and 1,2-dinonanoyl-sn-glycero-3-phosphocholine (DNPC) form nanotubules of diameter 50-60 nm and lengths between 10 to greater than 100 micrometers. These nanotubules were found to be stable when stored at 4C, but transformed into an interconnected helical ribbon phase at or above room temperature. In an effort to more fully characterize these phases, we have synthesized isotopically labeled DNPC (deuterated-DNPC). Mixtures of deuterated-DNPC with DC8,9PC were characterized by electron microscopy, scanning

calorimetry and infrared spectroscopy. This research was supported by NIH grant DE12599.

**9:45 AM EE6.4**
SELF-ASSEMBLY OF MICELLAR TUBULES OF THE AMPHIPHILIC DECYL ESTER OF D-TYROSINE AND THEIR ENZYMATIC POLYMERIZATION MEASURED AT THE QUARTZ CRYSTAL MICROBALANCE (QCM) SURFACE. Tiean 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 and surface binding of these aggregates as a function of increasing pH values from 3 to 7. Using the Sauerbray equation to estimate the rigid QCM bound surface mass, we estimate a $pK_{app}$ of 8.3 for the equilibrium involving both the deprotonation of the $\alpha$-NH2 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 inexpensive 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. (Acknowledge Seed Money Grant from Res. Fdn. at UML to Center for Intelligent Biomaterials)

**10:30 AM *EE6.5**
POLYMERIZATION OF LIQUID CRYSTALLINE ASSEMBLIES. David F. 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 appropriate. The lecture will focus on the latter case with particular emphasis on a strategy that relies on the formation of a self-organized assembly from reactive amphiphiles, followed by polymerization of the amphiphiles within the assembly.

**11:00 AM *EE6.6**
CYTOMIMETIC STRATEGIES FOR BIOMATERIAL DESIGN. Elliot L. Chaikof, 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 events and interfacial transport will likely prove to be powerful strategies for improving the clinical performance characteristics of a variety of artificial organ systems.

**11:30 AM EE6.7**
STRUCTURE OF POLYMERIZED LIPOSOMES AS MUCOSAL DELIVERY VEHICLES FOR COMPLEX MOLECULAR DRUGS AND VACCINES. Likan Liang, Elya Bolotin, Kyle Bucher, Fan Ma, Denise Bucher, Kathy Keck, David Fast, Dan Markovic, Hansi Dean, Robert Brey, 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 most liposome compositions 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-di (2,4 octadecyldienoyl)-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 experimentation were 150-200 nm unilamellar vesicles (>90%) and could be effectively charged with hydrophilic complex molecules ranging in molecular weight from 5K - 150 K. Correlation of liposome structure based on polymer content to biological outcome following mucosal administration was assessed for several protein drugs and vaccines. Oral administration of liposomes in mice resulted in serum bioavailability 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 boostable 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 (>80% polymer content) may fail to release drugs systemically.

**11:45 AM EE6.8**
POLYMERSOMES AND BIOMEMBRANES: 2D MATERIAL COMPARISONS. D.E. Discher, J.C-M. Lee, B.M. Discher, Y-Y. Won*, D.S. Ege, F.S. Bates*, D.A. Hammer, University of Pennsylvania, *University of Minnesota.

The multifaceted structure of biomembranes presents some interesting challenges for synthetic analogs. Beneath a relatively impermeable phospholipid bilayer, biomembranes often possess a cross-linked network with an important role in cell resilience and stability. An example, the ~6-fold spectrin network of the red cell, has been directly shown in one line of our studies to elastically sustain shear strains of up to ~200%. Despite this evident solidity, the network is soft, under considerable thermal motion, and with a compressibility comparable to its shear rigidity but much softer than the overlying lipid bilayer's. In steps towards mimicking certain features of such a multilayer structure, giant vesicles were formed from amphiphillic diblocks: PEO-PEE and cross-linkable PEO-PBD (~3 to 4 kD). As in stealth liposomes, the PEO block on these novel 'polymersomes' is shown to serve as a sort of synthetic glycocalyx that generically opposes adhesion and adsorption. The polymer membranes, when not covalently cross-linked, are fluid and have an area compressibility only slightly softer than that of phospholipid vesicles. Despite this softness, vesicle pressurization shows that the polymersome membranes can be far tougher, sustaining ~five-fold greater areal strain before rupture. Preliminary results with cross-linked PEO-PBD polymersomes indicate further enhancement of such toughness as well as rigidity. The polymer results tentatively suggest, however, some advantage of the composite biomembranes to maintaining solidity while remaining soft in in-plane shear.