CHAPTER 5

Structural characterization of $A\beta_{17-42}$ peptide dimer by potential of mean force analysis: Insights from molecular dynamics simulations

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5.1. Abstract:

Recent experiments with $A\beta_{1-42}$ peptide have indicated that the initial dimerization of $A\beta_{1-42}$ monomers to form amyloid dimers stand out as a key event in the generation of toxic oligomers. 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. In the present study, the process of $A\beta_{17-42}$ peptide dimerization which is known to play an important role in the plaque formation was evaluated in terms of PMF, which provided free energies along the reaction coordinates. The global minima structure of the $A\beta_{17-42}$ peptide dimer at the minimum distance of separation was isolated from the calculated free energy profile indicating a strong tendency of the monomers to associate and form the dimer. Further, the interactions involved in the formation of the dimer structure were examined and the global minima structure was used for the identification of protein-protein interfaces and the residue-residue interactions vital for generation of the dimer complexes. The simulation results elucidated hydrogen bonding to be a critical factor for the stability of the dimer structure. The results thus provide an atomistic insight into the bonding and non-bonding interactions involved in the dimerization process of $A\beta_{1-42}$ peptide along with the spontaneous formation of several basic structural units of dimer structure, thereby offering a key contribution to enhance our fundamental knowledge about AD and to design inhibitors to disrupt the $A\beta_{1-42}$ peptide dimers.

5.2. Introduction:

Till date numerous computational approaches have been applied to investigate the dimerization process of $A\beta_{1-42}$ peptide [79-83]. 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 on the process of dimerization [83]. However, the free energy calculation of the dimerization process for the full length $A\beta_{1-42}$ peptide remains unexplored. In the present study, the monomer structure of $A\beta_{17-42}$ extracted from the solid state pentamer structure (PDB ID: 2BEG) [125] was used to construct the initial dimer model structure. $A\beta_{17–42}$ peptide is produced from the cleavage of amyloid

precursor protein by α- and γ-secretases and is observed in amyloid plaques which are composed of amyloid fibrils [202]. It has been reported that the $A\beta_{17–42}$ peptide structures form U-shaped protofilaments similar to those of $A\beta_{1-40}$ or $A\beta_{1-42}$ peptide, which is supported by computational study [203]. Additionally, in the (pentamer structure) 2BEG model, the residues 18-42 form a strand-turn-strand motif that contains two parallel, in-register-sheets that are formed by residues 18–26 and 31–42 [125]. Since $A\beta_{17–42}$ peptide is a key fragment of $A\beta_{1-42}$ peptide, the formation of its U-shape protofilament is likely to be very similar to that of $A\beta_{1-42}$ peptide. Justification for this idea is that $A\beta_{17–42}$ peptide contains the two hydrophobic stretches that dominate the aggregation and fibrillization of $A\beta_{1-42}$ peptide as well as the turn region. Because of its convenient secondary structural properties; the dimerization study using the 2BEG model provides a more accurate representation of the process of dimerization. Although oligomerization studies of $A\beta_{17-42}$ peptide have been carried out previously; detailed structural characterization of dimerization of $A\beta_{17-42}$ peptide still remains to be investigated. The present study primarily focuses on the investigation of the early dimerization process of $A\beta_{17-42}$ peptide corresponding to the hydrophobic segment of the full length $A\beta_{1-42}$ peptide in order to get a full view of the interactions involved in the process.

In this study, US simulations [173] were utilised to estimate the association energy of the $A\beta_{17-42}$ peptide dimer. At the initial phase of the investigation, evaluation of the process of dimerization in terms of PMF [172] was carried out, which was computed as a function of inter-chain distances between the entire C-α atoms of the monomers. Here one of the monomers served as a reference, while the other monomer was placed at both increasing and decreasing centre-of-mass (COM) distance from the reference with its position maintained by a biasing potential at two different sets. These COM distances represent so-called "sampling windows," wherein independent simulations were conducted to generate an ensemble of structures along the reaction coordinate. Furthermore, to monitor the dimerization process, the conformer with the most populated cluster on the basis of the free energy profile, was isolated from the PMF plot at the minimum monomer-monomer separation, and the protein-protein interactions between the two monomers was investigated. To gain insight into the bonding and non-bonding interactions along with interface residues early events of $A\beta_{17-42}$ peptide self-assembly, the PDBsum server [180] was used.

5.3. Materials & Methods:

5.3.1. Preparation of initial $A\beta_{17-42}$ peptide dimer model:

The initial monomer structure of $A\beta_{17-42}$ peptide to construct the dimer structure was created by deleting the corresponding number of peptide chains from the pentamer structure of $A\beta_{17-42}$ (PDB ID: 2BEG) [125]. 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.

The system was minimized in two stages to ensure the stability wherein, it was first subjected to 500 steps of steepest decent minimization followed by 500 steps conjugate gradient minimization. The system was first constrained by 50 kcal/mol/$Å^2$ harmonic potential to remove the bad contacts. The whole system (monomer with water) was subsequently minimized using 1,000 steps of steepest decent minimization without the harmonic restraints at NVT ensemble. The system was then gradually heated from 0 to 300 K without under atomic restraints over a timescale of 20 ps. The system was switched to NPT ensemble and equilibrated for 100 ps without applying any restraint. The bonds to the hydrogen atoms were constrained using the SHAKE algorithm [167]. Subsequently MD was performed under constant pressure-temperature conditions (NPT) with temperature regulation achieved using Berendsen weak coupling method [168] (0.5 ps time constant for heat bath coupling and 0.2 ps pressure relaxation time) and calculated electrostatic forces using the PME procedure [190].The equilibrated $A\beta_{1-42}$ peptide after equilibration was used to generate the possible dimeric structures using the PatchDock [175] web server.

5.3.2. Construction of dimer structure using docking:

The conformer representing the most populated clusters after each individual equilibration was used to generate the possible $A\beta_{17-42}$ peptide dimer structures. Using the PatchDock [175] web server the selected conformer was docked to the copy of itself, and the best energy dimer was chosen in terms of minimum free energy and maximum contact surface area. This server applies the concept of geometric-based docking algorithm to select the optimum candidate with the RMSD clustering to remove the redundant models. Each model was given a score which implies docking transformation of one of the monomer which optimally fit with the other monomer

inducing both wide interface areas and small amounts of steric clashes. In our current study, a default RMSD of 4 Å was considered. The $A\beta_{17-42}$ peptide dimer with the maximum atomic contact energy and minimum global energy was selected.

