STSM: **Characterization of the binding affinity of anti-*Streptococcus uberis* antibodies**

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Previous work
Design and production of antibodies for the detection of *Streptococcus uberis*[1]

- Streptococcus uberis (S. uberis) is an important environmental pathogen causing mastitis in dairy cattle.

- Combining in silico bioinformatic analysis and solid phase peptide synthesis using Fmoc chemistry, a peptide was synthesized to mimic the adhesion protein of *S. uberis*, which is promoting the attachment of bacteria to epithelial cells.

- After purification with RP-HPLC, the peptides were conjugated with a larger carrier protein (KLH) and used for immunization of rabbits to produce specific antibodies.

- The separation of *anti-S. uberis* antibodies from rabbit blood antisera was carried out with affinity chromatography, using the synthetic peptides as affinity ligands.

- The purified antibodies showed high specificity (using ELISA and immunofluorescence assay (Fig.1)) towards *S. Uberis.*
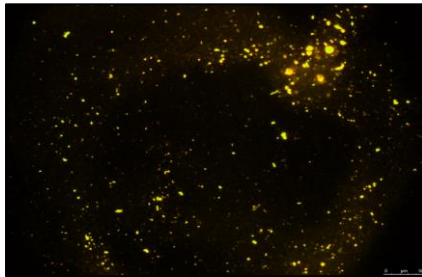

Figure 1. Immunofluorescence analysis of binding the purified *anti-S. uberis* antibodies labelled with DyLight®550 to *S. uberis* cells.

1. Mihklepp,K., Kivirand,K., Nikopensius,M., Peedel,D., Utt,M., and Rinken,T., Design and production of antibodies for the detection of *Streptococcus uberis. Enzyme and Microbial Technology* 2017. 96: 135-142.
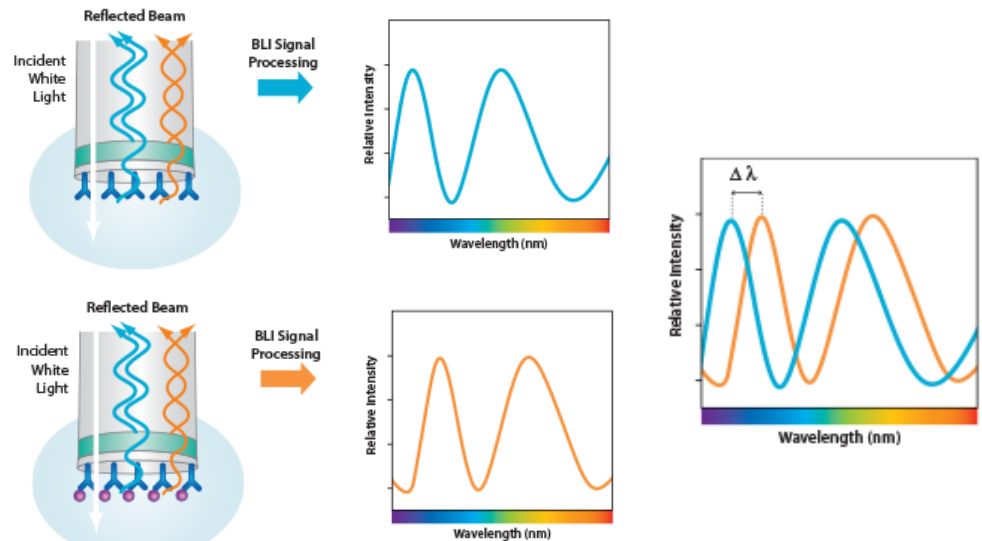
STSM host institution: Prof. S. Ricard-Blum and Prof. A. Miele, Institute of Molecular and Supramolecular Chemistry and Biochemistry, UMR 5246, University Lyon 1, France

AIM of the STSM

- To learn the Bio-layer interferometry (BLI) technique

- Characterize the binding affinity of original anti-*Streptococcus uberis* antibodies
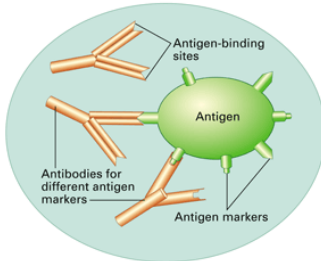
What is BLI?

