Chapter 1

Motivation and Outline of the thesis

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1.1. Motivation of the present work:

Parkinson's disease (PD) is the second most common neurodegenerative disorder following Alzheimer's disease (AD), which affects about 1% of the population over 60 years old [1]. The onset of PD results from the interplay of genetic and environmental elements, rendering its origin complex. Factors such as pesticide exposure pose risks, while behaviours like physical activity and smoking may increase the risk of developing PD disease [1]. This disorder can be both sporadic and familial and some genetic forms are due to mutations in the SNCA gene [2], encoding for the protein α-Synuclein (α-Syn). PD pathological marks are the prominent death of the dopaminergic neurons in the substantia nigra pars compacta and the presence of proteins and lipid inclusions, termed Lewy bodies (LB) [3, 4] in the surviving neurons in Parkinsonian brains. In pathological conditions, it causes other non-motor symptoms such as anosmia (loss of smell) [5], constipation [6], sleep disorders [7], and depression [8] that occur prior to impairments of cognitive functions [9]. LB, pathological hallmark are the cytoplasmic protein aggregates containing ubiquitin and fibrils of α-Syn, shelter in the substantia nigra and various regions of the brain [10-12]. The principal component associated with LB is α-Syn, which tends to be misfolded and aggregated [13]. This misfolding of α-Syn results in the formation of oligomerization and later the LB, which causes the occurrence of PD [14] as shown in **Figure 1.1**.

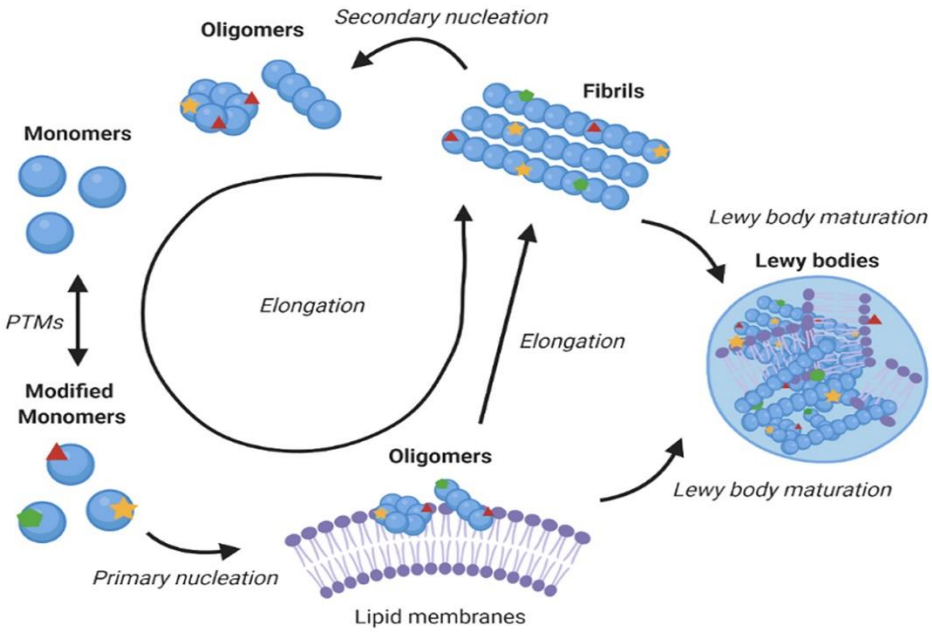

Figure 1.1. *The involvement of Lipid Membranes in α-Syn Aggregation and its Association with PD (Taken from [14])*

The main constituent of LB is an aggregated fibrillar β-sheet-rich form of α-Syn. The aggregation process of α-Syn was widely studied in the past [10-16] as the protein is unfolded in its native state. Still, in pathological conditions, it tends to aggregate, forming oligomeric species. α-Syn is a small protein abundantly expressed in the brain situated in synaptic terminals. The occurrence of PD and dementia with LB is associated with the conversion of oligomers into amyloid fibrils. Reports [17] have suggested that protein is proposed to interact with biological membranes and membrane proteins that play a significant role in synaptic plasticity and neurotransmitter release. Also, α-Syn promotes the assembly of the SNARE complex *in vivo* and *in vitro* via the formation of a multimer at the synaptic vesicle surface. The association of α-Syn to the lipid bilayer is believed to be important for its biological function in regulating synaptic vesicles, as it has been observed to be essential for SNARE complex assembly in the presynaptic membrane [17]. The detailed mechanisms by which membranes enhance the aggregation of α-Syn remain unclear. Still, a potential role for helical intermediates in this process has been suggested by recent NMR (Nuclear Magnetic Resonance) studies of vesicle binding modes of α-Syn. Disease linked to α-Syn mutants [18] as well as by a study of inhibitors such as trifluoroethanol (TFE) induced α-Syn aggregation [19]. Point mutations associated with familial forms of PD such as A30G, A30P, A53T, E46K, H50Q, G51D, and A53E in the N-terminal region have been identified and mutations of A30P, A53T, E46K, H50Q were found to accelerate the aggregation of α-Syn [20, 21]. It has also been reported [22] that the A30P mutant of α-Syn was found to have a transient change near the mutation site to form a kink-like conformation, which would influence the self-assembly of α-Syn. It is noted that changes in α-Syn can affect its function of regulating membrane fusion and thus inhibit synaptic transmission and its impacts may also contribute to various neuronal disorders to acquire a more clear understanding of α-Syn, which is critically essential [23].

