

Effects of Optical Anisotropy on Waveguide Spectroscopy and its Measurement allows Elucidation of Conformational Changes in Supported Lipid Bilayers

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INTRODUCTION: Supported lipid bilayers (SLB) have recently received a high degree of interest for the functionalisation of biosensor interfaces and the importance of quantitatively investigating peptide-membrane interaction is increasingly appreciated.[1] Functionalisation of biosensor interfaces with SLB requires knowledge not only about their formation, but also about how the biosensor itself responds to the presence of a bilayer and conformational changes within it. Alignment of lipids orthogonal to the substrates, as in the case of SLB formation, significantly changes the effective refractive index of the lipids as probed by linearly polarised evanescent fields. The assumption of an isotropic SLB refractive index leads to incorrect determination of film thickness of lipid membranes and similarly how the estimated mass uptake will vary. Here these effects are investigated and quantified using the technique of Dual Polarisation Interferometry (DPI).[2]

METHODS: DPI measurements were done using Analight® BIO200 (Farfield Scientific Ltd., UK). By exciting the SiO_xN_y waveguide chip with two orthogonal polarisations, TE and TM waveguide modes, two separate measurements of fringe shifts were made as a function of time during liposome adsorption. DMPC, POPC and POPC:POPS (80:20 w/w) uni-lamellar vesicles, were prepared by extrusion through polycarbonate filters with 100 nm pore diameter in 10 mM HEPES, 150 mM NaCl with and without CaCl₂ (1mM and 2mM) at pH 7.4. The phase shifts were analyzed both using a standard one-layer model for isotropic films and modeled allowing for optical anisotropy within the film at a fixed thickness.

RESULTS: Larger phase responses are observed for DMPC liposomes layers than SLB (figure 1 (left)). SLBs formed with different protocols converge on similar phase change. SLB thickness resolved in a one-layer model for homogenous isotropic films yields a value up to 5 times the real value. In this case, thickness is a function of anisotropy (alignment/compression of the lipids). In case of POPC and POPC:POPS, regardless of lipid mixture, the SLB birefringence reaches a significant mean value of ~0.021 after SLB formation with a calculated average refractive index $n \approx 1.47$ assuming a typically 4.7 nm thick SLB. Addition of 2 mM CaCl₂ to the buffer gives

rise to a pronounced maximum in the difference between TM and TE phase shifts during the liposome rupture phase (figure 2 (right)). Pronounced plateaus in the birefringence with increasing mass occur especially clearly for POPC without Ca²⁺ during infilling of first the vesicle layer before rupture and second the SLB before condensation of the SLB to its final density and alignment increases the birefringence.

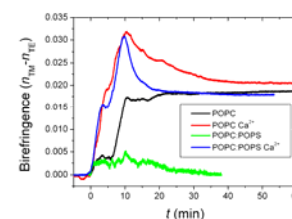
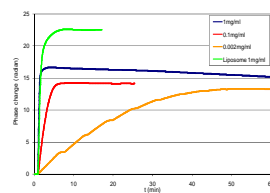


Fig. 1: TM phase changes for DMPC liposome and DMPC SLB depositions (left). Effect of calcium on the kinetics of POPC and POPC:POPS SLB formation (right).

DISCUSSION & CONCLUSIONS: Allowing for optical anisotropy in the analysis of DPI data enables determination of birefringence of SLBs together with thickness or refractive index. This enables the molecular arrangement and distribution to be determined, which provides mechanistic details for the SLB formation process that are complimentary to what is obtained by other methods such as QCM-D.[3] Specifically, it was shown that addition of CaCl₂ has a pronounced effect on the SLB formation kinetics monitored with DPI, likely due to higher lipid alignment and deformation of liposomes before and during the rupture phase.

REFERENCES: ¹S. Daniel, F. Albertorio, P.S. Cremer (2006) *MRS Bulletin*, **31**(7): 536-540. ²N.J. Feeman, L.L. Peel, M.J. Swann (2004) *J Phys: Condens Matter* **16** S2493-S2496. ³E. Reimhult, M. Zäch, F. Höök et al (2006) *Langmuir* **22**(7):3313-9.

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