The selected $A\beta_{17-42}$ peptide dimer was solvated in TIP3P water model [170] with a minimum distance of 10 Å to the border and then subjected to a two-step restrained minimization, followed by heating. Then the dimer was equilibrated for 100 ps. Such time was sufficient to obtain the stable dimer configuration. As our initial dimer had attained equilibration, so we ran production MD simulations for 10 ns. The dimer stabilized its conformation after 10 ns production run and their RMSD did not change significantly. Clustering was performed on a series of MD trajectories [204]. Trajectories were created from independent runs leading to a partitioning into six clusters. **Figure 5.1** shows the distribution of conformer population of $A\beta_{17-42}$ peptide dimer.

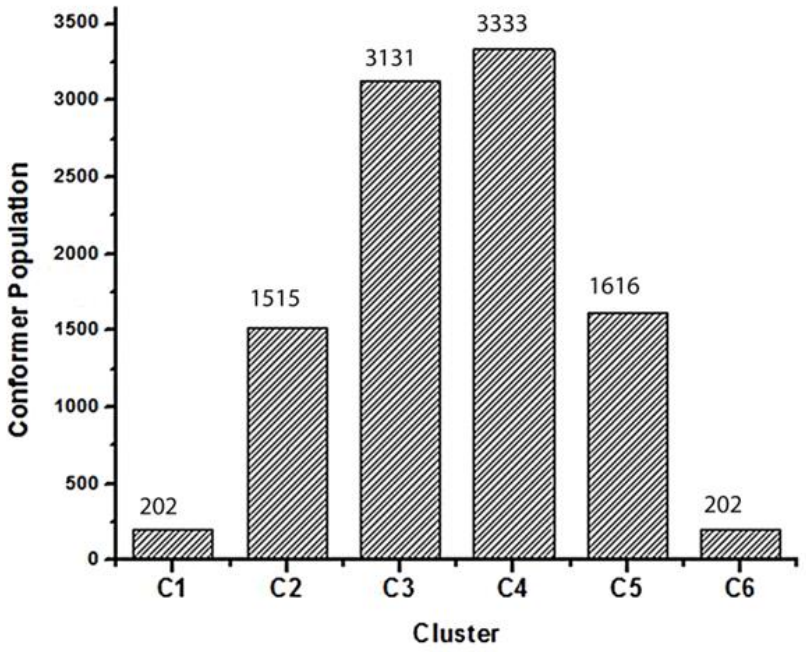

Figure 5.1. *Distribution of conformer population of $A\beta_{17-42}$ peptide dimer.*

5.3.3. PMF calculation:

In order to determine the free energy profile of the $A\beta_{17-42}$ peptide dimer, the US simulations with WHAM [174] was used. The reaction coordinate for the dimer was its distance between the entire C-α atom of the amino acids of monomer 1 and the monomer 2 and its space was divided into windows. For each independent simulation, the $A\beta_{17-42}$ peptide dimer was allowed to sample within that window only. The PMF was calculated by combining the data from each window which was achieved by applying a harmonic restraint of 2 kcal/mol/$Å^2$ to the reaction coordinate. To remove the non-equilibrium effects that may contaminate the PMF, the first few nanoseconds in each window were treated as an equilibrium phase, and as such were ignored for post-processing. Two sets of independent simulations were performed over the center of mass distance between the two monomers of $A\beta_{17-42}$ peptide dimer, one of decreasing inter-chain distances and the other of increasing inter-chain distances. The inter-chain distances for the $A\beta_{17-42}$ peptide dimer is shown in **Table 5.1.** The restart file of the previous step was used as the input file for the configuration in both the increasing and decreasing cases. After an increment of 1 Å, windows were obtained. For each window 10 ns, NPT MD run was performed and for the next window the resulting equilibrated structure was used as the starting co-ordinate. After every MD run, the VMD package was used for visualization of the trajectories generated [192]. At large separation of monomeric units in the dimer, the PMF data was normalized using centering and standard deviation method.

Table 5.1: *The inter-chain distances between the monomeric units of $A\beta_{17-42}$ peptide dimer.*

Dimer	Starting distance between COM M1-COM M2 (Å)	Decreasing inter-chain distance (Å)	Increasing inter-chain distance (Å)
$A\beta_{17-42}$-$A\beta_{17-42}$	4	4-1	4-25

5.3.4. Identification of interface residues and hot spot residues:

On the basis of the free energy profile, the conformer with the most populated cluster was isolated at the minimum monomer-monomer separation from the PMF plot and MD simulations were carried out. The trajectory of the last simulation run was selected from which we extracted the $A\beta_{17-42}$ peptide dimer structure with the most populated cluster for evaluating the protein-protein interfaces and the residue-residue interactions made across them using PDBsum server [180].

5.4. Results & Discussions:

5.4.1. Free energy analyses of $A\beta_{17-42}$ peptide dimer:

Dimer formation is the first step in the aggregation process of $A\beta_{1-42}$ peptide to form the toxic oligomers. The purpose of this study was to elucidate the various interactions involved between the monomeric units of the $A\beta_{17-42}$ peptide dimer to form a stable dimer structure and also to examine the conformational variability of the $A\beta_{17-42}$ peptide dimers. The conformer of dimeric complex from the cluster C 4, the most populated cluster, was taken as the initial structure for carrying out the PMF calculations. Figures displaying the cluster analysis can be seen in **Figure 5.2.** Atomistic MD simulations were carried out and PMF profile was constructed as a function of the RC as displayed in **Figure 5.2**. For $A\beta_{17-42}$ peptide dimer the numerical value of the PMF is set to 0 at a separation of 7.5 Å. This result indicates the presence of a minimum at a monomer-monomer separation of 7.5 Å, with a barrier to dissociation of 1.2 kcal/mol. This may be attributed to the fact that as the two monomers face a lower energy barrier of about 1.2 kcal/mol they may have a relatively strong range of transient interactions with each other to form the dimer. After the dissociation barrier of 1.2 kcal/mol, the two monomeric units do not show further interactions. As the two monomers attain an optimal distance from each other, they do not face any energy barrier wherein both of them can form a dimer spontaneously. As the inter-chain distance between the two monomers is lower than 7.5 Å the energy barrier drastically increases to ~ 2.5 kcal/mol due to the weak *van der Waals* force of attraction between the two monomers and eventually the PMF starts to increase.

According to the principle of statistical mechanics the possible homo-dimer complex with minimum energy among all the other conformations has the highest possibility to form. So in order to elucidate the interactions between the two monomeric units that may govern the dimer formation, we have isolated the global minima structure from the free energy profile and carried out further investigations.

