Experimental report

07/02/2017

Proposal: **INTER-325** **Council:** 4/2016

Title: Internal time on IN13

Research area:

This proposal is a new proposal

Main proposer: **Judith PETERS**

Experimental team: Judith PETERS

Local contacts: Judith PETERS

Samples: DMPC at pH and pH4

Instrument	Requested days	Allocated days	From	To
IN13	4	4	13/06/2016	17/06/2016

Abstract:

Report on June 2016 IN13 experiment Inter 325

Dynamics of DMPC under different pH conditions

In 2015 we have studied the effect of a stress on biological systems, precisely when the samples were not in their native environment measuring membranes of different extremophile and mesophile organisms at pH 7 and pH 4 [1]. We found that for some bacteria like *A. aeolicus* the membrane fragments were a lot more flexible at pH 7 than at pH 4, for other bacteria like *A. ferrooxidans* the membranes were just slightly more or even less flexible at pH 7. In order to better understand the measured effects of the pH on the dynamics of the membrane and the impact that proteins have on the flexibility of membranes at different conditions, we have now investigated the dynamics of a sample composed of only one kind of lipids to estimate their contribution within a natural membrane.

Therefore we have measured the scattered elastic incoherent neutron intensities of the phospholipid *1,2-dimyristoyl-snglycero-3-phosphocholine DMPC* (150mg, fully hydrated with $\mathrm{D_2O}$) for a temperature range between 20K and 310K at pH 4.5 and pH 7.4. Data were corrected by subtracting the scattering from the empty sample holder, by taking absorption into account and by normalizing the data to the lowest temperature (20K).

We analyzed first the summed intensities (Figure 1), which are inversely proportional to the mean square displacements (MSD), but have better statistics:

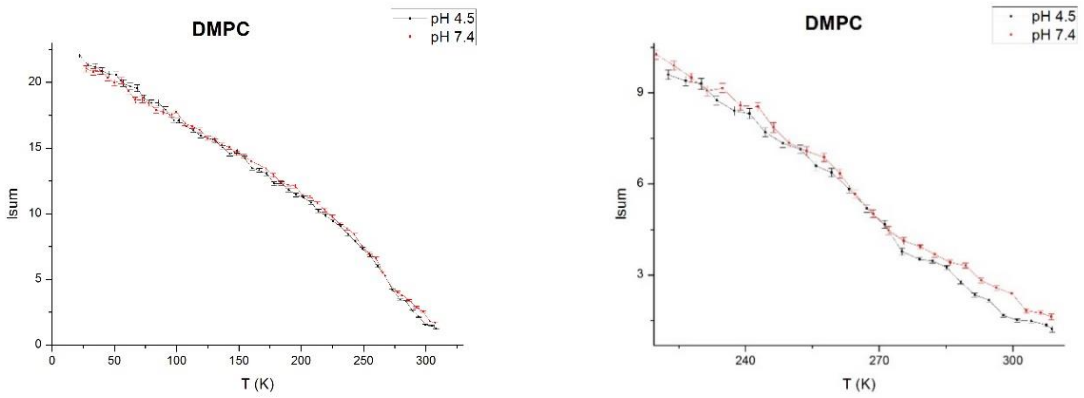

Figure 1: Summed intensities measured on IN13 for DMPC over the total temperature range (left) and at high temperatures (right).

Until around 150K the behavior seems to be independent of the pH value. Between 150K and 260K the summed intensities at pH 7.4 are systematically slightly higher and for more than 275K they are clearly above the ones at pH 4.5, which means that DMPC is less flexible at pH 7.4. This effect can be verified through the MSD that we calculated using the Gaussian approximation (Figure 2).

For temperatures below 285K the MSD at different pH values coincide within their error bars. For temperatures above 288K the MSD at pH 7.4 are clearly lower than at pH 4.5 which indicates that the membrane is less flexible. For both pH-values the change of the slope during the main phase transition at the temperature $\mathrm{T_M} \approx 297\mathrm{K}$ [2] is observable, whereupon the change is slightly weaker for pH 4.5. The observation that $\mathrm{T_M}$ did not change is in accordance with the results of Garidel et al. who measured: $\mathrm{T_M(pH4)} = 297.5\ \mathrm{K}$ and $\mathrm{T_M(pH7)} = 297.2\ \mathrm{K}$ [2].

