# CHAPTER 5

# STUDY OF THE EFFECT OF PEPTIDES ON DIMERIZATION AND AGGREGATION OF $AB_{1-42}$ PEPTIDE

# Study of the effect of peptides on dimerization and aggregation of $A\beta_{1-42}$ peptide

## 5.1. Abstract:

Alzheimer's disease (AD) is the most common progressive neurodegenerative brain disorder. It is characterized by the presence of extracellular aggregated fibrillary form of Amyloid-Beta (Aβ) peptide and intraneuronal neurofibrillary tangles caused by the hyperphosphorylation of tau protein. Monomeric form of Aβ-peptide in α-conformation is not toxic but it can undergo self aggregation to form β-conformation which is neurotoxic. The most promising approach 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, the aim of this work is 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, this 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. From the PMF profile, it has been noticed that the $A\beta_{1-42}$-CTerm Heterodimer (10.99 kcal $mol^{-1}$) complex has higher dissociation energy than the $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer (2.23 kcal $mol^{-1}$) complex. In this study, the findings from the PMF and BFE analysis of the two complexes, provides salient structural, binding and unbinding features and thermodynamics that support the ability of CTerm of HA in affecting the dimerization of $A\beta_{1-42}$.

## 5.2. Introduction:

Proteins are very important biomolecules that sustain life through their distinct functions. The 3-D structure of a protein is important in understanding the dynamics and function of the protein [555]. Proteins, under normal conditions, tend to fold into a relatively stable, native, three-dimensional structure with the help of chaperons. Protein folding to obtain stable conformation is correlated with the function of proteins. Therefore, the folding of a protein into its correct native conformation represents a

compromise between its thermodynamic stability and flexibility [556]. Though the native conformation is thermodynamically favorable, often it is found to be only slightly stable under various physiological conditions [557-560]. The failure in attaining the native conformation of proteins occurs commonly due to errors in molecular mechanisms in the cell processes such as translation, mutations, chemical, environmental or physical stress conditions, resulting in misfolded protein species. Cells in living organisms have devised an intrinsic protein quality control (PQC) system that consists of degradation pathways, a network of molecular chaperones, co-chaperones to control or remove the production of such misfolded proteins [561]. Under stress conditions, when the capacity of the PQC system gets overwhelmed, then this system fails to regulate the misfolded proteins. Aggregation of misfolded protein leads to the formation of pathogenic amyloids, causing amyloidosis, which is responsible for the occurrence of neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington disease etc. [559, 562-564]. Dementias are responsible for the greatest burden of neurodegenerative diseases. The number of patients suffering from AD is increasing every year. With the advancement of the disease, the patient suffering from AD starts having problems including memory loss, mood and personality changes, inability to communicate, increased anxiety and/or aggression, and taking a longer time to complete normal daily tasks [564]. As the patient's condition deteriorates, bodily functions are lost, ultimately leading to death [565-566].

AD is considered the most common neurodegenerative disorder [10-12]. The pathological hallmark of AD is amyloid plaques, similar to some other neurodegenerative diseases. The major constituent of amyloid plaque is found to be Aβ peptide [567, 568]. These amyloids exist as intracellular inclusions or extracellular plaques (amyloid). These amyloid deposits cause abnormal protein build-up in tissues and eventually lead to organ dysfunction and deaths. Aβ peptide is generated from the sequential cleavages of large membrane-spanning glycoprotein, the amyloid precursor protein (APP) [567, 568]. This Aβ peptide exists in two isoforms, $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide. Between the two isoforms, the aggregation of $A\beta_{1-42}$ is found to be more significant and toxic [584]. The $A\beta_{1-42}$ peptide initially exists as an unordered random coil but it has the propensity to misfold into β-sheets and aggregate to form neurotoxic oligomers that eventually mature into amyloid fibrils [569]. 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.

At present, research is being carried out to develop strategies to inhibit the amyloid fibril formation [570, 571]. Some studies report that few small molecules can disrupt preformed amyloid fibrils [572]. Inhibitors may prevent the amyloid aggregation by binding and stabilizing the native conformation of a protein or by binding to aggregation-prone regions of amyloidogenic peptides, thereby prohibiting self-assembly of the peptide [573]. Recent inhibition studies of amyloid fibril formation have helped to newly design a number of compounds or small molecules yet many of these have failed to make an impact as a drug at the clinical level. Hence, a more detailed understanding of inhibition strategies of amyloid aggregates is needed for the prevention of AD.

Human Serum Albumin (HSA) is one of the most abundant proteins present in blood plasma. It is a globular protein with a molecular weight of ~66.5 kDa [574]. The function of HSA is to transport hormones, fatty acids and various compounds through the bloodstream in the blood vascular system. The ultimate goal is to utilize HSA to improve drug delivery of novel pharmacological approaches to treat various human diseases [575, 576]. Aβ present in brain parenchyma is believed to play a prominent role in the pathogenesis of AD. Aβ is transported from the brain to the plasma via complex transport pathways at the blood-brain barrier (BBB) [577]. It has been reported that approximately 90–95% of plasma Aβ may be bound to albumin. Hence, replacement of serum albumin in plasma has been proposed as a favorable therapy for the cure of AD [578-581]. It has also been reported that albumin binds with Aβ-peptide impeding its aggregation [582, 583]. A recent study reported the interaction of C-Terminus (CTerm) domain of Human Albumin (HA) with Aβ-peptide (protein-protein interaction) with the help of *in silico* and *in vivo* techniques [584]. This study has revealed CTerm of HA has a specific binding affinity to Aβ-peptide, and also participates in the inhibition of Aβ-peptide assembly as well as favors the disassembly of preformed amyloid aggregates.

