Detection of Mannose-capped Lipoarabinomannan among Mycobacterial Pathogens via Diagnostic Electrochemical Biosensor

Tyler Sodia, Dylan Poch, Andrew J. Bonham

Metropolitan State University of Denver, Department of Chemistry & Biochemistry

METROPOLITAN STATE UNIVERSITY OF DENVER
Department of Chemistry and Biochemistry

## Introduction to ManLAM & *Mycobacteria*

Mycobacterium tuberculosis (M.tb) is one of the world's most prevalent bacterial pathogens. However, Nontuberculous Mycobacterium (NTM) are less recognized, yet just as impactful as *M.tb* while remaining a difficult public health risk. While there are over 140 NTM species identified, not all are pathogenic and those that are, tend to be opportunistic. Often, NTM disease is confused with that of a tubercle infection leaving diagnosis semi-unreliable. Additionally, the time spent to fully diagnose a mycobacterial disease is extensive and dependent on the species growth rate due to the necessity of culture. The duration of culturing and obtaining an isolate colony of NTM is highly variable. Furthermore, in 2014 the estimated expense for treatment and diagnosis of NTM was approaching $1.7 billion. Thus, designing a sensitive electrochemical DNA-based (E-DNA) biosensor for a diagnostic tool for NTM would dramatically decrease the timeline of diagnosis and therefore improve patient outcomes all while being much more cost effective. One possible avenue for improved detection lies in the cell envelope of various NTM species, which includes many complex glycolipids and glycopeptidolipids. Mannose-capped lipoarabinomannan (ManLAM) is one of the most prevalent of the glycolipids and presents as a novel biomarker for the sensitive detection of various NTM strains. The purpose of this research is to design and develop an electrochemical biosensor that is equipped to detect ManLAM in biological fluids at a very early stage of infection.

Introduction to Biosensors and Our Aptamer

Electrochemical DNA-based biosensors have been used to detect various biological molecules and analytes. An aptamer – a single stranded DNA oligonucleotide that binds a given target – is often incorporated as a key aspect in the sensing mechanism. Our sensors are optimized to undergo a conformational change upon interaction with their given target which is translated into a change in electrochemical current by way of an appended redox-active tag. The aptamer used here, BM2, was developed through Systematic evolution of ligands by exponential enrichment (SELEX)(1). Using a RNA folding prediction tool, *Quikfold* (2), a modified aptamer that fit the necessary confirmation "switch" was designed as a functional biosensor. Such a biosensor may ultimately allow rapid, on site diagnosis of Mycobacterial infection within the time constraints of patient-doctor interaction.
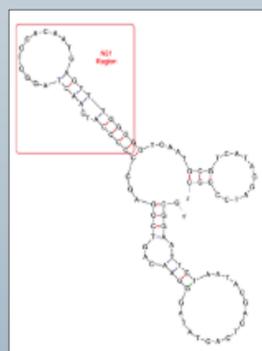
Predicted structure of the BM2 aptamer (1). N31 Region was the structure that was predicted to be essential in binding to ManLAM.
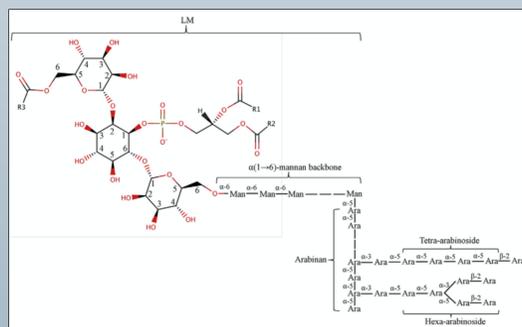
LAM "backbone". Variants in LAM structure are dependent on the nature of their caps linked to the tetra/hexa-arabinoside. Used with permission (3).

Methods & Techniques

- Quikfold, a free online computer software, aided in determination of the aptamer biosensor structure and design. Created several truncations of BM2 aptamer (i.e., full length, medium length, etc.) and ran electromobility shift assays (EMSA) to detect binding.
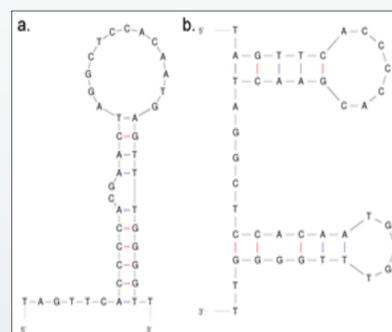
Quickfold predication of the first version of aptamer (final version below). (left) Predicted On state of the first sensor. (right) Predicted Off state of the first sensor.

- Fluorescence anisotropy was used to determine the binding affinity of our aptamer to target ManLAM.

- A gold electrode biosensor was prepared through electrochemical cleaning followed by biosensor attachment and a backfill of mercaptohexanol for passivation.

- Square Wave Voltammetry measures the electrochemical current produced from our ssDNA aptamer binding to ManLAM. This produces a change in the aptamers conformational state, favoring either the on or off conformation.

(bottom) Example Square Wave voltammogram produced by appended MB-aptamer to the gold electrode.

