

# PROBING REPEATED ANTIBODY-ANTIGEN INTERACTIONS BY THE USE OF Q-SENSE BIOTIN FUNCTIONALIZED SENSORS

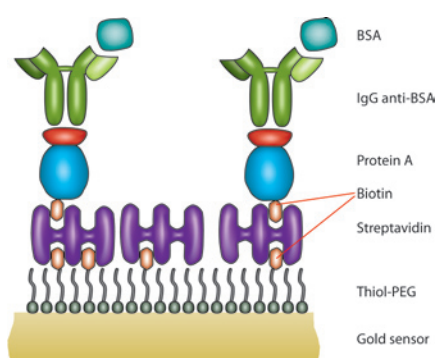
Antibodies and their interactions with different antigens are subjects to great interest both for the pharmaceutical industry and a wide variety of research areas. Screening tools targeting these interactions are therefore of great value and here QCM-D integrated with an autosampler was used to enable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

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,  $\Delta f$ , and includes both actual biomolecular mass and liquid medium associated with it. The energy dissipation change of the crystal oscillation,  $\Delta D$ , 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  $\mu\text{g/ml}$ ) while minimizing non-specific protein binding and enabling subsequent immobilization of biotinylated protein-A (25  $\mu\text{g/ml}$ ). 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.

The interaction between IgG-BSA (50  $\mu\text{g/ml}$ ) and BSA (100  $\mu\text{g/ml}$ ) was regenerated by subjecting the surface to NaOH (10 mM) that is known to

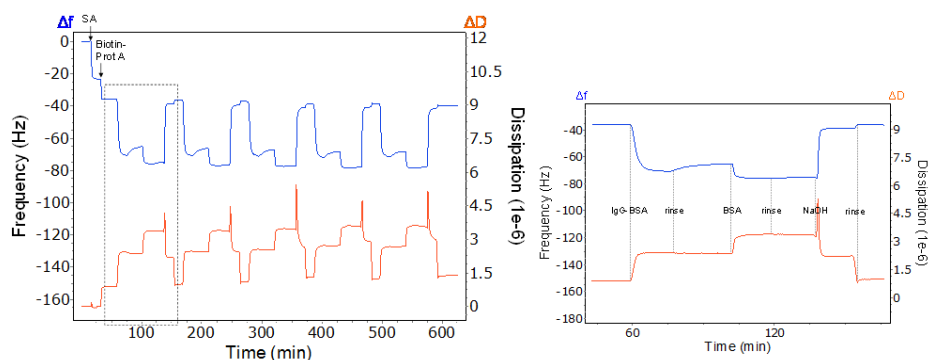


**FIGURE 1.** SA immobilized to a Q-Sense biotin functionalized sensor (biotin, see above) and subsequently bound biotin-Protein A, IgG anti-BSA (IgG-BSA) and BSA. The antibody-antigen (Ab-Ag) complex of IgG-BSA and BSA is later removed by NaOH regeneration in fig. 2

dissolve this interaction while keeping the protein-A intact.

## RESULT 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



**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.

BSA. 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.

### REFERENCES

This study was performed by Q-Sense, BiolinScientific AB. For additional information contact [info@q-sense.com](mailto:info@q-sense.com).

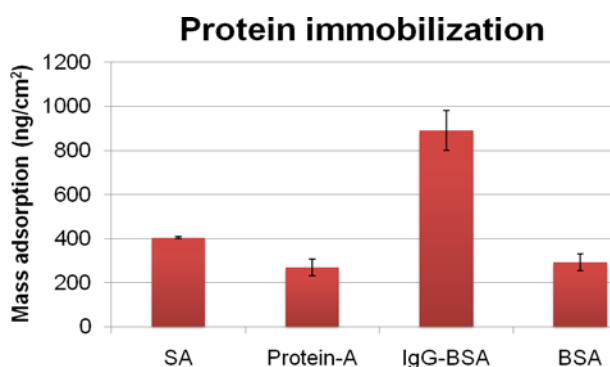


FIGURE 3. Adsorption levels for SA, protein-A, IgG-BSA and BSA

\* QTools is a viscoelastic modeling and curve fitting software provided by Q-Sense.

