CHAPTER 6

Cross-seeding interactions between Amyloid β and Tau protein can enhance aggregation?

Cross-seeding interactions between Amyloid β and Tau protein can enhance aggregation?

6.1. Abstract:

The two pathological hallmarks associated with AD include the accumulation of senile plaques and the generation of neurofibrillary tangles. Although it is a known fact that interaction of Tau with $A\beta_{1-42}$ peptide oligomers could destabilize the microtubule integrity and might lead to the formation of new aggregates, there is no reasonable explanation for the $A\beta_{1-42}$ peptide and Tau interaction in particular. In this Chapter, we have carried out PMF analysis in order to examine the cross-seeding interactions between $A\beta_{25-35}$ and $Tau_{273-284}$. The $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer; $Tau_{273-284}/Tau_{273-284}$ homo-dimer and $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer were constructed using PatchDock. We have used MD simulation with the US methodology to examine the cross-sequence interactions between homo-dimers and the hetero-dimer. Our study reveals that the formation of $A\beta_{25-35}/Tau_{273-284}$ heterodimers leads to a structural transition for both the peptides from compact form to extended form and that $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer formation are preferential over the $Tau_{273-284}/Tau_{273-284}$ and $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer formation.

6.2. Introduction:

The two pathological hallmark of AD are the aggregation of senile plaques [12, 13] and progressive accumulation of NFTs by Tau [27, 28]. Both the aggregation process occurs independently of each other, as NFTs develop intracellularly whereas senile plaques develop extracellularly. Recent studies have suggested that interaction of Tau with $A\beta_{1-42}$ peptide oligomers could destabilize the microtubule integrity and might lead to the formation of new aggregates [209]. Studies have revealed that $A\beta_{1-42}$ peptide may lead to Tau pathology via four different mechanisms [210]. 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. Here we have studied the interaction of Aβ and Tau fragment: $A\beta_{25-35}$ and $Tau_{273-284}$ by analyzing the free energy profile since both these segments of $A\beta_{25-35}$ and $Tau_{273-284}$ are noted as important regions of the full length proteins. Both these fragments tend to be aggregation prone and studies on oligomer conformation have been carried out before. $A\beta_{25-35}$ peptide is hydrophobic and is toxic in nature similar to the full length

$A\beta_{1-42}$ peptide [98, 99]. Enhancement of Tau phosphorylation by $A\beta_{25-35}$ peptide has been reported by Takashima and co-workers [96]. Recent work has shown that $Tau_{273-284}$ located in the second repeat (R2) of Microtubule binding region (MTBR) interacts more strongly with Aβ oligomers [97]. Although it is known that Aβ and Tau interact with each other, the mechanism behind their cross-seeding behavior is still unclear. So, in this study we have combined MD simulation with the US methodology to examine the cross-sequence interactions between $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer; $Tau_{273-284}/Tau_{273-284}$ homo-dimer and $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer.

6.3. Materials & Methods:

6.3.1. Preparation of initial $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer; $Tau_{273-284}/Tau_{273-284}$ homo-dimer and $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer:

The initial monomer structure of $A\beta_{25-35}$ peptide was extracted from the full length $A\beta_{1-42}$ peptide (1IYT) [211] from the Protein Data Bank [212]. We constructed the initial monomer structure of $Tau_{273-284}$ from the 2MZ7 NMR structure [213]. The monomeric structure was then solvated with TIP3P [170] water model with solvent buffer being 10 Å in all directions. To neutralize the negative charge of the monomer, appropriate numbers of sodium ions were added.Further minimization, heating and equilibration were carried out as described in Chapter 5 (section 5.3.1). The equilibrated $A\beta_{25-35}$ peptide and $Tau_{273-284}$ structure was used to generate the possible homo-dimers and hetero-dimer structures using the PatchDock [175] web server.

6.3.2. PMF calculation:

We have calculated the PMF [172] of the homo-dimer ($A\beta_{25-35}/A\beta_{25-35}$, ($Tau_{273-284}$/ $Tau_{273-284}$) and hetero-dimer ($A\beta_{25-35}/Tau_{273-284}$) using the US simulations [173] with WHAM as described in section 5.3.3. Two sets of independent simulations were performed over the COM distance between the two monomers, one of decreasing inter-chain distances and the other of increasing inter-chain distances at 1 Å intervals. The inter-chain distances for the homo-dimers and the hetero-dimer is shown in **Table 6.1**.

