accuracy. Additionally, phenomena such as splitting events (multiple spots) within a trajectory in a single frame are naturally analyzed by our proposed method.
The model system chosen to investigate these diffusion behaviors are on glass coverslip supported phospholipid bilayers (DLPC, POPC, DMPC and DPPC). Sub-nM solutions of an amphiphilic cationic carbocynanine dye (DiI) with varying hydrophobic chain lengths are equilibrated and movies of diffusing single molecules are acquired at the lipid-water interface by TIRF-microscopy.

413-Pos
Micropatterned Model Membranes Composed of Polymerized and Fluid Lipid Bilayers
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Substrate supported planar lipid bilayers (SPBs) are versatile models of the biological membrane on solid substrates. We have developed a methodology for generating SPBs composed of polymeric and fluid phospholipid bilayers by using a photo-polymerizable diacetylene phospholipid (DiynePC).(1) Polymeric bilayers could be generated with micro-patterns by the conventional photolithography, and the degree of polymerization could be controlled by modulating UV irradiation doses.(2)(3) After removing non-reacted monomers, fluid lipid membranes could be integrated with polymeric bilayers. The presence of preformed polymeric bilayer domains enhanced the incorporation of fluid bilayer membranes into the voids between them.(4) We could also immobilize biological membranes (sarcoplasmic reticulum (SR) membranes from rabbit skeletal muscle) by utilizing mixtures of SR membrane with a short chain phospholipid, 1,2-hexyanoyl-*sn*-glycero-3-phosphocholine (DHPC). These results clearly suggest the possibilities to reconstitute biological membranes on solid substrates for analyzing their properties in a structurally well-defined platform. In the present paper, I discuss on the unique features of the micropatterned composite model membranes and our recent approaches to construct more complex model biological membranes based on them.
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414-Pos
Self-Assembly in Phospholipid DNA - Protein Mixtures With Applications To Complex Formation in Cationic Liposome-Chromatin Systems
Lars Nordenskiöld[1], Viveka Alfredsson[2], Nikolay Berezhnoy[1], Nikolay Korolev[1], Björn Lindman[2], Chenning Lu[1,3], Dan Lundberg[4], Maria Miguel[4].
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We prepared complexes between cationic liposomes (CL) from a mixture of the neutral (DOPC) and the cationic (DOTAP) lipids with either nucleosome core particles (NCP) or chromatin arrays, prepared by in vitro over-expression. Microscopy showed the presence of distinct globules. Fluorescence labelling of the histone protein, DNA and lipid components showed complete co-localization under many conditions at the optical length scale, while separation of the histones from the DNA and lipids was sometimes found.
Cryo-EM confirmed array aggregates with excess of positively charged lipid forming ordered complexes of multilamellar lipids. For complexes with lower cationic charge (50% DOTAP and 50% neutral DOPC), there is an indication of less order. In most Cryo-EM samples the complexes seems to have a ''subunit size'' on the order of 100-200 nm, consistent with DLS data.
Synchrotron SAXS measurements were performed. The X-ray diffraction pattern demonstrates the lamellar Bragg peaks corresponding to an inter-lamellar separation of about 6-8 nm and sometimes presence of an in-plane DNA-DNA peak. This confirms a remarkably interesting phase behaviour for the system of DNA/protein (NCP or chromatin) with lipids. The SAXS and Cryo-TEM data clearly shows the formation of multilamellar aggregates with DNA sandwiched in between. This means that the DNA and the protein histone-octamer complex of the NCP and the chromatin arrays have dissociated. Hence, the question arises where the histone proteins are located, given the demonstrated co-localization at the length scale of the multilamellar complexes (a few hundred nm). One hypothesis is that the histone proteins are partially embedded in the bilayer and partially between the DNA domains. Alternatively, a few 50-100 nm size DNA-lipid complexes cluster, with histones associated in between, a type of associative phase separation.

415-Pos
Entropy Driven Structures and Interactions of Lipid Based Self Assemblies
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At present there is a surge of interest in biophysical research in elucidating collective interactions between cellular proteins, membranes and associated biomolecules leading to supramolecular structures, with the ultimate goal of relating structure to function. We present x-ray scattering data, osmotic stress experiments, cryo-electron microscopy, and optical imaging data, in self assembled systems of charged lipid bilayers and lipid-peptide complexes, which reveal unexpected structures and intermolecular forces not predicted by current electrostatic theories of charged systems. Those structures are reversible and are entropy driven due to the soft nature of the membrane interfaces. We show how membrane composition, charge density, spontaneous curvature, membrane bending rigidity and temperature control the structures and forces in those systems.

