Biolin Scientific
[Progress Together]

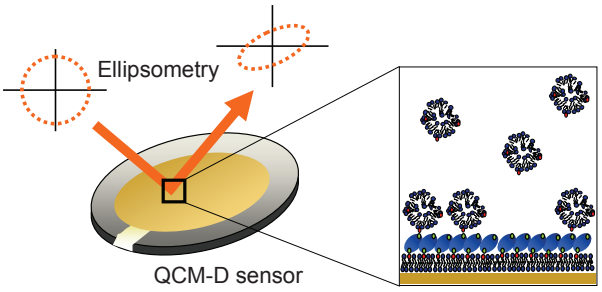
Q-Sense | [Application Note] 20

Combined QCM-D/ellipsometry setup for real-time characterisation of thin molecular films

Several surface sensitive techniques are able to monitor the formation and properties of thin molecular films on a sensor surface. As the complexity of the studied systems increases, one technique alone can often not provide all desired insight. Multitechnique approaches provide complementary information, and thus allow for improved interpretation and/or the identification of artifacts. Comparing data from several separate experimental setups, however, is not always straight forward due to differences in the experimental conditions. Therefore, merging several techniques into the same setup and monitoring events on the same surface is a promising approach. This application note presents how a combined experimental setup of Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) and ellipsometry can augment the measurement of molecular behavior at a sensor surface.

Background

Both QCM-D and ellipsometry can provide information about adsorption events on surfaces and the properties of the resulting films. In particular, both techniques can quantify adsorbed masses in real-time. Ellipsometry, being an optical technique, is sensitive to the mass of adsorbed molecules only, whereas the mass determined by QCM-D also includes solvent that is coupled to the film. Thus, a comparison of the masses measured by the two techniques provides information about the amount of solvent in the film. With such an approach, film swelling or collapse can easily be distinguished from adsorption/desorption events, changes in the solvent content of thin films can be monitored over time, and other structural or morphological changes of adsorbed films identified.



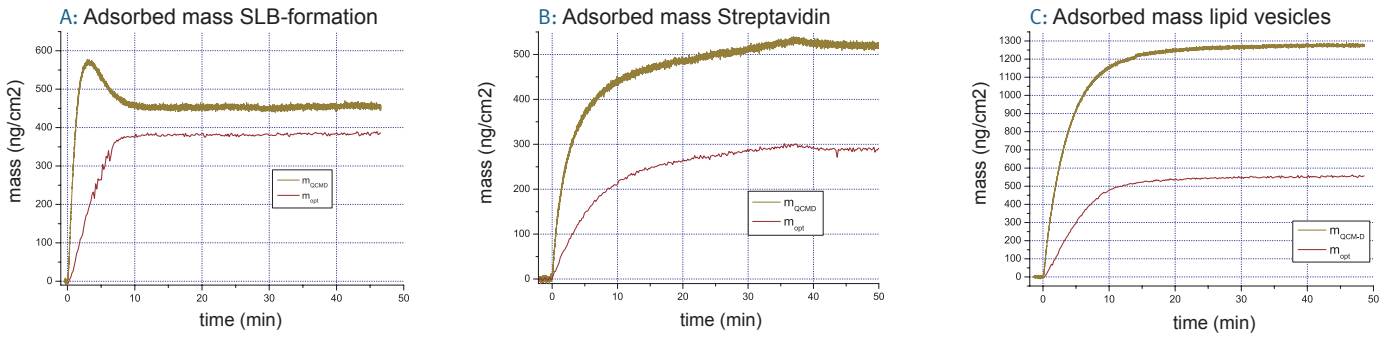
[Figure 1]: A setup combining QCM-D and ellipsometry was used to study a build up of lipid bilayer onto a QCM-D sensor, followed by streptavidin and vesicle binding.

Approach

Figure 1 shows a schematic figure of the combined QCM-D/ellipsometry setup. QCM-D data, changes in oscillation frequency of the sensor to acquire information on mass and dissipation data to acquire data on structural properties of the film is measured. Simultaneously, polarized light is reflected at the surface of the QCM-D sensor and the changes in its polarization state measured. Here, the formation of a supported lipid bilayer from small unilamellar vesicles (SUVs) containing 10% biotinylated lipids, followed by the specific binding of streptavidin, as well as biotinylated vesicles, were investigated.

