Optimization of gold nanostructured aptasensors for a multielectrode-array

Olivia Stuehr, Jamma Lucky, Amanda Ritz, Debashis Sen, Nicholas Volya, Robert A. Lazenby

Lazenby Lab @FSU

FLORIDA STATE UNIVERSITY

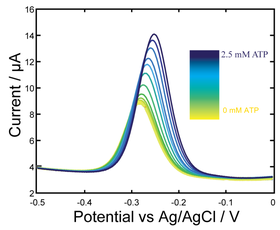
Department of Chemistry & Biochemistry

Electrochemical, Aptamer-Based Sensing

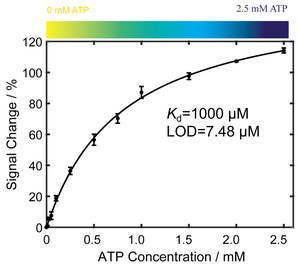
Why EAB Sensing?

- Reagentless
- Reversible analytical detection
- Highly specific
- Gives rapid results in real time

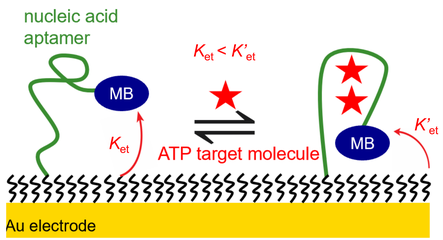
Square wave voltammograms (SWVs) for detecting ATP



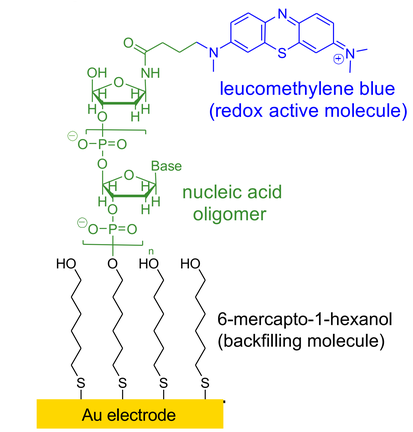
Langmuir isotherm calibration curve for quantifying sensor performance



Schematic of analyte detection



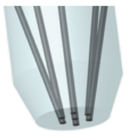
Components of an EAB sensor



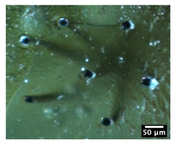
Project Aims

- To fabricate a **microelectrode array** for the simultaneous detection of multiple analytes using E-AB sensor platform and selective aptamer modification.

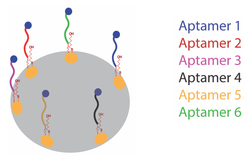
Schematic of anticipated sealed device



Surface of Pt array after polishing

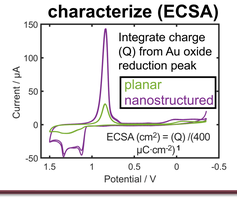


Schematic of Electrode Surface with multiple aptamers

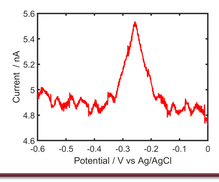


- To better understand the effects of **gold nanostructured microelectrodes** on EAB sensor performance. Once understood, these results can be applied to a microelectrode array.

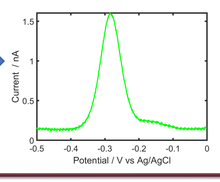
CV in 0.05 M H_2SO_4 to characterize (ECSA)



unmodified



nanostructured



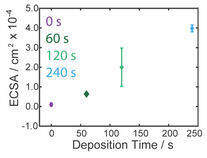
References

[1]Liu, Y.; Canoura, J.; Alkhamis, O.; Xiao, Y. *ACS Appl. Mater. Interfaces* **2021**, *13* (8), 9491–9499.

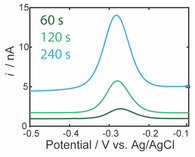
Nanostructured Microscale EAB Sensors

Surface area enhancement via pulsed waveform electrodeposition

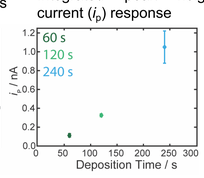
Electrochemical Surface Area



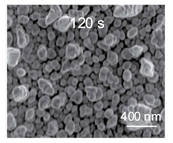
Dopamine detecting SWVs



Integrated peak height current (i_p) response

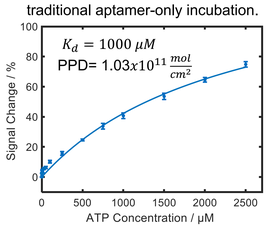


SEM Image of gold nanostructures on the UME surface

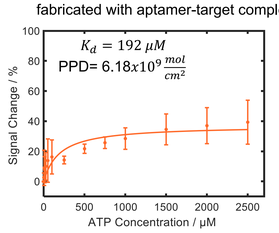


Aptamer-only vs Aptamer-target complex immobilization

EAB sensor titration of ATP with traditional aptamer-only incubation.



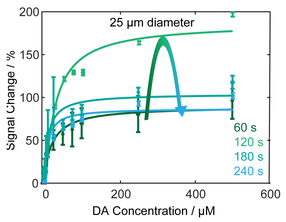
EAB sensor titration of ATP with fabricated with aptamer-target complex



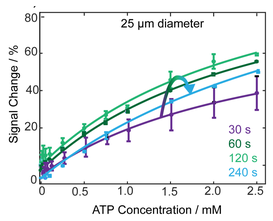
Aptamer-target immobilization[1] entails functionalizing a pristine electrode with target-bound aptamer probes. This led to a lower aptamer packing density, and decreased signal changes observed on the microscale. This decrease in signal change contradicts the trend reported in the literature[1].