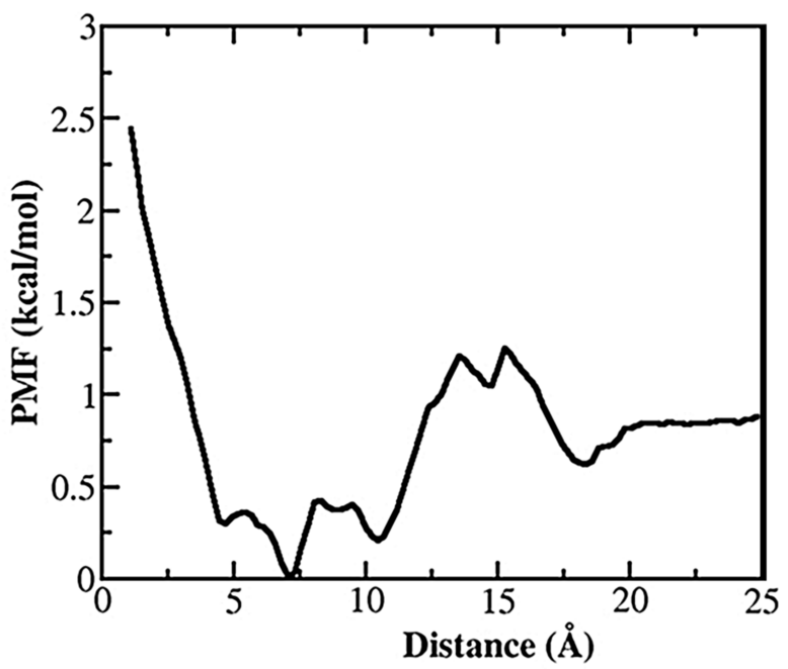

Figure 5.2. *Potential of mean force as a function of the reaction co-ordinates for the association of the $A\beta_{17-42}/A\beta_{17-42}$ peptide dimer (in kcal/mol).*

The conformational variability of $A\beta_{17-42}$ peptide dimer was subsequently investigated at different inter-chain distances as defined by the RC, r. As shown in **Figure 5.3**, different conformations were observed for the $A\beta_{17-42}$ peptide dimer at different inter-chain distances. From the conformational changes underwent by the $A\beta_{17-42}$ peptide dimer it can be clearly seen that as the two monomers lie close to each other, both the monomers tends to stay intact in their β-strand conformation. As the inter-chain distances between the two monomeric unit increases after 16 Å, one of the monomeric units was found to be in the random coil conformation instead of β-strand. And eventually the propensity of the dimeric unit to stay in its β-strand conformation reduces, as the inter-chain distance between the two monomers increases gradually.

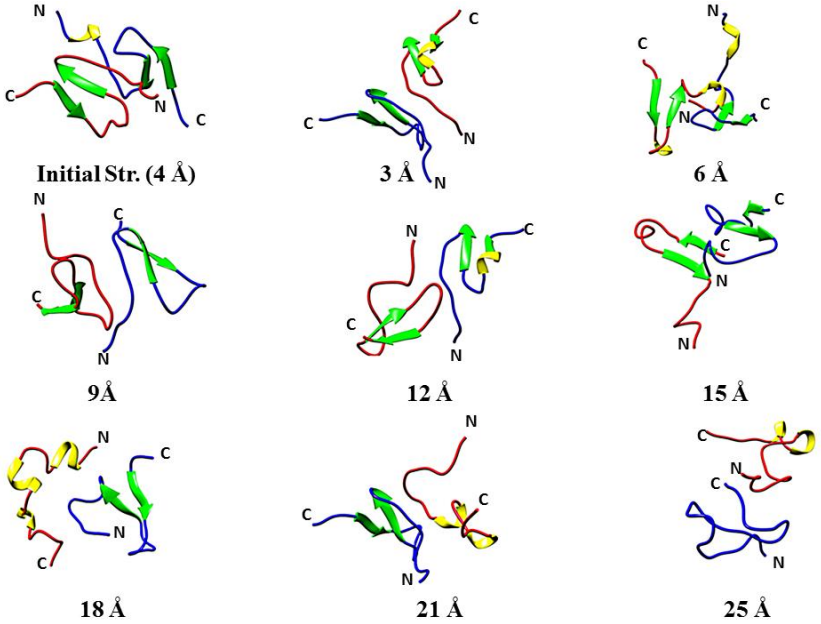

Figure 5.3. *Captured snapshots of the Aβ$_{17-42}$ peptide dimer at 300 K during the time course of simulation period at varying inter-chain distances in Å.*

To further investigate the effect of the inter-chain distances on the structural configuration of the Aβ$_{17-42}$ peptide dimer, the secondary structure preferences of each residue at 300 K was analyzed using the Kabsch and Sander algorithm [183] incorporated in the DSSP program. The residue index and the corresponding probable secondary structure of the dimers are shown in **Figure 5.4 and 5.5** at 300 K. The probability score graph with increased inter-chain distance (**Figure 5.4**) shows that majority of the residues displayed anti-parallel β-strands along with transition between helices and turns. From the graph we can observe that one of the monomeric units displayed a very low probability score for β-strands. On the other hand, from **Figure 5.5** we can see the probable secondary structures of the Aβ$_{17-42}$ peptide dimer with decreasing inter-chain distance; we can notice that both the monomeric units displayed a higher probability of β strands in comparison to that of Aβ$_{17-42}$ peptide dimer with increasing inter-chain distance (**Figure 5.4**). The results thereby showcase the anti-parallel β-strand population to be higher when the two monomers were close to each other. Earlier studies conducted by various researchers suggested the presence of secondary structural transitions from α-helix to β-strand at higher temperature [205, 206] in Aβ$_{1-42}$ peptides. Moreover, several studies have also reported loss of native α-helix structure to β-strand during fibril formation [207]. Thus, the formation of β-strand seems to an inevitable necessity in the generation of Aβ$_{1-42}$ dimer as a whole.