In this study, the interaction of $A\beta_{1-42}$ peptide with CTerm of HA have been studied in terms of Potential of mean force (PMF) [79, 585]. The conformational changes undergone by $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer have been studied with the help of Molecular Dynamics (MD) simulation approach. The BFE between monomeric units in $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer

complexes was also calculated. The contribution of individual residues contributing to the protein-protein interaction (PPI) for both the complexes has also been analyzed using PDBsum server [500, 501].

## 5.3. Materials and Methods

### 5.3.1. Molecular docking and the preparation of the initial structure:

#### 5.3.1.1. Preparation of receptor:

The micelle-bound human $A\beta_{1-42}$ monomeric structure, PDB ID: 1IYT [547] obtained from RCSB PDB [502, 503] was used as the receptor molecule for molecular docking.

#### 5.3.1.2. Preparation of ligand:

The structure of CTerm of HA, PDB ID: 5FUO (35 residues: 504-538) [586], obtained from RCSB PDB[549,550] was used as the ligand molecule for molecular docking.

#### 5.3.1.3. Preparation of $A\beta_{1-42}$-$A\beta_{1-42}$ homodimer complex:

Two different chain IDs have been assigned to the same monomeric structure of $A\beta_{1-42}$ peptide and saved it as two different .pdb files. These two monomeric structures of $A\beta_{1-42}$ peptide were docked to form a homodimer molecule using ClusPro, an online protein-protein docking tool [512]. From the ten model structures (cluster centers) obtained from ClusPro for the $A\beta_{1-42}$-$A\beta_{1-42}$ complex, the first docked model have been chosen for the study. This selection is based upon the rankings of docked model structures (as shown in **Figure 5.1)** depending on the number of highly populated clusters, cluster center, and the lowest energy weighted scores (as shown in **Table 5.1**).

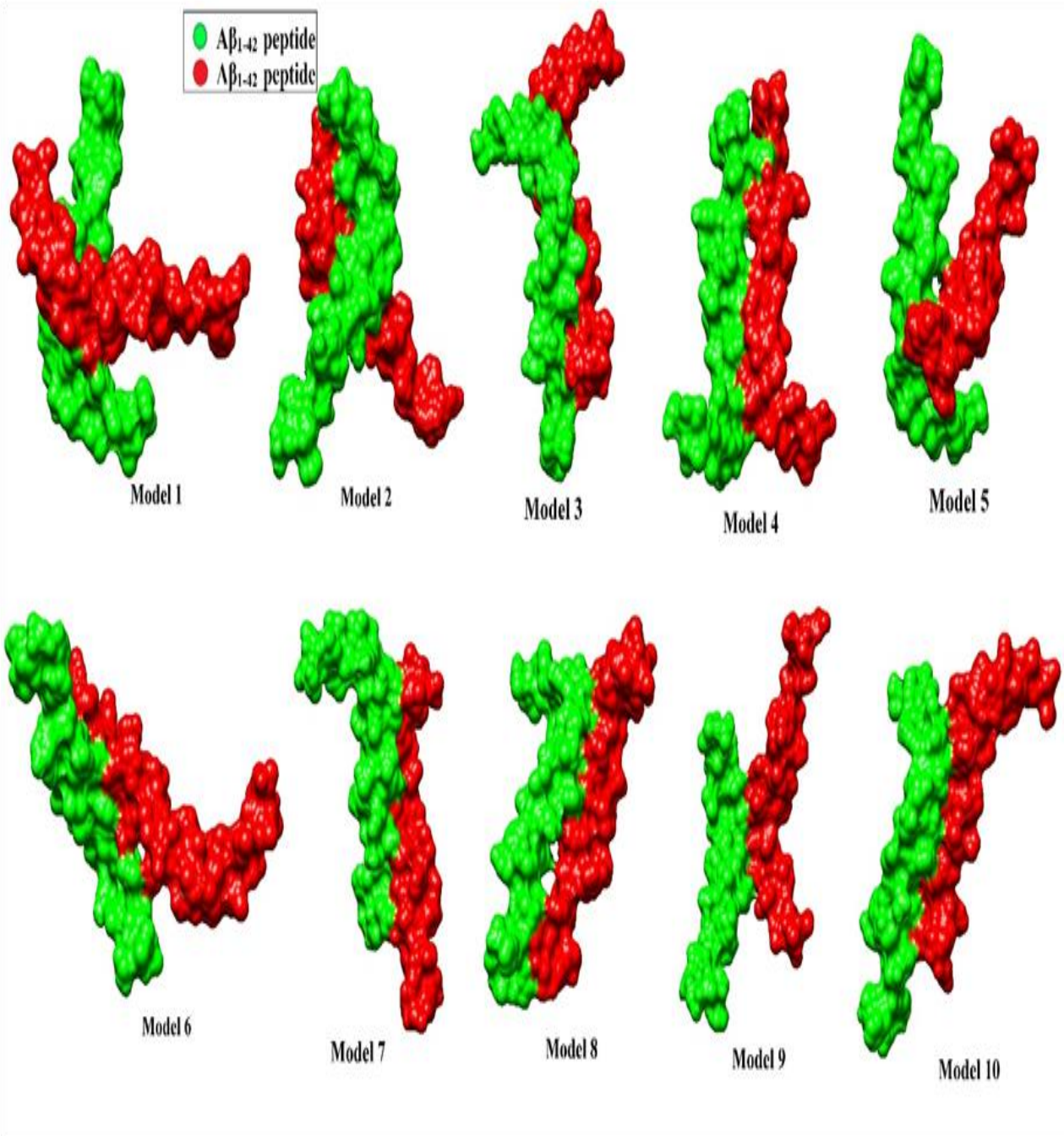


***Figure* 5.1.** *Top 10 representative docked models for (Aβ1-42 peptide + Aβ1-42 peptide) complex obtained from ClusPro online docking server.*

