Abstract | 2024

Parkinson's disease (PD), a complex and disabling neurological disorder, is caused by the degenerative process of nerve cells, or neurons, in the region of the brain known as the substantia nigra, which controls movement. Insoluble protein clumps, known as Lewy bodies (LB), are a pathogenic aspect of PD. α-Synuclein (α-Syn), a protein consisting of 140 amino acids, possesses an acidic C-terminal and a basic N-terminal and serves as the principal component of these fibrillar aggregates. α-Syn is known to self-associate to produce insoluble forms when it aggregates. α-Syn complex aggregation kinetics are characterised by a multi-step process that leads from soluble monomers to insoluble fibrillar aggregates via the formation of dangerous intermediate oligomeric species and protofibrils. α-synucleinopathy is characterised by the formation of circular cytoplasmic inclusions containing dense core-like structures surrounded by fibrils, known as LB, which serves as a hallmark of PD. The degeneration of neural cells is attributed to these misfolded proteins. The first Lewy body observed in PD typically occurs outside the basal ganglia. These cells are an essential component of the basal ganglia, which are in charge of animal motor coordination. Loss of these cells leads to the development of akinesia, stiffness, postural instability, and tremor in both humans and non-human animals. These symptoms worsen as the rate of neuronal loss in this area increases. Globally, the reported crude prevalence rates of PD range from 15 to 12,500 cases per 100,000 people, with reported incidences of 328 cases per 100,000 people. The second most common neurodegenerative illness, PD, is predicted to impact more than 7–10 million individuals globally by 2030. The burden this disease places on patients, their families, and caretakers not only increases with time but also develops severely over the course of time. A primary challenge associated with this condition is its tendency for misdiagnosis, making it easily overlooked. Although there is presently no cure for PD, there are a number of medications that can help manage symptoms and relatively preserve quality of life.

James Parkinson first described PD in 1817, but despite the complexity of the illness, the molecular sequence of events that ultimately led to PD has never been identified. A multitude of genes, environmental exposure, gene-environment interactions, and their combined impact on the ageing brain are all part of the multifactorial aetiology of the illness, which has both genetic and environmental causes identified. While most cases of PD are sporadic, the recognition of hereditary forms of the condition has been crucial for elucidating the cellular mechanisms involved in its pathophysiology. Currently, there is no known cure for PD, and the available options primarily focus on alleviating symptoms using commercially available herbal and synthetic medications.

Furthermore, the current treatments are unable to stop the progression or reverse it. The pathophysiology of PD has been associated with the dysfunction and aggregation of the neuronal protein α-Syn. This protein is predominantly present in soluble and membrane-bound states at the presynaptic terminal. It binds to synaptic vesicles (SVs) to control both its normal activity, which is involved in neurotransmission, and aggregation. Apart from aggregating in association with membranes, α-Syn can also aggregate independently in solution. The existence of these two distinct aggregation pathways poses challenges in developing therapeutic agents that effectively target both pathways.

In the initial part of the thesis, the interaction between α-Syn and phospholipid membrane bilayers was extensively explored using all-atom molecular dynamics (MD) simulations. When α-Syn is in its membrane-bound state, it adopts specific α-helical structures that prevent the secondary α-helical structure from transitioning into a coiled shape. Throughout the simulation period, subtle conformational changes were observed in α-Syn, particularly in the NAC region extending from the membrane surface. These changes are believed to enhance the aggregation propensity of α-Syn in its membrane-bound state.

Biophysical characterization of conformational changes in proteins has predominantly been studied in dilute and less dense media. However, proteins execute their biological functions within highly crowded cellular environments. The impact of crowding conditions within cells on the equilibria and transition rates of various protein conformations remains poorly understood. Interestingly, α-Syn exhibits a stronger crowding effect in the presence of ethanol. The effects of different ethanol concentrations on the structural conformation of α-Syn were analyzed. It was observed that conformational changes and fluctuations in α-Syn gradually decreased with increasing ethanol concentration, the crowding agent. Our findings suggest that the predominant higher helical conformation in α-Syn, observed at higher ethanol concentrations, plays a role in preventing fibrillation. This structural characteristic may induce a conformation that limits fibrillation, as proposed earlier. As α-Syn is an intrinsically disordered protein (IDP), the structural binding pockets of IDPs and their adaptability present challenges for conventional druggable strategies. To get a better sample of conformers, we have performed MD simulations on the α-Syn protein, considering its extremely dynamic character. Recent studies suggest that the druggability feature of α-Syn will make it a more desirable target for drug development in the near future. Hence, the most probable druggable conformers are based on MD structural parameters such as radius of gyration (Rg), solvent-accessible surface area (SASA), and secondary structure. It was evident that the conformers with lower values of Rg have a greater number of binding

pockets. Conformers with a lower Rg value, a lesser solvent-accessible surface area, and a higher standard secondary structure content are more likely to possess druggable features. In the second part of my thesis, the effects of α-Syn mutations such as A30G, A30P, A53E, A53T, E46K, G51D, and H50Q on the N-terminal domain were discussed. A comparative analysis was conducted between the recently identified heterozygous A30G α-Syn mutant in its free state and the A30G α-Syn mutant in its membrane-bound state. In its free form, A30G α-Syn is destabilized due to a decrease in the secondary helical structural content. A30G α-Syn in a membrane-bound state is attached evenly to membranes as a single entity; different areas of α-Syn exhibit various interaction modes. The structural variation of A30G α-Syn in membrane-bound and in free form as a function of simulation time depicts the dynamics along with the folding, unfolding, and helical formation in the structure. The MD analysis of the other α-Syn mutants (A30P, A53E, A53T, E46K, G51D, and H50Q) determined the order of their ability to adapt to the biological membranes and the rate of change in the conformation of the α-Syn mutant. The H50Q α-Syn has the most stable conformation among other mutants and therefore influences the aggregation propensity of the α-Syn structure.

