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Label-Free Multiplexed Analysis Using Photonic Crystal-Based Biosensors

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Abstract. Label-free bioanalytical methods have been widely employed in biomedical research, in particular, in drug screening and discovery, diagnostics, and proteomics. Photonic crystals (PCs) represent a modern alternative to surface plasmon resonance (SPR) techniques. Imaging of PC surface modes has been demonstrated as a promising label-free approach allowing for multiplexed detection. Surface modification of PC sensors is an important stage determining the effectiveness of the analysis of biomolecule interactions. Here, we describe the results of the development of a label-free PC-based sensor, the key steps of the modification and functionalization of the PC surface with proteins, as well as the evaluation of its suitability for sensing via 2D imaging of binding events. Our data demonstrate the efficiency of the designed PC-based sensor for analysis of proteins interactions and pave the way for the engineering of a label-free biosensing platform based on PCs.

1. Introduction

Photonic crystals (PCs) have been demonstrated to be promising label-free multiparametric sensors being a good alternative to the widely used surface plasmon resonance (SPR) approach for detection of biomolecule interactions in modern diagnostics and drug discovery [1, 2]. PC are periodic multilayer stacks of dielectrics with a periodic modulation of their refraction indices on the scale of the wavelength of light [3]. Imaging of PC surface modes ensures analysis of multiple interactions occurring at the sensor surface. PC-based imaging sensors are characterized by a longer surface wave propagation and finer spatial resolution resulting in a higher sensitivity compared to the SPR-based approach [3]. Two-dimensional (2D) imaging of binding events at the PC sensor surface with a picometer resolution, which provides them with the clear comparative advantages against the similar SPR imaging techniques, has been presented earlier [4].

Surface modification is an important stage in the engineering of biosensors that directly affects the effectiveness of the analysis of biomolecule interactions [5]. SPR-based sensors usually employ the gold or silver films and, therefore, require using thiol chemistry. Their surface cannot be completely regenerated, which limits their use and increases the cost of analysis. In contrast, the PC outer silicon surface is chemically stable, can be fully regenerated, and allows using surface chemistry techniques to



attach the desirable functional groups to the sensor for immobilization of analyte-binding moieties and subsequent coupling of biomolecules to assemble bioanalytical complexes.

In this study, we describe an approach to surface chemical modification and functionalization of PC sensors using capture molecules: protein G (immunoglobulin G (IgG)-binding protein) and neutravidin (biotin-binding protein). We also present the results of the proof-of-the-concept of label-free detection of model analytes (IgG) with the use of a microfluidic PC imaging sensor enabling the high-resolution 2D imaging of individual binding events.

2. Materials and Methods

2.1 Fabrication of a photonic crystal sensor

One-dimensional PC was prepared by alternated deposition of titanium dioxide (TiO₂) and silicon dioxide (SiO₂) layers with refractive indices at $\lambda = 500$ nm of 1.48 and 2.27, respectively, onto the surface of a glass substrate with a refractive index of 1.52 at 500 nm by electron-beam evaporation and plasma ion-assisted deposition [6].

2.2 Photonic crystal surface modification and functionalization

Primarily, the PC surface was cleaned using a UV-cleaner. A sensor with a pre-cleaned surface was incubated in a 1% acetone solution of (3-aminopropyl)triethoxysilane (APTES) overnight. Then, the excess APTES was removed, and the chip surface was additionally treated at 90° for 1 h. Surface activation was performed for 1 h while shaking using 2.5% glutaraldehyde buffered with 0.02 M phosphate (pH 7.4) with subsequent triple washing off the excess of the activation agent [3].

Afterwards, protein G or neutravidin was spotted. The protein coupling efficiency was analyzed using the A280 protein approach by means of a Spark™ 10M multimode microplate reader (Tecan, Switzerland).

2.3 Photonic crystal surface modification and functionalization

Analysis of interactions of analytes (goat IgG and biotinylated goat IgG) and spotted proteins was performed in the microfluidic mode as described earlier [6].

