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Q-Sense | [Application Note] 21

# Probing repeated antibody-antigen interactions by the use of Q-Sense biotin functionalized sensors

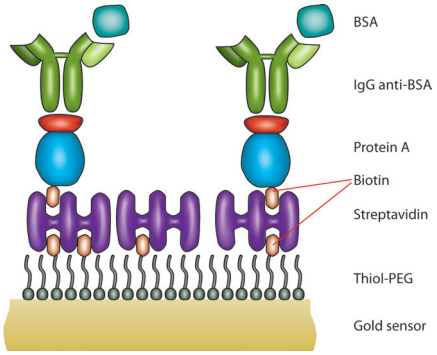
Antibodies and their interactions with different antigens are subjects to great interest both for the pharmaceutical industry and a wide variety of research areas. Screening tools targeting these interactions are therefore of great value and here QCM-D integrated with an autosampler was used to enable multiple, stable cycles of antibody-antigen interactions.

## Introduction

Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) is an acoustic, surface sensitive technique that measures changes in mass and viscoelastic properties in real-time at a surface. The coupled mass at the sensor surface is related to the resonance frequency change of the crystal oscillation, Δf, and includes both actual biomolecular mass and liquid medium associated with it. The energy dissipation change of the crystal oscillation, ΔD, is related to the viscoelastic properties of the material adsorbed to the sensor.

## Experimental

Q-Sense biotin functionalized sensors were used to specifically immobilize streptavidin (SA, conc. 25 µg/ml) while minimizing non-specific protein binding and enabling subsequent immobilization of biotinylated protein-A (25 µg/ml). Protein-A binds to the heavy chain Fc region of immunoglobulin (Ig) antibodies and this enabled the assay illustrated in fig. 1 to be created with bovine serum albumin (BSA) and IgG anti-BSA (IgG-BSA). Phosphate buffered saline (PBS) was used as running and diluting buffer.

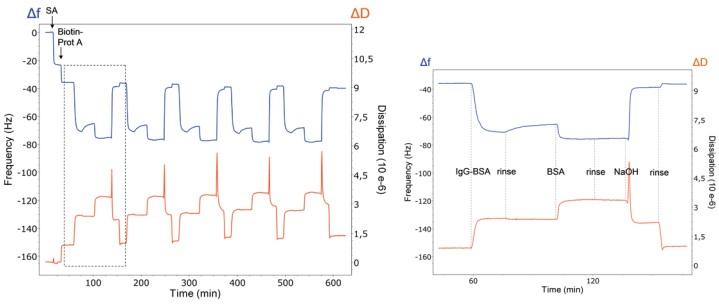



**[Figure 1]:** SA immobilized to a Q-Sense biotin functionalized sensor (biotin, se above) and subsequently bound biotin-Protein A, IgG anti-BSA (IgG-BSA) and BSA. The antibody-antigen (Ab-Ag) complex of IgG-BSA and BSA is later removed by NaOH regeneration in fig. 2

The interaction between IgG-BSA (50 µg/ml) and BSA (100 µg/ml) was regenerated by subjecting the surface to NaOH (10 mM) that is known to dissolve this interaction while keeping the protein-A intact.

## Results and discussion

The assay described (fig. 1) was performed using the Q-Sense E4 Auto with excellent immobilization and regeneration results (fig. 2). Handling time was reduced to less than 1 h by using the E4 Auto setup, while the measurements were performed overnight for more than 10 h. Regeneration of the immobilization of IgG-BSA and BSA with NaOH did not effect the stability of the SA or protein-A and five regeneration/immobilization cycles could be done in sequence with reproducible amounts of immobilized protein (see fig. 2 and 3). During regeneration, the IgG-BSA and BSA were released likely due to the drastic change in ionic environment at NaOH exposure, which disturbs the charge dependent interactions between protein-A, IgG-BSA and BSA.




**[Figure 2]:** Complete measuring sequence for the 7th overtone (a), the dashed box is magnified (b) and shows the Ab-Ag regeneration sequence that was repeated 5 times using the Q-Sense E4 Auto system.

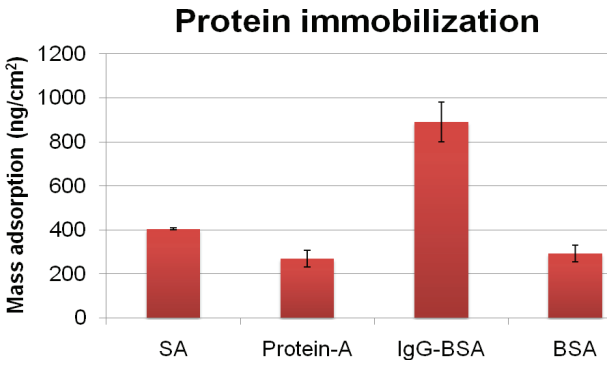
One interesting aspect is that the IgG-BSA was immobilized from a diluted polyclonal antibody serum solution containing a wide variety of proteins. Despite this complex test solution the measurement setup enabled the IgG-BSA and BSA interaction which indicate low non-specific protein binding and that the immobilized protein-A still was active.

Adsorbed mass was modeled using viscoelastic modeling in QTools* with results shown in fig. 3. SA, protein-A and BSA have similar molecular weights of 53, 50 and 66 kDa respectively and is also reflected in the adsorbed mass. The used IgG-BSA has unspecified molecular weight but is typically > 150 kDa and this is also indicated by the higher mass adsorption of the antibody.

## Conclusion

Q-Sense biotin functionalized sensors proves to enable specific immobilization of streptavidin and subsequent biotinylated biomolecules while minimizing non-specific adsorption. This immobilization strategy can be used to study biomolecular interactions, here shown successfully for protein-A, IgG-BSA and BSA.

The Q-Sense E4 Auto setup enabled repeated antibody-antigen interactions to be screened for over 10 h with excellent reproducibility. Automatic sample handling greatly reduced the required handling time. This methodology could be used in drug discovery as a screening tool, potentially presenting new viscoelastic information not easily obtainable from other methods.




**[Figure 3]:** Adsorption levels for SA, protein-A, IgG-BSA and BSA
* QTools is a viscoelastic modeling and curve fitting software provided by Q-Sense.

**References:**

This study was performed by Biolin Scientific AB.

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E-mail:info@biolinscientific.com
biolinscientific.com