CHAPTER 2

INTRODUCTION AND REVIEW OF LITERATURE

Introduction and Review of Literature

2.1. Protein folding and misfolding:

The structure of a protein determines its functional characteristics. The order of amino acids also reveals the structure of a protein. In addition to the different components that constitute the protein folding mechanism, the amino acid sequence of a protein is crucial in passing on the information necessary for native folding and function. Nonetheless, a given sequence can generate numerous large conformations. Also, the intermediate conformational stages that are reached by different sequences are the primitive conformational stages for lowering additional conformational stage complexity [18]. Due to the functional state of a protein, the native interactions that exist between amino acid residues are the lowest-energy ones. And this "lowest level energy" controls the protein folding energy landscape [19]. The "properly" folded polypeptide chains are obtained through a pool of smaller intermediate states during the folding process. In the energy landscape, the sequence of a protein consists of a small number of states, and the energy level is also quite low **(Figure 2.1)**. This energy level concept, when combined with the phenomenon of natural selection permits the evolution of a large number of amino acid sequences that facilitate the quick and efficient folding of proteins [18, 20].

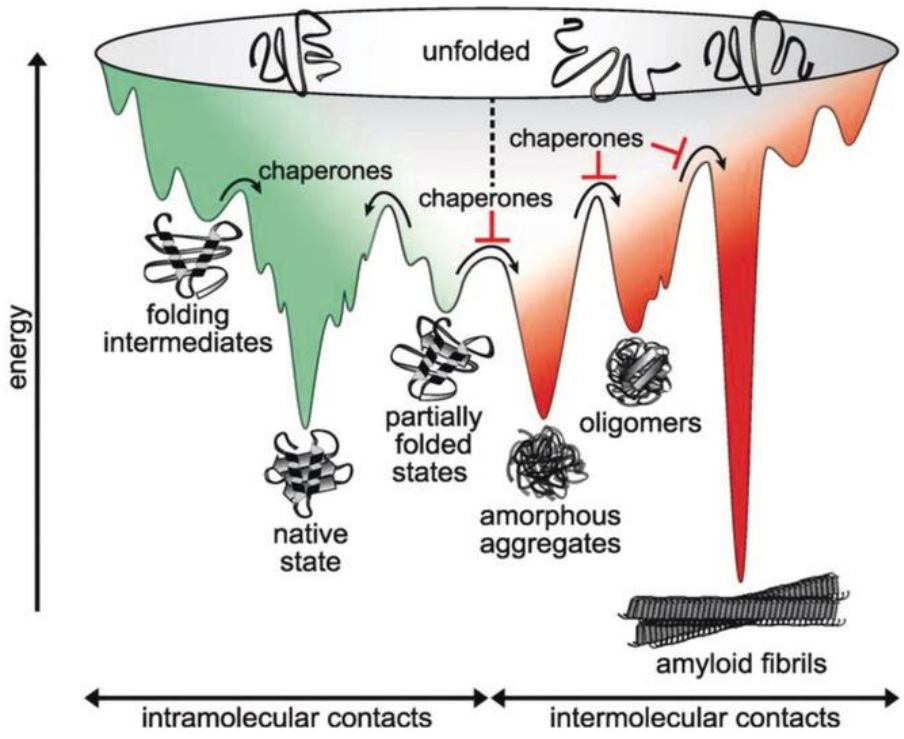

Figure 2.1. *Energy landscape of protein folding and misfolding. (Taken from [20]).*

Under certain conditions, the energy landscape enables misfolded proteins to reach a functional state, as opposed to native proteins. Prior to our current understanding, various diseases develop when a protein degrades to a level of energy that is not regarded to be functional. This is because the protein develops a novel and hazardous function during the misfolding process; otherwise, the capacity to conduct regular tasks is lost.

2.2. Protein aggregation:

Misfolded proteins have a tendency to aggregate, resulting in protein aggregation. Protein aggregation can have harmful effects on patients suffering from a variety of conditions, including prion diseases, amyloidosis, and other protein deposition disorders [20-21]. The protein aggregation is well associated with the different protein folding, molecular chaperones and also the stability. In fact, the protein aggregation mechanism is quite significant, yet its predominance is generally overlooked in protein folding experiments [22]. Protein aggregation is becoming increasingly acknowledged, as indicated by numerous research evaluations [23-29]. The term 'aggregation' often refers to pathogenic protein aggregates that contain insoluble precipitate production. This creation is in fact the opposite of the insolubility in the native state, which is mostly the result of protein concentrations exceeding the solubility limit. It is also attributable to the establishment of intermolecular associations, which is related to the synthesis of native oligomers. However, the initial substance that may be generated during pathological aggregation is soluble aggregates that become insoluble as their size increases. **Figure 2.2** depicts a flowchart describing the path of protein aggregation and **Figure 2.3** depicts the factors that affect the protein aggregation mechanism.

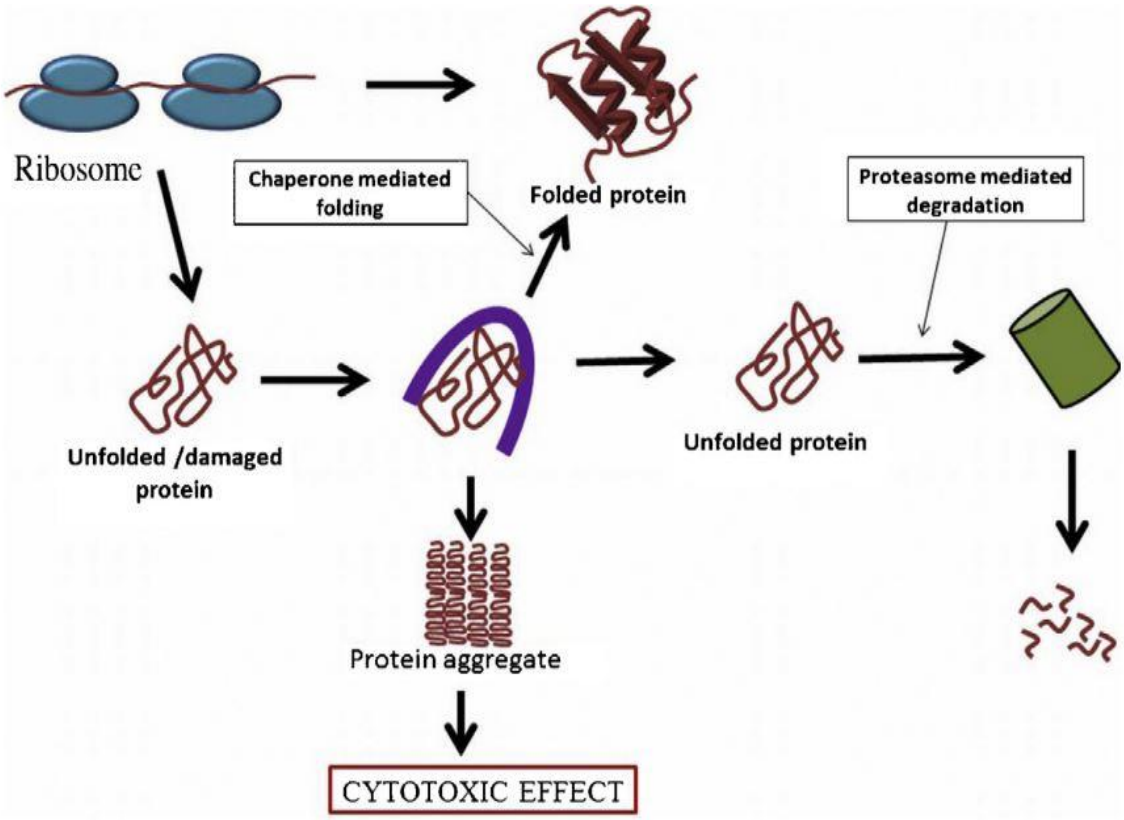

Figure 2.2. *Schematic representation of protein aggregation mechanism (Taken from [22])*

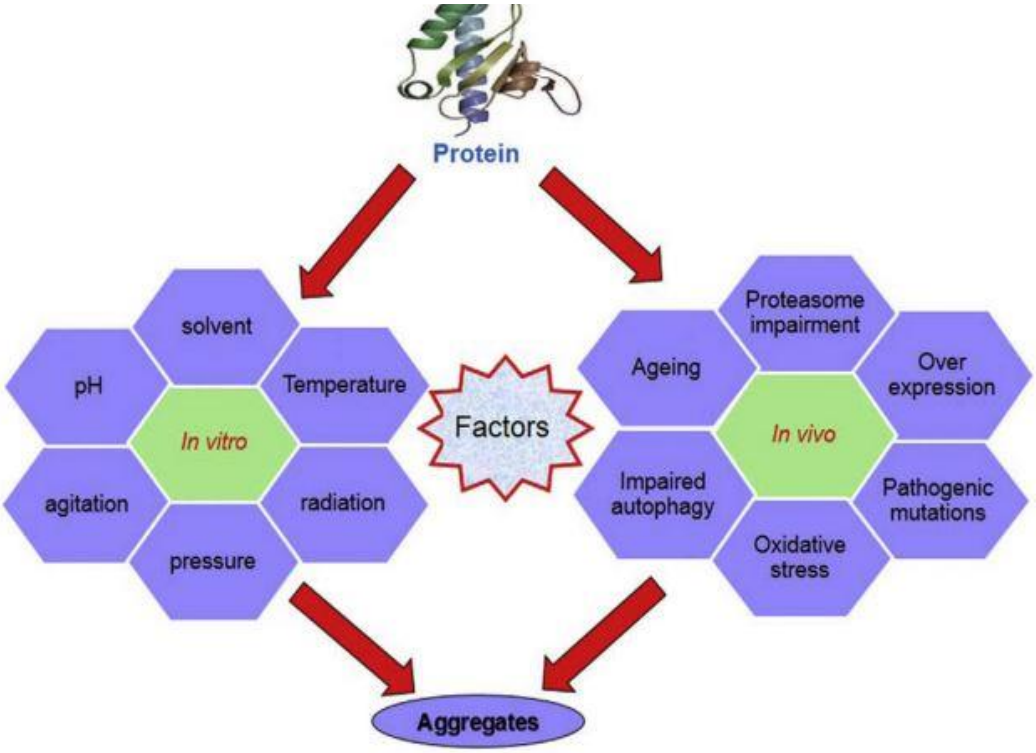

Figure 2.3. *Factors affecting the protein aggregation mechanism (Taken from [22])*

Therefore, it is vital to classify protein aggregation into distinct categories, such as *in vivo*, *in vitro*, orderly, and disordered [30]. Ordered aggregates are comprised of both *in vivo* and *in vitro* amyloid fibrils, whereas disordered aggregates are comprised of *in vivo* inclusion bodies. Disordered *in vitro* aggregates form during denaturant-unfolded protein refolding at high protein concentration or at high protein concentration under weakly native circumstances, which are generally referred to as the folding aggregates. Under some conditions, these folded native proteins can combine, resulting in salting out and isoelectric precipitation (net charge is zero). In accordance with their solubility in the buffer, these precipitates can be distinguished from their pathogenic aggregates under a variety of natural settings. In contrast, pathogenic aggregates may be capable of dissociating and dissolving in the presence of a detergent or denaturant at a higher concentration. This aggregation process involves either the native or unfolded state. Inclusion bodies and other aggregates are created as a result of the hydrophobic aggregation of the denatured and unfolded states of proteins during the mechanism of protein folding. Recent investigations indicate, however, that aggregation may proceed from the exact partially folded intermediates [30]. Multiple causes and environments enable aggregation, which is a crucial component that aids the intermediate population. Consequently, these characteristics contribute to the determination of aggregation tendency. Diverse studies indicate that the temporary aggregation that occurs during *in vitro* protein refolding may be mistaken for transient intermediates [22, 31-34]. In relation to the aggregating procedure, numerous questions remain unanswered. These questions are about the nature of the species that induces the aggregation mechanism, the various factors responsible for aggregation in detail describing the protein aggregates structure, the inter-molecular interactions specificity, the cause of formation of ordered and disordered aggregates, the environmental factors responsible for aggregation, and the prevention of the aggregation mechanism. In order to avoid sequences with a high propensity for aggregation, evolution of proteins has occurred, as evidenced by the data. Short peptide sequences that are more susceptible to β-sheet formation and also contain numerous hydrophobic residues have a strong tendency to form aggregates and amyloid fibrils. It is known that the polar flanking residues enhance the solubility limit, which can sterically avoid the interactions that result in a lack of aggregation and amyloid formation. A plethora of neurodegenerative disorders, such as AD and PD, are caused by protein aggregation. Neurotoxicity occurs from hazardous gains of function in multimeric aggregates of primarily monomeric and disordered proteins at the molecular

level. They oscillate between structurally distinct states on the microsecond and nanosecond timeframes. These proteins, known as **Intrinsically Disordered Proteins (IDPs)**, are difficult to examine using conventional biophysical techniques.