Briefly, analyte solutions in 0.02 M phosphate buffer (pH 7.4) were run through a microfluidic cell. 0.02 M phosphate buffer (pH 7.4) was also used as a running buffer to fill the microfluidic cell. The PC surface was initially quenched using 0.1 M glycine in 0.02 M phosphate buffer pH 7.4. The chip surface was additionally blocked to prevent nonspecific binding of the protein analytes using a 1 or 2% bovine serum albumin (BSA) solution in 0.02 M phosphate buffer (pH 7.4). To follow proteins interactions, the spot layout of the chip image placed in front of the camera was prepared. The desired areas of interest corresponding to the protein spots applied were selected, and signals in these areas were recorded and monitored.

3. Results and Discussion

Before the protein spotting, the PC sensor surface was aminosilanized by APTES deposition and activated with glutaraldehyde to provide effective protein coupling. Primary amine groups of the APTES layer reacted with glutaraldehyde to form Schiff bases, which enabled protein coupling to the sensor surface via primary amines of the proteins (Figure 1) [3]. To ensure oriented immobilization of IgG, a protein-protein approach of noncovalent immobilization was employed. Spotting of protein G or neutravidin was performed to ensure oriented attachment of IgG molecules to the chip surface. Protein G interacts with the Fc fragments of IgG molecules, enabling the exposure of their Fab parts away from the sensor surface to enhance the capture capacity of the assembled bioanalytical complex [7]. Neutravidin is a biotin-binding protein that can be used for immobilization of biotinylated IgG molecules while ensuring low nonspecific binding compared to the other proteins of the avidin family [8]. The efficiency of protein coupling to the activated surface was found to be no less than (85±6)%, which indicated sufficient coupling of the spotted proteins with the pre-activated surface of the PC sensor.

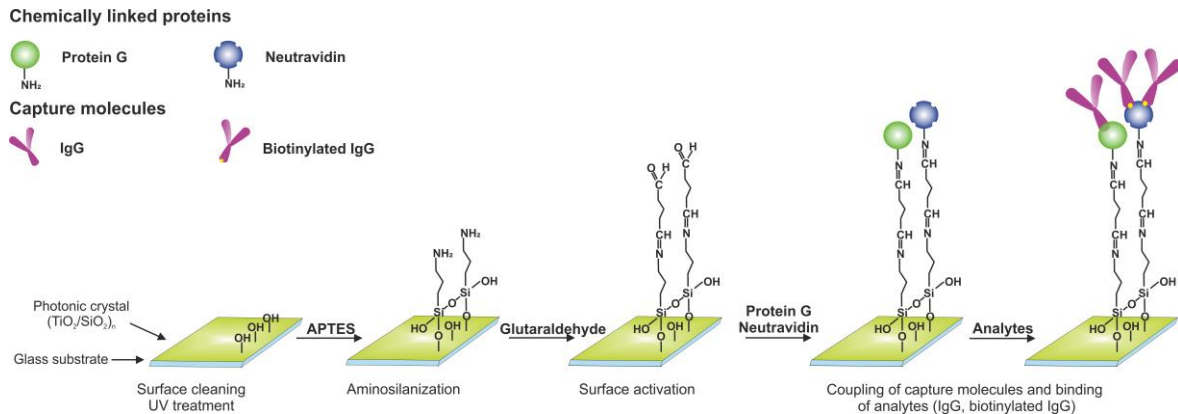


Figure 1. The scheme of surface functionalization of a photonic crystal sensor with protein G and neutravidin, and consequent analyte binding. Abbreviations: APTES, (3-aminopropyl)triethoxysilane; IgG, immunoglobulin G.

