Computational investigation on the role of C-Terminal of human albumin on the dimerization of Aβ_{1-42} peptide

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ABSTRACT

Alzheimer’s disease (AD) is characterized by the presence of Amyloid-beta (Aβ) peptide, which has the propensity to fold into β-sheets under stress forming aggregated amyloid plaques. Nowadays many studies have focused on the development of novel, specific therapeutic strategies to slow down Aβ aggregation or control preformed aggregates. Albumin, the most abundant protein in the cerebrospinal fluid, was reported to bind Aβ impeding its aggregation. Recently, it has been reported that C-terminal (CTerm) of Human Albumin binds with Aβ_{1-42}, impairs Aβ aggregation and promotes disassembly of Aβ aggregates protecting neurons. In this computational study, we have investigated the effect of CTerm on the conformational dynamics and the aggregation propensity of Aβ_{1-42} peptide. We have performed molecular dynamics simulations on the Aβ_{1-42}-Aβ_{1-42} homodimer and Aβ_{1-42}-CTerm of albumin heterodimer using the AMBER force field ff99SBildn. From the Potential of mean force (PMF) study and Binding free energy (BFE) analysis, we observed the association of Aβ_{1-42} peptide monomer with itself in the form of homodimer to be stronger than its association with the CTerm in the heterodimer complex. The difference in the number of residues in the Aβ_{1-42} peptide monomer (42 AAs) and CTerm (35 AAs) may be probable reason for the difference in association between the monomeric units in corresponding homodimer and heterodimer complexes. But even then CTerm shows a significant effect on the dimerization of Aβ_{1-42} peptide. Our findings therefore suggest that CTerm can be used for the disassembly of Aβ_{1-42} peptide monomer.

Keywords: Molecular dynamics simulation; Amyloidosis; Amyloid plaques; Potential of mean force.

1. 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 [1]. 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 [2]. Though the native conformation is thermodynamically favorable, often it is found to be only slightly stable under various physiological conditions [3-6]. 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 [7]. 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. [5, 8-10]. Dementias are responsible for the greatest burden of neurodegenerative diseases. According to WHO, Alzheimer’s disease is the most common form of dementia and may contribute to 60–70% of cases. World Health Organization (WHO) also reported in their fact sheets that Worldwide around 50 million people have dementia, and there are nearly 10 million new cases every year representing approximately 60-70% of dementia cases, affecting large numbers of elderly Worldwide. 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 [10]. As the patient’s condition deteriorates, bodily functions are lost, ultimately leading to death [9].

Alzheimer’s disease is considered the most common neurodegenerative disorder [10-12]. The pathological hallmark of Alzheimer’s disease is amyloid plaques, similar to some other neurodegenerative diseases. The major constituent of amyloid plaque is found to be Amyloid-Beta (Aβ) peptide [9, 11-13]. 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. Amyloid-Beta (Aβ) peptide that is generated from the sequential cleavages of large membrane-spanning glycoprotein, amyloid precursor protein (APP) [14,15]. This Aβ peptide exists in two isoforms, Aβ1-40 and Aβ1-42 peptide. Between the two isoforms, the aggregation of Aβ1-42 is found to be more significant and toxic [16]. The Aβ1-42 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 [17]. Despite a high degree of sophistication,
probing the conformational changes of Aβ_{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 [18, 19]. Some studies report that few small molecules can disrupt preformed amyloid fibrils [20]. 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 [18]. 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 [21]. The function of HSA is to transport hormones, fatty acids and various compounds through the blood stream 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 [22]. Aβ present in brain parenchyma is believed to play a prominent role in the pathogenesis of Alzheimer’s disease (AD). Aβ is transported from the brain to the plasma via complex transport pathways at the blood-brain barrier (BBB) [23]. 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 [24-28]. It has also been reported that albumin binds with Aβ-peptide impeding its aggregation [29,30]. 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. 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 favours the disassembly of preformed amyloid aggregates [31].

In this study, we have studied the interaction of Aβ_{1-42} peptide with CTerm of HA in terms of Potential of mean force (PMF). We have also studied the conformational changes undergone by Aβ_{1-42}-Aβ_{1-42} Homodimer and Aβ_{1-42}-CTerm Heterodimer with the help of Molecular Dynamics (MD) simulation approach. We also calculated the Binding free energy (BFE) between monomeric units in Aβ_{1-42}-Aβ_{1-42} Homodimer and Aβ_{1-42}-CTerm Heterodimer complexes. The contribution of individual residues contributing to the protein-protein interaction (PPI) for both the complexes has also been analyzed using PDBsum server [32].

