Biomolecular interaction analysis using the Insplorion Acoulyte for combined NPS and QCM-D measurements

The combination of two label-free, surface sensitive measurement techniques based on different physical principles- Insplorion’s NanoPlasmonic Sensing (NPS) and Q-Sense quartz crystal microbalance with dissipation monitoring (QCM-D) enables detailed studies of biomacromolecular interactions at solid-liquid interfaces. Here, this is demonstrated by investigating the adsorption of lipid vesicles on a titanium dioxide surface and the consecutive, peptide mediated, structural transformation of the adsorbed vesicles into a supported lipid bilayer (SLB).

Introduction
Clarifying the mechanistic details of the dynamic interaction processes that macromolecular assemblies undergo at interfaces in biological systems is of great importance for a wide range of fundamental and applied research fields. Here, it is demonstrated that combined NPS and QCM-D measurements can be used to help study interaction processes and provide insight into the mechanistic details of such processes. The complementarity in the measured quantities (“dry” (optical) vs. “wet” (acoustic) mass) as well as the different probing depths of the two techniques (60-250 nm for QCM-D and ca. 30 nm for NPS) is used to obtain detailed information on the vesicle adsorption and peptide induced structural transformation of the vesicles into a SLB.

Experimental Procedure
The experiments were performed using TiO2 coated Acoulyte sensors.
Two different biomacromolecular events were investigated: (i) the monotonic adsorption of zwitterionic lipid vesicles (67 nm diameter DOPC) and (ii) the amphipathic, α-helical (AH) peptide induced rupture of the surface adsorbed vesicles into a SLB.

Results
The adsorption of DOPC vesicles caused an initial decrease in the frequency (f), an initial increase in the energy dissipation (D) together with a monotonic red-shift in the NPS signal (Δλpeak). After stabilisation, the shifts in f and D were -90 Hz and 6.8 x 10^-6 (Fig. 2A), respectively, which are indicative of a saturated adlayer of small vesicles.

The overshoot behavior observed
in both the f and D signals is most likely due to a “rocking and rolling motion” of the vesicles in the loosely packed adlayer (at medium coverage). This observation is corroborated by the simultaneous NPS measurement (Fig. 2B) which indicates monotonic vesicle adsorption. The NPS signal stabilises almost simultaneously as the f and D signals i.e. the overshoot is observed before full coverage is reached and is most likely not attributed to the loss of lipid mass but rather to a complex hydrodynamic response associated with coupled solvent. When the vesicles start to rupture, lipid and solvent mass is lost and a dense SLB is formed causing an increase in f and a decrease in D. The f and D values after stabilisation are indicative of the formation of a SLB.

The NPS signal rapidly red-shift upon the addition of the AH peptide followed by a slight blue-shift before reaching a stable signal (Fig. 3B). The red-shift is caused by the binding of the AH peptide to the vesicles and the movement of lipid mass closer to the sensor surface- first because the vesicles swell and become less rigid and then because of vesicle rupture and SLB formation. The small, subsequent, blue-shift is due to lipid mass being lost from within the sensing volume and the release of the AH peptide from the bilayer.

Conclusions
Here a model system comprised of vesicle adsorption and the structural transformation of adsorbed vesicles into a SLB was used to demonstrate how complex biomacromolecular interactions can be deciphered by using combined NPS and QCM-D measurements. The integration of NPS and QCM-D provides a versatile tool for studying dynamic processes in biomolecular thin film.

Figure 2: QCM-D (A) and NPS (B) data for the adsorption of DOPC vesicles on a TiO2 surface. Redrawn from reference [1].

Figure 3: QCM-D (A) and NPS (B) data for AH peptide induced vesicle rupture and lipid bilayer formation. Redrawn from reference [1].

This study was performed by Prof. Nam-Joon Cho and coworkers at Nanyang Technological University. The results were originally published in reference [1].

Reference