

## Optical nanoimmunosensor based on periodic array of resonant nanopillars for real time detection of Okadaic Acid

Paula Ciaurriz<sup>1</sup>, Fátima Fernández<sup>1</sup>, Iñaki Cornago<sup>1</sup>, Edurne Tellechea<sup>1</sup>, Ana L. Hernández<sup>2</sup>, Rafael Casquel<sup>2</sup>, Francisco J. Sanza<sup>2</sup>, Miguel Holgado<sup>2</sup>, Clarence Deffaud<sup>3</sup>, Alice Baron<sup>3</sup>

<sup>1</sup>Cemitec, Polígono Mocholí, Plaza Cein 4, Noain, Spain

<sup>2</sup>Centre for Biomedical Technology, Universidad Politécnica de Madrid, Madrid, Spain

<sup>3</sup>Biotem, Parc d'Activités Bièvre Dauphine, Alphonse Gourju 885, Apprieu, France

[pciaurriz@cemitec.com](mailto:pciaurriz@cemitec.com)

The field of optical sensors is continuously evolving towards the search of new transducers. Recently, a new transducer based on periodic array of resonant nanopillars (RNP) has been described [1,2]. Particularly, RNP composed by two Bragg reflectors ( $\text{SiO}_2/\text{Si}_3\text{N}_4$ ) and a central cavity of  $\text{SiO}_2$  have shown very interesting results, being suitable for label-free molecule detection [3] and also for real-time monitoring by using a common spectrophotometer [4].

Here we present the detection of okadaic acid (OA) – a toxin produced by several species of marine dinoflagellates and responsible of the syndrome known as diarrhetic shellfish poisoning – by using a RNP biosensing system based on monoclonal antibodies (Ab), in a competitive working format. The surface is biofunctionalized with an OA competitor (BSA-OA), for the subsequent binding with the antibody, which signal is inhibited by the presence of OA. The label-free and real-time signal is monitored by using a microfluidic cell and a spectrophotometer.

Employing laser interference lithography, we fabricated RNP of different pitches and heights [5] and most sensitive nanopattern was selected to develop the biosensor. By using an IgG-antigen model, several biofunctionalization strategies were evaluated: activation and silanization processes were assessed to achieve covalently and stable immobilization of bioreceptor on transducer. Besides RNP real time response, the biofunctionalization was tracked by scanning electron microscopy (SEM).

Optimized functionalization protocol was applied for detection of OA. After immobilization of BSA-OA, we measured optical changes upon flowing solutions with different OA concentrations and calibration curve was evaluated. Smooth regeneration of antibody-antigen interaction allows reusing the surface for measuring several samples. Results show an OA limit of detection close to 5 ppb revealing the sensitivity of RNP assay as well as its rapidity (25 min/sample). These features constitute our OA sensor as a competitive analytical tool, since usually OA is determined by more complex and expensive techniques as HPLC.

This biosensing technology will be part of the Chemical Detection Unit of FP7 Enviguard project whose aim is the development of a biosensor device for real time and in-situ measurement of chemical pollutants and biohazards (viruses, microorganism and toxins) to be used as an early warning system in aquaculture.

- [1] M. Holgado, C.A. Barrios, F.J. Ortega, F.J. Sanza, R. Casquel, M.F. Laguna, M.J. Bañuls, D. López-Romero, R. Puchades, A. Maquieira, *Biosens. Bioelectron.* 25 (2010) 2553–2558.
- [2] M.F. Laguna, F.J. Sanza, J. Soria, M. Jara, Á. Lavín, R. Casquel, A. López, T. Suarez, M. Holgado, *Sens. Actuators B Chem.* 203 (2014) 209–212.
- [3] A.L. Hernández, R. Casquel, M. Holgado, I. Cornago, F.J. Sanza, B. Santamaría, M. Maigler, F. Fernández, A. Lavín, M.F. Laguna, *Opt. Lett.* 40 (2015) 2370–2372.
- [4] F. Fernández, P. Ciaurriz, I. Cornago, R. Casquel, A.L. Hernandez, F.J. Sanza, M.F. Laguna, M. Holgado, *Sens. Actuators B Chem.* 244 (2017) 323–326.
- [5] I. Cornago, A.L. Hernández, R. Casquel, M. Holgado, M.F. Laguna, F.J. Sanza, J. Bravo, *Opt. Mater. Express.* 6 (2016) 2264.

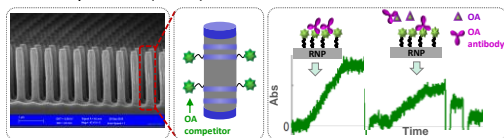


Figure 1: SEM image of RNP; biofunctionalized RNP; real time detection of OA (competitive assay).