2.3. Intrinsically disordered protein and its link to protein aggregation:

Intrinsically Disordered Proteins (IDPs) are proteins that do not have a proper three-dimensional structure but have a significant effect on vital processes such as translation, transcription, cell signaling, and cell cycle regulation. The majority of IDPs experience protein aggregation. The physicochemical properties of IDPs/IDRs are typically negatively correlated with those of aggregation-prone sequences [32-34]. By executing the various processes, it is possible to adopt diverse interactions with varying outcomes from a single polypeptide [35-36]. Upon binding to their partners, IDPs undergo a transition from disorder to order [37-39]. Although they are functional, they cannot rapidly adopt a well-defined, globular 3-D orientation. However, through a variety of conformations, they can fluctuate rapidly, resulting in a continuous conformational space that ranges from extended statistical coils to a disordered collapsed globule [36]. It is also known that they influence the assembly of structured macromolecular machines [38-42]. In addition to IDPs, there are also Intrinsically Disordered Regions (IDRs) containing disordered sequences. They have the capacity to serve as hubs for the various protein interaction-based networks [43-44]. However the protein abundance is being regulated tightly in order to establish the signaling in space and time and for the mutations involved in IDP that further connect to the various diseases [45-47]. Consequently the order-to-disorder transition state can be controlled by numerous folded proteins which can bring about the many biological functions [48-49]. The main goal of the post translational modification (PTM) involved in the IDP is either to stabilize or destabilize the sole components of the secondary structure elements [50-52]. These IDPs can bind transiently to the numerous interacting partners in the regulatory networks that are dynamic in nature. However these networks might contain the ability for having certain complex information that can execute by carrying out the signaling functions [53]. The PTMs permits the interaction of IDPs and binding partners that helps in working them as switches and rheostats [36, 54-58]. Several neurodegenerative illnesses manifest from these IDPs as a result of the protein misfolding mechanism.

2.4. Neurodegenerative disease:

Neurodegenerative diseases are a large group of conditions characterized by progressive structural and functional deterioration of the Central Nervous System (CNS) or Peripheral Nervous System (PNS). Due to the recent increase in the elderly population, the number of neurodegenerative disorders is constantly growing [59]. The patho-physiology of these illnesses is diverse, ranging from memory and cognitive deficits to impairments in movement, speech, and breathing [60-63]. An in-depth study of the origins and processes of each illness will pave the path for successful therapies [64-66]. The aggregation of insoluble proteins as extracellular or intracellular deposits is a common feature of all neurodegenerative disorders, and it generally comprises of IDPs or Intrinsically disordered protein regions (IDPRs). Numerous proteins, including α-Synuclein (αS), Tau, and the islet amyloid polypeptide (IAPP), are largely unstructured in solution and are frequently described as natively unfolded or intrinsically disordered. However, it is believed that many of these proteins fold into well-defined structures upon interaction with specific binding partners. Proteolysis of larger, normally folded proteins can also generate intrinsically disordered structures, such as the Aβ peptide and the gelsolin amyloidogenic fragment. IDPs/IDPRs have been linked to a variety of human illnesses [67-69], notably neurodegenerative diseases [70, 71]. IDPs and IDPRs are functional proteins or protein portions that lack organized three-dimensional structures [72, 73]. They have the ability to bind to numerous partners, allowing them to act in regulation, signaling, and control. **Table 2.1** summarizes the numerous neurodegenerative disorders that result from the misfolding of proteins. Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS), Fronto-temporal dementia (FTD), and Spinocerebellar ataxias (SCA) are only a few examples of neurodegenerative illnesses.

Table 2.1. *Neurodegenerative diseases associated with the protein misfolding mechanism (Modified from [71]).*

Types of Neurodegenerative diseases	Genes responsible for the diseases	Primary component	Origin of site
Parkinson's Disease (PD)	α-Synuclein *SNCA* Parkin *PINK1* *UCHL1 DJ1*	α-Synuclein	Intra-cellular Lewy Bodies
Alzheimer's Disease (AD)	APP Presenilin 1 Presenilin 2	Aβ peptides (Aβ40, Aβ42) Hyperphosphorylated Tau	Extra-cellular senile plaques Intra-cellular neurofibrillary tangles
Dementia with Lewy Bodies (DLB)	-------	α-Synuclein	Intra-cellular Lewy Bodies
Huntington's Disease	*IT15* (huntingtin)	Mutant huntingtin	-------
Polyglutamine Diseases	---------	---------	Cytoplasmic and nuclear inclusions
Familial amyotrophic lateral sclerosis	*SOD1*	Mutant SOD1	Intra-cellular inclusions
Spinocerebellar ataxias (SCA1-3, 7)	ataxins	Mutant ataxin	---------
Spinal and bulbar muscular atrophy	Androgen receptor	Mutant androgen receptor	---------

2.5. Alzheimer's Disease (AD):

AD is a progressive neurological disorder which has been increasing at an alarming rate over the years, effecting large numbers of people worldwide [74]. It causes degeneration and death of brain cells. The formation and accumulation of Amyloid-Beta (Aβ) peptide, commonly referred to as senile plagues in the brain, and flame-shaped neurofibrillary tangles of the microtubule-binding protein tau [75,76], which is said to be associated with oxidative stress in the brain and peripheral nervous system, are the two major histopathological biomarkers of AD [77]. AD is defined by dementia: a progressive loss in cognitive, behavioral, and social abilities, which significantly impairs daily functioning. Age is one of the risk factors for the development of AD. It primarily affects adults over the age of 65, with younger people being affected only in rare cases [78, 79].

2.5.1. Background of AD:

Dr. Alois Alzheimer discovered AD in 1906 [80]. The disease was first identified in a 51 year old lady, Mrs. Auguste Deter where Dr. Alzheimer found shrinkage of the cerebral cortex and atrophied brain cells coupled with senile plaques and Neurofibrillary Tangles (NFTs) in her brain at autopsy which eventually became the pathological characteristic of AD [81, 82]. Memory deterioration, aphasia, speech difficulties, psycho-social incompetence, and disorientation worsened over her final years. Dr. Alzheimer discussed Mrs. Auguste Deter's condition in 1907 at the 37th Conference of South-West German Psychiatrists in Tubingen, and Krapelin, his supervisor, named AD in 1910 in the eighth edition of Psychiatrie [83]. Alzheimer's and dementia are often used interchangeably, yet they are different. Memory loss or other mental abilities that influence daily life are called dementia. In the late 1960s, AD was termed dementia [84, 85]. In 1964, researchers discovered the mutation that causes hereditary AD [86, 87]. These investigations identified AD as a distinct disease and showed that removing other dementia causes and monitoring symptom progression may diagnose AD. In 1984, George Glenner and Cai'ne Wong sequenced β-amyloid, the main component of Alzheimer's brain plaques [88]. Tau, the second pathological characteristic of AD, was discovered in 1986 [89]. In 1987 the APP gene was found on chromosome number 21 that produces Beta (β) amyloid. In 1987, the National Institute on Ageing (NIA) and the Alzheimer's Association began the first AD medication clinical trial with Pfizer [90]. AD is complicated, making clinical trials difficult. **Figure 2.4** compares neurons in normal and AD brains.

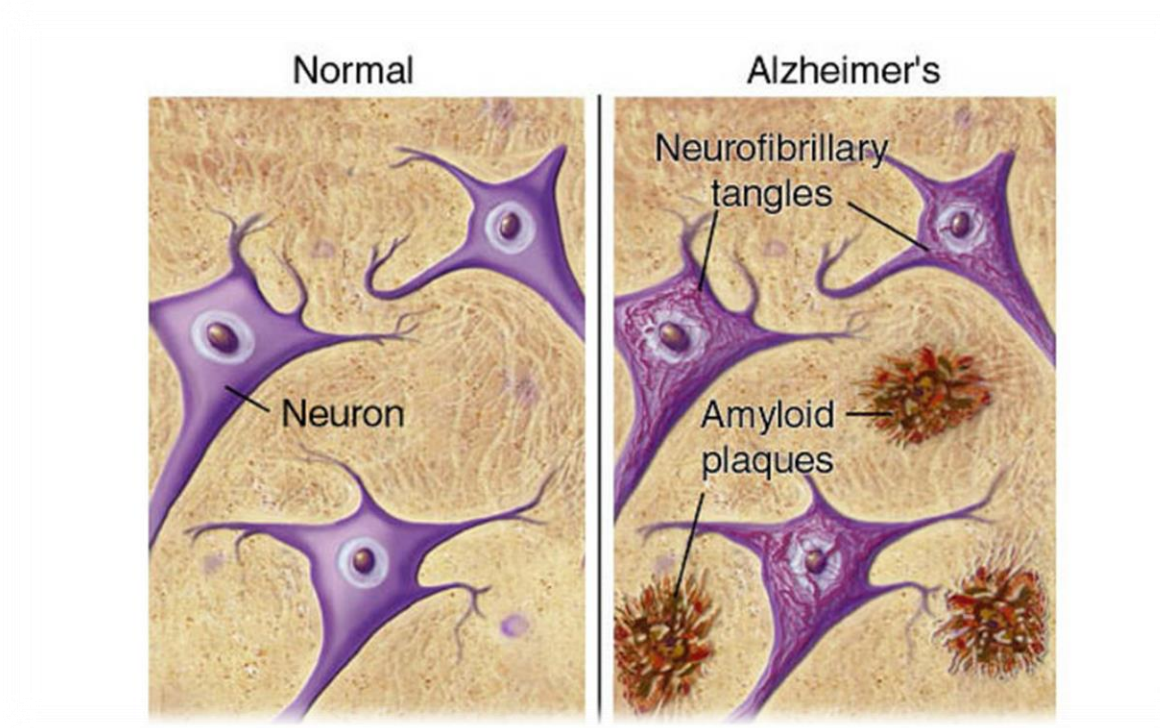

Figure 2.4. *Normal brain vs. Alzheimer's disease brain (taken from [91]).*

2.5.2. Occurrence and symptoms of AD:

Around 60% of those with dementia live in low and middle-income countries, according to the World Health Organization (WHO), which estimates that there are approximately 55 million cases of dementia globally (data checked in April 2023) [92]. Approximately 10 million new cases are reported annually. Multiple kinds of brain injuries and diseases can induce dementia. AD, the most common form of dementia, may be responsible for 60–70% of cases. Deaths attributable to dementia have increased steadily over time, more than doubling between 1990 and 2016 because of population growth and ageing. Deaths from AD dementia have also increased [93, 94]. According to a study on AD dementia mortality in Europe, the number of deaths from AD in individuals under the age of 50 (as indicated by diagnostic codes) more than doubled between 1994 and 2013 (41,255 deaths vs. 86,822 deaths). In 2013, the age-standardized death rate due to AD in Europe was 45.2 per 100,000. Alzheimer's Disease Facts & Statistics, 2023, an annual report from the Alzheimer's Association, reveals that more than 6 million Americans are living with AD. This number is projected to reach

approximately 13 million by 2050. By 2023, AD and other forms of dementia will cost the US economy $345 billion. These costs could approach $1 trillion by 2050 [93, 94].

