CHAPTER 1

MOTIVATION AND OUTLINE OF THE THESIS

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1.1. Motivation of the present work:

In the elderly population around the world, neurodegenerative disorders are a leading cause of disability and mental decline. Alzheimer's Disease (AD) and Parkinson's Disease (PD) are the most frequent neurodegenerative conditions [1, 2]. AD is the most prevalent form of dementia in the world, accounting for 60–80 percent of all dementia cases and impacting an estimated 24 million individuals worldwide [3, 4]. According to a report from the Alzheimer's Disease Association, AD has affected around 6.2 million Americans till the year 2022. AD primarily affects individuals aged 65 years and older. Utilizing a nationwide research study by J. Lee et. al., 2023 in India between 2017 and 2020, it was discovered that 7.4% of those 60 years and older were thought to have dementia (8.8 million individuals).

Neurodegenerative disorders develop when nerve cells in the brain or peripheral nervous system gradually lose their functioning and die [5-7]. Although there are treatments that can help with some of the physical or mental symptoms of neurodegenerative diseases, there is presently no way to stop their progression and there are no known cures. Age significantly raises the probability of developing a neurodegenerative disease. Neurodegenerative diseases may affect an increasing number of people in the coming decades, particularly as life expectancy rises. In order to improve therapy and preventive measures, it is better to understand the etiology of neurodegenerative diseases. The interplay of a person's genes and environment affects how susceptible they are to neurodegenerative diseases, according to experts [8]. For instance, even if a person has a genotype that predisposes them to PD, their environmental exposures may alter whether, when, and how severely they are impacted.

According to the National Institute on Aging, there is currently no preventive treatment for AD [4]. Current research has shown that early disease detection can lead to more effective treatment options. While there are no effective treatments for AD and the only existing pharmaceuticals are analgesic in nature, it is crucial to discover drugs that prevent the formation of misfolded $A\beta_{1\text{-}42}$ peptides [9, 10]. The typical role of $A\beta_{1\text{-}42}$ peptide, which is produced through successive cleavage of Amyloid Precursor Protein (APP), is poorly understood. No discernible decrease of physiological function has been detected in the absence of $A\beta_{1\text{-}42}$ peptide. Several possible functions of $A\beta_{1\text{-}42}$ peptide

have been described in earlier studies, including activation of kinase enzymes, protection against oxidative stress, and modulation of cholesterol transport [11, 12].

PD is the second most prevalent neurodegenerative disorder after AD [5]. According to the Parkinson's Foundation, nearly one million People are affected by PD. PD is projected to have a prevalence of 0.3% in the general population, 1.0% in those older than 60 years of age, and 3.0% in those aged 80 years or older [6]. Similarly, the ultimate reason for the onset of PD is yet unknown. In the affected region of the brain, misfolded proteins are associated with PD. These proteinaceous deposits are caused by amyloid fibrils containing 140 amino acid residues of the presynaptic α-Synuclein (αS) protein. It is the primary factor related with the start of PD and a major contributor to the formation of aberrant neuronal protein aggregates known as Lewy Bodies (LBs) [12]. It has been observed that αS is an Intrinsically Disordered Protein (IDP) because it lacks a distinctive secondary structure conformation. Since the precise mechanism by which αS fibrils are formed is unclear, numerous studies are focusing on the mechanism of αS aggregation. There is currently no effective treatment for one of the most difficult disabling condition, PD.

Though the AD and PD has been identified since many decades, it is still incurable due to its complex pathogenesis. Thus, inhibiting the aggregation of the causative proteins of these two diseases to prevent formation of oligomers and fibrils has been considered as a potential goal in their therapies. Medicinal herbs, nutraceuticals, pharmacological techniques, nano-pharmaceuticals, and gene therapy are used as treatment strategies for AD and PD [15-17]. Therefore, it can be seen that research is evolving in this field to find out better inhibition strategies or approaches to prevent or cure AD and PD.