Table 6.1: *The inter-chain distances for the homo-dimer $A\beta_{25-35}/A\beta_{25-35}$, $Tau_{273-284}/Tau_{273-284}$ and the hetero-dimer $A\beta_{25-35}/Tau_{273-284}$.*

Dimer	Starting distance between COM M1-COM M2 (Å)	Decreasing inter-chain distance (Å)	Increasing inter-chain distance (Å)
$A\beta_{25-35}/A\beta_{25-35}$ homo-dimer	11	11-1	11-30
$Tau_{273-284}/Tau_{273-284}$ homo-dimer	15	15-1	15-30
$A\beta_{25-35}/Tau_{273-284}$ hetero-dimer	13	13-1	13-30

6.4. Results & Discussions:

6.4.1. Free energy analysis of $A\beta_{25-35}$ peptide with $Tau_{273-284}$ peptide:

To date, the simultaneous accumulation of plaques and tangles in AD is a mystery. By computing the free enrgy profile of $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer complex we have demonstrated that a stable complex can form between the two proteins. The PMF graph present evidence that $A\beta_{25-35}$ peptide has a strong tendency to bind strongly to $Tau_{273-284}$ peptide and thus we can assume that this hetero-dimer formation may be a precursor event to later self-aggregation of both the proteins. **Figure 6.1** illustrates the PMFs for the $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer, $Tau_{273-284}/Tau_{273-284}$ homo-dimer and $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer.

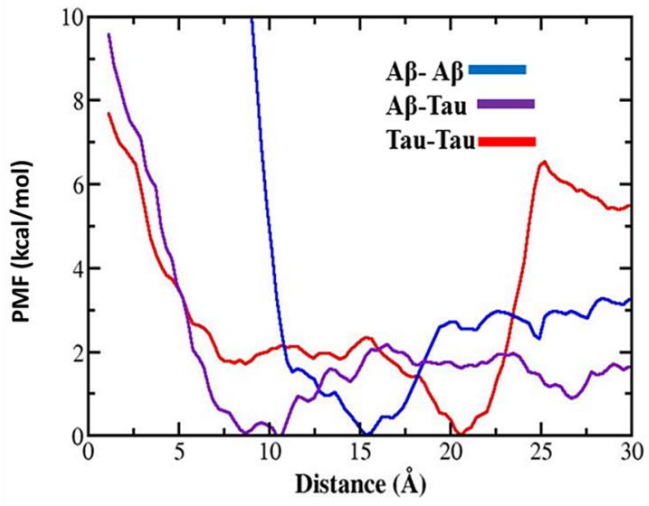

Figure 6.1. *Potential of mean force as a function of the reaction co-ordinates for the association of the homo-dimer ($A\beta_{25-35}/A\beta_{25-35}$, $Tau_{273-284}/Tau_{273-284}$) and hetero-dimer ($A\beta_{25-35}/Tau_{273-284}$) (in kcal/mol).*

The PMF profile of $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer showed presence of a minimum at a separation of 8 Å and 11 Å. The energy barrier between $A\beta_{25-35}$ and $Tau_{273-284}$ peptide with increasing inter-chain distances was found to be around 2 kcal/mol indicating that the two peptide fragment exhibits a mild *van der Waals* force of repulsion. On contrary, the energy barrier shoots drastically up to more than 9 kcal/mol indicating a very strong *van der Waals* force of repulsion as the distance was gradually decreased to a distance of 1 Å. $A\beta_{25-35}/A\beta_{25-35}$ homo-dimers was found to have a main basin of attraction at around the inter chain distance of 21 Å and $Tau_{273-284}/Tau_{273-284}$ homo-dimers to have a main basin of attraction at around the inter chain distance of 15 Å. From the values of the global minimum we can say that $A\beta_{25-35}/Tau_{273-284}$ hetero-dimers have the highest possibility to interact with each other and thus form dimers.

6.4.2. Protein-Protein interaction study of the optimized $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer and $A\beta_{25-35}/A\beta_{25-35}$ and $Tau_{273-284}/Tau_{273-284}$ homo-dimers:

In the PMF plot we noticed the lowest energy conformers of the homo-dimers of $A\beta_{25-35}/A\beta_{25-35}$ and $Tau_{273-284}/Tau_{273-284}$ and $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer at the minimum of separation. We have isolated those lowest energy conformers and carried out the protein-protein interaction studies in the PDBsum server [180]. The interface statistics of the $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer and $A\beta_{25-35}/A\beta_{25-35}$ and $Tau_{273-284}/Tau_{273-284}$ homo-dimers are shown in **Table 6.2**. In case of $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer, the interface area for each monomer was found to be around ~250-300 $Å^2$ and the $Tau_{273-284}/Tau_{273-284}$ homo-dimer had the interface area in the range of ~350 $Å^2$. In contrary the interface area for each monomer of $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer was found to be more than 400 $Å^2$ (**Table 6.2**).

