Application Note # 160

Biosensor for Bacteria Detection from Powdered Milk

Contamination of food by pathogenic bacteria is a serious threat to human health and thus biosensors for fast and accurate food quality control are extensively studied.

Multi-Parametric Surface Plasmon Resonance (MP-SPR) based biosensor was developed to detect Salmonella Typhimurium in dairy products. Direct label-free detection of bacteria by using a capture antibody was further improved utilizing bio-catalyzed precipitation. For control samples the limit of detection (LOD) was 10^2 CFU/mL and for real samples (powdered milk) LOD was 10^3 CFU/mL, demonstrating a high sensitivity of the biosensor.

Introduction

Currently used methods for *Salmonella* detection include culturing, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Farka et al. 2016). These methods are generally time-consuming, they require complex sample pre-treatment and trained personnel, thus more robust and easy-to-use methods are being actively developed.

Surface Plasmon Resonance (SPR) is a well-established method utilized to measure binding affinity and kinetics. Innovative Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments can perform measurements in an exceptionally wide angular range (40-78 degrees) and at more than one wavelength, thus allowing a wide range of applications from small molecule interactions to real-time detection of bacteria, cells and viruses.

MP-SPR is unparalleled method for biosensor development, whether your sensor is based on MP-SPR detection or whether you are developing new portable (point-of-care) biosensors. Advantages of MP-SPR are especially high sensitivity and label-free detection. It also allows development of a sensor directly on your material-of-choice: metal electrodes for electrochemistry, plastics for well-plate assays, cellulose for printed biosensors, glass for traditional chemistry, nanoparticles for SERS, etc. avoiding assay transfer step. Sensor slides can be easily modified *in situ* and *ex situ* providing a wide range of possibilities for functionalization (CVD, ALD, spin coating, dip coating, etc.). Additionally, MP-SPR allows measurements of real samples (milk, 100% serum, urine, sea-water, etc.), unlike many traditional SPR instruments. After MP-SPR measurement, the sensor surface can be further characterized with other methods such as AFM. This is enabled thanks to the oil-free operation of MP-SPR, using a prism coated with an optical elastomer.

Materials and methods

In this study, the measurements were performed with BioNavis MP-SPR Navi™ 210 VASA instrument at 20 µL/min flow-rate. Carboxymethyl dextran (CMD 3D) sensor slides were cleaned with a solution of 2 M NaCl and 10 mM NaOH for 5 min, followed by the activation of carboxylic groups using a mixture of EDC (200 mM) and NHS (50 mM) for 7 min. The capture antibody (and BSA for the reference channel) was introduced (10 µg/mL in 50mM acetate buffer, pH 4.5) before blocking the remaining reactive groups by ethanolamine (1 M, pH 8.5, 5 min). For additional blocking, BSA (2 mg/mL in HBS-P) and 1% powdered milk in HBS-P were injected.

The powdered milk (Laktino) was diluted in the HBS-P buffer to the concentration of 1%. Varying concentrations of *Salmonella enterica* (subsp. *enterica serovar Typhimurium*) cells were added to the sample after culturing and heat treatment (80°C/30min). Concentrations of treated bacteria are expressed as CFU/mL corresponding to viable cells before the treatment.

The bacteria were injected for 10 min, followed by 10 min injection of the horseradish peroxidase antibody conjugate (HRP-Ab2) and 10 min of precipitation substrate solution (HRP) (Figure 1). The bio-catalyzed reaction converted 4-chloro-1-naphthol to insoluble benzo-4-chlorocyclohexadi-enone. The limit of detection (LOD) was evaluated based on the signal-to-noise ratio, where the measurable minimum signal level has to be three times higher than the noise level.

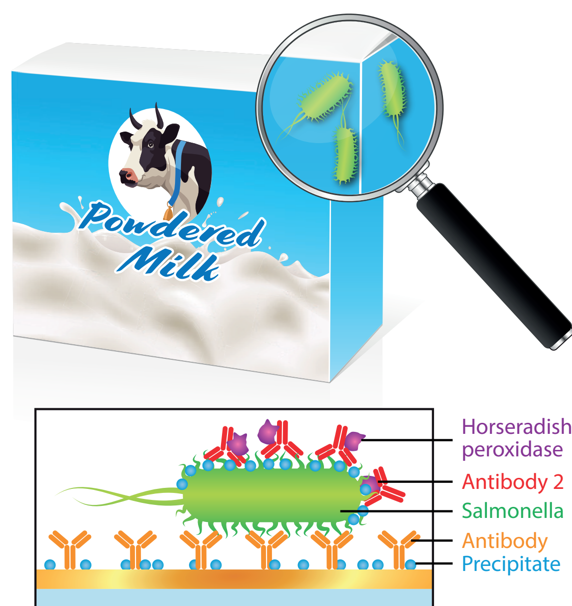

Figure 1. *Salmonella* detection from powdered milk using Multi-Parametric Surface Plasmon Resonance (MP-SPR). Binding of horseradish peroxidase antibody 2 (HRP-Ab2) specifically on *Salmonella* and formation of precipitate.

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Results and discussion

The biosensor was first developed on a Biacore 3000 SPR instrument where LOD of 10^4 CFU/mL was achieved. To further improve the biosensor, a bio-catalyzed precipitation reaction was selected for sensitivity enhancement. However, horseradish peroxidase requires the use of ethanol. The Biacore instrument is not compatible with alcohols, unlike BioNavis MP-SPR instruments which provide excellent resistance to organic solvents. Thus, MP-SPR Navi™ 210A VASA was selected for further research.