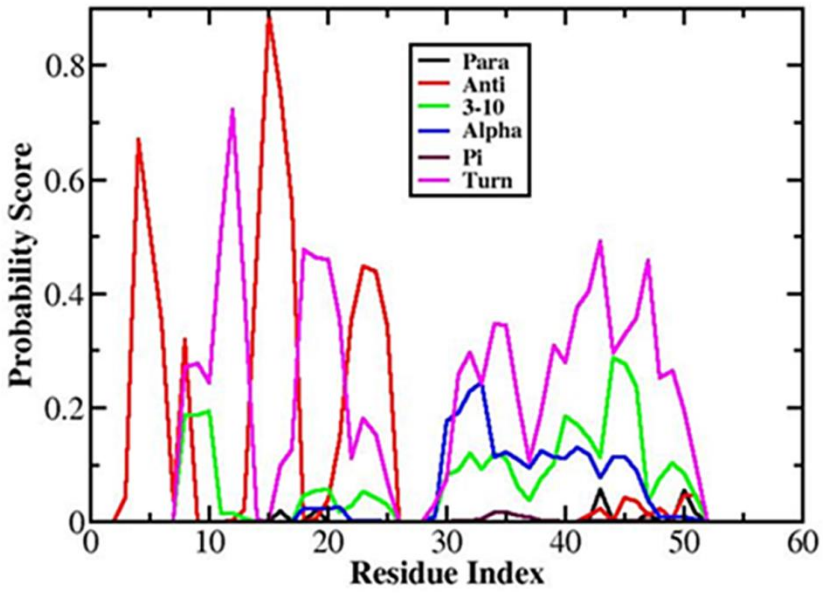

Figure 5.4. *Probability score of secondary structure for each residue of the $A\beta_{17-42}$ peptide dimer at 300 K at increasing inter-chain distance.*

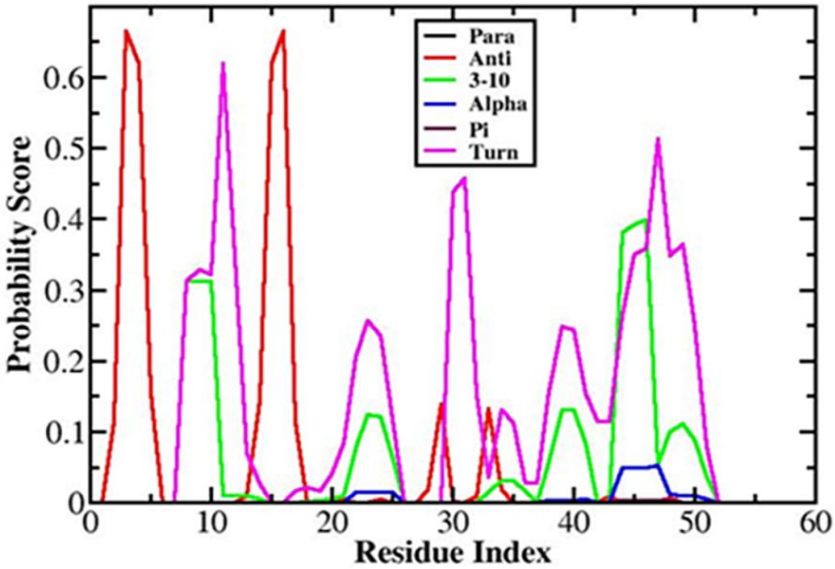

Figure 5.5. *Probability score of secondary structure for each residue of the $A\beta_{17-42}$ peptide dimer at 300 K at decreasing inter-chain distance.*

Throughout the simulation run, the CHC regions of the monomers were found to largely retain their secondary structures. However, the monomers are quite flexible and therefore sampling of the conformational space is an important aspect to characterize the $A\beta_{17-42}$ peptide dimer structures. To measure the structural convergence, the RMSD values of the C-α atom from their initial position were analyzed from the trajectories of the dimers with both increasing and decreasing inter-chain distance. During the simulation of the $A\beta_{17-42}$ peptide dimer with increasing inter-chain distance the RMSD values for the two monomers were observed to be around 2.8 Å (**Figure 5.6.A**). Alternatively, when the inter-chain distance was decreased from 4 Å to 1 Å, a lower value of RMSD was observed and found to be settled around 1.9 Å for both the monomers (**Figure 5.6.B**). The high fluctuation of RMSD value in the first case may be attributed to the conformational switching of the monomers from β-strands to coils and helix after an inter-chain distance of 12 Å as shown in **Figure 5.3,** caused by the bonding and non- bonding interactions between the individual monomeric units of the $A\beta_{17-42}$ peptide dimers.

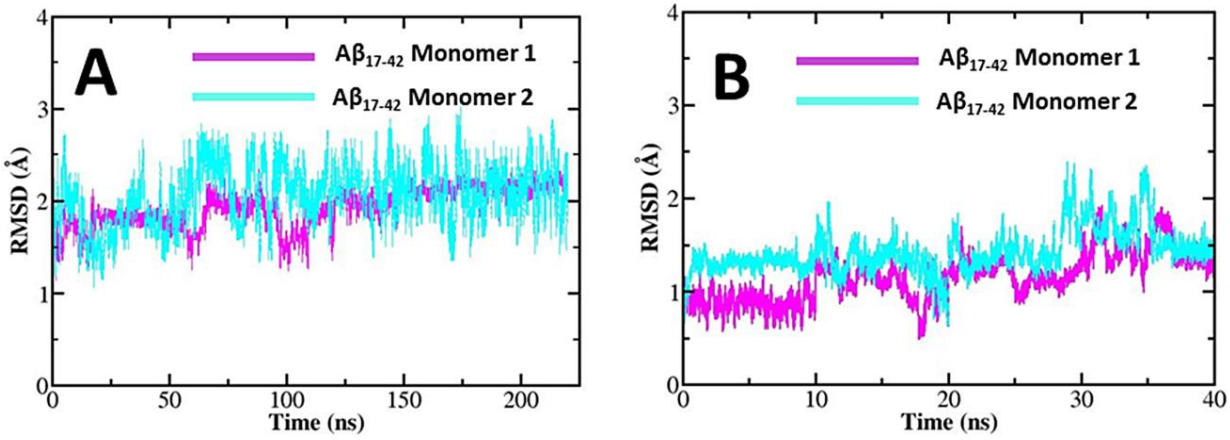

Figure 5.6*. Backbone RMSD vs time course of simulation period for the $A\beta_{17-42}$ peptide dimer in explicit solvent: A) At increasing inter-chain distance; B) At decreasing inter-chain distance.*

Furthermore, detailed analysis with respect to the energetics from the simulation trajectories of the $A\beta_{17-42}$ peptide dimer was also carried out and the results are shown in **Figure 5.7. Figure 5.7.A** shows the energetics of the monomers at increasing inter-chain distance and **Figure 5.7.B** shows the energetics of the monomers at decreasing inter-chain distance. Overall, both the electrostatic interaction for the increasing inter-chain distance and the decreasing inter-chain distance remained the same in the range of

~ - 3600 kcal/mol as shown in **Figure 5.7**. Furthermore, the *van der Waals* force of attraction for the $A\beta_{17-42}$ peptide dimers was found to decrease with decreasing inter-chain distance, while the $A\beta_{17-42}$ peptide dimers exhibited more or less constant *van der Waals* forces of attraction at increasing inter-chain distance. This is in agreement with the results obtained from the PMF plot wherein a higher energy barrier was observed with decreasing inter-chain distances and is identified to be caused by the decreasing *van der Waals* force of attraction between the two monomers.