2. MATERIALS AND METHODS

2.1 Molecular docking and the preparation of initial structure.

2.1.1 Preparation of receptor

The micelle-bound human Aβ_{1-42} monomeric structure, PDB ID: 1IYT [33] obtained from RCSB Protein Data Bank (Berman et al., 2000) was used as the receptor molecule for molecular docking.

2.1.2 Preparation of ligand

The structure of C-terminal of Human albumin (CTerm), PDB ID: 5FUO (35 residues: 504-538) [34], obtained from RCSB Protein Data Bank [35, 36] was used as the ligand molecule for molecular docking.

2.1.3 Preparation of Aβ_{1-42}-Aβ_{1-42} homodimer complex

We assigned two different chain IDs to the same monomeric structure of Aβ_{1-42} peptide and saved it as two different .pdb files. These two monomeric structures of Aβ_{1-42} peptide were docked to form a homodimer molecule using ClusPro, an online protein-protein docking tool [37]. From the ten model structures (cluster centers) obtained from ClusPro for the Aβ_{1-42}-Aβ_{1-42} complex, we have chosen the first docked model for our study. This selection is based upon the rankings of docked model structures (as shown in Figure S1) depending on the number of highly populated clusters, cluster center, and the lowest energy weighted scores (as shown in Table S1).

2.1.4 Preparation of Aβ_{1-42}-CTerm heterodimer complex

The pdb format of Human Albumin (PDB ID: 5FUO) was taken from RCSB PDB and visualized using UCSF Chimera [38]. Recently, it has been reported that CTerm retains HA binding property [39], so we selectively isolated peptide region of 504-538 residues from HA. CTerm peptide, 504-AETFTFHADICTLSEKERQIKKQTALVELVKHKPKamide^{33}, containing the hydrophobic domains reported to be Aβ binding sites [40] 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 Aβ_{1-42} peptide using ClusPro and top ten docked models (Figure S2) were obtained from which top first docked model was selected for our study. As discussed in section 2.1.3, in this case, the selection of the best suitable docked model based on Table S2.

2.2 Molecular Dynamics (MD) simulation of Aβ_{1-42}-Aβ_{1-42} Homodimer and Aβ_{1-42}-CTerm Heterodimer complexes.

To perform MD simulation, the required coordinate and topology files of Aβ_{1-42}-Aβ_{1-42} Homodimer and Aβ_{1-42}-CTerm Heterodimer complex structures have been built using AMBERff99SBildn [41, 42] force field and with the Leap module of the AMBER 14 software package [43]. As per reports of recent studies, the structural ensembles of intrinsically disordered proteins (IDPs) are strongly dependent on their force field [44]. The uneven energy landscapes of IDPs are capable of revealing force field deficiencies, thus contributing to force field development. We have observed that there is no ideal force field to study IDPs. However, presently available literature shows that ff99SBildn and ff99SB force fields have been used in many studies to analyse the salient structural features of IDPs [44-48]. In addition, ff99SBildn is the advanced force field of ff99SB for IDP. Hence, we have used ff99SBildn force field to carry out this particular study. Solvation of these complexes was done with TIP3P (transferable intermolecular potential with 3 points) water molecules [49] with solvent buffer being 10 Å surrounding the complexes from all directions.

The MD study was carried out using a standard procedure, which includes heating dynamics followed by density, equilibration, and production dynamics. The two complexes were
neutralized with appropriate number of counter ions, followed by
two steps energy minimizations with first subjected to 500 steps of
steepest descents minimization and then 500 steps of conjugate
gradient (CG) minimization. The systems were gradually heated
from 0-300 K in constant volume (NVT) conditions, after which
the density procedure was carried out. The equilibration of the
protein systems was conducted in NPT conditions (300 K and 1
atm pressure) for 1 ns. To ensure successful equilibration of the
systems, the temperature, energy and pressure graphs were plotted
and analysed. Next, a 50 ns MD production run for the
equilibrated structures of both the systems using the Particle Mesh
Ewald (PME) algorithm [50] the time step of 2 fs was performed.
A cut-off of 8 Å to treat the nonbonding interactions (short-range
electrostatic and van der Waals interactions) during the simulation,
while the long-range electrostatic interactions were treated with
the PME method. All the bonds present in the systems were
constrained with the SHAKE algorithm [51]. The pressure and
temperature (0.5 ps of heat bath and 0.2 ps of pressure relaxation)
were held constant by the Berendsen weak coupling algorithm [52]
throughout the simulation process. For each system, the trajectory
snapshots were recorded every 10 ps for further analysis.

