Impact of Molecular Surface Charge on Electrical Impedance Spectroscopy Biosensing

Y. Ram[a], T. Yoetz-Kopelman[b], A. Freeman[b], and Y. Shacham-Diamand[a]
[a] Department of Physical Electronics, Faculty of Engineering, Tel Aviv University, Tel Aviv, Israel
[b] Department of Molecular Microbiology and Biotechnology, Faculty of Life Science, Tel Aviv University, Tel Aviv, Israel

1. Introduction

- Electrical Impedance Spectroscopy (EIS) is a powerful and sensitive electrochemical tool for detecting analytes by capturing them by an immobilized binding protein [1].
- Using a redox couple, changes in the impedance of the system can be detected. These changes reflect changes in the flux, as a result of changes in surface charge.

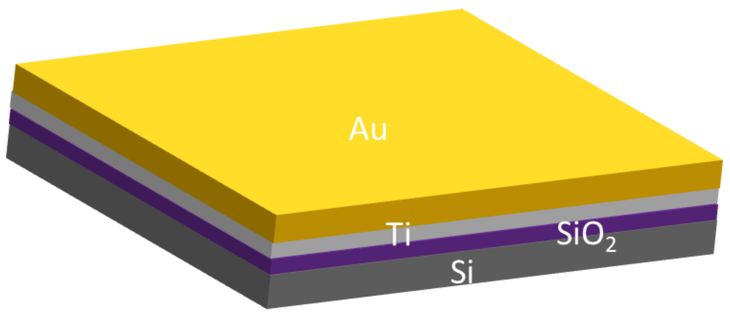
2. Objectives

• Exploiting the impact of surface charge modification of immobilized Avidin on biodetection of biotinylated proteins.
• Getting a physical understanding of the detection mechanism and offering a physical model.

3. Experimental Details and Methods

3.1 Working Electrode Preparation

Gold/Ti (300 nm/20 nm) was deposited by RF sputtering silicon wafer with a 500 nm thick thermal oxide layer.



3.2 Electrochemical Cell

- Counter electrode – Pt
- Reference electrode – Ag/AgCl
- Redox couple - $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$
- Electrolyte – HEPES + KNO_3

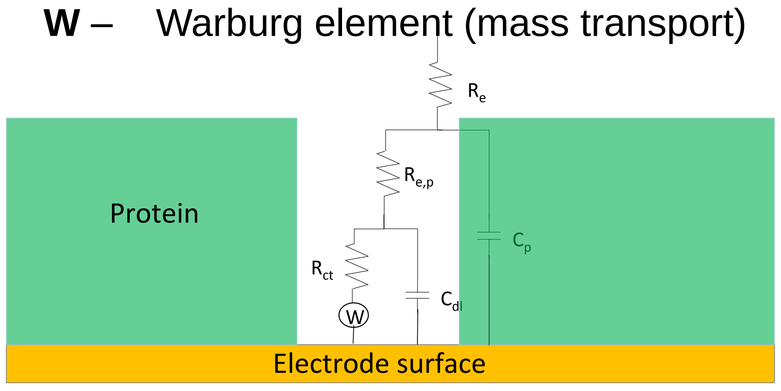
3.3 Binding Proteins and Analytes

- The Avidin-Biotin interaction was chosen as model for this study.
- Avidin and its modified form [2] were immobilized on the surface and served as biotin binding layers.
- A biotinylated Horseradish peroxidase served as the analyte (2^{nd} layer).
- Blocking steps and SAM were not required.

3.4 Equivalent Electrical Circuit [3]

The measured data was fitted to the following circuit:

R_e – Solution resistance
$R_{e,p}$ – Solution resistance between proteins
C_p – Protein capacitance
C_{dl} – Double layer capacitance
R_{ct} – Charge transfer resistance
W – Warburg element (mass transport)



4. Results and Discussion

4.1 ELISA test

To ensure Avidin retains its specificity for biotin binding, a standard ELISA was performed in a 24 well plate with transferable gold discs. Result are presented in Fig. 1:

