Characterization of Electrochemical Aptamer-Based Sensors in Complex Media

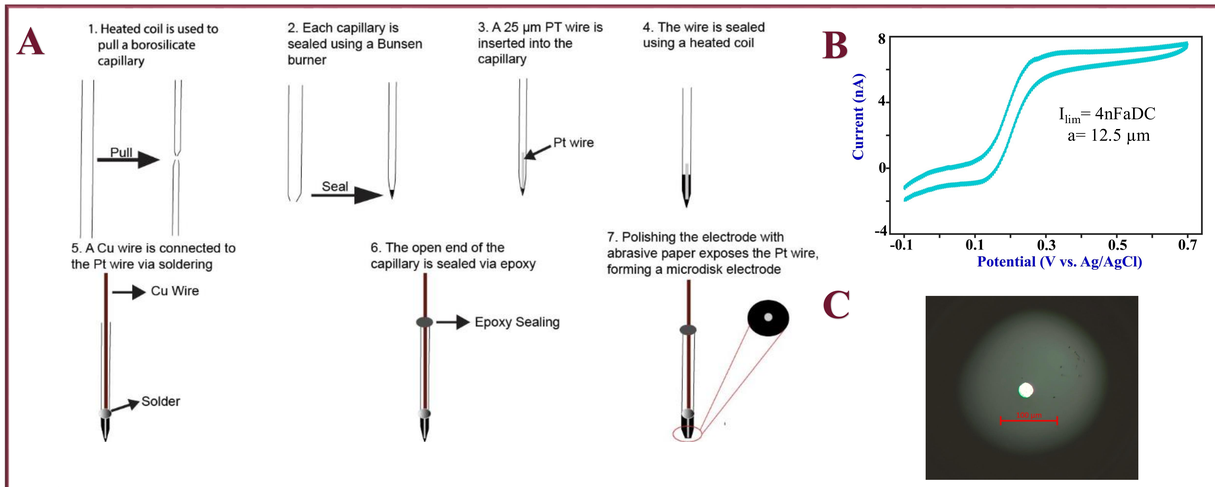
Brett Jung, Robbie Huff, Debashis Sen, Robert A. Lazenby

Department of Chemistry & Biochemistry

Introduction

- Electrochemical biosensors enable rapid, sensitive, and selective detection of biomolecules. Dopamine and glucose biosensors were fabricated to quantify analytes relevant to neurological disorders and diabetes.
- Project aims:
  - Fabricate Pt microelectrodes.
  - Fabricate dopamine and glucose E-AB sensors.
  - Characterize dopamine and glucose sensors.

Microelectrode Fabrication



- **Figure A.** Microelectrode fabrication.
- **Figure B.** Cyclic voltammogram of the bare Pt microelectrode before Au deposition, demonstrating baseline electrochemical response. This Pt microelectrode features an optimal peak current of approximately 7 nA.
- **Figure C.** Optical image of the polished Pt microelectrode tip. The 100 µm scale bar indicates the micrometer-scale geometry and confirms defined microdisk exposure.

Introduction to E-AB Sensors

- Our lab focuses on sensors that contain aptamers which are single stranded DNA/RNA molecules.
- Sensor components: aptamer, thiol linker, mercaptohexanol, redox molecule, and Au coated Pt electrode.

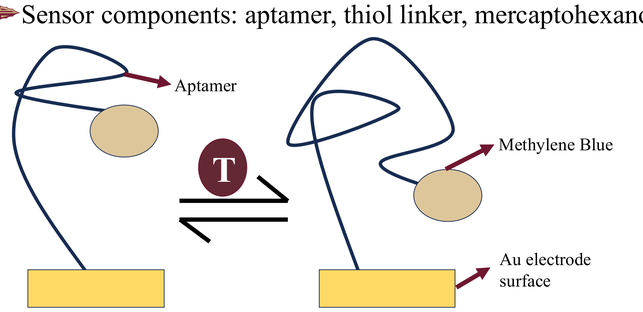


Figure D. E-AB sensing mechanism. The aptamer is immobilized on a gold electrode surface through a thiol linker and is labeled with a redox reporter. In the absence of target, the aptamer adopts a conformation that positions the redox molecule at a defined distance from the electrode. Upon target binding (T), the aptamer undergoes a conformational change that alters the distance and electron transfer efficiency between methylene blue and the gold surface. This structural rearrangement produces a measurable change in electrochemical signal.

