

Capturing of Biotinylated Molecules on the Attana[®] Biotin Sensor Chip Surface

SUMMARY

The Attana[®] Biotin Sensor Chip surface has been evaluated in terms of functionality and performance. As follow are the key properties of the Attana Biotin Sensor Chip surface

- Provides fast and easy capture of biotinylated molecules
- Robust and durable
- Versatile and reliable performance
- Controllable surface density

INTRODUCTION

Attana biosensors are based on the quartz crystal microbalance (QCM) technique, which is a highly sensitive mass sensor, capable of measuring mass changes in the nanogram range.¹ The piezoelectric quartz crystal that constitutes the sensor can be functionalised by a variety of surface chemistries to obtain surfaces suitable for protein immobilisation and biomolecular interaction studies.² This technical note describes the functionality and performance of the Attana Biotin Sensor Chip surface for capturing of biotinylated molecules using streptavidin.

The Attana Biotin Sensor Chip is designed for versatile and effective capturing of biotinylated molecules (i.e. ligands) on the sensor surface using the homotetrameric protein streptavidin (SA) ($M_w \sim 52$ kDa). **Fig. 1** illustrates the general principle and a typical sensorgram. Streptavidin is the non-glycosylated form of avidin with a near-neutral pI, as opposed to avidin which has a pI of ~ 10.5 . The binding affinity of SA to biotin is similar to that of avidin to biotin, which is the strongest non-covalent biological interaction known, with a K_d of 10^{-15} M³.

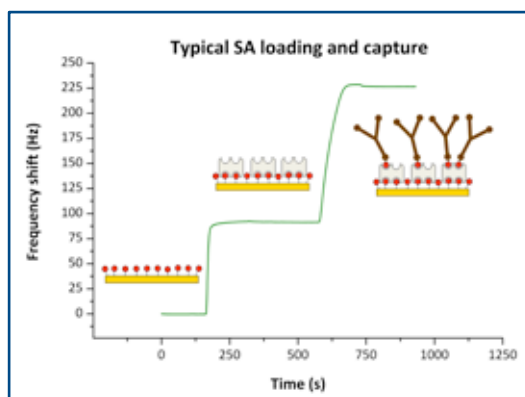


Figure 1: A typical sequence of loading an Attana Biotin Sensor Chip surface with SA and capturing of a biotinylated rabbit antibody (10 μ g/mL). The red dots signify biotin, the SA is shown in grey and the antibody is displayed in brown.

The objective of this technical note is to demonstrate the capabilities of the Attana Biotin Sensor Chip surface in terms of SA loading, capacity of capturing biotinylated species and an approach to control the ligand density on the surface. In addition, the reproducibility of the SA loading and capturing of biotinylated molecules is shown.

METHOD

First, the Attana Biotin Sensor Chips were allowed to stabilise in the biosensor system at a running buffer flow rate of 100 μ L/min. The running buffer used was either HBS-T buffer (10 mM HEPES, 150 mM NaCl, 0.005% Tween[®] 20), pH 7.4 or PBS-T buffer (10 mM phosphate, 2.7 mM KCl, 137 mM NaCl, 0.005% Tween[®] 20), pH 7.4. After lowering the flow rate to 20 – 25 μ L/min, the biotin surfaces were saturated with SA typically using a concentration and contact time of 100 μ g/mL and 120 – 150 s, respectively. The biotinylated biomolecules to be captured were diluted in running buffer to a concentration of 1-10 μ g/mL. The experiments were carried out on the Attana[®] 100 and the A100[®] C-Fast biosensor systems.

