Application Note

Supported Lipid Bilayer formation followed at low- and high-fundamental frequencies

Summary

The process of supported lipid bilayer (SLB) formation from adsorbed liposomes is a robust biophysical system that is used in laboratories all over the world. Here, it is used to test AWSensors Quartz Crystal Microbalance with Disipation measurement (QCMD) equipment and high fundamental frequency QCMD sensors. It is shown that the AWSensors QCMD system correctly and quantitatiely reports the frequency and dissipation changes associated with the SLB formation on high- and low-fundamental frequency SiO_2-coated sensors. Some differences between the two types of sensors are highlighted.

Quartz crystal microbalance with dissipation measurement, or QCMD, has become a popular technique for research in such disparate fields as material science, biophysics, electrochemistry, and immunosensing. [1] One of the reasons for the wide range of applicability and popularity of QCMD is its ability to provide information about molecular organization (topology and geometry) at solid/liquid interfaces. Specifically, it was shown how the combination of frequency and dissipation could distinguish between different surface-immobilized lipidic assemblies: adsorbed liposomes and supported lipid bilayers (SLBs; Figure 1).[2] This allowed the process of SLB formation from liposomes on SiO_2-coated QCMD sensors to be followed in situ.[2] Subsequent studies further showed how the combination of frequency and dissipation measurements on various overtones could be used to study adsorbed liposome deformation[3,4] and detect mutations through the analysis of DNA conformation and length.[5,6]

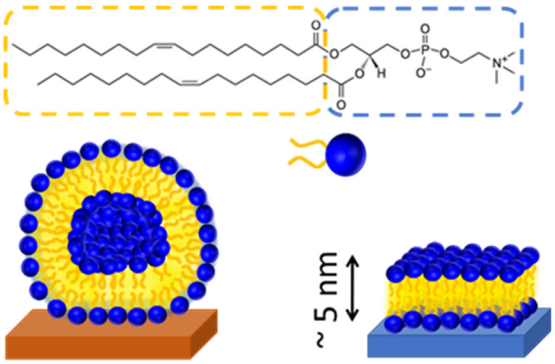

Figure 1. A lipid molecule (dioleoylphosphatidylcholine, top), it's schematic representation (middle), and solid-supported lipid systems (liposomes and lipid bilayers, bottom).

Formation of SLBs from adsorbed liposomes on SiO_2 is a process that is very robust and that has been reproduced across numerous international laboratories. Since it is also very demanding on the instrument with respect to its ability to measure small, rapidly changing values of the dissipation, it is useful for benchmarking QCMD instruments. We therefore use it here to illustrate the capabilities of the Advanced Wave Sensors QCMD instruments.

Advanced Wave Sensors is a company that designs, develops, and manufactures ultrasensitive QCMD systems. Our QCMDs provide frequency and dissipation measurements on multiple overtones, from n = 1 to n = 13 for 5 MHz sensors,

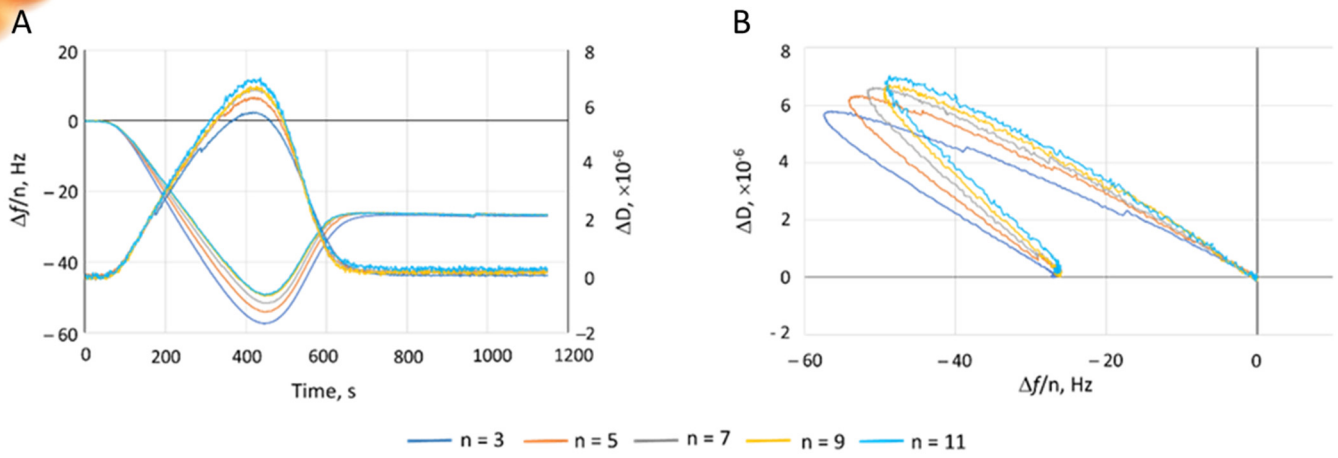

Figure 2. A typical SLB formation experiment performed with a 5 MHz SiO2-coated sensor and 50 nm extruded DOPC liposomes in a 10 mM HEPES buffer pH 7.4 containing 150 mM NaCl and 2 mM CaCl2. (A) Frequency and dissipation vs. time. (B) Dissipation plotted as a function of frequency. Data for n = 1 and n = 13 are not shown.

