ESRF	**Experiment title:** Structural and morphological modifications induced by the interaction of MBP with charged and neutral phosphlipid monolayers	**Experiment number**: SC-1715
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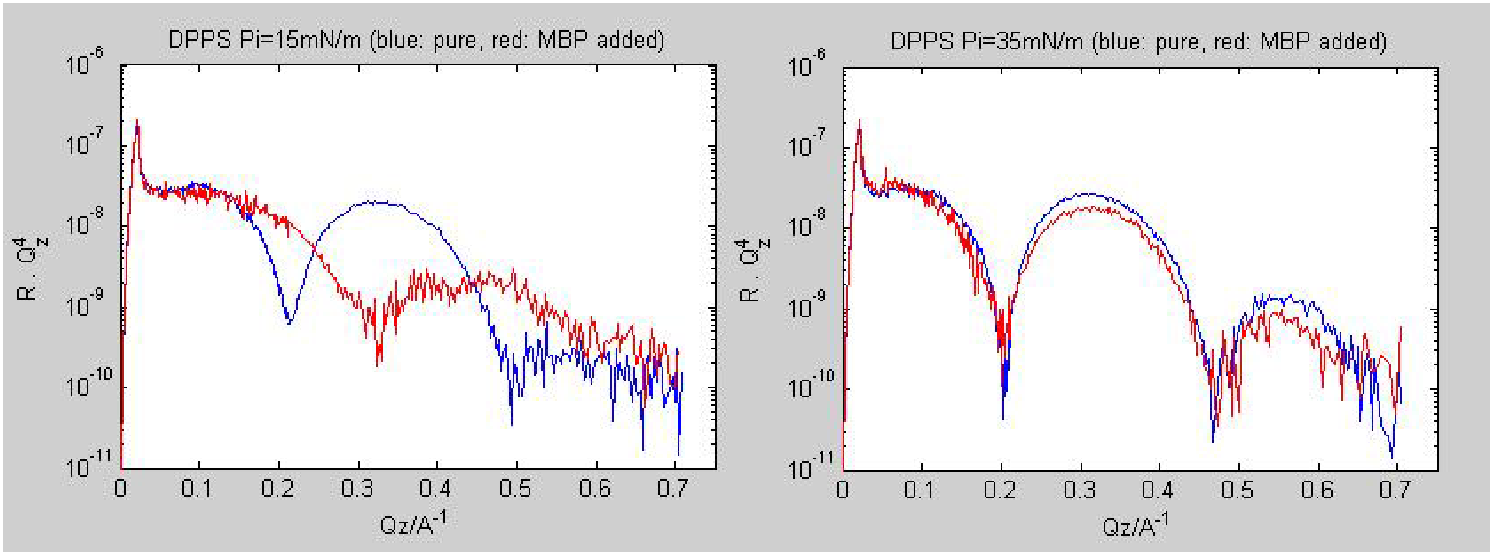
The present experiment of reflectivity on phospholipid monolayers at air-water interface aimed to give further insight in the behaviour of the model membrane in presence and in absence of Myelin Basic Protein (MBP), as MBP is believed to be responsible of the formation and preservation of central nervous system [1,2].
Reflectivity experiments were performed with λ=1.55Å in several conditions of surface tension and environment, first on the pure lipid monolayer and then after exposure to MBP for about 15 minutes before film compression. We tested both charged (DPPS) and uncharged (DPPC) phospholipid as model membranes. While uncharged phospholipid showed no alteration of reflectivity pattern upon insertion of MBP in subphase, implying no interaction between DPPC and MBP, on the contrary charged DPPS films showed a different behaviour, as shown in the following figure.



In details, DPPS on pure water subphase (pH=6.7, unbuffered) were studied with surface tension of 15 and 35 mN/m before and after addition of MBP in two different concentration (2 mg/l and 4 mg/l respectively). Next, the pH of the subphase was changed to 11.3 and finally we checked the effect of subphase ionic strength on the mechanism of protein interaction by means of $CaCl_2$ 0.1M. Typical reflectivity profiles are shown in the figure below.

In the following table we present the main parameters of the monolayers as obtained by the best fit of the reflectivity profile using the Parrat[3,4] approach:

Surface Π (mN/m)	***Note***	***Alkyl chain thicknes (Å)***	***Polar heads thickness (Å)***	***Film surface (cm^2)***
15	Pure water	16.6	10.6	335
15	MBP 4mg/ml	10.0	10.5	460
35	Pure water	19.3	8.4	203
35	MBP 2mg/ml	18.7	9.6	231
35	MBP 4mg/ml	18.7	9.7	245
15	pH11	15.4	11.1	565
15	MBP 2mg/ml pH11	(monolayer)	8.5	683
35	pH11	17.6	9.8	411
35	pH11+MBP2mg/ml	17.4	11.4	265
13	$CaCl_2$	14.8	12.8	180
13	$CaCl_2$+MBP2mg/ml	16.8	9.4	505

In absence of MBP the phospholipid layer could be well modelled by the usual bilayer structure, with, starting from the water surface, the polar heads with higher electron density, and thickness about 10 Å while the upper layer, consisting of alkyl chains (thickness about 16Å) shows lower density. In comparing data relative to the film on pure water and after addition of MBP, a general trend also confirmed by preliminary measurements, is that in order to keep the pressure constant a higher area per molecule is required. This strongly suggests that some degree of intercalation of the protein in the film occours.
As shown in the table, exposure to MBP yields a reduction of the thickness of the alkyl chains layer, while the polar head layer is almost unaffected by the intercalation at low pressure, while at high pressure a moderate thickness increase is observed. Moreover, for Π=15mN/m after interaction the film results much less structured than before, at higher pressure the film stays well structured. This could be rationalised by assuming that at low P the protein enters directly into the layer, while at high pressure MBP is confined in the water underneath the polar head layer.
In order to modulate the long range electrostatic interaction between the charged polar heads of DPPS and MBP we tested a salt containing subphase (0.1M $CaCl_2$). The results appear contradictory: we observe a reduced effect of MBP on phospholipid monolayer structure, even if the area per molecule increases in the same fashion as with the pure water subphase.
Finally, similar results were also obtained by using a buffer in the subphase to increase the pH to 11.3: at high pressure no major effect is observed, whereas at low pressure the addition of MBP results in a completely unstructured film composed of a single layer, most likely because MBP itself is denatured at the air-water surface at such extreme value of pH.

REFERENCES

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