**Table S1.** List of top 10 docked clusters of (Aβ<sub>1-42</sub> + Aβ<sub>1-42</sub>
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         |
| 2       | 92      | Lowest Energy  | -130.5         |
| 3       | 69      | Center         | -123.9         |
| 4       | 60      | Lowest Energy  | -124.5         |
| 5       | 54      | Center         | -100.8         |
| 6       | 45      | Lowest Energy  | -112.9         |
| 7       | 40      | Center         | -102.8         |
| 8       | 38      | Lowest Energy  | -111.6         |
| 9       | 38      | Center         | -102.5         |
| 10      | 34      | Lowest Energy  | -107.6         |
| 11      | 34      | Center         | -101           |

**Table S2.** List of top 10 docked clusters of (Aβ<sub>1-42</sub> 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         | -596           |
| 2       | 181     | Lowest Energy  | -1035.9        |
| 3       | 170     | Center         | -916.3         |
| 4       | 170     | Lowest Energy  | -1009.5        |
| 5       | 132     | Center         | -933.3         |
| 6       | 132     | Lowest Energy  | -1030.5        |
| 7       | 78      | Center         | -971.6         |
| 8       | 78      | Lowest Energy  | -1123.5        |
| 9       | 72      | Center         | -973.8         |
| 10      | 72      | Lowest Energy  | -1037.9        |
| 11      | 65      | Center         | -907.4         |
| 12      | 65      | Lowest Energy  | -1043          |
| 13      | 63      | Center         | -926.8         |
| 14      | 63      | Lowest Energy  | -1012.7        |
| 15      | 52      | Center         | -1036.9        |

### 2.3 PMF calculation.

Molecular dynamics (MD) simulations coupled with umbrella
sampling (US) [53] method and the Weighted Histogram Analysis
Method (WHAM) [54] were used to calculate the potential of
mean force (PMF) [55] for Aβ<sub>1-42</sub>-Aβ<sub>1-42</sub> Homodimer and Aβ<sub>1-42</sub>
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 coordinate. The reason behind this is
that the energy barrier of interest is many times the size of kT 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 [56]
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 Aβ<sub>1-42</sub>-Aβ<sub>1-42</sub> Homodimer and Aβ<sub>1-42</sub>-CTerm
Heterodimer into different series and then applied restraint over
the samples to remain close to the centre of window, provided the
end point overlaps. Biasing potentials was 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 Aβ<sub>1-42</sub> peptide monomeric units in Aβ<sub>1-42</sub>-Aβ<sub>1-42</sub>
Homodimer complex and Aβ<sub>1-42</sub> peptide and CTerm in the Aβ<sub>1-42</sub>
CTerm Heterodimer complex was carried out by changing the
centre of mass (CoM) distances (in two different directions by
increasing and decreasing) between the two monomers in Aβ<sub>1-42</sub>
Aβ<sub>1-42</sub> Homodimer and Aβ<sub>1-42</sub>-CTerm Heterodimer complexes.
The distance between CoMs of the Aβ<sub>1-42</sub> peptides in Aβ<sub>1-42</sub>
Homodimer and Aβ<sub>1-42</sub> 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. We have computed the PMF as function of reaction
coordinate for both the complexes.

### 2.4 MMPBSA/GBSA Binding free energy calculation.

The relative binding free energy (BFE) analysis for the (Aβ<sub>1-42</sub>
Aβ<sub>1-42</sub>) Homodimer and (Aβ<sub>1-42</sub>-CTerm) Heterodimer complex
was carried out using MMPBSA.py script of the AMBER 14 suite.
This script is based on the Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/GBSA) and
Molecular Mechanics/Generalized Borne Surface Area (MM/GBSA) algorithms. The MM-PBSA/GBSA methods [57–65] were used to calculate the binding free energy ($\Delta G_{\text{binding}}$) and the contributions of electrostatic and van der Waals in the formation of complexes. All the trajectories were taken into consideration for the PMM-PBSA/GBSA calculations. Thereafter, MM-PBSA/GBSA analysis was performed on the three components of the complex systems: (i) the protein (Aβ$_{1-42}$) (ii) the ligand (Aβ$_{1-42}$) in case of Homodimer or CTerm in case of Heterodimer and (iii) the complex (Aβ$_{1-42}$+Aβ$_{1-42}$ or Aβ$_{1-42}$-CTerm). For each of these components, the interaction energy and solvation free energy were calculated and the averages of these results were considered to ascertain an estimate of the ligand-binding free energy. The binding free energy (BFE) was calculated using Eqn. [1]:

