MULTICHANNEL IMPEDIMETRIC BIOSENSOR PLATFORM FOR LABEL-FREE AFFINITY ASSAYS USING ELECTRICALLY CONDUCTIVE FUNCTIONAL POLYMERS

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ABSTRACT

In this work we report on a multichannel label-free impedimetric sensor platform that is suitable for high-throughput analysis of affinity binding. The sensor is based on electrochemical impedance spectroscopy (EIS) and a surface modification of gold electrodes using a carboxy functionalized conductive polymer (polypyrrole). Concentrations of Biotinylated Bovine Serum Albumin (bBSA) down to 1 ng/ml were achieved using this setup.

KEYWORDS

Impedimetric biosensor, Conductive polymer, Polypyrrole, Electrochemical impedance spectroscopy, Microfluidics

INTRODUCTION

There are a number of techniques available for label-free affinity assays, the most commonly used methods include gravimetric systems such as quartz crystal microbalances or surface acoustic waves, surface plasmon resonance as well as electrochemical techniques. Recently EIS has been more intensively studied as an alternative method [1, 2]. EIS monitors the impedance (resistance and reactance) of a surface by applying an AC signal with variable frequency and small-amplitude to an electrode system while measuring the resulting current (Figure 1a). The real and imaginary parts (i.e., resistance and reactance) of the impedance are plotted as a function of frequency in a Nyquist diagram (Figure 1b). The changes in the current provide means to assess the electrical properties of the layers on top of the electrodes. These changes can clearly be seen in the Nyquist diagram as a curve shift in both axis.

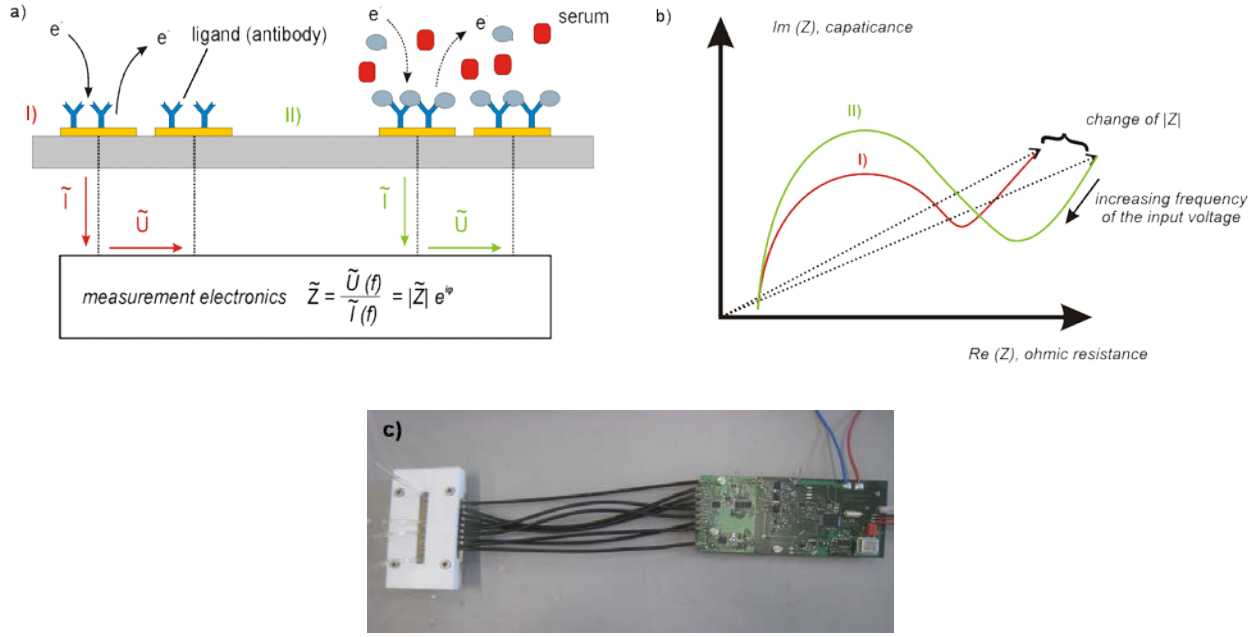

Figure 1 a) Setup of EIS in a biosensor– a pair of electrodes is used to measure the increasing inhibition of the charge transfer through a surface interface by the specific binding of an analyte (such as a protein) to its surface. b) The change of impedance in the complex plane can be correlated to the presence of the analyte in the liquid sample above the electrodes. c) Measurement electronics and the flow cell with housing.

EXPERIMENTAL

We have designed a custom made EIS-based measurement cell consisting of a polymer microfluidic flow cell with 8 independent channels (each measuring 4 × 1 × 0.1 mm), polymer housing, planar gold electrodes and custom made electronics (Figure 1c).

