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A disposable evanescent wave fiber optic sensor coated with a molecularly imprinted polymer as a selective fluorescence probe

Xuan-Anh Ton [a], Victor Acha [b], Paolo Bonomi [a], Bernadette Tse Sum Bui [a,*], Karsten Haupt [a,*]

[a] CNRS Enzyme and Cell Engineering Laboratory, Compiègne University of Technology, Rue Roger Couttolenc, CS 60319, 60203 Compiègne Cedex, France
[b] HydrISE Unit, Institut Polytechnique LaSalle Beauvais, BP 30313, 60026 Beauvais, France

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ABSTRACT

We have developed a disposable evanescent wave fiber optic sensor by coating a molecularly imprinted polymer (MIP) containing a fluorescent signaling group on a 4-cm long polystyrene optical waveguide. The MIP is composed of a naphthalimide-based fluorescent monomer, which shows fluorescence enhancement upon binding with carboxyl-containing molecules. The herbicide 2,4-dichlorophenoxyacetic acid and the mycotoxin citrinin were used as model analytes. The coating of the MIP was either performed *ex-situ*, by dip-coating the fiber with MIP particles synthesized beforehand, or *in-situ* by evanescent-wave photopolymerization on the fiber. The sensing element was interrogated with a fiber-coupled spectrofluorimeter. The fiber optic sensor detects targets in the low nM range and exhibits specific and selective recognition over structural analogs and non-related carboxyl-containing molecules. This technology can be extended to other carboxyl-containing analytes, and to a broader spectrum of targets using different fluorescent monomers.

1. Introduction

Molecularly imprinted polymers (MIPs) are biomimetic synthetic receptors possessing specific cavities designed for a target molecule. Produced by a templating process at the molecular level, MIPs are capable of recognizing and binding their targets with specificities and affinities comparable to those of natural receptors (Arshady and Mosbach, 1981; Alexander et al., 2006). These tailor-made synthetic receptors have considerable advantages over biological receptors due to their greater chemical and physical stability, and have become an interesting alternative as recognition elements for biosensors (Haupt and Mosbach, 2000; Wulff, 1995). In particular, MIPs have been widely used for optical sensing, especially using fluorescence (Dickert and Thierer, 1996; Henry et al., 2005; Ton et al., 2012; Wolfbeis, 2005). Target binding can be monitored by directly measuring a fluorescent analyte, or, if the analyte is not fluorescent, by a competitive assay with a fluorescent analog as a probe. Alternatively, non-fluorescent analytes can be monitored if a fluorescent monomer is incorporated into the MIP that shows a change in its fluorescence properties when analyte binding occurs (Haupt and Mosbach, 2000), namely fluorescence quenching (Takeuchi et al., 2005; Wagner et al., 2013), fluorescence shift (Matsui et al., 2000) or fluorescence enhancement (Leung et al., 2001; Kubo et al., 2003; Ton et al., 2013; Wan et al., 2013). Among these, fluorescence enhancement is the most interesting, as it is more specific and less prone to false-positive results.

There are only a few examples of fluorescence sensors with signaling MIPs, most of them in fiber optic format. Optical fibers are of interest as they offer many advantages such as ease of miniaturization and integration, limited loss of light even over long distances, and low cost (Marazuela and Moreno-Bondi, 2002). One example used lanthanide luminescence, combined with a MIP-based fiber optic sensor to measure the hydrolysis product of the nerve agent Soman (Jenkins et al., 1999). More recently, Nguyen et al. reported a MIP-based fiber optic sensor for the detection of cocaine. The signaling monomer was acrylamidofluorescein and the limit of detection (LOD) of the sensor was 2 μM (Nguyen et al., 2012). In these examples, the polymer coating procedure was complex, requiring multistep pretreatments of the fibers (polishing, surface activation, silanization) before the polymer could be covalently attached. Very recently, we reported the development of a new type of fluorimetric fiber optic sensor that carries a micrometer-sized MIP tip photopolymerized *in situ* at one end (Ton et al., 2013). No pretreatment of the fiber was necessary and the tip was fabricated in just a few seconds. The signaling MIP contained a naphthalimide fluorescent monomer (Doussineau et al., 2009; Niu et al., 2004), specially designed to exhibit fluorescence enhancement when interacting with carboxyl groups.

* Corresponding authors. Fax: +33 3 4420 3910.
E-mail addresses: jeanne.tse-sum-bui@utc.fr (B. Tse Sum Bui), karsten.haupt@utc.fr (K. Haupt).

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This sensor could detect the herbicide 2,4-dichlorophenoxyacetic acid in the nM range.

With the objective of developing an economic, disposable but sensitive fiber optic sensor based on a MIP, we turned towards fiber optic waveguides allowing for evanescent-wave measurements (Taitt et al., 2005; Wolfbeis, 2004). An evanescent field is generated when total internal reflection of light occurs in an optical fiber coated with a lower refractive index material. This field propagates perpendicularly to the fiber surface into the medium, decaying exponentially with distance. If a fluorescent recognition element like a MIP is coated onto the fiber, the fluorophores immediately adjacent to the surface (typically within 100–1000 nm) (Rogers et al., 1992) are excited by the evanescent wave, and part of the emitted fluorescent light is coupled back into the fiber and can be measured. This system has the advantage of a low background even in complex samples, as the molecules not bound to the MIP contribute very little to the measured signal. For example, a fiber optic immunosensor based on evanescent wave measurements was developed for the detection of the mycotoxin fumonisin B_1 (FB_1) (Thompson and Maragos, 1996). Anti-FB_1 antibodies were covalently attached to the surface of an 800 µm core fiber, and monitoring of FB_1 was performed by a displacement assay with fluorescently labeled FB_1. Evanescent-wave fiber optic biosensors have also been used for the detection of *Escherichia coli* in food samples, cocaine in urine, trinitrotoluene in environmental waters, ricin in water and urine, hormones in plasma, and others (Taitt et al., 2005).