$$\Delta G_{\text{binding}} = \Delta G_{\text{complex}} + [\Delta G_{\text{receptor}} + \Delta G_{\text{ligand}}]$$  \hspace{1cm} (1)

where, $\Delta G_{\text{binding}}$ is the total binding free energy. For the system Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer, $\Delta G_{\text{complex}}$, $\Delta G_{\text{receptor}}$ and $\Delta G_{\text{ligand}}$ represent free energy contributions from Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer complex, Aβ$_{1-42}$ peptide and Aβ$_{1-42}$ peptide respectively. Similarly for the system Aβ$_{1-42}$-CTerm Heterodimer, $\Delta G_{\text{complex}}$, $\Delta G_{\text{receptor}}$ and $\Delta G_{\text{ligand}}$ represent free energy contributions from Aβ$_{1-42}$-CTerm Heterodimer complex, Aβ$_{1-42}$ peptide and CTerm respectively.

3. RESULTS
3.1 PMF profile.
We have conducted a PMF study by running a series of MD simulation with the umbrella sampling (US) methodology to examine the degree of association between monomeric units in the Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer complex and Aβ$_{1-42}$-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 1. As illustrated in Figure 1, we can see the presence of a minimum PMF value of Aβ$_{1-42}$-Aβ$_{1-42}$ 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 Aβ$_{1-42}$-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, we observe the disassociation energy value for Aβ$_{1-42}$-CTerm Homodimer to be ~3 times more than Aβ$_{1-42}$-Heterodimer complex. The snapshots of (Aβ$_{1-42}$ peptide + Aβ$_{1-42}$ peptide) Homodimer complex and (Aβ$_{1-42}$-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 S3 and S4.

3.2 Binding free energy (BFE) Analysis.
The BFE calculations for Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer and Aβ$_{1-42}$-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. Table 1 and 2 summarize the values of binding free energy that had been determined for Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer complex and Aβ$_{1-42}$-CTerm Heterodimer complex along with the energy terms.

From Table 1 and 2, we observed that all the derived components for the BFE analysis contributed to the binding of two units of Aβ$_{1-42}$ peptide in Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer and units of Aβ$_{1-42}$ peptide and CTerm in Aβ$_{1-42}$-CTerm Heterodimer complexes respectively. The values of $\Delta G_{\text{GB,TOT}}$ and $\Delta G_{\text{PB,TOT}}$ for Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer complex were observed to be -48.91 kcal mol$^{-1}$ and 2.96 kcal mol$^{-1}$. In the case of Aβ$_{1-42}$-CTerm Heterodimer complex, we observed $\Delta G_{\text{GB,TOTAL}}$ and $\Delta G_{\text{PB,TOTAL}}$ to be -31.62 kcal mol$^{-1}$ and 13.91 kcal mol$^{-1}$ respectively. From the BFE analysis, we observe that the $\Delta G_{\text{GB,TOT}}$ and $\Delta G_{\text{PB,TOT}}$ values of Aβ$_{1-42}$-Aβ$_{1-42}$ Homodimer complex to be more negative than the Aβ$_{1-42}$-CTerm Heterodimer complex. This indicates that the components of Aβ$_{1-42}$-CTerm Heterodimer...
3.3 Contribution of individual residues in the formation of Aβ1-42-Aβ1-42 Homodimer complex and Aβ1-42–CTerm Heterodimer complex.

We have also studied the contribution of the individual amino acid residues to the overall PPI of the Aβ1-42–Aβ1-42 Homodimer and Aβ1-42–CTerm Heterodimer complex using PDBsum server (as depicted in Figure 2). The residues contributing mainly in the PPI between two units of Aβ1-42–Aβ1-42 Homodimer complex were found to be PHE, ARG, LYS, MET, GLY, ILE, ALA, LEU, GLN, SER, HIE and VAL as shown in Figure 2. Similarly, the prime residues contributing in PPI between the two units of Aβ1-42–Aβ1-42 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 2. The summary of interactions observed in Aβ1-42 – Aβ1-42 Homodimer and Aβ1-42–CTerm Heterodimer complexes were tabulated in Table 3 and 4 respectively. Additionally, interactions of residues of Aβ1-42–Aβ1-42 peptide and Aβ1-42–CTerm Heterodimer complex, obtained from Ligplot+ software [66] were shown in Table S3 and S4 respectively.

