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Short Communication

Polyoxometalate nanostructured gold surfaces for sensitive biosensing of benzo[a]pyrene

Dimitri Mercier[a,b], Maroua Ben Haddada[a,b], Maria Huebner[c], Dietmar Knopp[c], Reinhard Niessner[c], Michèle Salmain[d,e], Anna Proust[d,e], Souhir Boujday[a,b,f,*]

[a] Sorbonne Universités UPMC Univ Paris 6, UMR CNRS 7197, Laboratoire de Réactivité de Surface, F-75005 Paris, France
[b] CNRS, UMR 7197, Laboratoire de Réactivité de Surface, F-75005 Paris, France
[c] Chair for Analytical Chemistry and Institute of Hydrochemistry, Technische Universität München, Marchioninistr. 17, Munich, Germany
[d] Sorbonne Universités, UPMC Univ Paris 6, UMR 8232, IPCM, F-75005 Paris, France
[e] CNRS, UMR 8232, IPCM, F-75005 Paris, France
[f] Nanyang Technological University, Centre for Biomimetic Sensor Science, 50 Nanyang Drive, Singapore 637553, Singapore

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ABSTRACT

We report the design of a polyoxometalate-nanostructured immunosensor for benzo[a]pyrene (B[a]P) detection. The organic–inorganic hybrid polyoxometalate (POM) $(NBu_4)_3[PW_{11}O_{39}\{(SiC_6H_4NH_2)_2O\}]$ carrying two amine functions was covalently attached to a functionalized gold substrate to achieve a nanometric organization of amine groups at its surface. Pyrenebutyric acid (PBA) was subsequently grafted to amine groups to create the sensing layer. The detection of B[a]P in the indirect competitive format was carried out using a monoclonal anti-B[a]P antibody whose binding to the immunoprobe was monitored with a quartz crystal microbalance with dissipation measurement (QCM-D). The performances of the POM-nanostructured biosensor were compared to a reference sensor constructed from a cysteamine self-assembled monolayer. QCM-D measurements displayed significant input from POM-nanostructuration. Both the accessibility of the analogue on the surface and the analytical performances were enhanced showing a promising effect of this strategy of nanostructuration for the biosensing of small molecules.

1. Introduction

Building up analytical devices to quantify small-sized analytes in various matrices in a fast and sensitive way is still an important challenge to which immunosensing devices provide a relevant response. However, while immunosensors enable rapid and low cost analyses, their sensitivity still requires improvement. In the case of low molecular weight targets, the detection is generally performed in the indirect competitive format: an analogue of the analyte is immobilized on the sensor surface that captures the antibody, and the presence of the target in the sample is evidenced by a decrease in the amount of bound antibody [1]. Thus, the greatest challenge in this format is the control of the surface functionalization to achieve a reliable, reproducible and efficient sensing layer [1–4] as well as the relative affinity of the antibody for the analogue and the target analyte [5]. The key parameters are consequently the organization and the accessibility of the analogue molecules on the transducer surface, which can be optimized by surface nanostructuration. Very few methods enable patterning and controlled reactivity of surfaces at nanometric resolution. Lithographic techniques enable surface patterning with a resolution above 50 nm [6–9]. Colloids, either gold [10–12] or oxide nanoparticles [13], decrease the organization down to 5 nm. Below, at the 1-nm scale level, the potential of polyoxometalates (POMs) as nanostructuration agents deserves to be addressed. POMs are discrete nanoscale oxoclusters of the early transition metals displaying a great variety of molecular structures [14,15]. A growing attention is paid to these molecular oxides due to their multiple properties and related applications [16]. Deposition of POMs on surfaces has proved to lead to self assembled 2D-ordered arrays of POMs. The availability of organic–inorganic hybrid POMs including reactive residues [16,17] enables their covalent attachment to surfaces to form stable, dense, and well-ordered monolayers. In view of application

* Corresponding author at: Laboratoire de Réactivité de Surface, UMR CNRS 7197, Université Pierre et Marie Curie – Paris 6, 4 Place Jussieu, 75252 Paris cedex 05, France. Tel.: +33 144276001; fax: +33 144276033.
E-mail address: souhir.boujday@upmc.fr (S. Boujday).