Over the course of several years, the progression of AD symptoms is gradual. Sometimes, these symptoms are misdiagnosed as those of other conditions or initially attributed to ageing. The progression of symptoms differs between individuals. In some instances, other conditions, such as infections, strokes, and delirium, can worsen symptoms [95].

Generally, the symptoms of AD are divided into 3 main stages. The different stages of AD are discussed below:

(i) Early symptoms: As AD progresses, a number of additional symptoms may emerge, such as dysphagia, difficulty altering position or moving without assistance, weight loss – sometimes severe, urinary incontinence, bowel incontinence, and gradual speech loss.

(ii) Middle-stage symptoms: As AD progresses, memory difficulties will worsen. In addition to having difficulty recognizing family and friends, a person with the disease may also have difficulty remembering the names of familiar individuals. Additional symptoms may include increasing confusion and disorientation, such as getting lost, obsessive, repetitive, or impulsive behavior, delusions (false beliefs), feeling paranoid and suspicious of guardians or family members, aphasia, disturbed sleep, etc. Some persons also exhibit certain vascular dementia symptoms. At this stage, a person with AD typically requires assistance with daily duties.

(iii) Later symptoms: The symptoms of AD worsen as it progresses, which can be upsetting for the patient as well as their caretakers, friends, and family. Hallucinations and delusions may come and go during the course of the illness, but they may get worse as the condition worsens. Patients with AD are occasionally violent, demanding, and untrusting of others. Additional symptoms that may develop as AD worsens include dysphagia, difficulty changing positions or moving without help, often significant weight loss, urine incontinence, bowel incontinence, and a progressive loss of speech.

2.5.3. Occurrence of AD in India:

India, which has 1.37 billion people and makes up 18% of the global population as of 2019, is predicted to surpass China as the world's most populous country in 2023. The population is ageing quickly as well. About 15.4% of the world's population will be 60 or older by 2050, when India's population (319 million) will have increased to about 20% of its current level [96]. This demographic chart demonstrates how India's life expectancy has steadily climbed from 42.9 years in 1960 to 70.4 years in 2020. Using nationally representative data collected in India between 2017 and 2020, J. Lee et al.'s 2023 study found that 8.8 million people aged 60 and older (7.4%) were believed to have dementia. Dementia was more common in rural than in urban regions (8.4% vs. 5.3%), and it was more common in women than in men (9.0% vs. 5.8%). If frequency stays the same, 16.9 million people globally might suffer dementia in 2036 as a result of India's growing elderly population [96].

2.5.4. Causes of AD:

In an endeavour to determine the disease's origins, researchers have examined the factors that can increase or decrease a person's likelihood of developing AD. After extensive research, numerous influential factors on AD have been identified, and they are discussed here.

(i) Age: Ageing is the largest known risk factor for developing AD. Despite the fact that AD is not a component of normal ageing, the majority of AD cases are observed in individuals of 65 years or later. About 5% of people between the ages of 65 and 74 are affected by AD. The group with the highest risk increases to 50 percent for those aged 80 and older [91].

(ii) Genetics: Gene *Apolipoprotein E* (*ApoE*) has also been associated with an increased risk of AD. Forty to sixty-five percent of AD patients have at least one copy of the allele 4 of the *ApoE* gene. The *ApoE* gene participates in multiple processes, including brain development, maintenance, and repair. In addition, it distributes cholesterol and helps maintain lipid levels in the brain. According to *ApoE*, this locus accounts for over 65 percent of the inherited risk [97, 98]. This is an uncommon occurrence, as just 5% of AD patients had an early onset. Rare familial AD can be caused by mutations in three genes:

APP on chromosome 21, Presenilin 1 (PSEN1) on chromosome 14, and PSEN2 on chromosome 1 [99]. These three genes are mutated, which increases the amount of Aβ, which then forms senile plaques and destroys neurons. Less than 10% of AD patients under the age of 65 are affected, and inheritance of a single chromosomal mutation increases the likelihood of developing AD.

(iii) Concomitant illnesses: According to some hypothesis, cardiovascular diseases (CVD) accelerate the disease's progression when present and share several risk factors with AD [100]. Both the indirect effects of cardiovascular disease, which predisposes the brain to neurodegeneration, and the direct effects of vascular variables on neuronal mortality have been proposed as causes of disease development [101]. Although the precise mechanisms by which CVD is believed to contribute to or cause AD's pathogenesis remain unclear, lipid disruption is considered a key factor. Many risk factors for type 2 Diabetes (also known as Diabetes Mellitus type 2; DM2) and AD are similar [102], and it has been suggested that AD is a "type 3" member of the diabetes family of disorders [103, 104]. Even diabetes medications have been linked to reduced neuropathology in AD [104].

2.5.5. Amyloid-Beta (Aβ) peptide:

The most widely accepted theory for the origin of AD focuses on the Amyloid-Beta (Aβ) peptide, a 40–42 amino acid intrinsically unstructured protein that results from the sequential cleavage of the APP, a type 1 integral cell surface membrane protein that resembles a signal transduction receptor. **Figure 2.5** shows a schematic pathway of proteolytic cleavage of APP to Aβ peptide [105]. The parent protein, which has 695-770 amino acids, is implicated in familial AD due to mutations in the gene that codes for it and runs through both amyloidogenic and non-amyloidogenic routes. It is expressed in several cells with uncertain functions. Although its exact purpose is unknown, APP is thought to be essential for neuron development [107, 108]. Aβ peptide was discovered to be the main component of amyloid plaques in the middle of the 1980s [109]. In the year 1992, Hardy and Higgins proposed their amyloid cascade hypothesis in one of their reviews that has been highly cited [110]. Selkoe and Hardy et al. were the first to propose that Aβ peptide is the major risk factor of AD and all other phenomenon such as tau phosphorylation, vascular damage, neuronal death and finally death follows in a

sequential order, from the over-production of Aβ peptide [111, 112]. Without compromising the underlying evidences that suggest Aβ peptide to be the causative agent of AD, the research community has accepted the amyloid cascade hypothesis. However, substantial amount of evidence is increasing that shows different amyloid species with varying degrees of toxicities in different reaction pathways. One of the recurring criticisms of the amyloid cascade hypothesis is that it fails to explain why Alzheimer's is an age related disease.

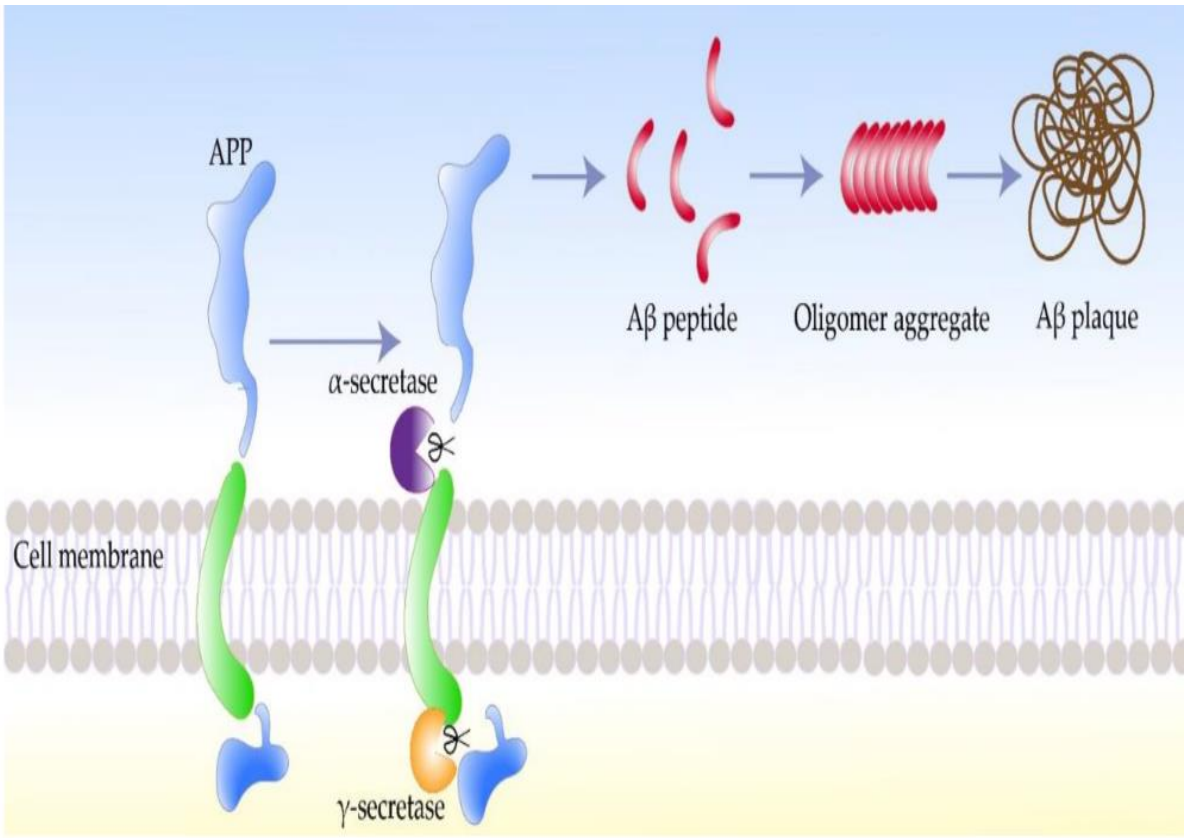

Figure 2.5. *The formation process of amyloid beta plaques from the cleavage of the APP: a hypothetical pathway (Taken from [113]).*

2.5.5.1. Production of Aβ peptide from APP:

The non-amyloidogenic pathway involves the proteolytic processing of APP by α-secretases and γ-secretases, which releases a soluble 16 amino acid P3 peptide fragment extracellularly [114]. The amyloidogenic process involves the cleavage of APP on the extracellular side of the cell membrane by β-secretase and the subsequent cleavage by γ-secretase on the intracellular side of the membrane to produce $A\beta_{1\text{-}42}$ or

$A\beta_{1-40}$ [114]. $A\beta_{1-42}$ is discovered to be the most neurotoxic of the two alloforms. The $A\beta_{1-42}$ peptide is more hydrophobic and more likely to fibrillate because it has two more amino acids in the C-terminal region [115, 116]. Human neurons are toxic to $A\beta_{1-42}$ peptide when it is intracellular. Although being neurotoxic, the $A\beta_{1-42}$ peptide only causes intracellular cytotoxicity in human neurons and not in other cell lines [117]. $A\beta_{1-42}$ peptide and $A\beta_{1-40}$ peptide are known to disrupt long-term potentiation (LTP) in neurons [118]. In an *in vivo* research, it was discovered that neurons from transgenic mice expressing mutant versions of APP or PSEN associated to familial AD (FAD) had broken synapses and lost dendritic spines [119]. Since inhibition of γ-secretase accelerates several signs of Synaptic damage, $A\beta_{1-42}$ peptide is linked to these abnormalities. Presynaptic terminal density declines and spine loss occur before amyloid plaques are formed and LTP is impaired, indicating that oligomeric or prefibrillar $A\beta_{1-42}$ peptides have an adverse effect on neurotoxicity. Further studies that focused on the role of soluble oligomeric $A\beta_{1-42}$ peptide in Synaptotoxicity and LTP suppression [120] also lend support to this.