RESULTS

Streptavidin loading and capturing

The Attana Biotin Sensor Chip surfaces were prepared for capturing by exposing them to SA. When saturating the surface with SA, the frequency shift is normally around 90 Hz, **Fig. 2**. Subsequent capturing responses of three different biotin-tagged molecules (bovine serum albumin (bt-BSA), rabbit anti-mouse Fc specific antibody (bt-RAM-Fc) and an IgG specific Protein A-like protein (bt-PAL)), on SA-loaded surfaces, are also shown in **Fig. 2**. The injection parameters were chosen to obtain saturation levels for bt-BSA and bt-RAM-Fc, respectively, whereas for bt-PAL they were adjusted to reach a quarter of the maximum capturing capacity as part of a density control experiment. These results show that the Attana Biotin Sensor Chip surface possess reproducible capacities, on a chip-to-chip basis, with respect to SA loading and capturing of biotinylated species, both at saturated and non-saturated levels.

Capturing of Biotinylated Molecules on the Attana[®] Biotin Sensor Chip Surface

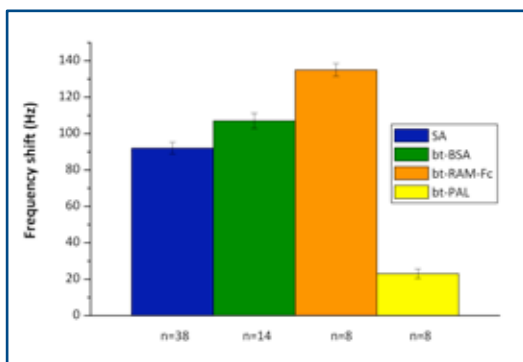


Figure 2: Streptavidin loading and capturing of bt-BSA, bt-RAM-Fc and bt-PAL. The frequency shifts correspond to the saturation levels in all cases except that of the bt-PAL, in which the capture level is about a quarter of the maximum. The number of samples (n) is shown below each column and the error bars are given with one standard deviation.

The reproducibility of the performance of a single sensor chip was investigated by capturing the bt-RAM-Fc and then cycling a sequence including injections of (1) a mouse anti-myoglobin antibody and (2) myoglobin followed by (3) a regeneration step. Data from such an experiment is shown in **Fig. 3** and as seen, the interactions display a high degree of conformity. Initial outliers with a higher response than the following ones may occasionally arise. The occurrence of such outliers may in many instances be suppressed or avoided altogether by subjecting the surface to a few repetitions of the regeneration procedure before the interaction study begins. This is often referred to as post-capture conditioning. Outliers may still appear to some extent during the first few cycles and should then be treated as part of the conditioning and hence be omitted from the study.

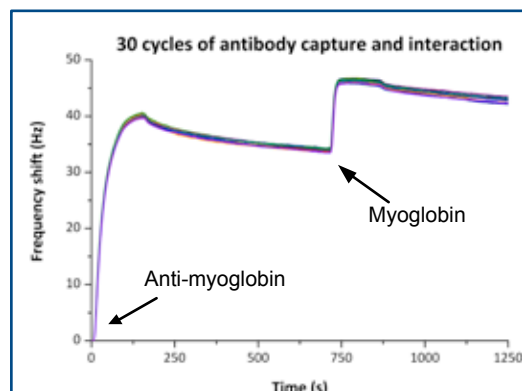


Figure 3: An SA-loaded Attana Biotin Sensor Chip surface, which has previously captured bt-RAM-Fc, exposed to 30 repetitions of mouse anti-myoglobin and myoglobin injections, both at 10 µg/mL. Each cycle was ended by a regeneration step involving HCl 100 mM and NaOH 20 mM during 75 s and 40 s, respectively (not shown). The first cycle was excluded and considered to be part of the post-capture conditioning.