The prepared PC sensor with spotted proteins was placed onto a prism under a Kretschmann-like configuration. The PC was illuminated with a parallel light beam, with a polarization of $+45^\circ$. Being reflected, the light beam passes through a -45° polarizer to the plane of incidence, and the signal is recorded by a colour camera. The PC supports p-polarized surface modes at 500 nm with a refractive index of 1.38 and an in-prism excitation angle of 65.18° . The surface excitation mode at 500 nm is located between the maximum responsivities for the blue and green pixels of the colour camera. Changes in the adsorption layer (adlayer) thickness cause a shift of the PC surface mode peak and, thus, affect the ratio of the green and blue components in the colour pixel response. This makes it possible to obtain a 2D image showing the spectral shifts of the PC surface mode resonances along the sensor surface and ensures the possibility of signal recording as a change in the adlayer thickness at the PC surface within the selected area of interest (Figure 2).

A typical sensorgram demonstrating the changes in the adlayer thickness during analyte running is shown in Figure 2b. The protein spot titration with IgG analytes resulted in a subsequent gain of the adlayer thickness with increasing protein analyte concentration. The signal plateauing indicates surface saturation of the selected protein G spots with the analytes injected.

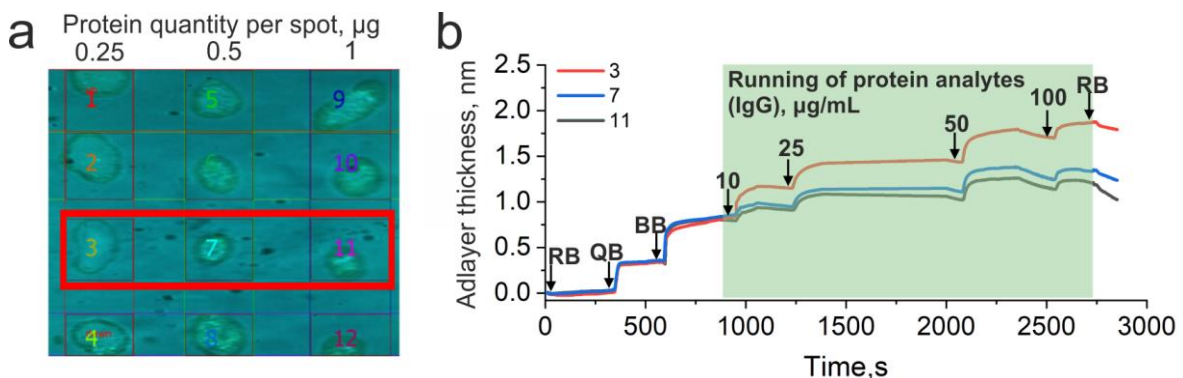


Figure 2. The proof-of-the-concept of label-free detection of analytes using functionalized photonic crystal sensor. (a) The protein G spot layout at the chip surface. (b) A typical sensorgram of analyte interaction with protein spots in the selected area (selected spots are framed red in panel a). The sensorgrams registered over the selected spots 3, 7, and 11 are shown in red, blue, and grey lines, respectively. Abbreviations: RB, running buffer (0.02 M phosphate buffer, pH 7.4); QB, quenching buffer (0.1 M glycine in 0.02 M phosphate buffer, pH 7.4); BB, blocking buffer (1-2% bovine serum albumin in 0.02 M phosphate buffer, pH 7.4); IgG, immunoglobulin G (analyte).

4. Conclusions

We have demonstrated that the developed approach of PC - surface chemical modification ensures effective immobilization of spotted proteins with the efficiency of protein coupling to the activated surface exceeding 85% and ensuring oriented coupling of IgG. The sensorgrams showed a gain of the adlayer thickness after running of IgG within the selected spot, which indicated binding between the spotted proteins and analytes. Reliable detection of IgG analytes was achieved in μg range, but a further decrease in detectable concentration is possible. The data demonstrate the possibility of label-free analysis of interactions of model proteins and pave the way to future engineering of a multipurpose biosensing platform based on PCs.

5. Acknowledgements

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