CHAPTER 2

INTRODUCTION & REVIEW OF LITERATURE

2. Introduction & review of literature:

Proteins are built of hundreds or thousands of smaller units called amino acid residues which are connected in long chains by peptide bonds. To impart biological function, a protein undergoes proper folding and acquires its native 3-dimensional (3-D) structure and the native folding of protein is the most energetically favorable state. The primary amino acid sequence of a polypeptide chain holds the information needed for proper folding [14]. If proteins misfold, cellular pathways recognize and degrade the misfolded proteins thus inhibiting them from causing any impairment [15]. Thus, to maintain vital cell functions, correct folding of polypeptide chains and rapid degradation of misfolded ones are crucial. If a cell fails to degrade misfolded proteins, it may form aggregates and can form large insoluble deposits, which can progress to amyloids. Amyloid, first described by Rudolf Virchow in 1854, is typically composed of long straight unbranched fibrils, built up of parallel protofilaments having a cross β-sheet structure [16].

Figure 2.1 illustrates atomic resolution structures of a cross-β amyloid fibril formed by an 11-residue fragment of the protein transthyretin, TTR (105-115) [17]. These fibrils have the classic amyloid morphology, being 100-200 Å in diameter and typically 1-3 μm in length.

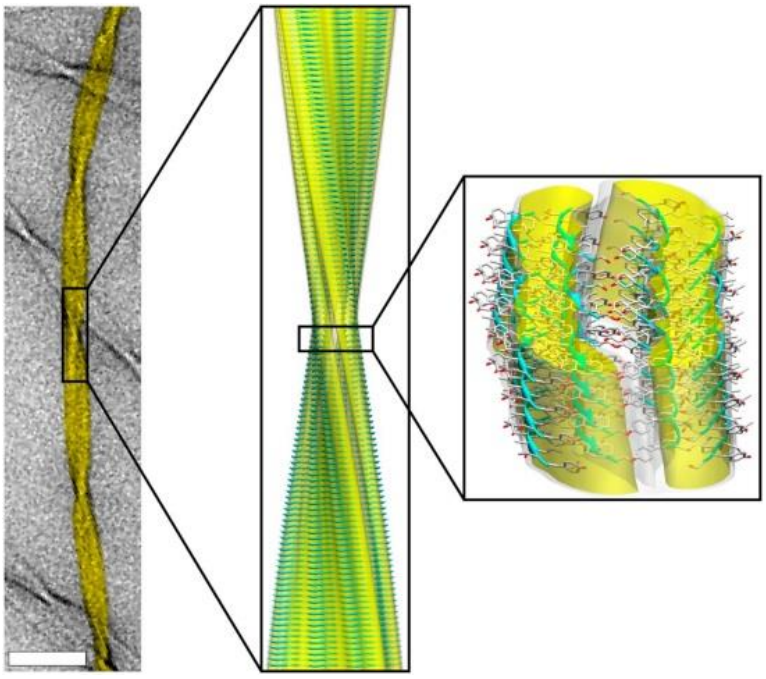


Figure 2.1. *Atomic structure and assembly of an amyloid fibril (Taken from [17]).*

Amyloidosis is characterized by accumulation of amyloids which signify a diverse family of both systemic and tissue-specific diseases. While the ability of proteins to form amyloid fibers is suggested to be a general feature, more than 25 proteins are known to spontaneously form amyloids and disease in humans till date [18]. Many neurodegenerative diseases are caused due to protein deposition and misfolding. **Table 2.1** gives a list of neurodegenerative diseases caused due to protein misfolding.

Table 2.1: *Neurodegenerative diseases linked to protein misfolding.*

Disease	**Neuropathology**	**Proteins**
Alzheimer's disease	Senile plaques, CAA, NFTs	Aβ and Tau
Amyotrophic lateral sclerosis	Intracellular inclusions	SOD1
Creutzfeldt-Jakob's disease	Prion plaques	PrP
Frontotemporal dementia	NFTs, Pick bodies, TDP43-bodies	Tau, TDP-43, ubiquitin
Huntington's disease	Intracellular inclusions	Huntingtin
Lewy body dementia	Lewy bodies, senile plaques	α-synuclein, Aβ
Parkinson's disease	Lewy bodies	α-synuclein
Polyglutamine disease	Nuclear and cytoplasmic inclusions	Polyglutamine containing proteins

AD, one of the most common forms of dementia is caused due to amyloids that are formed by the intrinsically disordered Aβ peptide [4, 5]. Amyloid formation that leads to the deposition of senile plaques is conceptualized as a complex process of protein aggregation, involving the misfolding of Aβ peptide into soluble and insoluble assemblies [12, 13]. The conformational transition of the monomeric Aβ peptide from its α-helical and/or unordered structure to the extended β-sheets, promotes self-aggregation finally leading to Aβ peptide oligomer formation, which then serve as seeds/nuclei for accelerated fibril growth as illustrated in **Figure 2.2.**

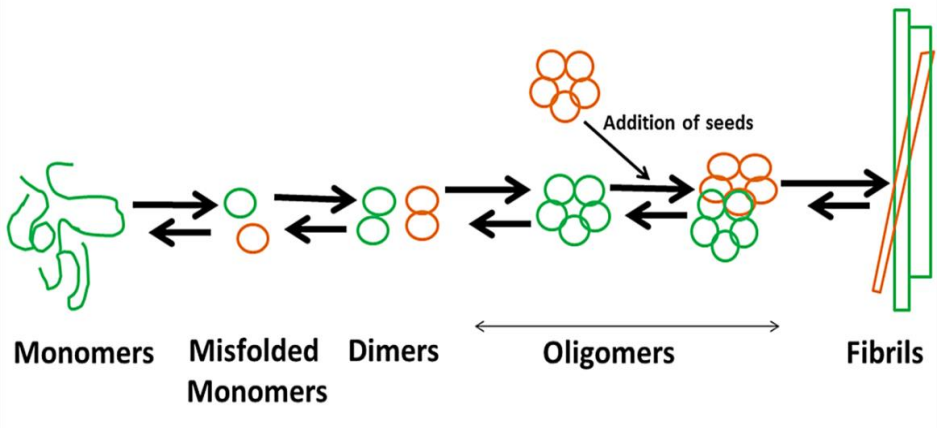


Figure 2.2. *Schematic representation of $A\beta_{1-42}$ peptide aggregation pathway from monomer to mature fibrils.*