Membrane interactions are also potent modulators of α-Syn's propensity to self-assemble into amyloid fibrils enhanced by several orders of magnitude in some cases through the presence of lipid vesicles [24-27]. Previous studies have shown that α-Syn occurs *in vivo* in a state of equilibrium between the membrane-bound and cytosolic forms, with membrane partition being strictly controlled [18]. During aggregation, the α-Syn and membrane interactions emphasize the significance of the interplay between different functional modes of α-Syn and its aggregation mechanism, resulting in PD [28]. Compared to the cytosolic form, the membrane-bound form has a greater propensity to aggregate, and the aggregates created within the membrane function as "seeds" to quicken the aggregation of the free cytosolic α-Syn [29]. Furthermore, changes in

the lipid composition or chemical modification of membrane lipids may also result in structural modifications to the membrane that facilitate aggregation. One such structural modification involves oxidative modification in lipids and the protein that was found to play a role in the aggregation in the membrane. Also, a common phenomenon is that in aged brains there is an increase in oxidative damage to the lipid membrane. Given its greater tendency for aggregation and seeding capacity, the membrane-bound form may represent a nucleating species. Membrane lipid oxidation may encourage α-Syn aggregation by either creating an environment that is favorable for α-Syn self-assembly or by causing oxidative alterations of α-Syn that alter the protein's structure and increase its susceptibility to aggregation. Several studies support the emerging view that the N-terminal region of α-Syn plays an anchoring role in membrane interactions by modulating the physiological and pathological roles of α-Syn [30]. In recent times, there are no available disease-modifying drugs for a larger population of approximately 10 million individuals suffering from synucleopathies. A critical stage in the oligomerization and cytotoxicity processes is interactions between α-Syn and lipids in the neuronal cell membrane [31]. Therefore, strategies to eliminate α-Syn from the membrane, quicken its breakdown and clearance, prevent aggregation, or lessen its synthesis might be useful therapeutic modalities.

1.2. Outline of the Thesis:

Chapter 2 gives us a description of IDP, Neurodegenerative disorder-PD, Epidemiology, Diagnosis, Pathology, Symptoms and causes of PD, membrane interaction with α-Syn protein and their aggregation mechanism, the effect of post-translational modification of α-Syn, therapeutic strategies including peptidomimetic and aminosterols inhibitors that interfere with membrane interaction.

Chapter 3 elaborately on the Molecular Dynamics (MD) simulation methods and different computational tools that are utilized in this thesis work.

Chapter 4 presents the conformational stability of membrane-bound α-Syn to understand the α-Syn-membrane interactions that may be useful for developing a new therapeutic approach for treating PD and other neurodegenerative disorders.

Chapter 5 characterizes the co-solute properties of the crowded intracellular environment along with the excluded volume effect to understand the α-Syn dynamics in cells. We have also demonstrated the isolation of the most probable conformer of α-Syn from structural molecular

dynamic analysis based on some critical aspects that emphasize the nature of druggability as a potential drug target.

Chapter 6 elaborates on the effects of A30G mutation in α-Syn on its association with lipid membrane and in free solution. Our findings suggested that the structure of A30G α-Syn in solution as a free monomer was noticed to be mostly unfolded, but it did show a preference for helical conformation, which may be important in the aggregation of α-Syn into fibrils.

Chapter 7 compares the conformational dynamics of α-Syn mutants (A30P, A53E, A53T, E46K, G51D, and H50Q) and their subsequent aggregation propensity in their membrane-bound state. From the MD trajectory analysis, it was evident that membrane-bound H50Q α-Syn showed the highest flexible region in the NAC domain that infers diverse effects on aggregation propensity.

Chapter 8 puts forward the potential binding position of these two drugs, NPT100-18A and NPT200-11, on α-Syn and the impact of these two drugs on the α-Syn and lipid membrane interactions at an atomistic level. Similarly, we have analyzed the effect of aminosterol inhibitors (Squalamine and its derivative Trodusquemine) on the protein-membrane interaction.

Chapter 9 provides the conformational characteristics of the truncated CTD α-Syn (1-99 and 1-108) that affect its interaction with the membrane and subsequently have an impact on the aggregation. From our findings, the truncated CTD can be suggested to modulate the α-Syn aggregation by interfering with the binding of the α-Syn protein to the membrane and providing support for the pathogenic function of CTD truncation in PD development.

Chapter 10 presents the effect of post-translational modification on the α-Syn pathogenicity. This study gave an insight into the role of phosphorylation in Tyrosine 39 (pY39), Serine 87 (pS87), and Serine129 (pS129) in protein-membrane interaction and its subsequent aggregation behaviour.

Chapter 11 presents the overall conclusion of the present study and also highlights the future aspect of the investigation.