Using the MP-SPR instrument, a biosensor was successfully developed and the bio-catalyzed precipitation enhanced the limit of *Salmonella* detection (LOD) from 10^4 to 10^2 CFU/mL (Figure 2). The binding level was high in the channel containing the capture antibody and the bacteria, whereas only a minor amount of precipitate was formed in the reference channel (BSA treated). *Salmonella* binds multiple HRP-Ab2 conjugates which improves sensitivity of the biosensor exponentially with the increasing concentration of microbes. The HRP-Ab2 conjugate is specific to *Salmonella*, providing also improved selectivity compared to direct binding assay. The cross-reactivity of the developed biosensor was tested with Gram-negative bacteria *E. coli* K-12 which showed negligible binding. Optical microscopy and AFM images of an MP-SPR sensor slide were used as reference, and both confirmed bacteria attachment and precipitate formation on the biosensor surface (Figure 3).

The total analysis time by MP-SPR was 60 minutes which is significantly shorter than other methods used for detection of bacteria, such as cultivation (~days), ELISA (~10 h) and PCR (~hours).

Binding of bacteria is interesting not only for biosensor development but also for material sciences. MP-SPR has been utilized to characterize functional "self-defence" anti-microbial implant coatings (Cado et al., 2013). Coating releases anti-microbial peptides by stimulation with pathogens. Here, MP-SPR measures the build-up of a multilayer and quantifies adsorbed mass.

See also cancer cells (MCF7) detection from blood using a target peptide in MP-SPR instrument, Application Note #154.

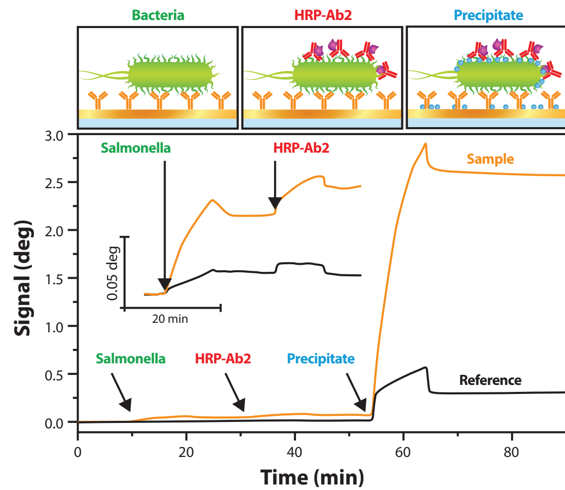

Figure 2. Detection of *Salmonella* is based on antibody (HRP-Ab2) binding on bacteria and bio-catalyzed precipitation by horseradish peroxidase. Binding of several HRP-Ab2 on one bacteria cause exponential amplification of the signal.

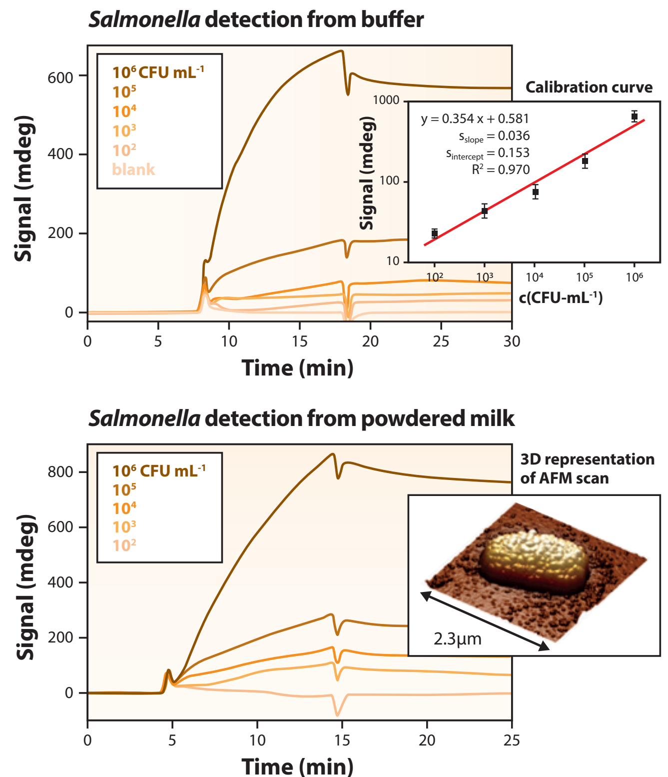

Figure 3. Bio-catalyzed precipitation caused by different concentrations of bacteria in buffer (on top) and bacteria detection from real-sample, powdered milk (below). AFM images confirmed bacteria attachment and formation of precipitates.

Conclusions

MP-SPR proved itself as a highly sensitive and selective biosensor to detect bacteria from food samples. MP-SPR is invaluable for assay development in food and feed safety, environmental safety, clinical diagnostics, border and process control, for example.

The key benefits to use MP-SPR in biosensor development are:

- Compatibility with organic solvents
- Easy modification of sensor surfaces
- Capability to work with crude samples

Original Publication:

Farka et al., *Anal. Chem.*, 2016, 88 (23), pp 11830–11836

Reference:

Cado et al., *Adv. Funct. Mater.* 2013, 23 (38), pp 4801–4809

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 220A NAALI, and 210A VASA with additional wavelength (-L).

Sensor surfaces: CMD, Au, other metal, or inorganic coating

Software: MP-SPR Navi™ Control, DataViewer, and TraceDrawer for MP-SPR.

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