Kinetics of amyloid fibril formation is well represented by a sigmoidal shape with a nucleation phase/lag phase followed by a rapid growth phase, followed by a saturation phase (**Figure 2.3**) [19]. A brief review of the disease, highlighting the Aβ peptide, its structural characterization and other salient features is discussed in this chapter. The current status of research in these areas is also reviewed briefly.

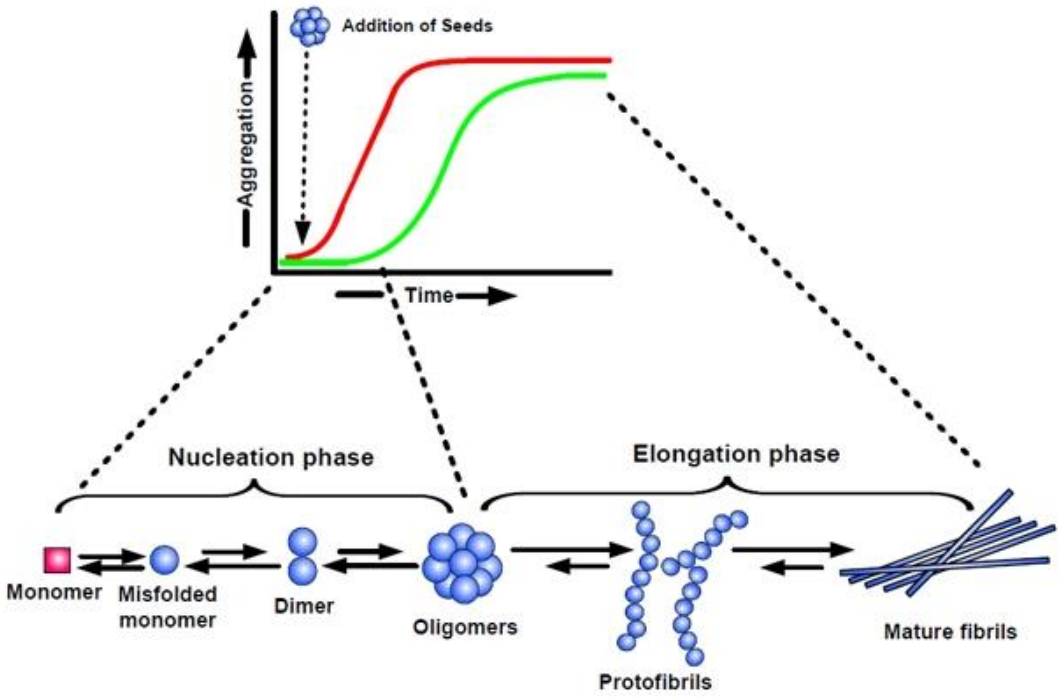


Figure 2.3. *Nucleation-dependent polymerization model of amyloid aggregation (Taken from [19]).*

2.1. Alzheimer's disease:

2.1.1. Overview of Alzheimer's disease:

AD, the most common form of dementia, affects approximately 44 million individuals worldwide. More than 10 million cases per year have been reported from India [20]. In most patients of AD, symptoms usually appear in the mid-60s and increases in over 85 years old and are classified as sporadic AD [21]. It ranges in severity from the mild stage to the severe stage, when the person becomes completely dependent on others to carry out the simplest tasks. The disease characterized by memory loss and other intellectual abilities interfere with day to day activities. With its complexity, till date it is unlikely that any drug or other mediation can successfully cure it and thus it demands in-depth research and development to elucidate the mechanism behind it [22].

2.1.2. The history of Alzheimer's disease:

In 1906, German neurologist and psychiatrist Dr. Alois Alzheimer discovered AD for the first time [23]. The disease was first observed in a 51 year old lady, Mrs. Auguste Deter where Dr. Alzheimer found shrinkage of the cerebral cortex and atrophied brain cells along with senile plaques and NFTs in her brain at autopsy which later became the pathological hallmark of AD [24, 25]. She was reported with problems of memory impairment, aphasia, difficulty in speaking, psychosocial incompetence and disorientation, which progressed gradually over the remaining years of her life worsening cognitive function. Dr. Alzheimer discussed the condition of Mrs. Auguste Deter in 1907 during the 37th Conference of South-West German Psychiatrists in Tubingen, and Krapelin, his supervisor, coined the name Alzheimer's disease in 1910 in the eighth edition of his book *Psychiatrie* [26].

Often the terms Alzheimer's and dementia are used interchangeably and although the two are related, they are not the same. Dementia is a general term for the loss of memory or other mental abilities that affect daily life. AD was considered as dementia until the late 1960's when studies revealed a connection between senile plaques and NFTs and cognitive decline [27, 28]. Furthermore, in 1964, researchers showed that AD is not similar to normal aging and identified the mutation leading to the hereditary form of the disease [29, 30]. These studies exposed AD as a separate disease and revealed that diagnosis of AD could be achieved by eliminating other causes of

dementia and monitoring progression of the symptoms. In 1984, researchers George Glenner and Cai'ne Wong reported β-amyloid as the chief component of Alzheimer's brain plaques and thus sequenced it [31]. In 1986, the second pathological hallmark of AD, the tau protein was identified [32]. The gene on chromosome 21 that codes APP from which beta amyloid is formed was traced down in the year 1987. The National Institute on Ageing (NIA) and the Alzheimer's association teamed up with a pharmaceutical company known as Pfizer and started the first clinical trial of a drug on AD in the year 1987 [33]. Unfortunately, due to its complex nature, AD remains a difficult disease to progress with clinical trials. **Figure 2.4** shows neurons in normal brain vs neurons in AD patients.

