Details of the excellence in research work for the Sun Pharma Science Foundation Research Fellowships 2024

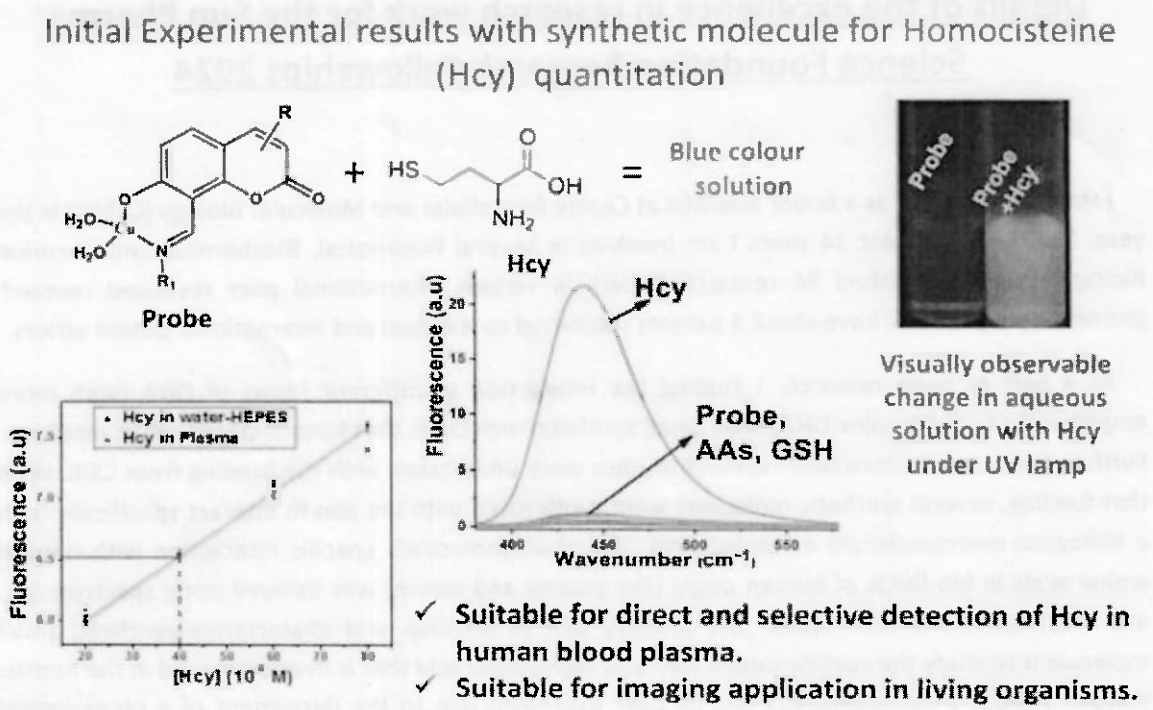
I started my carrier as a Junior Scientist at Centre for Cellular and Molecular Biology (CCMB) in the year, 1990. For the past 34 years I am involved in several Biophysical, Biochemical and Chemical Biology studies. Published 94 research papers in various international peer reviewed research journals. To my credit I have about 3 patents submitted to National and International patent offices.

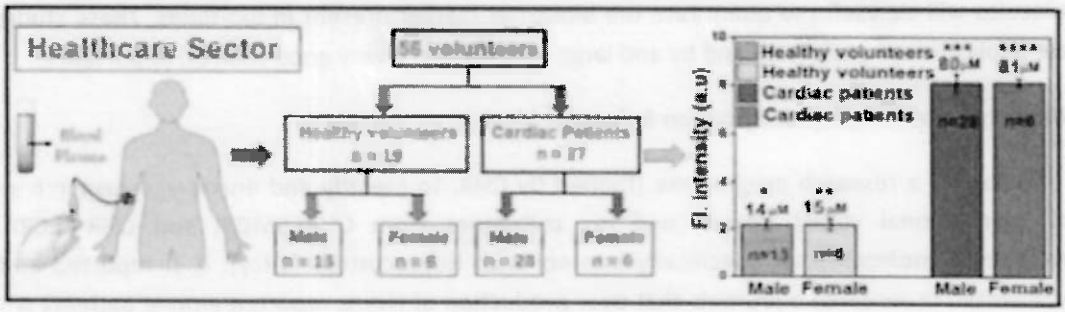
As a part of basic research, I studied the interaction of different forms of DNA (with more emphasis on G-quadruplex DNA) with small synthetic molecules that have applications in medicine. Further, few more translational research studies were undertaken with the funding from CSIR. With that funding, several synthetic molecules were synthesised with the aim to interact specifically with a biological macromolecule or amino acid. The small molecule's specific interaction with natural amino acids in bio-fluids of human origin (like plasma and serum) was assayed using spectroscopic and electrochemical techniques. The primary aim to develop and characterize synthetic small molecule is to study the specific interaction of it with amino acid that is over expressed in the human system. Usually a bio-molecule/s will be over expressed due to the derailment of a physiological process in the system. The spectroscopic or electrochemical assays performed with the synthetic molecules will be useful to quantitate the biological marker present in bio-fluids. These studies will have applications in medicine and by and large they will have very good societal importance.

