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## Elastic scattering studies of aligned DMPC multilayers on different hydrations

Marcus Trapp  
*Institut de Biologie Structurale, France*

Fanni Juranyi  
*LNS, ETH Zurich*

Moeava Tehei  
*University of Wollongong, moeava@uow.edu.au*

Lambert van Eijck  
*Institut Laue Langevin, France*

Bruno Demé  
*Institut Laue Langevin, France*

*See next page for additional authors*

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### Abstract

Biological membranes, consisting mainly of phospholipids and proteins, are organized in a bilayered structure which exhibits dynamical behaviour within time regimes ranging from 10-12 s with the motion of alkyl chain defects and 1 s corresponding to collective excitations of the bilayer [1]. With the prominent role hydration plays on the structural phase behaviour of phospholipids membranes, it is essential for a better description of membranes to understand also the influence of hydration on the dynamics of membrane systems. In the present study we have performed neutron scattering investigations on highly oriented DMPC-d54 multilayers at two different relative humidity (rh) levels. Our results reveal the strong influence of hydration on the local membrane dynamics i.e. head group dynamics.

### Keywords

oriented model membranes, elastic neutron scattering, dynamics, hydration effects, CMMB

### Disciplines

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### Authors

Marcus Trapp, Fanni Juranyi, Moeava Tehei, Lambert van Eijck, Bruno Demé, Thomas Gutberlet, and Judith Peters

# Elastic scattering studies of aligned DMPC multilayers on different hydrations

Marcus Trapp<sup>a</sup>, Fanni Juranyi<sup>b</sup>, Moeava Tehei<sup>c,d</sup>, Lambert van Eijck<sup>e</sup>, Bruno Demé<sup>e</sup>, Thomas Gutberlet<sup>f</sup> and Judith Peters<sup>a,e,g,\*</sup>

<sup>a</sup> *Institut de Biologie Structurale J.-P. Ebel, UMR 5075, CNRS-CEA-UJF, F-38042 Grenoble Cédex 9, France*

<sup>b</sup> *LNS, ETH Zürich & Paul Scherrer Institut, CH-5232 Villigen, Switzerland*

<sup>c</sup> *Australian Institute of Nuclear Science and Engineering (AINSE), Menai, NSW, Australia*

<sup>d</sup> *School of Chemistry and Centre for medical Bioscience, University of Wollongong, Wollongong, NSW 2522, Australia*

<sup>e</sup> *Institut Laue Langevin, F-38042 Grenoble Cédex 9, France*

<sup>f</sup> *JCNS at FRMII, D-85747 Garching, Germany*

<sup>g</sup> *Université Joseph Fourier, F-38042 Grenoble Cédex 9, France*

## Abstract

Biological membranes, consisting mainly of phospholipids and proteins, are organized in a bilayered structure which exhibits dynamical behaviour within time regimes ranging from  $10^{-12}$  s with the motion of alkyl chain defects and 1 s corresponding to collective excitations of the bilayer [1]. With the prominent role hydration plays on the structural phase behaviour of phospholipids membranes, it is essential for a better description of membranes to understand also the influence of hydration on the dynamics of membrane systems. In the present study we have performed neutron scattering investigations on highly oriented DMPC-d54 multilayers at two different relative humidity (rh) levels. Our results reveal the strong influence of hydration on the local membrane dynamics i.e. head group dynamics.

Keywords: oriented model membranes, elastic neutron scattering, dynamics, hydration effects

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\* Corresponding author: Judith Peters, Université Joseph Fourier, F-38042 Grenoble Cédex 9, France, Tel.: +33 476 20 75 60, Fax: +33 476 20 76 88 email: [peters@ill.fr](mailto:peters@ill.fr)

## 1. Introduction

Biological membranes consist not only of different kinds of lipids, but also of membrane proteins and molecules like e.g. cholesterol and ethanol [2]. To investigate the physical and structural behaviour of such complex systems, lipid membranes consisting of only one type of lipid such as 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) serve as suitable model systems.