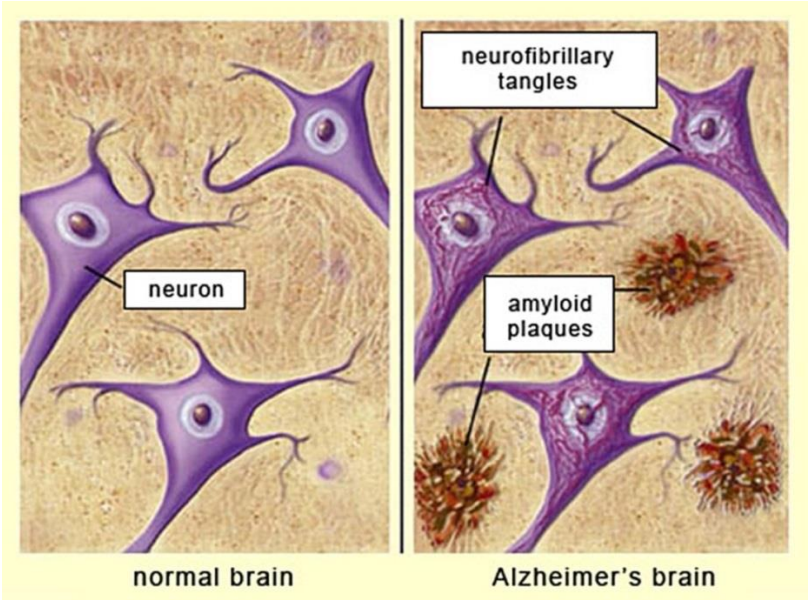


Figure 2.4. *Normal brain vs Alzheimer's disease brain (taken from [34]).*

2.1.3. Disease presentation:

AD progresses with age leading to death within 10-15 years. There are three main stages of the disease, ranging from mild form to its severity. Each stage has its own peculiar symptoms and challenges [34]. **Figure 2.5** illustrates the stages and symptoms of AD.

Scientists are trying to unravel the complex brain changes involved in the progression of AD. The damage causes the brain to lose neurons and synapses in the cerebral cortex, atrophy of the hippocampus, temporal and parietal lobes, as well as accumulation of large number of senile plaques and NFTs in the cytoplasm of neurons in the entorhinal cortex. There are two different constituents of the plaques, neurotic and

diffuse. Neurotic plaques are spherical structures that contain neurites, which are surrounded by an abnormal protein known as amyloid. Diffuse plaques lack neurites and have an amorphous appearance. As the number of plaques and tangles increases, healthy neurons begin to function less effectively. Neuron death, particularly in the hippocampus, restricts the patient's ability to form new memories.

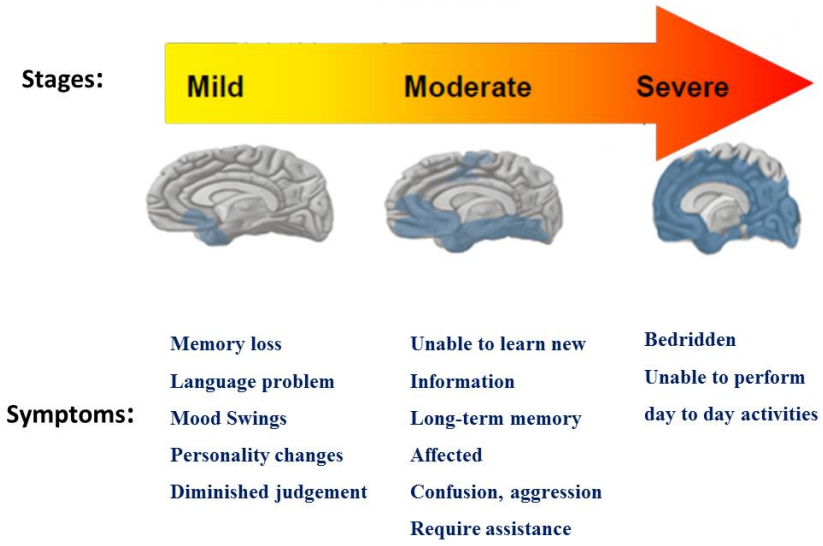


Figure 2.5. *Different stages and the associated symptoms in AD.*

2.1.4. Known factors playing a role for AD:

In order to identify the causes of AD, studies have examined the factors that can increase or decrease the risk of an individual's potential to develop the disease. Many years of research have narrowed down on number of primary impacting factors of AD which is discussed as follows.

(i) **Age:** The single greatest known risk factor for developing AD is advancing age. Although AD is not a part of normal ageing, most cases of AD are seen in older adults of ages 65 years or above. Around 5% of people are afflicted by AD between the ages of 65 and 74. The group with the highest risk increases to 50 percent with adults above 80 years. It is exceptional for AD to occur before age 60 which is a rare genetic form [34].

(ii) **Genetics:** Researchers have also identified a gene called Apolipoprotein E (ApoE) with an impact on AD risk. Approximately, 40-65% of AD patients carry at least one copy of the ε4 allele of the ApoE gene. The ApoE gene has many functions like brain development, growth, maintenance and repair. It also functions as a distributor of cholesterol and helps in maintaining lipid levels

within the brain. ApoE acclaims to account for approximately 65% of the genetics risk [35, 36]. Early-onset of AD is rare, accounting for about 5% patients with this disease. Familial AD, whilst rare, occurs due to mutations in the gene APP on chromosome 21, Presenilin 1 (PSEN1) on chromosome 14 and PSEN2 on chromosome 1 [37]. Mutations on these three genes escalate the level of Aβ which later aggregate to senile plaques leading to neuronal deaths. It affects less than 10 percent of AD patients of age below 65 and if one chromosome mutation is inherited, the person is most likely to develop AD.