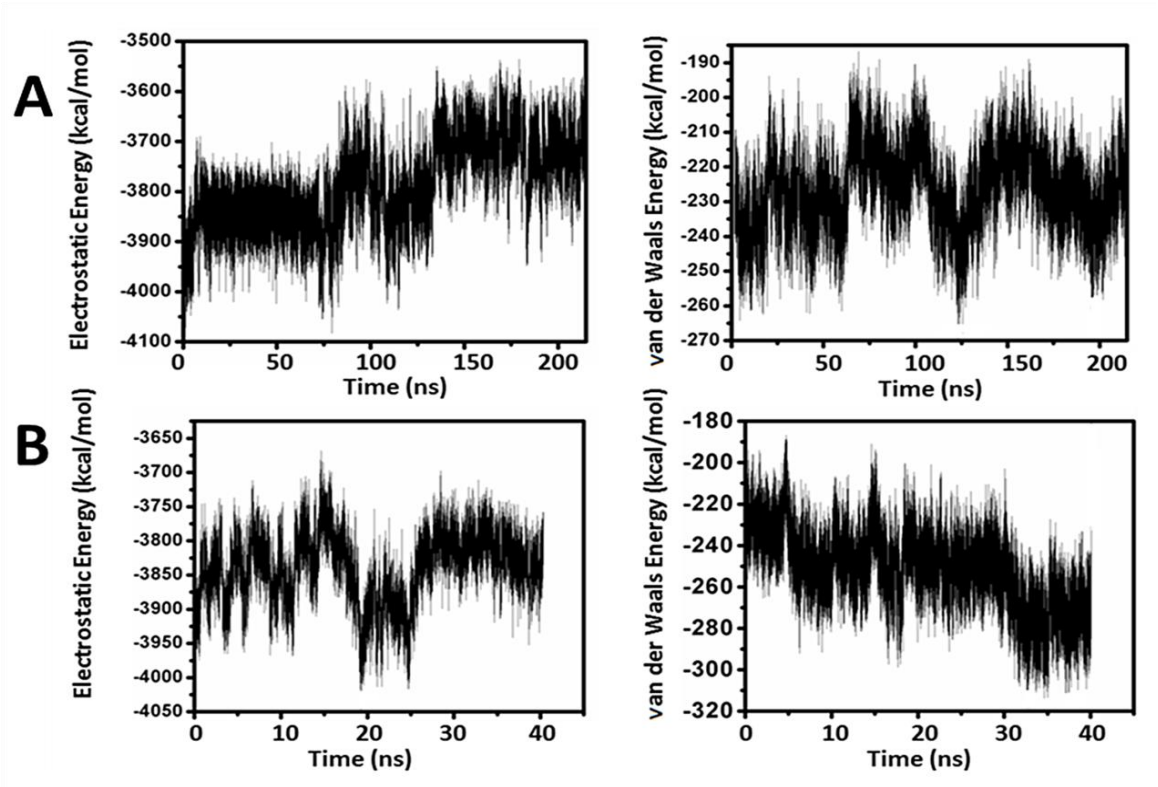

Figure 5.7*. Electrostatic Energy vs time course of simulation period and Van der Waals Energy vs time course of simulation period for the $A\beta_{17-42}$ peptide dimer with A) increasing inter-chain distance; B) decreasing inter-chain distance.*

5.4.2. Conformational dynamics of the optimized $A\beta_{17-42}$ peptide dimer structure from the free energy profile:

From the PMF plot (**Figure 5.2**) the optimized $A\beta_{17-42}$ peptide dimer structure was isolated at minimum monomer-monomer separation of 7.5 Å from the free energy profile. On the basis of energetics, this optimized $A\beta_{17-42}$ peptide dimer structure is found to represent the most probable $A\beta_{17-42}$ peptide dimer structure that may have formed. Hence, further studies on this structure were considered to be actually beneficial in the understanding of the actual dimerization process. The conformational dynamics of the optimized $A\beta_{17-42}$ peptide dimer structure was thereby analyzed by carrying out further MD simulations and is represented in the **Figure 5.8**. From **Figure 5.8**, it may be observed that most of the residues in $A\beta_{17-42}$ peptide dimer at certain regions (Residue index: 18-23 and 30-34) showed transitions to β-strand and the secondary structure propensity of the $A\beta_{17-42}$ peptide dimers observed herein was found to be in good agreement with previous evidences (26, 27, 29).

Additionally, the total energy and potential energy for the optimized $A\beta_{17-42}$ peptide dimer structure was also analyzed. **Figure 5.9** shows both the total energy and potential energy to have a negative value thereby confirming the stability of the $A\beta_{17-42}$ peptide dimer structure. Moreover, the stable structure for the $A\beta_{17-42}$ peptide dimer is suggestive of the fact that the time period of simulation is good enough to provide the appropriate results.

In order to study the various interactions that stabilize the dimerization process, the inter-molecular and intra-molecular hydrogen bonds of the $A\beta_{17-42}$ peptide dimer at their minimum distance of separation was calculated. For the calculation of the hydrogen bonds, the cut off for angle and distance was set to 120^0 and 3.5 Å respectively. **Figure 5.10.A** shows the total number of hydrogen bonds between the two monomers, wherein two distinct cases were considered: Aβ monomer 1 as an acceptor and monomer 2 as a donor followed by Aβ monomer 1 as a donor and monomer 2 as an acceptor. The total number of inter-molecular hydrogen bond was found to be around 15. **Figure 5.10. B** shows the total number of hydrogen bonds among the residues of each monomer separately and the total number of intra-molecular hydrogen bonds was found to be in the range of 10.