***Table 5.1.*** *List of top 10 docked clusters of ($A\beta_{1-42}$ peptide + $A\beta_{1-42}$ peptide) complex along with their members based on their weighted score from ClusPro online docking server.*

| Cluster | Members | Representative | Weighted Score |
|---|---|---|---|
| 1 | 155 | Center | -103.4 |
| 1 | 155 | Lowest Energy | -130.5 |
| 2 | 92 | Center | -123.9 |
| 2 | 92 | Lowest Energy | -123.9 |
| 3 | 69 | Center | -114 |
| 3 | 69 | Lowest Energy | -124.5 |
| 4 | 60 | Center | -123.4 |
| 4 | 60 | Lowest Energy | -123.4 |
| 5 | 54 | Center | -100.8 |
| 5 | 54 | Lowest Energy | -112.9 |
| 6 | 45 | Center | -102.8 |
| 6 | 45 | Lowest Energy | -118.7 |
| 7 | 40 | Center | -102 |
| 7 | 40 | Lowest Energy | -111.6 |
| 8 | 38 | Center | -102.5 |
| 8 | 38 | Lowest Energy | -121.4 |
| 9 | 38 | Center | -101.3 |
| 9 | 38 | Lowest Energy | -107.6 |
| 10 | 34 | Center | -101 |
| 10 | 34 | Lowest Energy | -114.6 |

### 5.3.1.4 Preparation of $A\beta_{1-42}$-CTerm heterodimer complex:

The pdb format of Human Albumin (PDB ID: 5FUO) was taken from RCSB PDB and visualized using UCSF Chimera [530]. Recently, it has been reported that CTerm retains HA binding property [586], so we selectively isolated peptide regions of 504-538 residues from HA. The CTerm peptide,

$^{504}$AETFTFHADICTLSEKERQIKKQTALVELVKHKPKamide$^{538}$, containing the hydrophobic domains reported to be Aβ binding sites [587] have been selected and saved as different .pdb file using UCSF Chimera. After assigning the chain ID to the CTerm, it was then docked with the target $A\beta_{1-42}$ peptide using ClusPro and top ten docked models (**Figure 5.2**) were obtained from which top first docked model was selected for our study. As discussed in *Section 5.3.1.3*, in this case, the selection of the best suitable docked model based on **Table 5.2**.

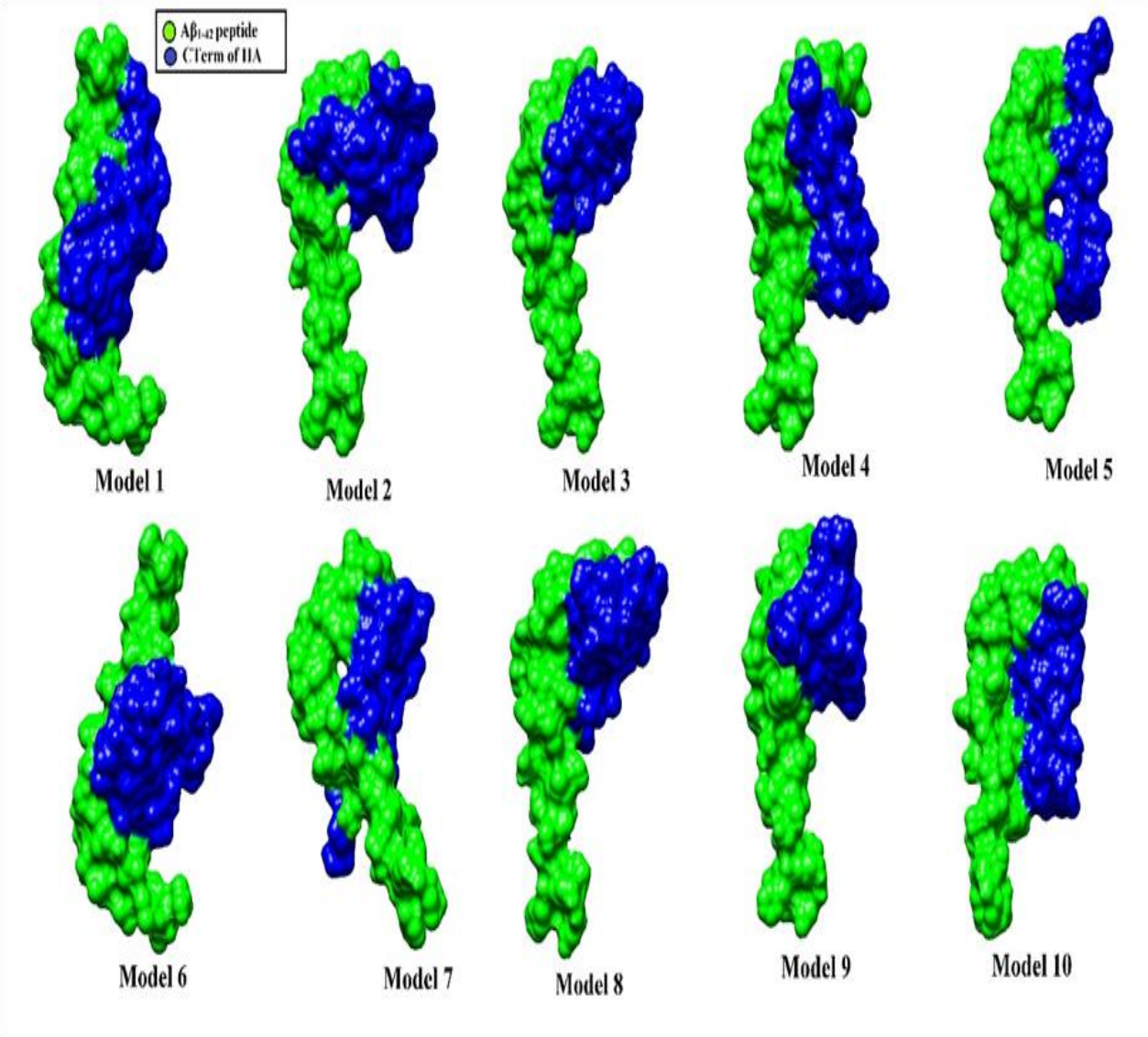


***Figure 5.2.*** *Top 10 representative docked models for ($A\beta_{1-42}$ peptide + CTerm) complex obtained from ClusPro online docking server.*