(iii) **Concomitant diseases:** Cardiovascular diseases (CVD) is thought to share many risk factors with AD, and also proposed to enhance the disease progression when present [38]. Suggested mechanisms for disease progression have include indirect effects from CVD, which predisposes the brain to neurodegeneration, as well as the direct effect from vascular factors on neuronal death [39]. The mechanisms by which CVD is thought to cause AD, or participate in pathogenesis is still not clear, however lipid dysfunction is believed to play a major role. Type 2 diabetes (diabetes mellitus type 2; DM2) also shares many risk factors with AD [40] and it has even been suggested that AD is a 'type 3' of the diabetes family of diseases [41, 42]. Treatments for diabetes have even indicated a reduction in AD neuropathology [43].

2.1.5. Alzheimer's disease diagnosis:

Doctors can diagnose "possible Alzheimer's dementia," "probable Alzheimer's dementia," or some other problem causing memory complaints. To diagnose Alzheimer's, doctors' deals with mental and behavioral tests along with physical examinations which are done in 90% of AD patients. It involves measuring the decline of memory, speech, understanding skills, orientation and functional abilities. In addition, doctors may perform brain scans such as Magnetic resonance imaging (MRI) and Positron emission tomography (PET), to give a diagnosis of possible or probable AD [44, 45]. It's important to note that AD can be conclusively diagnosed only after death, with an examination of brain tissue in an autopsy.

2.1.6. Amyloid cascade hypothesis: Aβ peptide in cause of AD:

The most commonly supported hypothesis for the cause of AD relates to Aβ peptide, an intrinsically unstructured protein composed of 40-42 amino acids which is formed after the sequential cleavage of the APP, a type 1 integral cell surface membrane protein which resembles a signal transduction receptor. **Figure 2.6** shows a schematic pathway of proteolytic cleavage of APP to Aβ peptide [46]. The parent protein, composed of 695-770 amino acids, undergoes both amyloidogenic and non-amyloidogenic pathways expressed in many cells of unknown function and implicated in familial AD due to mutations in the gene that code for it [47]. Although its function is not completely understood, APP is suggested to be critical for neuron growth [48, 49].

In the mid 1980's Aβ peptide was identified as the major constituent in amyloid plaques [50]. In the year 1992, Hardy and Higgins proposed their amyloid cascade hypothesis in one of their reviews that has been highly cited [51]. 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 [52, 53].

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.

