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Photonic biosensors for mycotoxin detection in milk

Contamination of food and agricultural products by various types of toxigenic molds (fungi) is a serious and global problem. Fungal toxins, in particular, mycotoxins produced by several species of fungi are naturally present in food species such as nuts, grains, maize, rice, soya. Among different categories of mycotoxins, aflatoxins (AF) produced by toxigenic strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* are recognized to be the most toxic/carcinogenic compounds. Particularly, Aflatoxin M1 (AFM1) found in milk is one of the most frequent and dangerous toxins for human health. The acceptable maximum level of AFM1 in milk according to EU regulation is 152 pM and 76 pM, for adults and infants, respectively. Therefore, methods for fast and effective detection of AFM1 are indeed crucial. Moreover, massive testing has a non-negligible economic impact, thus the method should also be cheap and available in poor developing countries, where the level of contamination is high.

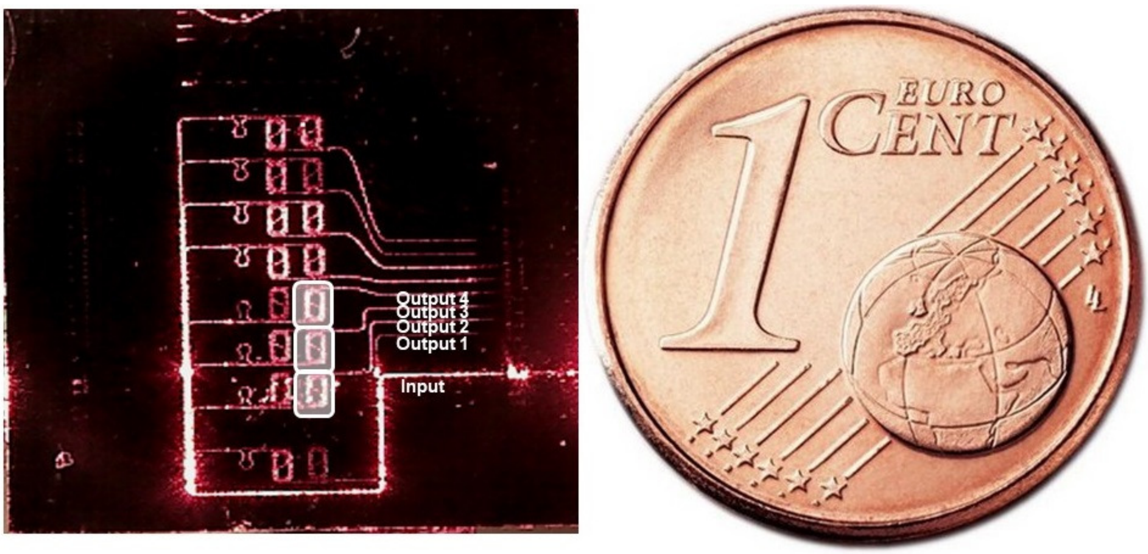

Fig. 1. Si3N4 photonic chip with illuminated aMZIs.

In search of an economic, reliable and portable device to detect AFM1 in milk in less than 1 hour reaching a limit of detection comparable with available commercial systems, the European project SYMPHONY (www.symphony-project.eu/ grant number 610580) has developed a biosensing system, based on an integrated photonic sensor, interfaced with a complex microfluidic stage to purify and concentrate the milk samples. Being highly sensitive and integrated into a small chip, the photonic sensor fulfills the requirements. The sensor is a refractometer (i.e. it measures changes in the refractive index) and is based on an array of eight asymmetric Mach–Zehnder interferometers (aMZI) to allow for multi-analyte sensing. It is fabricated by the single-stripe Si3N4 TriPlex technology. Sensing windows are opened on three aMZIs to sense the environment changes over the sensor (Fig. 1). In the figure, the last aMZI covered by SiO_2 plays the role of an internal reference for the laser (a VCSEL) and temperature fluctuations. We have tested the performances of our photonic sensors, by characterizing simultaneously the volume Sensitivity (S) of the three uncovered aMZIs. To calculate this parameter, we have monitored in real-time the phase shift of the aMZIs, when saltwater solutions of various concentrations flow on the sensor. For more than 60 different sensors a mean value for the sensitivity S≈(1250 ± 150) nm/RIU is achieved, while for the limit of detection LOD≈$(1.2 \pm 0.3)\times10^{-6}$ RIU is calculated. These values are in agreement with the state of the art biosensing devices.

