ESRF	**Experiment title:** **Lipid-mediated interactions between membrane inclusions**	**Experiment number**: 02-01-732
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Report:

The main goal of these measurements was to determine the interaction potential between inclusions in lipid and surfactant bilayers. We used the SAXS technique to determine the structure factor $S(q)$ of the inclusions in the plane of the membranes. A detailed analysis using concepts from classical liquid theory will be needed to obtain the interaction potential $V(r)$ from $S(q)$, but the quality of the data is very satisfactory and should yield the sought-for information. As a preliminary conclusion, the membrane-mediated interaction is repulsive, and its amplitude changes with the composition of the membrane.

The samples of doped lamellar phases were prepared following the protocols detailed in the proposal[1]. They were held in 100 μm thick flat glass capilaries and we selected the areas of homeotropic orientation (lamellae paralel to the glass walls). The scattering experiments were performed in normal incidence on the capillary walls (i.e. the incident beam was parallel to the layer normal z), so the scattering vector q was mainly contained within the (x,y) plane of the layers. The wavelength was $\lambda = 1.127$ Å and the sample-detector distance 27 cm. The available q-range was about 0.05-0.9 $Å^{-1}$. The typical exposure time was 300 s.

The form factor of the pore $|F(q)|^2$ was obtained from the atomic coordinates of a molecular dynamics simulation of a Gramicidin pore in a bilayer[2]. The structure factor $S(q)$ was described using an analytical hard disk model[3]. The fits are quite good (Figure 1a), and they yield a pore density (Figure 1b) in perfect agreement with the value estimated from the P/L ratio and the areas per Gramicidin and DLPC molecules. However, the fitting values for the effective hard-disk radius R_{HD} decreases systematically with the concentration (Figure 1c). This is a tell-tale sign of a "soft" repulsive interaction[4], and can be easily understood as follows: at low concentration (and therefore pressure), the particles are kept at a distance roughly corresponding to the hard core plus the soft repulsion. As the pressure increases, they are brought closer together and overcome the repulsive barrier. At high enough concentration, the particles only "see" the impenetrable core.

[1] Li, C., D. Constantin, and T. Salditt. 2004. Biomimetic membranes of lipid-peptide model systems prepared on solid support. *J. Phys. Cond. Matt.* 16:S2439-S2453.
[2] de Groot, Bert L., D. Peter Tieleman, Peter Pohl, and Helmut Grubmüller. 2002. Water Permeation through Gramicidin A: Desformylation and the Double Helix: A Molecular Dynamics Study. *Biophys. J.* 82:2934–2942.
[3] Rosenfeld, Y. 1990. Free-energy model for the inhomogeneous hard-sphere fluid in *D* dimensions: Structure factors for the hard-disk (*D*=2) mixtures in simple explicit form. *Phys. Rev. A* 42:5978-5988.
[4] Constantin, D., *et al*. 2007. Interaction of Alamethicin Pores in DMPC Bilayers. *Biophys. J.* 92:3978–3987.