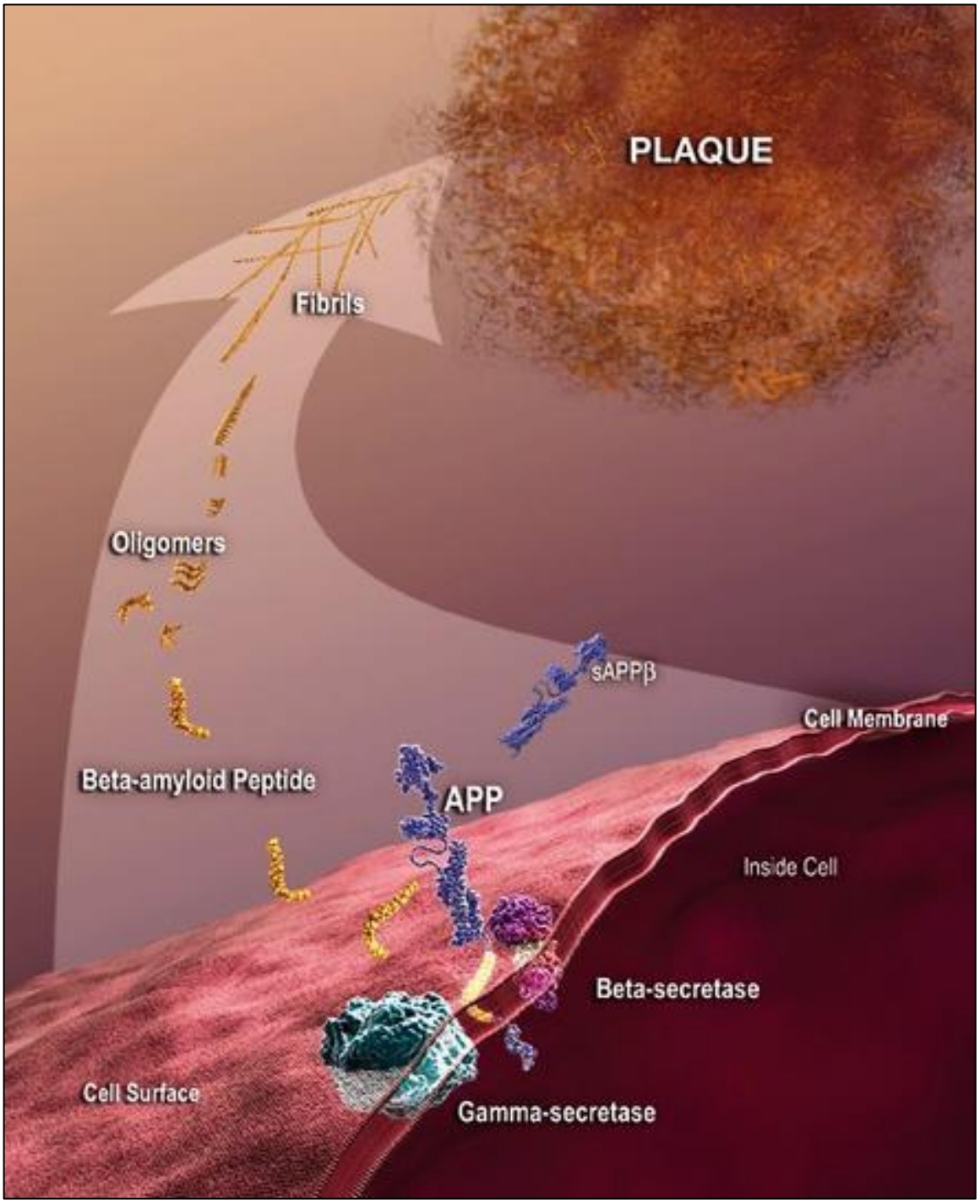


***Figure 2.6**. From Amyloid Precursor Protein to Amyloid β peptide: a hypothetical pathway (Taken from [46]).*

2.1.7. Production of Aβ peptide from APP:

The non-amyloidogenic pathway involves proteolytic processing of APP by α-secretase followed by γ-secretase which leads to the extracellular release of soluble P3 peptide fragment of 16 amino acids [54]. The amyloidogenic pathway involves cleavage of the APP on the extracellular side of the cell membrane by β-secretase followed by γ-secretase on the intracellular side of the membrane to generate $A\beta_{1–42}$ or $A\beta_{1–40}$ [54]. Of the two alloforms, $A\beta_{1–42}$ is found to be the most neurotoxic form. With two extra amino acids in the C-terminal region, $A\beta_{1-42}$ peptide is more hydrophobic and fibrillates more easily [55, 56].

2.1.8. Neuronal toxicity of $A\beta_{1–42}$ peptide:

Intracellular $A\beta_{1-42}$ peptide is neurotoxic for human neurons. Although $A\beta_{1-42}$ peptide is neurotoxic, it is selectively intracellularly cytotoxic to human neurons and not to other cell lines [57]. Disruption of Long-term potentiation (LTP) in neurons is known to be caused by $A\beta_{1-42}$ peptide and $A\beta_{1-40}$ peptide [58]. In *in vivo* study, neurons from transgenic mice expressing genes encoding mutant APP or PSEN linked to familial AD (FAD) were found to exhibit damaged synapses and loss of dendritic spine [59]. $A\beta_{1-42}$ peptide is associated in these defects because inhibition of γ-secretase advances some of the indications of synapse damage. Decrease in presynaptic terminal density and spine loss occurs prior to the deposition of amyloid plaques and deficits in LTP, which specifies the influence of oligomeric or prefibrillar $A\beta_{1-42}$ peptide on neurotoxicity. This is also supported by other works that specifically revealed the impact of soluble oligomeric $A\beta_{1-42}$ peptide in synaptoxicity and inhibition of LTP [60].

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

The Aβ monomers are about 1.0 ± 0.3 nm in size with a molecular weight of 4329.9 and 4514.1 Da for $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide, respectively [61]. Structural studies have shown that during the slow nucleation phase, α-helical or random coil $A\beta_{1-42}$ monomers change conformation and adopt partial β structure [62-64]. It is well established that following the initial misfold, the self-assembly of $A\beta_{1-42}$ peptide to form the first toxic molecule is the initial process resulting in neurotoxicity. Experimental evidence has reported biasness towards appearance of β-strand in the CHC region and the C-terminal region of $A\beta_{1-42}$ monomers [65]. Far-UV CD spectra studies were also carried out for $A\beta_{1–40}$ and $A\beta_{1–42}$ monomers wherein random coils were the dominated

characters [66]. The challenges and limitations faced by the experimental techniques for studying the aggregation-prone $A\beta_{1-42}$ monomers have stimulated the use of computational techniques to investigate the conformational dynamics of these peptides comprehensively. The approaches of various computational studies [67-70] have thus been complimentary to study the initial conformational changes of $A\beta_{1-42}$ peptide and to identify its transient states. Although many simulation works have been carried out on the monomeric structure of $A\beta_{1-42}$ peptide, the full characterization of its structure remains a major challenge.

Scope of this work

In the work described in **Chapter 4,** we have characterized the structural features of the probable initial seed structure of $A\beta_{1-42}$ peptide that might eventually lead to the aggregation using fully unrestrained MD folding simulations. In this particular study, we followed the secondary structure development in $A\beta_{1-42}$ peptide starting from its initial linear structure to its folded 3-D structure. Furthermore, structural organization of the $A\beta_{1-42}$ peptide was studied at higher temperatures.

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

Without detailed knowledge of the structure and assembly pathways, till now it has not been possible to classify the soluble $A\beta_{1-42}$ peptide species. Meanwhile soluble $A\beta_{1-42}$ peptide aggregates are generally referred to as protofibrils or oligomers [71]. They are claimed to be "on-pathway" intermediates to amyloid fibril formation and are believed to eventually get converted to fibrillar structures. $A\beta_{1-42}$ peptide aggregates to different forms which are found to be neurotoxic. The mechanism of toxicity may differ from one another.

The smallest oligomer, $A\beta_{1-42}$ peptide dimers, isolated from neuritic amyloid deposits have been reported to exhibit their toxic behavior to the neurons in the presence of microglia [72]. During the dimerization process, $A\beta_{1-42}$ peptide, initiating from either random coiled or partially unfolded monomers, aggregate and form cross-β fibrils rich structure, wherein each monomer interacts with its adjacent monomer to form a dimer [73-75]. Once dimers are formed, they serve as building blocks for monomers to form oligomers. Thus a dimer provides the first opportunity to investigate the inter-molecular interactions. Ever since the flexibility of $A\beta_{1-42}$ peptide makes the aggregation process complicated to investigate, the structural rearrangements due to inter-molecular interactions are believed to be an essential step in the fibrillation pathway [76].