Saturated phospholipids like DMPC show two phase transitions: 1. a pre-transition from the  $L_{\beta}$  gel phase to the  $P_{\beta}$  ripple phase (DMPC at 286 K) and 2. the main phase transition from the  $P_{\beta}$  ripple phase to the liquid-crystalline  $L_{\alpha}$  phase (DMPC at 296 K) where the mobility of the alkyl chains is enhanced with respect to the gel phase [3]. For chain deuterated lipids which were used in this study the temperature of the main phase transition is shifted by about 3K to lower temperatures [4].

Lowering the water content reduces the repeating distance of the bilayers. In addition, structural investigations [5] show that a lower degree of hydration causes a shift of the main phase transition to higher temperatures. The effect can be observed by X-ray or neutron diffraction following the shift in the distance of the Bragg peaks of the lipid bilayers. The relation  $d = \lambda / 2 \cdot \sin(\theta)$  ( $\lambda$ =X-ray or neutron wavelength,  $\theta$ =scattering angle) allows to calculate the repeating distance  $d$ . For a fully hydrated DMPC bilayer, the repeating distance  $d$  is around 63 Å [6]. While the influence of hydration on the structural properties of membranes is well investigated [5], its influence on dynamic behaviour is rather poor.

The diffusive motions of lipids within the bilayer have been explored by quasi-elastic neutron scattering [1,7,8]. More recently collective motions of the lipid bilayer were also studied with inelastic neutron scattering [9]. However, the hydration effects on the lipid bilayer properties were not enclosed in these investigations. In this study, we thus performed neutron scattering investigations on highly oriented DMPC multilayers at two different relative humidity (rh)

levels with the aim to fill this gap. Our results reveal the strong influence of hydration on the local membrane dynamics.

## **2. Materials and methods**

### **2.1 Sample preparation**

To probe dynamics of lipid membranes in and out of plane, oriented samples can be prepared on very smooth surfaces such as silicon wafers or mica sheets. Solid supported bilayer systems also allow the preparation of large amount of sample (approx. 150-200mg) required in inelastic and quasi-elastic neutron scattering experiments [10]. Another advantage of this preparation method is the very low mosaic spread (below  $0.5^\circ$ ). Each Si-wafer was of size of about 30mm x 40mm which fitted perfectly the dimensions of the flat gold coated aluminium sample cells used for the experiments.

Alkyl chain deuterated 1,2-dimyristoylphosphatidylcholine-d54 (DMPC-d54) was purchased from Avanti Polar Lipids (Alabaster, USA) and used without further purification. The DMPC powder was dissolved in a 3:1 chloroform-trifluoroethanol (TFE) mixture following a protocol described in [11]. This solution was then kept at  $-20^\circ\text{C}$  overnight.

About 30mg of lipid was deposited on a single wafer. After the deposition the wafers were dried for two days in a desiccator. One sample was rehydrated from pure  $\text{D}_2\text{O}$  with relative humidity of 100%, another one was rehydrated from a saturated salt solution ( $\text{D}_2\text{O} + \text{NaCl}$ ) with reduced relative humidity of 75%. Six such wafers were placed in the sample cell and sealed to achieve an amount of about 200 mg hydrated lipid bilayers per sample. The weight of both samples was monitored and no change in weight before and after the neutron experiments was observed.

## 2.2 Elastic neutron experiments

The level of hydration and the evolution of the repeating distance (d-spacing) of the bilayers with temperature were checked by neutron diffraction measurements on the small momentum transfer diffractometer D16 at the Institut Laue-Langevin (ILL), Grenoble, France [12]. After heating to 330K to allow annealing of defects, scans were performed to obtain the repeating distance of the membranes at three different temperatures, namely 330K, 310K and 280K. During the temperature changes data were taken to identify the temperature of the main phase transition. An example of the evolution of the d-spacing with temperature is shown in Fig. 1.