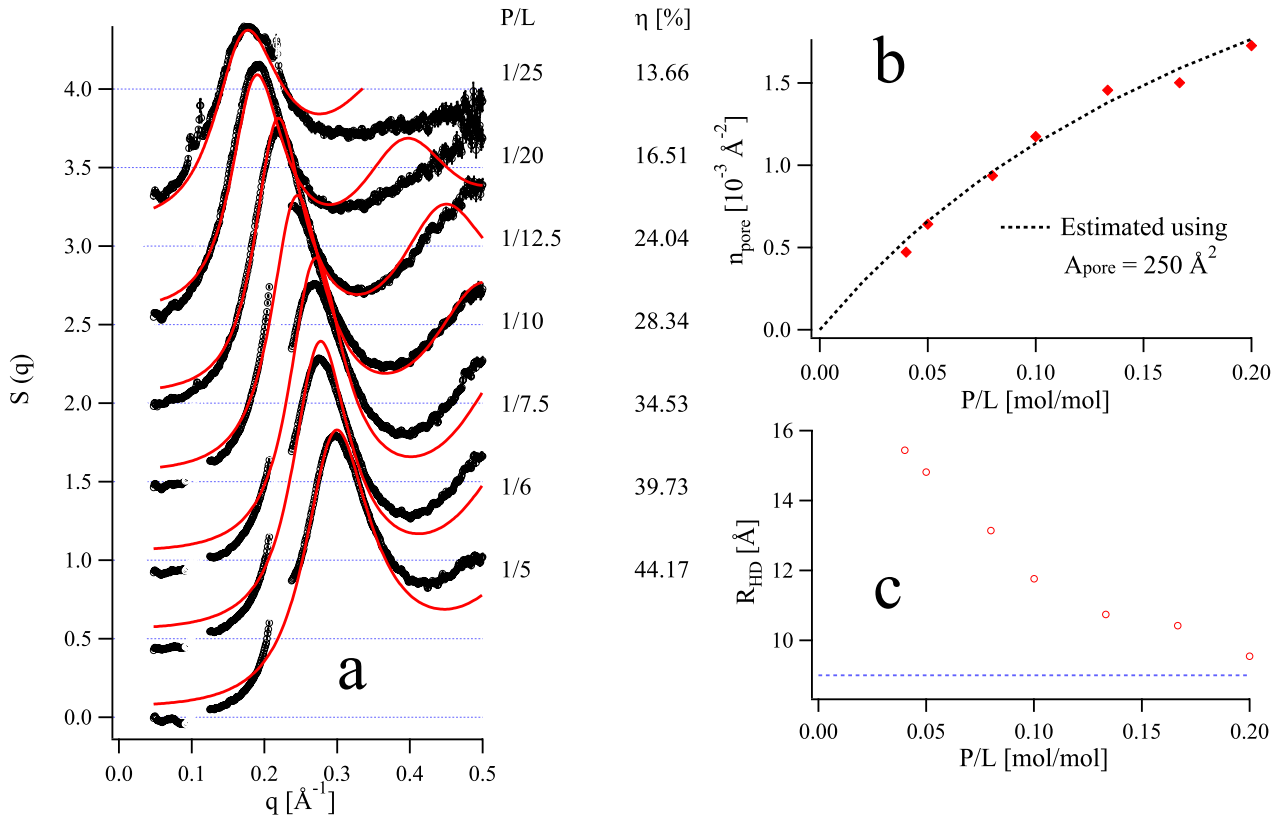

Figure 1: (a) Structure factors (symbols) and hard-disk fits (solid lines) for the Gramicidin/DLPC system, at different peptide/lipid (PL) molar concentrations indicated alongside the curves The theoretical surface fraction of Gramicidin pores, η, is also given. (b) Pore density obtained from the fits in (a) (symbols) and theoretically expected values (dotted line). (c) Effective values for the hard disk radius obtained from the fits in (a) (symbols) and geometrical pore radius (dotted line). The variation in R_{HD} with the pore concentration is a sign of repulsive interaction.

We illustrate this tendency by tracing the effective hard-disk radius R_{HD} as a function of the pore density n_{pore} for the three systems investigated. In spite of its simplicity, this plot gives a clear idea of the change in interaction due to the different bilayer composition (Figure 2).

In particular, the effective pore radius in DDAO bilayers changes very little with the concentration, remaining close to the geometric radius. This is a sign of moderate interaction, since the system is rather well described by the hard-core model.

More elaborate analysis of the data is in progress, aiming to obtain the interaction potential $V(r)$ from a simultaneous fit of all curves[4].

The results should contribute to the understanding of the membrane-mediated interaction between membrane proteins, highly relevant in processes such as protein sorting[5].

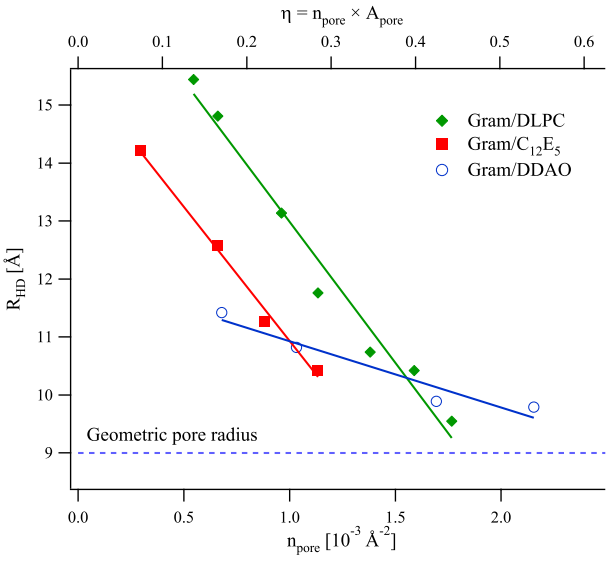

Figure 2: Effective hard disk radius of the gramicidin pore as a function of the density in bilayers with three different compositions. The lines are just guides for the eye.

[5] Killian, J. A. 1998. Hydrophobic mismatch between proteins and lipids in membranes. *BBA – Reviews on Biomembranes.* 1376:401-416.