# Lable-free Enzyme Sensing with a $Si_3N_4$ Grated Waveguide Optical Cavity

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**Abstract:** We report the label-free, sensitive detection of PepN enzyme using a $Si_3N_4$ grated waveguide optical cavity covered with an immobilized, selective (antibody) receptor layer. The receptor-enzyme reaction was monitored in real-time.
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## 1. Introduction

An important property of a grated waveguide (GWG), consisting of a waveguide with a grated section, is the occurrence of sharp fringes in the transmission spectrum near the stop-band edges. It is well known that these oscillations are due to Fabry-Perot resonances of Bloch modes propagating in the cavity defined by the grated section [1]. Any small structural changes in the environment of the GWG, which disturb the evanescent field of the GWG propagation mode, will lead to a shift of its transmission spectrum. Such an effect could be used to detect a bulk refractive index change [2], or nano-displacements of a cantilever suspended above the GWG [3]. In this research, we report label-free sensing of PepN enzyme, the major Suc-LLVY-AMC-hydrolyzing enzyme in Escherichia coli, where the spectral shift of the GWG response is due to the antibody-antigen interaction leading to growth of an ad-layer on it. The GWG setup for the enzyme sensing experiment is shown in Fig. 1a. Figure 1b shows a characteristic transmission spectrum of the GWG, highlighting the (sharpest) peak used for the sensing measurements.

## 2. Materials and experimental methods

The $Si_3N_4$ grated waveguides were fabricated using laser interference lithography as described in [3]. To detect a target biomaterial, such as PepN enzyme in this case, its antibody needs to be immobilized on the surface of the GWG device. We followed the standard immobilization process developed by Imenz b.v. [4]. The process consists of five steps [5]. The first two steps, 1) surface cleaning and activation and 2) silanization, were carried out in a cleanroom. Then a polydimethylsiloxane (PDMS) chamber (2x2x0.2 $cm^3$) with removable cap was prepared, cleaned by ethanol and placed directly on the device surface. This chamber served as a closed environment (to prevent bulk index changes of the liquid owing to evaporation) for liquid-phase reactions in the next steps of the bio-sensing experiment. After each step, the cap of the chamber was opened and the surface of the device was cleaned with a buffer solution, rinsed thoroughly with de-ionized water to remove unbound molecules, then dried with

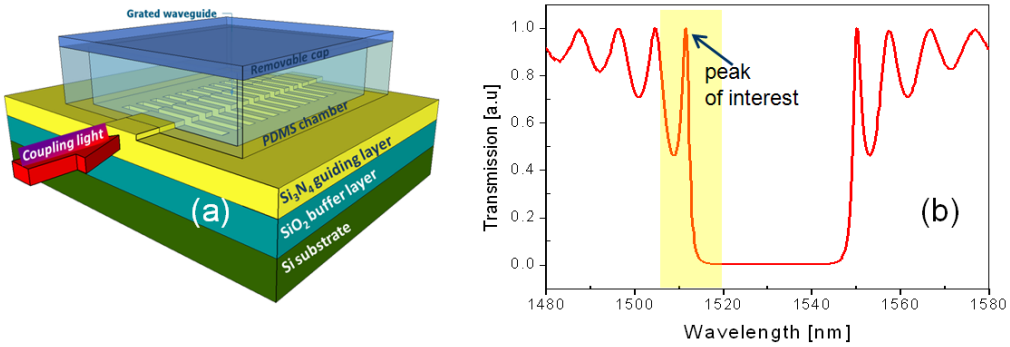


Fig.1. 3D schematic of the $Si_3N_4$ grated waveguide (GWG) device with a PDMS chamber serving as a closed environment for accurate monitoring of the antibody-enzyme interaction (a) and a characteristic transmission spectrum of the GWG (b).

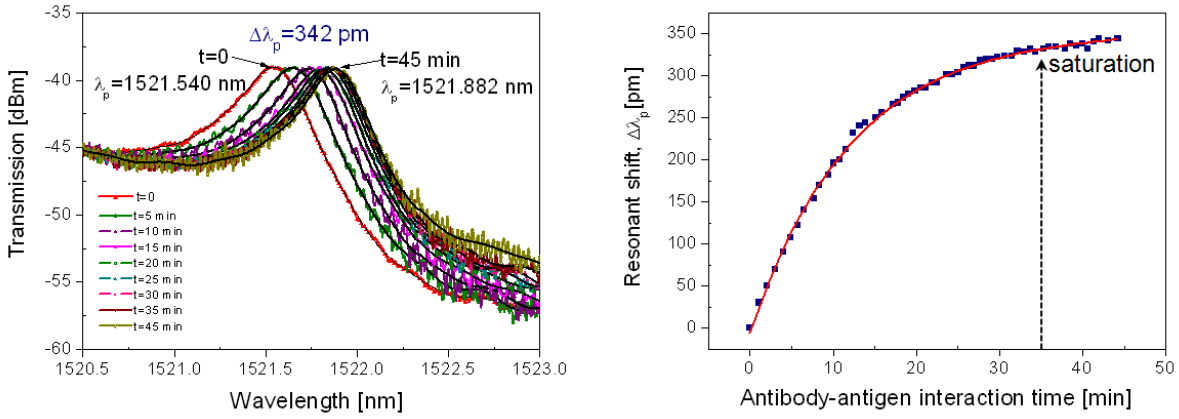


Fig. 2. Transmission curves monitored following antibody-antigen interaction time (a) and its spectral shifts (b), the binding reaction saturates after ~35 min.

nitrogen gas and optically characterized. Once the antibody was immobilized on the GWG surface and the blocking/washing/drying step right after that was treated, the cell-free extracted (CFE) PepN solution was applied and the antibody-antigen interaction was optically monitored through output transmission spectra of the GWG.

## 3. Results and discussion

Figure 2a shows the real-time measurement of the spectral shifts during the antibody-antigen interaction (*colored curves*). Noise was removed from the spectra using low pass filtering in the Fourier domain (*black curves*) to enable an accurate determination of the change, $\Delta\lambda_p(t)=\lambda_p(t)-\lambda_p(t_o)$, of the wavelength of the peak maximum, $\lambda_p$. Small changes on the GWG surface, caused by the antibody-enzyme interaction, lead to spectral shifts of the resonant peak, $\Delta\lambda_p$, as shown in Fig. 2b. It was found that the effect of the binding reaction was exponentially proportional to time according to $\Delta\lambda_p(t) \sim 342 \text{ pm}.\,(1-e^{-(1.3e-3)t/s})$; the reaction saturates after ~35 min. The total shift was approximately 342 pm, corresponding to the growth of an ad-layer of ~2 nm.

## 4. Conclusions

We have demonstrated the label-free enzyme sensing with a $Si_3N_4$ grated waveguide optical cavity. The enzyme is selectively recognized by its corresponding antibody immobilized on the surface of the sensor, and monitored in real-time. Owing to its simple fabrication and effective operation the sensor has potential as an element of a sensitive, on-chip multi-sensing system for label-free detection of a variety of bio-molecules.

## 5. Acknowledgements

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