In the same manner, the total number of interface residues in case of $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer was found to be 4 & 6; $Tau_{273-284}/Tau_{273-284}$ homo-dimer was found to be 7 & 5 and for $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer 8 & 7 (**Table 6.2**). Additionally, **Table 6.2** shows the number of non-bonded contacts stabilizing the complex, wherein in case of the $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer it was found to be highest in number (135). $A\beta_{25-35}/A\beta_{25-35}$ and $Tau_{273-284}/Tau_{273-284}$ homo-dimer was found to contain a low number of non-bonded contacts; 88 and77, respectively. The significant intensities of non-bonded contacts and interface area of the $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer indicate that the Tau fragment has a high affinity to bind $A\beta_{1-42}$ monomers relative to Tau monomers.

Table 6.2: *The interface plot statistics of the homo-dimers (Aβ25-35/Aβ25-35, Tau273-284/Tau273-284) and hetero-dimer (Aβ25-35/Tau273-284) as predicted by the PDBsum server.*

Chain	No. of interface residues	Interface Area (Å^2)	No. of salt bridges	No. of hydrogen bonds	No. of non-bonded contacts
Aβ25-35	8	406			
Tau273-284	7	465	0	1	135
Aβ25-35	4	293			
Aβ25-35	6	244	0	0	88
Tau273-284	7	342			
Tau273-284	5	376	0	1	77

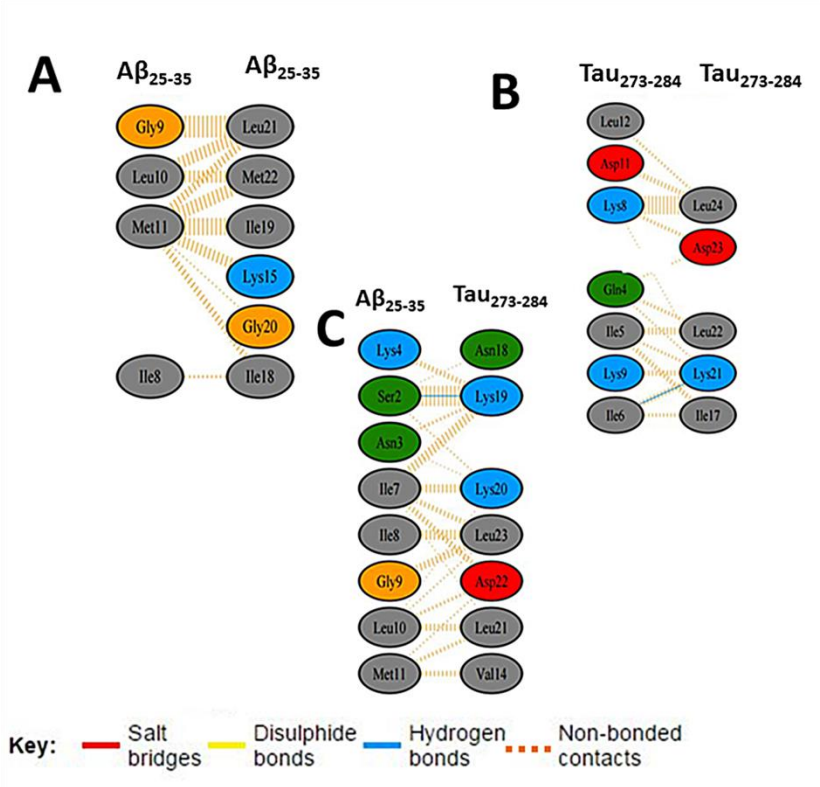

Figure 6.2. *The interface residues showing different interactions as predicted by the PDBsum server of A) Aβ25-35/Aβ25-35 homo-dimer; B)Tau273-284/Tau273-284 homo-dimer; C) Aβ25-35/Tau273-284 hetero-dimer.*

Figure 6.2 shows the important residues involved in the interaction of the homo-dimer and the hetero-dimer with hydrogen bonding and non-bonded contacts. **Figure 6.2.A** shows the interacting residues of $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer and **Figure 6.2.B** shows the interacting residues of and $Tau_{273-284}/Tau_{273-284}$ homo-dimer. In both the cases we can see involvement of hydrophobic residues in the interaction. **Figure 6.2.C** shows the interacting residues of $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer where more number of hydrophobic residues are prominent. The high distribution of hydrophobic residues in the $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer suggests that the hydrophobic interaction plays an important role in the cross-seeding interaction of $A\beta_{25-35}/Tau_{273-284}$ peptide.

