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Electrochemical DNA-Based Biosensor for the Detection of DNase I Activity for Potential Application for CRISPR-Cas Based Biosensor

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Electrochemical DNA-based Biosensor for the Detection of DNase I activity with Potential for Application for CRISPR-Cas based Biosensor

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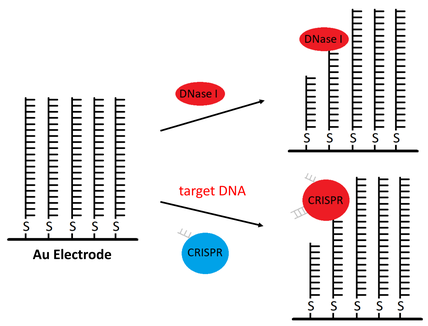
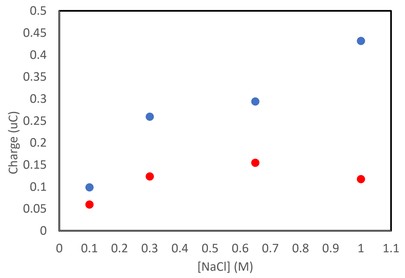
Introduction

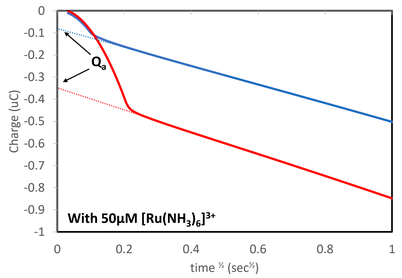
- DNase I activity is an important marker for many diseases.
- Existing assays to measure DNase I activity are expensive and time consuming.
- Electrochemical biosensors are an attract alternative to existing assays.
- Developed biosensor will be tested with DNase I and CRISPR-Cas enzymes. This may result in an easy to use and sensitive platform for the detection of nucleic acids.



DNA Deposition on Electrode Surface



- At higher NaCl concentrations more DNA was deposited on the electrode surface.
- Less electrostatic repulsion between DNA molecules at higher ionic strength resulted in more DNA on the electrode surface.
- Before DNase I, blue, after DNase I, red.
- More DNA deposited is beneficial for DNase I biosensor.



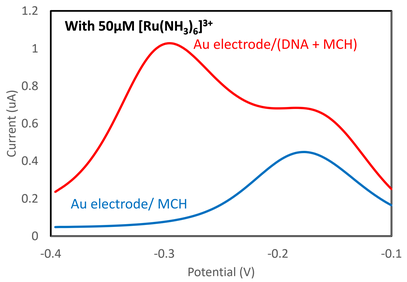
- Blue curve is CC from Au electrode/MCH.
- Red curve is CC from Au electrode/(DNA + Mercaptoheanol).
- Increase in Q_a value indicates successful deposition of DNA on electrode surface.

Electrochemical Methods

The equation for the total charge is shown below (1) and includes the charge generated by the diffusive species, the double-layer charge, and the charge generated by the absorbed species[2]. This allowed for confirmation of the DNA lost to DNase I cleavage in a quantifiable manner.

$$Q = \frac{2nFA\sqrt{D_0}C_0^*}{\sqrt{\pi}}\sqrt{t} + Qdl + Qa \quad (1)$$

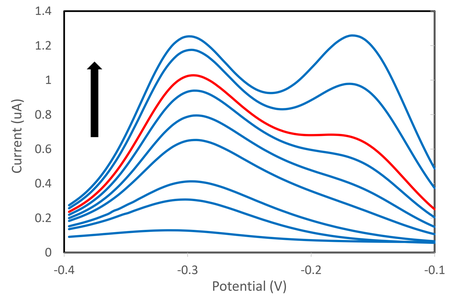
Q- the integrated current, n – the number of the electrons in the redox indicator, F - the Faraday's constant, A - the area of the electrode, D_0 – the diffusion coefficient, C_0^* – the bulk concentration, $\sqrt{t}$ - the slope, Q_{dl} – the charge of the double layer, Q_a – the charge of the absorbed species.



- The sweeping potential allows resolution of adsorbed and diffusive species with almost no contribution of the double-layer capacitance.
- Method is very sensitive and easy to analyze data.