2.5.5.2. Functions of Aβ peptide:

Aβ is best known as the misfolded peptide that is involved in the pathogenesis of AD. Because of this popularity, the evidence that it fulfils various key physiological functions has been largely overlooked. It has been discovered in every vertebrate that has been investigated to this day, and its molecular sequence demonstrates a remarkable degree of conservation. Aβ is present throughout their whole life cycle. This hypothesis has been reinforced by evidence of functions that are advantageous for the brain, which demonstrates that these characteristics are indicative of a factor that makes a major contribution to a person's biological fitness. It is hypothesized that Aβ plays several important roles in the body, including warding off infections, mending holes in the Blood-Brain Barrier (BBB), hastening healing after trauma, and controlling synaptic activity. *In vitro* and *in vivo* studies have shown that the cellular production of Aβ rapidly increases in response to a physiological challenge and often decreases upon recovery, providing evidence for the beneficial roles of Aβ in the body [150, 151]. These studies have also shown that Aβ plays a role in the recovery process. These responsibilities are confirmed even further by the unfavourable results of therapeutic trials that aimed to reduce levels of Aβ in order to cure AD.

2.5.5.3. Monomers of $A\beta_{1-42}$ peptide:

The Aβ monomers have a molecular weight of 4514.1 Da for the $A\beta_{1-42}$ peptide and 4329.9 Da for the $A\beta_{1-40}$ peptide, respectively, and are about 1.0 ± 0.3 nm in size [121]. Structure-based research has demonstrated that α-helical or random coil conformation of $A\beta_{1-42}$ monomers changes their conformation and adopts partial structure during the slow nucleation phase [122-124]. It is generally known that the initial mechanism leading to neurotoxicity is the self-assembly of the $A\beta_{1-42}$ peptide to generate the first hazardous molecule after the initial misfolding. The Central Hydrophobic Core (CHC) region and the C-terminal area of $A\beta_{1-42}$ monomers exhibit bias towards the development of the β-strand, according to experimental findings [125]. Random coils were the dominant features in analyses of the far-UV CD spectra for the $A\beta_{1-40}$ and $A\beta_{1-42}$ monomers [126]. The difficulties and constraints that experimental methods for analyzing the aggregation-prone $A\beta_{1-42}$ monomers encountered have prompted the employment of computational methods to thoroughly examine the conformational dynamics of these peptides. To examine the first conformational changes of the $A\beta_{1-42}$ peptide and to determine its transitory states, multiple computational studies and methodologies [127–130] have thus been utilized. The monomeric structure of the $A\beta_{1-42}$ peptide has been the subject of numerous modelling studies, but fully characterizing its structure remains a significant difficulty.

2.5.5.4. Dimerization of $A\beta_{1-42}$ peptide:

Until now, it has been impossible to classify the soluble $A\beta_{1-42}$ peptide species without in-depth knowledge of their structure and assembly pathways. In contrast, aggregates of soluble $A\beta_{1-42}$ peptides are typically referred to as protofibrils or oligomers [131]. They are believed to be "on-pathway" intermediates in the formation of amyloid fibrils and to ultimately transform into fibrillar structures. $A\beta_{1-42}$ peptide aggregates to neurotoxic forms that are distinct from one another. The mechanism of toxicity may vary between substances. $A\beta_{1-42}$ peptide dimers, the smallest oligomer isolated from neuritic amyloid deposits, have been reported to manifest neurotoxic properties in the presence of microglia [132]. During the dimerization process, $A\beta_{1-42}$ peptide aggregates and forms a structure abundant in cross- fibrils, with each monomer interacting with its adjacent monomer to form a dimer [133, 134]. Once dimers are formed, monomers use them as

building blocks to construct oligomers. Consequently, a dimer offers the first opportunity to study intermolecular interactions. Since the flexibility of $A\beta_{1-42}$ peptide makes it difficult to investigate the aggregation process, it is believed that structural rearrangements caused by intermolecular interactions are a crucial step in the fibrillation pathway [134].

2.5.5.5. Oligomers and fibrils of $A\beta_{1-42}$ Peptide:

Oligomers are said to be the hazardous agent, and it is believed that they are generated during the beginning stages of $A\beta_{1-42}$ peptide self-assembly [135-137]. The dynamic equilibrium that exists between these species can be inferred from the presence of $A\beta_{1-42}$ peptide oligomers that are contained within plaques. Within human neurons, $A\beta_{1-42}$ peptide oligomers have been discovered to be present [138]. Using total internal reflection fluorescence microscopy [139], researchers were able to determine the oligomerization status of the $A\beta_{1-42}$ peptide on the membrane by detecting individual Aβ species on the surface of murine hippocampus neurons. Although there have been an increasing number of studies conducted to understand the oligomeric structures of $A\beta_{1-42}$ peptide, [140, 141] a conclusive X-ray diffraction or 3-D NMR structure of an $A\beta_{1-42}$ peptide oligomer has not yet been determined [142]. This is despite the fact that there have been an increasing number of studies carried out to understand these structures. The structure of $A\beta_{1-42}$ peptide oligomers was anticipated to have a hydrophobic core and a hydrophilic surface in an MD simulation study that was carried out by Yu and colleagues in an environment consisting of lipid membranes [143]. Eisenberg and his colleagues developed a new kind of amyloid oligomer that has a mature cross-β structure. In this structure, the side chains of amyloid molecules enter neighbouring β-sheets to keep the molecules attached to one another. TABFO stands for toxic amyloid-beta fibrillar oligomer [144]. TABFOs are not short protofilaments and so are unable to seed the formation of new amyloid fibrils, despite the fact that they have a structural likeness to amyloid fibrils. Numerous studies have shown that oligomeric structures formed by $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides are comparable but not identical. The C-terminal end of the $A\beta_{1-42}$ peptide contains two additional amino acids, which results in a wider variety of interactions [145]. These findings have been presented in a number of studies. In spite of this, developing inhibitors that target one or more oligomers continues to be difficult since atomic-level resolution of oligomer structures is not yet available. Although though

structural studies on $A\beta_{1-42}$ peptide oligomers have been conducted, there is still very little knowledge on the beginning steps of oligomerization. In spite of the fact that $A\beta_{1-42}$ peptide has a poorly defined monomeric structure, researchers have recently been able to derive the structural knowledge of amyloid fibril from a variety of experimental studies that provide information on molecular fold and intermolecular packing (β-sheet formation and organization). This has been made possible by recent advances in the field. The "cross-β" structure of amyloid fibril is determined by fibre diffraction experiments. Its structure is characterized by the assembly of $A\beta_{1-42}$ peptide molecules into β-sheets, with β-strands orientated in a direction that is perpendicular to the long axis of the fibril [146-149].

2.5.6. Effects of Mutations Linked with Inherited AD:

Around 90–95% of AD cases are sporadic, whereas 5–10% are Familial Alzheimer's Disease (FAD) cases, which typically occur before the age of 65. Three genes have so far been found to be the source of FAD mutations, one of which codes for the protein APP. However, only 1% of FAD instances have been linked to APP gene mutations. The PS1 and PS2 genes [152, 153], which are the proteolytic subunits of the γ-secretase complex responsible for producing the C-terminus of Aβ, are mutated in the vast majority of FAD patients. In both the Aβ sequence and outside it, the APP has FAD mutations. Outside-Aβ mutations encourage the breakdown of APP by proteases and either increase total levels of Aβ (Swedish mutation) or the ratio of $A\beta_{1-42}$ to $A\beta_{1-40}$ (French mutation) [154–156]. Around 150 variants in APP, PS1 and PS2 have been found to date, and they all have an impact on how well APP is processed, either by enhancing cleavage effectiveness or by favouring $A\beta_{1-42}$ formation [157]. The gene dosage effect in individuals with trisomy 21 is the most notable illustration of higher Aβ concentration [158]. Current research demonstrates that duplication of the APP gene locus can also cause FAD [159]. In conclusion, all FAD mutations that do not alter the amino acid sequence of Aβ itself still favour the formation of Aβ amyloids through elevated intracellular Aβ concentrations or a higher proportion of $A\beta_{1-42}$, the form of Aβ that is most likely to form amyloids. As a result, seeds of $A\beta_{1-42}$ may cause $A\beta_{1-40}$ to fibrillize *in vivo*, which is consistent with the idea that an elevated $A\beta_{1-42}$/ $A\beta_{1-40}$ ratio may be connected to the majority of sporadic AD cases [160]. Several FAD-linked amino acid substitutions have been found in the Aβ sequence itself. Some, such are the

Dutch (E22Q), Italian (E22K), Iowa (D23N), Flemish (A21G), and Arctic (E22G) mutations, primarily affect residues 22 and 23. Arctic, Dutch, Italian, and Iowa variations of Aβ have been shown to congregate more quickly than Aβ wild type [159-162]. The Flemish mutation decreases fibrillization, yet in cell culture, intermediates of the Aβ variety bearing this mutation have been found to be both more soluble and lethal than wild-type Aβ [163]. The arctic mutation serves as an illustrative example of how these changes affect $A\beta_{1-42}$ levels, inclination to assemble *in vitro*, and protofibril fraction [158, 164]. Being heterozygous means that all FAD patients with amino acid substitutions in Aβ generate a mixture of wild-type and mutant Aβ. The two Aβ peptide variants appear to have distinct fibrillization mechanisms. In *in vitro* aggregation reactions with equimolar amounts of wild-type and mutant Aβ, it has been reported that wild-type Aβ is excluded from protofibrils and fibrils; furthermore, the morphology and size distribution of aggregation intermediates seem to differ for both Aβ forms [164], whereas, for example, human and mouse Aβ coaggregate and form heteropolymers [165]. The known Aβ variants with mutations at positions 22 and 23 and wild-type Aβ appear to have different neurotoxic properties from $A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers. The difference between Aβ variations and the wild type with equal length in cell culture is greater than the difference between wild-type $A\beta_{1-42}$ and wild-type $A\beta_{1-40}$ in terms of toxicity. The aggregation propensity of Aβ correlates with the hydrophobicity and β-sheet propensity of residues 41 and 42, as demonstrated by variation of these residues [166, 167].

2.5.7. Inhibitors of $A\beta_{1-42}$ peptide aggregation and current treatment of AD:

Despite the fact that there is no cure for AD, there are some symptomatic medications that are still only a hope. Further doom has been added to the picture by the failure of two Phase III therapeutic trials involving two Aβ-targeting monoclonal antibodies, bapineuzumab and solanezumab, in individuals with mild-to-moderate AD [168]. The genetic evidence makes it abundantly evident that Aβ drives the illness process, making it an appealing goal to reduce its creation or promote its clearance in the brain. The development of medicines intended to stop $A\beta_{1-42}$ peptide aggregation has been the focus of intensive medicinal chemistry research in recent years [169]. Several substances that contain carbohydrates [170, 171], polyamines [172, 173], chaperones

[174], metal chelators [175], osmolytes [176], and RNA aptamers [177] have all been proposed as potential inhibitors of Aβ fibrillogenesis. Moreover, it has been shown to disassemble pre-formed amyloid fibrils utilizing tiny organfluorine molecules and light. Over time, interest has grown in the biological use of degrading enzymes [178, 179], anti-aggregating molecules [180], nanoparticles [181–183], and antibody molecules [184] as treatments for disease. Some of the agents that have been produced include tacrine hybrids [185], benzylphenoxypyridine and pyrimidines [186], 3-Aminopyrazole derivatives [187], symmetric triazine derivatives [188], and Resveratrol derivatives [189]. A lot of work has gone into finding medications to treat this disease and research in this field is still going on.

