the wild-type protein, face the apolar environment of the lipid bilayer. We then measured the thermodynamic stability of the wild-type protein and of each of the sequence variants by chemical denaturation. We only made these measurements when the proteins appeared to be at reversible equilibrium between folded and denatured states. We also characterized the folded states of each protein by fluorescence spectroscopy and by a functional assay. Those characterizations revealed additional information about how the lipid bilayers may accommodate an arginine.

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Chain Length Effect on the Association of Fluorescent amphiphiles with lipid bilayer membranes

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The lack of quantitative, kinetic and thermodynamic knowledge regarding the interaction of amphiphiles with lipid bilayers, and the biological and pharmacological relevance of this subject, prompted our group towards a detailed study of those processes [1, 2]. Here we present a detailed study of the interaction of two homologous series of fluorescent amphiphiles (containing one or two acyl chains with different lengths) with a membrane in the liquid disordered phase (POPC). The kinetic rate constants for insertion, desorption, and the corresponding equilibrium partition constants, were obtained. The study was performed as a function of temperature, and the thermodynamic parameters were also obtained.

One of the homologous series studied is a phospholipid labeled with the fluorescent group 7-nitrobenzo-2-oxa-1,3-diazol-4-yl (NBD) in the polar head group and with different lengths of the two acyl chains (NBD-diC_nPE; with n=6, 8, 10, 12 or 14). The other homologous series consists of fatty amines labeled with NBD in the amine group an different *acyl* chain lengths (NBD-C_n; with n=8, 10, 12 or 16).

In contrast to the expectation based on the current model for the transition state in the insertion/desorption process [3, 4], we found a strong dependence between the rate of insertion and the acyl chain length, for both homologous series. The interpretation and implications of the results obtained are discussed.

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Effect Of The Acyl Chain Length On The Translocation Rate Of Amphiphilic Molecules In Liquid Disordered And Liquid Ordered Lipid Bilayers

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Passive transport across cell membrane is a significant route for the permeation of xenobiotics through tight epithelia, such as the vascular endothelium that constitutes the Blood Brain Barrier. One of the most important processes for permeation is transmembrane translocation, which depends on the structure of the permeating molecule and on the properties of the lipid bilayer.

In this work we report on the translocation of two homologous series of fluorescent amphiphiles between the two leaflets of lipid bilayers, in the liquid disordered phase (POPC) and in the liquid ordered phase (SpM:Chol 6:4), using established methods [1]. Both series are labeled with the probe 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) in the polar portion and have acyl chains of different length. One of the series is a phospholipid derivative (NBD-diCnPE; n=6,10 or 14) and the other is a fatty amine (NBD-Cn; n=8, 10, 12, 14 or 16). Along these homologous series, the hydrophilic group is maintained and the hydrophilic/hydrophobic ratio is changed *via* the length of the *acyl* chain. The work was done at different temperatures and the thermodynamic parameters were obtained.

For the fatty amine homologous series, the translocation rate constants recovered show a strong dependence on the length of the *acyl* chain for both lipid phases, being very fast for NBD-C8 and almost 3 orders of magnitude slower for the two longer *acyl* chains. A different behavior was found for the phospholipid homologous series, where the translocation was essentially independent on the *acyl* chain length, showing that for this series the solubilization of the polar head group in the center of the bilayer is the higher energetic barrier in the translocation process.

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1803-Pos Board B647

Unraveling the Role of Protein-Proteins Interactions of Annexin at the Membrane

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Protein-membrane interactions are a vital mechanism of propagating signals both across the membrane and between cells. One method of signal propagation is the formation of lipid microdomains that allow the preferential clustering of specific lipid types and proteins. To address this type of signal propagation, we investigated how lipid microdomains form in response to annexin binding to model membranes. Annexins bind to negatively charged (e.g., phosphatidylserine [PS]) membranes in a calcium-dependent manner and lead to the formation of PS-enriched microdomains in supported planar bilayers. Two distinct mechanisms of signal propagation via protein-lipid binding are addressed. First, we hypothesize that proteins can transmit binding information via the ordering of the lipid acyl chains upon binding. Alternatively, we predict that when a protein binds a specific lipid preferentially, protein-protein interactions are enhanced on a membrane surface. The role of lipid acyl chain ordering and protein-protein interactions as distinct mechanisms of signal propagation through lipid binding will be illustrated.

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Artificial Phospholipid Bilayers On Nano-patterned Gold Surfaces For Biosensing

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As cell surface mimics, supported lipid bilayers are suitable as functional overlayers that enable the study of binding interactions that occur at cell surfaces. These interactions are relevant to cell-cell interactions, and pharmacological applications. Their use however, is limited by the types of surfaces they can reliably be assembled on. We demonstrate the assembly of artificial phospholipid bilayers on gold substrates patterned with a regular array of nano-holes. The lipid layers are characterized by imaging and force indentation using an atomic force microscope. We also demonstrate a biosensor that combines nano-hole arrays, and lipid bilayers.

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Fission Of Lipid Nanotube By Osmotic Pressure

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Formation of new membrane compartments, such as transport vesicles in cells, is finalized by scission of the membrane connection between the vesicle and the parent membrane. To avoid leakage of the vesicle contents, fission has to pass through so called hemifission state, where inner monolayer of membrane neck self-merge while outer preserves its continuity. Creation of hemifission is coupled to generation of high membrane curvature by specialized protein machinery. To reveal the intrinsic behavior of lipid bilayer in this process we studied protein-free fission of membrane nanotubes (NT) subjected to osmotic stress. As expected, lowering of the osmolarity of the external solution caused NT expansion while increasing of the osmolarity produced NT narrowing. We found that osmotic pressure could squeeze NT to a critical radius where non-leaky fission occurred spontaneously. Furthermore, when we progressively increased the amount of cholesterol in the NT membrane to augment its rigidity, the value of the critical radius remained unchanged (corresponding to the lumenal radius of approximately 2 nm). Thus we conclude that membrane rearrangements leading to non-leaky membrane fission can be initiated by a critical narrowing of the membrane tubule.

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Measurement Of Mechanical Parameters Of Lipid Bilayer Form The Deformation Of Membrane Nanotube In Electric Field

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The effect of a longitudinal electric field on the shape of a lipid nanotube formed in an electrolyte solution is considered experimentally and theoretically. Application of a moderate (50-250mV) potential difference between two ends of the nanotube caused the tube expansion so that its shape deflected from the initial cylindrical to the parabolic one. The magnitude of this deviation depends on 1) the potential difference applied, 2) initial lateral tension and 3) bending modulus of the nanotube membrane. This deviation can be quantified as an effective radius of the nanotube determined by the mechanical parameters of the nanotube membrane and the magnitude of the applied electrical field. From the dependence of this radius on the potential difference the values of