Evaluation of nanostructured microscale EAB sensor performance

DA detecting EAB sensors



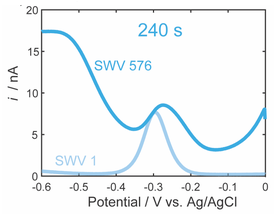
ATP detecting EAB sensors



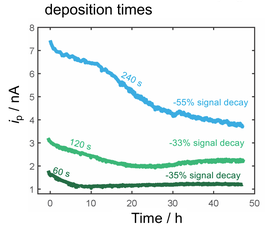
Increasing the deposition time initially increased then decreased the signal change for both DA and ATP detecting microscale sensors. The highest signal change is seen with 120 second deposition for both target analytes.

Effects of gold deposition on microscale EAB sensor signal decay

Comparing square wave voltammetry between 1st run and 578th run on a 240s deposited sensor



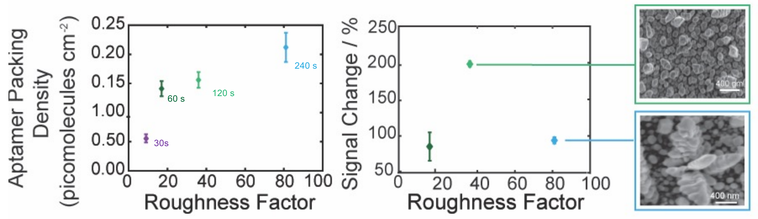
Comparing signal decay between sensors with varying deposition times



Microscale sensors' stability can be evaluated by interrogating the sensors in PBS every 5 minutes for 48 hours. High signal decay and poor stability indicate an unstable monolayer on the microscale EAB sensor surface.

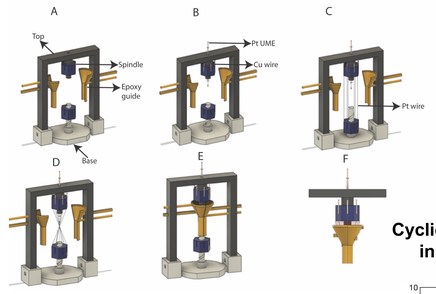
Effects of gold deposition on aptamer probe packing density of the microscale EAB sensor surface

As deposition time increases there is a general increase in aptamer packing density. However, there is a optimal aptamer packing density that gives the highest sensor performance.



Microelectrode Arrays

Fabrication of UME Array via 3D Printed Molds



Array fabrication process via 3D-Print:
A: 3D-printed assembly, B: Assembly with central Pt UME and Cu leads in place, C: Assembly with Pt wire soldered to Cu leads, D: Assembly with Pt wires anchored to base by epoxy, E: Closed assembly, F: Externally sealed array to be internally sealed with epoxy

Expected Limiting Current: I_{lim}= *4nFDac**

$$I_{lim} = 6.2725 * 10^{-9}\ nA$$

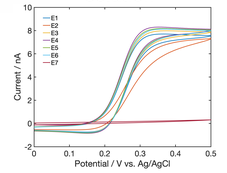
Why 3D PRINT ARRAY?

Allows even spacing of 4-7 Pt wires

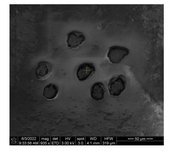
Result in electrodes with well behaved voltammetry

Offer platform for sensing multiple biomolecules simultaneously

Cyclic voltammogram (CV) in 2mM Ferricyanide solution



SEM Image of Array Surface



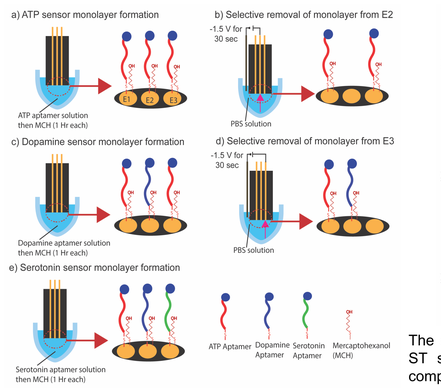
The CV of individual electrodes recorded in ferricyanide solution show well behaved CV with expected limiting current.

Challenges: Reproducible fabrication of array with even inter-electrode spacing and better connectivity of the individual electrodes within the array

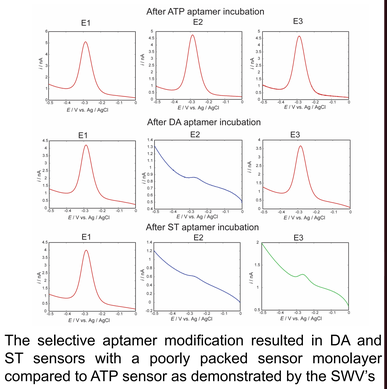
Selective aptamer modifications in the microelectrode array for simultaneous detection of multiple analytes

After the fabrication of the array, individual electrodes within the array can be selectively modified with different aptamers. This will allow the array to be used as a multiplex biosensing platform.

Schematic of selective aptamer modification



SWVs of the fabricated sensors throughout selective aptamer modification process



The selective aptamer modification resulted in DA and ST sensors with a poorly packed sensor monolayer compared to ATP sensor as demonstrated by the SWV's

Future Work

- To fabricate electrode arrays characterized by precise inter-electrode spacing and robust connection of the individual electrodes within the array by adjusting certain fabrication parameters.
- To repeat more experiments on the selective aptamer modification method using the fabricated array to have sensor with stable monolayer.
- To carry out the simultaneous detection of ATP, DA and ST using the array.

Acknowledgements

FSU Startup Funds

Lab website:
https://www2.chem.fsu.edu/~lazenby/