2.6. Parkinson's disease (PD):

Parkinson's disease (PD), the second most prevalent neurodegenerative disease after AD, is characterized by dopaminergic neuron loss in the substantia nigra pars compacta portion of the brain [190, 191]. PD is caused primarily by the accumulation of intracellular inclusions known as Lewy Bodies (LB) [192]. These deposits can spread from cell to cell in a prion-like manner and hence can lead to non-motor symptoms including stiff posture, erratic pacing, and resting tremor. The precise remedy for PD has been a larger area of investigation in the medicine and pharmaceutical industries. There has always been a search for natural compounds that might inhibit αS oligomerization and fibrillation, perhaps reducing the toxicity of preformed aggregated species. Many smaller organic and inorganic molecules have been reported to act as possible therapeutic agents for PD which are discussed in the therapeutic strategies [193, 194].

2.6.1. Background of PD:

James Parkinson described PD, a neurological condition, in 1817 [195]. Sylvius de la Boe wrote rest tremor and Sauvages wrote festination [196, 197]. Traditional Indian literature from 1000 BC and ancient Chinese sources also describe PD [198-199]. Charcot coined the term "Parkinson's disease" since PD sufferers are not feeble or tremorous [200]. In his "Manual of Diseases of the Nervous System," Londoner William Gowers studied PD demography. He mentioned his 80 patients in 1880s. He accurately diagnosed male predominance condition with minimal modifications and analyzed its joint abnormalities. The French neurological school reported additional clinical

descriptions and pathologic research of PD. Richer and Meige described the clinical and structural progression of Parkinsonian impairment [201]. Richer supplied information about the most important PD artwork and statues. Babinski noted on the disease-related motor irregularities [202]. Brissaud originally identified PD as substantia nigra injury [203]. In the 1920s, Tretiakoff, Foix, and Nicolesco continued midbrain pathologic studies related to the condition [204-205]. Greenfield and Bosanquet completed the most extensive pathologic investigation of PD and clear brain stem lesions in 1953 [206]. Hoehn and Yahr's key article on PD morbidity and clinical progression created the internationally known stage system. Time-tested staging distinguishes unilateral (Stage I) from bilateral illness (Stages II–V). Postural reflex dysfunction (Stage III) was another clinical turning point [207]. However, Blocq, Marinesco, and Friedrich Lewy's pathological definition of the disease revealed its characteristic, Lewy bodies (LB) [208-212]. Based on these findings, PD is caused by the self-assembly of eosinophilic aggregates in the LB of the brain, which causes neurons in the substantia nigra to die. Ehringer and Hornykiewicz showed that dopamine is a neurotransmitter in the brain area that causes PD [213-215].

2.6.2. Occurrence and symptoms of PD:

The age group of 60 years and above has the highest prevalence of PD, which is approximately 1 percent. This percentage graph rises steadily until it reaches 5% among individuals aged 85 and older [216-219]. PD is a degenerative disease that can affect individuals for over two decades. Despite the fact that the disease develops 10 to 15 years before the emergence of symptoms, multiple deaths can undoubtedly result from PD-related issues. These include swallowing difficulties that lead to food aspiration into the lungs, which can result in pneumonia and other pulmonary complications. Moreover, mobility problems enhance the risk of fatal falls [218, 220]. All of these fatalities have a substantial impact on individuals with PD.

The majority of patients at the clinical stage of the disorder display hypokinesia, bradykinesia, resting tremor, stiffness, and postural instability. These symptoms result in 80% neuronal degeneration in the afflicted brain region. Nonetheless, some cases manifest 15 years after the disease's onset [221-222]. As the absence of dopaminergic neurons causes the onset of motor symptoms, the effect can be recovered by modulating

with dopamine replacement therapy such as L-3,4-dihydroxyphenylalanine (L-DOPA) and various neurosurgical procedures such as Deep Brain Stimulation (DBS), which can send otherwise absent electrical inputs [223-226]. There is no effective treatment for this sickness, despite the significant development of medicines. **Figure 2.6** illustrates that PD is characterized by both motor and non-motor symptoms.

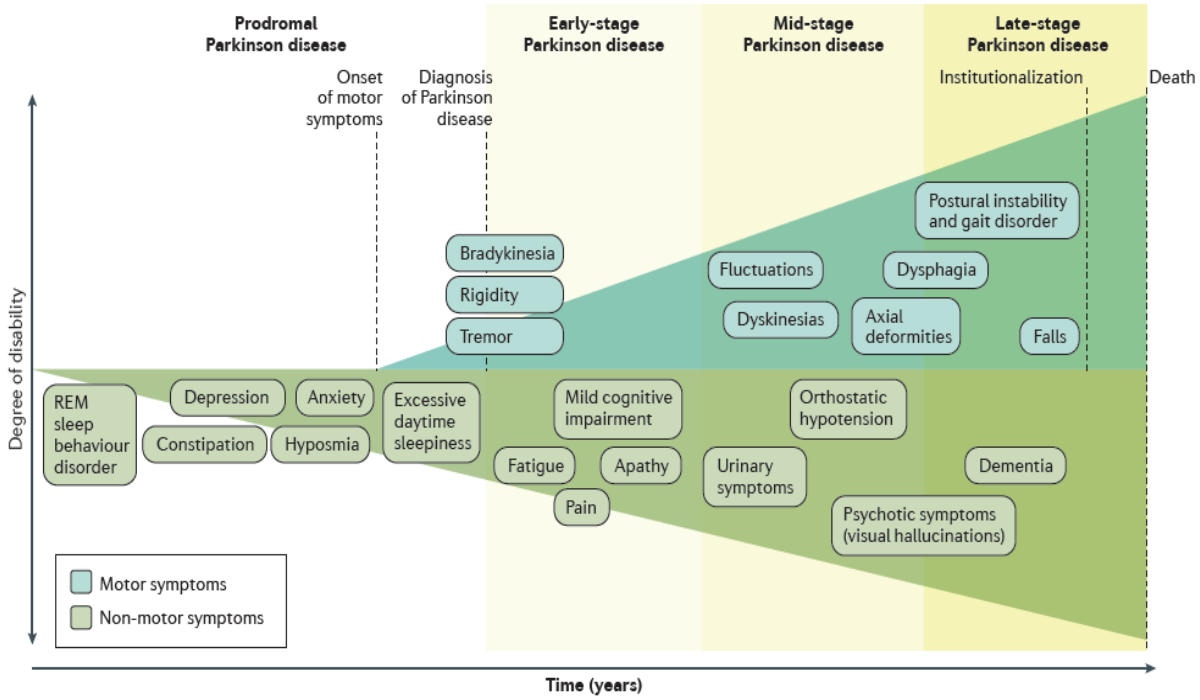

Figure 2.6. *Clinical symptoms associated with Parkinson's disease progression (Taken from [227]).*

2.6.3. Occurrence of PD in India:

In India in 2019, an estimated 7,71,000 (95% UI 6,35,000–9,19,000) persons had PD, and an estimated 45,300 (95% UI 38,600–52,800) deaths occurred attributable to PD. In 2019, the Disability-Adjusted Life Years (DALY) rate of PD varied by two-thirds amongst states, with Goa having the highest rate. From 1990 to 2019, the crude and age-standardized prevalence of PD increased in India, with the increase in crude prevalence being larger. The crude DALY rate of PD grew greatly throughout the same period, although the age-standardized rate did not change significantly. PD was uncommon among younger age groups in 2019. In both males and females, the prevalence increased significantly in later age groups, particularly among those older than 50 years [228].

2.6.4. Causes of PD:

Certain nerve cells (neurons) in the brain are affected by PD, which leads to their slow degeneration or death. The death of neurons in the brain that are responsible for the production of dopamine, a chemical messenger, is the root cause of many of the symptoms. Reduced levels of dopamine lead to abnormal brain activity, which in turn leads to decreased movement and other PD symptoms. [229].

PD has no established etiology, however, various factors appear to play a role, including:

- **Genes:** Researchers have identified certain genetic variants that are connected to PD. On the other hand, these instances are extremely unusual and only occur in exceptional cases when a substantial number of family members have PD. Despite this, there are specific gene variants that appear to increase the risk of PD, although at a risk that is quite small for each of these genetic markers.
- **Environmental triggers:** The risk of acquiring PD in the future may be slightly increased if an individual is exposed to certain substances or environmental variables.
- Researchers have also seen that PD patients' brains undergo significant changes, although the reason is unclear [229]. These alterations include:
- **The presence of Lewy bodies:** One of the microscopic sign of PD is the buildup of certain substances within brain cells. These are referred to as Lewy bodies, and researchers think they offer a significant tip on the cause of PD.
- **α-Synuclein found within Lewy bodies:** αS, a naturally occurring and widely dispersed protein, is thought to be the most major component of Lewy bodies, despite the presence of other substances. It is found in clumped form in all Lewy bodies, which cells cannot break down. For PD researchers, this is currently a focus area.

Risk factors: Some of the risk factors for PD include-

- **Age:** Young adults rarely experience PD. The likelihood that it may appear increases with age, usually in middle or later life. People typically get the illness at 60 years of age or later. Genetic counseling may be useful in helping a young person with PD make decisions regarding family planning. For an elderly person

with PD, work, social situations, and drug side effects are particular and necessitate specific attention.

- **Heredity:** Having a close relative with PD increases the risk of developing the disease. However, unless there are numerous relatives with PD, the risk remains modest.
- **Age:** PD is more prevalent among men than among women.
- **Exposure to toxins:** Prolonged exposure to herbicides and pesticides may increase the risk of developing PD.

2.6.5. Alpha-Synuclein (αS) protein:

αS protein is the precursor protein responsible for the development of PD [230-232]. It is an unidentified protein that was discovered in 1990. It was first described in the electric ray Torpedo, which has a homologue in rats and functions as a protein in the central nervous system. As it was mostly detected in Synaptic vesicles and close to the nucleus, it was designated Synuclein [233-257]. The non-amyloid-beta component (NAC) of the AD patient's brain plaques was observed to comprise approximately 140 amino acid residues. These were believed to have a greater similarity with torpedo Synuclein. In addition, another two homologues of approximately 134 amino acid residues were identified as Beta Synuclein (βS) [258] in the human proteome. In addition to these two proteins, another Synuclein called Gamma Synuclein (γS) [259-260] with around 127 amino acid residues was discovered in humans. In addition, it was discovered in subsequent years that a zebra finch homologue of αS was associated with song learning and brain plasticity [261]. Therefore, it was postulated that the natural folded form of αS adopts a helical secondary structure upon interacting with lipids [262]. However, the two major findings made by αS researchers reached the international stage. The mutation of alanine to threonine residue at position 53 (A53T) was discovered in the laboratory of Mihael Polymeropoulos [231]. The existence of Lewy bodies in the brains of PD patients was another discovery made by Maria Spillantini and her colleagues [232]. Both of the finds were made in 1997. Based on these two studies, both the hereditary component and protein misfolding were hypothesized to be the primary causes of the illness process. Also discussed is the existence of mutations (A30P, A30G, A53T, E46K, H50Q, and G51D), which elevate αS protein levels by threefold, thereby encouraging the development of a severe form of PD. In addition, it suggests that αS

plays an important role in the onset and course of PD [263-267]. **Figure 2.7** depicts the schematic representation of the αS aggregation mechanism.