i. Homocysteine levels quantification in human plasma:

As part of a research programme (funded by CSIR, to identify and encourage research studies with translational value), myself and my colleagues from CSIRCSMCRI and CSIR-CECRI have developed a molecule that specifically interact with Homocysteine (Hcy). It is reported and well documented in research a journals that over production of Hcy is reported among patients who are suffering from cardiovascular diseases. A coumarin modified synthetic molecule was designed that has specificity towards Hcy even in presence of other amino acids. It was characterized and quantified to find the concentration of Hcy in human plasma using fluorescence technique. Efforts gave good results to quantify Hcy in bio-fluids using electrochemical sensors. Studies were done with several clinical samples to evaluate Hcy levels, especially in human plasma of cardiac patients.

The data obtained in this study was published in the recent issue of Chem comm. (A unique water soluble probe for measuring the cardiac marker homocysteine and its clinical validation.(2022) (***Snehasish Debnath,Ratish R. Nair,Riya Ghosh, Gaddam Kiranmai,Narsini Radhakishan, Narayana Nagesh* and Pabitra B. Chatterjee*. Chem Comm., 58, 9210 - 9213.***). The structure of probe and and the fluorescence spectral quantification carried out is shown in figure 1.





Schematic representation showing Hcy levels measured in the blood samples of cardiac patients and healthy volunteers. In all cases (*, **, ***, and ****) $P < 0.05$. Each data point represents the average of triplicates.

Figure 1. The quantification of Hcy in human plasma. The level of Hcy in male and female patients were documented above.

ii. L-Cysteine level quantification in vitro:

Similarly, studies were extended to quantitate yet another amino acid namely cysteine (Cys), with another small synthetic molecule (synthesised in our laboratory) that specifically interacts with Cys. The amino acid L-cysteine, harbouring a thiol group, provides a high redox activity in cell metabolism, plays a crucial role in protein folding, functions as a catalytic residue of several enzymes and serves as a building block of 5-L-glutamyl-L-cysteinylglycine (GSH) and as a donor compound of sulphur, which is required for the synthesis of Fe/S clusters, biotin, coenzyme A and thiamine. Cysteine is a very crucial and important amino acid. Hyperhomocysteinemia and low levels of cysteine associated with various diseases like cardiovascular diseases (CVD), ischemic stroke,

neurological disorders, diabetes, cancer like lung and colorectal cancer, renal dysfunction-linked conditions, and vitiligo. The specific identification of the synthetic molecule through its interaction with Cys, is useful to quantitate the over expressing amino acids in biological fluids. Cysteine level estimation *in vitro* is done through fluorescence. The studies with clinical samples are in progress. The main intension in synthesizing a specific synthetic molecule is to develop a handy device that will quantitate specific amino acids in bio-fluids through fluorescence and electrochemical methods. The results obtained in these studies is published in an international journal namely, Chemistry- An European Journal (***A Hydrogen Bonded Non-Porous Organic-Inorganic Framework for Measuring Cysteine in Blood Plasma and Endogenous Cancer Cell. (2024) Ghosh, Riya, Pradhan, Debjani, Debnath, Snehasish, Mansingh, Arushi, Nagesh, Narayana*, Chatterjee, Pabitra*. Chemistry - A European Journal,DOI: 10.1002/chem.202401255, e202401255***.). The details of structure of probe to detect Cysteine in biological fluids and the possible mechanism of enhanced fluorescence is shown figure 2.

