mobile cations. Binding of one type of a mobile cation is affected significantly by the presence of a second type. While magnesium ions are tighter binders than sodium ions they can be partially repelled from RNA with a moderately high concentration of monovalent ions. This finite system behaves more as a strong electrolyte consistent with Debye Huckel arguments than as a polyelecrolyte that condenses ions around it.

3111-Pos Board B158

The Correlation Between Folding And Activity Of The 10-23 Deoxyribozyme Studied By 3-color ALEX-FRET

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The 10-23 deoxyribozyme is one of the most well-known deoxyribozymes with RNA-cleaving activity, whose folding is typically controlled by the concentration of Mg^{2+} ions. We carried out a systematic study of folding vs. activity of this enzyme and found that they are strongly correlated. We also investigated the effect of single base mutation on folding and activity, and found that the core region plays an important role in the folding and the enzymatic activity of the 10-23 deoxyribozyme.

3112-Pos Board B159

Transition Metal Complexes and the B-to-Z DNA Transition: Investigating the Role of Geometry and Hydration

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A combination of charge-charge interactions with the DNA backbone and site-specific hydrogen bonds to phosphates and base pairs accounts for the unusual ability of $[Co(NH_3)_6]^{3+}$ to drive the B-to-Z transition. We have used circular dichroism (CD) spectroscopy to analyze effects of other stable cobalt, chromium and platinum complexes on the conformation of the DNA copolymer poly[d(G-C)]. We previously reported that a number of octahedral complexes, with hydrogen bonding ligands similar to those in $[Co(NH_3)_6]^{3+}$, also induce the transition. Cationic charge plays a major role in determining their effectiveness, with transition midpoints $\leq$ 10 μM for +3 complexes, but $\geq$ 500 μM for a +1 complex. A series of new +2 complexes with octahedral or square planar geometry have been tested. The transition midpoint was about 100 μM for the octahedral $[Co(NH_3)_5NO_3]^{2+}$. However, the square planar $[Pt(NH_3)_4]^{2+}$ and $[Pt(en)_2]^{2+}$ failed to induce the transition even at 1500 μM, supporting the hypothesis that hydrogen bonding groups in three mutually *cis* positions facing the DNA molecule are required. A number of prior studies have highlighted the importance of hydration in the B-Z conformational equilibrium and osmotic stress measurements in our lab and Donald Rau's showed that addition of an osmolyte such as sucrose induced the transition at even lower $[Co(NH_3)_6]^{3+}$ concentrations. New results indicate that the transition mediated by $[Co(NH_3)_5Cl]^{2+}$ is even more osmotically sensitive. We will continue this line of investigation to explore why more water molecules appear to be displaced by the binding of the +2 complex compared to the +3 complex. Supported by a Towson University Faculty Development and Research Committee grant, a Towson University Undergraduate Research Grant (to B. Ha) and by the Towson University Department of Chemistry.

3113-Pos Board B160

Real-time Optical Assay For Monitoring Nucleic Acid Strand-exchange And Cleavage

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A simple, real-time optical assay has been developed to monitor nucleic acid strand-exchange and DNA/RNA cleavage reactions. The method takes advantage of the property of some guanine-reach oligonucleotides to adopt monomolecular quadruplex conformations in the presence of certain cations. The quadruplex structure is characterized by a significant absorption signal in the long-wavelength range of the ultraviolet region where other secondary structures are transparent. The ''signal'' oligonucleotide is incorporated into a reactant duplex, which is released into solution upon catalysis. The release is accompanied by fast quadruplex formation and the reaction is monitored by optical methods. We describe the use of this assay to monitor (i) strand exchange catalyzed by the HIV-1 nucleocapsid protein (NC), (ii) RNA cleavage by a DNAzyme in the presence of NC and (iii) DNA cleavage by restriction endonucleases. The reactions were studied as a function of temperature, ionic strength and the concentration and sequence of the substrate molecules. The strand-exchange data were analyzed in terms of activation energies and two alternative pathways (''dissociative'' and ''sequential displacement''). The role of NC in strand exchange and RNA secondary structure invasion by the DNAzyme were evaluated. Principles involved in selection of specific recognition sites by DNA-binding proteins will also be discussed.

