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Role of Confined Water on the Compressibility Modulus of Lipid Monolayers

## Maria Frias, Cecilia Salcedo, Andrea Cutro, Anibal Disalvo.

Biointerphases and Biomimetics, CITSE, University Santiago del Estero-CONICET, Santiago del Estero, Argentina.

The studies on lipid monolayer properties have been done by compressing the lipid spread in a large water surface up to its collapse. In this procedure, a surface pressure vs area per molecule isotherm can be obtained. In this methodology, it is assumed that the lipids spread at huge areas are considered as being in a gas phase and when they are compressed at a temperature below the critical one (the transition temperature) a condensed phase appears. The thermodynamic treatment is compared with a real gas in which the condensation is analyzed as a consequence of the manifestation of the intermolecular forces between the lipids. In other words, lipid interacts between them as gas particles in vacuum.

A more realistic approach to account on monolayer behavior is that lipids, even at large areas, are in contact with the water phase. Upon compression, the energy input is not merely used to make work of compression but to overcome the friction of hydrated lipid molecules with the water (stationary) phase. Thus, the lipids drag water during its compression, force its reorganization and/or a displacement work can occur. With this picture, the whole thermodynamic of lipid membrane is reevaluated. For this purpose, we analyzed the compression of the monolayer from a state identified as a free-force monolayer state up to the collapse for lipids with different hydration degrees (mainly PCs and PEs). The comparison of these compression curves with the branch of the isotherm obtained by compressing the lipids from the gaseous state allow to conclude that the water arrangements, mainly confined water and hydration water, determines the hysteresis and synergistic effects of membrane response.

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### Adhesion-Induced Domain Formation in Multicomponent Membranes Jan Steinkühler<sup>1</sup>, Reinhard Lipowsky<sup>1</sup>, Peter Hildebrandt<sup>2</sup>,

#### Rumiana Dimova<sup>1</sup>.

Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, <sup>2</sup>Institut für Chemie, Technische Universität Berlin, Berlin, Germany.

Lipid vesicles containing a relatively small number of molecular components provide simple model systems for cell membranes, which contain a large number of lipid and protein components. Such multicomponent membranes can undergo phase separation into two coexisting liquid phases and, thus, exhibit liquid domains within a liquid matrix. The associated phase diagrams depend on a number of parameters such as the overall membrane composition as well as on pH and temperature of the aqueous medium. Here, we address the influence of an adhesive substrate surface onto the phase behavior of the membranes. Specifically, we study giant unilamellar vesicles (GUVs) that can be prepared from different lipid mixtures and have sizes similar to those of cells. We control the adhesion by electrostatic membrane-surface interactions using a novel setup which allows tuning of the adhesion energy by an externally applied potential; see Fig. 1. With our single-vesicle approach, we can explore the phase behaviour of multicomponent membranes for different adhesion energies. One important objective is to identify distinct composition regimes for the phase separation in the bound and unbound part of the adhering vesicle membranes.

Figure -Side views (xz scans) of a one-component GUV (about 60 µm in diameter) obtained by confocal microscopy. Fluorescence of the GUV membrane is shown in green and the adhesion surface as a red line. Left: no applied potential. Right: 1V applied potential. The change in adhesion strength can be clearly seen.

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### Intermembrane Forces and Membrane Deformation Observed via Dehydration and Osmotic Pressure

Jacob J. Kinnun<sup>1</sup>, K.J. Mallikarjunaiah<sup>2</sup>, Luis A. Palacio<sup>1</sup>,

Michael F. Brown<sup>3</sup>, Horia I. Petrache<sup>1</sup>.

<sup>1</sup>Department of Physics, Indiana University-Purdue University Indianapolis, Indianapolis, <sup>2</sup>Department of Chemistry & Biochemistry,

University of Arizona, Tucson, AZ, USA, <sup>3</sup>Departments of Physics and

Chemistry & Biochemistry, University of Arizona, Tucson, AZ, USA.