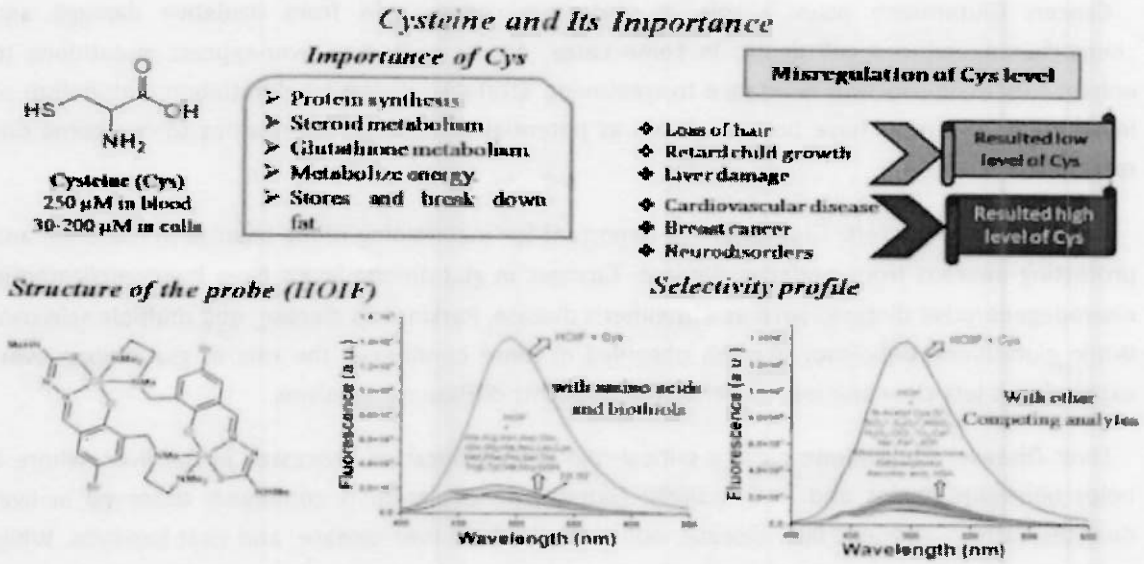


Figure 2a. Structure of the probe used for quantification of Cysteine in blood plasma. Its emission spectra

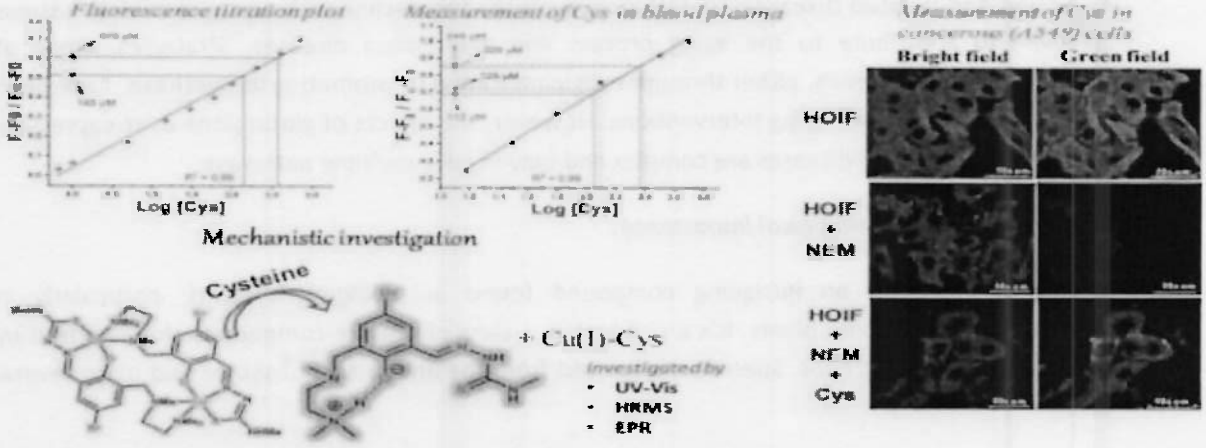


Figure 2b.The standard graph for the Cysteine (Cys) quantification. Measurement of Cys in A549 cells using fluorescence microscope.

