Experimental report

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Proposal: 9-13-661 **Council:** 4/2016

Title: Localisation of Hyaluronic Acid inLipid Membranes

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DMPC (C36H72NO8P)
Hyaluronicacid

Instrument	Requested days	Allocated days	From	To
D17	2	2	21/11/2016	23/11/2016

Abstract:

Osteoarthritis (OA) is the most common arthropathy in Western civilisation. It is caused primarily by the degeneration of cartilage which leads to increased friction in the joints. Hyaluronic acid (HA) is the main component of the synovial fluid which serves as a lubricant within the joints. In healthy conditions, the cartilage is decorated with surface active lipid linings. In a first investigation we exposed model linings to HA solution and observed an increase in the repeat distance of the oligolamellar lipid membranes by a factor of four. In the proposed experiment we now want to localise HA within the lipid model membranes and identify the mechanism responsible for the stabilisation of the swollen bilayers.

Localization of Hyaluronic Acid in Lipid Membranes

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The aim of this proposal was the localization of hyaluronic acid (HA) and the polyelectrolyte polyallylamine hydrochloride (PAH) in lipid membranes. For this purpose, we investigated the polymer-induced swelling behavior of contrast-matched lipid oligobilayers consisting of hydrogenated 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) mixed with chain deuterated DMPC (d-54).

Oligolamellar DMPC layers were prepared by spin-coating from chloroform solution onto cleaned silicon blocks. The samples were pre-characterized by X-ray reflectivity (XRR) using a home-built set-up at the Helmholtz Zentrum Berlin (HZB). For all three samples (P1, P2, P3) the measurements showed well-defined Kiessig fringes and also a Bragg peak indicating well-ordered oligobilayers (see Figure 1). From the X-ray reflective data we determined lamellar repeat distances between 52.7 and 53 Å and 8 to 10 oligobilayers for each sample.

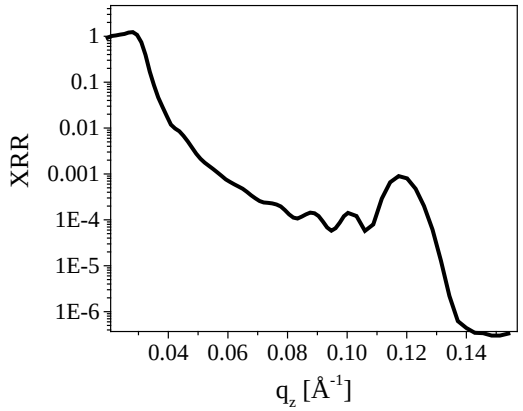

Figure 1: *XRR data of P3 measured against air. The overall thickness can be calculated from the position of the Kiessig fringes whereas the d-spacing of an individual lamella is deduced from the position of the Bragg peak.*

As lipids we chose mixtures of DMPC and DMPC d-54 in order to contrast match the lipid head groups and the tail region. If the scattering length density (SLD) of the liquid fronting is then adjusted to the same value by an appropriate selection of H_2O/D_2O mixtures, Kiessig oscillations and Bragg peaks should vanish. Bragg peaks will only reappear if hydrogenated HA or PAH, added to the solution, adsorbs onto the lipid headgroups and/or penetrates the lipid tailgroup regime. Kiessig oscillations will no more be observed due to the pronounced swelling of the films upon polymer incubation [1, 2]. If we solely use deuterated DMPC layers as originally proposed, it will be difficult to distinguish intruded HA (or PAH) from an increase in water content. The use of PAH offers the advantage of shorter equilibration times compared to HA while showing the same swelling and stabilization of the lipids layers.

For HA we used a molecular weight (MW) of 769 kDa and for PAH we chose MWs of 15 and 58 kDa. As a reference measurement prior to incubation with HA (or PAH) all samples were characterized against pure D_2O. In a second step, we changed the liquid fronting to contrast matched H_2O/D_2O as described above. After those measurements, we added HA (or PAH) in

concentrations of 3 g/L corresponding to the HA content observed in healthy mammalian joints. All incubations took place at a temperature of 17 °C, which is below the main phase transition temperature of chain deuterated DMPC. Upon incubation in D_2O we observed for all samples (P1, P2, P3) a shift of the Bragg peak to lower values of q_z indicating the expected increase in lamellar thickness to about 65 Å due to intrusion of water. Bragg peak as well as Kiessig oscillations remained well pronounced so that there is no indication of a loss of lipid layers (see Figure 2a). After reaching equilibration we mixed D_2O with H_2O in order to contrast match the liquid fronting to the SLD of the lipid layer. For all samples the signals vanished yielding a q_z^4 decrease in intensity (see Figure 2a). After adding PAH with a MW of 15 kDa the Bragg peak reappeared indicating incorporation of PAH into the lipid layer. The position of the Bragg was now observed at even lower values of q_z due to polymer-induced swelling (see Figure 2b).

