

Characterisation of the structure of PEG-supported lipid bilayers

[S.Kaufmann¹](#), [K.Kumar¹](#), [M.Textor¹](#), [E.Reimhult¹](#)

¹ETH Zurich, Zurich, Switzerland.

INTRODUCTION: Supported lipid bilayers (SLB) provide a basis for biotechnological applications as they constitute a simple model of cell membranes. They are of particular interest as components of future generations of biosensors based on transmembrane proteins. Two of the current limitations of supported lipid bilayers in biosensor applications are their sensitivity to air exposure and the limited aqueous space between the sensor substrate and the membrane available for large membrane proteins.

Supported membranes resting on a hydrophilic polymer spacer decouples the membrane from the surface and provides increased aqueous space, but are generally more complicated to assemble than supported lipid membranes resting on an inorganic support.¹ Recently it has been shown that poly(ethylene glycol) (PEG) can be incorporated into the membrane of liposomes through lipid molecules end-functionalized with a PEG chain and spontaneously fused to supported PEG-lipid bilayers (PEG-SLB) on glass.² These membranes have been shown to possess a remarkable stability in air and would be based on the length of the PEG-chains provide enough space between the SLB and the substrate to allow incorporation of functional transmembrane proteins. However, the structure of the PEG-SLB has not been characterized and important questions like whether the PEG brush is present on both sides of the membrane, its thickness, density and the kinetics of formation of PEG-bilayers have not been properly addressed. We present a comparison of the kinetics of PEG-SLB formation for different PEG molecular weights and densities as well as data on the location and thickness of the PEG brush.

METHODS: Large unilamellar lipid vesicles were prepared by extrusion through polycarbonate membranes with 100 nm pores from 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (POPC) lipids mixed with a 0-5 mol% of 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Poly(Ethylene glycol)) MW550, 2000 or 5000] (PEG(MW)-PC) lipids. Adsorption kinetics of PEG-lipid vesicles and PEG-SLB formation were recorded by quartz crystal microbalance with dissipation monitoring (QCM-D, E4, Q-Sense, Sweden). Fluidity of PEG-SLB was also confirmed by fluorescence recovery after photobleaching (FRAP). Force-distance curves

were recorded with atomic force microscopy (AFM, Multimode, Digital Instruments, USA) equipped with 0.5 μm in diameter colloidal or standard tips with spring constant 0.12 N/m.

RESULTS: QCM-D measurements typically showed PEG-SLB formation following the general phases identified for pure POPC SLB formation (Fig. 1 (left)), but with the relative and absolute rate of the different phases affected by the PEG-lipid molar fraction and PEG molecular weight. Higher dissipation and mass was recorded for PEG-SLB than for normal SLB.

Force-distance measurements demonstrated that the thickness of e.g. a PEG(2000)-SLB layer is approximately 10-12 nm and follows first a de Gennes-type compression followed by linear compression and “jump-ins” signifying membrane rupture events (Fig. 1 (right)).

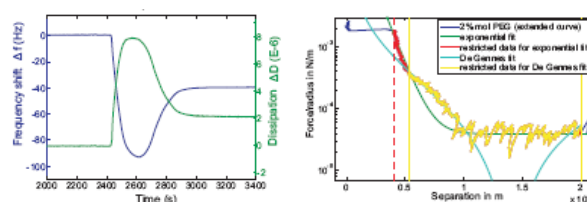


Fig. 1: QCM-D kinetics for formation of a PEG(2000)-SLB also confirmed by FRAP (left). Force-distance curve for compression of PEG(2000)-SLB with colloidal tip-AFM(right).

DISCUSSION & CONCLUSIONS: The QCM-D measurements indicate that a highly hydrated SLB is formed through a mechanism similar to POPC SLBs, but with higher barriers to vesicle adsorption and rupture, in particular the former. The thicknesses observed by AFM and the initial compliance to a behaviour typical for compression of a PEG brush strongly indicates that the PEG is only present on the distal leaflet of the PEG-SLB.

REFERENCES: ¹ M. Tanaka and E. Sackmann (2005) *Nature* **437**(7059):656-663. ² F. Albertorio, A.J. Diaz, T.L. Yang et al (2005) *Langmuir* **21**(16):7476-7482.

ACKNOWLEDGEMENTS: We thank the Swiss Competence Center for Materials Science and Technology (CCMX) for financial support and Dr S. Lee for support with the colloidal AFM measurements.