6.4.3. Conformational dynamics of the optimized $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer and $A\beta_{25-35}/A\beta_{25-35}$ and $Tau_{273-284}/Tau_{273-284}$ homo-dimers:

The conformational details of the lowest energy conformers of homo-dimers and the hetero-dimers at the optimal inter-chain distances are shown in **Figure 6.3. Figure 6.3.A** shows the lowest energy conformer of $A\beta_{25-35}/A\beta_{25-35}$ homo-dimer, where we can see that one of the monomers is in coil form whereas the other monomer retains α-helix in some of its region. In case of $Tau_{273-284}/Tau_{273-284}$ homo-dimer (**Figure 6.3.B**) both the monomer retain α-helical region which indicate that $Tau_{273-284}$ is less disordered in comparison to $A\beta_{25-35}$ peptide. As we observed two global minimum in the PMF plot for $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer, respective lowest energy conformers at 8 Å and 11 Å are shown in **Figure 6.3.C**. In both the conformers, both the monomers are in random coil form. We can say that $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer underwent much more disorder in the course of the association process to form the hetero-dimers.

Similar results were obtained from studies done by Tanh and his group wherein they have showed that $A\beta_{25-35}$ peptide and $Tau_{273-284}$ peptide interact with each other to promote hetero-oligomer formation; with the difference that herein we have studied the hetero-dimer formation in terms of the free energy profile [214]. Our free energy profile for the $A\beta_{25-35}/Tau_{273-284}$ hetero-dimer also suggested a strong association between the two monomers. Guo and his group also reported that $A\beta_{25-35}$ and $Tau_{273-284}$ peptide interact with each other to form soluble complex [215].

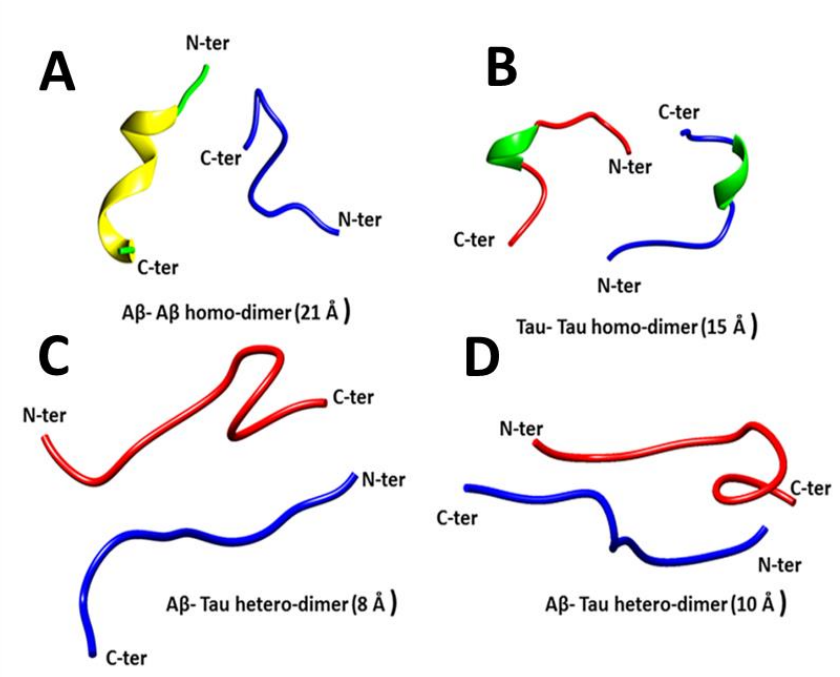

Figure 6.3. *Snapshots at 300K during the time course of simulation period at optimal inter-chain distances of A) $A\beta_{25\text{-}35}/A\beta_{25\text{-}35}$ homo-dimer; B) $Tau_{273\text{-}284}/Tau_{273\text{-}284}$ homo-dimer; C&D) $A\beta_{25\text{-}35}/Tau_{273\text{-}284}$ hetero-dimer.*

6.4.4. Residue-Residue interaction profile of the optimized $A\beta_{25\text{-}35}/Tau_{273\text{-}284}$ hetero-dimer structure:

Figure 6.4 shows interacting residues between the two chains at optimum distance from each other that was calculated using CMA [178]. As the inter chain distance lies at an optimum value, the stearic hindrance between the monomeric chains is likely to decrease, thus aiding in the conformational transitions of the monomers that further associate to form the extended dimer. **Figure 6.4.A** and **6.4.B** show the residues involved in the homo-dimer formation of $A\beta_{25\text{-}35}/A\beta_{25\text{-}35}$ and $Tau_{273\text{-}284}/Tau_{273\text{-}284}$ respectively. From the figure we can see that a few numbers of surface hydrophobic groups are available for interaction. On the other hand, from **Figure 6.4.C** we observed that the $A\beta_{25\text{-}35}/Tau_{273\text{-}284}$ hetero-dimer is stabilized by a larger number of hydrogen bonding network that is formed between backbone atoms of hydrophobic residues such as leucine and valine and hydrophilic charged residues such as lysine.