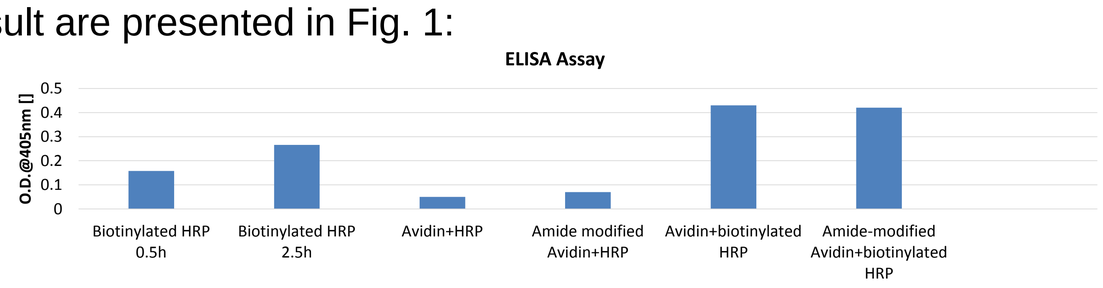


Fig. 1: ELISA assay conducted on the gold electrode

The data of Fig.1 show that both Avidin and the modified Avidin exhibits similar Biotin binding capability.

4.2 CV

CV for Avidin and its modified form before and after the binding of biotinylated HRP was conducted in comparison to bare gold (Fig. 2). The slope of the curve at the linear area represents the DC resistance, presented in Fig. 3. As the molecule becomes more negative, the resistance increases. The rate of change, however, before and after HRP binding was the same.

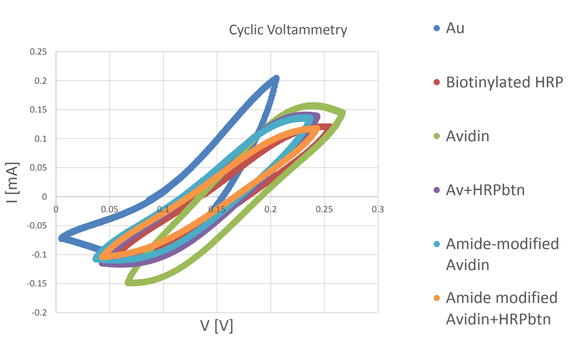


Fig. 2: Voltammograms of different protein formations and bare gold

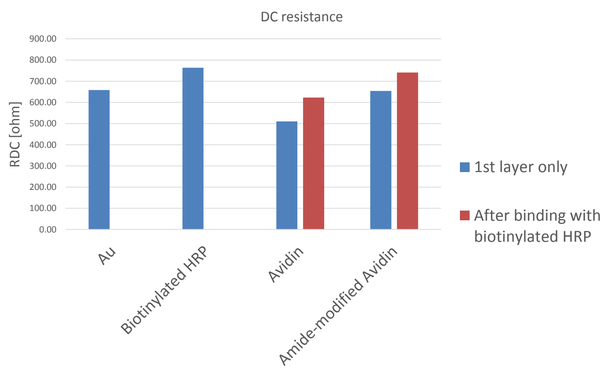


Fig. 3: DC resistances of the voltammograms in figure 2

4.3 EIS

EIS was performed, and the total resistance, which is equivalent to $R_{tot}= R_e+ R_{e,p}+ R_{ct}$, was evaluated by the electric model. The results are presented in Fig. 4:

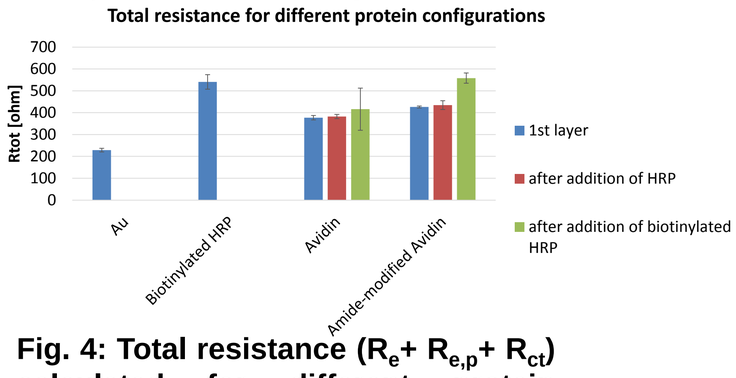


Fig. 4: Total resistance ($R_e+ R_{e,p}+ R_{ct}$) calculated for different protein configurations

The change after the binding of b-HRP is more significant when modified Avidin is used for sensing.

