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Detection of Target Molecules Using Synthesized Aptamer-Based Sensors

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Abstract

The objective of this study is to develop an aptasensor that can quantitatively detect specific target molecules. Aptamers are relatively short DNA sequences that have been separated using the general SELEX cycle. The aptamers used in this experimental procedure have been synthesized to bind selectively to the targeted molecule myoglobin. The aptamer was attached to an electrode surface, and results of the cyclic voltammetry and electrochemical impedance spectroscopy experiments conducted will be presented.

Aptamers

Aptamers are artificial single-stranded DNA or RNA sequences that fold into secondary and tertiary structures that bind to certain targets with extreme specificity. The ability to have a high selective affinity gives it a resemblance to chemical antibodies. Aptamers are generally produced using the systematic evolution of ligands by exponential enrichment (SELEX) approach with repeated rounds of in vitro selection. First, the library containing many single-stranded DNA or RNA sequences is incubated with the chemical target of interest to start the first cycle of selection. This is followed by iterative cycles of absorption, recovery of bound DNA or RNA, and amplification (Iliuk, 2011).

Cyclic Voltammetry

The Cyclic voltammetry is an extremely useful technique for analytical measurements. It measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted. Cyclic voltammetry is conducted by cycling the current of a working electrode and measuring the resulting potential (Cyclic Voltammetry Libretexts). When the analyte is added to the solution it is transported to the electrode surface by diffusion. When reduction occurs, current increases until it reaches a peak. Then, all M+ ions are exposed to the surface of an electrode and have been reduced to M. As the concentration of M increases, the distance the M+ ions have to travel also increases. Therefore, the current

continually decreases with time (Cyclic Voltammetry Libretexts).

Electrochemical Impedance Spectroscopy

The electrochemical impedance spectroscopy (EIS) response is very nonlinear. Electrochemical impedance is the response of an electrochemical system to an applied potential from an AC circuit. The frequency dependence of electrochemical impedance can reveal underlying chemical processes that are otherwise hard to detect (Reece, n.d.). The electrochemical impedance produced from the transfer of the molecules from the ground state to the excited state causes an excitation signal to occur that the EIS Gamry instrument program can detect. Unlike ohm's law which calculates the resistance of an electrical system to the potential, impedance is a measure of the ability of a circuit to resist electrical flow without the limitations that are usually found with resistance (Basics of Electrochemical Impedance Spectroscopy, n.d.).

The expression for $Z(\omega)$ is shown as $Z(\omega) = E/I = Zreal + Zimaginary$. Where impedance is $Z(\omega)$, E is the cell potential, and I is the current. If the real part of this equation is plotted on the X-axis and the imaginary part is plotted on the Y-axis of a chart, a "Nyquist Plot" is obtained. In this plot, the Y-axis is in the negatives and each point on the Nyquist Plot is the impedance at one frequency. Another frequently used presentation of EIS results is the Bode Plot. The impedance is plotted with log frequency on the X-axis and both the absolute values of the impedance, expressed as $|Z|=Z0$, and the phase-shift on the Y-axis. The Bode Plot does not show frequency information as the Nyquist plot presents (Basics of Electrochemical Impedance Spectroscopy).

Experimental Procedure

1) Collecting myoglobin signals in solution on a voltammogram with aptamers via cyclic voltammetry.

2) Collecting myoglobin signals with aptamers via electrochemical impedance spectroscopy.

Results

Appendix A.

Behind the Scenes at Bowers

The cyclic voltammetry of the aptamer-labeled gold electrode placed in the myoglobin solution gave no signal responses. Thus, the next step to the experiment was to obtain the electrochemical impedance of the myoglobin solution. EIS gave positive results for the detection of Myoglobin in the buffer solution.

Future Work

Now that the EIS results of the myoglobin showed positive results, the next step is to conduct EIS on the myoglobin solution using varying concentrations.

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Appendix A.

