Surface acoustic wave technology as a tool for functional characterization of new compounds

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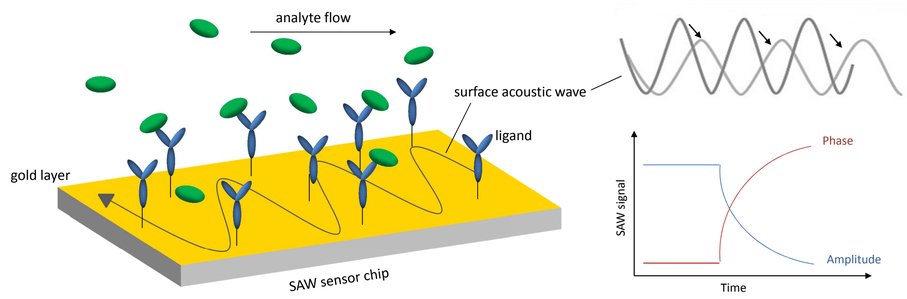
INTRODUCTION

Surface acoustic wave (SAW) biosensors allow the users to detect label-free binding events in the liquid phase, giving information on affinity (K_D), kinetics (k_{on} and k_{off}), viscoelastic effects and conformational changes. Therefore, one of the interaction partners (**ligand**) is immobilized on a sensor chip. After **analyte interaction**, changes in the surface-bound material and configuration result in a **modified oscillation** of the surface acoustic wave. The phase of the wave is shifted on mass changes. Viscoelastic and conformational characteristics are indicated by a change in amplitude. Both effects can be differentiated and are detected independently for interaction analysis.

Principle of SAW biosensors



OBJECTIVE

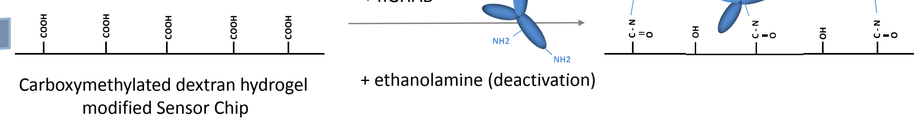
SAW biosensors have broad applications in different scientific fields. However, their pharmaceutical applications in drug R&D as well as in QA/QC are rather limited. Therefore, we want to exploit different pharmaceutical applications of the SAW biosensor. Preliminary data is presented below.

Application I: **Functional quality control of different chemically-modified somatropin batches**. Different batches of chemically-modified somatropin will be evaluated for their binding to the human growth hormone antibody (hGHAB) and later-on the human growth hormone binding protein (hGHBP).

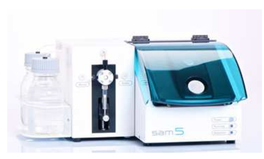
Application II: Functional quality control of small molecules and peptides. SAW responses are proportional to the molecular weight of the bound analyte, which makes binding of small molecules and peptides onto an immobilized ligand problematic. A way to overcome this problem is to change the orientation, *i.e.* immobilize the small molecule or peptide. However, since the amount of functional groups is rather limited, alternative methods has to be followed instead of the standard amine coupling. Our first results with opioid peptides and delta opioid receptor (DOR) membrane preparations are presented below.

