UMSAEP (UM–UWC) ACADEMIC EXCHANGE PROGRAM PROJECT REPORT 2024

Proposed Project: Nanorod functionalized aptasensor array for the simultaneous determination of interferon-gamma and tumor necrosis factor alpha tuberculosis biomarkers.

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UM Host: Professor Xiyun Richard Guan
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Period of Visit: April 1, 2025 - June 30, 2025

Proposed project aims and objectives

The original project aim was to develop an aptasensor electrode array capable of simultaneously detecting IFN-γ and TNF-α. The electrode array will be modified with novel MSA-capped $GeCeO_2$ nanorods (NRs). The NRs will allow for fast charge transfer and provide large surface area for the attachment of the respective aptamers.
The original project objectives were as follows:

- To use the electrode array to develop, characterise, and optimise the MSA-$GeCeO_2$ NRs based electrochemical aptasensor array using electrochemical techniques.
- Utilize the MSA-$GeCeO_2$ NRs functionalized aptasensor array to detect IFN-γ and TNF-α in buffer using square wave voltammetry (SWV) and chronoamperometry (CA) and recording the calibration profile for each electrochemical technique.
- Studying the stability, selectivity (interference studies) and reproducibility of the aptasensor array.
- Use the aptasensor array to detect IFN-γ and TNF-α in real samples such as serum, plasma and whole blood.
- Comparison of the performance of the aptasensor array with a commercial assay such as ELISA.

Adjustments to Project Scope

Upon initiating the research at the host lab (Prof. Xiyun Guan's group), several strategic adjustments were made:

- The target biomarkers were changed from IFN-γ and TNF-α to ESAT-6 and MPT64, due to project alignment and reagent availability.

- The nanomaterial was changed from the initially proposed MSA-$GeCeO_2$ NRs to MSA-CeO_2, after electrochemical characterization revealed significantly better conductivity behavior of MSA-CeO_2 for biosensing applications.
- The planned simultaneous detection of two TB disease biomarkers was modified to the detection of only ESAT-6, due to the unavailability of multi-channel potentiostat.

Aptasensor Development and Experimental Challenges

The ESAT-6 aptamer used in this study was labeled with ferrocene (Fc) as a redox marker, with the original intent of using its redox peak for detection via SWV. However, characterization of the aptasensor did not reveal the ferrocene redox signal, despite multiple troubleshooting strategies such as:

- Optimization of EDC/NHS coupling ratios to enhance aptamer immobilization.
- Thermal treatment (heat/freeze) of aptamer prior to immobilization to potentially expose the Fc tag.
- UV–Vis spectroscopy, which confirmed the DNA peak at 260 nm but showed no Fc absorbance, suggesting either low concentration or tag degradation.

The same issues occurred when testing the MPT64 aptamer labeled with anthraquinone, suggesting a broader issue with redox label visibility, potentially due to label detachment or internalization during aptamer folding. To verify aptamer immobilization, the aptasensors were tested using external redox probe (ferricyanide). These results confirmed successful aptamer attachment to the surface, leading to the decision to pursue external redox-based detection.

Equipment Breakdown and Method Adaptation

Progress was temporarily halted when the potentiostat broke down. Prof. Guan arranged an alternative potentiostat; however, it lacked SWV functionality. Consequently, the detection method was switched to chronocoulometry (CC), the only viable option available. This required recharacterization of the nanomaterial and aptasensor system, and repetition of several experiments originally done with SWV.

Aptasensor Optimization and Final Detection Results

Using chronocoulometry, I optimized:

- Aptamer concentration
- Aptamer incubation time
- Blocking conditions (BSA concentration, with/without ethanolamine)

Initial detection trials followed a traditional approach: drop casting individual concentrations of ESAT-6 on separate aptasensors, followed by incubation and CC measurement. This approach yielded inconsistent calibration profiles, likely due to inter-electrode variability and nonuniform target interaction. To resolve this, I implemented an in-solution detection strategy, where increasing concentrations of ESAT-6 were spiked directly into the electrolyte (PBS containing $KFe(CN)_6^{4-/3-}$)

containing the aptasensor. This approach yielded linear detection profiles with strong correlation coefficient values, confirming that the aptasensor was sensitive to ESAT-6 binding under optimized conditions.