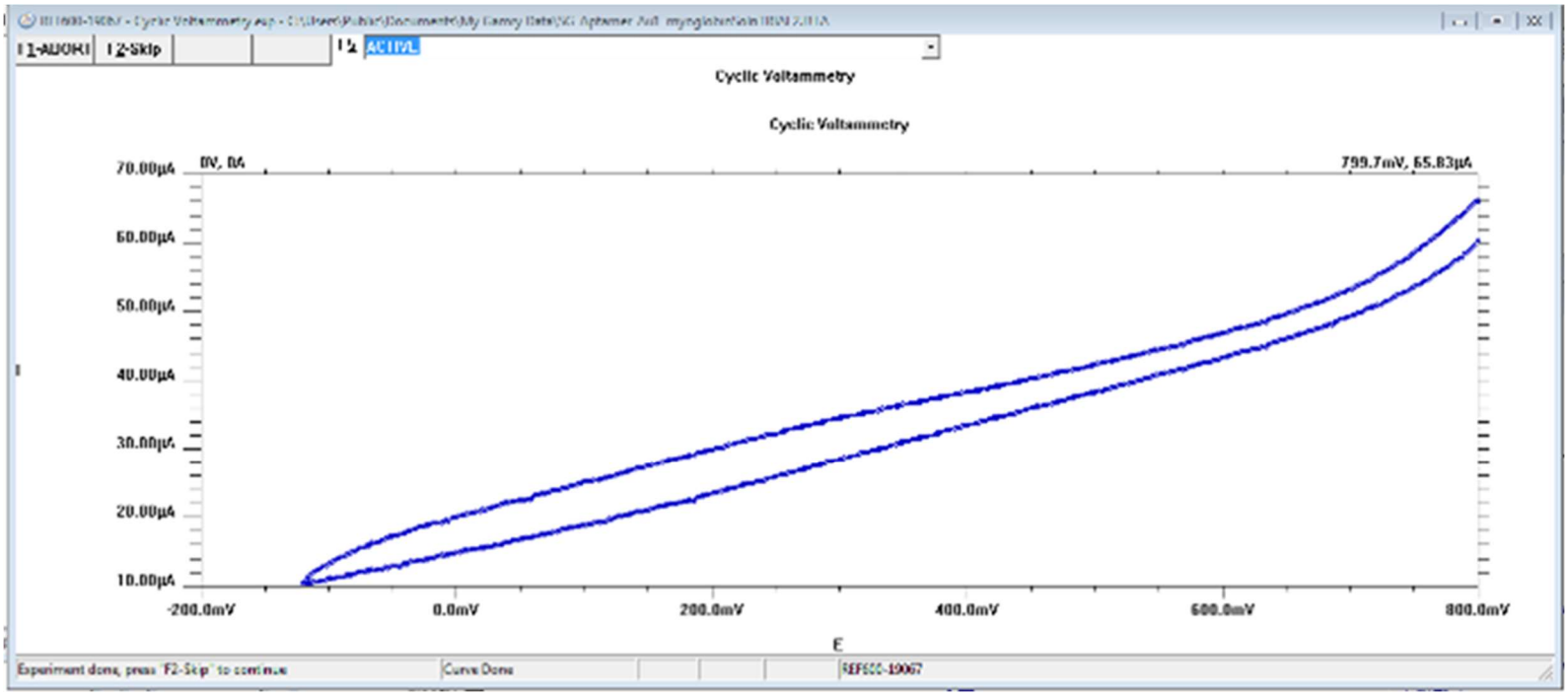

Figure 1. Cyclic voltammogram of the aptamer-labeled gold electrode in the myoglobin solution. There is no apparent signal that has been detected by the Gamry instrument.

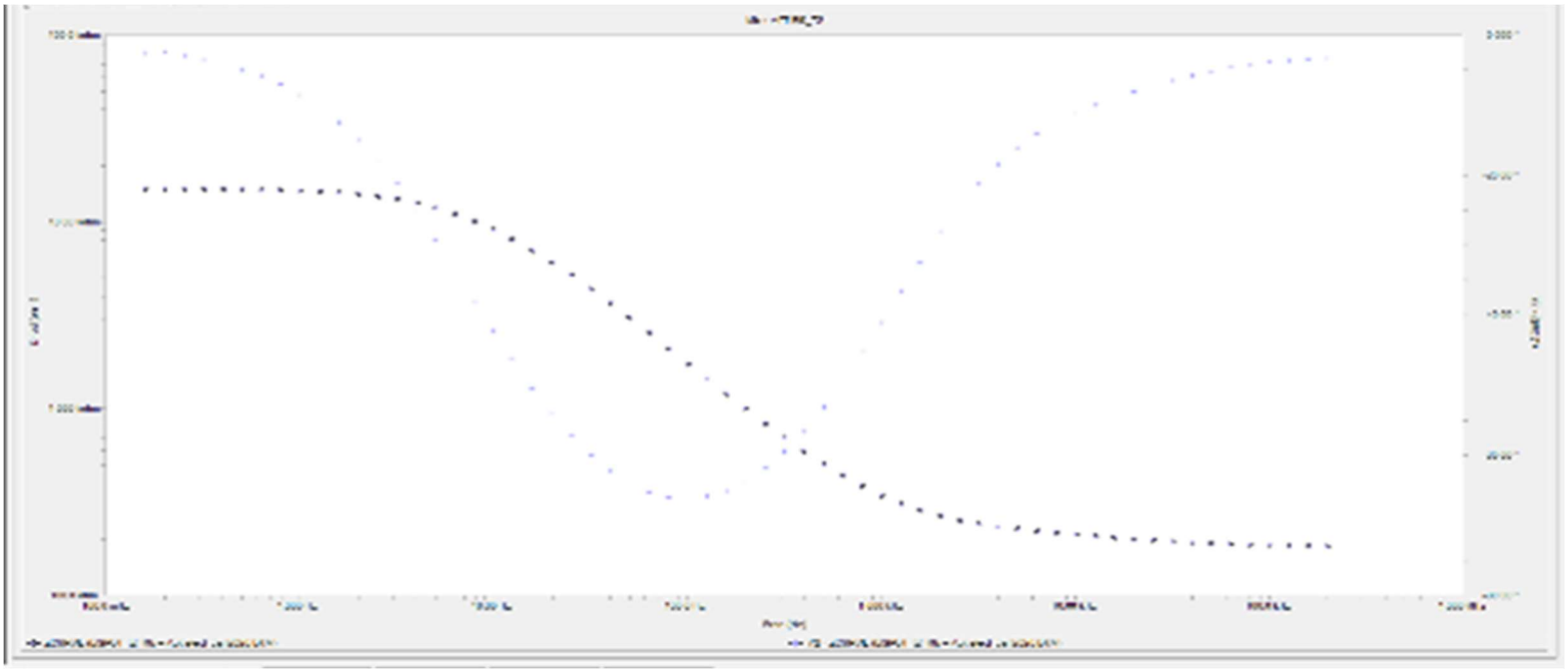

Figure 2. Myoglobin with aptamer-labeled electrode Bode plot. The Gamry instrument was able to detect electrochemical impedance signals from the myoglobin solution.

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Cyclic Voltammetry Libretexts – https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Instrumental_Analysis/Cyclic_Voltammetry

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