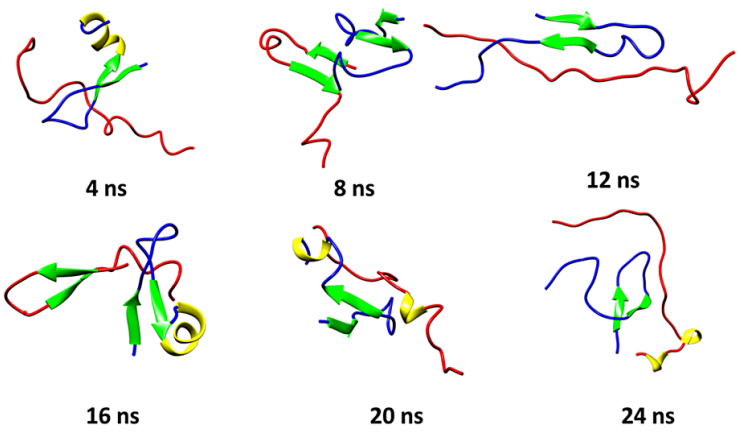

Figure 5.8. *Captured snapshots of the $A\beta_{17-42}$ peptide dimer at 300 K during the time course of simulation period at constant (7.5 Å) inter-chain distances.*

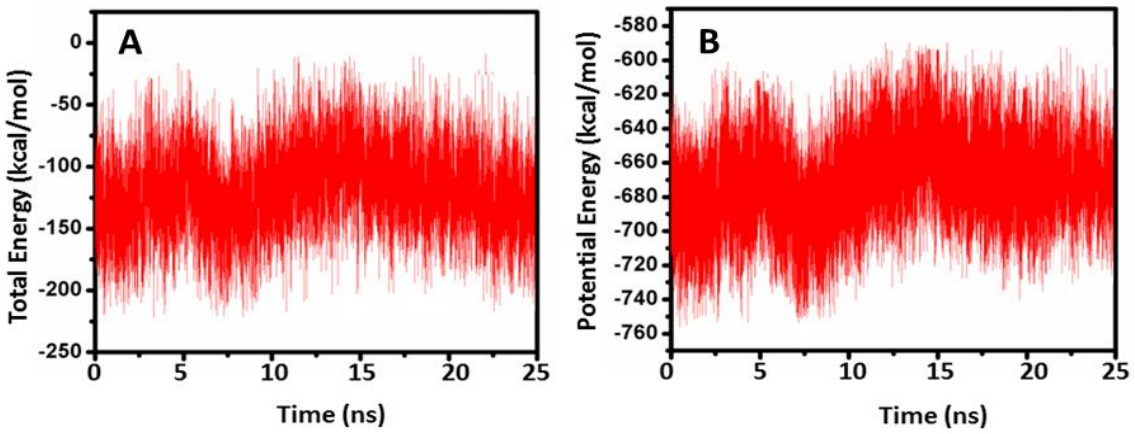

Figure 5.9. *A) Total Energy vs time course of simulation period; B) Potential Energy vs time course of simulation period for the $A\beta_{17-42}$ peptide dimer at constant inter-chain distance.*

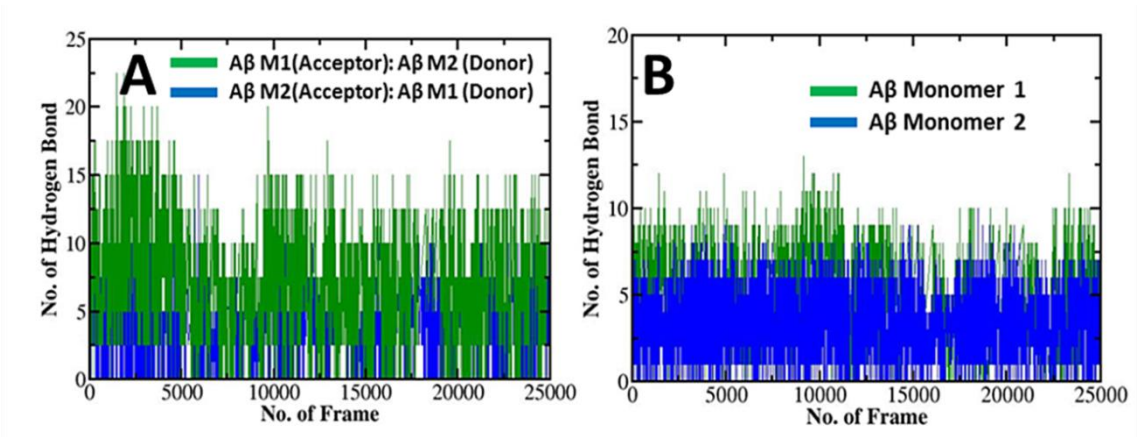

Figure 5.10. *Total number of A) inter-molecular hydrogen bonds; B) intra-molecular hydrogen bonds vs total number of frames for the $A\beta_{17-42}$ peptide dimer at constant inter-chain distance.*

We have further carried out inter-molecular and intra-molecular hydrogen-bonding analysis and the details are provided in **Table 5.2 & 5.3** respectively. The acceptor and donor residues and the corresponding atom that has formed the respective hydrogen-bond along with the can be seen from the tables. From **Table 5.2.A** we can see that oxygen of Asp at position 7 of monomer 1 forms hydrogen bond with nitrogen of Gly at position 35 of monomer 2 for the longest time period of 0.2448 ns. Similarly, when we change the acceptor and donor monomers, we can see that oxygen of Ala at position 40 of monomer 2 form hydrogen bond with nitrogen of Ile at position 15 for the longest time period of 0.3037 (**Table 5.2.B**). In case of intra-molecular hydrogen-bonds, oxygen of Ala at position 14 forms hydrogen bond with nitrogen of Ala at position 5 of monomer 1which persist for the longest duration of 0.5637 (**Table 5.3.A**). In case of monomer 2 the hydrogen bond that forms between oxygen of Ser35 and nitrogen of Lys38 continued for 0.3009 ns (**Table 5.3.B**). Although the number of inter-molecular hydrogen bonds is more, the time period to which the intra-molecular hydrogen bonds remained intact was higher than of inter-molecular hydrogen-bonds which indicate that intra-molecular hydrogen bonds are much more stable.

Table 5.2: Inter-molecular hydrogen bonding analysis of $A\beta_{17-42}$ peptide dimer.

A. $A\beta_{1-42}$ Monomer 1 (Acceptor) : $A\beta_{1-42}$ Monomer 2 (Donor)

Acceptor	Donor	Fraction
ASP_7@OD1	GLY_35@N	0.2448
SER_10@OD1	LYS_38@NZ	0.2217
GLY_13@O	ILE_42@N	0.0840
ILE_15@O	ALA_40@N	0.0812
VAL_24@O	ALA_31@N	0.0716
ALA_26@OXT	VAL_28@N	0.0602
GLY_9@O	LYS_38@NZ	0.0459
ILE_15@O	GLY_43@N	0.0406
ALA_26@O	VAL_28@N	0.0392
ILE_15@O	GLU_32@N	0.0263
GLY_9@O	ASN_37@ND2	0.0254
VAL_23@O	ILE_42@N	0.0252
VAL_24@O	PHE_30@N	0.0195
SER_10@OG	GLY_39@N	0.0141
ALA_14@HA	ILE_41@CA	0.0130
MET_19@O	PHE_29@N	0.0126

B. $A\beta_{1-42}$ Monomer 2 (Acceptor) : $A\beta_{1-42}$ Monomer 1 (Donor)

Acceptor	Donor	Fraction
ALA_40@O	ILE_15@N	0.3037
ILE_41@O	ILE_15@N	0.0839
VAL_28@O	ALA_26@N	0.0301
ALA_40@O	ILE_25@N	0.0224
GLU_32@OE1	GLY_17@N	0.0152
ILE_41@HA	ALA_14@CA	0.0132
ILE_51@O	LEU_18@N	0.0132
ALA_52@OXT	LYS_12@NZ	0.0131

Table 5.3: Intra-molecular hydrogen bonding analysis of $A\beta_{17-42}$ peptide dimer.