III. Glutahione (GSH) and Spermine:

a. Glutathione and its physiological importance:

Glutathione is a vital antioxidant in the body, playing a crucial role in detoxification and protecting cells from oxidative stress. Glutathione deficiency has been associated with various diseases like Cancer, Neurological disorders (like Alzheimer's and Parkinson's diseases), Liver disease, Aging and Age-Related Diseases, and health conditions, the consequences of glutathione over expression are less clear-cut and may vary depending on the context.

Here are a few points regarding glutathione over expression and its potential disease associations:

Cancer: Glutathione plays a role in protecting cancer cells from oxidative damage and chemotherapy-induced cell death. In some cases, cancer cells may over-express glutathione to enhance their survival and resistance to treatment. Strategies targeting glutathione metabolism or inhibiting its synthesis have been explored as potential therapeutic approaches to overcome this resistance.

Neurological Disorders: Glutathione is important for maintaining redox balance in the brain and protecting neurons from oxidative damage. Changes in glutathione levels have been implicated in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. While glutathione deficiency is often observed in these conditions, the role of glutathione over-expression is less clear and may depend on the specific disease mechanisms.

Liver Disease: Glutathione plays a critical role in detoxification processes in the liver, where it helps neutralize toxins and free radicals. Glutathione depletion is commonly observed in liver diseases such as alcoholic liver disease, non-alcoholic fatty liver disease, and viral hepatitis. While increasing glutathione levels through supplementation or over-expression could theoretically be beneficial in these conditions, the effectiveness and safety of such approaches require further investigation.

Aging and Age-Related Diseases: Glutathione levels tend to decline with age, and oxidative stress is believed to contribute to the aging process and age-related diseases. Strategies aimed at enhancing glutathione levels, either through supplementation or promoting its synthesis, have been proposed as potential anti-aging interventions. However, the effects of glutathione over-expression on aging and age-related diseases are complex and may involve multiple pathways.

b. Spermine *and its physiological importance:*

Spermine is indeed an intriguing compound found in biological systems, particularly in mammalian cells and some plants. It's a polyamine, a class of organic compounds characterized by multiple amine (-NH2) groups. Spermine is derived from the amino acid ornithine and plays several vital roles in cellular function.

As a biomarker, spermine has drawn attention due to its association with various physiological and pathological conditions. It has been studied in the context of cancer, neurodegenerative

diseases, and aging. Changes in spermine levels have been linked to alterations in cell proliferation, apoptosis (programmed cell death), and DNA stability.

In cancer research, spermine levels have been found to be altered in different types of tumors. While some studies suggest that elevated levels of spermine may promote cancer cell growth and survival, others indicate that reduced levels could be indicative of tumor progression. Therefore, spermine levels could potentially serve as a diagnostic or prognostic biomarker for certain cancers.

In neurodegenerative diseases like Alzheimer's and Parkinson's, alterations in polyamine metabolism, including spermine, have been observed. These changes may contribute to neuronal dysfunction and cell death in these conditions. Studying spermine levels could provide insights into the underlying mechanisms of these diseases and potentially lead to the development of novel therapeutic strategies.

Furthermore, spermine has been implicated in the aging process. Levels of spermine decline with age, and this decline has been associated with age-related physiological changes and diseases. Understanding the role of spermine in aging could provide valuable insights into the biological processes underlying aging and age-related diseases.

Overall, spermine serves as an intriguing biomarker due to its involvement in various cellular processes and its potential implications for human health and disease. However, further research is needed to fully elucidate its role as a biomarker and its therapeutic potential in different pathological conditions.

c. Detection of Glutathione and Spermine in solutions:

Detecting glutathione levels in solution can be done through various methods, each with its own advantages and limitations. Here are a few common techniques:

Colorimetric Assays: There are colorimetric assays available that utilize the reaction between glutathione and certain chemicals to produce a color change. One example is the Ellman's reagent (5,5'-Dithiobis(2-nitrobenzoic acid), DTNB) assay. Glutathione reacts with DTNB to produce a yellow-colored compound, which can be detected spectrophotometrically at 412 nm. The intensity of the color is proportional to the concentration of glutathione in the solution.

Fluorescence Assays: Fluorescent probes can be used to detect glutathione levels. These probes often contain a fluorophore that becomes fluorescent upon reacting with glutathione. For example, monochlorobimane (MCB) reacts specifically with glutathione to form a highly fluorescent product, S-glutathionyl adduct. The fluorescence intensity can be measured using a fluorometer.