In order to create an EIS-based biosensor it is necessary to add a biosensing layer which ensures the exclusive binding of the target to the surface, hence only specific signal changes are measured. In our work we used a carboxy functionalized conductive polymer: polypyrrole (PPy) with pyrrole-3-carboxylic acid (Pa). By means of the carboxy functional groups of the layer we immobilized streptavidin which specifically binds to biotinylated analytes such as

biotinylated bovine serum albumine (bBSA). The latter has been used as an exemplary target (Figure 2).

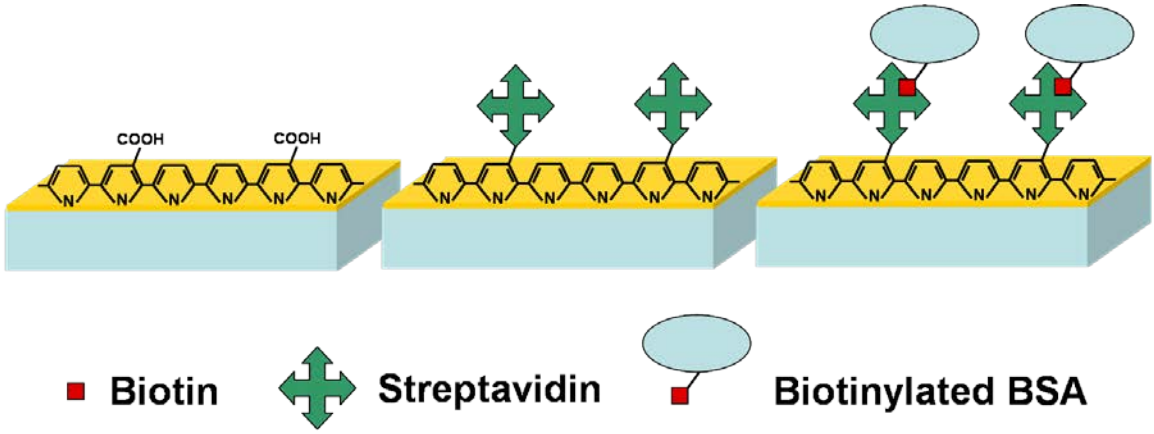

Figure 2 - Schematic representation of the assay procedure. Firstly pyrrole and Pa monomers (in 3:1 volume ratio) are electropolymerized onto the gold electrodes (800 mV during 40 s at 40 mV/s). In the following step streptavidin was immobilized by means of active ester chemistry. Finally, biotinylated BSA in different concentrations was used as exemplary analyte.

The conductive polymer layer has two important functions in this system; it serves as a shielding layer against unspecific adsorption whilst providing functional groups to which biomolecules (e.g. antibodies) can bind. Compared to strongly insulating surface modifications (such as those based on thiol-gold chemistry, [3]) electrically conductive polymers have a minimal effect on the sensor impedance (Figure 3).