416-Pos
Frustrated Phase Transformations in Supported, Interdigitating Lipid Bilayers
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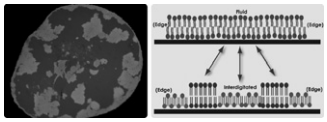
In free bilayers, the fluid to gel main phase transition of a monofluorinated phospholipid (F- DPPC) transforms a disordered fluid bilayer into a fully-interdigitated monolayer consisting of ordered acyl tails. This transformation results in an increase in molecular area and decrease in bilayer thickness. We show that when confined in patches near a solid surface, this reorganiza- tion proceeds under constraints of planar topography and total surface area. One consequence of these constraints is to limit the complete formation of the energetically-favored, interdigi- tated gel phase. The non-interdigitated lipids experience enhanced lateral tension, due to the expansion of the growing interdigitated phase within the constant area. The corresponding rise in equilibrium transition temperatures produces supercooled lipids that vitrify when cooled further. Ultimately, this frustrated phase change reflects a coupling between dynamics and thermodynamics, and gives rise to an unusual phase coexistence characterized by the presence of two qualitatively different gel phases.



417-Pos
Effect of Smooth Bacterial Lipopolysaccharide on the Behavior of DPPC Films
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The airspaces are lined with a DPPC-rich film called pulmonary surfactant, named for its ability to maintain normal respiratory mechanics by reducing surface tension at the air/liquid interface. Inhaled airborne particles containing smooth bacterial lipopolysaccharide (s-LPS) might incorporate into the surfactant monolayer. In this study, we evaluated the effect of s-LPS on the behavior of DPPC films by using epifluorescence microscopy combined with a surface balance. In addition, we investigated whether LPS effects could be counteracted by surfactant protein A (SP-A), which is a LPS binding protein, with the peculiarity that this protein is associated to the surfactant monolayer. Thus SP-A is in the initial defence barrier against inhaled airborne particles. Our data show that s-LPS injected in the aqueous subphase penetrated into DPPC films to form mixed DPPC/s-LPS monolayers. Low amounts of s-LPS fluidized the DPPC monolayer, as demonstrated by fluorescence microscopy and changes in the compressibility modulus. This promoted early collapse and prevented the attainment of high surface pressures. The interaction of SP-A with DPPC/s-LPS film further fluidized the monolayers and facilitated the extraction of s-LPS at surface pressures where SP-A was expelled from the mixed films, suggesting that SP-A is an LPS scavenger. A better understanding of the biophysical properties of lung surfactant monolayer and its susceptibility to LPS inhibition is important for the development of new surfactant formulations for respiratory diseases.

418-Pos
Membrane Curvature Modeling and Lipid Organization in Supported Lipid Bilayers
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Membranes in the cell exist in a wide range of shapes and provide for compartmentalization and transport throughout the cell. Curvature plays an important role in this cellular organization and even the organization of lipids within the membrane itself. Supported lipid bilayers (SLB) continue to be an important means of measuring the thermodynamic and mechanical properties of phospholipid membranes, but on some supports, the proximity of the solid surface may modify the behavior of the adsorbed bilayer. To overcome this problem, we use a technique for spin coating lipids on the substrate that creates multilamellar stacks of membranes[1] where the influence of the substrate on upper layers is weakened. The substrates we have used are nanoscopically patterned with steps and these features induce curvature in the membranes that appear to be step-height dependent. This provides a platform for adhesion, mobility and organizational studies. We show that multilamellar SLB on patterned substrates exhibit curvature induced phase separated domain organization and increased lateral lipid mobility. Molecular dynamics of coarse-grained supported lipid bilayers[2] are used to simulate membranes supported on corrugated surfaces and we discuss and compare the behavior with experimental systems. We show that substrate corrugation height, adhesion energy, and mechanical moduli can be controlled to predict adsorbed membrane curvature. Furthermore we model lipid mixtures in the regions of substrate induced curvature to show the relationship between bending energies and phase separation.
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419-Pos
Concerning a Percolation Concept of Zeta Potential and Electrokinetic Phenomena in Bio-Systems
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The electrokinetic or zeta potential is the basic notion in theory of electrokinetic phenomena. It is defined as electric potential in the interfacial electrical double layer (EDL) at the location of the ''slipping plane'' versus a point in the bulk fluid away from the interface. Many interfacial/electrokinetic phenomena in biological systems can be interpreted in terms of the membrane electrical properties of the cells involved. However, the conventional definition of the zeta potential and a ''slipping plane'' are based on two unrealistic assumptions: (1) that one can realize a procedure of the ''infinitesimal'' change of the continuous layer thickness without changing its topology, during both thinning and growth, and (2) that the interface is ideally homogeneous. These assumptions are already denied with many experimental facts in modern interface science. Instead of the infinitesimal variations of the continuous layer thickness, one has to account topologic transitions from continuous to discrete EDL structures at the interface [1]. During EDL thinning, the hydrodynamic flow stops at a percolation level (effective thickness), corresponding to a discrete film. It brings us to a real and exact meaning of a mythic ''slipping plane''. The zeta potential is the potential corresponding to beginning of percolation of the EDL, or shortly, the ''zeta potential is the percolation potential''. This new paradigm gives effective heuristic keys for quantitative analyzing electrokinetic processes in real disperse systems with heterogeneous interfaces, in part, biointerfaces, such as transport processes in cellular ion channels, cell electrophoresis, electroosmosis, adhesion and fusion [2-3]. Refs.:
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420-Pos
Steady-State Electrochemical Determination of Lipidic Nanotube Diameter Utilizing An Artificial Cell Model
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By exploiting the capabilities of steady-state electrochemical measurements, we have measured the inner diameter of a lipid nanotube using Fick's first law of diffusion in conjunction with an imposed linear concentration gradient of electroactive molecules over the length of the nanotube. Fick's law has been used in this way to provide a direct relationship between the nanotube diameter and the measurable experimental parameters change in current and nanotube length. Catechol was used to determine the change in current attributed to its flux out of the nanotube.
Comparing the nanotube diameter as a function of nanotube length revealed that membrane elastic energy was playing an important role in determining the size of the nanotube and was different when the tube was connected to either end of two vesicles or to a vesicle on one end and a pipette tip on the other. We assume that repulsive interaction between neck regions can be used to explain the trends observed. This theoretical approach based on elastic energy considerations provides a qualitative description consistent with experimental data.