The third part of the thesis describes the two peptidomimetic inhibitors (NPT100-18A and NPT200-11) that could potentially interact with α-Syn and affect the interaction of α-Syn with the membrane. It was shown how the two peptidomimetic inhibitors affected the interaction between the membrane and α-Syn. The two inhibitors were optimised using density functional theory (DFT) simulations. The results showed that NPT100-18A and NPT200-11 had respective nucleophilicity (N) values of 3.90 and 3.86 and electrophilicity (ω) values of 1.06 and 1.04. It was also observed that the NPT100-18A inhibitor molecules were distributed shallower in the membrane (from -20 Å to 20 Å), indicating that the molecules are oriented parallel to the membrane surface. On the other hand, the NPT200-11 inhibitor molecule was discovered to be distributed somewhat deeper (-30 Å to 35 Å) within the membrane. Based on the ligplot analysis, the interactions observed between NPT100-18A and the membrane bilayer included ol143, ol146, ol172, pe172, ol275, ol164, ol141, ol218, ps157, ps142, pe217, and ol143. Similarly, NPT200-11 showed interactions with the membrane bilayer, including ol243, ol141, ol146, pe145, ps142, ol1820, ol143, ol171, and ps157. Developing a disease-modifying approach to treat PD and other neurodegenerative disorders may be aided by the findings of this study on α-Syn-membrane interactions in the presence of two peptidomimetic inhibitors. Another aminosterol inhibitor molecules

(Squalamine and Trodusquemine) are believed to inhibit the early stages of aggregation, which displaces α-Syn from the lipid vesicle surfaces. The inhibitor molecules Squalamine and Trodusquemine were determined to have an electrophilicity (ω) of -0.84 and -0.68, and a nucleophilicity (N) of 3.25 and 3.18 respectively. The band gap between HOMO and LUMO ($\Delta E_{HOMO-LUMO}$) of the inhibitor molecules Squalamine and Trodusquemine were calculated to be -126.9 and -138.3 respectively. The α-Syn in the presence of the Trodusquemine molecule (ΔG_{bind} = -33.6 kcal/mol) was shown to be energetically favourable as compared to the Squalamine molecule (ΔG_{bind} = -11.09 kcal/mol). From the conformational snapshots of the α-Syn-Squalamine complex, the α-Syn showed stability and maintained its integrity, which was evident. Also, the binding of Trodusquemine was found to be stronger with the C-terminal region of α-Syn.

The C-Terminal Domain (CTD) of α-Syn functions as a guard to control the normal functioning of α-Syn. But if the CTD is truncated, the N-terminal of α-Syn may interact with the membrane, altering the normal function of α-Syn, which will all have an impact on the aetiology of PD. In this study, the conformational dynamics of CTD-truncated α-Syn (1-99 and 1-108) monomers and their effect on the protein-membrane interactions were demonstrated. α-Syn (1–108) was observed to have more intermolecular hydrogen bonds between the membrane and the non-amyloid β-component (NAC) region and fewer extended strands than the other truncated monomer, according to the MD analyses. It was also found to be more stable. The binding environment of the truncated CTD α-Syn (1-99 and 1-108) in the bilayer is substantially changed since Helix-N of the truncated CTD α-Syn (1-99) ends up at a deeper depth of 8 Å underneath the lipid head group phosphates than the truncated CTD α-Syn (1-108).

Post-translational modifications (PTM) in proteins affect interactions with membranes and synaptic processes. Phosphorylation at specific sites at pS87 and pS129 influences α-Syn function and neuropathology by regulating aggregation, oligomerization, and vesicle control. Earlier studies suggested that mimicking phosphorylation at pS129 may have an inhibitory effect on α-Syn aggregation and thus control α-Syn neuropathology. Also, the phosphorylation at position pS87 is increased in synucleinopathies, inhibits α-Syn oligomerization, and affects the interaction between α-Syn and the membrane. Tyrosine 39 phosphorylation is vital for α-Syn's role in synaptic vesicles, impacting normal and abnormal functions. Based on the MD investigation, we have shown that α-Syn's interaction with the

lipid membrane is significantly impacted by phosphorylation at the Tyrosine 39 site. pY39 α-Syn triggers aggregate formation by interfering with the helix-2 binding site and initiating α-Syn unfolding. The phosphorylated pS87 α-Syn exhibited poor flexibility and fluctuations, whereas the amyloidogenic NAC region demonstrated reduced interaction with the membrane and with the other α-Syn structural areas. Furthermore, pS87 phosphorylation alters α-Syn conformation, lowering lipid vesicle affinity. Disruption of the helix-2 binding area, therefore, has the potential to influence binding to the lipid membrane and assume control of interactions with other proteins or vesicles. The NAC region also shows little connection with other protein areas and is not incorporated into the lipid bilayer, according to the examination of intermolecular hydrogen bonds. Our results highlight key characteristics of pS129 alterations that prevent α-Syn from aggregating. Our MD simulation study highlights pS129 phosphorylation's role in stabilizing the secondary helical structure of α-Syn by reducing hydrophobic binding sites.