Numerous computational approaches have been applied to elucidate the dimerization process of $A\beta_{1-42}$ peptide [77-81]. An efficient discrete molecular dynamics (DMD) study on the dimer formation by $A\beta_{1-42}$ peptide was found to focus on three positively charged amino acids in mediating the $A\beta_{1-42}$ peptide oligomer toxicity [82]. Dimerization of the full-length $A\beta_{1-42}$ peptide in explicit aqueous solutions has further emphasized the specificity of hydrophobic regions of the monomers in the process of dimerization [83]. However, the structural characterization of $A\beta_{1-42}$ peptide dimer at the atomistic level and the dimerization mechanism by which $A\beta_{1-42}$ peptides co-aggregate still remains unclear. Therefore, there is a motivation to examine various interactions along with the factors involved in the formation of dimers that may help in the advancement of various design strategies for the development of inhibitory approaches which target the dimerization process at an early stage.

Scope of the present work

In the work described in **Chapter 5,** we have carried out dimerization study on $A\beta_{17-42}$ peptide in terms of PMF to understand the bonding and non-bonding interactions involved in the formation of a dimer. Since $A\beta_{17-42}$ peptide has also been reported to be present in the plaques and has been found to form U-shaped protofilaments similar to those of full length $A\beta_{1-42}$ peptide, its convenient secondary structural properties provide accurate representation of the process of dimerization.

2.1.11. Cross-seeding interaction of $A\beta_{25-25}$ peptide and $Tau_{273-284}$:

The two pathological hallmark of AD are the aggregation of senile plaques and progressive accumulation of NFTs by Tau. Tau is expressed in adult human brain in six different isoforms consisting of two functional domains whose major role is to bind and stabilize the microtubules facilitating axonal transport. Hyper phosphorylation or deficiency in de-phosphorylation of Tau promotes the aggregation of Tau into NFTs [84-86]. Although mutations of Tau isoforms have been reported to induce neurodegenerative diseases, so far no distinct studies have showed the simultaneous appearance of senile plaques and NFTs in AD. It seems that both the aggregation process occurs independently of each other, as NFTs develop intracellularly whereas senile plaques develop extracellularly. Nevertheless, the senile plaques are the dominant because mutations in APP which lead to production of senile plaques cause autosomal dominant AD. On the other hand, mutations in Tau promote autosomal Frontotemporal dementia (FTD) but not AD. While a “loss of function” hypothesis is often invoked to

explain the role of Tau aggregation in AD, but does not address the role of $A\beta_{1-42}$ peptide in AD, or explain exactly how could $A\beta_{1-42}$ peptide interact with Tau [87]?

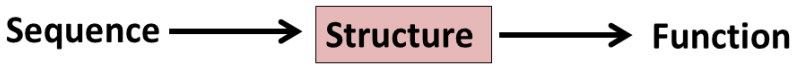
According to the amyloid cascade hypothesis, $A\beta_{1-42}$ peptide aggregation leads to AD which is β-strand rich oligomer that eventually forms fibrillar aggregates [1-3]. While this $A\beta_{1-42}$ peptide is produced extracellularly, intracellular $A\beta_{1-42}$ peptide oligomers also exist. These oligomers are reported to interact with a variety of proteins including Tau. Recent studies have suggested acceleration of Tau NFTs formation by the $A\beta_{1-42}$ peptide [88-90]. Interaction of Tau with $A\beta_{1-42}$ peptide oligomers could destabilize the microtubule integrity [91] and formation of new aggregates or enhancement of NFT generation [92]. Four different mechanisms have been identified through which $A\beta_{1-42}$ peptide may lead to Tau pathology [93-95]. These studies underscore the need for an in-depth understanding of the interaction between $A\beta_{1-42}$ peptide and Tau in order to understand the mechanism through which Aβ-Tau complex leads to AD pathology. Enhancement of Tau phosphorylation by $A\beta_{25-35}$ has been reported by Takashima and co-workers [96]. Recently a theoretical work has shown that $Tau_{273-284}$ located in the second repeat (R2) of microtubule binding region (MTBR) interact more strongly with $A\beta_{1-42}$ peptide oligomers [97]. These 273-284 fragments of Tau also play an important role in aggregation of Tau. Since it is known that the amyloidogenic peptides undergo polymerization into fibrils and do have in common similar sheet intermediates, it is conceivable that amyloid fibril formation may occur via beta sheet interactions. Although it is known that $A\beta_{1-42}$ peptide and Tau interact with each other, but exactly how an $A\beta_{1-42}$ peptide could induce subsequent aggregation with Tau is unclear. The mechanism through which Aβ-Tau complexes lead to AD pathology and the interactions between $A\beta_{1-42}$ peptide and Tau is an important aspect to explore.

Scope of the present work

In **Chapter 6,** we describe the result of our study on the interaction of $A\beta_{25-35}$ and $Tau_{273-284}$ peptide in terms of free energy analysis. Both these segments of $A\beta_{1-42}$ peptide and Tau are marked as important regions of the full length proteins. Both these fragments play crucial role in aggregation and studies on oligomer conformation and aggregation, tendency of both the fragments have been suggested before [98, 99]. $A\beta_{25-35}$ peptide is hydrophobic and is toxic in nature similar to the full length $A\beta_{1-42}$ peptide [100, 101]. Both the segments are mainly made of hydrophobic amino acids.

2.1.12. Intrinsic disordered regions in $A\beta_{1-42}$ peptide:

Our traditional view of protein structure and function is the structure–function paradigm represented as



According to the paradigm, a protein folds into a stable 3-D structure and imparts its biological functions. However, almost 20 years ago it was suggested that many proteins or regions of proteins lack a stable 3-D structure, and are rather intrinsically disordered, coined as IDPs/IDRs [102, 103]. The word "intrinsically" indicates a sequence dependent characteristic [104]. The thermodynamic definition of disordered regions in a protein is the random coil structural state. The structural disorder, which is prevalent in all organisms, is found to play roles in cellular signaling [105] and regulation, and thus IDPs are implicated in diseases [106] and represent important drug targets [107].