3114-Pos Board B161

Adsorption Of DNA And PAMAM Dendrimers - At Silica Surfaces And Model Membranes

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The objective in non-viral gene delivery is to enable the passage of DNA over membranes using *e.g.* cationic agents as a way of replacing viral vectors as gene carriers. The study presented here forms part of a larger project, Neonuclei that aims to design a module for DNA packaging, *e.g.* a transcription competent DNA-based particle. The cationic agent used for *in vitro* condensation of DNA is the PAMAM dendrimer of generation 4, highly monodisperse in both size and constitution and with primary amines as functional groups. Upon mixing of the two, DNA undergoes a transition from a semi-flexible coil to a more compact globule due to the electrostatic interaction present, providing protection against DNase activity and also inhibiting the genetic expression.

Neonuclei aims not only to design a module for DNA packaging but also to reveal how this module interacts with the cell and its membranes. The eukaryotic nucleus is surrounded by a double lipid membrane and the intranuclear space itself also contains phospholipids, not in connection with the nuclear envelope. Lipids within nuclei are thought to play a role in cellular signaling and to be linked to the function of the nucleus, possibly stabilizing the chromatin structure.

Here, the interaction between cationic PAMAM dendrimers and DNA is studied with regard to the presence of macroscopic surfaces using *in situ* null ellipsometry, quartz crystal microbalance with dissipation as well as neutron reflectometry. In addition to using bare silica surfaces as substrates, measurements were performed using model membranes composed of deposited DOPC bilayers on solid surfaces. The adsorbed amount, solvent content as well as the layer thickness and the lateral molecular distribution of an adsorbed film exemplifies the important information obtained.

3115-Pos Board B162

Static and Dynamic Light Scattering applications in Protein Crystallogenesis

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In this communication we give many examples about different uses of Static (SLS) and Dynamic Light Scattering (DLS) in a protein crystallography laboratory. Although the advantages of both techniques are well documented in the literature their use in the crystallographic community is almost limited to determining the sample polydispersity index (PDI). Nevertheless, both dispersion techniques can be powerful tools in helping protein crystallogenesis. Here we illustrate some applications of both techniques to test protein, virus, and protein/lipid/detergent solutions. We illustrate how useful and easy is to determine -aside from the PDI- the oligomerization state, the molecular weight, to find pH-dependent aggregations, to predict crystallization conditions through the Second Viral Coefficient, and to measure detergent's CMC and lipid-detergent micelles sizes. In sum, we encourage crystallographers to exploit their DLS/SLS equipments in order to maximize the information about the state and conditions of the protein solution prior to set-up crystallization experiments.

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Membrane Structure III

3116-Pos Board B163

Cholesterol Reverts The Relative Susceptibility Of Sphingomyelin And Phosphatidylcholine To Solubilization By Triton X-100. A P31-NMR Study

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Solubilization of bilayers made of sphingomyelin (SM) requires much less Triton X-100 (TR) than solubilization of bilayers made of phosphatidylcholine (PC). By apparent contrast, partial solubilization of biomembranes results in PC-rich mixed micelles, in which the SM/PC ratio is lower than 1.0, in coexistence with detergent-resistant membranes (DRM), rich in SM (SM/PC>1.0) and cholesterol. Regardless of the question of whether DRM are