www.fortebio.com/

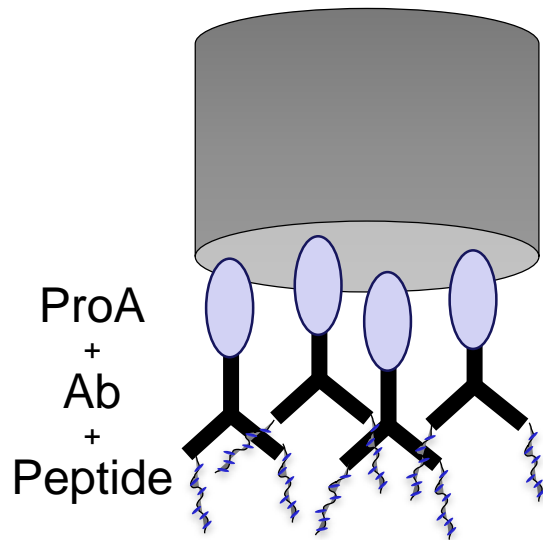## Materials
Four batches of antibodies
Synthetic peptide[1]: C(Npys)SAPVYLGVSTE

1. Mihklepp,K., Kivirand,K., Nikopensius,M., Peedel,D., Utt,M., and Rinken,T., Design and production of antibodies for the detection of *Streptococcus uberis. Enzyme and Microbial Technology* 2017. 96: 135-142.

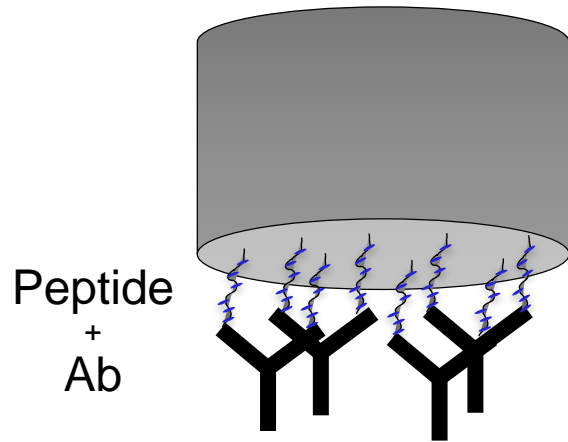# ProA biosensor

All of the antibody batches bound to protein A

➢ Ab concentration from 15 nM to 500 nM

ProA
+
Ab
+
Peptide

Ab1
Ab4

50 nM immobilized Ab solution on protein A sensors
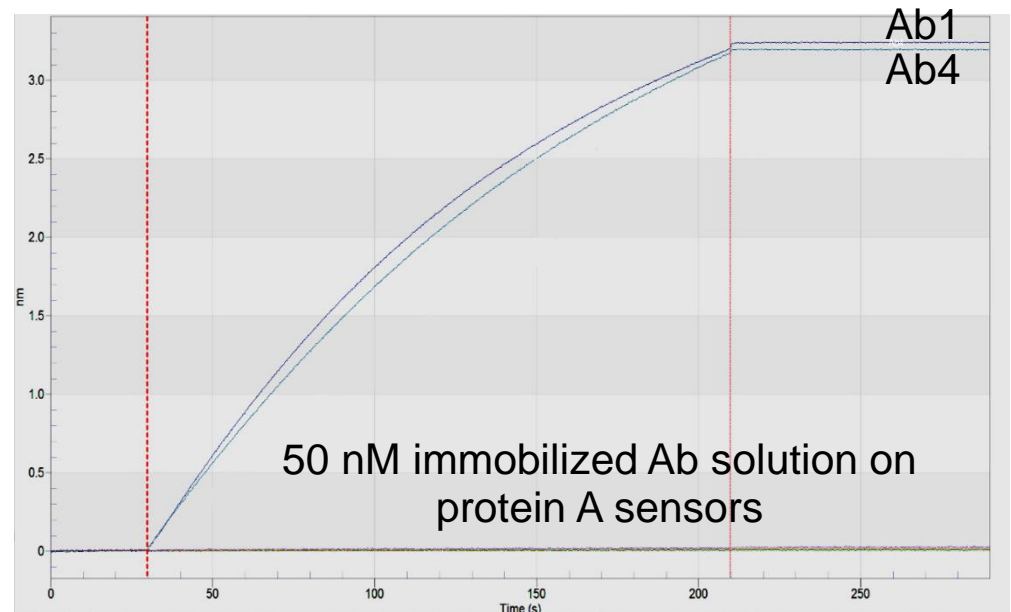
No change in signal (no displacement) in case the peptide was attached to the immobilized Ab

