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Title:

Fiber Optic SPR biosensing platform for multiplex DNA quantification and identification

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Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text/diagrams into it.)

Accurate identification and quantification of bacteria and viruses is essential in food quality and safety as well as in healthcare and biotechnology. Although many different assays already exist for detecting specific DNA sequences of multiple pathogens, qPCR followed by high resolution melting (HRM) analysis remains the current golden standard. However, qPCR is still largely incompatible with the concept of point-of-care (POC) testing due to the cost and the complexity.

In this work, we present real-time simultaneous detection of two related bacteria, *Mycobacterium bovis (M. Bovis)* and *Mycobacterium avium subspecies paratuberculosis (MAP)*, two frequently encountered pathogens in life stock, using the fiber optic surface plasmon resonance (FO-SPR) biosensor (Fig. 1A). FO-SPR is an in-house developed platform with the proven capacity to monitor DNA melting profile and discriminate single nucleotide polymorphisms (SNPs) at high resolution by implementing DNA functionalized gold nanoparticles (Au NP) as labels[1,2]. To further realize simultaneous quantification and cycle-to-cycle identification of DNA products, the established FO-SPR melting assay was combined with the PCR reaction (Fig. 1B and C).
Thus, each FO-SPR melting cycle was preceded with the PCR amplification of DNA free in solution, resulting thereby in monitoring of melting curves in each reaction cycle of the melting curve bioassay. Multiplexing capacity was demonstrated by using two different sets of hybridization probes and primers, each targeting a region of interest in different bacterial species for functionalizing the FO-SPR sensor tip and Au NP labels or for PCR amplification, respectively. Moreover, the same assay could detect SNP mutations in both target sequence simultaneously.

In conclusion, we demonstrate FO-SPR ability for multiplex simultaneous quantification and identification of DNA targets, together with the possibility of target detection directly in turbid media, thereby proving the real potential of this platform to be useful as POC biosensors.

[1] J, Pollet, K. Janssen, K. Knez, J.Lammertyn, *Small*, 2011, ***7***, *1003-1006*
[2] K. Knez, K. Janssen, D. Spasic, P. Declerck, L. Vanysacker, C. Denis, T. Tran, J. Lammertyn, *Analytical Chemistry*,2013, **85**, 1734-1742.

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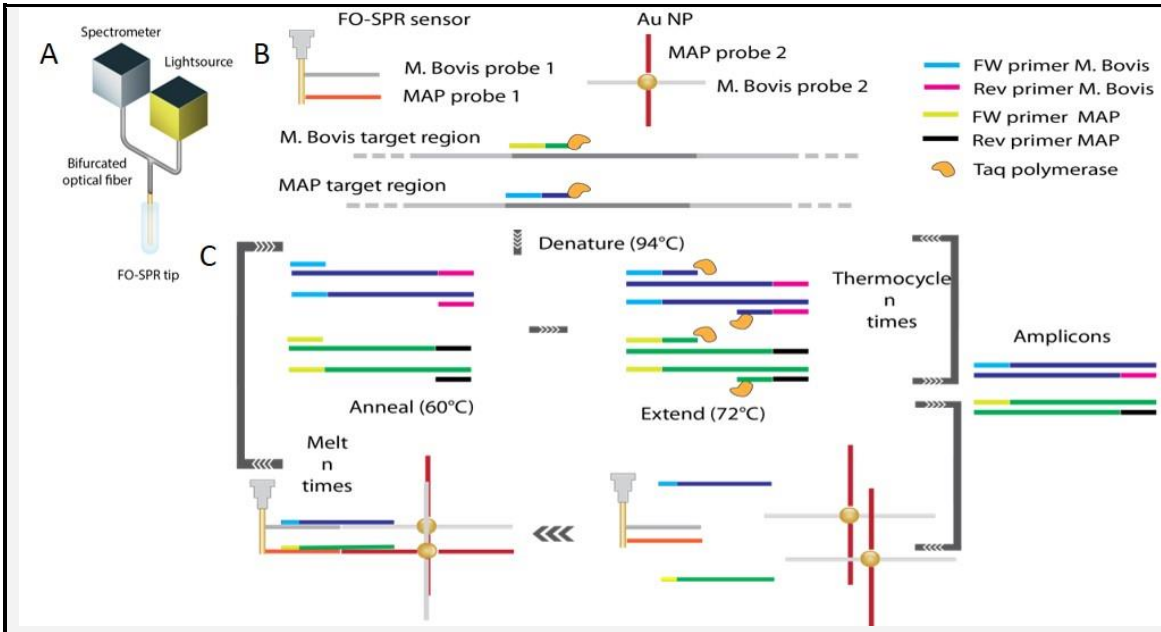

Figure 1: Schematic overview of the FO-SPR quantitative multiplex PCR. A) Different components of the reaction. B) PCR primers specific for Mycobacterium Avium Paratuberculosis (MAP) and Mycobacterium Bovis (M. Bovis) will be used to amplify a target region during a standard PCR reaction. The amplicons of both MAP and M. Bovis will be able to hybridize during the PCR reaction to complementary probes on the FO-SPR sensor and Au NPs. During the normal thermo cycling used to denature the PCR amplicons for the PCR reaction, the FO-SPR sensor can register the T_m of the PCR amplicons and as a result perform a melting analysis, which can be used to identify different targets. C) Representation of the Fiber Optic set-up.