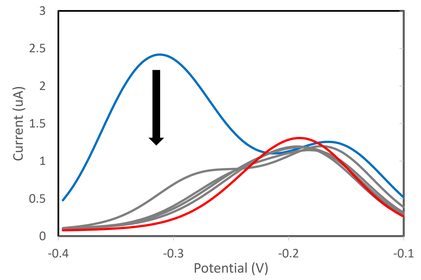
Optimization of the Biosensor with Non-Covalent Redox Tag

$[[Ru(NH_3)_6]^{3+}]$ Dependance



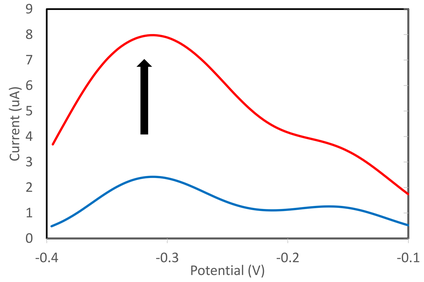
- $[Ru(NH_3)_6]^{3+}$ concentrations were varied from 5uM to 100uM.
- 50uM $[Ru(NH_3)_6]^{3+}$ chosen for further experiments.

$[Ru(NH_3)_6]^{3+}$ is Bound Electrostatically



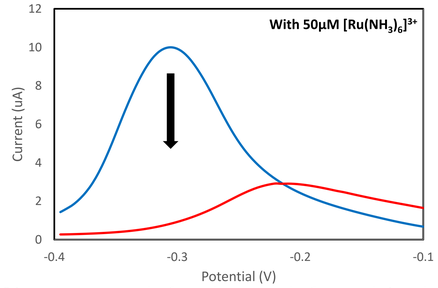
- NaCl concentration was varied from 0 to 50mM.
- Addition of NaCl decreased the peak of absorbed $[Ru(NH_3)_6]^{3+}$ indicating that the redox tag is bound due to electrostatic interactions.
- Low ionic strength buffer was chosen for further experiments.

SWV Parameter Optimization



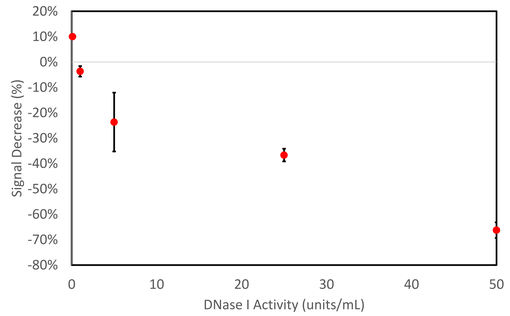
- Changing SWV parameters such as step, amplitude, and frequency allowed increase of the signal.
- Blue curve, old parameters, and red curve, new parameters.

DNase I Cleaves the DNA on the Electrode



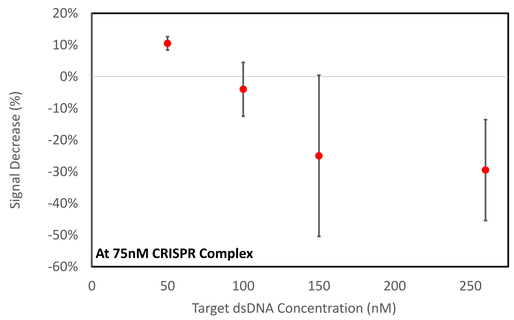
- SWV peak currents before, blue, and after, red, incubation with DNase I.
- Difference before and after used to solve for calibration curve.

Biosensor Signal vs. DNase I Activity



- The biosensor signal depends on the concentration of DNase I.
- Detection limit is about 0.1units/mL.
- The calibration curve is in progress.

Biosensor Signal vs. CRISPR target DNA



- The biosensor signal depends on the concentration of target DNA to activate nonspecific trans nuclease activity.
- The calibration curve is in progress.

Conclusions

- ✓ In this work, the interaction of $[Ru(NH_3)_6]^{3+}$ with DNA electrode was studied using CC and SWV. The latter method revealed the shift of standard potential when $[Ru(NH_3)_6]^{3+}$ is bound to DNA as well as the electrostatic nature of this interaction.
- ✓ A sensitive and easy to use DNase I biosensor was developed and utilizes $[Ru(NH_3)_6]^{3+}$ as a non-covalently bound redox tag.
- ✓ The detection principle was used in combination with CRISPR-Cas12a to measure HPV virus DNA and has a potential for CRISPR-Cas based biosensors for nucleic acid detection.

References

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