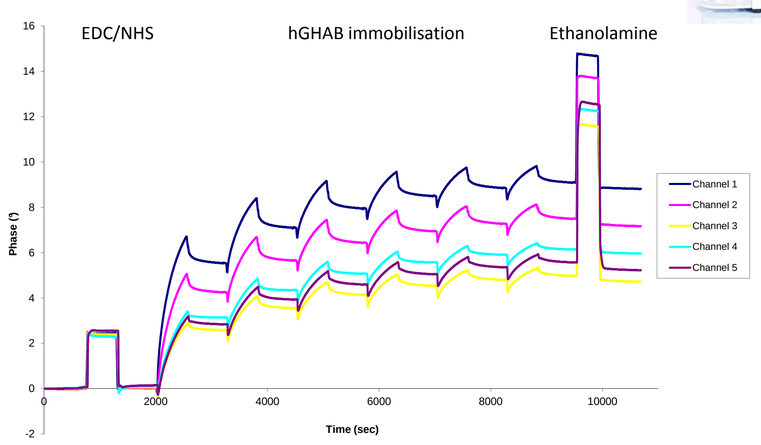
APPLICATION I

1. Random immobilization of hGHAB via amine coupling

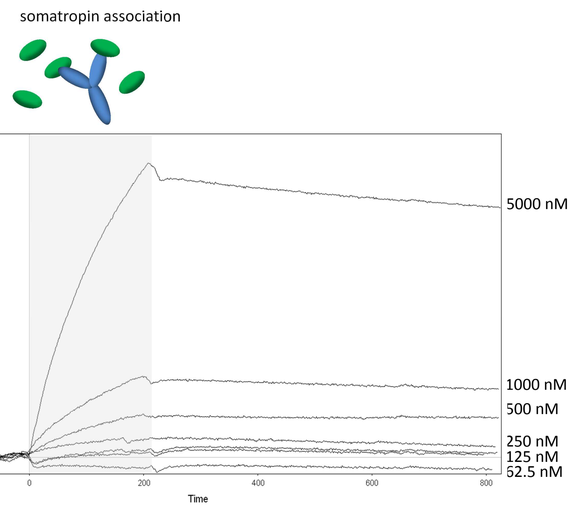


Online immobilization on the SAM5 biosensor





2. Binding experiment between somatropin and immobilized hGHAB

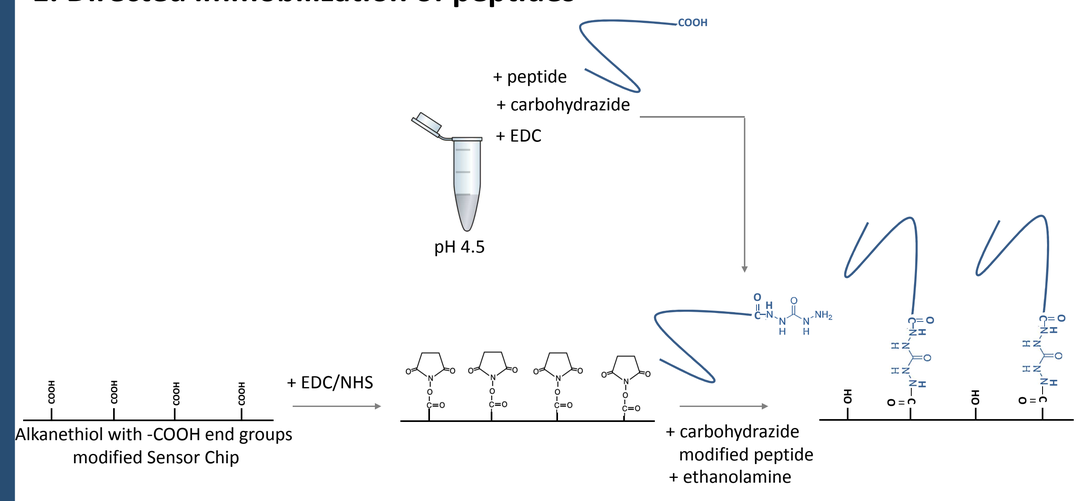


The binding characteristics were analysed (Scrubber2):

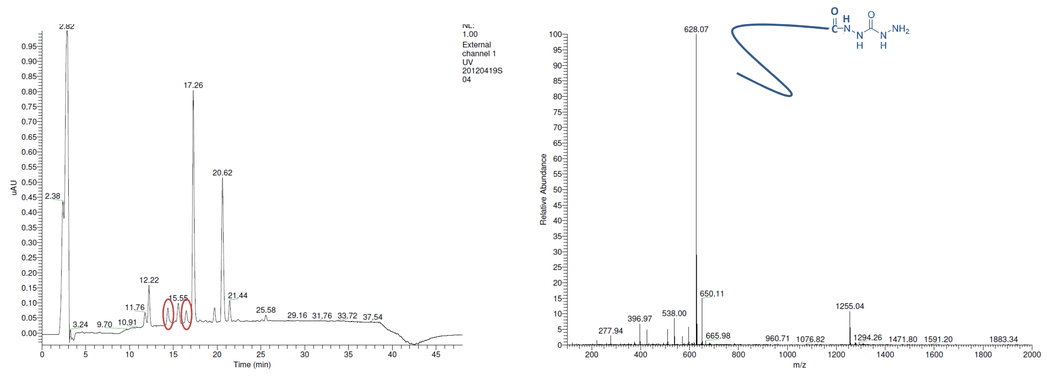
K_D	114.76 nM
k_{on}	1491.0 M^{-1} s^{-1}
k_{off}	1.711E-4 s^{-1}

APPLICATION II

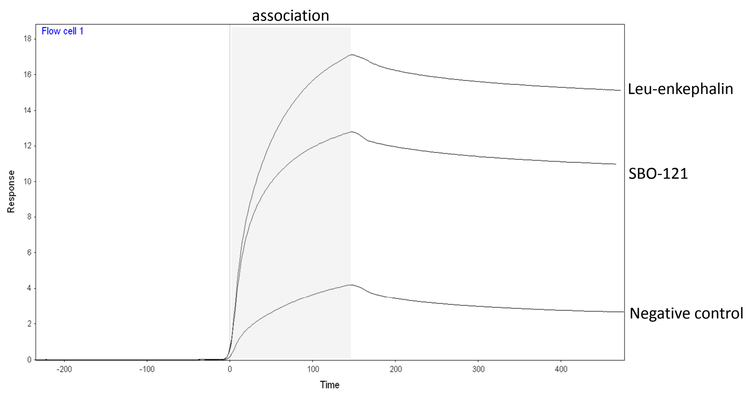
1. Directed immobilization of peptides

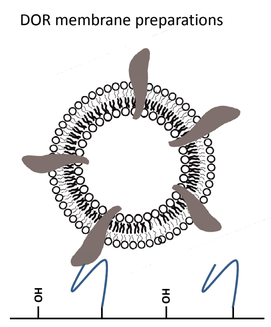


2. Quality control of the carbohydrazide modified Leu-enkephalin (LC/MS)



3. Binding experiment DOR membrane preparations and immobilized opioid peptides





CONCLUSION

Application I: **Functional quality control of different NOTA-somatropin batches**.
Immobilization of hGHAB and initial binding experiments were successful. Although the affinity of the hGHAB for somatropin was not reported, the measured K_D value is within the expected affinity for antibodies to their antigens. In future experiments, the affinity of different NOTA-somatropin batches for hGHBP will be evaluated.

Application II: Functional quality control of small molecules and peptides.
A new immobilization method was evaluated. Leu-enkephalin and a new peptide (SBO-121) were both modified with carbohydrazide on the *C*-terminus. QC-analysis (LC/MS) of modified Leu-enkephalin showed that only small fractions were carbohydrazide-modified, so further reaction optimization needs to be performed. However, an initial binding experiment showed promising results.

References:
Gronewold, T.M.A. (2007). Surface acoustic wave sensors in the bioanalytical field: recent trends and challenges. *Anal. Chim. Acta*, 603, 119-128.
Schlesinger, M.; Simonis, D.; Schmitz, P.; Fritzsche, J.; Bendas, G. (2009). Binding between heparin and the integrin VLA-4. *Throm. Haemost.*, 102, 816-822.