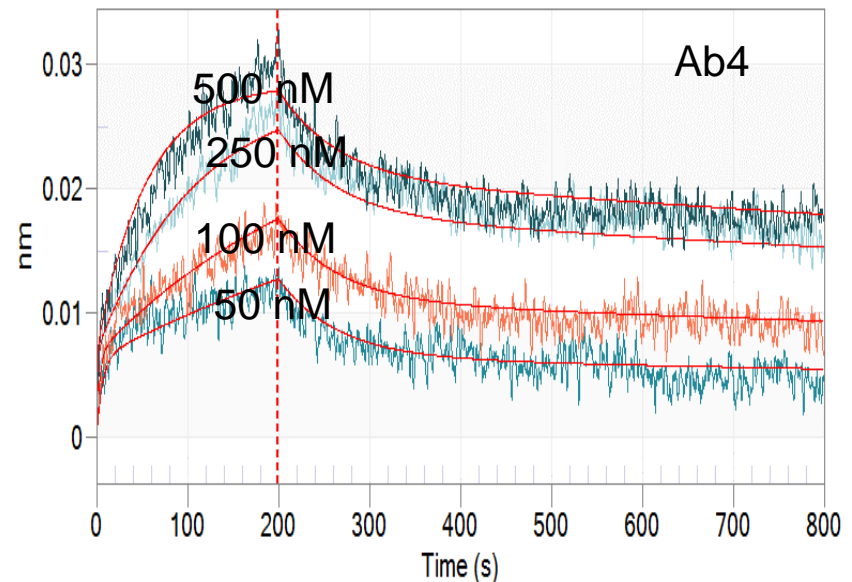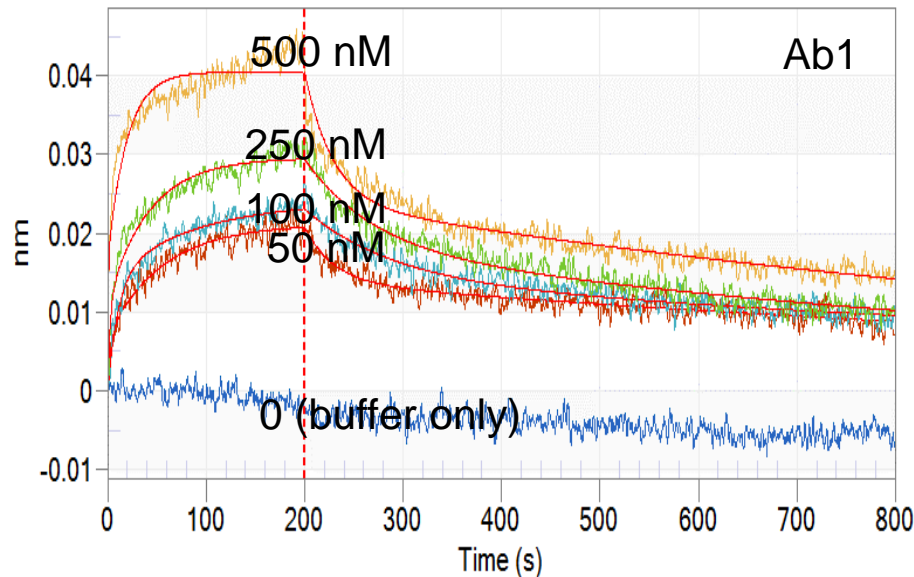➢ Peptide concentration from 100 nM to 1 mM

AR2G biosensor

Peptide immobilized *via* its N-terminus

Results:

- Ab1 → specific binding
- Ab2 → no binding
- Ab3 → non-specific binding
- Ab4 → specific binding

Peptide
+
Ab

Ab1 and Ab4 usable for bio-detection of *S. Uberis* bacteria

Sincere gratitude

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T. Rinken, K. Mihklepp from Biosensor laboratory at the University of Tartu

Prof. **M. Min** from research laboratory for Communicative Electronics at the Tallinn University of Technology

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Thank you for listening