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Q-Sense | [Application Note] 29

Improved understanding of lipid membranes through combined QCM-D and EIS measurements

A current trend in the field of sensing is to use instruments in which several detection principles can be combined in one experiment. Methods that provide a combination of structural and functional information on biomolecules can provide crucial insights into the understanding of biomolecular functions.

Introduction

It is of great interest in many fields to investigate how peptide and protein interactions change the properties of a lipid membrane. It is well known that membrane damage is fatal to cells and bacteria. Therefore, a common mechanism of toxins is to disrupt the membrane or interfere with the transport of ions across the membrane. Typically, antibacterial peptides incorporate into bacterial membranes and destroy the ion gradient between the inside and the outside of the bacterium.

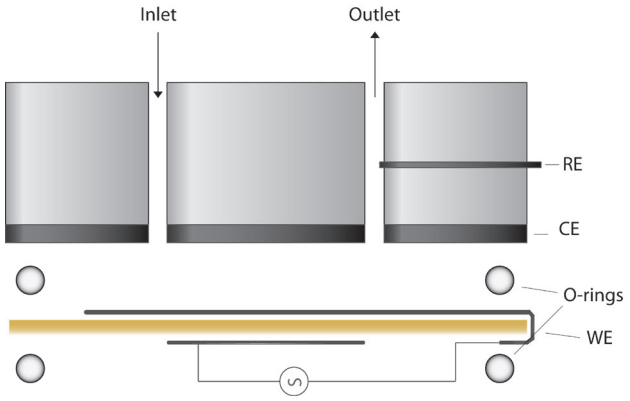
Approach and experimental setup

This application note reviews two examples of experiments where QCM-D and Electrical Impedance Spectroscopy (EIS) were combined on the same sensor in real-time by the use of Q-Sense Electrochemistry module (figure 1). The first example deals with the disruption of a lipid membrane through the catalytic action of the enzyme PLA2, found in insect and snake venom. PLA2 cleaves phospholipids into the corresponding lysolipids and fatty acids by catalyzing the hydrolysis of a phosphoester bond in the lipid molecule. This cleavage disrupts the membrane.

The second example demonstrates the insertion of transmembrane pores in a model membrane by the addition of the peptide Gramicidin D. The combination of QCM-D and electrochemistry is especially attractive in these examples. The effect on the membrane can be sensitively detected by the electrochemical method, whereas the strength of the QCM-D method lies in its ability to characterize the membrane's viscoelastic (soft or rigid) properties.

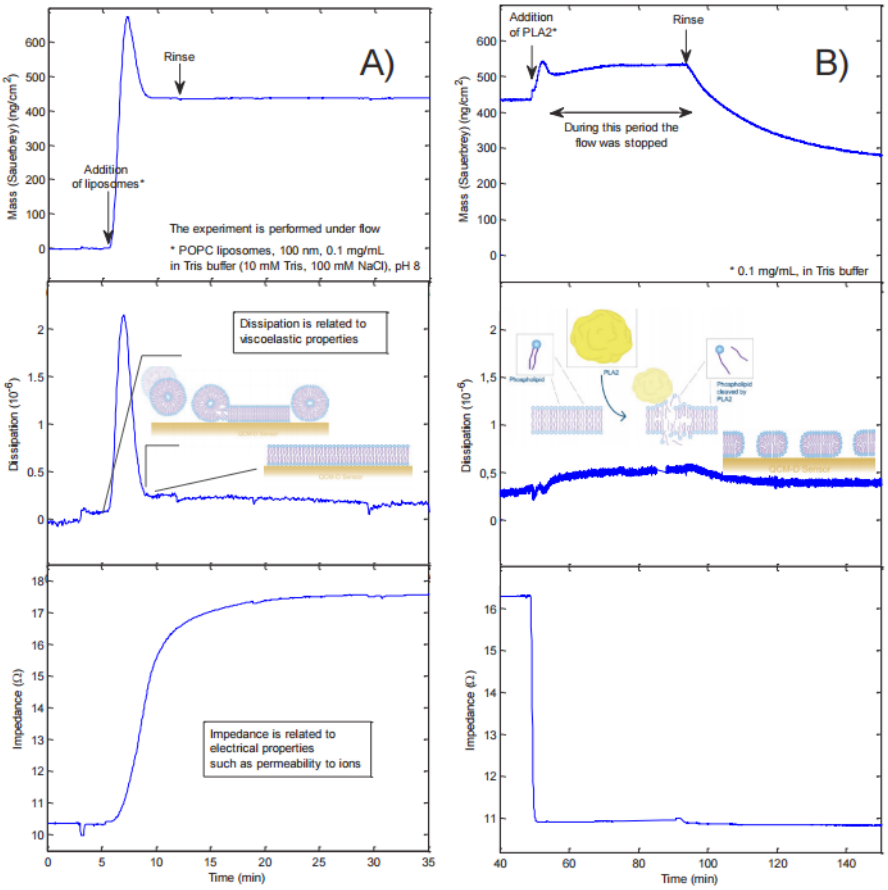
Results and discussion

The formation of good quality lipid membranes was first ascertained by both QCM-D and EIS. The formation of a lipid bilayer on the QCM-D sensor was detected in real time by both frequency and dissipation shifts and by single frequency impedance spectroscopy, as demonstrated in Figure 2A.