CONTROLLING CAPTURING LEVEL

In some applications of biosensor analysis, it is advantageous to reduce the amount of captured ligand on the surface in order to avoid issues with for instance mass transport limitations and avidity effects.^{4,5} Mass transport problems are a result of sample depletion or build-up in the liquid layer close to the surface, during the association and dissociation phases, respectively. In other words, when the mass transport is limited, the sample concentration in the vicinity of the surface is not the same as that of the bulk solution, which naturally leads to the risk of faulty result interpretation. Increasing the flow speed and lowering the ligand surface density are both useful tools to avoid mass transport limitations. Generally, there are two approaches to gain control of the resulting surface density of the biotinylated ligand; either the concentration or the contact time can be varied during the injection. The latter approach is demonstrated herein. In **Fig. 4**, bt-PAL was injected employing three different contact times; 20 s, 40 s and 100 s. As seen, the ligand capturing rate is more or less linear and therefore this approach presents a very powerful means of controlling the surface density in this case. Response levels obtained from subsequent capturing of rabbit antibodies reflect the different surface densities well. A comparison between the dissociation phases in the different sensorgrams reveals that higher bt-PAL surface density results in lower off-rates for the captured antibodies. This behaviour is most likely explained by a combination of

Capturing of Biotinylated Molecules on the Attana[®] Biotin Sensor Chip Surface

avidity effects and rebinding, both of which are reduced at a lower concentration of the ligand on the surface. If the objective was to study the interaction between the bt-PAL and the antibody, it would therefore be essential to assure a low surface density of the bt-PAL in order to obtain accurate results. If, on the other hand, the aim was to capture as much of the antibody as possible and/or to obtain a low off-rate, a high density of the bt-PAL would be desirable.

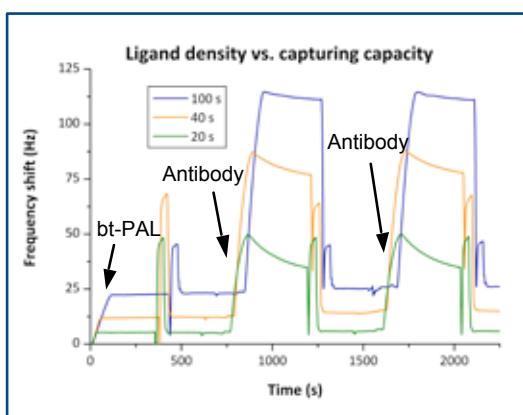


Figure 4: An SA-loaded Attana Biotin Sensor Chip surface, exposed to bt-PAL (1 $\mu\text{g}/\text{mL}$) during 20 s, 40 s and 100 s. A rabbit antibody (5 $\mu\text{g}/\text{mL}$) was subsequently captured and removed again. The regeneration solution (glycine 10 mM, pH 3.0), used to remove the antibody, was also injected before any antibody capture took place to desorb any loosely bound bt-PAL. Negative control buffer injections were carried out prior to the antibody injections.

CONCLUSIONS

The Attana[®] Biotin Sensor Chip surface was proven suitable for capturing of several different biomolecules, while preserving their functionality. A very reproducible capturing capacity of both SA and biotinylated molecules was demonstrated on a chip-to-chip basis. Such robustness, in turn, grants a high degree of control with respect to adjusting the ligand surface density, a quality that was also displayed. In addition, the surface was shown to be durable and withstand many cycles of interactions and harsh regeneration conditions without impairing its functionality.

REFERENCES

1. O'Sullivan, C. K.; Guilbault, G. C. Commercial quartz crystal microbalance – theory and applications. *Biosensors & Bioelectronics* 1999, 14, 663-670.
2. Davis, F.; Higson, Seamus P. J. Structured thin films as functional components within biosensors. *Biosensors & Bioelectronics* 2005, 21, 1-20.
3. Green, N. M. Avidin. *Advances in Protein Chemistry* 1975, 29, 85-133.
4. Christensen, L. L. H. Theoretical analysis of protein concentration determination using biosensor technology under conditions of partial mass transport limitation. *Analytical Biochemistry* 1997, 249, 153-164.
5. Myszka, D. G. Kinetic analysis of macromolecular interactions using surface plasmon resonance biosensors. *Current Opinion in Biotechnology* 1997, 8, 50-57.

Balancing **Power** and **Simplicity** in Molecular Interaction Studies

www.attana.com