421-Pos
Statistical Thermodynamic Determination of Chemical Potential From Hamiltonian For Sterol Superlattice Domains in Phospholipid Bilayers
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Multiple experiments suggest that at certain mol fractions ($\chi_{sterol} = C_r$) sterol molecules, cholesterol (chol) and ergosterol (erg), form superlattice (SL) structures occupying particular acyl chain sites in a phospholipid bilayer. We have successfully tested a model against our own nystatin-erg channel data and fluorescence measurements of sterol concentration [1]. Using Kirkwood's coupling parameter method we previously obtained partial agreement with chemical potential data at $\chi_{sterol} = 0.4$. Here we report the results of a more appropriate statistical thermodynamic analysis to determine the chemical potential for our model. We obtained the density of states (DOS) using a binning procedure and the recently developed Wang-Landau algorithm [2]. The form of the DOS dictated a numerical summation for the partition function. Piecewise linearity of the statistical thermodynamic Helmholtz Energy yielded plateaus in the chemical potential consistent with experimental data for $0.20 \leq \chi_{sterol} \leq 0.60$ [3].
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422-Pos
Micro- and Nanoscale Devices For Controlling Two-Dimensional Chemistry
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There is an ever increasing need for methods to perform chemical reactions involving individual molecules, or small molecular assemblies in applications ranging from single enzyme dynamics to molecular electronics. To meet these demands, a transition from traditional 3D, to 2D or 1D reactor systems that reduces the dimensionality, and hence exponentially reduces the number of interacting particles would be beneficial. Low-dimensional systems, unlike bulk ensembles, exhibit some degree of order and can be made on small foot-prints using nanofabrication techniques. However, whereas chemistry can easily be performed in e.g. test tubes, and droplets, initiating and controlling chemistry with the same ease on planar surfaces has been a tremendous challenge. We here present a 2D micro-/nano-fluidic technique with such a capability, and where reactant-doped molecular liquid crystal lipid films spread and mix on patterned amphiphilic substrates. These substrates can be micro- and nanopatterned photoresist, or even micropatterned Teflon. Eventually, all reactants are present in two dimensions, mimicking a situation in the lipid bilayer of cells or cell compartments.
Phospholipid monolayer films are spread and contain complementary DNA strands modified with a lipophilic anchor and with a fluorescent dye. By using resonance energy transfer, we monitor the hybridization of the complementary strands, and are able to detect the double-stranded DNA in flowing streams on lanes as small as 250 nm wide, with as few as 900 molecules in the cross section. Our results show that the density and number of different reactants, can be controlled within liquid crystal films confined to patterned substrates. The technology introduced here provides a platform for nanochemistry with the potential for kinetic control where molecules with 2D orientational order can be synthesized, controlled, routed, and probed. Therefore, this technology could become a model system for dynamic biological surfaces.