Intermembrane interactions and forces that govern membrane structure can modulate lipid-protein interactions and thus affect cellular functions [1]. Here we address material properties of the membrane via structural deformation due to external stress using small-angle X-ray scattering (SAXS) and solid-state <sup>2</sup>H NMR spectroscopy. These techniques have been extensively used to study structural changes of membrane bilayer dispersions through application of osmotic pressure. However, distinguishing the effects of osmotic stress on intermembrane forces (separation force) versus membrane deformation requires further investigation [2]. We subjected model membranes (DMPC) in the liquid-crystalline state to dehydration and high osmotic pressures (up to 25 MPa). Using SAXS we were able to directly measure the interlamellar spacings and compare the results to solid-state <sup>2</sup>H NMR order parameters [1,3]. This approach allowed us to gauge the strength of intermembrane forces for a given hydration state and estimate the area per lipid and structural deformation at the molecular level. Under high osmotic pressure or low hydration we found large area deformations of up to 15% [1]. Also, we verified that the intermembrane force decays exponentially as a function of intermembrane distance as described by the hydration force theory [2]. However, temperature variation revealed decay constants of much larger than a single water molecule, possibly suggesting the existence of forces besides the hydration force. To provide insight into this we have introduced the osmotic coefficient (ratio of work of removing water to thermal energy) which distinguishes regimes of forces. These findings show significant area deformation of membranes and provide insight into the forces that govern intermembrane interactions. [1] K.J. Mallikarjunaiah et al. (2011) BJ 100, 98-107. [2] V.A. Parsegian et al.. (1979) PNAS76, 2750-2754 [3] H.I. Petrache and M.F. Brown (2007) Meth. Mol. Biol. 400, 341-353.

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Phenomenological Elasticity Theory Approach to Bolalipid Membranes Timur R. Galimzyanov<sup>1</sup>, Petr I. Kuzmin<sup>2</sup>, Sergey A. Akimov<sup>2</sup>.

<sup>1</sup>Theoretical Physics & Quantum Technologies, NUST "MISiS", Moscow, Russian Federation, <sup>2</sup> Laboratory of Bioelectrochemistry, A.N. Frumkin Institute of Physical Chemistry and Electrochemistry of Russian Academy of Sciences, Moscow, Russian Federation.

Extremophilic bacteria reveal exceptional stability and vitality under the high temperature, pressure and environment acidity. Such features are considerably provided by properties of their membranes composed of bolalipids. Therefore bolalipids appears to be very intriguing and promising object for the investigations. As opposed to ''conventional'' lipids bolalipids are composed of two polar head connected by two hydrocarbonic tails. As a rule they pierce through the whole membrane. At present the investigation of bolalipids is at its opening stage of the gathering of the various experimental and computer simulations data. However, for the effective progress in the investigation of the bolalipid membranes the elasticity theory should be developed. Similar theory applied to the conventional lipid membranes gives results that are in an excellent agreement with the experimental data. That is why we have chosen the way of the adaptation of the effective elasticity theory of lipid membranes to the case of bolalipids.

In the present work we constructed the phenomenological elasticity theory of the bolalipid membranes. The set of all feasible types of deformations are found and the number of the possible experiments for the determining of the elastic moduli are suggested. Theory takes into account the presence of the two configurations of bolalipids in the membrane: U-shape and O-shape configurations. The way of the experimental determination of the ratio of these configurations is proposed.

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# Interaction of Phosphatidylinositol-4,5-Bisphosphate with Potential Clustering Agents Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Cholesterol

Zachary T. Graber<sup>1</sup>, Arne Gericke<sup>2</sup>, Edgar E. Kooijman<sup>3</sup>.

Department of Chemistry, Kent State University, Kent, OH, USA,

2 Department of Chemistry, Worcester Polytechnic Institute, Worcester, MA,

USA, <sup>3</sup>Department of Biology, Kent State University, Kent, OH, USA. Phosphatidylinositol-4,5-bisphosphate  $[PI(4,5)P_2]$  is an important signaling lipid in the cell plasma membrane, playing an important role in many diverse signaling processes. It is important to gain an understanding of how PI(4,5)  $P<sub>2</sub>$ 's role in these signaling processes is regulated. For example, it has been proposed that regulation by  $PI(4,5)P_2$  is based on its lateral localization within the plasma membrane, with multiple pools of  $PI(4,5)P_2$  used for different signaling purposes. In vitro studies have indicated that both  $Ca^{2+}$  and cholesterol have the capacity to promote formation of  $PI(4,5)P_2$  clusters in model membranes. To shed light on this we have examined the interaction of  $PI(4,5)P_2$  with  $Ca^{2+}$ ,  $Mg^{2+}$ , and cholesterol using solid-state MAS <sup>31</sup>P NMR. The solid state 31P-NMR allows us to examine the differential effects of  $Ca^{2+}$ ,  $Mg^{2+}$ , and cholesterol on the 4 and 5 phosphomonesters of  $PI(4,5)P_2$  independently. We examined phosphatidylcholine multilamellar vesicles containing near physiological concentration of PI(4,5)P<sub>2</sub> in the presence of micro and millimolar concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup>. The 4- and 5-phosphates of PI(4,5)P<sub>2</sub> were both found to shift downfield in the presence of  $Ca^{2+}$  and  $Mg^{2+}$ , indicating increased