***Table 5.2.*** *List of top 10 docked clusters of ($A\beta_{1-42}$ peptide + CTerm) complex along with their members based on their weighted score from ClusPro online docking server.*

| Cluster | Members | Representative | Weighted Score |
|---|---|---|---|
| 1 | 181 | Center | -896 |
| 1 | 181 | Lowest Energy | -1035.9 |
| 2 | 170 | Center | -916.3 |
| 2 | 170 | Lowest Energy | -1009.5 |
| 3 | 132 | Center | -933.3 |
| 3 | 132 | Lowest Energy | -1030.5 |
| 4 | 78 | Center | -971.6 |
| 4 | 78 | Lowest Energy | -1123.5 |
| 5 | 72 | Center | -973.8 |
| 5 | 72 | Lowest Energy | -1037.9 |
| 6 | 65 | Center | -907.4 |
| 6 | 65 | Lowest Energy | -1043 |
| 7 | 63 | Center | -926.8 |
| 7 | 63 | Lowest Energy | -1012.7 |
| 8 | 52 | Center | -1036.9 |
| 8 | 52 | Lowest Energy | -1036.9 |
| 9 | 49 | Center | -931 |
| 9 | 49 | Lowest Energy | -1001.3 |
| 10 | 46 | Center | -935.9 |
| 10 | 46 | Lowest Energy | -1100.6 |

## 5.3.2 Molecular Dynamics (MD) simulation of $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer complexes:

To perform MD simulation, the required coordinate and topology files of $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer complex structures have been built using AMBERff99SBildn force field and with the Leap module of the AMBER 14

software package. As per reports of recent studies, the structural ensembles of intrinsically disordered proteins (IDPs) are strongly dependent on their force field [588]. The uneven energy landscapes of IDPs are capable of revealing force field deficiencies, thus contributing to force field development. It has been observed that there is no ideal force field to study IDPs. However, presently available literature shows that AMBERff99SBildn and AMBERff99SB force fields have been used in many studies to analyze the salient structural features of IDPs [588-590]. In addition, ff99SBildn is the advanced force field of AMBERff99SB for IDP. Hence, the AMBERff99SBildn force field has used to carry out this particular study. Solvation of these complexes was done with TIP3P (transferable intermolecular potential with 3 points) water molecules [480] with solvent buffer being 10 Å surrounding the complexes from all directions.

The MD study was carried out for 50 ns time period using a standard procedure, as discussed in *Section 4.3.2*.

### 5.3.3 Potential of Mean Force (PMF) calculation:

Molecular dynamics (MD) simulations coupled with Umbrella Sampling (US) [493, 590] method and the Weighted Histogram Analysis Method (WHAM) [494, 591] was used to calculate the potential of mean force (PMF) [493] for $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer. The use of PMF is to calculate free energy along a definite reaction coordinate and this free energy profile helps in the identification of transition states, intermediates and relative stabilities of the end points. However, this cannot generate accurate PMF as just by running the MD simulation to generate free energy along reaction coordinates. The reason behind this is that the energy barrier of interest is many times the size of $k_bT$ and hence the MD simulation will either remain in the local minimum it started in or cross to different minima but very rarely sample the transition state. US sampling approach is used with WHAM [494] as it helps in attainment of the transition states of the interest samples which otherwise with solely running of MD simulation would restrict the interest samples in the local minima or cross it to different local minima. US separated the reaction coordinate, for both the $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer into different series and then applied restraint over the samples to remain close to the center of the window, provided the end point overlaps. Biasing potentials were added to the Hamiltonian to confine the

molecular system around the selected regions of phase space. The biasing potential is usually a harmonic potential that keeps the system near a specified value in the reaction path. This was done in a number of windows along the reaction path. In each window, equilibrium simulations were performed and the biased probability distribution (histogram) was obtained. The WHAM is then used to determine the optimal free energy constants for the combined simulations.

The PMF calculation for the study of degree of association for two $A\beta_{1-42}$ peptide monomeric units in $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex and $A\beta_{1-42}$ peptide and CTerm in the $A\beta_{1-42}$-CTerm Heterodimer complex was carried out by changing the Center of Mass (CoM) distances (in two different directions by increasing and decreasing) between the two monomers in $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer complexes. The distance between CoMs of the $A\beta_{1-42}$ peptides in $A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$ and CTerm was changed with time from 1 to 23 Å spanning different configurations. At each window of umbrella sampling, the system was carried out for a 5 ns time period of MD simulation with harmonic potentials to maintain the angle and CoM between the two molecules near the desired values. The PMF has been computed as a function of reaction coordinate for both the complexes.

### 5.3.4. MM-PBSA/ GBSA Binding free energy calculation:

The relative BFE analysis for the ($A\beta_{1-42}$-$A\beta_{1-42}$) Homodimer and ($A\beta_{1-42}$–CTerm) Heterodimer complex was carried out using MMPBSA.py script of the AMBER 14 suite as discussed in *Section 3.1.3*. All the trajectories were taken into consideration for the MM-PBSA/GBSA calculations. Thereafter, MM-GBSA/PBSA analysis was performed on the three components of the complex systems: (i) the protein ($A\beta_{1-42}$) (ii) ligand ($A\beta_{1-42}$ in case of Homodimer or CTerm in case of Heterodimer) and (iii) the complex ($A\beta_{1-42}$+$A\beta_{1-42}$ or $A\beta_{1-42}$+CTerm).