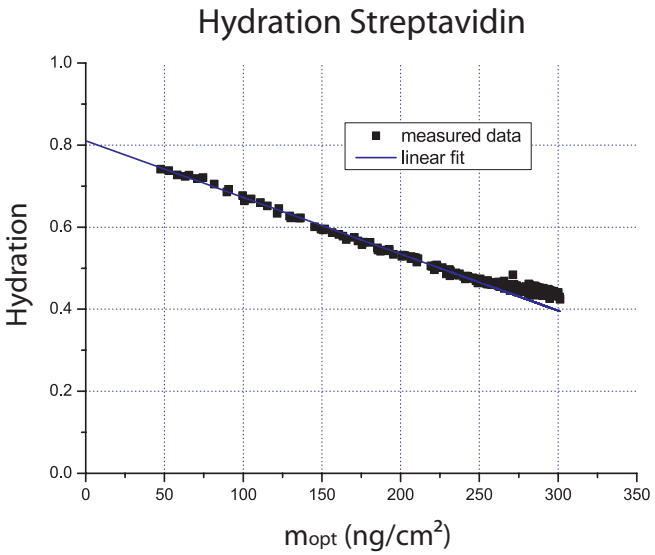
Results & discussion

Figure 2 shows the adsorbed mass upon exposure of a silica-coated QCM-D sensor to SUVs, followed by streptavidin and additional vesicles. First, a supported lipid bilayer was formed from vesicles that attached to the QCM-D sensor (Fig 2A). The bi-phasic behavior of the QCM-D mass provides a direct indication that vesicles initially adsorbed intact and then ruptured to form a planar lipid bilayer. This transition is not readily visible from the ellipsometry data, which only shows a monotonous mass increase during bilayer formation. Second, streptavidin bound to the biotin



[Figure 2]: Masses, as detected by ellipsometery (mopt) and by QCM-D (mQCM-D), upon (A) formation of supported lipid bilayer, (B) subsequent binding of streptavidin and (C) intact vesicles.

groups that are present on the supported lipid bilayer, as indicated by the mass increases in Fig 2B. Finally, biotinylated vesicles were again added to the streptavidin layer. The absence of a biphasic behaviour in this step (Fig 2C) provides a first indication that adsorbing vesicles remained intact. As expected, the masses determined by QCM-D (m_{QCM-D}) were consistently higher than the ellipsometric masses (m_{opt}), because QCM-D senses the solvent that is dynamically coupled to and trapped in the film. A rather small mass difference between QCM-D and ellipsometry was observed for the supported lipid bilayer (Fig 2A), consistent with expectations for such a planar and solvent-poor structure.



[Figure 3]: Amount of coupled solvent (H=1-mopt/mQCMD) for streptavidin binding as function of the optical mass (=coverage).

This can be compared to binding of the solvent filled, intact vesicles, where solvent contributes more than 50% to the QCM-D mass (Fig 2C).

The amount of solvent in the adsorbed film can been defined as:

$$H = 1 - \frac{m_{opt}}{m_{QCM-D}}$$

The evolution of this parameter over time is shown in Fig. 3 for the streptavidin binding step. The amount of solvent is displayed as a function of the optical mass, which is proportional to the surface coverage of the adsorbed species. The dependency is linear over almost the entire range of binding.

Conclusions

Combination of QCM-D and ellipsometry gives real-time, complementary data about the amount of solvent associated with molecular films. In addition to the data presented in this application note, QCM-D can provide information on mechanical properties of thin films (viscoelasticity), while ellipsometry can measure optical film properties (refractive index). Furthermore, the layer thickness can be determined with both techniques, and results compared. Thus, parallel QCM-D and ellipsometry analysis in the same setup can provide a detailed understanding of molecular events taking place at a surface.

References:

Bingen, P, Wang, G, Steinmetz, N. F, Rodahl, M, Richter, R. P. Anal. Chem. in press, and work by R. Richter and S. Stahl at CIC biomaGUNE, Spain, in collaboration with Q-Sense AB.

Biolin Scientific
[Progress Together]

E-mail:info@biolinscientific.com
biolinscientific.com