Electrochemical DNA Biosensors for Detecting Nontuberculous Mycobacteria (NTM).

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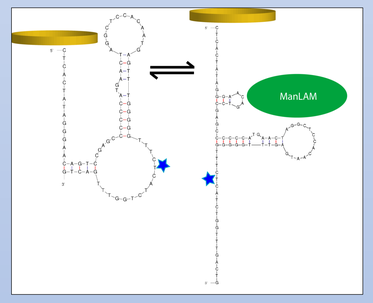
Introduction E-DNA Biosensors

Mycobacterial infection and mortality rates remain high; an estimated 10 million cases of Tuberculosis emerge each year. However, less studied Mycobacterium species are becoming a growing concern: species of nontuberculous Mycobacterium (NTM). Over the last 30 years, NTM-based lung disease cases have grown to outnumber TB infections in many regions. [1] The "gold standard" for NTM diagnosis involves a weeks-to-months-long microbiological culture-based method. [2] Here, we are developing a novel electrochemical DNA (E-DNA) biosensor to detect NTM for a more efficient diagnostic tool than the current, time-intensive methods.

Previously, we have investigated Mannosylated lipoarabinomannan (ManLAM) as a molecular target to use against our sensor. However, to increase the efficacy of our biosensor, we are simultaneously pursuing a new target: glycopeptidolipids (GPLs). GPLs are absent from TB and may consequently increase the specificity of the sensor. The biosensor will contain an electrode-bound DNA aptamer that is thiol-bounded to the surface of a gold-plated electrode. We are developing an aptamer that changes conformational shape upon binding to its molecular target (GPL) leading to a subsequent change in proximity of the redox moiety (methylene blue) with the electrode surface. The expected outcome of this project is to develop an E-DNA GPL-based sensor that could be used in the collection of environmental NTM samples as well as to create a rapid, point-of-care diagnostic tool to improve outcomes of patients suffering from NTM infection.

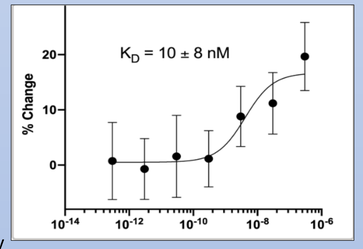
Previous Work (ManLAM E-DNA Biosensor)

E-DNA-based biosensors have previously shown reliable detection of a myriad of target proteins/drugs down to picomolar concentrations.[3] These sensors hold great promise in efficient point-of-care (POC) clinical diagnostics. Previously, we have developed an E-DNA biosensor using an ssDNA aptamer sequence that changes conformational shape upon binding to the target ManLAM.



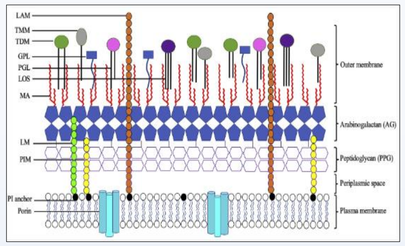
(upper-left) Schematic of the final version of our previous biosensor showing methylene blue moiety (blue star) attached to internal thymidine. Shows isoenergetic conformational states: (left) non-binding and (right) binding-capable state.

Dose-response curve of our previous biosensor for ManLAM (bottom right).

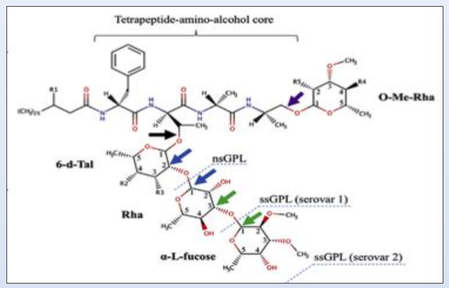


Glycopeptidolipids (GPLs)

GPLs are a glycosylated molecule with a tetrapeptide core abundant in many pathogenic NTM species. [4]



(top) Typical extracellular matrix of a mycobacterial cell wall. Of note, LAM molecules are shown as orange circles, and GPL is shown as blue rectangles. (bottom) Molecular structure of GPL. Used with permission.[4]

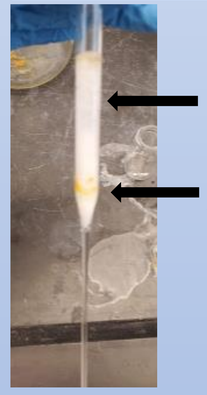


Note: GPL structure varies amongst NTM species. The magnitude of glycosylation from various hexose sugars onto the 6-d-Tal residue results in multiple serovar specific classifications .[5]

Why GPL?

Our lab is in the process of optimizing the ManLAM biosensor; however, engineering a novel E-DNA biosensor for the detection of GPL molecules would increase selectivity of our diagnostic tool. Having a GPL-based biosensor would enable detection below the genus level (i.e. *Mycobacterium avium* Complex – MAC*)* due to a variety of serovar chemistry. Additionally, our lab has reported numerous biosensors using peptide-based biomarkers; as GPL has a peptide backbone, it makes for an ideal target.

Methods & Techniques



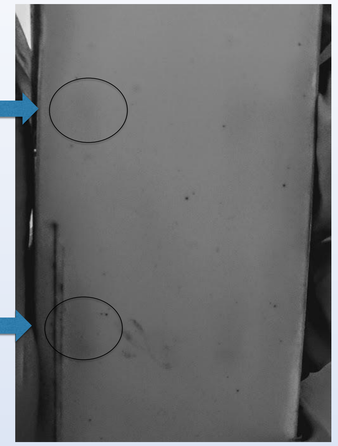
(top) Silica column used to purify GPL from lipid extracts.

- Silica column chromatography was used to purify GPL from total lipid extracts of NTM species isolated from clinical samples (obtained from Dr. Jennifer Honda, National Jewish Health).
- Confirmation was done with thin-layer chromatography (TLC) using silica gel 60 plates after testing several solvents.
- Systematic Evolution of Ligands by Exponential Enrichment (SELEX) will be done with GPL isolates to create an aptamer for incorporation into biosensor.

Results and Challenges

- While unable to successfully purify GPL as of yet, TLC plates have shown separation of resuspended lipid extracts.

- Initial results (left) from TLC plates did not show significant GPL identification when using a Chloroform, Methanol and H20 solution (30:8:1).
- After overnight charring with 10% cupric sulfate & 8% phosphoric acid, bands in the TLC plate became apparent.



(right) TLC plate ran on *M.tb* (H37Rv) lipid extract suspended with 2% methanol in chloroform. Blue arrows indicate separation of lipomannans and other cell wall constituents.

Further Directions

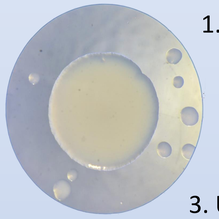


Image of NTM culture (2x).[6]

1. Finish purification of GPL from the total lipid extracts of clinical samples.
2. Run SELEX to form an aptamer sequence for biosensor incorporation.
3. Use Square Wave Voltammetry to analyze and optimize biosensor response to GPL in buffer conditions.
4. Run trials against live microbes as well as serum infected with NTM, through collaboration with Dr. Jenn Honda at National Jewish Health.

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Acknowledgements

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