## 5.4. Results and Discussion:

### 5.4.1 Potential of Mean Force (PMF) profile:

A PMF study has been performed by running a series of MD simulation with the umbrella sampling (US) methodology to examine the degree of association between

monomeric units in the $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex and $A\beta_{1-42}$-CTerm Heterodimer complex. For both the complexes, the PMF profile in the water at room temperature as a function of reaction coordinate has been depicted in **Figure 5.3**. As illustrated in **Figure 5.3**, we can see the presence of a minimum PMF value of $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex at a separation of 22 Å with barriers to dissociation of 7.03 kcal $mol^{-1}$ and 20.53 kcal $mol^{-1}$ at 1.23 Å and 22.73 Å respectively. For the $A\beta_{1-42}$-CTerm Heterodimer complex, the presence of minimum PMF value was found at a separation of 22 Å with barriers to dissociation of 13.37 and 10.12 kcal $mol^{-1}$ at 2.26 Å and 22.92 Å respectively.

From the PMF plot, it has been observed the dissociation energy value for $A\beta_{1-42}$-CTerm Homodimer to be ~3 times more than $A\beta_{1-42}$-$A\beta_{1-42}$ Heterodimer complex. The snapshots of ($A\beta_{1-42}$ peptide + $A\beta_{1-42}$ peptide) Homodimer complex and ($A\beta_{1-42}$-CTerm) Heterodimer complex at discrete distance of separation (in Å) during the course of simulation from 1 Å to 23 Å were shown in **Figure 5.4 and 5.5.**

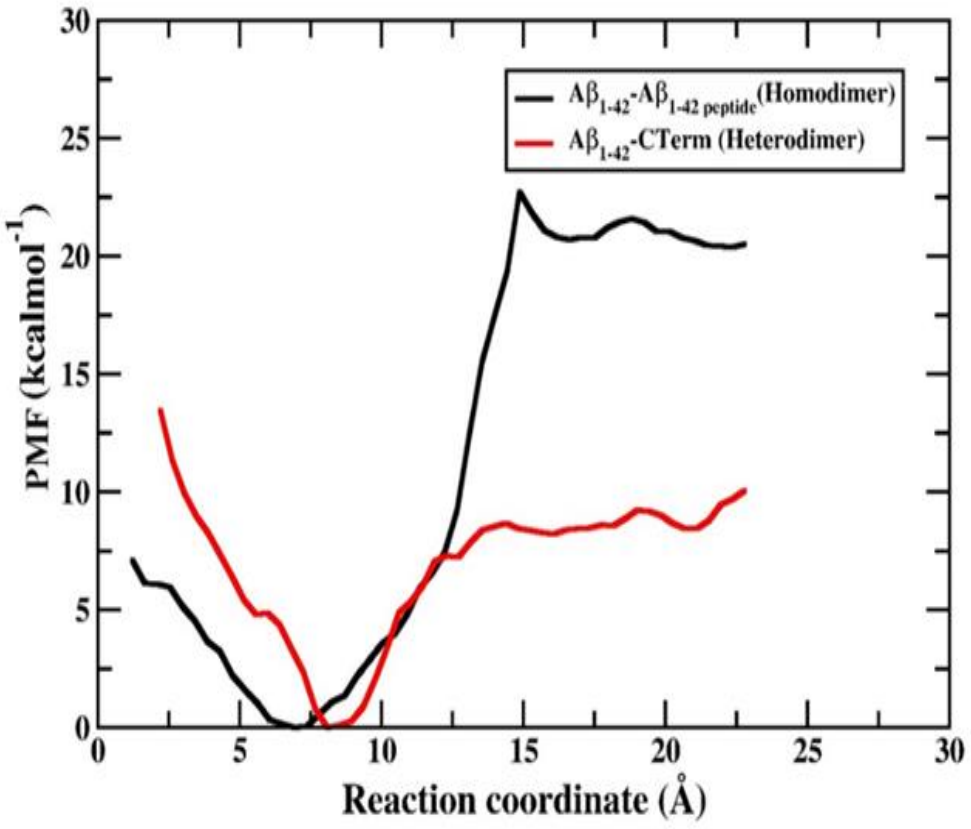

***Figure 5.3.*** *Potential of Mean force as a function of the reaction coordinates for theshowing the association of $A\beta_{1-42}/A\beta_{1-42}$ (homodimer) and $A\beta_{1-42}$ /CTerm (heterodimer) (kcal mol$^{-1}$).*