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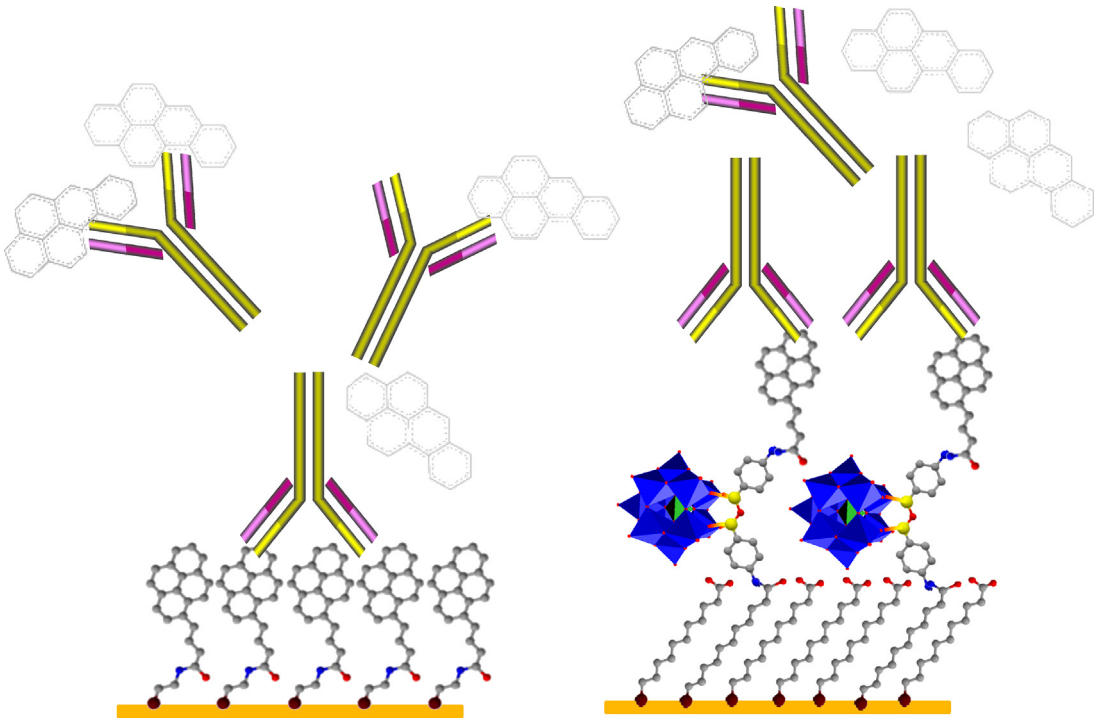

Fig. 1. Schematic representation of the reference immunosensor (left) and nanostructured immunosensor (right).

in the biosensor field, we have chosen to tether the homobifunctionalized $[PW_{11}O_{39}\{(SiC_6H_4NH_2)_2O\}]^{3-}$ anion to gold substrate prefunctionalized with a carboxylic acid terminated self-assembled monolayer (SAM) of thiol. This POM carries two amine functions, the first devoted to its reaction with the acid-terminated SAM, while the second enables further grafting of molecules. The sequential steps of immobilization of this POM were investigated and optimized using polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS), X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM) in a recent work described by some of us [18]. The binding of POMs to the surface was successful and led to a nanostructured layer of organized amine functions. Eventually, we constructed a model immunosensor for which a monoclonal anti-rabbit IgG antibody was immobilized on a quartz crystal sensor. This immunoprobe efficiently recognized rabbit IgG and the POM nanostructuration avoided non-specific adsorption [18]. We now apply this strategy to the detection of a member of the polycylic aromatic hydrocarbons (PAHs) family. PAHs are a class of chemicals resulting from the incomplete combustion of organic substances such as fossil fuels [19]. Benzo[a]pyrene (B[a]P), whose concentration often correlates well with the total PAHs contents in environmental samples, is an identified carcinogen and displays endocrine-disruptive activity. A limited number of label-free immunosensors have been set up to detect and quantify B[a]P in water samples. All these immunosensors operate in the competitive format with electrochemical [20], optical [21], or piezoelectric [22] transduction modes, due to very small size of this molecule (252.31 g mol^{-1}). In this work, we constructed a POM-nanostructured surface and used it to immobilize pyrenebutyric acid (PBA), an analogue of B[a]P recognized by the same antibody with a lower affinity. Then we assessed the efficiency of the sensing layer using quartz crystal microbalance in the 0–10 μM range of B[a]P. As we intend to establish the benefit of surface nanostructuration in terms of analogue accessibility and sensor sensitivity, the nanostructured sensing layer was compared to a reference layer built from an amine-terminated SAM on gold (see Fig. 1).

2. Experimental

2.1. Chemicals

Cysteamine (CEA), mercaptoundecanoic acid (MUA), N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), benzo[a]pyrene (B[a]P) and 1-pyrenebutyric acid (PBA) were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). The mouse monoclonal anti-PAH antibody (mAb) 22F12 was prepared at the Institute of Hydrochemistry, TU Munich [5]. The polyoxometalate (POM) $(NBu_4)_3[PW_{11}O_{39}\{(SiC_6H_4NH_2)_2O\}]$ was prepared following the procedure we previously described [23]. A stock solution of B[a]P (1 mM) was prepared in absolute ethanol. Standard solutions of B[a]P (1–10 μM) were prepared in PBS/ethanol 9:1 which had been thoroughly degassed under Argon (for 15 min) before use in the QCM-D experiments. Experiments were carried out at room temperature unless otherwise stated.

2.2. Surface functionalization and nanostructuration

Self-assembled monolayers formation: Gold-coated sensor chips were immersed in a solution of CEA in water (1 mM, 10 mL) during 12 h for the "reference immunosensor" or in a solution of MUA in absolute ethanol (1 mM, 10 mL) during 3 h for the "POM-nanostructured immunosensor". Then substrates were washed twice with the same volume of water or EtOH and dried under nitrogen flow.

Nanostructuration with POM: The MUA-modified substrates were chemically activated using the following procedure: 150 μL of NHS (20 mM) and EDC (10 mM) in Milli-Q water were deposited on the MUA-modified surface during 90 min, then substrates were rinsed with Milli-Q water and dried under nitrogen flow. The activated surfaces were immediately immersed in a solution of POM (0.5 mM, 10 mL) in acetonitrile during 3 h, then were rinsed twice with the same solvent and dried under nitrogen flow.