liquid-ordered domains (rafts) that exist in the intact membrane prior to the addition of TR, the cause of formation of DRM is not fully understood. Our working hypothesis was that the formation of DRM is due to the established strong binding of SM to cholesterol. This results in the formation of SM=cholesterolcomplexes with a large negative spontaneous curvature, which stabilizes it against solubilization. This interpretation implies that under conditions of incomplete solubilization the solubilized (micellar) fraction arising from bilayers composed of PC and SM, will be rich in SM whereas in cholesterol-containing mixtures, the solubilized fraction will be rich in PC. To test these predictions, we have determined the SM/PC ratio in mixed micelles, using high resolution ^{31} P-NMR, to which non-solubilized phospholipids do not contribute, because of being broadened beyond detection. The results were as expected, thus supporting our working hypothesis (SM/PC$>$ 1.0 in the solubilized fraction of SM-PC liposomes; SM/PC$>$ 1.0 in the solubilization of liposomes made of PC, SM and cholesterol), This supports the hypothesis that the detergent-resistance of SM is due to the relative stability and large negative spontaneous curvature of the SM-cholesterol complexes. The results also demonstrate the strength of ^{31}p-NMR spectroscopy in the investigation of the partial solubilization of specific membrane phospholipids without having to separate the solubilized from non-solubilized fractions.

3117-Pos Board B164

Simple Phenomenological Model and Phase Behavior of Ternary Mixtures of Saturated and Unsaturated Lipids and Cholesterol

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We present a phenomenological theory for the phase behavior of ternary mixtures of cholesterol and saturated and unsaturated lipids, one which describes both liquid and gel phases. It leads to the following description of the mechanism of the phase behavior. In a binary system of the lipids, phase separation occurs when the saturated chains are well ordered, as in the gel phase, simply due to packing effects. In the liquid phase the saturated ones are not sufficiently well ordered for separation to occur. The addition of cholesterol, however, increases the saturated lipid order to the point that phase separation is once again favorable. Our theory addresses this last mechanism, the means by which cholesterol-mediated ordering of membrane lipids leads to liquid-liquid immiscibility. It produces, for the system above the main chain transition of the saturated lipid, phase diagrams in which there can be liquid-liquid phase separation in the ternary system but not in any of the binary ones, while below that temperature it yields the more common phase diagram in which a gel phase, rich in saturated lipid, appears in addition to the two liquid phases.

3118-Pos Board B165

Cholesterol-phospholipid Interactions: New Insights From Surface X-ray Scattering Data

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In cell membranes, cholesterol-enriched domains are presumably involved in a wide variety of cellular processes. Although a number of conceptual models exist, there is no consensus on the molecular mechanism of cholesterol-phospholipid interactions. Here we report on a systematic study of cholesterol-phospholipid interactions in lipid monolayers using Langmuir isotherms, epifluorescence microscopy, synchrotron X-ray reflectivity (XR), and grazing incidence X-ray diffraction (GIXD) techniques. Lipid monolayers consisted of cholesterol-DPPC mixtures with various cholesterol mole fractions (χCHOL from 0 to 1). XR results demonstrate that cholesterol tends to stay in the acyl chains region of DPPC with its hydroxyl group in a proximity to carbonyl groups of the phospholipid. Increase in cholesterol content promotes ordering of the phospholipid acyl chains. Moreover, X-ray and Langmuir isotherm data used in a complimentary manner indicate that in cholesterol-lipid mixture cholesterol molecule craves to grab an additional 10 Å2 of molecular area from the acyl chains directly above the phospholipid headgroups. These results provide a reasonable explanation for the well documented ''condensing effect'' of cholesterol in lipid mixtures. At high cholesterol concentrations the phospholipid headgroups tilt significantly, but even then appear to be incapable of providing an additional 10 Å2 required to enclose the cholesterol molecules. Interestingly, the critical cholesterol concentration at which phospholipids still shield cholesterol molecules is the same as that at which the phase transition from α- to β-region observed with the epifluorescence microscopy. GIXD data yield DPPC crystalline order only in the mixtures with χCHOL below 0.15. At higher χCHOL, cholesterol seizes the places of the acyl chains in the DPPC crystalline lattice at the same stoichiometry as cholesterol and DPPC in the mixture. Diffraction pattern of such mixtures yields a short-range hexagonal packing order with d-spacing increasing as a function of the χCHOL.

