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Impedimetric Detection of COVID Proteins on Functionalized Boron Doped Diamond Electrodes – is the Redox Marker Necessary?

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Summary:
Boron doped diamond (BDD) electrodes were functionalized with receptors specific to COVID-19 viral proteins. It was shown that the concentration of the protein can be quantitatively estimated using electrochemical impedance spectroscopy (EIS). Detection was successful using the standard methodology with external redox marker, as well as without the marker. Pros and cons of both strategies are discussed, emphasizing the differences in measurements protocols, electrode stability, and reliability of the results.

Keywords: Boron Doped Diamond, Biosensor, COVID, Electrochemical Impedance Spectroscopy,

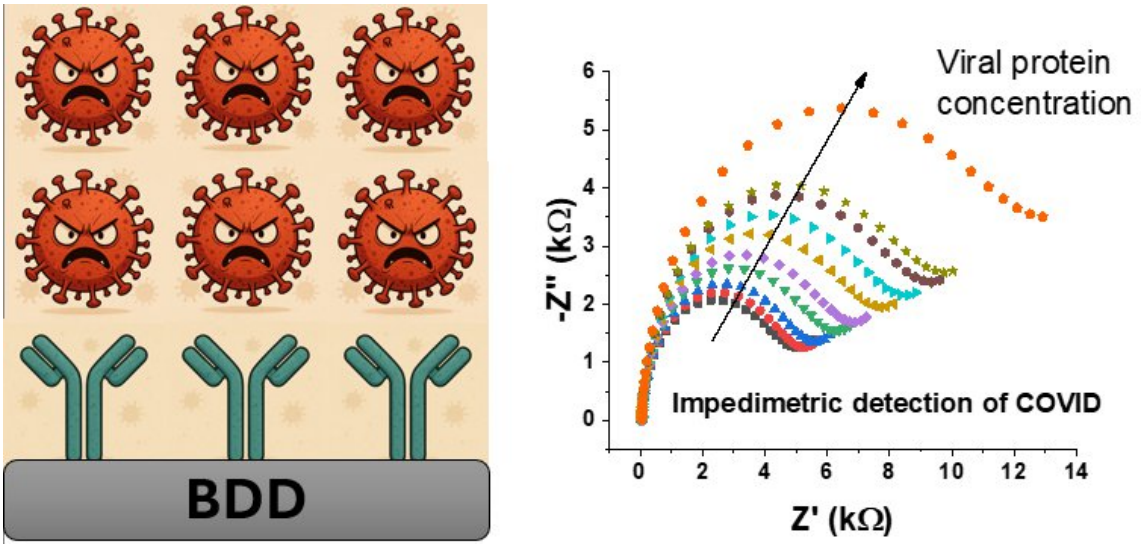

Fig. 1 Cartoon of receptor-functionalized boron doped diamond (BDD) electrode and application of electrochemical impedance spectroscopy (EIS) for quantitative detection of COVID viral proteins.

Background, Motivation an Objective

Detection of viral proteins is essential for quick and reliable diagnostics. COVID-19 and Influenza still remain the most abundant causes of viral infections. There is a variety of approached including optical, spectroscopic, colorimetric, and electrochemical. Despite a substantial effort in recent decades, reliability and cost-efficiency of viral sensors is still to be improved.

Electrochemical methods offer the possibility of cheap and fast detection of viral proteins, provided the electrodes are functionalized with specific receptors, and the measurement protocol is properly optimized (Fig. 1). Typically, to realize such experiments redox-active compounds (such as hexaferrocyanides (II/III) or hexaamineruhenium (II/III)) are added to the analyte solutions prior the measurement serving as amplifiers of the chemical signal. According to this approach, receptor-antigen binding event is reflected as an increase in the charge transfer resistance of the modified electrode, which can be converted to the protein concentration by linear function [1].

Although this method is well-established and gives decent results, it possesses intrinsic drawbacks, which might affect the quality of measurement and make detection unreliable. Besides on obvious inconvenience of adding external compound, redox marker can interact with both the receptor and target protein. Therefore, changes in the impedance might originate not only in the receptor-antigen binding event, but also those unintended interactions. Moreover, stability of the electrode can be compromised by those interaction. This work discusses pros and cons of using the external redox marker in electrochemical biosensing research on the example case of COVID-19 protein detection using boron doped diamond (BDD) functionalized with angiotensin convertase enzyme (ACE2).

Methods

Boron doped diamond electrodes are prepared using microwave-assisted chemical vapor deposition process and functionalized with ACE2 receptors against COVID proteins according to the methods elaborated in the work [2]. The successful formation of nanostructured boron doped diamond and biomolecules functionalization of receptors are confirmed accordingly.

Detection of the receptor binding domain (RBD) protein of COVID-19 is realized by electrochemical impedance spectroscopy in neutral aqueous solution with or without external hexaferrocyanide (II/III) redox couple in 1 mM concentration. For simplicity, detection signal is assumed to be the imaginary part of the impedance at 1 Hz frequency. Fitting of equivalent circuit is not necessary.

Limit of detection was calculated according to the approach advised in the work [3]:

$$LOD = \frac{3.3\,SD}{a} \quad (1)$$

Where the slope a is taken from three first points of the low concentration edge of the linear range, and the standard deviation SD is the sum of squared errors from the linear fit of these three points.

Results

The BDD-ACE2 sensor response covers several orders of magnitude of the RBD concentration from 1 pg/L to 1 mg/L. The linear range spans from 0.05 µg/L to 1 mg/L regardless whether the external redox marker is used. However, LOD value is significantly lower (Figure 2.) for the non-marker case, and the slope is greater – these two factors are beneficial for sensor performance.

Disadvantage of the non-marker approach is larger initial impedance of the electrode and higher deviation from the response linearity, which originates from 1/f noises. Moreover, stability and repeatability of the response is higher compared to the standard protocol.

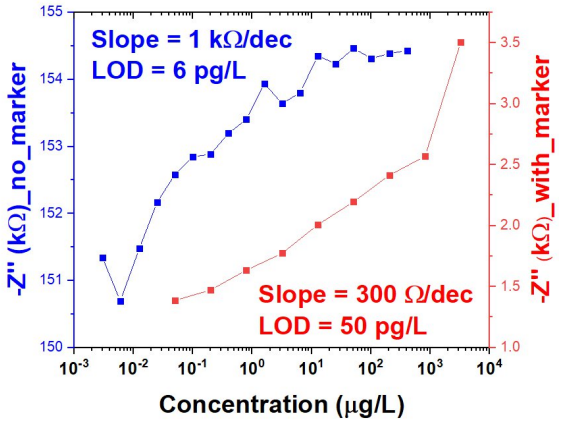

Fig. 2. Dependence of imaginary part of the impedance for RBD protein detection in the variants with or without the redox couple. Electrolyte is 1 X Tris buffered saline (pH = 7.2).

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