Biolin Scientific
[Progress Together]

Q-Sense | [Application Note] 2

Structural change of adsorbed protein layer

This application note illustrates how the dissipation factor together with the frequency response enables mass measurement of soft films as well as the ability to study structural changes in the film. In combination with ellipsometry measurements it is also possible to determine the amount of coupled water.

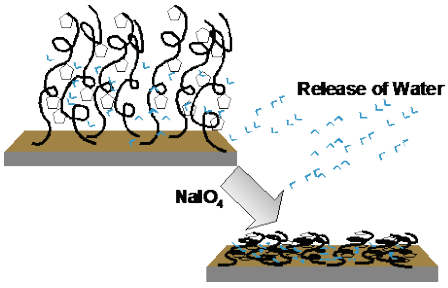
Introduction

The merit of the QCM-D technique lies primarily in the simplicity and sensitivity (ng/cm^2) by which an adsorbed mass, Δm, can be deduced from a change in resonance frequency, Δf. For rigid, evenly distributed and thin adsorbed layers, the linear Sauerbrey relation between Δf and adsorbed mass, Δm_{QCM}, is a good approximation. However, for sufficiently non-rigid ("soft") adsorbed layers, the Sauerbrey relation is not valid. The physical explanation for the failure of the Sauerbrey relation ($\Delta f \propto \Delta m$) derives from the propagation of the shear acoustic wave in the ad-layer: a soft and/or thicker ad-layer does not fully follow the shear oscillation of the sensor crystal. In other words: outer parts of the layer do not follow the oscillation of the sensor.

An important additional fact in this context is that water may couple to immobilized molecular layers and is thus sensed as an additional mass. Such water is referred to as coupled water below. This means that the layer is sensed as a viscoelastic "hydrogel" composed of macromolecules and coupled water.

These factors in combination with use of optical techniques, e.g. ellipsometry (ELM) or surface plasmon resonance (SPR), provide a platform for unique new information about adsorbed molecular layers. With a theoretical model (included in software QTools from Q-Sense) that handles the elastic and inelastic components of the shearwave propagation through the film, unique information can be obtained.

To demonstrate these possibilities, a model system has been studied in which the viscoelastic properties can be varied in situ, by (bio)chemical means, namely the adsorption of the mussel adhesive protein, Mefp-1 (Mw 120 kD). Mefp-1 is especially attractive for this purpose since it has an open flexible conformation that can be changed easily by cross-linking using for instance, $NaIO_4$ (Figure 1). As substrate, we have used electrically inert, non-polar methylterminated surfaces on which Mefp-1 is known to form an elongated flexible layer upon spontaneous adsorption.



[Figure 1]: Injection of $NaIO_4$ results in a collapse of the protein film and coupled water is released.

Results

Information about thickness and amount of water coupled to thin protein films (nm) can be determined and correlated to their viscoelastic properties using the Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring technique combined with ELM. The frequency, f, and the energy dissipation, D, responses of the QCM-D (Figure 2) was modeled using a Voigt based viscoelastic model, representing the protein film as a homogeneous film with an effective thickness, density and complex shear modulus. The best fit was achieved for values in Table 1 below.

Modelling of the QCM-D data shows that cross-linking of surface bound Mefp-1 using NaIO4 results in a decrease in the effective acoustic thickness from ~22 nm to ~7 nm, accompanied by a three-fold increase in shear viscosity and a five fold increase in shear elastic modulus. These results were confirmed by ELM measurements, demonstrating a decrease in effective optical thickness from ~21 nm to ~5 nm during the cross-linking reaction, accompanied by an increase in effective refractive index from ~1.35 to ~1.4, signaling transformation from an elongated and hydrated to a contracted and compact protein film. Without

using mathematical modelling the film thickness was calculated to 12 nm, which clearly shows that the Sauerbrey relation is not valid for the elongated protein structure before cross-linking. Thus, our results demonstrate that for acoustically thin films inducing significant energy dissipation, direct con-version of the frequency shift to mass uptake using the Sauer-brey relation results in a significant underestimation of the adsorbed mass (protein + water).

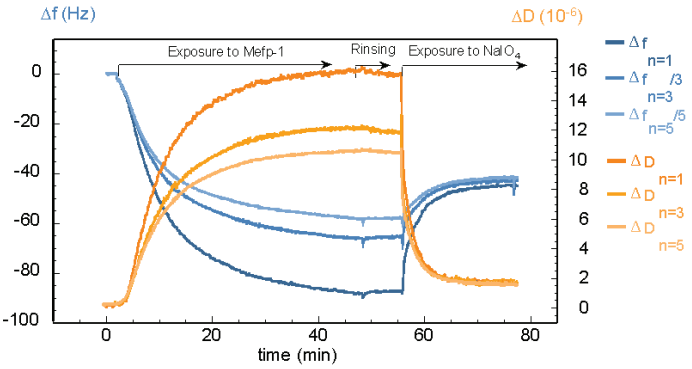
By further comparing the mass uptake data from the optical and the acoustic techniques, it is shown that a significantly larger amount of water is coupled to the elongated, compared to the cross-linked film of adsorbed Mefp-1. Before cross-linking, the corresponding mass uptake according to the QCM data is ~1168 ng/cm^2, whereas it was only ~730 ng7cm^2 after the cross-linking reaction. Compared to the ELM data, indicating a mass uptake of ~135 ng/cm^2 prior to and ~130 ng/cm^2 after the cross-linking reaction, it means that 90 % of the coupled mass sensed via the QCM-D frequency shift prior to cross-linking consists of coupled solvent (primarily water), whereas afterwards this number is only ~65%.

Conclusions

Since QCM-D not only measures changes in f, related to adsorbed mass, but also changes in dissipation (D), structural changes such as cross-linking and folding/unfolding can be monitored. In this application note an elongated protein layer collapses when exposed to NaIO4 and the thickness decreases from 22 nm to 7 nm. The kinetics of the structural change is easily followed in real time with QCM-D.

References:

Variations in coupled water, viscoelastic properties and film thickness of a Mefp-1 protein film during adsorption and cross-linking: a QCM-D, ellip-sometry and SPR study. Analytical Chemistry 2001, 73, 5796-5804 F Höök, B Kasemo, T Nylander, C Fant, K Sott, H Elwing.



[Figure 2]: Frequency and dissipation responses during spontaneous adsorption of the protein layer followed by cross-linking. The dissipation changes from very high values (soft layer) to low values (compact layer).

	Measured parameter	Prior to cross-linking	After cross-linking
QCM-D	Δm, sauerbrey, n=3 (ng/cm^2)	1168	730
	Thickness, sauerbrey n=3 (nm)	11.3	6.9
	Thickness, modelling (nm)	22.4	7.3
	Viscosity (e-3 N s m^{-2})	1.8	6
	Elasticity (e4 N m^{-2})	6.6	30
ELM	Δm (ng/cm^2)	135	130
	Thickness (nm)	21	5
	Refractive index	1.35	1.40

[Table 1]: Mass and thickness values, obtained with QCM-D and Ellipsometry, for the mussel adhesive protein layer prior to, and after, cross-linking.

Biolin Scientific
[Progress Together]

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