3119-Pos Board B166

The Physical Properties Of Model Membranes Containing POPC, POPE And Sterol: A Deuterium NMR Study

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We have investigated the effect of sterol on the physical properties of lipid membranes containing 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE). POPC/POPE/ergosterol, POPC/POPE/cholesterol, and POPC/POPE membranes were studied as a function of temperature using deuterium nuclear magnetic resonance (^{2}H NMR), with POPC and POPE deuterium labeled alternatively. It is found that the presence of ergosterol or cholesterol disorders gel-phase POPC/POPE (1:1) membranes, whereas orders lc-phase membranes. The modulation of lipid orders by ergosterol is less dramatic than that by cholesterol. In addition, the presence of ergosterol or cholesterol modulates the interaction between POPC and POPE. It is of interest that in POPC/POPE/cholesterol both lipid components display identical M_1(T) curves, which does not observed in POPC/POPE/ergosterol and POPC/POPE.

3120-Pos Board B167

Effects of Cholesterol and Unsaturated DOPC Lipid on Chain Packing of Saturated Gel-phase DPPC Bilayers

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Wide angle x-ray scattering (WAXS) from oriented lipid multilayers was used to study the effect of adding cholesterol (Chol) or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) to gel-phase 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers. Small quantities (X<0.10 mole fraction) of both molecules disrupt the tight packing of tilted chains of pure gel-phase DPPC, forming a more disordered, untilted phase. The addition of larger quantities of DOPC causes the sample to phase-separate into a gel phase, characterized by a narrow WAXS peak, and liquid disordered phase, characterized by wide, diffuse WAXS scattering. In contrast, two WAXS peaks indicative of two coexisting phases were not observed in Chol/DPPC mixtures (X_{Chol} = 0.07 to 0.40). Instead, Chol caused a gradual increase in the width of the WAXS peak, consistent with a gradual change from a more gel-like to a more liquid-like state rather than passing through a region of two phase coexistence. Our WAXS data include a huge amount of information. A new method of analysis suggests that WAXS data may provide definitive results relating to the disagreements between previously published phase diagrams for Chol/DPPC.

3121-Pos Board B168

Role Of Membrane Cholesterol Content In The Activity Of Cyclooxygenase-2 (COX-2) In MCF-7 Human Breast Cancer Cells

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Cyclooxygenase-2 (COX-2) and its product PGE_2 are known to increase both angiogenesis and resistance to apoptosis (promoting tumor growth) and to enhance the penetration of cancer cells into adjacent tissues (causing metastasis). Thus, knowing how the activity of COX2 is regulated at the cellular level has implications for breast cancer therapeutic strategies. The goal of this research is to unravel a new molecular mechanism for regulating the activity of COX-2. The proposed molecular mechanism may be elucidated by using the sterol superlattice model. In plasma membranes, arachidonic acid (AA) is released by phospholipase A2 (PLA2). Cyclooxygenase then converts AA to prostaglandins (e.g., PGE_2). The activity of PLA2 is known to vary with membrane cholesterol content in an alternating manner, showing a local minimum at critical sterol mole fractions (C_r) for maximal superlattice formation. Hence, it is logical to hypothesize that the activity of COX (including the isoform COX-2) also varies with cholesterol content in a biphasic manner. In this study, the cholesterol content in MCF-7 human breast cancer cells was decreased systematically by using methyl-beta-cyclodextrin. A biphasic change in COX-2 activity, as monitored by the Cayman COX-2 assay with minor modifications, was observed at certain cell cholesterol content C_{rcell}. The cholesterol content near C_{rcell} could serve as a fine-tuning mechanism to regulate COX-2 activity and PGE_2 production, and consequently, cancer cell growth and metastasis. (supported by DOD, NSF and PDOH)

3122-Pos Board B169

Free Energy Of Cholesterol Transfer In Lipid Bilayers With Varying Degree Of Saturation

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