Elastic temperature scans in the range of 280K – 330K were performed on the cold neutron backscattering spectrometer IN16 at ILL at an energy resolution of  $\Delta E = 0.9\mu\text{eV}$  (full width half maximum) and an accessible Q-range of  $0.19 - 1.93 \text{ \AA}^{-1}$  [13]. The investigated temperature range covers both the main phase transition from the  $P_\beta$  ripple to the liquid-crystalline  $L_\alpha$  phase which occurs around 296K for DMPC at full hydration and also the pre-transition from the  $L_\beta$  gel phase to the  $P_\beta$  ripple phase about 10 degrees below the main phase transition.

Special care was taken on the orientation of the sample to probe motions around the chain correlation peak at  $Q=1.48 \text{ \AA}^{-1}$  parallel and perpendicular to the membrane surface. Via the relation  $Q=4\pi\sin(2\theta)/\lambda$  the angle of the sample with respect to the incoming beam was calculated to be  $135^\circ$  for the orientation parallel to the membrane surface and  $45^\circ$  for the perpendicular orientation, respectively. From the obtained data an empty cell was subtracted and the data were normalised by the scattering of a 1mm Vanadium sample.

### 3. Results and discussion

From the shift of the main phase transition temperature to higher temperatures with decreasing water content (see section 1 and Fig. 1 in [5]), it is possible to evaluate the water content between the phospholipid surfaces. We can extract the parameter  $R_w = n_w/n_A$  where  $R_w$  expresses the molar ratio of water ( $n_w$ ) and amphiphile ( $n_A$ ) [14,15]. Fig. 2 (using the left y-axis) shows the summed elastic intensities measured for the parallel orientation at  $135^\circ$  with respect to the incoming beam for the two samples on IN16. At the temperature of the main phase transition a drastic decrease in the elastic intensity occurs. The phase transition temperature moves to higher temperatures for the sample with lower water content. The calculated  $R_w$  for the higher hydrated sample is  $R_w \approx 6$  (transition temperature: 305K) and  $R_w \approx 4$  for the less hydrated sample (transition temperature: 310K). Pfeiffer et al. find for DMPC multilayers a value of  $R_w \geq 12$  for fully hydrated membranes [15].

To characterise the local dynamics from the elastic neutron scattering intensity, the so called mean square displacements (msd)  $\langle u^2 \rangle$  were calculated. The elastic scattering function  $S(Q, \omega=0)$  can be approximated by  $S(Q, \omega=0) \approx \exp(1/6 \langle u^2 \rangle Q^2)$  [16]. From the slope of a linear fit to a semi-logarithmic plot of the data one can extract the  $\langle u^2 \rangle$ . Below the temperature of the main phase transition, coherent scattering arising from the ordering of the alkyl chain, gives rise to the so called “chain correlation peak” around a characteristic Q value of  $1.48 \text{ \AA}^{-1}$ . Therefore the fit range was limited to  $0.18 \text{ \AA}^{-2} \leq Q^2 \leq 1.33 \text{ \AA}^{-2}$  in our data analysis. As shown in Fig. 2 (using the right y-axis) the  $\langle u^2 \rangle$  show transitions at 305 K and 310K for the higher and lower hydrated sample, respectively. At first sight the evaluated mean square displacements are very large above temperature of the main phase transition, but  $\langle u^2 \rangle$  in the same order of magnitude have been observed in a previous study which aimed to investigate the influence of the myelin basic protein (MBP) on the dynamics of model membranes (DMPA in this study) [17].

A recently published molecular-dynamics simulation performed on fully hydrated DMPC

bilayers in the  $L_\alpha$  phase (at 303K) also shows very large  $\langle u^2 \rangle$  at this temperature, even larger than the two partially hydrated samples used in our study [18]. Hence we attribute the difference in the values of the mean square displacements to the different hydrations of the samples, ranging from the highest  $\langle u^2 \rangle$ -value for the simulation ( $\approx 8 \text{ \AA}^2$  for the centre of mass) to the sample with the lowest hydration of  $R_w \approx 4$  ( $\approx 2.9 \text{ \AA}^2$ ). The authors differentiate three different diffusion regimes in time: 1. a ballistic region where  $\langle u^2 \rangle \sim t^2$ ; 2. a subdiffusive domain where  $\langle u^2 \rangle \sim t^\beta$  with  $\beta < 1$  and 3. a domain of Fickian diffusion with  $\langle u^2 \rangle \sim t$ . According to this simulation, the time window of IN16 of about 700 picoseconds probes length scales in the subdiffusive regime. A detailed analysis of quasi-elastic data taken in both the  $L_\beta$  gel and liquid-crystalline  $L_\alpha$  phase is still in progress [19].