Table 1. Calculated binding free energy MM-GBSA and MM-PBSA values for the (Aβ1-42 – Aβ1-42) Homodimer complex.

| Method of BFE calculation | Complex | Ligand | ΔΔG_{bind} |
|--------------------------|---------|--------|------------|
| **MM-GBSA**              |         |        |            |
| Energy components        |         |        |            |
| ΔvdW                     | -474.63 | 8.91   | -177.97    |
| ΔEel                     | -6141.52| 31.07  | -3083.25   |
| ΔEgb                     | -1544.19| 20.57  | -790.23    |
| ΔESURF                   | 49.21   | 0.66   | 32.92      |
| ΔE_{△hh}                 | -6616.14| 29.46  | -3261.22   |
| ΔG_{△hh}                 | -1494.98| 20.41  | -757.31    |
| GB_{TOTAL}               | -8111.12| 17.49  | -4018.53   |
| **MM-PBSA**              |         |        |            |
| Energy components        |         |        |            |
| ΔvdW                     | -474.63 | 8.91   | -177.97    |
| ΔEel                     | -6141.52| 31.07  | -3083.25   |
| ΔEgb                     | -1599.23| 20.89  | -810.54    |
| ΔENPOLAR                 | 748.27  | 3.96   | 424.16     |
| ΔEDISPER                 | -559.66 | 3.76   | -361.16    |
| ΔG_{gas}                 | -6616.14| 29.46  | -3261.22   |
| ΔG_{sol}                 | -1410.62| 20.82  | -747.54    |
| PB_{TOTAL}               | -8026.76| 19.27  | -4008.76   |

Table 2. Calculated binding free energy MM-GBSA and MM-PBSA values for the (Aβ1-42 – CTerm) Heterodimer complex.

| Method of BFE calculation | Complex | Ligand | ΔΔG_{bind} |
|--------------------------|---------|--------|------------|
| **MM-GBSA**              |         |        |            |
| Energy components        |         |        |            |
| ΔvdW                     | -482.37 | 10.17  | -207.36    |
| ΔEel                     | -5964.20| 28.43  | -2671.06   |
| ΔEgb                     | -1205.89| 18.33  | -705.13    |
| ΔESURF                   | 42.15   | 0.57   | 23.12      |
| ΔG_{gas}                 | -6466.57| 25.32  | -2887.41   |
| ΔG_{sol}                 | -1163.74| 18.51  | -682.02    |
| GB_{TOTAL}               | -7610.32| 18.70  | -3560.43   |
| **MM-PBSA**              |         |        |            |
| Energy components        |         |        |            |
| ΔvdW                     | -482.37 | 10.17  | -207.35    |
| ΔEel                     | -5964.20| 28.43  | -2671.06   |
| ΔEgb                     | -1228.36| 18.77  | -696.52    |
| ΔENPOLAR                 | 697.23  | 3.31   | 352.28     |
| ΔEDISPER                 | -500.11 | 3.57   | -277.00    |
| ΔG_{gas}                 | -6646.58| 25.3199| -2878.41   |
| ΔG_{sol}                 | -1031.23| 18.8606| -621.25    |
| PB_{TOTAL}               | -7477.81| 2.6568 | -3499.66   |

ΔE_{el} = electrostatic energy as calculated by the MM force field; ΔE_{vdw} = van der Waals contribution from MM; ΔE_{gb} = total gas phase energy (sum of ELE, VDW, and INT); ΔG_{gas} = the electrostatic contribution to the polar solvation free energy calculated by PB; ΔE_{sol} = non-polar contribution to the solvation free energy calculated by an empirical model; ΔG_{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.

Table 3. Interface statistics of Aβ1-42–Aβ1-42 peptide Homodimer complex.

| Aβ1-42 | Aβ1-42 |
|--------|--------|
| 14     | 12     |
| 862    | 939    |
| 1      | -      |
| 4      | 56     |

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### Table 4. Interface statistics of Aβ_{1,42}–CTerm Heterodimer complex.

|                | No. of interface residues | Interface area | No. of salt bridges | No. of disulfide bonds | No. of hydrogen bonds | No. of non-bonded contacts |
|----------------|---------------------------|----------------|---------------------|-----------------------|-----------------------|---------------------------|
| Aβ_{1,42}      | 11                        | 746            | 2                   | -                     | 3                     | 47                        |
| CTerm          | 12                        | 728            | 2                   | -                     | 5                     | 50                        |

4. CONCLUSIONS

In this study, we have demonstrated the association of monomeric units in Aβ_{1,42}–Aβ_{1,42} peptide Homodimer and Aβ_{1,42}–CTerm peptide Heterodimer complexes using Potential of Mean force and Binding free energy analysis. We found the dissociation energy for the Aβ_{1,42}–Aβ_{1,42} peptide Homodimer complex to be higher than the Aβ_{1,42}–CTerm peptide Heterodimer complex.