High-Performance Liquid Chromatography (HPLC): HPLC coupled with a suitable detection method, such as UV-vis or fluorescence detection, can be used to quantify glutathione levels in solution. This method provides high sensitivity and specificity for glutathione detection but requires specialized equipment and expertise.

Enzyme Assays: Glutathione reductase can be used to enzymatically reduce oxidized glutathione (GSSG) to its reduced form (GSH), which can then be detected using methods like those mentioned

above. The rate of reduction can be correlated with the initial concentration of glutathione in the solution.

Electrochemical Assays: Electrochemical sensors can also be employed for the detection of glutathione. These sensors utilize specific recognition elements, such as enzymes or antibodies, immobilized on the electrode surface to selectively capture glutathione molecules, leading to changes in electrical properties that can be measured.

d. Detection of Spermine in solutions:

Detecting spermine typically involves specialized laboratory techniques due to its presence in low concentrations and its chemical structure. Here are some common methods used for detecting spermine:

High-Performance Liquid Chromatography (HPLC): This technique separates and identifies individual compounds in a mixture based on their interaction with a stationary phase and a mobile phase. Spermine can be separated from other compounds and quantified using HPLC.

Mass Spectrometry (MS): Mass spectrometry is a technique used to identify the chemical composition of a sample by measuring the mass-to-charge ratio of ions. Spermine can be detected and quantified using MS, either alone or in combination with other techniques like chromatography.

Fluorescence Spectroscopy: Spermine can fluoresce when exposed to specific wavelengths of light. Fluorescence spectroscopy can be used to detect and quantify spermine by measuring the intensity of emitted fluorescence.

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA is a common immunoassay technique used to detect the presence of a specific protein or small molecule in a sample. While less common for spermine detection, ELISA kits specific for spermine may be available for research purposes.

Polymerase Chain Reaction (PCR): PCR can be used to detect and quantify nucleic acids associated with spermine metabolism or regulation, such as the genes involved in spermine synthesis or transport.

Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR spectroscopy can provide information about the structure and composition of molecules, including spermine, by analyzing the nuclear properties of atoms within the molecule.

Electrochemical Assays: Electrochemical sensors can also be employed for the detection of Spermine. These sensors utilize specific recognition elements, such as enzymes or antibodies, immobilized on the electrode surface to selectively capture spermine molecules, leading to changes in electrical properties that can be measured.

These techniques can be applied to various types of samples, including biological fluids, tissues, and cell extracts, depending on the specific research or diagnostic needs. It's important to choose the most appropriate method based on factors such as sensitivity, specificity, sample type, and available resources. Additionally, consulting with a laboratory specialist or research scientist experienced in spermine detection can provide valuable guidance in selecting and implementing the most suitable detection method.

Keeping the significance of GSH in human metabolism, a small synthetic molecule that has specific interaction with GSH in solution. The specific interaction followed by enhancement of its fluorescence is used to quantitate GSH in solution. Further, electrochemical device to detect the level of GSH in solution was also tested and it was giving good results. Studies are in progress to make a desktop and handy device to check the levels of GSH in solution. Similarly efforts are in progress to check the levels of another biomarker i.e Spermine in biological fluids.