In order to compare the results obtained from the mean square displacement (using the right y-axis in Fig.2) with the summed elastic intensities, the intensities shown in fig. 2 (using the left y-axis) were summed over the same Q-range ( $0.43 \text{ \AA}^{-1} \leq Q \leq 1.16 \text{ \AA}^{-1}$ ) which was used in the evaluation of the msd's. The effect is stronger for the summed intensities due to adding up the signals from all detectors in the used Q-range. From hydration dependent studies of e.g. bacteriorhodopsin [20] it is known that with increasing hydration the msd's show a steeper slope. The guide lines in figure 2 show a slight trend as expected but in order to make qualitative conclusions about the slope of the msd's in the liquid phase, a larger temperature range has to be covered.

Rheinstaedter et al. [21] analyzed the elastic intensity at various Q-values of DMPC to map out the transition of the different molecular components from immobile to mobile as a function of temperature. The effect of hydration on the membrane dynamics was neglected in their analysis. Our study reveals the strong influence hydration has on the membrane systems, not only on the structure but also on the dynamics. Similar to hydrated protein powders [22] also phospholipids show a dynamic transition in the mean square displacements  $\langle u^2 \rangle$  and a

shift of the temperature of the main phase transition depending on their hydration. The transition for proteins occurs around 200K and is often called “glass transition”. In the case of membranes this transition is due to the structural transition into the liquid-crystalline  $L_{\alpha}$  phase. In contrast to proteins where the individual amino acid is hydrated to allow local motions of the proteins in phospholipid bilayers only the hydration of the hydrophilic head group of the phospholipid triggers the dynamic response of the hydration shielded hydrophobic alkyl chains. In conclusion special care should be taken for the hydration control to avoid a mixing of effects, which could be partly due to the hydration state of the sample.

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Fig. 1: Diffraction data taken on D16 at ILL. Data are shown for three different temperatures: 280K (straight line), 310K (broken line) and 330K (broken line with crosses). The sample was hydrated from a saturated salt solution ( $D_2O + NaCl$ ) resulting in a relative humidity of 75%.

Fig. 2: Summed elastic intensity (Q-range:  $0.43 \text{ \AA}^{-1} - 1.16 \text{ \AA}^{-1}$ ) measured at  $135^\circ$  (left y-scale, filled squares  $R_w \approx 4$ , empty circles:  $R_w \approx 6$ ) and mean square displacements  $\langle u^2 \rangle$  (right y-scale, filled diamonds  $R_w \approx 4$ , empty triangles:  $R_w \approx 6$ ). Drawn lines are guides to the eyes to follow the evolution of the mean square displacements. Data taken on the cold neutron spectrometer IN16 at ILL (energy resolution:  $0.9 \text{ \mu eV}$ ). Intensities are normalised to the lowest temperature.

