

ANALYZING ENZYMATIC ACTIVITY ON FUNCTIONALIZED SURFACES

This application note demonstrates how Quartz Crystal Microbalance with Dissipation (QCM-D) can be used to study enzyme-protein interactions on functionalized biological surfaces in real time. Specifically we study how functionalised surfaces can be used to control antibody binding and orientation. Further, the specific enzymatic degradation of antibodies is investigated. These concepts are illustrated by studying the defense mechanisms adapted by *Streptococcus pyogenes* and how IdeS enzyme cleaves immunoglobulin proteins.

QCM-D is a surface sensitive technique, which provides real time information on mass and structure of molecular layers. Simultaneous measurements of both mass and structural properties give a thorough understanding of molecular adsorption and interaction under a variety of experimental conditions. Molecular events and processes such as adsorption, degradation, interactions, cross-linking, cell attachments and swelling can be investigated using QCM-D.

Here we show how QCM-D enables real time investigation of the two defense mechanisms of *S. Pyogenes* against the immune system.

INTRODUCTION

Immunoglobulin G (IgG) are central proteins in the immune system and consist of two antigen-recognizing Fab fragments, linked through a hinge region to the reaction-triggering Fc fragment. Fc fragments are recognized by the immune system, which initiates immune responses.

S. Pyogenes, a common human bacterial pathogen, has developed two defense mechanisms to escape the human immune system. First it expresses cell-wall receptors that binds to Fc

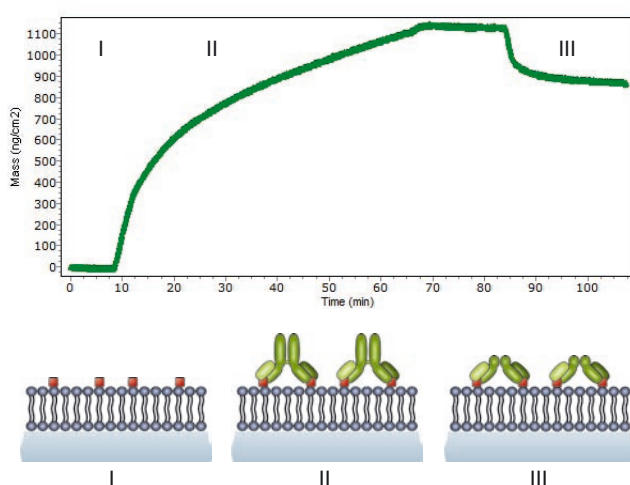


FIGURE 1. Modeled surface mass density throughout the binding of anti-biotin on a biotinylated lipid bilayer and cleavage by IdeS.

I: Biotinylated lipid bilayer.
 II: Anti-biotin antibodies bound to surface.
 III: Addition of IdeS resulting in cleavage of antibodies, detected as reduction of surface mass density of layer.

fragments. Secondly, *S. Pyogenes* secretes an extracellular enzyme called IdeS. IdeS efficiently and specifically¹ cleaves IgG antibodies in the hinge region removing Fc fragments.

APPROACH

To mimic the events at the bacterial surface during an immune response, two modeled surfaces were created *in situ*. Antibodies were captured exposing either the Fab or Fc fragment.

First, to mimic a bacterial membrane, a biotinylated lipid bilayer was applied on silicon dioxide coated QCM-D sensors. Anti-biotin antibodies were bound to the surface with their biotin-recognizing Fab fragments, thus exposing

the Fc fragment (fig 1). Secondly, to mimic the cell wall receptors that bind Fc fragments, an oriented protein A surface was created on gold coated QCM-D sensors. To this, the same antibodies were captured with their Fc fragment, hence this time exposing Fab fragments (fig 2). When antibodies were captured to the surfaces, IdeS was introduced into the chamber. Just before antibodies were injected into the chamber, the QCM-D measurement was initiated.

RESULTS AND DISCUSSION

The raw data from the QCM-D experiment was modelled, which provided a quantitative analysis of the events on the surface.

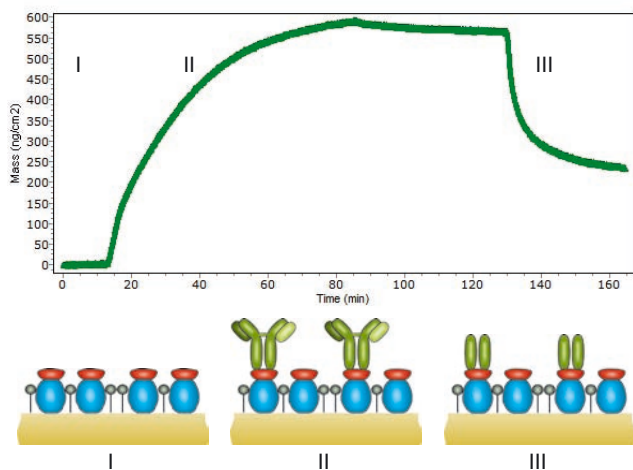


FIGURE 2. Modeled surface mass density throughout the binding of antibodies on protein A surface and cleavage by IdeS.

I: Protein A surface.
 II: Antibodies bound with Fc fragment to protein A surface.
 III: Addition of IdeS resulting in cleavage of antibodies, detected as reduction of surface mass density of layer.

Figure 1 shows the modeled mass of the experiment on the lipid bilayer surface along with an illustration of the scenario. When IgG was injected into the chamber, antibodies bound to the biotin in the lipid bilayer. This was seen as a mass increase. IdeS was then introduced and antibodies were immediately cleaved, detected as a 30% mass release from the surface. Since the Fc fragment represent 1/3 of the weight of IgG, it can be concluded that the antibodies were correctly oriented at the surface and that IdeS successfully cleaved IgG at the hinge region.

Figure 2 shows the modeled mass change for the experiment on the protein A surface along with an illustration of the scenario. Here the mass increase upon capturing of antibodies was lower as compared to the lipid-based setup, indicating a lower antibody surface coverage. Enzymatic degradation by IdeS of antibodies on protein A resulted in a 60% mass release from the surface. The Fab fragments represent 2/3 of the total antibody weight. From this it was concluded that the antibodies were bound through the Fc fragments and that IdeS efficiently cleaved at the

hinge region, this time removing the Fab fragments.

CONCLUSIONS

The QCM-D analysis showed in real time how antibodies can be captured and analyzed on model pathogen surfaces. The QCM-D setup enables *in situ* functionalisation of the sensor surfaces for antibody capturing in two separate directions. The study also demonstrated how enzyme-protein interactions can be followed in real time.

REFERENCE

1. Von Pawel-Rammingen, U, Johansson, B.P and Björck, L (2002) IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G, *The EMBO Journal*, 21, 1607-1615.

Measurements by Q-Sense AB in collaboration with Chemical Physics, Chalmers University of Technology, Sweden. IdeS enzymes provided by HansaMedical, Sweden. Protein A self assembly kit provided by Orla Protein Technologies, UK.