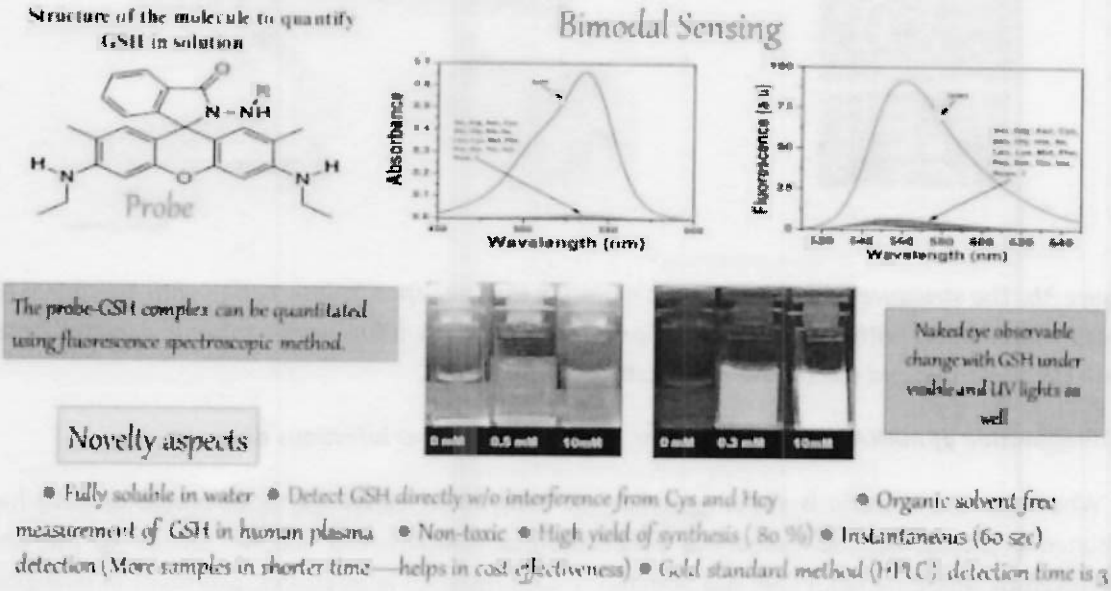
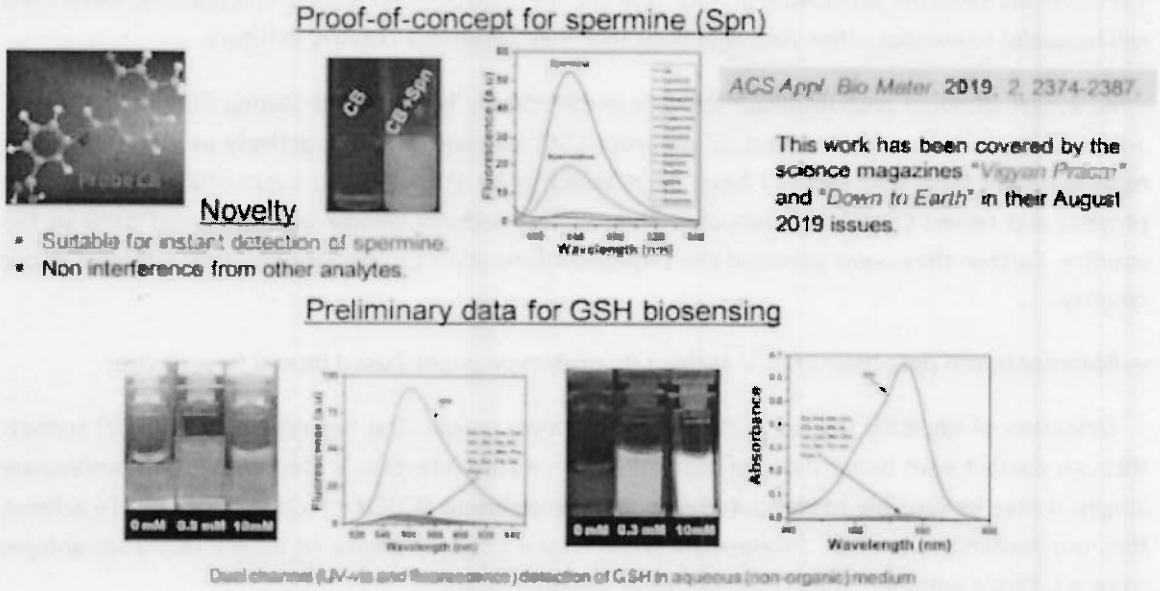


Figure 3a. The structure of small synthetic molecule that has specific interaction with GSH. Its specific interaction with GSH in the presence of various other amino acids is depicted here.