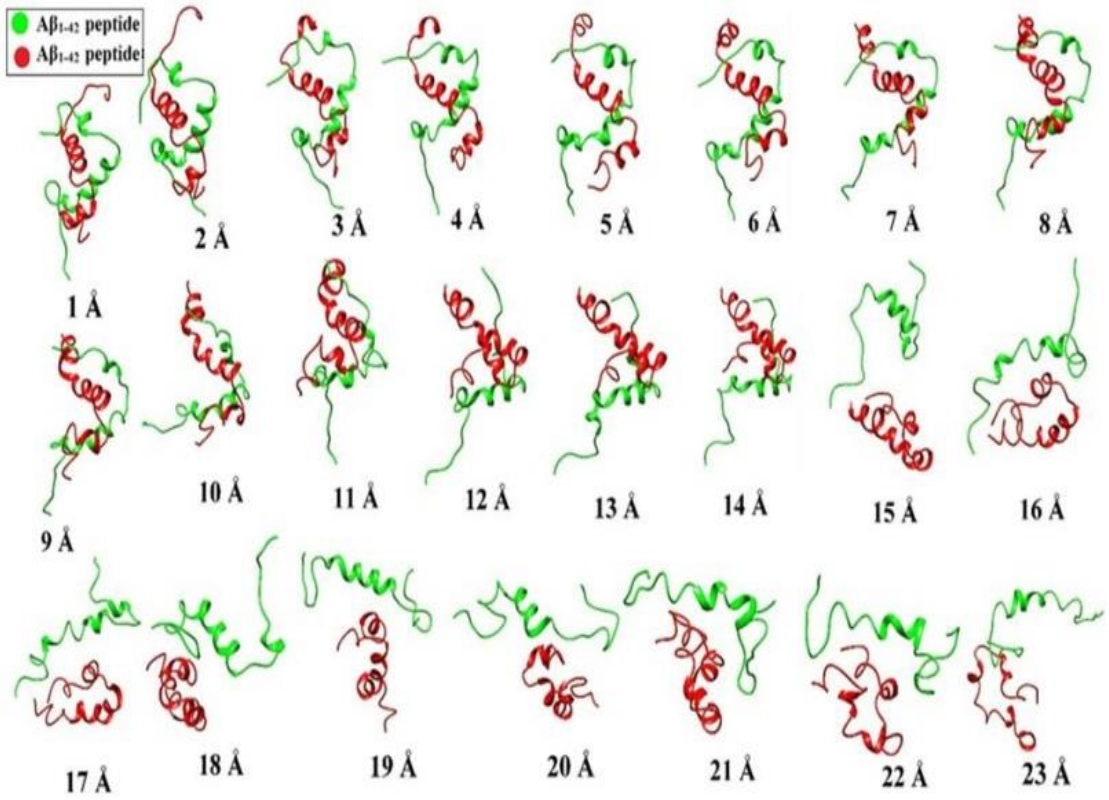


***Figure 5.4.*** *Snapshots of ($A\beta_{1-42}$ peptide + $A\beta_{1-42}$ peptide) Homodimer complex structures at discrete distance of separation (in Å) during the course of simulation from 1 Å to 23 Å.*

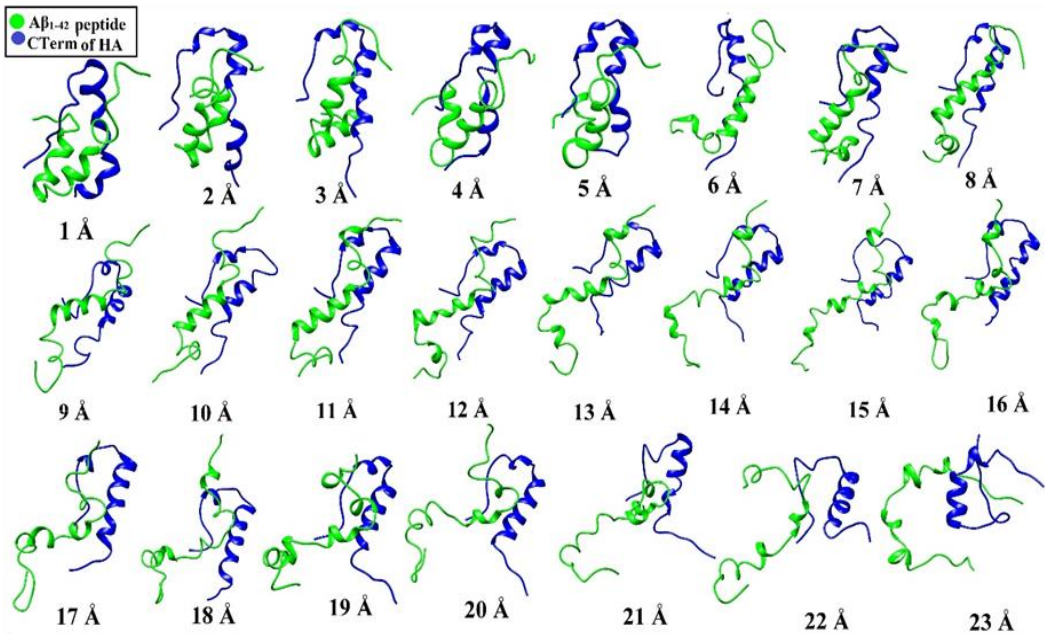


***Figure 5.5**. Snapshots of (Aβ1-42+CTerm) Heterodimer complex structures at discrete distance of separation (in Å) during the course of simulation from 1 Å to 23 Å.*

## 5.4.2. Binding Free Energy (BFE) Analysis:

The BFE calculations for $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer complexes were done using MM-PBSA/GBSA methods. The values here represent only the relative binding free energy rather than absolute or total binding energy, as MM-PBSA/GBSA uses a continuum solvent approach to determine the binding free energies of the systems. **Tables 5.3 and 5.4** summarize the values of binding free energy that had been determined for $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex and $A\beta_{1-42}$-CTerm Heterodimer complex along with the energy terms.

From **Tables 5.3 and 5.4**, it has been observed that all the derived components for the BFE analysis contributed to the binding of two units of $A\beta_{1-42}$ peptide in $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and units of $A\beta_{1-42}$ peptide and CTerm in $A\beta_{1-42}$-CTerm Heterodimer complexes respectively. The values of $\Delta G_{GB\_TOTAL}$ and $\Delta G_{PB\_TOTAL}$ for $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex were observed to be -48.91 kcal $mol^{-1}$ and 2.96 kcal $mol^{-1}$. In the case of the $A\beta_{1-42}$-CTerm Heterodimer complex, it has been observed $\Delta G_{GB\_TOTAL}$ and $\Delta G_{PB\_TOTAL}$ to be -31.62 kcal $mol^{-1}$ and 13.91 kcal $mol^{-1}$ respectively.

From the BFE analysis, it has been observed that the $\Delta G_{GB\_TOTAL}$ and $\Delta G_{PB\_TOTAL}$ values of $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex to be more negative than the $A\beta_{1-42}$-CTerm Heterodimer complex. This indicates that the components of $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex are tightly bound than the components in $A\beta_{1-42}$-CTerm Homodimer complex.

