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論文の内容の要旨

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.

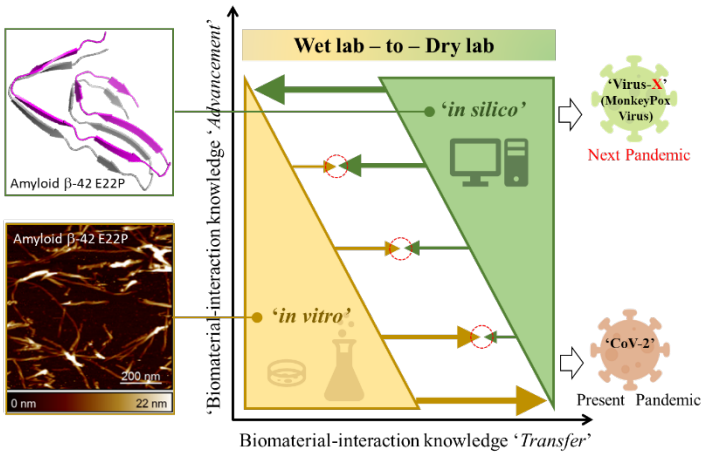
The second chapter is based on the interaction between ***protein*** and ***nucleic acid***. It is titled *"Co-operative interaction between RNA polymerase and Recombinase for Reverse Transcriptase-based improved isothermal nucleic acid amplification assay"*. This chapter talks about the bio-interaction 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 22^{nd} 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

論文審査の結果の要旨

本研究では、in vitro 実験と計算機による分子ドッキング(in silico)実験を統合的に行い、生体物質間相互作用の構造多様性に基づく生体物質間相互作用の探索と、その制御について報告している。

第 1 章では、蛋白質（HIV-1 Reverse Transcriptase (RT)）機能を阻害する低分子天然化合物を同定した。既存の HIV-1 疾患治療法の代替療法として、インドの伝統的自然療法である「アーユルヴェーダ」の概念を活用し、Argemone mexicana の葉の水抽出物に阻害活性を見出した。本研究を医薬品開発へ発展させるために、in silico アプローチを用いた阻害の分子機構の理解が期待できる。

第 2 章は、タンパク質(RNA ポリメラーゼ)と核酸の相互作用による逆転写酵素ベースの改良型等温核酸増幅法について報告している。異なる機能性タンパク質を相互作用させ、感染症の新規診断法を開発している。RNA ポリメラーゼ酵素とリコンビナーゼ酵素の生体分子間相互作用を理解することにより、RICCA（RNA isothermal co-assisted coupled amplification）と呼ばれる新しい手法が開発された。低コピー数のウイルス RNA を直接簡便に検出することに成功している。プライマー設計の最適化など、in silico アプローチを活用することで、より高度に応用することができる。

第 3 章は、ペプチド間の相互作用についてである。アルツハイマー病の原因であるアミロイドベータ 42（A β 42）の分子構造の多様性について解析した。ペプチド鎖の 1 つのアミノ酸置換(E22P)によって凝集特性が変化し、強毒化する事を明らかにした。電気泳動と原子間力顕微鏡(AFM)観察により、凝集ダイナミクスを解析し、E22P は剛直で周期性が低い線維を形成する事が分かった。分子ドッキング分析(in silico)によって β シート内のアミノ酸残基数や重合角度等、線維構造の詳細を明らかにした。構造転移の分子基盤を理解し、線維形成のモデルを提案できた。

第 4 章では、ペプチドと核酸の相互作用に基づいて Aβ42 の毒性コンフォマーを制御するための核酸アプタマーの探索と評価を行った。A β 42 を特異的に認識する核酸アプタマーを、in vitro 競争的選択法を用いて同定した。選択したアプタマー候補は、野生型・毒性型それぞれの標的に対して高い結合親和性を示した。選択したアプタマーが A β 42 の凝集を抑制する機能を持つ事が、in vitro, in silico 両方の研究で明らかとなった。　第 5 章では、以上の結果を総括し、将来の展望について述べている。

以上、本論文は、バイオマテリアルによる機能制御について多角的に検討し、有意義な成果を得ているものであり、学術的に貢献するところが大きい。

よって博士（マテリアルサイエンス）の学位論文として十分価値あるものと認めた。