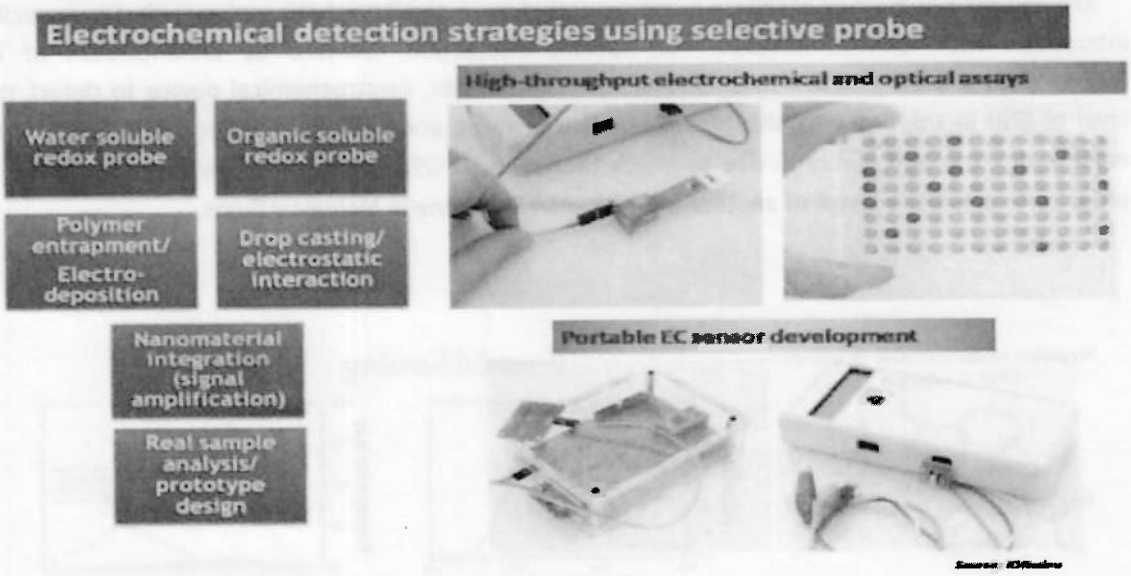


Figure 3b. The structure of small synthetic molecule that has specific interaction with Spermine. Its specific interaction with Spermine in the presence of various other amino acids is depicted here. Both spectroscopic and electrochemical methods were shown.

iv. Preparation of mRNA based vaccines for COVID-19 and other infectious diseases:

When the whole world is suffering under the clutches of COVID-19, Government of India has initiated a project for the indigenous preparation of mRNA based vaccine (similar to the one produced by Moderna Ltd.,) CSIR has selected, 7 CSIR research laboratories, proficient in biological and chemical studies, and given the responsibility to develop a mRNA based COVID-19 vaccine (using the technology developed by Moderna Ltd.,). CSIR-CCMB was made a nodal lab and I am the nodal scientist for this project. The aim is to develop an indigenous platform for making mRNA based vaccines. We have successfully developed a platform for the preparation of mRNA based vaccine. The developed vaccine was tested in Hamsters and it exhibited good results. The platform developed will be useful to combat other viral infections that may pester our country in future.

As a part of social responsibility, the whole CSIR family has initiated testing COVID-19 samples across India. In this project, about 12 different CSIR laboratories have actively participated (as a representative from CSIR-CCMB I have been asked to be a nodal PI to successfully complete the project) and tested COVID-19 infection among several lakhs of people from different parts of the country. Further they were provided the required information to combat COVID-19 infection in our country.

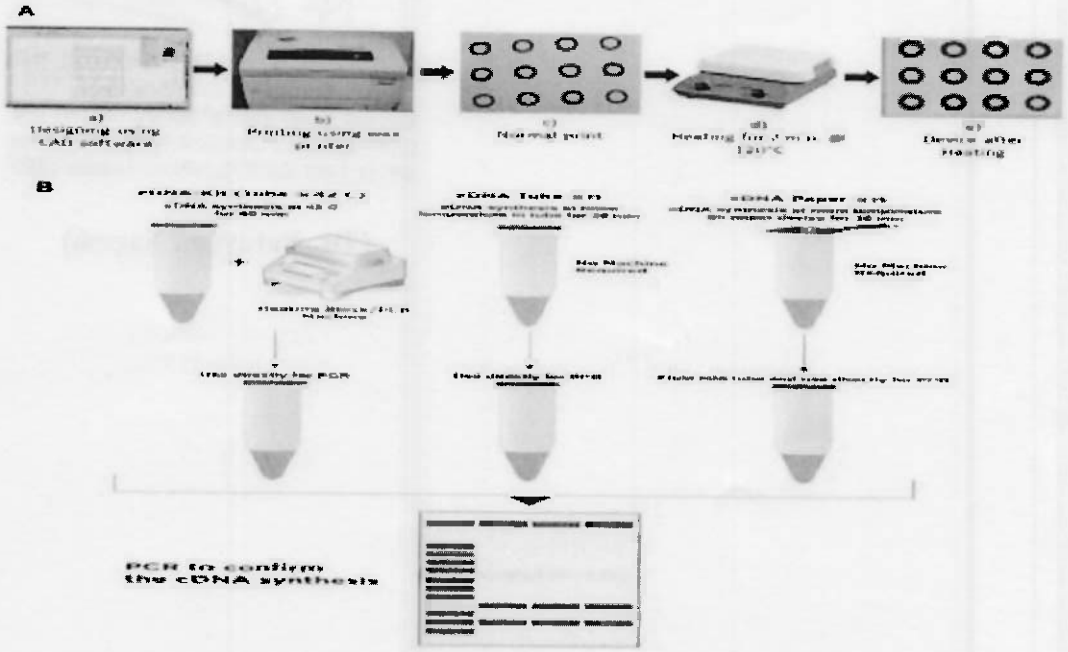
v. Aptamer based detection of HCV antigen on prototype paper-based lateral flow device:

