

## QCM-D study of molecular conformational effects on protein - DNA interactions

Estrogen receptors are transcription factors activated by specific ligands. Upon ligand binding the receptors undergo structural changes and subsequently bind to specific DNA sequences. The characteristics of the ligand – receptor binding have previously been identified. However, the conformational effect of ligand binding on the receptor – DNA complex, believed to influence gene transcription, is not yet understood. In this context the QCM-D technique is unique as it reflects viscoelastic characteristics of biomolecular films, which in turn can be related to molecular conformation.

### Introduction

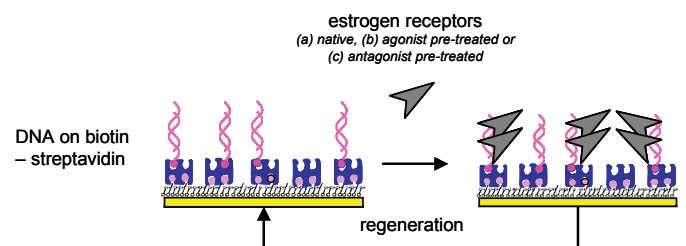
Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring is a surface sensitive technique for assessment of mass and viscoelastic properties of thin films. It is commonly used for evaluation of binding and interactions of biological molecules in liquid environment. Since the QCM-D working principle is based on lateral movement of a quartz sensor, both deposited biomolecules and associated water, are coupled to the sensor motion. Therefore, the output data describes hydrated films, where frequency of the sensor movement ( $\Delta f$ ) reflects film mass, and damping of the movement ( $\Delta D$ ) correlates to viscoelastic properties. In contrast, surface analytical techniques based on optical principles, such as Surface Plasmon Resonance (SPR), can only sense the biomolecules and omit the surrounding medium. By complementing QCM-D data with information from SPR, parameters such as film hydration and density can be accurately calculated. Furthermore, with the QTools\* modeling software film thickness, viscosity and elasticity can be determined. This application note describes capability of QCM-D technology to characterize protein – DNA interactions.

### Approach

DNA was presented on a biotin – streptavidin sandwich (Figure 1). Subsequent binding of three conformations of estrogen receptors was evaluated:

- (a) native
- (b) pre-incubated with ligands promoting binding to DNA (agonist)
- (c) pre-incubated with ligands partially inhibiting binding to DNA (antagonist).

The DNA functionalized surfaces could be regenerated by removal of bound receptors in situ enabling multiple protein binding experiments (Figure 1).



**[Figure 1]:** DNA was presented on a biotin – streptavidin sandwich to study interaction with estrogen receptors. Bound receptors could be removed to regenerate the DNA-presenting substrate.

### Results and discussion

QCM-D sensed differences in binding behavior of estrogen receptors to DNA depending on the protein pre-treatment with ligands (Figure 2). Largest shift in  $\Delta f$ , thus largest bound receptor mass, was observed for native proteins, followed by agonist ligand pre-treated receptors and antagonist ligand pre-treated receptors, respectively. Shifts in  $\Delta D$  exhibited the reversed order. Binding of the native receptors resulted in the lowest  $\Delta D$ , indicating the most rigid film, compared to DNA – receptor complexes formed with the pre-treated proteins.

These trends were highlighted by direct comparison of rigidity to mass ratios in  $\Delta D$  vs.  $\Delta f$  plots (Figure 3). A large  $\Delta D/\Delta f$  ratio indicates an extended structure or loose binding between interacting molecules. Accordingly, the highest ratio, i.e. steepest  $\Delta D/\Delta f$  slope, was observed for the DNA complex with the antagonist pre-treated receptors, while the lowest was typical for native receptors (Table 1).

	$\Delta D/\Delta f$	hydration	density	Thickness/ viscosity/ elasticity
DNA-native receptor	+	+	+++	+++
DNA-agonist receptor	++	++	++	++
DNA-antagonist receptor	+++	+++	+	+

[Table 1]: Summary of DNA-estrogen receptor complex properties calculated from QCM-D and SPR data. Growing trends are indicated with increased number of "+".

Interpretation of QCM-D data was complemented and confirmed with SPR analysis. In accordance with the QCM-D, the largest mass increase was detected for binding of native receptors, followed by agonist and antagonist pre-treated proteins, respectively (Figure 4).