In the present work, we use 4-cm long injection-molded tapered polystyrene waveguides (Geng et al., 2006; Lim, 2003), combined with a MIP, as a disposable fiber optic sensor, to detect the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and the mycotoxin citrinin (Fig. 1A). Monitoring of 2,4-D is of major concern as it is widely used and represents a threat for human health due to its endocrine disruption properties (Bus and Hammond, 2007). Citrinin is a nephrotoxic mycotoxin produced by several species of *Aspergillus*, *Penicillium* and *Monascus* fungi and occurs mainly in stored grains but also in other plant products (Bazin et al., 2013). The polystyrene fibers are generally coated with capture antibodies for the sensing of microorganisms and toxins in a competitive format and are incorporated in a multi-channel evanescent wave fiber optic biosensor packaged in a portable instrument called RAPTOR, developed by the U.S. Naval Research Laboratory (Anderson et al., 2000; Lim, 2003). Here, we coated the polystyrene fibers with 'plastic antibodies', that is, 2,4-D and citrinin-specific MIPs (Fig. 1B). The MIPs not only have specific recognition sites for capturing their respective template

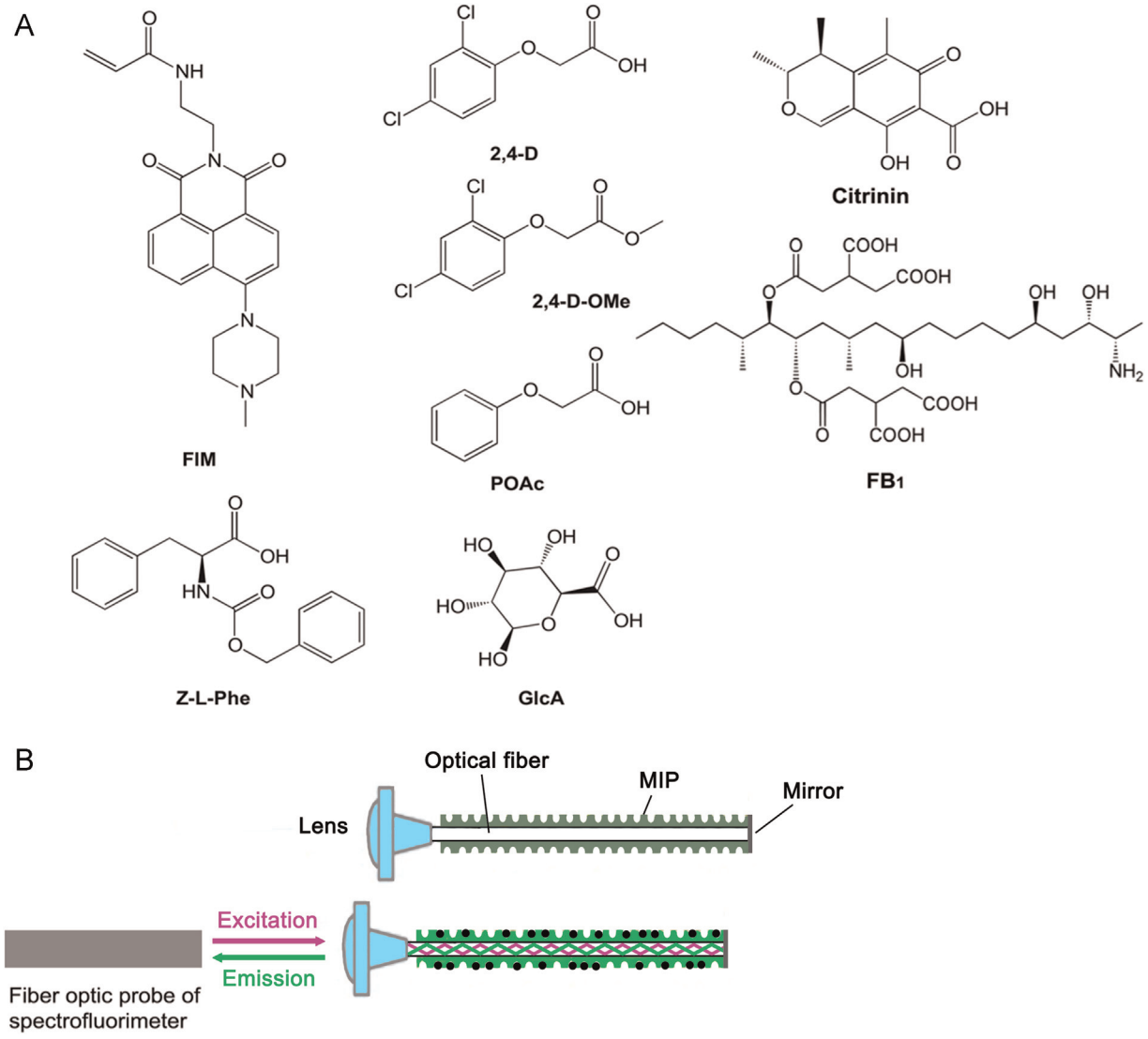

Fig. 1. (A) Structures of compounds used in this study. (B) Schematic drawing of the polystyrene evanescent wave fiber optic waveguide coated with fluorescent MIP particles. Excitation with the fiber optic probe of a spectrofluorimeter and collection of emitted fluorescence light use the same fiber.