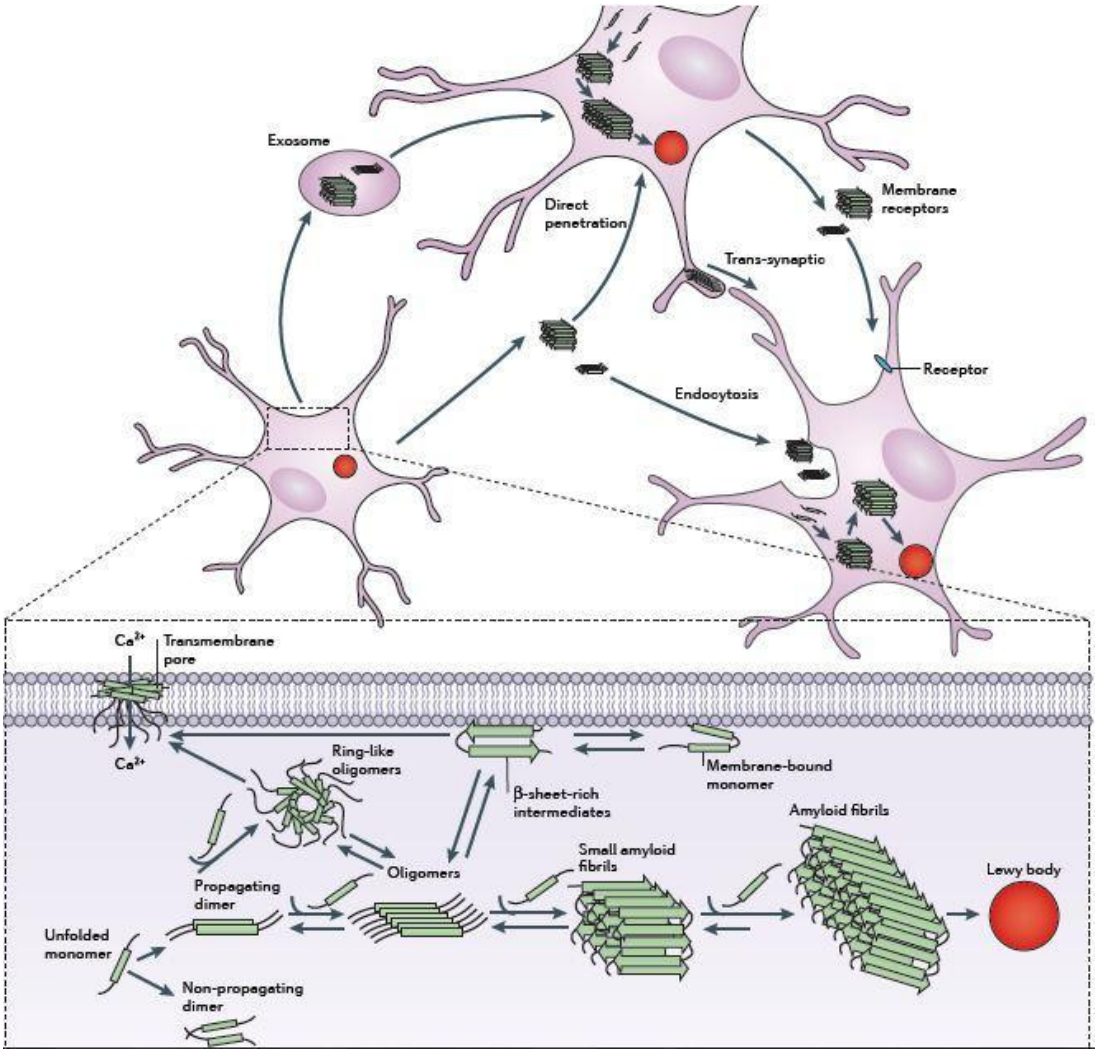

Figure 2.7. *Schematic representation for mechanisms of αS aggregation and propagation (Taken from [12])*

The αS protein has 140 amino acid residues that are found in vertebrates, but it doesn't have any tryptophan or cysteine residues [268]. αS, on the other hand, is coded by a single gene with seven exons on the human genome's 4th chromosome [269]. αS is only found in the presynaptic nerve terminals of the brain [257, 270], which is different from the other parts. The sequence of αS can be broken down into three main areas: the positively charged N-terminal region, which is made up of residues 1 to 60, the hydrophobic non-amyloid beta component, also known as the fibril core region, which is made up of residues 61 to 95, and the negatively charged C-terminal region, which is

made up of residues 96 to 140 [268]. The sequence of αS is made up of seven repeats of amino acids that are highly conserved and have the consensus sequence KTKEGV. The parts of the membrane that interact are shown by the presence of this consensus sequence [271]. Hyperphosphorylated S129 is thought to be the most common type of αS protein [231, 264, 267, and 272]. αS is an intrinsically disordered protein (IDP) [262, 273], which means it doesn't have a clear secondary structure. However, its structure is more compact than that of a simple random coil [262]. Even though it doesn't have a stable secondary structure, there seems to be a long-range interaction between the NAC domain and the C-terminal region. The interactions between molecules are a little bit random and tend to form a compact, unfolded ensemble [264]. Reports also say that αS can interact with acidic phospholipids in a way that gives it a stable helical conformer [275, 276]. Two α-helices from residues 3-37 or 45-92 and a single elongated helix from residue 94 show that the protein is in a helical shape [277]. Because of the conserved motif (KTKEGV), αS can interact with the polar residues of the N-terminal region (S, E, K) that face the hydrophilic environment, while the hydrophobic residues get inserted into the membrane. The boundary between hydrophobic and polar domains is formed by the negatively charged surface of the phospholipid bilayer [278]. The positively charged residues in the N-terminal region interact with this negatively charged surface. But it seems that only the first 94 residues interact with the membrane. The C-terminal region of αS, which is part of the intramolecular chaperone, moves around a lot. It acts as a scaffold when it interacts with other proteins and is driven to the binding region of the membrane [279]. **Figure 2.8** shows how αS can be divided into its three structural parts. Recent studies suggest that αS protein can exist in all of its forms, including as an unfolded monomer, a dimer, and a tetramer. The species is most common in its tetrameric form, which is found in living cells. It has been seen that this highly ordered tetramer promotes the formation of α-helical secondary structure and a sudden drop in the tendency to clump [280, 281]. So, it is very important to find ways to make the secondary structure of αS more stable so that it doesn't want to stick together as much.

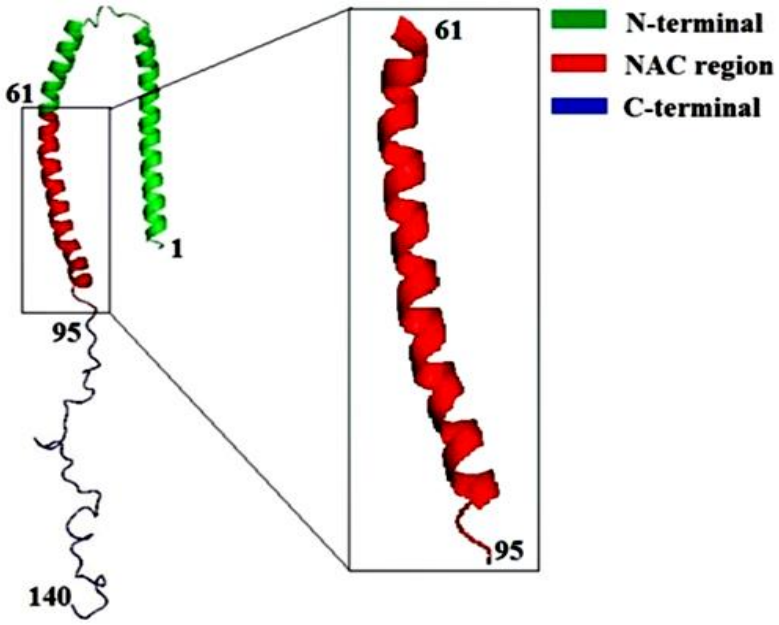

Figure 2.8. *Schematic representation of the 3-D structure of αS along with the three different structural components.*

2.6.5.1. Functions of α-Synuclein:

In the field of neurology, the function of αS remains an outstanding mystery. However, αS plays a crucial role in the cycling of Synaptic vesicles, mobilization, altering the vesicle pool size, and endocytosis [271]. It has been proposed that αS influences Synaptic transmission by boosting vesicle clustering without altering the efficiency or kinetics of vesicle fusion in response to calcium triggering. This effect happens when αS polymerizes during membrane binding. αS may impede vesicle trafficking, however, by promoting vesicle clustering. Although it is known that high amounts of non-aggregated αS impede SNARE-independent vesicle docking via contact with acidic lipids [271], SNARE is not required for vesicle docking. Multiple conformations have been observed for αS, which can simultaneously exist in a dynamic equilibrium between the monomeric, oligomeric, and higher order aggregated states [246]. However, αS fibrils can interconvert to numerous hazardous oligomers, allowing them to operate as a destructive source particle. The use of recombinant proteins is required for research focusing on the pathological conversion of αS [291-294]. In addition, it has been hypothesized that cells can only interact with whole particles and not their constituent monomers. Therefore, it seems more appropriate to normalize the

concentration of each species according to their size. The issue may be mitigated, however, if the effect of αS fibril is compared to that of protein fibrils and monomers and oligomers are analyzed using the same method. This protein is also crucial for mitochondrial failure, oxidative stress, and reactive aldehydes.

2.6.5.2. Association of α-Synuclein protein with PD:

The majority of the essential features of the αS protein have been covered in earlier sections. Numerous reviews on αS contain the majority of the knowledge regarding its structural form, synaptic plasticity, and cognitive function [268, 278, 281]. To date, the role of αS has been the subject of debate. Despite the fact that numerous neuroproteins are distributed uniformly throughout neurons, αS is restricted to presynaptic nerve terminals and is absent from the cell body, dendrites, and extra-synaptic locations along the axon [282-283]. In addition, αS plays an important role in neuronal function since it is extensively expressed in numerous neuronal subtypes. After arriving at peripheral membrane synapsis proteins and integral membrane proteins of the Synaptic vesicle [284], αS protein localizes in forming Synapses. As the Synuclein family is only present in vertebrates [271], the presence of αS at the presynaptic terminal is irrelevant to Synaptic development and function. However, the localization of αS is problematic since it lacks a particular transmembrane domain or a traditional lipid anchor capable of directing it there. This may be owing to the fact that αS requires the N-terminal region for membrane interaction. However, this contact between the αS and membrane is minimal, as the extracted protein often resembles a water-soluble and unfolded form. Due to its mobility, αS can function both in the presence and absence of membrane, i.e. in free solution, despite the absence of interaction between αS and membrane [284]. Significantly strong connection exists between αS and Synaptic vesicles, while the interaction between αS and lipidic membrane is not demonstrated. This annotation is readily explained by the αS protein's preference for high membrane curvature, indicating that Synaptic vesicles behave like the tiniest biological membranes [168]. The protein αS interacts with tiny vesicles via lipid rafts, providing evidence that this association benefits neurons [284]. Aside from neuron connections, αS [285] has also been associated with the absorption of cardiolipin, acyl chain composition, and fatty acid metabolism. αS's involvement as a neurotransmitter and in the construction of the Synapse is dependent on its interaction and location with the membrane. However, the

knockout mouse models of αS recover dopamine faster than Wild-Type (WT) mice following recurrent stimulation, and as a result, dopamine levels in the striatum are decreased [286]. Aside from these, the presynaptic neuronal boutons appear to be smaller in mice lacking all three Synucleins [287]. Consequently, the maintenance role of nerve terminals is more significant than neurotransmitter release [246]. The role of αS as an intramolecular chaperone has already been hypothesized, and it is not necessary for knockouts of cysteine string protein alpha (CSP alpha) to have a comparable phenotype. Those are the levels of SNARE SNAP-25 that are significantly impacted [288]. The majority of studies suggest that αS can govern the development of SNARE complexes in a fine-tuning-determining manner [246]. The interaction of αS with another protein is also a possible αS function for αS that is not mutually exclusive. According to reports, the multimeric form of αS promotes microtubule polymerization by interacting with tubulin and also improves the local structure of the cytoskeleton [289]. In addition to this contact, there is a strong interaction between αS and the small GTPases rab3a and rab8 that regulate the GTP-dependent binding of αS to membranes [290-291]. In addition to the protein, another partner of αS discovered in LB is Synphilin-1, which was first reported as filling nerve terminals [292]. However, both interacting partners encourage the creation of non-reactive aggresomes, so neutralizing the toxicity of the other. The dynamics of aggregation are thus redirected towards a less damaging state [293-295].