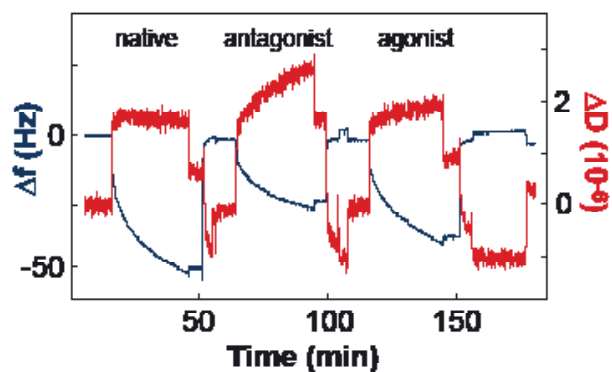
Comparison of QCM-D and SPR data provided accurate quantification of film parameters, such as water content and density. As expected, the complex between the antagonist pre-treated receptor and DNA was the most hydrated with the lowest density (Table). Other film parameters, such as thickness, viscosity and shear elasticity, could be accurately quantified with QTools modeling software.

Modeling showed that DNA film thickness was lower than expected, pointing towards a tilted on random DNA conformation. Subsequent binding of the receptors doubled the total film thickness simultaneously increasing density, viscosity and shear elasticity indicating a high density packing and tight binding of the receptors. The increase in thickness, density, shear elasticity and viscosity were least drastic for antagonist pretreated receptors, which was consistent with the higher water content and less close-packed nature of these films (Table). Thus the QTools quantification verified the qualitative trends from the  $\Delta D$  vs.  $\Delta f$  plots.

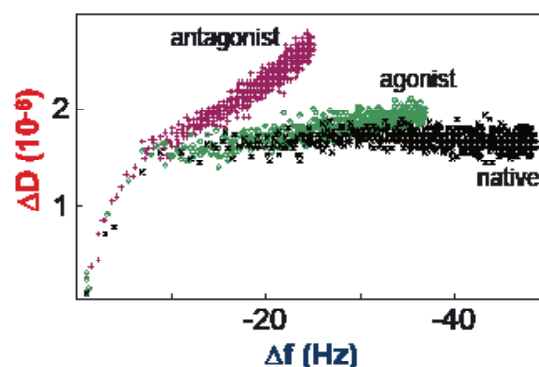
\*) QTools is an analysis software included in your Q-Sense QCM-D system; it is used for viscoelastic modeling of QCM-D data.

## Conclusions

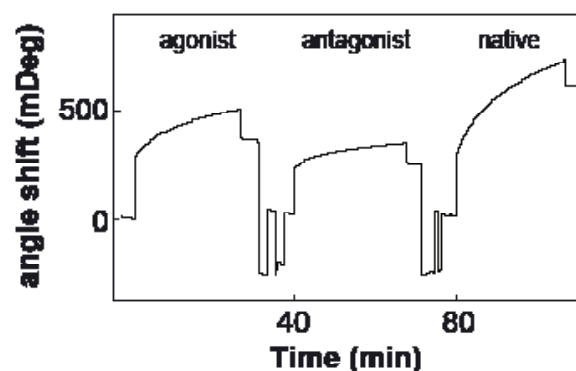
QCM-D analysis provided conformational information on estrogen receptor - DNA complexes, which are believed to be of importance in understanding subsequent gene transcription. The distinct viscoelastic properties of these biomolecular films were dependant on receptor conformation. Antagonist pre-treated receptors resulted in water-rich and less well-structured films compared to agonist pre-treated receptors. Conformational differences were qualitatively extracted from  $\Delta D$  vs.  $\Delta f$  plots and could be confirmed and quantified by QTools modeling.



[Figure 2]: QCM-D data of estrogen receptor binding to DNA. Changes in  $\Delta f$  (blue) and  $\Delta D$  (red) upon incubation with native, antagonist and agonist pre-treated receptors, respectively, and regeneration between additions.



[Figure 3]: QCM-D data for estrogen receptor binding to DNA from Figure 2 presented as changes in  $\Delta D$  as function of  $\Delta f$ .



[Figure 4]: SPR data of estrogen receptor binding to DNA. Subsequent incubation with agonist, antagonist and non-pretreated receptors, respectively, and regeneration between additions.

## References:

Understanding ligand binding effects on conformation of Estrogen Receptor DNA complex: A combinational QCM-D and SPR study. Biophysical Journal 2007, 92, p. 4415-4423. Peh W Y X, Reimhult E, Teh H F, Thomsen J, Su X.