***Table 5.3.*** *Calculated binding free energy MM-GBSA and MM-PBSA values for the ($A\beta_{1-42}$- $A\beta_{1-42}$) Homodimer complex.*

| Method of BFE calculation | Energy Components | Complex | Ligand | Receptor | $\Delta\Delta G_{bind}$ |
|---|---|---|---|---|---|
| | | Energy values (kcal $mol^{-1}$) | Energy values (kcal $mol^{-1}$) | Energy values (kcal $mol^{-1}$) | Energy values (kcal $mol^{-1}$) |
| MM-GBSA | ΔvdW | -474.63 | -177.97 | -224.33 | -72.33 |
| | $\Delta E_{ele}$ | -6141.52 | -3083.25 | -3046.89 | -11.38 |
| | $\Delta E_{GB}$ | -1544.19 | -790.23 | -799.98 | 46.02 |
| | $\Delta E_{SURF}$ | 49.21 | 32.92 | 27.51 | -11.22 |
| | $\Delta G_{gas}$ | -6616.14 | -3261.22 | -3271.22 | -83.71 |
| | $\Delta G_{solv}$ | -1494.98 | -757.31 | -772.47 | -34.79 |
| | $GB_{TOTAL}$ | **-8111.12** | **-4018.53** | **-4043.68** | **-48.91** |
| MM-PBSA | ΔvdW | -474.63 | -177.97 | -224.33 | -72.33 |
| | $\Delta E_{ele}$ | -6141.52 | -3083.25 | -3046.89 | -11.38 |
| | $\Delta E_{PB}$ | -1599.23 | -810.54 | -827.92 | 39.24 |
| | ΔENPOLAR | 748.27 | 424.16 | 388.75 | -64.65 |
| | ΔEDISPER | -559.66 | -361.16 | -309.91 | 111.41 |
| | $\Delta G_{gas}$ | -6616.14 | -3261.22 | -3271.22 | -83.71 |
| | $\Delta G_{solv}$ | -1410.62 | -747.54 | -749.07 | 85.99 |
| | $PB_{TOTAL}$ | **-8026.76** | **-4008.76** | **-4020.29** | **2.96** |

***Table 5.4.****Calculated binding free energy MM-GBSA and MM-PBSA values for the ($A\beta_{1-42}$ - $CT_{erm}$) Heterodimer complex.*

| Method of BFE calculation | Energy Components | Complex | Ligand | Receptor | $\Delta\Delta G_{bind}$ |
|---|---|---|---|---|---|
| | | Energy values (kcal $mol^{-1}$) | Energy values (kcal $mol^{-1}$) | Energy values (kcal $mol^{-1}$) | Energy values (kcal $mol^{-1}$) |
| MM-GBSA | ΔvdW | -482.37 | -207.36 | -213.64 | -61.38 |
| | $\Delta E_{ele}$ | -5964.20 | -2671.06 | -2999.91 | -293.23 |
| | $\Delta E_{GB}$ | -1205.89 | -705.13 | -833.86 | 333.11 |
| | $\Delta E_{SURF}$ | 42.15 | 23.12 | 29.15 | -10.12 |
| | $\Delta G_{gas}$ | -6446.57 | -2878.41 | -3213.55 | -354.61 |
| | $\Delta G_{solv}$ | -1163.74 | -682.02 | -804.71 | 322.99 |
| | $GB_{TOTAL}$ | **-7610.32** | **-3560.43** | **-4018.27** | **-31.62** |
| MM-PBSA | ΔvdW | -482.37 | -207.35 | -213.64 | -61.38 |
| | $\Delta E_{ele}$ | -5964.20 | -2671.06 | -2999.91 | -293.23 |
| | $\Delta E_{PB}$ | -1228.36 | -696.52 | -856.57 | 324.74 |
| | ΔENPOLAR | 697.23 | 352.28 | 400.24 | -55.29 |
| | ΔEDISPER | -500.11 | -277.00 | -322.18 | 99.08 |
| | $\Delta G_{gas}$ | -6446.58 | -2878.41 | -3213.55 | -354.61 |
| | $\Delta G_{solv}$ | -1031.23 | -621.25 | -778.51 | 368.52 |
| | $PB_{TOTAL}$ | **-7477.81** | **-3499.66** | **-3992.07** | **13.91** |

***Abbreviations****: $\Delta E_{ele}$ = electrostatic energy as calculated by the MM force field; $\Delta E_{vdW}$ = van der Waals contribution from MM; $\Delta E_{MM}$ = total gas phase energy (sum of ELE, VDW, and INT); $\Delta G_{PB}$ = the electrostatic contribution to the polar solvation free energy calculated by PB; $\Delta G_{surf}$ = non-polar contribution to the solvation free energy calculated by an empirical model; $\Delta G_{sol}$ = sum of non-polar and polar contributions to solvation; $PB_{TOTAL}$/ $GB_{TOTAL}$ = final estimated binding free energy in kcal $mol^{-1}$ calculated from the terms above.*

## 5.4.3. Contribution of individual residues in the formation of $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex and $A\beta_{1-42}$-CTerm Heterodimer complex:

The contribution of the individual amino acid residues to the overall PPI of the $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer complex has been studied using PDBsum server (as depicted in **Figure 5.6)**. The residues contributing mainly in the PPI between two units of $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer complex were found to be PHE, ARG, LYS, MET, GLY, ILE, ALA, LEU, GLN, SER, HIE and VAL as shown in **Figure 5.6.** Similarly, the prime residues contributing in PPI between the two units of $A\beta_{1-42}$-CTerm Heterodimer complex were found to be GLU, LEU, THR, ALA, PRO, HIE, ILE, LYS, ARG, VAL, ASP, GLN, PHE, MET and GLY as shown in **Figure 5.6**. The summary of interactions observed in $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer and $A\beta_{1-42}$-CTerm Heterodimer complexes were tabulated in **Table 5.5 and 5.6** respectively**.**

