

LIPID BILAYER FORMATION; A COMPARISON BETWEEN QCM-D, SPR AND AFM

INTRODUCTION

In order to increase the understanding of molecular adsorption or interaction studies, many laboratories use a multiple technique approach. Below it is shown how QCM-D, SPR and AFM have been used to understand the formation of lipid bilayers on SiO₂ surfaces.

RESULTS

Keller et al. have shown that lipid vesicles exposed to a surface, form different structures: monolayer, bilayer or intact vesicles, depending on the properties of the surface. It is known that vesicles form a bilayer on a SiO₂ (very hydrophilic) surface. However, very little was known of *how* this bilayer was formed. SPR measurements showed a monotonic increase of lipids on the surface but QCM-D measurements showed that the total adsorbed mass reaches a peak and then levels off to a level closely corresponding to the theoretical mass of a lipid bilayer (see **Figure 1**). The QCM-D dissipation signal shows a marked peak and then almost returns to zero indicating a significant phase transition in the adsorbed layer. Keller et al. therefore concluded that the vesicles initially adsorb intact and do not rupture and form a bilayer until a critical surface concentration is reached. The difference in QCM-D and SPR mass was attributed to water trapped inside and between adsorbed vesicles. Using QCM-D and SPR in parallel thus gives a detailed understanding

of the complete adsorption process.

This picture of vesicle adsorption on SiO₂ surfaces was further confirmed by AFM measurements as shown in **Figure 2**. AFM was used to look at the local morphology of the vesicles and the lipid bilayer at various stages of the adsorption process.

CONCLUSIONS

This application note shows that by using QCM-D in combination with SPR and/or AFM a detailed understanding of molecular events taking place on surfaces is attained. It is a common approach among research groups

working with surface science to use multiple techniques to understand molecular processes taking place on different surfaces. QCM-D and SPR together gives the possibility to extract the water content of molecular films and recently, a simple way of extracting the exact water content of adsorbed molecular layers has been given. Both techniques present real time data. AFM is an excellent technique to reveal at the molecular level how molecules have adsorbed and/or clustered at the surface which complements the real-time mass data achieved by QCM-D/SPR and the overall structural properties of adsorbed layers acquired by QCM-D.

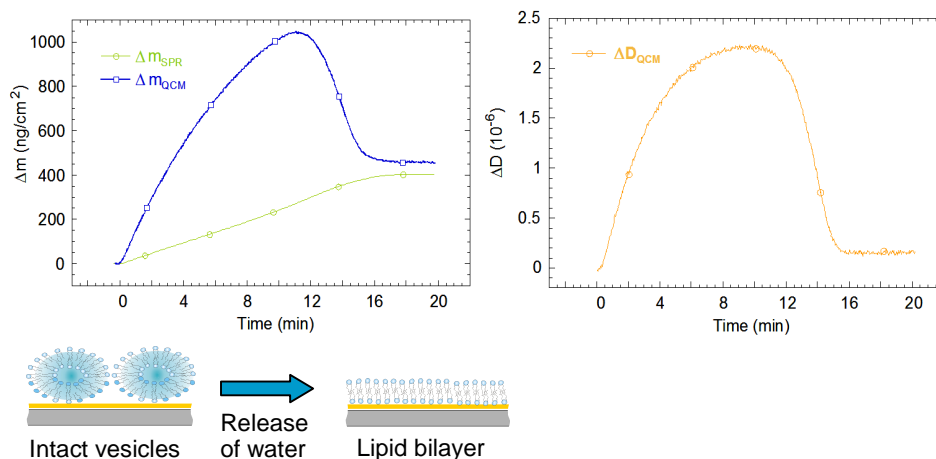


Figure 1. QCM-D and SPR responses during formation of a lipid bilayer. Since QCM-D senses coupled water, the phase transition from intact vesicles to a lipid bilayer is clearly seen as a decrease in mass (release of water). SPR shows additional mass as more lipid molecules adsorbs directly to the surface of the SPR chip. Simultaneously the dissipation of the crystal increases when intact vesicles adsorb in the beginning of the adsorption. The dissipation at the end is close to its original values since the lipid bilayer is thin and closely packed.

REFERENCES

Further information about this subject can be found in several publications; the approach above; to compare the QCM-D response with that of SPR, was first shown in *Formation of Supported Membranes from Vesicles* in Physical Review Letters Vol. 84, Number 23 (2000) 5443-5446 by C Keller et al.

The complete AFM work is presented in *Pathways of Lipid Vesicle Deposition on Solid Surfaces: A Combined QCM-D and AFM Study* in Biophysical Journal, Volume 85, November 2003, p. 3035 –3047 by R Richter et al.

How to extract exact water content of immobilized molecular

layers by using QCM-D in combination with for example SPR is presented in *Characterization of DNA Immobilization and Subsequent Hybridization on a 2D Arrangement of Streptavidin on a Biotin-Modified Lipid Bilayer Supported on SiO₂* in Analytical Chemistry 2003, 75, 5080-5087 by C Larsson et al.

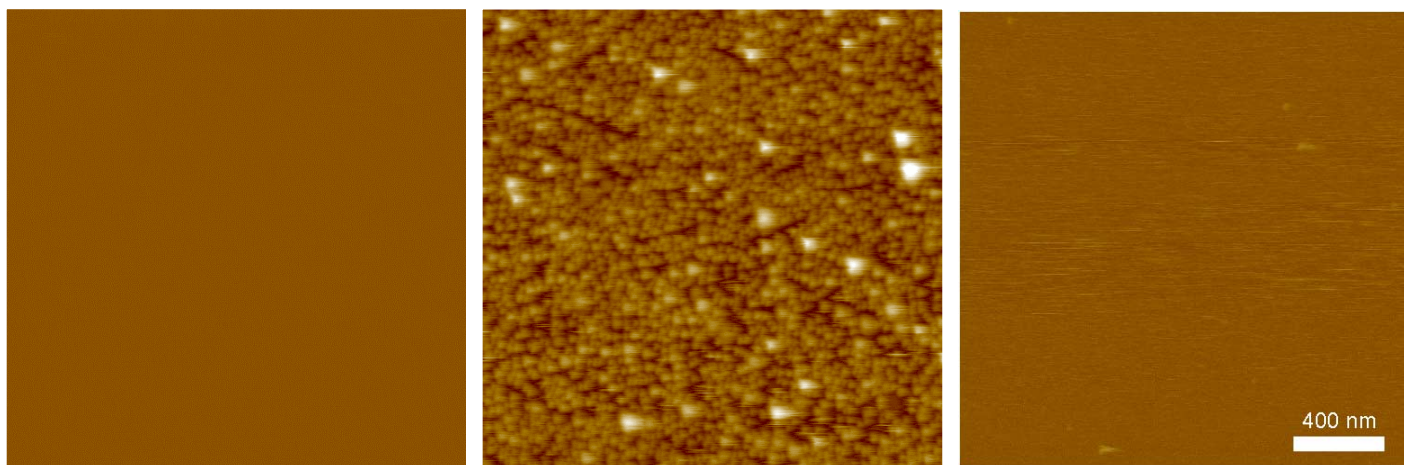


Figure 2. AFM responses during formation of a lipid bilayer on SiO₂. Left panel shows the SiO₂ surface prior to adsorption, middle panel intact vesicles at a surface concentration below the critical coverage needed for rupture and the right panel shows the lipid bilayer after completed adsorption. As can be seen in the right panel the lipid bilayer is extremely flat and covers the whole surface without defects. Z-scale image size (black-white) is about 40 nm.

