Antibody quantification and corresponding molecular hydration using Insplorion's Nanoplasmonic Sensing in combination with QCM-D

Insplorion's technology allows for the combination of two label-free characterization techniques, namely quartz crystal microbalance with dissipation measurement (QCM-D) and Nanoplasmonic Sensing (NPS), for the design of a biosensor to monitor water resources. By combining both transduction techniques, it is possible to estimate the adsorbed antibody surface coverage on the nanostructured sensors as well as their hydration percentage.

Introduction

Immunosensors, biosensors that use an antibody as the bioreceptor, are one of the most widespread classes of sensors as they offer great affinity toward an analyte.
Diclofenac (DCF) is a widespread painkiller used worldwide, being considered a water pollutant and hazardous to the aquatic environment. In this study, an immunosensor was designed for the detection of DCF [1]. Its performance was evaluated in buffer media and ultimately using water from the Seine and Marne rivers.

Experimental procedure

Insplorion Acoulyte sensors were functionalised for the immunoassay. Briefly, the top SiO_2 layer was incubated with GOPTS to create a silane layer with terminal epoxy groups, which were in turn used to bind amine-terminated PEG molecules. A solution of DCF where the carboxylic acid functional groups had been activated was prepared, and the sensor substrates were immersed therein, thus allowing the DCF to be grafted on the sensor surface. (Figure 1). The Insplorion Acoulyte, together with a QCM-D instrument was used to perform the assay. After the stabilisation of the QCM-D and NPS signals in a running buffer, monoclonal anti-DCF antibody (5 mg/L) was flown over the sensor surface at 25 µL/min at a temperature of 20 °C. The frequency change and dissipation were recorded from the QCM-D as well as the NPS signal with focus on the peak shift parameter.

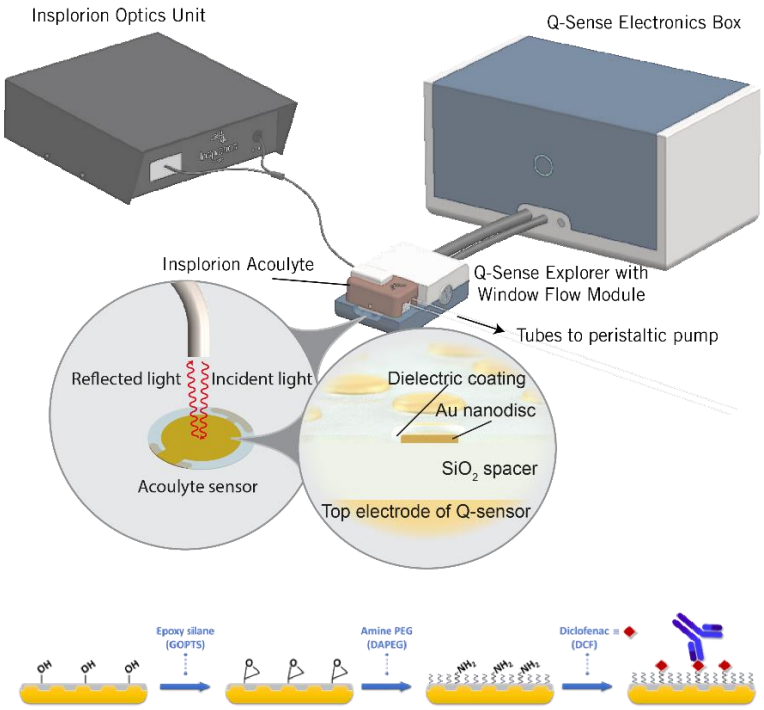

Figure 1: Insplorion system setup at the top. The bottom shows a schematic illustration of the sensor surface chemical modification and setup for the indirect competition assay used. Figure is not to scale.

Results

Injection of the antibody solution led to signal shifts in both QCM-D and NPS sensograms. Specifically, frequency decrease and dissipation augmentation were accompanied by an increase in the NPS signal upon antibody recognition.
Since a rigid antibody layer was formed onto the sensor surface, data treatment allowed the determination of the acoustic mass uptake from QCM-D data by applying the Sauerbrey equation. At the same time, the dry mass uptake was calculated to be 260 ng/cm^2 from the NPS signal, which agrees with the theoretical mass uptake for a

monolayer of antibody in a head-on orientation, as found in literature. This orientation is very likely, considering that PEG is known to avoid non-specific adsorption and DCF is immobilized onto the surface. The only other possible grafting orientation would be side-on, but that would have resulted in a smaller mass uptake. [2]

It is worth noting that the optical mass uptake characterises the antibody surface coverage more accurately as it only considers the antibody mass without interference from solvent molecules.