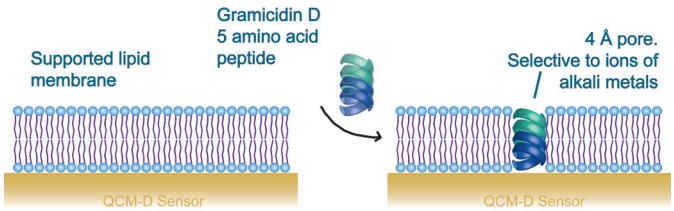


[Figure 1]: Schematic illustration of an electrochemistry module.

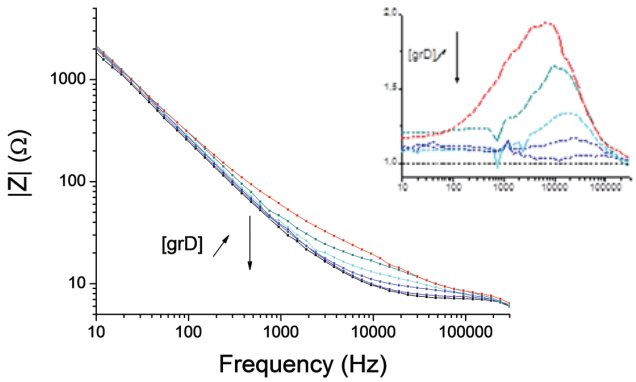
Bilayer formation follows the typical pathway via the adsoption of a critical mass of liposomes, which eventually ruptures and forms an extended lipid bilayer on the surface with the characteristic QCM-D responses $\Delta f = -26$ Hz and $\Delta D < 0.5$, indicating that a layer a few nanometers thick (typically 5 nm) and rigid (as indicated by the low ΔD) has formed on the sensor surface. Note that bilayer formation as monitored by QCM-D is already complete long before the impedance amplitude stabilizes. A plausible explanation for this observation is that the fusion and rupture of adsorbed liposomes is followed by a slower annealing process, resulting from increased ordering of the lipid molecules in the lipid membrane and improved electrical sealing of the membrane. The first example, involving membrane disruption, is shown in Figure 2B, where the action of the enzyme immediately alters the electrical properties of the membrane, as evidenced by the sharp decrease in the impedance amplitude. By QCM-D, the process is first characterized by mass uptake, followed by mass removal only after the system has been rinsed with buffer.



[Figure 2]: A) QCM-D and EIS results obtained for the formation of a supported lipid membrane on the SiO_2 -coated sensor surface in real-time. Figure adopted after [1] with permission from the authors and Reproduced by permission of The Royal Society of Chemistry. B) The effect of addition of the enzyme PLA2 to the lipid membrane. Courtesy of E. Briand



[Figure 3]: Schematics of a supported lipid membrane and the addition of pore forming peptide Gramicidin D.



[Figure 4]: Bode plots showing the insertion of Gramicidin D as a function of peptide concentration, redrawn after [1] with permission from the authors and Reproduced by permission of The Royal Society of Chemistry. In the inset, spectra of the modulus of impedance are normalized against the modulus recorded on the sensor.

In the second example, impedance spectra were recorded at different stages of an experiment involving a supported lipid membrane and the pore-forming peptide Gramicidin D, as schematically depicted in Figure 3. Bode plot from such experiments are shown in Figure 4. As expected, the resistivity of the membrane decreased after exposur to Gramicidin D. The insertion of the peptides is seen in the EIS spectra, while no changes are observed in the QCM-D signals (data not shown). This means that this modification of the resistivity is not linked to modification of the mechanical properties of the membrane, but most likely to the insertion of active Gramicidin D [1].

Conclusions

The combination of QCM-D and electrical impedance spectroscopy is a powerful tool to investigate structure-function relationships of proteins and peptides interacting with lipid membranes. In particular the function of ion channels can be followed while at the same time monitoring the viscoelastic properties of the lipid membrane.

References:

[1] Elisabeth Briand, Michael Zäch, Sofia Svedhem, Bengt Kasemo and Sarunas Petronis. Combined QCM-D and EIS study of supported lipid bilayer formation and interaction with poreforming peptides. Analyst, 2010, 135, 343–350.

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