

# DYNAMIC MEASUREMENTS OF BOUND WATER IN PROTEINS AND PROTEIN-RESISTANT POLYMERS: A COMBINED OWLS AND QCM-D STUDY

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**INTRODUCTION:** Water is known to play an important role in biological systems, but the time-dependent variation of the amount of bound water during adsorption processes has not been fully characterized. To elucidate the role of water in protein and polymer adsorption, comparative studies were carried out using two different, but complementary, *in situ* techniques: optical waveguide light mode spectroscopy (OWLS) and the quartz crystal microbalance with dissipation measurement (QCM-D).

To study the behaviour of bound water during protein adsorption, the model proteins human serum albumin (HSA), fibrinogen, immunoglobulin, and haemoglobin were adsorbed onto titanium-oxide coated substrates.

In addition, the role of water in adsorbed poly(L-lysine)-*g*-poly(ethylene glycol) (PLL-*g*-PEG) was examined. Such graft-copolymers, like other PEGylated coatings, have been shown to be remarkably resistant to protein adsorption [1] and the cationic poly(L-lysine) backbone allows for facile and rapid adsorption to metal oxide surfaces (such as titania and silica) that are negatively charged at neutral pH. This makes these polymers highly suitable for biosensing applications.

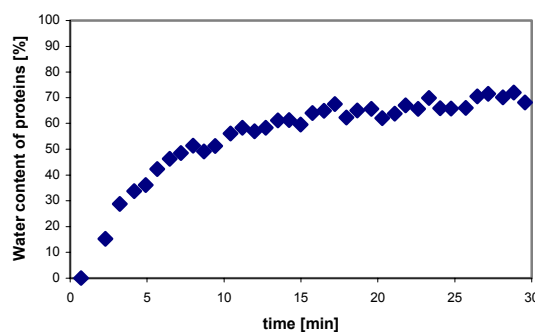
**METHODS:** The OWLS technique involves the incoupling of a He-Ne laser into a planar waveguide generating an evanescent field which allows for the direct online monitoring of macromolecule adsorption. It is highly sensitive (i.e.  $\sim 1$  ng/cm<sup>2</sup>) up to a distance of 100 nm above the surface of the waveguide. The mass of an adsorbed layer can be calculated from the thickness and refractive index values derived from the mode equations.

The QCM-D technique is based on the pulsed excitation of shear oscillations in piezoelectric quartz crystals. Changes in the resonance frequency of these shear oscillations as a function of time provide information about adsorbed masses, and the decay rate of the oscillation amplitude after the pulse gives insight into the viscoelastic nature (i.e., mechanical properties) of the adsorbed layer.

Whereas the adsorbed mass calculated from OWLS measurements reflects only the mass of the polymer or protein, the mass calculated from the QCM experiments contains an additional contribution from the mass of the water that is immobilized within the adlayer [2]. The two techniques thus provide complementary information.

Both the OWLS and QCM-D techniques have the advantages of being able to monitor real-time kinetics and of not requiring labelled samples.

**RESULTS AND DISCUSSION:** The water content was found to be characteristic for the investigated proteins. An example of a typical experiment, shown in the figure below, demonstrates the evolution of the water content of HSA during the adsorption process onto a TiO<sub>2</sub> surface. The water content of HSA is low during the initial phase of adsorption indicating a different conformation than at the end of the adsorption process.



Adsorption and water content of PLL-*g*-PEGs with different molecular weights and grafting ratios were also examined on several different metal oxide surfaces.

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