for *n* = 1 and *n* = 3 for 50 MHz sensors, and for the fundamental for the 100 MHz sensors. Further information can be found in the corresponding *Technology Note*[7] and product catalogue.

Figure 2 shows the process of SLB formation from liposomes on a 5 MHz SiO_2-coated sensor. Data were acquired with an AWS X1 QCMD system operating in tracking mode equipped with a temperature controller, a flow controller, and a Quick-lock™ flow cell for mounting 14 mm, wrapped electrode 5 MHz sensors. The measurements were performed at 23 °C. Liposomes were prepared by extrusion through 50 nm membrane filters as previously described,[8,9] in a buffer containing 10 mM HEPES, 150 mM NaCl and 2 mM $CaCl_2$, pH 7.4. The liposomes were injected into the cell at a concentration of ~ 0.2 mg/ml and a flow rate of 15 µl/min after acquiring a baseline in buffer. In the top panel of Figure 2, it can be seen that injection of the liposomes causes the frequency to decrease, and the dissipation—to increase, up to their respective extrema. The signals then return to their asymptotic values of -26.6 ± 0.2 Hz for the frequency shift and $(0.14 \pm 0.09) \times 10^{-6}$ for the dissipation, corresponding to a mass of 470 ± 4 ng/cm^2 (averaging is done over time and over signals obtained on the different overtones) calculated according to the Sauerbrey relationship, $\frac{\Delta f}{n} = -\frac{2f_0^2}{\sqrt{\varrho G}}\Delta m$,[10] where f_0 is the fundamental frequency, ϱ and G are the density and shear elastic modulus of quartz, respectively, and *n* is the overtone order. The bottom panel contains the same information but plotted in terms of the dissipation as a function of frequency (the so-called *Df* plots).

The responses shown in Figure 2 correspond to the classical signature of the SLB formation that proceeds via the adsorption of intact liposomes causing large shifts in frequency and dissipation, followed by their decomposition into a planar bilayer that exhibits minimal dissipation.[2,9] The values of the asymptotic shifts are in good agreement with the

literature.[2, 11] These results indicate that AWSensors QCMD systems accurately record small values of the dissipation and follow their changes.

The sensitivity of QCMD sensors is expected to improve with the fundamental frequency. This can be seen from the Sauerbrey relationship, which contains the f_0^2 term: the higher the fundamental, the smaller is the mass corresponding to a given frequency shift that is measured by the instrument. In practice, the sensitivity is limited by the noise,[12] but it does nevertheless improve with f_0.[13] For this reason, AWSensors introduced high fundamental frequency (HFF) sensors with frequencies of 50, 100, and 150 MHz.[14] Here, we use the SLB formation process to evaluate the performance of SiO_2-coated 100 MHz HFF sensors. The results are shown in Figure 3, together with the XPS analysis of the SiO_2 coating. The coating was prepared by magnetron reactive sputtering.

Examination of the frequency and dissipation changes visible in Figure 3 reveals very similar features to the process of SLB formation on the 5 MHz sensors (Figure 2), but also some differences. Specifically, the dissipation signal exhibits a peak in both the HFF and the regular, low-frequency sensors. In both cases, dissipation returns to a low value. However, the frequency changes are quite different: in the case of the HFF sensor, the signal attains an asymptotic value without reaching a minimum.

The asymptotic values of the frequency and dissipation shifts observed with the HFF sensors were – 10400 ± 90 Hz (corresponding to 440 ± 4 ng/cm^2) and (0.13 ± 2)×10^{-6}, respectively; the averaging is performed over the results of three separate experiments. These values also agree very well with those obtained with the 5 MHz resonators.