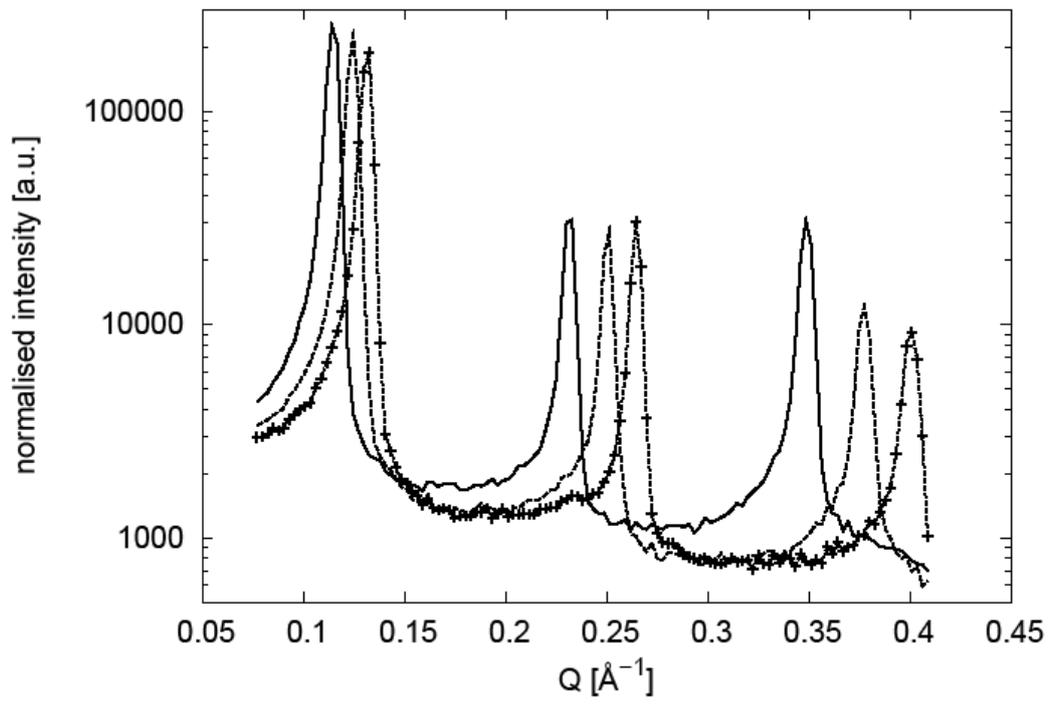


Fig. 1

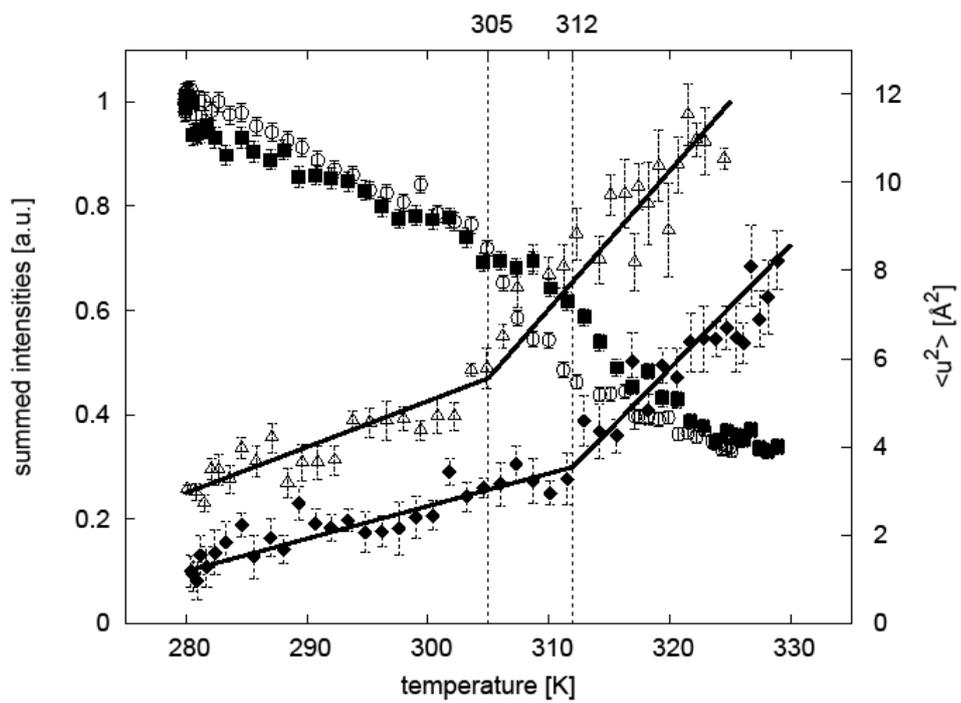


Fig. 2