Since the Insplorion Acoulyte enables the simultaneous recording of QCM-D and NPS signals in the same experimental conditions, comparison of mass uptakes from both was made possible. This was key in allowing the authors to calculate a hydration percentage of 75% for the antibody monolayer, which is consistent with previously published investigations.

The authors continued the study by performing competitive immunoassays for DCF in buffers and evaluating performance parameters such as dynamic range, limit of detection, and limit of quantification. The sensor was later tested with surface water from the Seine and Marne rivers.

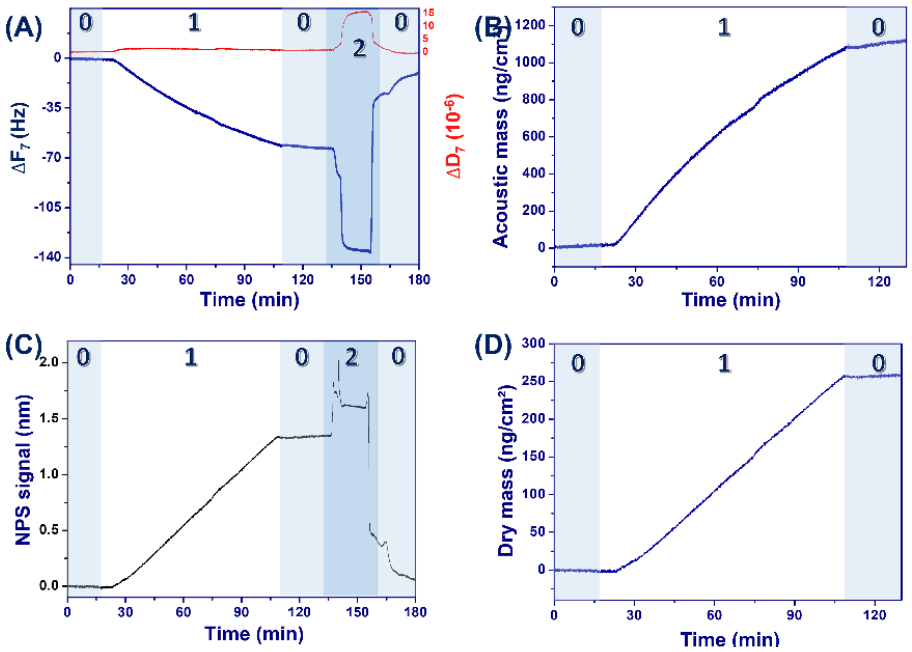

Figure 1: Adsorption of mAb by DCF-modified nanostructured quartz sensor: (A) QCM-D frequency (blue) and dissipation (red) shifts and (B) the corresponding acoustic mass obtained from the Sauerbrey equation. (C) LSPR peak shift Δλmax simultaneously recorded on the same substrate.

Conclusion

Insplorion technology was used to study antibody binding to diclofenac for immunosensing. Combining QCM-D and NPS transduction techniques was employed to determine antibody surface coverage as well as the hydration degree upon binding onto the sensor surface.

Further experiments can be performed to address baseline shifts, for instance with PEG molecules of lower molecular mass, in order to avoid reorganisation. Employing alternative strategies for surface functionalization, for example self-assembled monolayers (SAMs), can also be valid approaches for target immobilisation onto the substrate surface.

This work demonstrates the robustness of NPS and can be used to detect a variety of other small molecules and analytes, as well as demonstrating how powerful NPS can be in combination with other techniques such as QCM-D.

This study was performed by Prof. Souhir Boujday and coworkers at Sorbonne University, France.

References

[1] Mazouzi et al. (2021) *Design and Analytical Performances of a Diclofenac Biosensor for Water Resources Monitoring*. DOI: 10.1021/acssensors.1c01607

[2] Zhang et al. (2020) Antibody-Gold Nanoparticle Bioconjugates for Biosensors: Synthesis, Characterization and Selected Applications. DOI : 10.1016/j.bios.2020.11237