Using the well-defined transition between adsorbed liposomes and lipid bilayers as a model system, the results presented in Figure 3 show that the information provided by the HFF sensors compares well with that provided by the regular, 5 MHz sensors. Some subtle differences (absence of an extremum in the frequency) are also revealed, requiring further

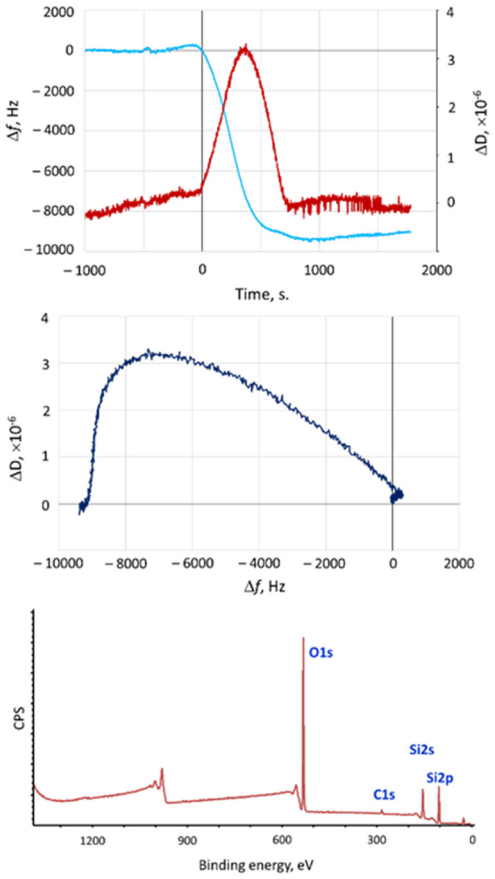

Figure 3. SLB formation monitored with an HFF sensor. The results were obtained with an A20+ system connected to an F20+ flow controller. Sonicated DOPC liposomes were used in this experiment, injected at time zero at a flowrate of 25 µl/min. Top and middle panels are as in Figure 2. The slope visible in the dissipation signal in the top panel before the liposome injection at 0s is due to the pressure changes from the pump. Bottom panel: an XPS spectrum of the coating demonstrating the presence of Si, O, and C elements. Quantitative analysis of the detailed spectra revealed 34, 57, and 9 atomic% of the three elements, respectively.

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investigation. We note that the absence of the extremum in the frequency shift during the SLB formation process has been reported previously for a different lipid system.[11] The magnitude of the peak is ultimately related to the lifetime of the surface-adsorbed liposomes during the SLB formation process.

Finally, we examined another well-established system: the binding of a protein, streptavidin, to biotinylated lipid bilayers (Figure 4). There is a decrease in the frequency to an asymptotic shift of -31.3 ± 0.4 Hz (relative to the bilayer), corresponding to a mass of 530 ± 7 ng/cm^2, which is consistent with the literature values.[15] Most notably, the dissipation signal exhibits a slight maximum, first reported in ref. 15 and subsequently discussed in detail in ref. 16 as being due to the hydrodynamic interactions. The asymptotic dissipation, $(1.05 \pm 0.09) \times 10^{-6}$, is somewhat larger than expected, probably due to residual protein aggregates that are sometimes present in streptavidin preparations.

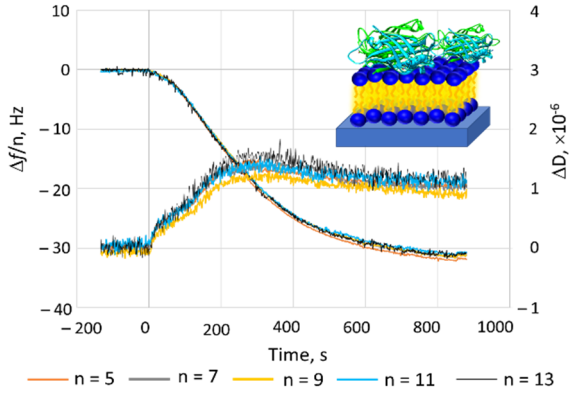

Figure 4. Binding of streptavidin to biotinylated lipid bilayers. Note the maximum in the dissipation signal. The inset shows, schematically, two streptavidin molecules bound to the bilayer, to illustrate that the size of the streptavidin molecules is comparable.

In summary, bilayer formation and streptavidin binding experiments verify that AWSensors QCMD systems monitor small changes in the dissipation reliably and accurately. The results obtained with the AWSensors QCMD systems quantitatively agree with the literature.

Acknowledgements

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Order Information

Product	Quantity	Reference
AWS X1	1	AWS X1 000031 A
AWS X1 Temperature Control Unit	1	AWS X1 000032 A
AWS X1 Flow Control Unit	1	AWS X1 000033 A
QCM 14 mm flow cell for AWS instruments	1	AWS CLS+ 000021 Q
HFF-QCM flow cell for AWS instruments	1	AWS CLS+ 000115 Q
14 mm, wrapped QCM sensor (Au/SiO_2, 5 MHz)	10	AWS SNS 000049 A
AWS HFF-QCM sensor (Au, 50 MHz)	10	AWS SNS 000077 A

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