The same type of experiments was performed for both HA 769 kDa and PAH 58 kDa. In both cases, the lipid films were successfully contrast matched to the liquid fronting, but no reoccurrence of Bragg peaks was observed when polymer was added to the solution (see Figures 2c and 3a). To make sure that this was not due to a complete loss of lipid layers during the experiments we changed the SLD of the liquid fronting by adding D_2O to the PAH 58 kDa solution in order to again generate contrast between the liquid phase and the lipid film. The Bragg peaks emerging at lower values of q_z proved the integrity of the swollen lipid oligobilayers as expected (see Figure 3b).

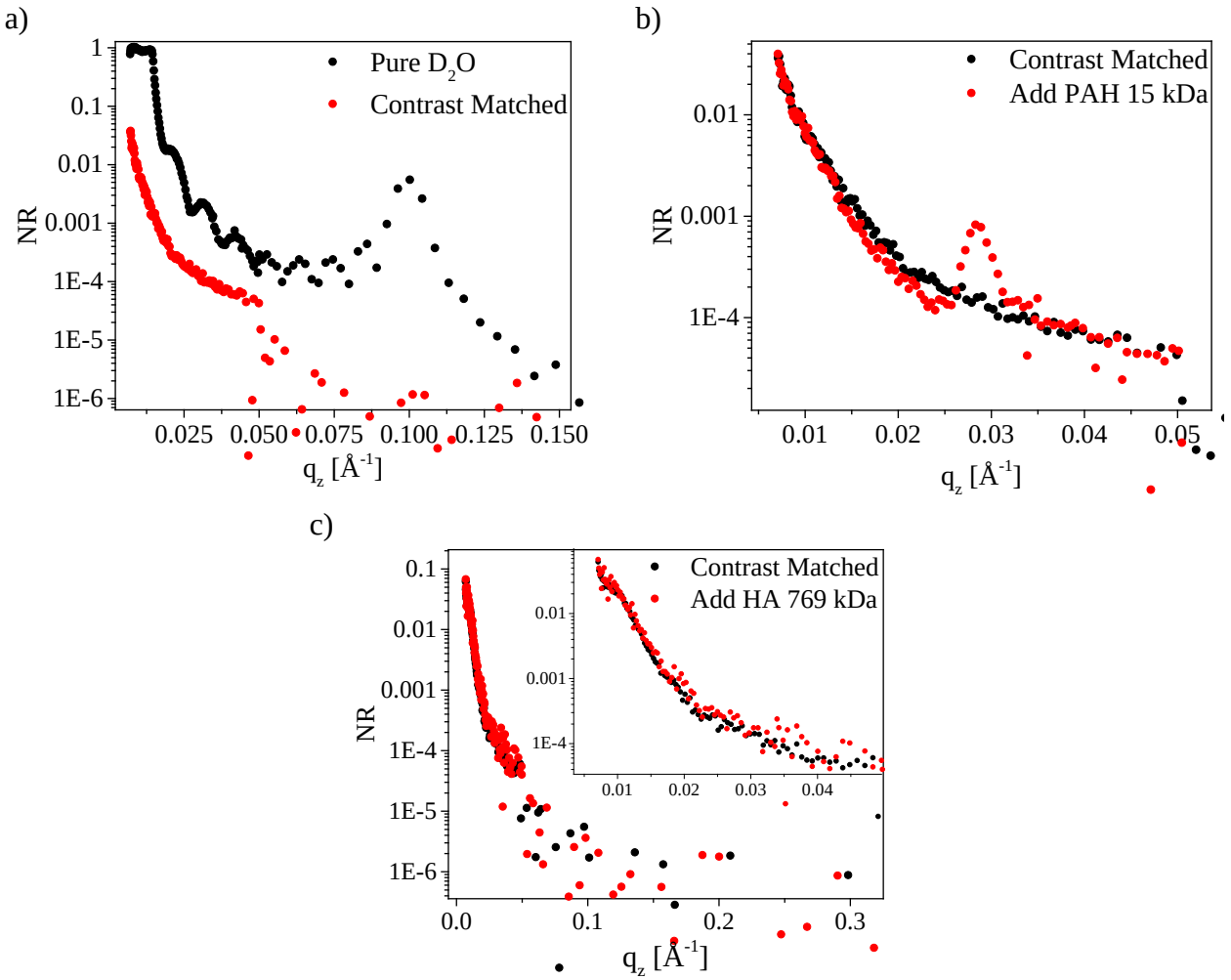

Figure 2: *a) NR measurements on P3 incubated in pure D_2O and in a mixture of H_2O and D_2O to contrast match the liquid fronting and the lipid layer. b) NR measurements on P3 contrast matched and after adding PAH 15 kDa. c) NR measurements on P2 contrast matched and after adding HA 769 kDa.*

a) b)

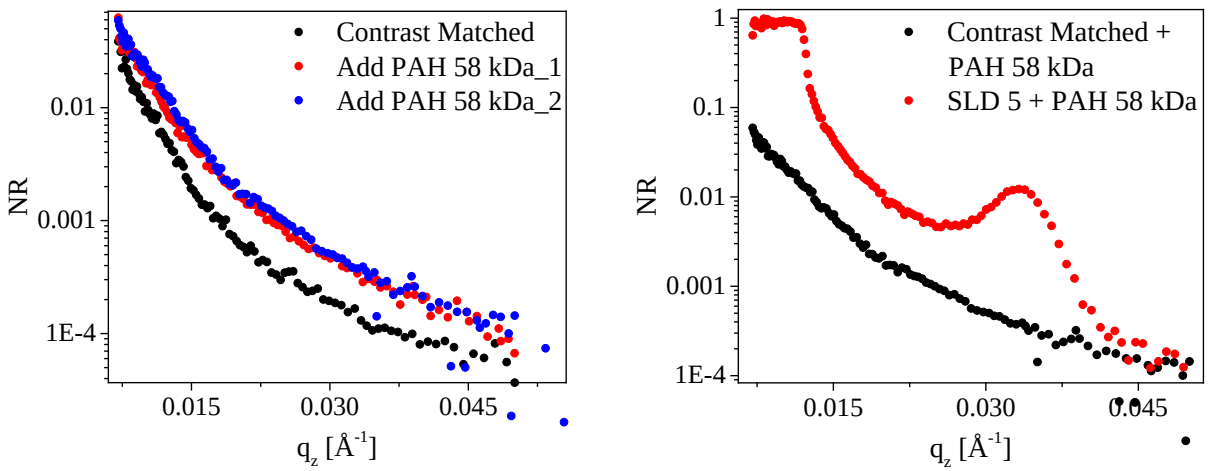

Figure 3: *a) NR measurements on P1 contrast matched and after adding PAH 58 kDa. b) P1 incubated in a PAH 58 kDa solution with an SLD different from the SLD of the lipid layer.*

In summary, we have successfully proven the incorporation of PAH 15 kDa in lipid oligobilayers by contrast matching. The detailed internal structure of the films is still being investigated, in particular, whether the polymer adsorbs to the lipid headgroups and/or penetrates the lipid tailgroup regime. In future experiments it will have to be investigated why such incorporation process has not been observed for HA 769 kDa and PAH 58 kDa. One possible reason would be significantly longer intrusion times for polymers of higher molecular weight so that the process could not be observed within the beamtime granted.

[1] M. Kreuzer, M. Strobl, M. Reinhardt, M.C. Hemmer, T. Hauß, R. Dahint, R. Steitz, Impact of a model synovial fluid on supported lipid membranes, Biochim. Biophys. Acta, 1818 (2012) 2648-2659.
[2] F. Schwörer, M. Trapp, M. Ballauff, R. Dahint, R. Steitz, Surface-Active Lipid Linings under Shear Load—A Combined in-Situ Neutron Reflectivity and ATR-FTIR Study, Langmuir, 31 (2015) 11539-11548.