Detection of hepatitis C virus (HCV) infection is very crucial. The hepatitis C virus (HCV) spreads through contact with blood that has the virus in it. If HCV infection is identified in the preliminary stages, it may be possible to combat liver damage and spread of HCV infection to others. To achieve this, our research team has developed a paper based dot blot device to detect HCV core antigen using a suitable aptamer. The achievements in this project are:

1. Development of a prototype paper-based dot-blot device detection of HCV core antigen using aptamers in place of antibodies. The paper based dot-blot device was shown to identify the level of HCV core antigen. This data obtained from this part of study is communicated in the form of a manuscript, to an international journal.

2. Further, a simple paper-based device for RNA extraction and room-temperature reverse transcription with minimum reagents and with need of any equipment. This part is published in New Biotechnology journal.

The title of the manuscript published in *New Biotechnology* is ***"Ready-to-Use Vertical Flow Paper Device for Instrument-free RoomTemperature Reverse Transcription. (2022) Thomas Michael Shiju, Chaturvedula Tripura, Pritam Saha, Arushi Mansingh, Venkatapathy Challa, Ira Bhatnagar, Narayana Nagesh, and Amit Asthana. New Biotechnology, 68, 77-86"***. A brief description of the study is shown in figure 4.



Fabrication of paper-based device and testing methodology for cDNA synthesis, (A) Flow chart demonstrating the detailed process of fabrication of paper based devices. Wax printing of paper-based device: (a) CAD designing, (b) printing design on filter papers using Xerox solid ink printer (c) normal wax print on the surface of the paper, (d) heating of wax printed filter paper, (e) filter paper after heating, (B) Flow chart for setting up the process for cDNA synthesis, using the kit method (cDNA-Kit), in the tube (cDNA-Tube), and on paper (cDNA-Paper). The kit method utilizes a PCR machine/heat bock while the tube and paper-based methods are instrument-free. The efficiency of cDNA synthesis was tested by PCR.

Figure 4. Paper device used for performing the instrument free RT reverse transcription.

vi. Nano-particles preventing the bio-fouling of micro-organisms on cotton fabrics are synthesized:

Nano-particles preventing the bio-fouling of micro-organisms on cotton fabrics are synthesized. The synthesised material was coated on various fabrics and tested for its efficiency in bringing down the microbial population on the surface of the cloth or material. The mechanism followed is chemically coated nano-particles are embedded in the cotton fabrics to stop bio-fouling of micro-organisms. This study is in progress along with scientists at CSIR-CGCRI. At CSIR-CCMB, we have studied the growth of various pathogenic and non-pathogenic micro-organisms on the surface of

cotton or synthetic fabrics. Further we have also checked and found the suitability of synthetic chemical spread on the fabric using various human skin cell lines. The applications of this study are mostly seen in Indian Army, where soldiers residing at different places, difficult environmental conditions and under various geographical regions may carry several kinds of micro-organisms on their cloth surface. The dress materials are treated with nano-particles having synthetic material will combat the bio-fouling of micro-organisms.

The details of this technology developed to stop bio-fouling of micro-organisms on various cloth surfaces is communicated to suitable international journal.

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