For prevention or treatment of AD, considering the etiology of aggregation progress of Aβ, a number of inhibitors including small molecule inhibitors like natural polyphenolic compounds, flavonoids, alkaloids etc. have gained importance in the recent times [15]. The designed inhibitors control the formation of Aβ oligomers and fibrils, either by stabilizing the monomeric Aβ peptide or destabilizing the oligomeric structures. Ionic strength-dependent studies of amyloid formation have suggested that ions can influence the kinetics and thermodynamics of the aggregation process [16]. Replacement of serum albumin in plasma has been proposed as a favorable therapy for the cure of AD.

It has also been reported that albumin binds with Aβ-peptide impeding its aggregation. Designing of peptides as inhibitors for treatment of AD is also a very recent approach [17].

For prevention or treatment of PD, literature studies have shown that macromolecular crowding is one of the essential cellular environment elements that can influence the aggregation mechanism of αS [18, 19]. Designing of peptides as inhibitor has gained new momentum as inhibition strategy for PD. Another evolving inhibition approach in this field is the development of natural compounds as small molecule inhibitors for PD treatment [18, 19].

Therefore, these inhibition strategies have been investigated for prevention of AD and PD using computational approach. The computational techniques provide an alternative approach in determining the protein-ligand interactions at an atomic level, which, otherwise, are difficult to elucidate using experimental techniques. These recent research works on finding new therapeutic strategies for prevention of AD and PD have motivated me to focus my research on inhibition strategies of the causative proteins of these two diseases and therefore the theme of my thesis work was set as the inhibition approaches of Amyloid-β and α-Synuclein amyloidogenic aggregation: an *In-silico* study.

Hence, an effort has been made to address two broad areas about this particular topic in this thesis work: (1) To study the inhibition approaches of Aβ amyloidogenic aggregation and (2) To study the inhibition approaches of αS amyloidogenic aggregation. To carry out this work we have mainly used Molecular Dynamics (MD) simulation with the AMBER 14 software package.

1.2. Outline of the Thesis:

Chapter 2 introduces AD and PD, their symptoms, causes and also discusses their respective causative proteins in detail.

Chapter 3 gives a description about the various computational techniques and the key principle of Molecular Dynamics (MD) simulation and other computational tools, including web servers and software used in this thesis.

Chapter 4 features the study of the effect of ionic strength on the $A\beta_{1-42}$ peptide aggregation. In this study, different concentrations of Sodium Chloride (NaCl) solution

have been used in the surroundings of the $A\beta_{1-42}$ peptide in a computational environment to analyze the effect of ionic strength on the aggregation propensity of $A\beta_{1-42}$ peptide.

Chapter 5 presents the study on the effect of peptides on dimerization and aggregation of $A\beta_{1-42}$ peptide. The C-Terminal domain of Human Albumin (HA) has been used as a peptide for studying its role in the dimerization and aggregation of $A\beta_{1-42}$ peptide. This study can be helpful in understanding the fibrillation process and dimerization of $A\beta_{1-42}$ peptide.

Chapter 6 discusses the role of small molecule inhibitor in preventing aggregation of $A\beta_{1-42}$ peptide. In this study, the role of Resveratrol, a natural polyphenolic compound, present in grapes, tea and wine, was studied using computational approaches to understand its effects on the aggregation properties of $A\beta_{1-42}$ peptide.

Chapter 7 discusses the effect of crowding agents on αS aggregation. This study involves the understanding of the conformational dynamics of αS protein in the presence of a crowding agent, Polyethylene Glycol (PEG).

Chapter 8 is about the study of the effect of peptides on αS aggregation. Recently, two novel peptides K84s and K102s were found to have inhibition effects on the αS aggregation. However, the molecular details of the role of these two peptides K84s and K102s and the sites of their respective interaction with αS have not been investigated. Hence in this study, the influence of two peptides K84s and K102s on the structural and conformational dynamics of αS were studied.

Chapter 9 discusses the study of the role of small molecule inhibitor in preventing the aggregation of αS. Here, Oleuropein aglycone (OleA) have been used to check its anti-amyloidogenic properties on the αS protein.

The summary and the future prospects of this thesis are discussed in **Chapter 10.**