The AFM1 sensing is performed by measuring the phase shift of the output signal, caused by the binding of AFM1 on the functionalized aMZI surface, which yields in a material density and, consequently, in refractive index changes near the sensor surface. To have a specific detection of AFM1, a functionalization process, based on antigen-binding fragments (Fab') is applied to the surface of the sensing arm of an aMZI (Fig. 2). The main advantage of using Fab', with respect to the whole antibodies, is the possibility to obtain a higher surface density of well-oriented Fab' fragments, which yields a higher biosensor sensitivity as well as a lower limit of detection (LOD_{AFM1}) of AFM1.

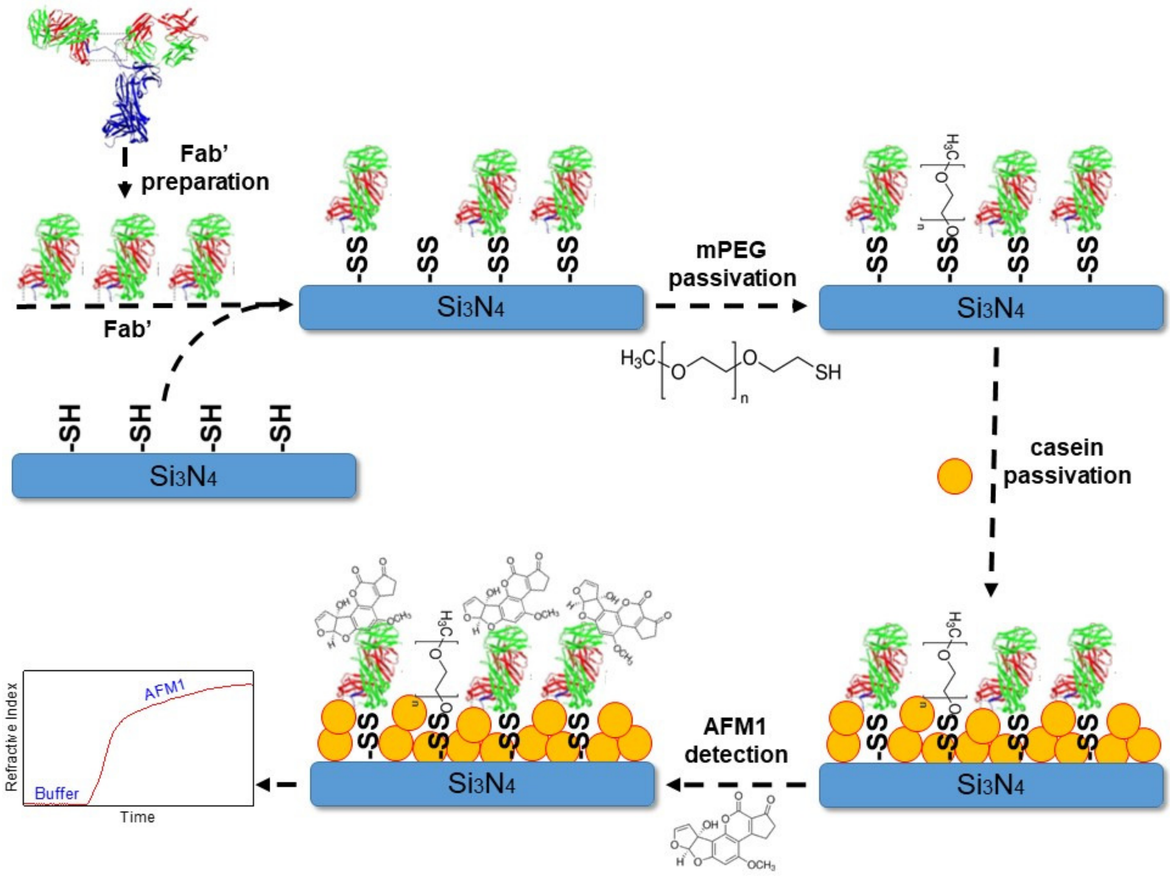

Fig. 2. Schematics of the surface functionalization principle. Note that the molecule sizes are not scaled and are not corresponding to the real proportions.

In a time as short as a few minutes, the minimum concentration of AFM1 detected by our aMZI sensors is 48 pM in purified and concentrated milk samples, prepared with a proprietary technology developed in SYMPHONY, starring the high specificity of our platform. These results show that integrated and portable photonic biosensors are viable solutions for lab-on-a-chip devices in food safety analyses. The Dutch company Surfix (surfix.nl) is commercializing the whole sensor platform.

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Publication

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