Summary of Achievements

Despite several challenges and experimental redesigns, the following were successfully achieved:

- Developed and characterized an MSA-CeO_2-based aptasensor for ESAT-6 detection.
- Overcame limitations with redox-labeled aptamer detection by switching to external redox probe (ferricyanide) methodology.
- Adapted the detection technique from SWV to chronocoulometry due to equipment constraints.
- Optimized aptasensor parameters (aptamer concentration, incubation time, blocking).
- Demonstrated reproducible and linear detection of ESAT-6.
- Although the initial goal of dual biomarker detection and real sample analysis could not be achieved during the limited visit window, this project laid the groundwork for a functional aptasensor platform that will be further developed and validated at my home institution.

Benefits of the Visit and Project

This international research visit marked my first time in the United States, providing invaluable international exposure that significantly enhanced my research skills, professional development, and global perspective. Working in Prof. Xiyun Guan's laboratory allowed me to gain hands-on experience with advanced electrochemical techniques and adapt to new experimental challenges, such as instrumentation changes and method redesign. These experiences strengthened my problem-solving abilities and technical confidence. I also built collaborative relationships with fellow researchers, initiating connections that may lead to future joint publications, mentorship or research exchanges. Beyond the laboratory, the visit was personally enriching, I had the opportunity to explore diverse destinations such as St. Louis, Kansas City, Chicago, and the scenic Lake of the Ozarks. New friendships were formed, and the cultural exchange and camaraderie I experienced made the visit both memorable and transformative. Overall, the visit laid a strong foundation for future international collaboration and continued research progress upon returning to my home institution.

Impact of Visit and Planned Outputs

The research visit had a significant impact on the progression of my project and overall research development. Despite encountering unexpected technical and methodological challenges, I was able to develop, optimize, and validate a novel chronocoulometry-based aptasensor platform for the detection of ESAT-6. The visit served as a foundation for this work and provided critical preliminary data. I plan to continue the remaining aspects of the project at my home institution, including additional validation studies and target detection under real-sample conditions. The

final goal is to prepare and submit a manuscript based on the work initiated during the visit.

Acknowledgement
I would like to express my sincere gratitude to Prof. Uphoff and the entire UMSAEP committee for granting me this opportunity. I am especially thankful to my supervisor, Prof. Emmanuel Iwuoha, for his kind consideration, guidance, and encouragement, which made this visit possible. I also wish to thank Prof. Xiyun Guan, my research host at the University of Missouri Columbia, for his scientific insights, and generous support during my time in his laboratory. I am truly grateful to Prof. Uphoff and Judy for organizing all logistics related to my stay and ensuring everything ran as smoothly as possible. I would also like to extend heartfelt appreciation to Prof. Guan's students; Shuo, Sathish, Alisa, Rana, Yuan, and Anudha, for their valuable assistance in the lab, technical support, and warm friendship. Their presence created a collaborative and enjoyable working environment, and they played a meaningful role in making my stay both productive and memorable. Finally, I acknowledge with gratitude the broader UMSAEP initiative for fostering research collaboration and capacity-building, and for giving South African scholars the chance to grow through international exposure and academic exchange.

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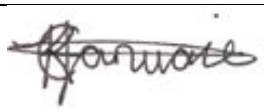
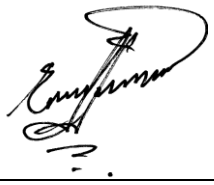
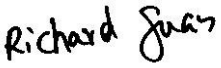
Dr. Kaylin Januarie: 	21 July 2025
Prof Emmanuel Iwuoha: 	21 July 2025
Prof Xiyun Richard Guan: 	23 July 2025