Square Wave Voltammogram
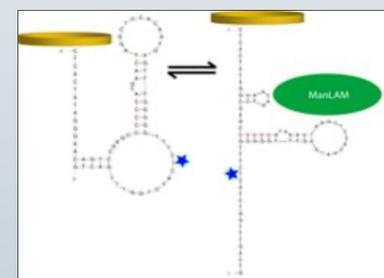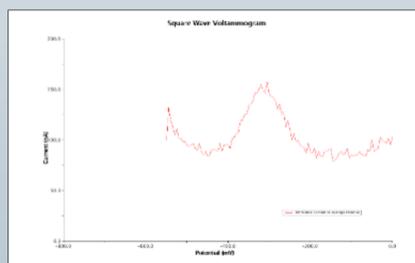
(top) Schematic of final version of the biosensor with 5'-thiol linkage to gold electrode and methylene blue moiety (blue star) attached to internal thymidine. (left) non-binding state and (right) binding-capable state with green ManLAM predicted binding site.

Results

- Early EMSA attempts did not show evidence of ManLAM binding due the lipidated nature of ManLAM and complications with optimizing buffer conditions and fluorescent indicators.
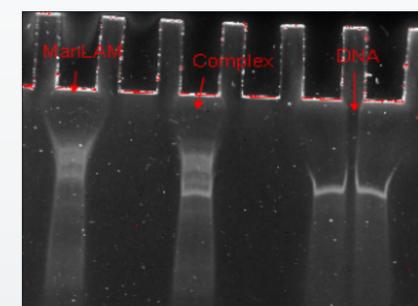
- EMSA stained with SYBR Green. ManLAM was stained (top).
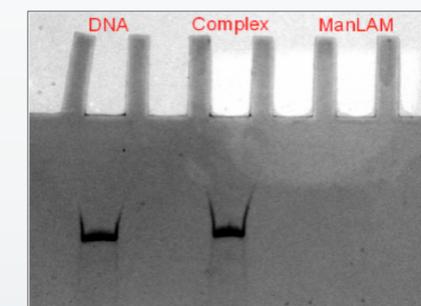
- EMSA stained with Methylene Blue. ManLAM was not stained; however, little to no evidence of binding (top).

$K_D = 15 \pm 10$ nM
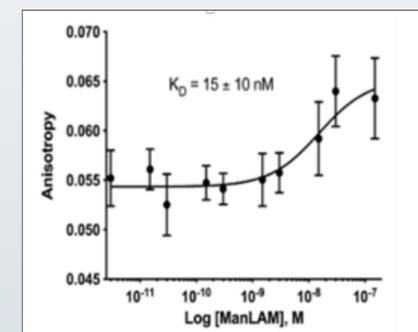
- Anisotropy results (left) yielded much better results than EMSA. The increase in the anisotropy value as a function of ManLAM concentration is indicative of a binding interaction between the aptamer and ManLAM.

$K_D = 10 \pm 8$ nM
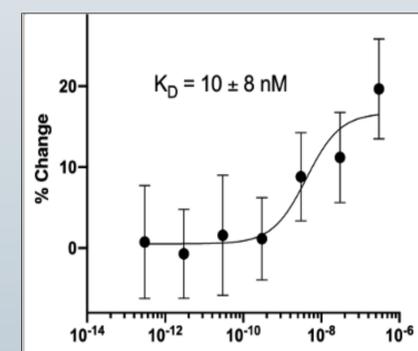
- Data obtained from our preliminary trials infer a direct change in electrochemical current with increased concentrations of added ManLAM (left).

Challenges & Future

- Optimize sensor response while working on reproducibility of either signal ON or signal OFF. Test biosensor against other LAM variants that are present in the envelope of other NTM species *(PiLAM, AraLAM)*.

- Build aptamer sequence and biosensor with high affinity to glycopeptidolipids (GPLs) that are also highly abundant in the cell envelope of various NTM species (poster by Dylan Poch). This will increase selectivity and hopefully result in detection past the genus level.

References Cited

(1) Sun, X.; Pan, Q.; Yuan, C.; Wang, Q.; Tang, X.-L.; Ding, K.; Zhou, X.; Zhang, X.-L. A Single SsDNA Aptamer Binding to Mannose-Capped Lipoarabinomannan of Bacillus Calmette–Guérin Enhances Immunoprotective Effect against Tuberculosis. *J. Am. Chem. Soc.* 2016, 138 (36), 11680–11689. https://doi.org/10.1021/jacs.6b05357.
(2) Zuker, M. Mfold Web Server for Nucleic Acid Folding and Hybridization Prediction. *Nucleic Acids Res.* 2003, 31 (13), 3406–3415. https://doi.org/10.1093/nar/gkg595.
(3) Tran, T.; Bonham, A. J.; Chan, E. D.; Honda, J. R. A Paucity of Knowledge Regarding Nontuberculous Mycobacterial Lipids Compared to the Tubercle Bacillus. *Tuberculosis* 2019, 115, 96–107. https://doi.org/https://doi.org/10.1016/j.tube.2019.02.008.
(4) Strollo, S. E.; Adjemian, J.; Adjemian, M. K.; Prevots, D. R. The Burden of Pulmonary Nontuberculous Mycobacterial Disease in the United States. *Ann. Am. Thorac. Soc.* 2015, 12 (10), 1458-1464. https://doi.org/10.1513/AnnalsATS.201503-173OC.

Acknowledgements