INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

Tuberculosis (TB) remains a global health concern, impacting millions worldwide. Early and confirmatory diagnosis is the key to control the transmission in addition to ensuring better treatment outcomes. To understand how short oligonucleotide probes called “aptamers” can be beneficial towards addressing the drawbacks of traditional antibody based detections, we designed a systematic workflow for development of DNA aptamers as biorecognition molecules against two secretory biomarkers of TB, Ag85B and MPT64; found in the bodily fluids of TB patients. Initially, we identified a 69-nucleotide-long DNA aptamer using a modified “Cut-off filtration based assembly” (COFA) SELEX strategy, which demonstrated high specificity of binding to the target Ag85B. We further probed the binding interactions to validate the nature of the protein-DNA binding. We tested its analytical performance by fabricating a fluorescence-based aptasensor using FAM-labelled aptamer as the donor and GO as quencher. Remarkably, the Ag85B aptasensor showed specific binding to Ag85B as compared to other secretory proteins used as controls (like MPT64 and ESAT6) in spiked binding buffer and serum samples. Moreover, we also designed a Systematic Mimotope-guided Aptamer Refinement Technique, i.e. “SMART” approach for repurposing old aptamers by linear epitope prediction, against MPT64 biomarker and incorporated them into pre-structured libraries for aptamer development. Additionally, we proposed a methodology of using MPT64 mimotopes as target molecules for SELEX and develop high-affinity aptamers. Furthermore, we synthesized and characterized gold nanoclusters as a sensitive nanoprobe to incorporate with our MPT64 aptamer and build a futuristic aptasensing nanoplatform. In conclusion, our study offers new insights into the intricacies of designing high-affinity aptamers via the combination of computational tools as well as experimental novel SELEX methodologies. This will help us understand the mechanistic details of aptamer-target interactions and help us utilize the same to develop sensitive aptasensing detection platforms for TB; in a quest to address the existing diagnostic challenges.

Keywords: Tuberculosis, aptasensor, SELEX, diagnosis, Ag85B, MPT64