Aptamer and DNA hybridization assays on gold fiber optic sensors with nanoparticle signal enhancement

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Abstract: Detection of allergens and micro bacteria on a fiber optic SPR sensor is performed by means of an oligonucleotide bio-recognition surface. In this work, the DNA surface is optimized by mixing with PEG monolayers to suppress unspecific and enhance specific detection. Furthermore, due to the surface characteristic of DNA functionalized gold nanoparticles the bioassay sensitivity is increased and a mean of real-time multiplexing is introduced.

Point-of-care (POC) diagnostic tests for are expected to deliver fast and sensitive detection of DNA and/or protein targets. However, nowadays, detection of molecular targets alone, without their quantification and/or identification is often insufficient^{1,2}. Furthermore, reliable interpretation of the results largely depends on suppressing unspecific binding and carefully designing the surface for maximal specific detection. Although, different assays have been developed to meet these requirements, including quantitative PCR (qPCR)³ followed by high resolution melting analysis as well as ligation assays⁴, most of them are still largely incompatible with the concept of POC testing due to their cost or complexity. Furthermore, antibody sandwich assays, such as ELISA and lateral flow tests, are the industry standard, but lack kinetic data and they need multiple handling steps and a separate detector.

In this talk, the fiber optic (FO) SPR sensor will be presented as a flexible platform for detection of allergens using aptamers and qPCR. Here, we describe an aptamer-based bioassay for detecting one of the most important peanut allergens, Ara h1 and its comparison to the antibody based detection (Figure 1). Furthermore, the FO SPR sensor is applied for multiplex, real-time monitoring of DNA amplification and detection of single nucleotide polymorphisms (SNPs) in a single sample. The importance of balancing the amount of oligonucleotides as bio-recognition molecules on gold surfaces using PEG back filling will be emphasized in this talk through several examples describing different bioassays.

The FO-SPR sensor is a translation of the prism based SPR principle to an optical fiber. Although in comparison some sensitivity is reduced, the sensor surface can be used as a consumable bringing SPR sensing to the diagnostic field. As a proof of concept, an FO-SPR bioassay was developed for detecting Ara h1 protein in both buffer and food matrix samples using a DNA aptamer showing high specificity towards Ara h1. Using the selected aptamer, an A PEG mixed SAM layer was used to functionalize the gold surface and suppress nonspecific binding events. Finally, gold nanoparticles (Au NPs) functionalized with antibodies were implemented for signal amplification in a sandwich type bioassay.

Fig. 1. Overview of the Ara h1 immunoassay on the FO-SPR biosensor.

In this talk, the FO-SPR set-up will be also presented as a platform for monitoring the DNA melting profile. Here, DNA functionalized Au NPs were implemented as labels (Figure 2B), which allowed discriminating SNPs at high resolution^{6,7} due to the tight packing of DNA molecules on their surface. However, to realize simultaneous quantification and cycle-to-cycle identification of the reaction products, the FO-SPR melting assay was combined either with the PCR or with ligation chain reaction (LCR). To this end, appropriate backfilling of the sensor surface was applied to prevent sticking of the amplification enzymes during the thermal cycling.

The FO-SPR LCR assay amplifies DNA in exponential manner through multiple ligation cycles that progress through 3 succeeding phases: hybridization, ligation and melting phase (Figure 2C). Basically two

probes are ligated in presence of their target. After denaturing the probe target complex ligation is repeated with their complements. The detection limit of the FO-SPR melting assay was drastically improved compared to the direct melting assay using the LCR, whereas capacity for SNP detection was preserved (Figure 2 D,E). Furthermore, to achieve detection of multiple targets within the same sample, FO-SPR melting assay was combined with solution phase PCR using two sets of hybridization probes. In a proof-of-concept study, two DNA targets were used to discriminate *Mycobacterium bovis* from *Mycobacterium avium* subsp. *Paratuberculosis*, bacteria frequently encountered in life stock (Figure 2F). Backfilling of the gold sensor surface proved to be crucial here for maintaining the high resolution capabilities of the melting detection principle throughout the thermal cycling.

Fig. 2. (A) Schematic representation of the FO-SPR setup with all components. (B) Schematic representation of a FO-SPR melting assay (top panel) with DNA target (1) and DNA probes immobilized on the FO-SPR sensor (2) and on Au NPs (3). Gene probes hybridize at the FO surface, and are subsequently melted off by a gradual increase of the temperature, resulting in the FO-SPR sensorgram (bottom panel). (C) Schematic overview of the FO-SPR LCR: (1) Different components of the reaction. (2) LCR reaction where the forward and reverse probes are ligated only in the presence of the target sequence, resulting in an exponential amplification during multiple cycles. (3) The forward LCR product can, during the LCR reaction, form a complex with two complementary probes immobilized on the FO-SPR sensor and on Au NPs, allowing real-time monitoring of the reaction. (D) The derived calibration curve with C_t values from the FO-SPR LCR, spans 7 orders of magnitude for DNA concentrations. (E) Obtained signals for WT and MM target DNA using FO-SPR LCR assay. (F) FO-SPR multiplex PCR, which allows resolving the melting point of the two targets.

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