ABSTRACT

Studies on Functional regulation by Biomaterial interaction using an integrated in silico-in vitro approach

The knowledge of regulating the function of proteins or peptides directed by the biomaterial-interaction based on the structural diversity and conformational dynamics by integrating both *in vitro* laboratory-based experiments and *in silico* computational-based molecular docking experiments has been studied.

The thesis is organized into 4 major chapters:

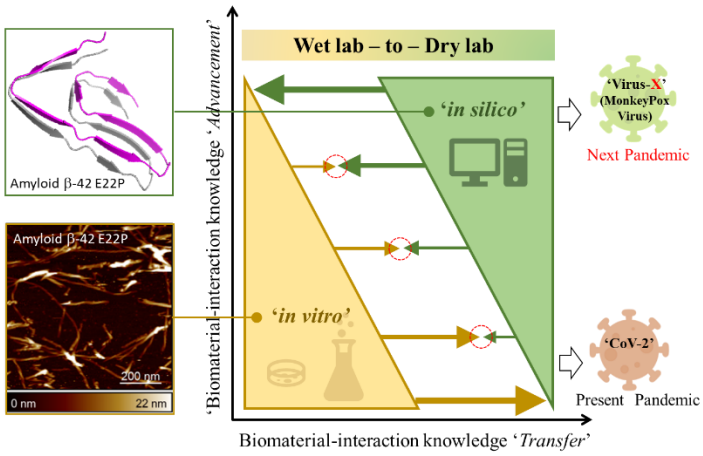
The first chapter is based on the interaction between ***protein*** and ***small molecule***. It is titled *"Identification and evaluation of small molecule-based natural compounds for inhibition of HIV-1 Reverse Transcriptase activity"*. This chapter talks about the use of small-molecule based natural-compounds that are derived from plants, as biomaterials that interact and regulate the function of reverse transcriptase (RT) enzyme which is responsible for causing HIV-1 (Human immunodeficiency virus-1) disease. For this purpose, few Indian plants (not native but abundantly grown as weeds in India) have been chosen and screened for identification of inhibitory activity to RT enzyme from HIV Type-1 using different Reverse Transcription Assays. The results suggest that water-extracts of leaves of *Argemone mexicana* plant strongly inhibited the DNA polymerase activity of HIV-1 RT, indicating they contain organic compounds that inhibit the enzyme activity. This study thus leads to the understanding of how small molecules based natural compounds as biomaterials can interact with the functional proteins and inhibit their activity.

The second chapter is based on the interaction between ***protein*** and ***nucleic acid***. It is titled *"Co-operative interaction between RNA polymerase and Recombinase for Reverse Transcriptase-based improved isothermal nucleic acid amplification assay"*. This chapter talks about the bio-interaction of different functional proteins that can co-assist and interact with each other and help to develop an improved diagnostic assay for infectious diseases. By understanding the biomolecular interaction of RNA polymerase enzyme and Recombinase enzyme, a new method has been developed, which is termed as RICCA (RNA isothermal co-assisted coupled amplification). We integrated the essentials of both types of isothermal amplification methods, i.e. RNA specific amplification and RPA (recombinase polymerase amplification) into a simple format of 'sample-in and answer-out' with a primary focus on the detection of low copy numbers of viral RNA directly from COVID-19 saliva samples without the need for any laboratory handling or sample preprocessing. We report the development of a completely homogeneous, isothermal, highly sensitive, and ultrarapid method for detecting virus RNA target sequences for the on-site (low resource settings) molecular diagnosis of COVID-19 and other infectious diseases. We further plan to advance this study by utilizing the *in silico* approach for optimization of primer designing that can be further utilized for the application of RICCA for new variants.

The third chapter is based on the interaction between ***peptide*** and ***peptide***. It is titled *"Understanding biophysical properties of oligomerization and fibril formation of amyloid beta 42 conformers"*. This chapter deals with the diversity of molecular conformation of small peptide, amyloid beta 42 (Aβ42), which is caused by a single amino-acid substitution in the peptide chain. This causes one conformer to be more toxic, i.e. E22P- Aβ42 (mutation at 22nd position of amino acid chain that changes Glutamic acid (E) to proline(P)) and the other one to be less-toxic i.e.E22- Aβ42 (wild-type). To understand how a single amino acid change in the sequence can lead to several folds increased toxicity and increased aggregation of the peptide leading to increased risk to disease, macromolecular analysis was made using SDS-PAGE followed by making a microscopic analysis of the aggregation dynamics of both the conformers using atomic force microscopy (AFM) studies. Our results disclose the formation of amorphous aggregates in E22P-Aβ42 that are stem-based network-like structures while formation of mature fibrils in E22-Aβ42 that are sphere-based flexible structures. A relative comparison is made between the biophysical properties of E22P-Aβ42 and E22-Aβ42 that reveals high stiffness, lesser periodicity, and higher rigidity in E22P-Aβ42. *In silico* studies were performed by molecular docking that revealed atomic scale details like number of beta sheets, and number of residues in beta sheets involved, and the dihedral angle between beta sheets involved in the formation of E22-Aβ42 and E22P-Aβ42. We propose a systematic model of fibril formation that helps in understanding the molecular basis of conformational transitions in the Aβ42 species. These findings will have significant implications to our understanding of the structural basis of toxicity caused by conformational diversity in Aβ42 species.

The fourth chapter is based on the interaction between ***peptide*** and ***nucleic acid***. It is titled *"Identification and evaluation of aptamer-based synthetic compounds for regulation of toxic conformer of amyloid beta 42"*. Based on the understanding of Chapter 3, this chapter was designed to identify aptamer-based synthetic compounds as biomaterials for specific recognition of both the conformers of Aβ42 using an advanced screening method. We identified novel 70-nt DNA aptamer sequences using in vitro competitive selection method SELCOS. Utilizing this advanced screening approach helps in identification of unique aptamer sequences that can specifically recognize their respective targets and not the competitive target. Our results revealed that the selected aptamer candidates (Apt-W1 for E22-Aβ42 and Apt-T2 for E22P-Aβ42) show high binding affinity to their respective targets. Further, their binding was confirmed by electrochemical evaluation, SDS PAGE and qPCR analysis. The *in silico* studies support the supposition that the number of beta sheets decrease on interaction of aptamer with Aβ42 indicating the inhibitory function of selected aptamer towards aggregation of Aβ42. We believe this work could be a promising research tool for further studies about toxicity caused by E22P-Aβ42, and thereafter regulate the toxicity.

From these studies in chapter 1-4, we can thus draw a general understanding of how integrating the *in vitro* and *in silico* studies can help in a better understanding of the biological systems. We therefore give a 'dry-lab concept', as shown in below figure to develop a platform that can 'integrate' the power of *in vitro* and *in silico* systems to regulate the function of proteins with the help of structure and biomaterial-interaction. This integration can help to gradually reduce the dependency of *in vitro* system on the *in silico* system and develop a fully functional in silico platform for the future.



The DRY-Lab concept

KEYWORDS: biomaterial interaction, *in silico-in vitro* approach, aptamer, amyloid beta, reverse transcriptase