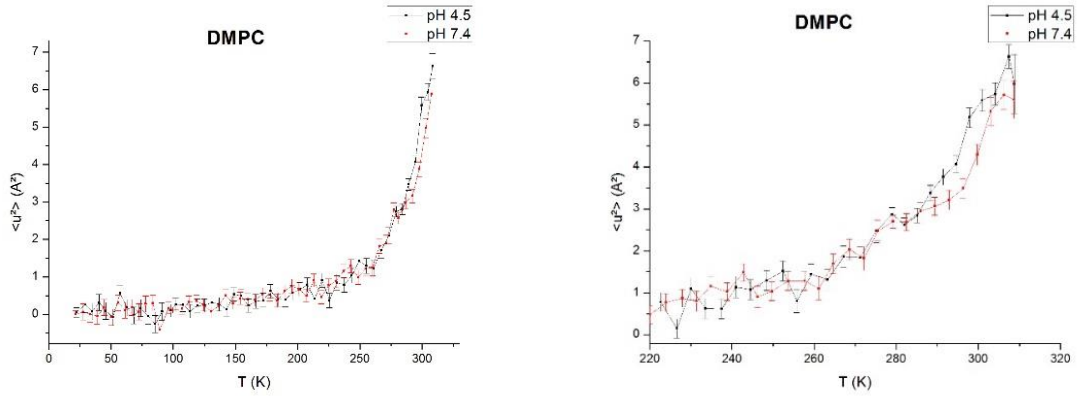

Figure 2: MSD measured on IN13 for DMPC over the whole temperature range (left) and at higher temperatures (right).

Furthermore, we calculated the effective force constant using the definition proposed by G. Zaccaï [3]:

$$< k_{eff} >= 0.00276\left(\frac{d < u^2 >}{dT} \right)^{-1} \qquad (1)$$

for a temperature range between 260K and the temperature of the main phase transition $\mathrm{T_M} = 297\mathrm{K}$.

pH	Temperature	$< k_{eff} >$ (N/m)	Error (N/m)
7.4	260K-297K	0.047	0.005
4.5	260K-297K	0.034	0.003

In 2014, we measured for DMPC at normal pH a force constant of 0.030 ± 0.004 N/m [4] (taking here into account a factor of 6 in the Gaussian approximation instead of 3). The force constants in both experiments are similar but not identical within error bars, differences might be due to different hydration level or preparation of the sample. As DMPC is a membrane lipid it is a lot more flexible than entire membrane fragments including proteins and therefore has a considerably lower force constant.

The flexibility of DMPC is higher at low pH which may be explained by the fact that electric charges are partly abolishing the ordered structure of the membrane lipids. As the differences in the force constants and in the summed intensities for the two pH values are rather small and the main phase transition is still observable at pH 4.5, we can however assume that at pH 4.5 the structure of DMPC is still mostly maintained. This is in agreement with the simulation of Santos et al. predicting that for pH values over pH 4 DMPC is completely deprotonated and therefore is only contributing significantly to titration profile at pH < 4 [5].

References

[1] J.Peters, M. Guiral, MT Giudici-Orticoni, Report CRG 2279.

[2] Garidel, P.; Johann, C.; Blume, A. Biophys. J. 1997, 72, 2196– 2210.

[3] J. Peters, M.T. Giudici-Orticoni, G. Zaccai and M. Guiral, Dynamics measured by neutron scattering correlates with the organization of bioenergetics complexes in natural membranes from hyperthermophile and mesophile bacteria, Eur. Phys. J. E36 (2013), 78.

[4] Trapp, M., Marion, J., Tehei, M., Demé, B., Gutberlet, T., & Peters, J. (2013). High hydrostatic pressure effects investigated by neutron scattering on lipid multilamellar vesicles. *Physical Chemistry Chemical Physics*, *15*(48), 20951-20956.

[5] Santos, H. A., Vila-Viçosa, D., Teixeira, V. H., Baptista, A. M., & Machuqueiro, M. (2015). Constant-pH MD simulations of DMPA/DMPC lipid bilayers. *Journal of chemical theory and computation*, *11*(12), 5973-5979.