In order to asses the functionality of this device, the modified Avidin was used for sensing different concentrations of b-HRP. The results are presented in Fig. 5:

The device was able to detect nM changes in the concentration of the analyte (b-HRP).

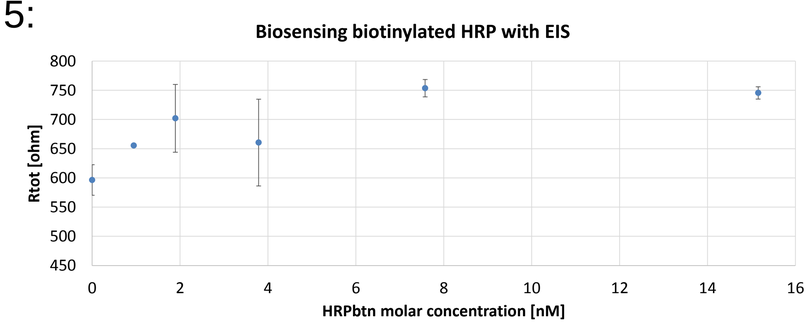
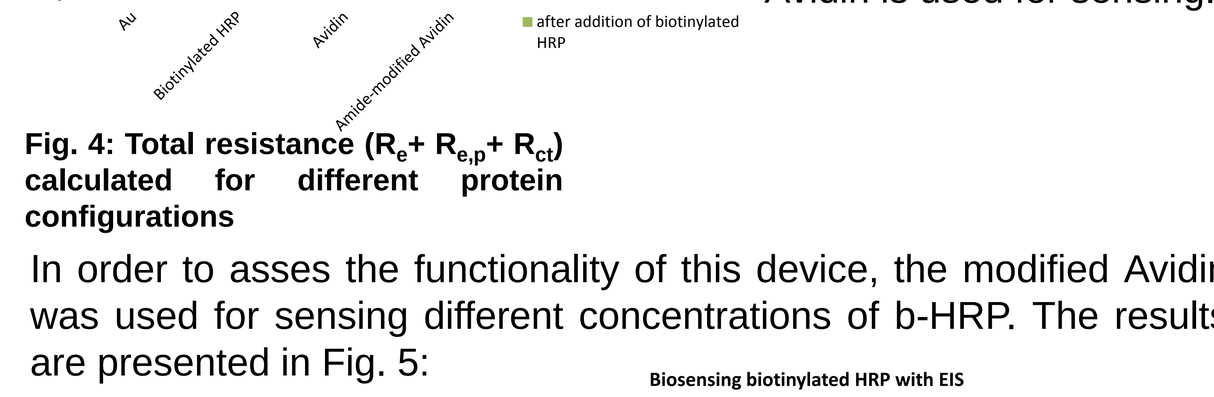


Fig. 5: Biosensing of biotinylated HRP with amide-modified Avidin

4.4 Physical model

The results indicate that EIS is highly dependent on the **charge** at the surface of the electrode. A physical model is presented to explain the detection mechanism:



The oppositely charged molecules attract each other, and charge transfer process is increased (resistance decreases).

When the charge becomes slightly negative, the force becomes repulsive and makes it harder for the redox probe to reach the electrode surface.

6. Conclusions

- **EIS is more sensitive than CV for monitoring of biorecognition events.**
- **Chemical modification of binding proteins can make a significant impact on the functionality of the sensing device with EIS.**
- **Sensitivity to biotinylated HRP concentration is achieved for the modified Avidin as binding molecule, since the change in surface charge after binding is higher in this case than the native Avidin.**

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References

1. Jonathan S. Daniels and **Nader Pourmand,** *Electroanalysis*. 2007 May 16; 19(12): 1239–1257 **2. Noa Cohen-Hadar, Shira Lagziel-Simis, Yariv Wine, Felix Frolow and Amihay Freeman ,** Biotechnology and Bioengineering, Vol. 108, No. 1, 2011
3. M. Stelzle, G. Weissmiiller, and E. Sackman, J. Phys. Chem. 1993,97, 2974-2981