1968-Plat

Ionization Properties of Mixed Lipid Membranes: A Gouy-Chapman Model of the Electrostatic-Hydrogen Bond Switch

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The dissociation state of phosphatidic acid (PA) in a lipid bilayer is governed by the competition of proton binding and formation of a hydrogen bond through a mechanism termed the electrostatic-hydrogen bond switch. This mechanism has been suggested to provide the basis for the specific recognition of PA by proteins. Even in bare lipid bilayers the electrostatic-hydrogen bond switch is present if the membrane contains lipids like phosphatidylethanolamine that act as hydrogen bond donors. In the present work we incorporate the electrostatic-hydrogen bond switch mechanism into the Gouy-Chapman model for a membrane that is composed of PA and a hydrogen bond-donating zwitterionic lipid. To this end, our model integrates into the Gouy-Chapman approach a recently suggested electrostatic model for zwitterionic lipids. Hydrogen bond formation is incorporated phenomenologically as an additional non-electrostatic interaction between the phosphomonoester headgroup of PA and the zwitterionic lipid headgroup. We express the energetics of the composite membrane in terms of a free energy functional whose minimization leads to a modified non-linear Poisson-Boltzmann equation that we have solved numerically. Our calculations predict the shift of the second pKa of PA as a function of membrane composition, following experimental observation. This shift is large and negative if PA is the minor component in the membrane, and it changes over four pH units as function of the mole fraction of PA in the membrane. In contrast, the shift of the second pKa of PA remains small and is always positive if the zwitterionic lipid is unable to act as hydrogen bond donor. Hence, we find that the electrostatic-hydrogen bond switch mechanism regulates the dissociation state of PA with much greater sensitivity than would be possible based on a pure electrostatic regulation through the membrane potential.

1969-Plat

Dynamics of Sedimentation and Deformability of GUVs in the Presence of an Electrostatic Surface Repulsion

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POPC Giant unilamellar vesicles (GUVs) with 1 mol% NBD-PE were prepared in different sucrose solutions by electroformation. These vesicles were then resuspended in glucose solutions with the same sugar concentrations to obtain isotonic conditions. We studied sedimentation velocities and vesicle deformation through SPIM and confocal microscopy. The results obtained show a relationship between area strain and surface tension that is in agreement with the elastic theory of membranes. The presence of an overall negative charge density in the surface of the glass and a net negative charge in the vesicle membrane leads to an electrostatic repulsive interaction. This is modeled using the linear Deryaguin approximation for an electrolyte solution. We find that the separation distances between GUVs and the glass surface, predicted by the theory, are in the same range than those measured by confocal imaging of sedimented vesicles. These distances range between 1.5-5 μm and influence the hydrodynamic interactions of vesicles moving close to surfaces.

Boundary element simulations were performed to model vesicle deformation during sedimentation within a viscous fluid (Stokés flow) as a function of gravitational pull. A force field is implemented that takes into account local tension, local curvature force, gravitational pull, and the electrostatic interaction with the surface. Vesicle sedimentation, deformation and membrane tension were evaluated as a function of g0, a dimensionless factor relating gravitational and curvature energies. Results from the simulations are in agreement with the experimental results and gave us additional information of the deformability and sedimentation dynamics.

1970-Plat

Determination of Tether Threshold Forces, Interlayer Viscosity, and Thermal Area Expansivity using a Magnetic Force Transducer

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Membrane tether experiments, which involve applying point forces to membranes to form thin tubes of the membrane, provide a powerful method to drastically alter membrane curvature and study membrane mechanics. To study the formation of membrane tethers, we have designed and built a magnetic force transducer (MFT) using microfabrication techniques. Our initial results indicate our ability to control the amplitude of the applied force up to 20 pN over a distance of 50 microns. We have used this device to precisely measure the tether formation force under dynamic loading conditions. Tether formation forces from microaspirated POPC giant unilamellar vesicles (GUVs) containing 0.5-5 mol% biotinylated lipid were found to range from 2 pN up to 19 pN, depending on the concentration of biotin. During the tether retraction phase the applied force is zero and a mechanical model can be applied to calculate the coefficient of interlayer viscosity that opposes relative motion between the inner and outer leaflets of the GUVs. Our results confirm earlier measurements that a significant portion of energy involved in dynamic tether formation and retraction goes into viscous loss. Finally, we noticed that activation of the MFT induced areal strain in the vesicles and studied this at different levels of current. By comparing these measurements to a thermal conduction model of the system, we determined the thermal area expansivity of SOPC GUVs to be 1.7x10-3/°C in the temperature range of 22 to 50°C.

1971-Plat

A simple Thermodynamic Model of the Liquid-Ordered State and the Interactions between Phospholipids and Cholesterol

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A theoretical model is proposed to describe the heat capacity function and the phase behavior of binary mixtures of phospholipids and cholesterol. The central idea is that the liquid-ordered state (Lo) is a thermodynamic state or an ensemble of conformations of the phospholipid, characterized by enthalpy and entropy functions that are intermediate between those of the solid and the liquid-disordered (Ld) states. The values of those thermodynamic functions are such that the Lo state is not appreciably populated in the pure phospholipid, at any temperature, because either the solid or the Ld state have much lower free energies. Cholesterol stabilizes the Lo state by nearest neighbor interactions, giving rise to the appearance of the Lo phase. The model is studied by Monte Carlo simulations on a lattice with nearest neighbor interactions, which are derived from experiment as much as possible. The calculated heat capacity closely resembles that obtained by calorimetry. The phase behavior produced by the model is also in agreement with experimental data. The simulations indicate that separation between solid and Lo phases occurs below the melting temperature of the phospholipid (Tm). Above Tm, only small Ld and Lo domains exist, but no phase separation.

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1972-Plat

Compositional Heterogeneity in Ternary Models for the Cell Membrane

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Ternary models for the cell membrane including cholesterol plus high- and low-melting temperature lipids exhibit rich phase behavior as a function of temperature and composition. Of particular interest is a region of coexisting ordered and disordered fluid phases thought to indicate how lipids organize to promote protein function in the cell membrane. We have used Forster resonance energy transfer (FRET) to investigate two ternary mixtures bSM (porcine brain sphingomyelin)/DOPC (1,2-dioleoyl-sn-glycero-3- phosphatidylcholine)/Chol and bSM/POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine)/Chol at high compositional resolution. We have confirmed liquid coexistence for bSM/DOPC/Chol at 15 and 25C that melts by 35C, but in contrast to one published study (Frazier et al.) we detected no fluid-phase compositional heterogeneity for bSM/POPC/Chol from 5-35C. If domains exist in the POPC-containing mixtures, they must be smaller than the approx. 5 nm sensitivity provided by the fluorescent lipid analogs we employed. We subsequently used electron spin resonance (ESR) to investigate local environment. Order and rotational diffusion parameters were extracted by simulating spectra measured at 9.5 GHz. Similar trends in ESR parameters were observed along approximate tieline trajectories through the expected liquid coexistence region where macroscopic phase domains occur in bSM/DOPC/Chol and where no macroscopic domains are seen in bSM/POPC/Chol, providing support for liquid-liquid heterogeneity in bSM/POPC/Chol.