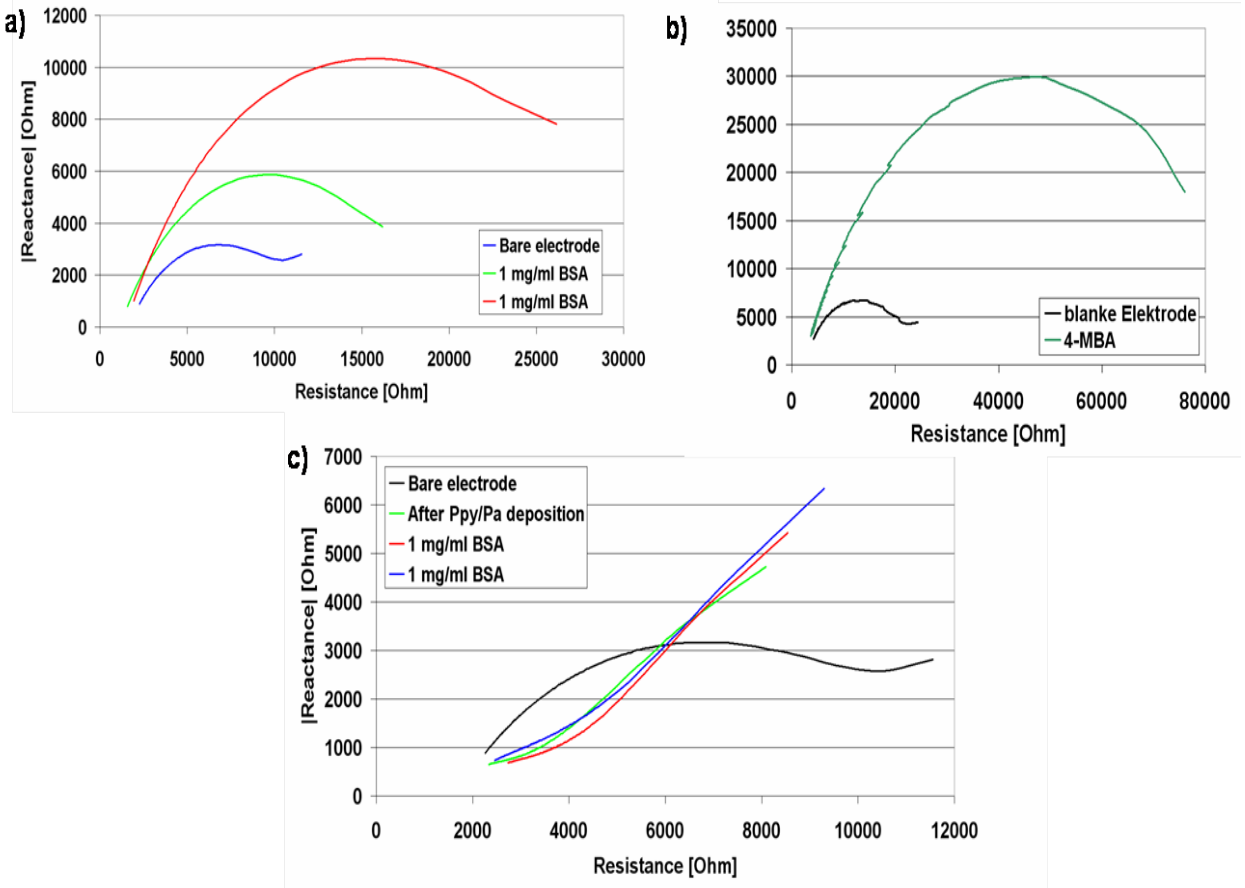

Figure 3 - Unspecific adsorption test using biotinylated BSA. a) On a bare electrode surface, the impedance increases greatly each time BSA is probed across the electrode. b) Compared to the conductive polymer the immobilization of an electrode with a self-assembled monolayer (4-mercapto benzoic acid) increases greatly thus reducing the overall sensor sensitivity. c) After polymerization of PPy/Pa on the electrode surface, the impedance shows no significant change upon BSA probing demonstrating that the PPy/Pa coating is suitable as shielding layer.

RESULTS AND DISCUSSION

Gold was structured by means of a lithography based etching process. PPy/Pa (molar ratio 3 to 1) was then electropolymerized on the electrode surface whereupon streptavidin was coupled to the carboxy functional groups by active ester chemistry followed by coupling of ethanolamine in order to block remaining unbound carboxy groups. Afterwards BSA in 1 mg/ml concentration was used to probe the extent of non-specific protein adsorption.

shows that an electrode without PPy/Pa will suffer from strong adsorption effects resulting in increasing impedance after each BSA sampling whereas the PPy/Pa covered electrodes show no significant change upon probing. In a final step, various concentrations of bBSA (which binds specifically to streptavidin) were probed across the sensor as an exemplary analyte. Concentrations down to 1 ng/ml were successfully measured with this setup (Figure 4).

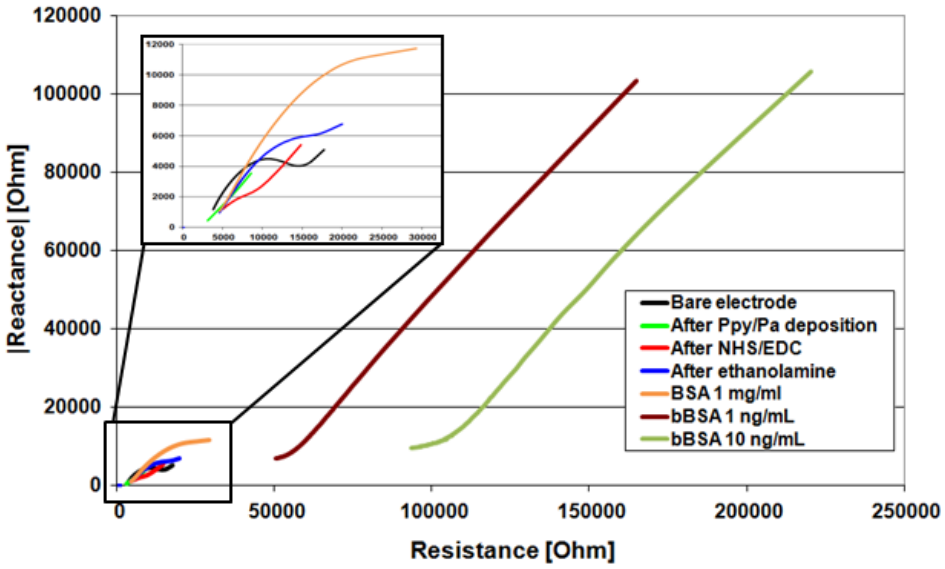

Figure 4 - Exemplary measurement, the bare electrode is coated with Ppy/Pa followed by streptavidin immobilization, ethanolamine blocking and BSA probing. Concentrations of bBSA down to 1 ng/ml were detected.

CONCLUSION AND OUTLOOK

Our system is a cheap, fast and flexible platform for affinity assays on multiple channels with a current limit of detection of 1 ng/ml of bBSA (measured as an exemplary affinity system). We will demonstrate that the electrically conductive PPy/Pa coating can be used as a generic low impedance biochemically functional interface layer for detection of various targets whilst shielding the surface against nonspecific binding.

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