The location of chlorhexidine in model membranes: the influence of cholesterol

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Chlorhexidine (CHX) is an anti-microbial most commonly used as an antiseptic agent in personal hygiene products such as soaps, contact lens solutions, and mouthwashes. It is effective against both gram-positive and gram-negative bacteria, yeast, and viruses with membranes, which leads to the theory that its mode of action is via membrane disruption. A probable biophysical mechanism of action was put forward by Komljenovic et al.; where we propose that chlorhexidine bends at its centre and inserts itself into the membrane like a wedge.[1] This can lead to instability in the integrity of the membrane, and lead to large pores that leak vital cytoplasmic fluids.

A series of Differential Scanning Calorimetry (DSC) experiments were conducted to study the phase behaviour of DMPC and DMPC membranes doped with cholesterol when exposed to CHX. CHX lowers the melting transition of the DMPC acyl chains, and the addition of cholesterol furthers this effect. Thus, cholesterol appears to facilitate chlorhexidine's penetration into the DMPC bilayer amplifying the effect of CHX at high cholesterol concentrations. In an effort to explain this result, neutron diffraction experiments were conducted to determine if cholesterol draws CHX deeper into the membrane in the presence of cholesterol, than without.

The neutron diffraction data was collected at the CNBC N5 beamline, using 2.37 Å wavelength neutrons. During the data collection, the samples were kept at a constant temperature of 37 °C and a constant humidity of using a saturate KNO_3 solution (90.7% RH). The phasing of the form factors were determined hydrating the samples with a series of D_2O/H_2O mixtures as outline in Kucerka et al.[2]

The data (figure 1) illustrates that the hypothesis of cholesterol pulling CHX into the DMPC bilayer is incorrect. In fact, the CHX label mass is located at the exact same depth with 1% cholesterol incorporated into the bilayer as the pure DMPC bilayer as shown by Komljenovic et al. in 2008. Further experiments with deuterium labeled cholesterol is required, to see if cholesterol is the effected molecule, possibly being displaced by the presence of CHX.

References

[1] Ivana Komljenovic, Drew Marquardt, Thad. A. Harroun, and Edward Sternin. Location of chlorhexidine in DMPC model membranes: a neutron diffraction study. *Chemistry and Physics of Lipids,* **2010***, 163*, 480-487

[2] Kucerka, N.; Marquardt, D.; Harroun, T. A.; Nieh, M.-P.; Wassall, S. R.; de Jong, D. H.; Schafer, L. V.; Marrink, S. J. & Katsaras, J.. Cholesterol in Bilayers with PUFA Chains: Doping with DMPC or POPC Results in Sterol Reorientation and Membrane-Domain Formation B*iochemistry,* **2010***, 49*, 7485–7493

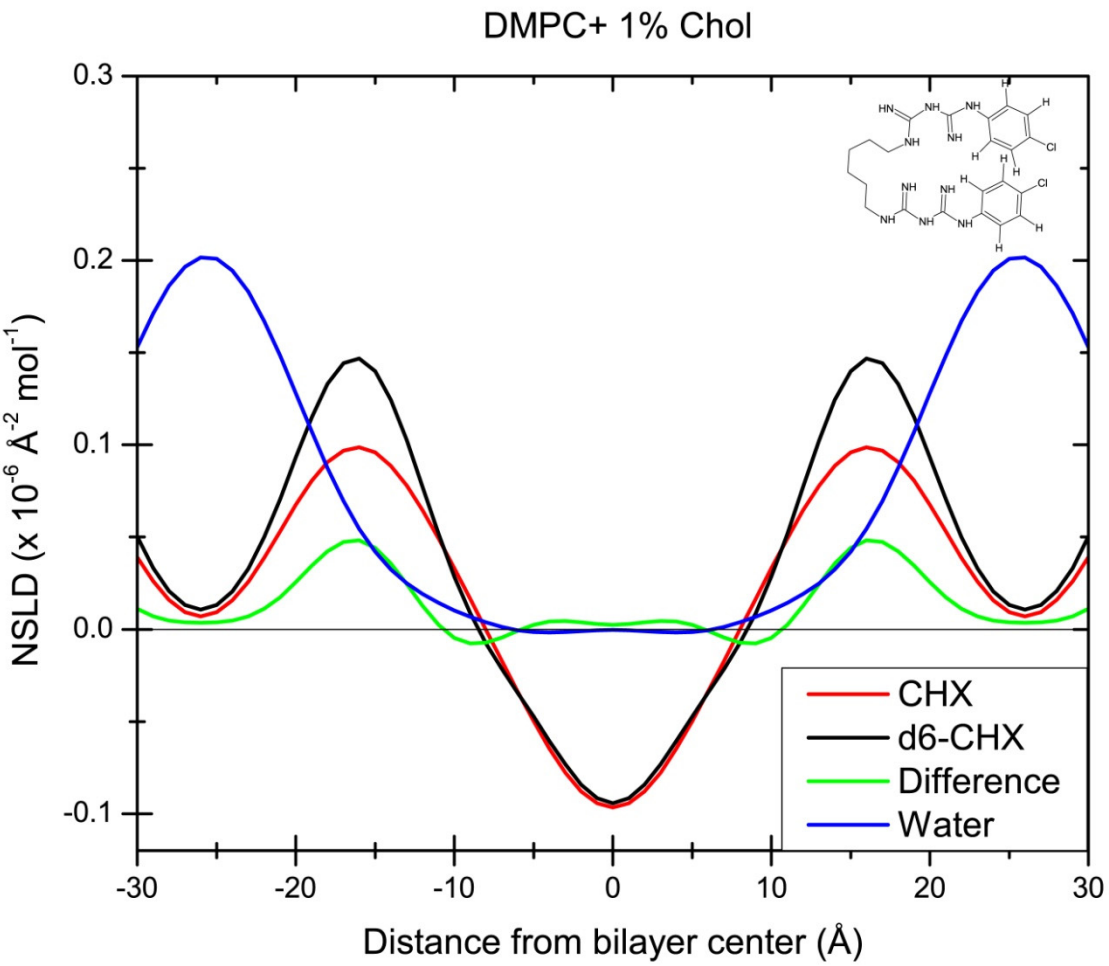

Fig. 1 SLD profile for labeled (black) and unlabeled (red) CHX in a DMPC bilayer doped with 1% cholesterol. The green line is the SLD difference between labeled and unlabeled CHX and the blue line is the water distribution in the sample. The CHX structure (top right) is the most probable orientation as determined by the data and molecular dynamic simulation.