A. Aβ Monomer 1

Acceptor	Donor	Fraction
ALA_14@O	ALA_5@N	0.5637
PHE_3@O	ILE_16@N	0.5170
ASP_7@O	LYS_12@N	0.2601
ASP_7@OD1	GLY_9@N	0.2122
ASP_7@OD2	SER_10@OG	0.2064
ILE_16@O	PHE_3@N	0.1654
SER_10@O	GLY_13@N	0.1580
ASP_7@O	ASN_11@N	0.1142
SER_10@OG	GLY_13@N	0.1102
ASN_11@O	GLY_13@N	0.1073
GLY_21@O	VAL_24@N	0.1058

B. Aβ Monomer 2

Acceptor	Donor	Fraction
SER_36@O	LYS_38@NZ	0.3009
ASP_33@O	LYS_38@NZ	0.2208
PHE_29@O	ALA_31@N	0.1389
ILE_42@O	MET_45@NZ	0.1116
ALA_31@O	ASP_33@NZ	0.1

5.4.3. Interaction study of the optimized $A\beta_{17-42}$ peptide dimer structure from the free energy profile:

In order to study the interactions between the monomers of $A\beta_{17-42}$ peptide dimer, we analysed the optimized $A\beta_{17-42}$ peptide dimer structure which forms the most stable complex with the minimum free energy respectively. The interface and possible interacting residues across the interface of the $A\beta_{17-42}$ peptide dimer were predicted using the PDBsum server [180]. The interface area for each of the monomeric unit involved in the interaction in the $A\beta_{17-42}$ peptide dimer was found to be in the range of ~700-800 $Å^2$ (**Table 5.4)**. In the same manner, the total number of interface residues involved in the interaction between the monomers present in the $A\beta_{17-42}$ peptide dimer was found to be ~12 for each of them. From **Figure 5.11,** it was observed that most of the interface residues involved in the interaction of the $A\beta_{17-42}$ peptide dimer complex was hydrophobic in nature. Electron cryo-microscopy, 3-D reconstruction, and integrative structural modelling methods to determine the molecular architecture of a fibril formed by $A\beta_{1–42}$ also shows that the two peptides forming the dimer interact with each other by packing their hydrophobic C-terminal β-strands [208]. Also, the $A\beta_{17-42}$ peptide dimer was found to be stabilized by molecular interactions such as hydrogen bonds and non-bonded contacts as shown in **Figure 5.11**. Total number of non-bonded contacts between the two monomers of $A\beta_{17-42}$ peptide dimer was found to be 67 and number of hydrogen bonds present between the monomers was found to be 9 (**Table 5.4**). This is in agreement with the above mentioned hydrogen bond analysis wherein the inter-peptide hydrogen bond number approximately appears to be the same with the inter-molecular hydrogen bonds present in the lowest energy conformer. Moreover, the non-bonded contacts between the two monomeric units are indicative of bringing the monomeric units together, allowing the backbone-backbone interactions of the monomers to initiate the dimer formation.

Table 5.4: *Interface Statistics of $A\beta_{17-42}$ peptide dimer as provided by the PDBsum server.*

Monomer	No.of Interface Residues	Interface Area (Å^2)	No. of Salt Bridges	No. of Hydrogen Bonds	No. of Non-bonded Contacts
M1	**12**	**791**	**1**	**9**	**67**
M2	**14**	**722**			