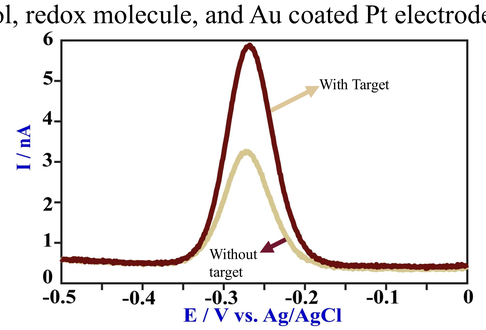
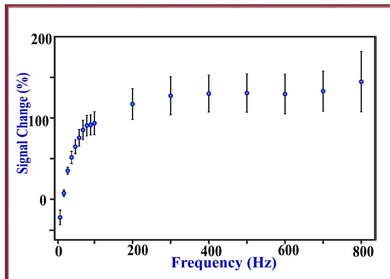
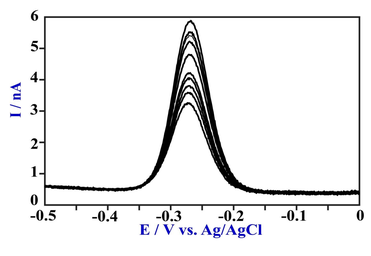


Figure E. Illustration of the electrochemical response with and without target binding. In the absence of target, the baseline current reflects the resting aptamer conformation and electron transfer rate. Upon target binding, the conformational change enhances electron transfer of the redox reporter, resulting in an increased current signal. This mechanism underlies the frequency sweeps, voltammograms, and calibration curves presented in this work, where signal magnitude directly correlates with analyte concentration.

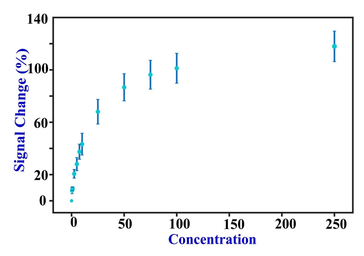
Dopamine and Glucose Sensor Characterization in PBS



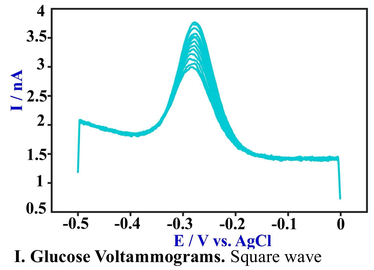
F. Dopamine Frequency Sweep. This graph represents a frequency sweep illustrating the optimal percent signal change for a saturated 100 µM dopamine solution in **Phosphate Buffer Solution(PBS)**.



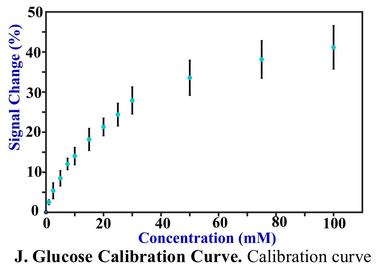
G. Dopamine Voltammograms. Square wave voltammograms demonstrating concentration-dependent signal enhancement for dopamine, consistent with direct redox-mediated electron transfer upon target binding.



H. Dopamine Calibration Curve. Calibration curve showing concentration-dependent signal increase followed by saturation, indicative of binding-limited E-AB sensor behavior



I. Glucose Voltammograms. Square wave voltammograms demonstrating concentration-dependent signal enhancement for glucose, reflecting analyte-induced modulation of the electrochemical interface



J. Glucose Calibration Curve. Calibration curve showing concentration-dependent signal increase with saturation behavior, consistent with E-AB sensing dynamics for glucose detection.

Data Collection in Complex Media

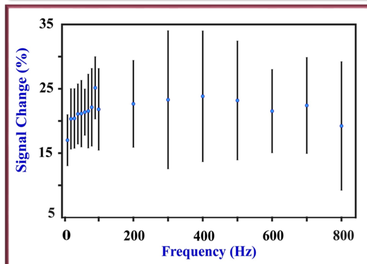


Figure K. Glucose Frequency Sweep in Cell Culture Media. Frequency sweep for saturated 1000 µM in **DMEM.**

- Frequency sweep in Dulbecco's Modified Eagle Medium (DMEM) produced a lower overall signal change than PBS. It also showed greater noise and larger variability across frequencies.
- The reduced signal magnitude likely arises from matrix effects associated with proteins, salts, and other biomolecules present in the media.
- Matrix components in complex media can disrupt aptamer-target binding and hinder electron transfer at the electrode surface.
- Nonspecific adsorption and partial surface fouling likely contribute to the increased noise and reduced measurement stability observed in complex media.
- The increased variability in DMEM makes it more difficult to identify a clear optimal operating frequency.
- Results indicate that sensor performance is more robust in PBS than in complex biological media.

Conclusion and Future Work

- Fabricated functional Au-modified Pt E-AB microsensors.
- Dopamine (µM) and glucose (mM) detected with saturating calibration behavior.
- Frequency response confirms aptamer-analyte interaction.
- Future work will assess matrix effects by comparing frequency response and calibration behavior in PBS versus cell media.

References

- Sen, D., & Lazenby, R. A. (2023a). Electrochemical biosensor arrays for multiple analyte detection. *Electrochemical Biosensor Arrays for Multiple Analyte Detection*, *4*(2). https://doi.org/10.1002/anse.202300047
- Sen, D., & Lazenby, R. A. (2023b). Selective aptamer modification of au surfaces in a microelectrode sensor array for simultaneous detection of multiple analytes. *Analytical Chemistry*, *95*(17), 6828–6835. https://doi.org/10.1021/acs.analchem.2c05335

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