As the IDPs exist as an ensemble of rapidly interconverting conformations, their structural and functional characterization is a special challenge. Although they cannot be directly characterized by X-ray crystallography, there are a variety of techniques that can report their highly dynamic structural state [102]. NMR and X-ray crystallography provide site-specific information, whereas far-UV CD, size-exclusion chromatography provides qualitative and global information. The current best structural descriptions of IDPs/IDRs are solved by a combination of experimental and computational approaches.

Development of methods to predict in advance the disordered regions in a protein is getting significant attention. The capability of a method to predict the aggregation propensity of a protein from its sequence will be useful to control the unwanted protein depositions through specific sequence targeted therapeutics.

Scope of my work

In **Chapter 7,** the disordered regions in native $A\beta_{1-42}$ peptide were predicted using the disorder predictors: AMYLPRED2 [108] and DisEMBL [109]. They are chosen owing to their physicochemical premises. Also, they are independent of sequence alignment**.**

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

Oligomers formed in the initial self-assembly process of $A\beta_{1-42}$ peptide are reported to be the toxic agent [110-112]. The occurrences of $A\beta_{1-42}$ peptide oligomers confined within plaques specify to the dynamic equilibrium between these species. In human neurons $A\beta_{1-42}$ peptide oligomers are found to be present intracellularly [113]. Determination of the oligomerization state of $A\beta_{1-42}$ peptide on the membrane by visualizing individual Aβ species on the surface of murine hippocampal neurons has been carried out previously using total internal reflection fluorescence microscopy [114]. While there has been an increasing number of studies carried out to understand the oligomeric structures of $A\beta_{1-42}$ peptide, [115, 116] a conclusive X-ray diffraction or 3-D NMR structure of an $A\beta_{1-42}$ peptide oligomer is yet to be determined [117].

MD simulation study carried out by Yu and colleagues in a lipid membrane environment predicted the structure of $A\beta_{1-42}$ peptide oligomers to have a hydrophobic core and hydrophilic surface [118]. Eisenberg and coworkers prepared a novel species of amyloid oligomer with mature cross-β structure where side chains penetrate adjacent β-sheets holding the sheets together. It has been named as toxic amyloid-β fibrillar oligomer (TABFO) [119]. Although, TABFO's share structural similarity with the amyloid fibrils, they are not short protofilaments and cannot seed new amyloid fibrils. Various reports have demonstrated similar but not identical oligomeric structures formed by $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides with two additional amino acids in the C-terminal end of $A\beta_{1-42}$ peptide leading to a more diverse set of interactions [120]. However, without atomic level resolution of oligomer structure, designing inhibitors targeting one or more oligomers remains a challenge. Although conformational studies on $A\beta_{1-42}$ peptide oligomers have been carried out, yet little is known about the initial stages of oligomerization.

While $A\beta_{1-42}$ peptide has a poorly defined monomeric structure, recent advances have allowed researchers to derive the structural knowledge of amyloid fibril from various experimental studies which provide information on molecular fold and intermolecular packing (β-sheet formation and organization). The "cross-β" structure of amyloid fibril is established by fiber diffraction studies, wherein $A\beta_{1-42}$ peptide molecules assemble into β-sheets with β-strands oriented perpendicular to the long axis of the fibril [121-124].

Figure 2.7 shows a structural model for Aβ fibrils formed by the 42-residue Aβ peptide, based on a set of experimental constraints from solid state NMR spectroscopy [125]. When these single protofilaments wrap around one another, they form a mature amyloid fibril. The β-sheet structure in the amyloid fibril was further established by the binding of β-sheet specific dyes such as thioflavin-T and Congo red [126]. It should be noted that X-ray diffraction method was used to measure the detailed cross-β structures of microcrystals of several short peptides forming amyloid fibrils by Sawaya *et al.* [127]. More detailed studies have demonstrated β-sheets with polypeptide chains to run roughly perpendicular to the fibril axis which is referred to as a cross-beta pattern [128]. MD simulations show strong hydrophobic interactions between non-polar residues of the β-strand that result in fibril core [129].

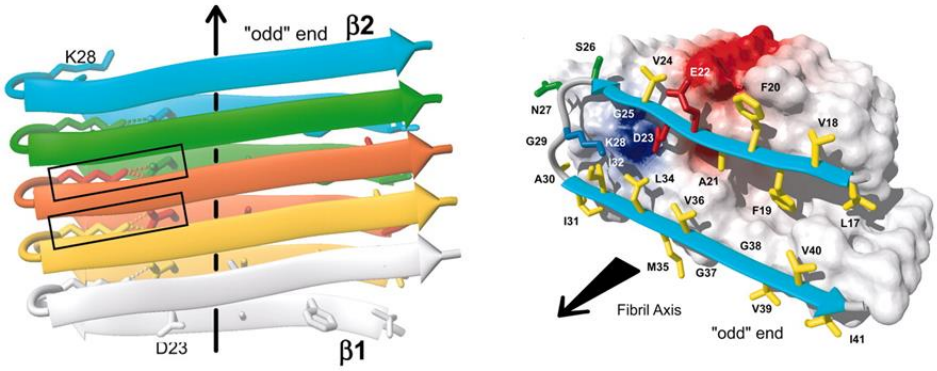


Figure 2.7. *The 3D structure of $A\beta_{1-42}$ fibril illustrating the intermolecular nature of the inter--strand interaction (Taken from [125]).*

β-sheet stacking in $A\beta_{1-42}$ peptide can be parallel, anti-parallel, made up of parallel dimers or parallel stacks of anti-parallel dimers [130]. Tycko and colleagues studied both the fibrils formed from antiparallel β-sheets and parallel β-sheets respectively and found antiparallel β-sheets to nucleate [131]. Both were found to be equally neurotoxic. Experimental results suggest that the observed amyloid-like crystals are thermodynamically stable, although kinetic trapping can be driven by electrostatic side chain interactions.