2.6.5.3. Mutations in α-Synuclein:

Gene mutations can lead to the development of rare family types of PD and other linked diseases. To date, about six point mutations have been found, including A30P, A30G, A53T, E46K, A53E, H50Q, and G51D. It has been observed that these mutations play a crucial role in amyloid formation and αS toxicity [296-315]. A53T was discovered in an Italian family [231], E46K in a Spanish family [267], A30P in a German family [316], G51D in a British family [317], H50Q in a Caucasian female English patient [263], and A53E in a Finnish family. The existence of these point mutations influences the oligomerization rate and production of fibrils in αS protein, according to studies. These six missense variants in αS (A30P, A53T, E46K, A53E, H50Q, and G51D) have been linked to family types of Synucleinopathies [231, 263, 265,267, 316, 318]. Mutants A53T, H50Q, and E46K were reported to increase fibril development [279, 319-320], whereas mutants A30P, A53E, and G51D slowed fibril formation [279, 321-322]. The

A53T, H50Q, and E46K mutations were shown to create larger objects, whereas the A30P, G51D, and WT mutations were observed to produce fewer and smaller objects. These observations complement the mutants' literary perspective. Several investigations utilizing recombinant proteins *in vitro* have demonstrated that mutants E46K [323], H50Q [322, 324], and A53T [299, 325-326] had a greater tendency to assemble than WT αS. Despite the fact that an increased quantity of fibrils may also be observed in numerous cell types [327-328]. The mutants A30P [299, 325] and G51D [265] were found to aggregate *in vitro* to the same or a smaller degree than the WT αS. It was observed that these mutants create the same number of fibrils in their cells. Recently, it was shown that the types of oligomers generated by the mutants A30P and A53T towards the conclusion of the lag phase [330] were considerably dissimilar. Among the variations of αS and WT, A30P, G51D, and WT do not recruit into the rapidly developing fibrils of E46K, H50Q, or A53T. A30G is the most recently discovered mutation. This is one of the most noteworthy findings. However, mutants A30P, G51D, and WT αS are less likely to generate their own fibrils and incorporate one another into these objects. This implies that mutants H50Q, A53T, and E46K on one side, and A30P, WT, and G51D on the other side, are capable of forming two distinct types of fibrils. Previous research has demonstrated that mutants E46K and A53T created distinct fibrils compared to WT αS [331-333], but mutant A30P fibrils have a similar shape compared to WT fibrils [331-332].

2.6.5.4. Aggregation of α-Synuclein:

The propensity for fibrillation of αS is critical for the course of PD, as fibrillar forms of αS are characteristic of PD and other Synucleinopathies. Thus, the prerequisites for fibrillation are crucial for the development of novel therapies. According to reports, the shape and staining features of the filament formation generated by αS and its disease mutant forms are identical to those of diseased brains [323, 335]. Consequently, the use of the recombinant protein as a model system for assessing *in vitro* αS aggregation is validated. Different morphologies, such as fibrils, soluble oligomers, and insoluble amorphous aggregates, can be observed based on the aggregation of αS under different experimental conditions [336]. Ongoing studies investigate the variables responsible for the production of pathological insoluble aggregates of αS. It has been claimed that αS can spontaneously assemble with a considerable propensity [337]. It has been observed

that the monomeric form of αS forms fibrils when incubated at 37°C with neutral pH in a condition-dependent manner that can increase the process [336]. At an acidic pH, however, the incubation of αS results in the production of an aggregate morphology that leads to the formation of amorphous aggregates as opposed to fibrils [338]. The aggregation kinetics of αS exhibit a sigmoidal curve characterized by an early lag phase, exponential phase (fibril growth phase), and final plateau after which fibril formation occurs. The αS aggregation mechanism is essentially a nucleation-dependent mechanism involving a partly folded intermediate that is responsible for oligomerization and fibrillation [339, 340]. Unanswered till now is the question of what forces and conditions cause the transformation of αS from a random coiled shape to a primarily β-pleated sheet structure [341]. Although a model for the fibrillation propensity of αS has been proposed, much remains unknown. Fibrils, amorphous aggregates, and soluble oligomers are generated from an aggregation-prone intermediate derived from the unfolded monomeric form of the protein [112, 336]. Numerous studies indicate that the constructed model demonstrated a correlation between fibrillation and incomplete folding [112]. Therefore, fibril production is encouraged by the generation of intermediates that are partially folded. Several studies [339-348] have been conducted to discover the factors responsible for reducing the aggregation propensity of αS as opposed to the factors that increase this propensity.

2.6.5.5. Important factors for the aggregation mechanism of α-Synuclein:

αS protein misfolding and aggregation are caused by a number of different circumstances, including: (i) mitochondrial dysfunction and (ii) oxidative stress have a crucial role in the course of both kinds of the disease. It is also characterized by the loss of neurons and the production of fibrillar aggregates, similar to various other neurodegenerative conditions such as AD and prion illnesses. The creation of such protein aggregates or organised prefibrillar, oligomer, or protofibrils [334, 335] may contribute to cell death. However, fibrils can be neuroprotective in later phases. On the other hand, the mechanism of fibrillation is currently unknown. Conversely, postmortem analysis of PD brains reveals a decline in complex I activity for the mitochondrial respiratory chain. The discovery of family genes such as PINK1, HtrA serine peptidase 2 (Omi/HtrA2), and DJ-1 mutations revealed that mitochondrial malfunction is a major

contributor to the PD pathway. DJ-1 protein distribution appears to be predominantly cytoplasmic, with a lesser pool of mitochondrial and nuclear-associated protein. In addition, the mutation produced by DJ-1 disrupts the protein's function by destabilizing or altering its subcellular location. Each DJ-1 mutation associated with PD exhibited a decreased nuclear localization that favors mitochondrial localization [349]. On the other hand, it is unclear whether the toxicity associated with the enhanced mitochondrial localization is the result of a loss of access to binding partners in various cellular compartments or an increase in mitochondrial activity. In addition to these variables, oxidative stress is implicated in the progression of disease by: a) Inhibiting complex I, which promotes the formation of reactive oxygen species (ROS); b) The dopamine (DA) neurons may be considered a fertile environment for the production of reactive oxygen species (ROS) due to the fact that dopamine (DOP) metabolism generates hydrogen peroxide and superoxide radicals; c) DJ-1 protein may protect cells from oxidative stress that triggers death, and its removal makes cells more susceptible to oxidative stress [334].

2.6.5.6. Proto-fibrils of α-Synuclein:

Biophysical studies suggest that the αS fibrils might not be the species that causes disease [350, 351]. The "*protofibril hypothesis*" says that proto-fibrils are made of temporary small units of β-sheet that contain oligomers of αS, which are more toxic than the insoluble fibrillar form. Growths in cell-free systems have shown that proto-fibrils are hollow cylinders that can let Synthetic vesicles through [20, 352]. Researchers have seen that the structure of αS fibrils can be either straight or twisted, and their diameters range from 5 to 18 nm [232, 353]. Pathological factors [354-355] speed up the process by which unstructured monomers change into β-sheets and then join together to form small protofibrillar structures. So, factors like mutations, interactions with bilayer membranes [360], or exposure to metal ions are needed for annular proto-fibrils to form.

In vitro studies show that the two known mutations of αS can speed up the rate at which proto-fibrils form [353]. Even though doing the same thing might help figure out which *in vivo* animal models can produce consistent results.

αS fibrils, on the other hand, are known to be polymorphic, but it is still not clear how their cross-structures are different. Some signs suggest that different strains cause

different Synucleinopathies, and that these αS fibrils cause inflammation [361]. With these things in mind, more work can be done to make better structural models of the fibrillar structure of αS fibrils. And these describe the relationship between clinical isolates as a way to understand how diseases get worse [362-363].

2.6.5.7. Membrane interactions of α-Synuclein:

Oligomers can make the cell membranes more permeable, which is a well-known reason that αS harms cells [364-426]. Different oligomers can stop cellular membranes from doing their normal jobs by making structures that look like pores, which can lead to an abnormal flow of calcium and neurodegeneration [427]. αS oligomers can, however, interact with lipid membranes, which can increase the membrane's ability to conduct electricity and also form a pore complex in planar lipid bilayers [428, 429]. It is known that the mutants A53T and A30P make membranes more permeable, which can lead to the formation of pores in the plasma membrane of SH-SY5Y cells. This lets Ca^{2+} into the cells, which is a very important part of cell death [430]. Neuronal cells with WT or A53T mutants have a higher level of calcium inside the cell. So, there is a higher chance that annular pore-like oligomeric structures will form when calcium flows into the cell and cell membranes are broken [427]. Also, a study that used computer modeling and membrane simulation found that A53T mutant αS was able to get through the membrane 20% faster than WT αS [427].

Several studies have been done to figure out how αS sticks together in the presence of lipids. It has been said that monomers of αS that are attached to a membrane can join together faster than monomers that are not attached to a membrane. There have also been studies on how binding of the membrane stops the mechanism of aggregation [431]. Because of the formation of annular-shaped proto-fibrils (ring, spherical, or tubular), pores form in the cell membrane. This leads to the leakage of cellular contents, an increase in intracellular levels of potential cytotoxins, calcium, and dopamine [352]. A known theory called the "Amyloid pore-channel" says that when protofibrils are made, they get stuck in the lipid bilayers and form channels. Compared to the other mutants, A30P αS has less affinity for lipids and binds to membranes very weakly [316, 432, 433-436]. Also, there have been a lot of studies on how to stop αS [432-450], but the exact

way the aggregation pathway works is still not clear. So, the mechanisms of inhibition are still in a predicament.

2.6.6. Inhibitors of α-Synuclein aggregation and current treatment of PD:

Presently, there is no treatment for PD, and patients with PD are offered symptomatic management, which can be performed precisely with precise medication, exercise, close monitoring, education, adjustments, and occasionally surgery. Depending on the severity of the patient's symptoms, the choice of medical treatment begins with the diagnosis. Priority is given to a patient's ability to perform activities of daily living (ADLs). There are approximately five types of drugs used to treat the symptoms associated with PD in the current situation. Dopamine agonists, anticholinergics, levodopa, catechol-O-methyl-transferase (COMT) inhibitors, and monoamine oxidase B (MAO-B) inhibitors are examples of these [451].

Levodopa is considered the most promising target for treating the motor symptoms of PD. At the level of the neurotransmitter, efforts have been made to replace the missing dopamine. Levodopa is combined with carbidopa to prevent its peripheral metabolism, which may allow more levodopa to cross the blood-brain barrier (BBB). Long-term exposure to levodopa, such as five to ten years, results in various involuntary abnormalities in at least fifty percent of levodopa-consuming patients [452-454]. However, there are currently no treatments for slowing the progression of PD. The monoamine oxidase (MAO)-B inhibitors can be considered for the initial treatment of the early disease. These medications provide a moderate symptomatic benefit with adverse effect profiles. And, according to a Cochrane review, the long-term quality-of-life indicators have improved by 20-25% [454]. In comparison to levodopa, dopamine agonists such as ropinirole and pramipexole delay the onset of dyskinesia and provide a moderate symptomatic benefit. As suggested by a review of the Cochrane and PubMed databases from 1990 to 2008, these agents cause a 15% increase in adverse events such as somnolence, sleep onset, hallucinations, edoema, and impulse control disorders [455].

