INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Development of Cardiac Troponin-T (cTnT) aptasensors for point-of-care diagnosis of myocardial infarction.

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SHORT ABSTRACT

The main objective of this thesis is the development of aptasensors for cardiac troponin T (cTnT) for the diagnosis of myocardial infarction (MI). The thesis has been divided into six chapters, starting with a brief introduction expressing the motivation behind this work and outlining the work performed to address the primary objective defined for this thesis.

The first chapter described a detailed literature survey on the work, identified the research gaps based on the survey, and then defined the objectives to bridge a few critical gaps through the work described in this thesis.

The second chapter described the development of an aptamer database using 610 aptamer candidates for different targets reported in the literature. The database provides key information on the individual aptamers, including sequence, sequence length, GC%, buffer compositions, targets (general and specific), and target affinity. It has been created with an interactive user interface with options to update it for admin and end users. The front end of the database was designed using HTML, CSS, Bootstrap, and JavaScript. The database's backend or server-side was developed using Apache web server and MySQL database, and the scripting was performed with PHP. The Aptabase also hosts plugins like the GC calculator, which segregates the inserted sequence into its corresponding nucleotides for both ssDNA and ssRNA. The database was also equipped with google analytics to understand website traffic better.

Understanding the 3-D structure of nucleic acid aptamers is important for the rational design of aptamer-based constructs in various applications, including for developing aptasensors. The third chapter described a simple approach for 3D modelling of ssDNA aptamers through an ensemble of web applications. The procedure utilized 30 aptamers whose 3D XRD or NMR experimental structures are available for validation. As a first step, the primary sequences of ssDNA aptamers were transformed into 2D structures using six widely used web applications: RNA fold, Vector builder, RNA Structure, UNA fold, Centroid fold, and IP Knot. The generated 2D structures were

then passed through the RNA composer web application to generate 3D RNA structure, which in turn was converted to 3D DNA structures using various Visual Molecular Dynamics plugins that also include conversion of ribose sugar into deoxyribose sugar backbone and uracil to thiamine. The energy-minimized generated 3D structures were matched well with high accuracy to their experimental counterparts. This study identified that the Guanine residues are crucial in the aptamer 3D structure prediction and in algorithms that generate secondary structures. Further, the GC content (<50%), GC bond percentage (<60%), and G: C ratio (<1.12) act as limiting factors in predicting the 2D structures of aptamers. There were variations in the 2D structure predictions by the web applications, even though all these applications were a combination of the MFE, MEA, and McCaskill functions. Processing these structures through the web applications described above produced best-fit 3D structures with the experimental one, thus offering the present ensemble approach to predict the 3D structure of aptamers for various applications reliably.

The MI biomarker, cTnT, and the control protein for the experiment, smooth skeletal troponin T (sTnT), were cloned, expressed, purified, and finally, characterized through the work described in Chapter 4. The proteins were cloned and expressed into *E.coli* BL21 (DE3) pLysS bacterial cells. Various methods like CD spectroscopy, western blot, MALDI-TOF, and Zeta potential confirmed the structural integrity of the expressed proteins. We expressed these proteins with His-tag to facilitate their purification and immobilization, which is involved in some experiments in the latter chapters of this thesis work.

Chapter 5 describes the selection of ssDNA aptamers against cTnT following a centrifugal SELEX (c-SELEX) process, a variant of the conventional SELEX process. The c-SELEX works principally on centrifugation. The advantages of the c-SELEX over the conventional one are (a) less time consuming, (b) it requires less number of SELEX rounds, (c) the complete protein is exposed for interaction with the aptamer library, thereby increasing the chances of finding the best aptamer candidates, and (d) requirement of less amount of consumable items for the selection process. The cTnT protein purified in Chapter 4 was used as the target for the c-SELEX, whereas sTnT served as the control protein for the negative c-SELEX. Following the process of c-SELEX, we isolated four aptamer candidates, cT12, cT21, cT22, and cT33. The selected aptamers were then characterized for their specificity and binding affinity to the target. The sequences and free energy values determined by circular dichroism (CD) spectroscopy and Isothermal titration calorimetry (ITC) are presented in the table below.

Aptamer name	K_d value ITC	K_d value CD	Sequence (5' - 3')
cT12	14.42±1.5µM	0.4193±0.1561µM	CACCTAATACGACTCACTATAGACTTCGTATGCCAACAGCGATCCTAGATCGCGCAAGCTTGTTCGAGCCAG
cT21	10.7±0.056µM	0.17±0.2877µM	CACCTAATACGACTCACTATAGACTTCGTATGCCAACAGCGCACAGGGGACGCGCAAGCTTGTTCGAGCCAG
cT22	49.22±3.33µM	0.5888±0.2884µM	CACCTAATACGACTCACTATAGGCACAGGGGACGCACTTCGTATGCCAACAGCGCAAGCTTGTTCGAGCCAG
cT33	13.1±21µM	2.6143±0.1091µM	CACCTAATACGACTCACTATAGGCACAGGGGACGCACGGCGTATGCCAACAGCGCAAGCTTGTTCGAGCCAG

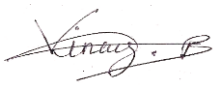
The tertiary structures of the selected aptamers were generated using the ensemble technique proposed earlier, and docking studies were performed as a shred of supporting evidence for the

specific interactions between the aptamers and cTnT. The developed aptamers were utilized in two independent yet interlinked approaches for cTnT detection described in chapters 6 and 7, respectively.

Chapter 6 described a conductive ink-based electrochemical paper platform for cTnT detection. In this approach screen printing technique, sensing platform, and conductive ink were optimized for their performance in the integrated sensing platform. The aptamer cT22 was immobilized on the screen-printed electrodes (SPE) by physical adsorption. The electrodes and their performance were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The LOD of the sensor, discerned from the CV experiment, was 280.03 pM, and the linear detection range was 1 nM - 100 nM. Thus the fabricated sensor could detect cTnT within a clinically significant range at a much lower level. Notably, the cTnT level in a healthy individual is < 5 nM, and its physiologically significant range under the ailing condition is 5 nM to 10 µM.

Chapter 7 described electrochemiluminescence-based lateral flow assay (LFA) for cTnT. There has been a continuous strive to develop highly sensitive and low-cost detection systems for MI to meet the demand for effective screening of patients in developing countries where the cases are predominant. In the current chapter, a modified LFA platform was devised. The screen printing technique of the electrodes was adopted from the previous work where a paper-based electrochemical platform was generated. Lumidot (CdSe@ZnS) nanoparticles tagged with cT33 aptamer were used as the receptor molecules, whereas the aptamer cT22 immobilized on the LFA platform acted as the capture probe. The ECL signal was generated when cTnT was captured by cT22 and tagged by the cT33-lumidot at an applied potential of 0.6V. The ECL-LFA strips could detect very low levels of cTnT in the ECL-based sensors. The LOD of the system was 42.71 pM.

The thesis concluded with a section, Conclusions and future direction, at the end of the chapters, describing the key findings and the scope for future work on improving the developed biosensors against cTnT and translating the proof of concept for real-world applications.



Vinay B (24-1-24)