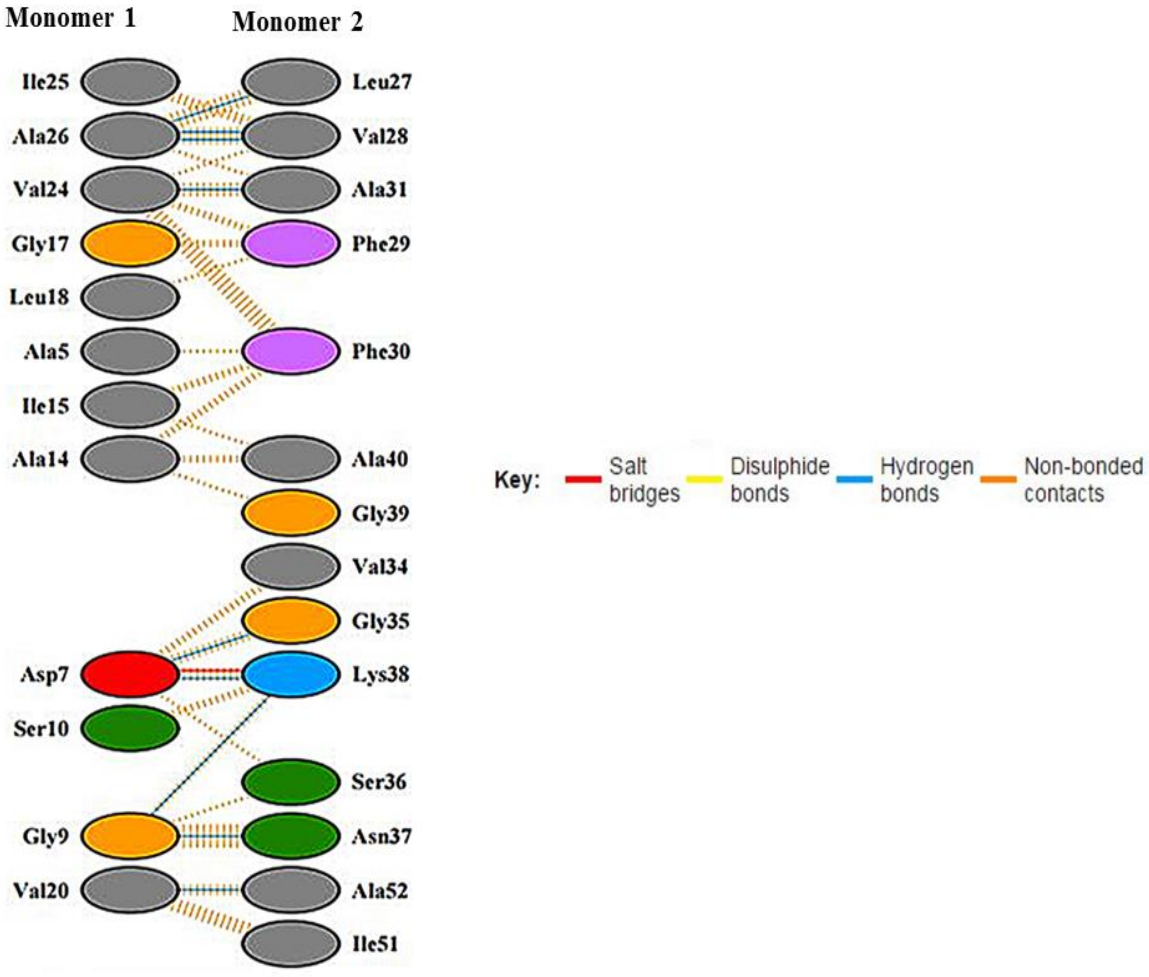

Figure 5.11*.The total number of interface residues in $A\beta_{17-42}$ peptide dimer as predicted by the PDBsum server.*

5.4.4. Cα-Cα distance map analysis:

The inter-chain interaction of the $A\beta_{17-42}$ peptide dimer (**Figure 5.12**) at the optimal distance (7.5 Å) was analyzed by using CMA [178]. It provides better information on protein structure by displaying detailed information about all atom-atom contacts between a given pair of contacting residues. Atom to atom contacts of each pair of amino acid residues involved in the interaction are displayed in the form of a contact map. In the present analysis, a contact area threshold above 8 $Å^2$ was considered to investigate the residue-residue interaction between the two monomers. As the inter chain distance lies at an optimum value, the steric hindrance between the monomeric chains is likely to decrease, thus aiding in the conformational change of the α-helical monomeric units to β-strands that further associate to form the resultant dimer . This is corroborated from the observed results wherein an increase in the inter chain distance to optimum value was found to significantly contribute towards an increasing percentage of β-strands thus validating our assumption.

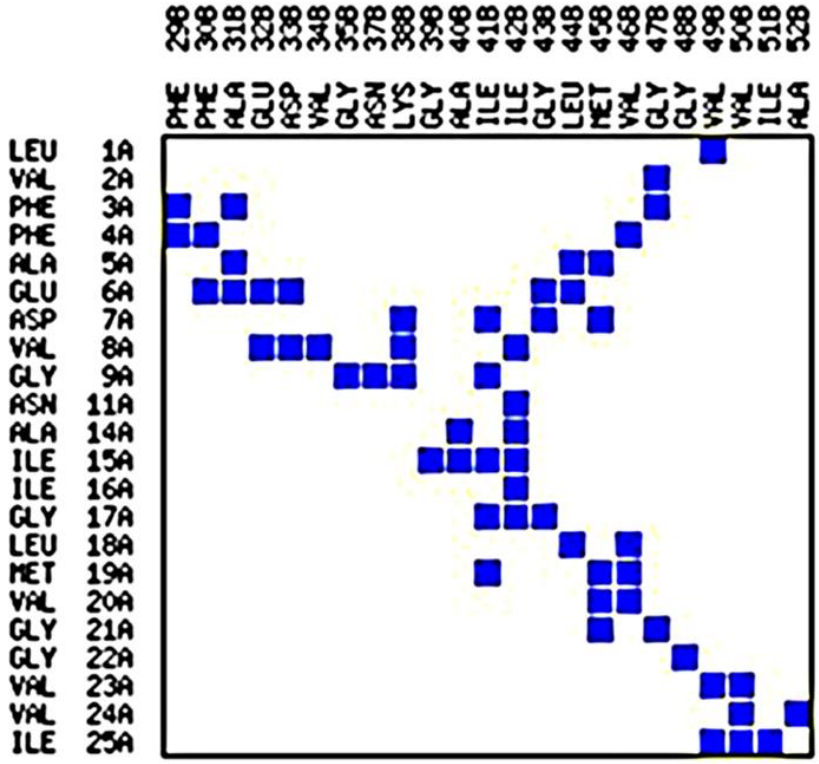

***Figure 5.12**. Cα-Cα contact probability map (inter-peptide) at 300 K of the $A\beta_{17-42}$ peptide dimer at optimal inter-chain distance.*

5.5. Conclusion:

The present study elucidates the dimerization process of the $A\beta_{17-42}$ peptide based on the MD simulations study. The PMF study carried out on the $A\beta_{17-42}$ peptide at varying inter-chain distances probes the key structural and thermodynamic features of the $A\beta_{17-42}$ peptide dimer that are likely to seed dimerization. The results that reported here are consistent with the structural characteristics of the $A\beta_{17-42}$ peptide dimer and reconcile the previous evidences about the importance of hydrophobic residues and the β-strands in the dimerization process. From the analysis of the PMF plot, the minimum at a monomer-monomer separation of 7.5 Å, with a barrier to dissociation of 1.2 kcal/mol was obtained. The optimized $A\beta_{17-42}$ peptide dimer structure which forms the most stable complex with the minimum free energy was subjected to protein-protein interaction studies and Cα-Cα contact map analysis, which demonstrated the interaction between the monomeric units to be governed primarily by the hydrophobic and hydrogen bonds. The resultant $A\beta_{17-42}$ peptide dimer was found with varying amount of β-strands. Moreover, the stable β-strand regions in the $A\beta_{17-42}$ monomeric units were also predicted at varying inter-chain distance from the secondary structure analysis. Additionally, the $A\beta_{17-42}$ monomeric units were specifically shown to have an increased β-strands propensity at the hydrophobic regions encompassing the CHC region and the simulation studies show this hydrophobic region encompassing the CHC region to be crucial in dimerization. Furthermore, specific hydrophobic residues were found to play a vital role in the formation of the dimer complex. The results thus describe the secondary structural changes of the monomers, wherein β-strands were found to be predominant at specific regions, followed by the interaction of the two β-strands to form the resultant dimer. Thus, the findings from this study provide detailed information for the complex process of early events of $A\beta_{1-42}$ aggregation and also a library of dimer structures that may be employed as targets to design inhibitors, which are capable of binding $A\beta_{1-42}$ peptide and are likely to contribute significantly towards the disruption of the dimerization process in combating AD.