Although surgery is recommended for patients with motor fluctuations and dyskinesias, these conditions cannot be optimally treated with drug modifications. Deep Brain Stimulation (DBS) has largely supplanted neuroablative lesion procedures as the

primary surgical option in terms of surgery. In some locations, notably the United States, the levodopa/carbidopa intestinal gel infusion is offered alongside clinical studies [456]. It is now common knowledge that the non-motor symptoms of PD are more problematic than the motor symptoms. In addition to dementia, hallucinations, rapid eye movement (REM), depression, sleep behaviour disorder, orthostatic hypotension, and constipation, these non-motor symptoms can be classified as autonomic, cognitive/psychiatric, and sensory [457]. Also known to fluctuate are the non-motor symptoms, including numbness, paresthesia/dysesthesia, sadness, pain, akathisia, and restless legs syndrome. Recognizing the non-motor symptoms of PD is necessary for appropriate care [457-459].

At the present time, there is no appropriate treatment for most neurodegenerative diseases, especially PD, which is one of the hardest to treat. Even though Levodopa has been used in a lot of drugs and medical treatments, it is still the best way to treat PD. Several medical treatments, such as deep brain stimulation that focuses on managing disease symptoms, might not be able to stop the loss of dopamine (DA) neurons, though they might be able to slow it down.

2.7. Interaction of $A\beta_{1-42}$ peptide and α-Synuclein:

$A\beta_{1-42}$ peptide and αS are aggregation-prone proteins that are frequently linked to AD and PD, two separate neurodegenerative illnesses. However, αS was discovered in conjunction with AD plaques years before it was connected to PD or the development of LBs. Today, it is widely known that a sizable percentage of AD patients (50%) also co-exhibits severe αS LB pathology. Unfortunately, patients with AD LB variants experience more symptoms sooner in the course of their illness. Basic research is starting to suggest that $A\beta_{1-42}$ peptide and αS may work in concert to encourage the accumulation and aggregation of one another. Despite the fact that the precise ways in which these proteins interact are yet unknown, mounting evidence shows that $A\beta_{1-42}$ peptide may be the primary factor in the pathophysiology associated with αS through interfering with protein clearance, inducing inflammation, boosting phosphorylation, or directly encouraging aggregation [460].

LBs, Aβ plaques, and neurofibrillary tangles do not coexist in AD-LBV brains with a frequency high enough to account for it. Instead, it has been proposed by researchers that Aβ, tau, and αS may encourage the aggregation or accumulation of one

another. This theory has been supported by numerous researches over the past ten years, and investigations have started to reveal the cellular and molecular mechanisms behind these interactions.

2.7.1. Synergistic interactions between Amyloid-β and α-Synuclein:

Aβ and αS pathology overlap in neurodegenerative diseases, thus researchers are studying their interactions. *In vitro* and *in vivo* research have begun to reveal probable pathways by which Aβ and αS interact, promising to improve our understanding of these inter-related neurodegenerative disorders [460].

Researchers first investigated Aβ-αS interactions using cell-free experiments. Recombinant human α-Syuclein (hSYN) incubated with $A\beta_{42}$ formed high-molecular-weight oligomers [461]. $A\beta_{42}$ caused αS oligomer formation, however $A\beta_{40}$ did not. In a cell culture paradigm, extracellular $A\beta_{42}$, but not $A\beta_{40}$, increased intracellular αS aggregates [461]. $A\beta_{40}$ and $A\beta_{42}$ directly interact with αS *in vitro* [20]. αS appears to modify $A\beta_{42}$ more structurally. $A\beta_{42}$ forms oligomers and precipitates after αS co-incubation, but $A\beta_{40}$ remains soluble. Given $A\beta_{42}$'s toxic and aggregate-prone features compared to other Aβ isoforms, αS's interaction with it may be important.

A mutation in Presenilin 1, which increased $A\beta_{42}$, also increased pathogenic phosphorylation and aggregation of αS in patients and cells. This result supports the concept that $A\beta_{42}$ is important in αS aggregation and identifies a mechanism: $A\beta_{42}$-induced phos-phorylation. 4% of normal brain αS is phosphorylated at Serine 129 (pS129-Syn). In Synucleinopathies like DLB, up to 90% of αS is phosphorylated at this location, suggesting a pathogenic function [462, 463]. *In vitro*, αS phosphorylation at Ser129 promotes fibril formation [30]. Thus, αS pathology may be increased by $A\beta_{42}$-induced phosphorylation.

In vitro studies show that αS interacts with tau, the second key pathogenic protein in AD. Tau requires cofactors to polymerize [464], while αS self-polymerizes. αS promotes tau polymerization and co-localizes with tau in inclusion bodies [461, 465]. Co-transfection of αS with tau produces intractable, cytotoxic αS aggregates [465]. Cellular seeding with αS fibrils produces cytotoxic neurofibrillary tangle-like inclusions.

Extracellular seeding of αS fibrils recruits soluble αS into insoluble LB-like inclusion bodies [466].

2.7.2. Direct interactions between Amyloid-β and α-Synuclein:

Aβ and αS rarely share a sub-cellular compartment in healthy cells, hence limiting their direct contact [461]. Many proteins, including Aβ and αS, can change location in pathological situations. Mitochondria contain Aβ and αS. Both proteins concentrate in lysosomes and autophagasomes [467]. Thus, injured cells may have direct protein interactions. *In vitro* investigations have shown most direct connections between Aβ and αS. αS induces Aβ structural alterations in cell-free experiments [462]. Aβ and αS form complexes and co-immunoprecipitate from AD-LBV patient brains and transgenic models, suggesting direct interactions [468] (as shown in **Figure 2.9**). This work showed that these two proteins can generate hybrid pore-like oligomers that promote calcium influx. Tau increases αS aggregation and toxicity, and both proteins co-localize in AD-LBV neurons, dystrophic neurites, and Lewy bodies [469]. If direct interactions between Aβ and αS contribute to AD-LBV pathogenesis, it will be vital to understand why they only occur in particular patients and brain regions.

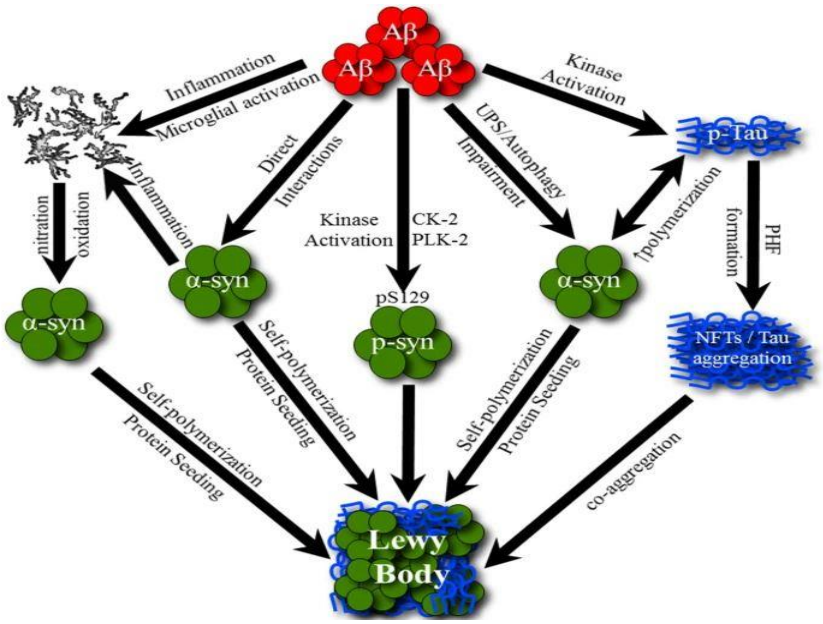

Figure 2.9. *Potential mechanisms linking Aβ and αS pathology (Taken from [460]).*

2.8. Scope of the Thesis:

[1] Numerous studies have emphasized the significance of ionic strength in influencing the tendency of $A\beta_{1-42}$ peptide for folding and aggregation. Hence, there was a need to understand the effect of ionic strength of the solution on the aggregation propensity of $A\beta_{1-42}$ peptide at the molecular level. Therefore, in **Chapter 4** of the thesis, a computational study have been performed for a clear understanding of the effect of ionic strength of the solution on the aggregation propensity of $A\beta_{1-42}$ peptide using Molecular Dynamics (MD) Simulation.

[2] One of the most promising approaches to combat AD is to prevent the self aggregation of Aβ-peptide. The design of peptides as inhibitors against Aβ-peptide aggregation has been gaining popularity. Therefore, it is important to study the role of peptides on dimerization and aggregation properties of $A\beta_{1-42}$ peptide. Recently, it has been reported that a peptide, C-terminal (CTerm) of Human Albumin (HA) binds to the $A\beta_{1-42}$ peptide and impairs the $A\beta_{1-42}$ aggregation and promotes disassembly of $A\beta_{1-42}$ aggregates. Hence, in **Chapter 5** of the thesis, a computational work has been designed using Potential of mean force (PMF) and Binding Free Energy (BFE) calculations, where the role of a peptide (CTerm of HA) is studied to understand its effects on the dimerization and aggregation of $A\beta_{1-42}$ peptide.

[3] Resveratrol (RSV), a polyphenolic compound is reported to have anti-aggregation property against Amyloid-β peptides. It is therefore significant to understand the mechanism of inhibition of $A\beta_{1-42}$ peptide aggregation by the RSV at the molecular level. Therefore, in **Chapter 6** of the thesis we have used Molecular docking along with Molecular dynamics (MD) simulation techniques to address the role of RSV in the inhibition $A\beta_{1-42}$ peptide aggregation.

[4] Macromolecular crowding is one of the essential cellular environment elements that can influence the aggregation mechanism of αS. Polyethylene Glycol (PEG) is one the most commonly used crowding agent. But the effect of macromolecular crowding on the structural and conformational properties of αS in the presence of PEG has not been investigated in molecular details. Therefore, in **Chapter 7** of the thesis, the role of PEG on the αS aggregation has been studied. The study involves the understanding of the changes in the structure and conformation of αS protein in the presence of the crowding agent, PEG.

[5] Designing of peptides as inhibitor has gained new momentum as inhibition strategy for AD and PD. 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 **Chapter 8** of the thesis, a study of the effect of peptides on αS aggregation has been performed. In this study, the influence of two peptides K84s and K102s on the structural and conformational dynamics of αS were studied.

[6] Recently, Oleuropein aglycone (OleA) has been reported to stabilize the monomeric structure of αS, subsequently favoring the growth of non-toxic aggregates. Therefore, understanding the conformational dynamics of αS monomer in presence of OleA is significant. Hence, in **Chapter 9** of the thesis, the effect of OleA on the conformational dynamics and the aggregation propensity of αS has been investigated using molecular dynamics simulation.

2.9. Main Objective of the Thesis:

[1] To study the inhibition approaches of Amyloid-β amyloidogenic aggregation

a) To study the effect of ionic strength on $A\beta_{1-42}$ peptide aggregation
b) To study the effect of peptides on dimerization and aggregation of $A\beta_{1-42}$ peptide
c) To study the role of small molecule inhibitor in preventing aggregation of $A\beta_{1-42}$ peptide.

[2] To study the inhibition approaches of α-Syuclein amyloidogenic aggregation

a) To study the effect of α-Synuclein aggregation using crowding agents
b) To study the effect of peptides on α-Synuclein aggregation
c) To study the role of small molecule inhibitor in preventing aggregation of α-Synuclein.