A primary property of amyloid fibrils is their ability to proliferate by addition of misfolded monomers from their surroundings. Likewise, polymorphism is another important property of amyloid fibrils [132]. Amyloid fibril polymorphs are different bundled arrangements of the basic amyloid protofilament structures. These polymorphs contain distinct molecular structures and thus can propagate themselves [133]. Many

experimental studies have tried to study the polymorphism of $A\beta_{1-42}$ fibrils [134] that review current information about variations in molecular structures that underlie amyloid polymorphism. Similarly, $A\beta_{1-42}$ peptide can lead to the formation of different molecular structures of $A\beta_{1-42}$ fibrils depending on specific growth conditions. Polymorphism may arise if the protofilaments in the fibril possess different orientations or if they exhibit different internal structures [134]. The fact that polymorphism may arise from the formation of differently structured fibril nuclei is supported by earlier evidence [135]. Certain nucleation event and critical nuclei may form leading to molecular-level polymorphism of $A\beta_{1-42}$ fibrils [136]. Changes in backbone orientation, backbone conformation and differences in the way of association of oligomers may lead to fibril morphologies. As it is a known fact that the mature amyloid fibrils are formed by the aggregation of the disordered monomers, there is a considerable change in the aggregation pattern and the fibril morphology. Although fibril polymorphs evoke different clinical characteristics and neuropathologies, the structural basis of the superstructures are yet not clearly understood.

Scope of the present work

In **Chapter 8,** we have investigated the structural dynamics of the toxic $A\beta_{1-42}$ peptide intermediates and analyzed the simulation trajectories to examine the interactions that stabilize the oligomers. Additionally, we subjected the equilibrated structure of the oligomers in PDBsum server to examine the protein-protein interactions. **Chapter 7** also presents structural details of the polymorphs of $A\beta_{1-42}$ fibril that are reported in Protein Data Bank. Additionally, we have examined the inter-molecular interactions that hold together the monomeric units in the respective polymorphs of $A\beta_{1-42}$ fibril.

2.1.14. Inhibitors of $A\beta_{1-42}$ peptide aggregation:

Although there is no cure to AD, symptomatic treatments do exist with currently available drugs which are a mere hope. Two Phase III clinical trial failures on two Aβ-targeting monoclonal antibodies, bapineuzumab and solanezumab, in patients with mild-to-moderate AD have added further gloom to the outlook [137]. From the genetic evidence it is very much clear that amyloid-β drives the disease process, so decreasing its production or stimulating its clearance in the brain is an attractive aim. In the past years intense research in medicinal chemistry has been carried out to develop therapeutics that aimed at preventing $A\beta_{1-42}$ peptide aggregation [138].

Till date, a large number of potential Aβ fibrillogenesis inhibitors have been suggested, for instance carbohydrate-containing compounds [139, 140], polyamines, [141,142] chaperones [143], metal chelators [144], osmolytes [145], and RNA aptamers [146]. Also disassembly of pre-formed amyloid fibrils using small organfluorine molecules and light has been reported. The biological application of anti-aggregating molecules [141], nanoparticles [147-150], degrading enzymes [151, 152], and affibody molecules [153] as disease-modifying remedies have gained interest over the years. Tacrine hybrids [154], benzylphenoxypyridine and pyrimidines [155], 3-Aminopyrazole derivatives [156], symmetric triazine derivatives [157] and resveratrol derivatives [158] are some of the developed agents. Significant efforts have been made to find drugs to combat with this disease.

Scope of the present work

Chapter 9, 10 and 11 demonstrates different approaches we have employed to inhibit the aggregation of $A\beta_{1-42}$ peptide at early and later stages. In **Chapter 9, 10 and 11** we used ss- oligonucleotide; $A\beta_{1-40}$ peptide an isoform of $A\beta_{1-42}$ peptide and 6-mer peptide (IGLMVV) respectively as potent inhibitor in the aggregation process of $A\beta_{1-42}$ peptide in the early and later stage.

2.2. Main objectives of the thesis:

[1] Despite a high degree of sophistication, probing the conformational changes of $A\beta_{1-42}$ peptide aggregation is challenging owing to the vast heterogeneity of the aggregates and the sensitivity of the process to different environmental conditions. Thus our first objective is set to characterize the salient structural features of initial seed structure of $A\beta_{1-42}$ peptide responsible for aggregation.

[2] A dimer provides the first opportunity to investigate the inter-molecular interactions that lead to the formation of toxic aggregates. Thus our second objective is addressed to reveal the interactions between the $A\beta_{17-42}$ peptide units during the process of dimerization.

[3] The two pathological hallmarks associated with AD include the accumulation of senile plaques and the generation of neurofibrillary tangles (NFTs) by Tau, but there is no reasonable explanation for the $A\beta_{1-42}$ peptide and Tau interaction in particular. Therefore, we framed the third objective to reveal the cross seeding interactions of $A\beta_{1-42}$ peptide.

[4] Our protein of interest $A\beta_{1-42}$ peptide is an IDP that misfolds and aggregates to form senile plaques leading to the AD. Thus our fourth objective is to examine the intrinsic disorderness and regions in the $A\beta_{1-42}$ peptide.

[5] While oligomers are the most critical players in the pathology of AD and fibril fragmentation are toxic as well, there is currently little information in atomistic level and the dynamics of their assembly. Thus our fifth objective is to examine the structure and stability of transient $A\beta_{1-42}$ peptide oligomers.

[6] Polymorphic structures of $A\beta_{1-42}$ peptide perhaps induce the difficulty in understanding the pathological mechanism of AD. In this context we set our sixth objective to study the $A\beta_{1-42}$ fibril polymorphism.

[7] Although there is no cure to AD, a large number of potential Aβ fibrillogenesis inhibitors have been suggested. Significant efforts have been made to find drugs to combat with this disease. Consequently we have set our last objective to design the inhibition methods for aggregation of $A\beta_{1-42}$ peptide at early & later stage.