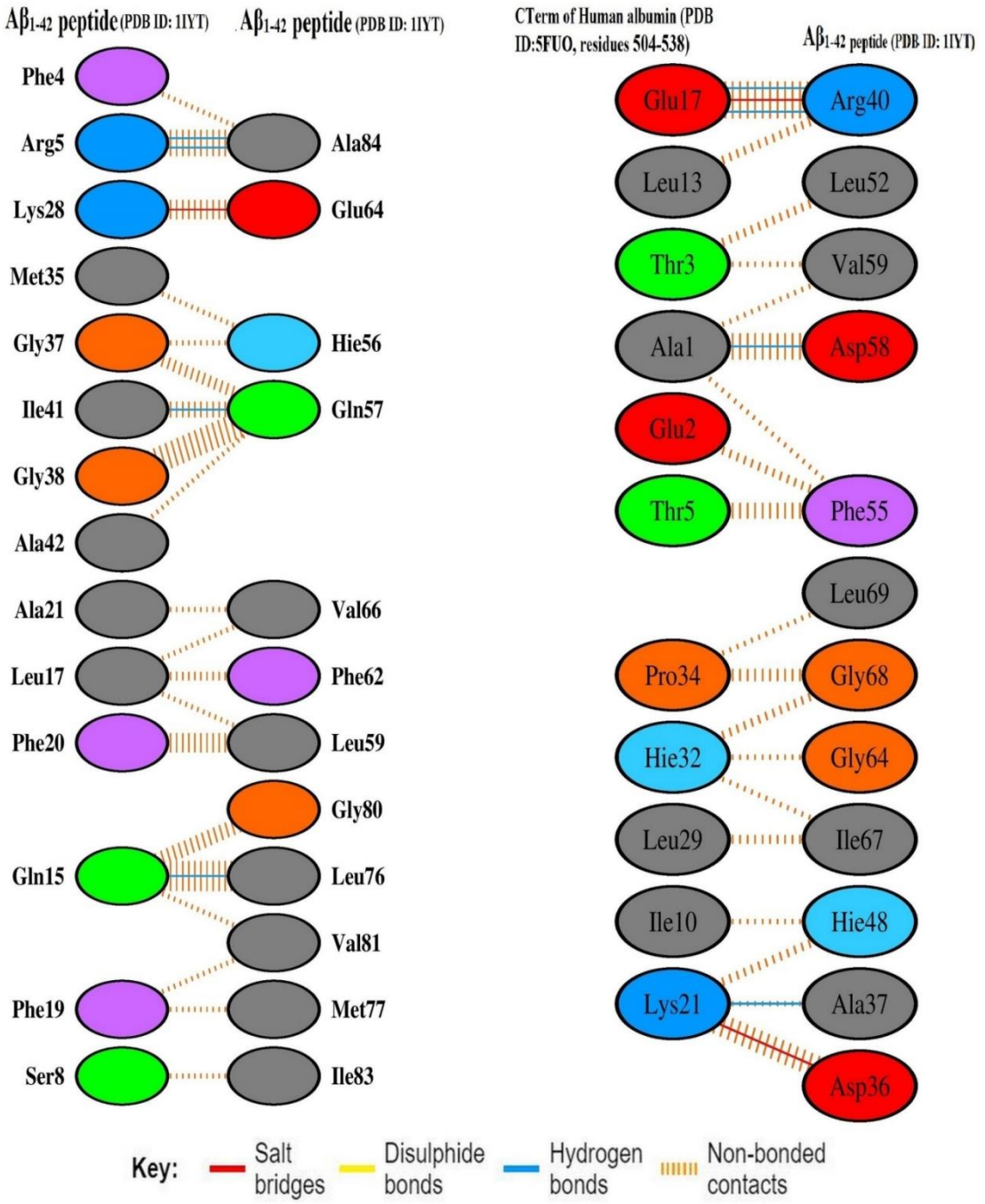


***Figure 5.6.*** *Schematic representation of interacting residues of Aβ1-42 peptide (PDB ID: 1IYT) with residues of Aβ1-42 peptide (homodimer state), and CTerm of human albumin (PDB ID: 5FUO) with residues of Aβ1-42 peptide (heterodimer state).*

***Table 5.5.*** *Interface statistics of $A\beta_{1-42}$-$A\beta_{1-42}$ peptide Homodimer complex.*

| System | No. of interface residues | Interface area | No. of salt bridges | No. of disulphide bonds | No. of hydrogen bonds | No. of non-bonded contacts |
|---|---|---|---|---|---|---|
| **$A\beta_{1-42}$** | 14 | 862 | 1 | — | 4 | 56 |
| **CTerm** | 12 | 939 | | | | |

***Table 5.6.*** *Interface statistics of $A\beta_{1-42}$-CTerm Heterodimer complex.*

| System | No. of interface residues | Interface area | No. of salt bridges | No. of disulphide bonds | No. of hydrogen bonds | No. of non-bonded contacts |
|---|---|---|---|---|---|---|
| **$A\beta_{1-42}$** | 11 | 746 | 2 | — | 4 | 50 |
| **CTerm** | 12 | 728 | | | | |

## 5.5. Conclusion:

In this study, the association of monomeric units in $A\beta_{1-42}$-$A\beta_{1-42}$ peptide Homodimer and $A\beta_{1-42}$-CTerm peptide Heterodimer complexes has been demonstrated using PMF and BFE analysis. It has been found that the dissociation energy for the $A\beta_{1-42}$-$A\beta_{1-42}$ peptide Homodimer complex to be higher than the $A\beta_{1-42}$-CTerm peptide Heterodimer complex. The CTerm peptide with a lesser number of amino acids than $A\beta_{1-42}$ peptide was found to associate significantly with $A\beta_{1-42}$ peptide in the $A\beta_{1-42}$-CTerm heterodimer complex. From these findings, it has been seen that CTerm of HA has the ability to affect the dimerization of $A\beta_{1-42}$ peptide and also to disassemble the $A\beta_{1-42}$ monomer.