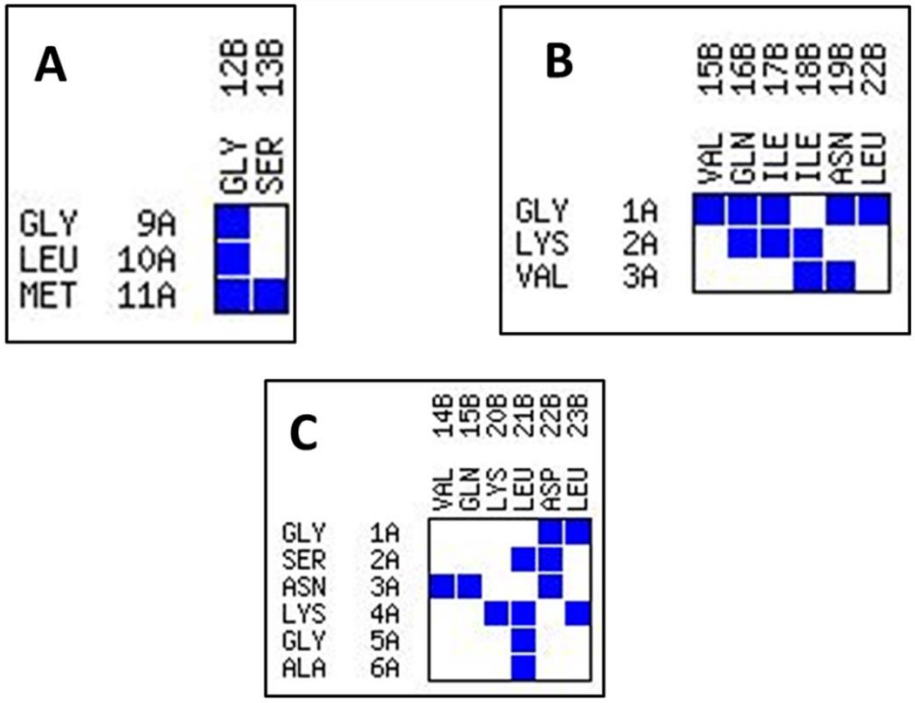

Figure 6.4. *Cα-Cα contact probability map (inter-peptide) at 300 K of the monomeric units of the A) $A\beta_{25\text{-}35}/A\beta_{25\text{-}35}$ homo-dimer ; B) $Tau_{273\text{-}284}/Tau_{273\text{-}284}$ homo-dimer; C) $A\beta_{25\text{-}35}/Tau_{273\text{-}284}$ hetero-dimer at optimal inter-chain distance.*

6.5. Conclusions:

Since $A\beta_{1\text{-}42}$ peptide is known to interact with other related peptides, we carried out the free energy analysis of $A\beta_{25\text{-}35}$ peptide with one important peptide known to be risk factor of AD, $Tau_{273\text{-}284}$. Our PMF study reveals that $A\beta_{25\text{-}35}$ peptide has a strong tendency to form $A\beta_{25\text{-}35}/Tau_{273\text{-}284}$ hetero-dimer complex and the hetero-dimer complex formation leads to a structural transition for both the peptides from compact form to extended form. The protein-protein interaction study showed that $A\beta_{25\text{-}35}/Tau_{273\text{-}284}$ heterodimer has high interface area scores over the $A\beta_{25\text{-}35}/A\beta_{25\text{-}35}$ and $Tau_{273\text{-}284}/Tau_{273\text{-}284}$ homo-dimers and the contact map analysis indicated that the hetero-dimer is stabilized by the hydrogen bonding network between backbone atoms of hydrophobic and charged residues. The findings from the current study thus suggest that the $A\beta_{1\text{-}42}$ peptide may bind to Tau monomers thereby forming hetero-dimers and later hetero-complexes and may accelerate aggregation. Also as $A\beta_{1\text{-}42}$ peptide interacts with the Tau protein it shifts the population of Tau by resulting in a decrease in the percentage of Tau monomers required to regulate the microtubule dynamics and consequently leading to toxicity by degradation of microtubules.