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Supplementary materials

Figure S1. Top 10 representative docked models for (Aβ$_{1-42}$ peptide + Aβ$_{1-42}$ peptide) complex obtained from ClusPro online docking server.

Figure S2. Top 10 representative docked models for (Aβ$_{1-42}$ peptide + CTerm) complex obtained from ClusPro online docking server.

Figure S3. Snapshots of (Aβ$_{1-42}$ peptide + Aβ$_{1-42}$ peptide) Homodimer complex structures at discrete distance of separation (in Å) during the course of simulation from 1 Å to 23 Å.

Figure S4. Snapshots of (Aβ$_{1-42}$+CTerm) Heterodimer complex structures at discrete distance of separation (in Å) during the course of simulation from 1 Å to 23 Å.
Table S3. Interactions of residues of Aβ_{42} peptide with Aβ_{42} peptide itself (in homodimer state) obtained from Ligplot® software.

List of atom-atom interactions across protein-protein interface

| no. name name no. Chain | no. name name no. Chain | Distance |
|-------------------------|-------------------------|----------|
| 1. 76 Nδ1 ARG 5 A << 1254 O ALA 84 B 3.09 |
| 2. 79 NH2 ARG 5 A << 1255 OXT ALA 84 B 2.83 |
| 3. 229 NE2 GLN 15 A << 1146 O LEU 76 B 2.85 |
| 4. 616 O ILE 41 A << 857 NE2 GLN 57 B 2.80 |

Hydrogen bonds

| no. name name no. Chain | no. name name no. Chain | Distance |
|-------------------------|-------------------------|----------|
| 1. 50 CE1 PHE 4 A << 1254 O ALA 84 B 3.72 |
| 2. 76 NH1 ARG 5 A << 1253 C ALA 84 B 3.51 |
| 3. 76 NH1 ARG 5 A << 1254 O ALA 84 B 3.09 |
| 5. 76 NH1 ARG 5 A << 1255 OXT ALA 84 B 3.38 |
| 6. 79 NH2 ARG 5 A << 1253 C ALA 84 B 3.68 |
| 7. 79 NH2 ARG 5 A << 1254 O ALA 84 B 3.81 |
| 8. 79 NH2 ARG 5 A << 1255 OXT ALA 84 B 2.83 |
| 9. 123 O SER 8 A << 1239 CD1 ILE 83 B 3.66 |
| 10. 217 N GLN 15 A << 1141 CD2 LEU 76 B 3.58 |
| 11. 219 CA GLN 15 A << 1135 CG LEU 76 B 3.84 |
| 12. 219 CA GLN 15 A << 1141 CD2 LEU 76 B 3.73 |
| 13. 233 O GLN 15 A << 1200 CG1 VAL 81 B 3.64 |
| 14. 221 CB GLN 15 A << 1135 CG LEU 76 B 3.70 |
| 15. 221 CB GLN 15 A << 1137 CD1 LEU 76 B 3.89 |
| 16. 224 CG GLN 15 A << 1146 O LEU 76 B 3.36 |
| 17. 224 CG GLN 15 A << 1193 O GLY 80 B 3.49 |
| 18. 227 CD GLN 15 A << 1146 O LEU 76 B 3.55 |
| 19. 227 CD GLN 15 A << 1193 O GLY 80 B 3.06 |
| 20. 228 OE1 GLN 15 A << 1193 O GLY 80 B 3.35 |
| 21. 229 NE2 GLN 15 A << 1146 O LEU 76 B 2.85 |
| 22. 229 NE2 GLN 15 A << 1193 O GLY 80 B 3.44 |
| 23. 263 CG LEU 17 A << 1006 CG2 VAL 66 B 3.68 |
| 24. 265 CD1 LEU 17 A << 893 CD1 LEU 59 B 3.54 |
| 25. 265 CD1 LEU 17 A << 943 CB PHE 62 B 3.66 |
| 26. 269 CD2 LEU 17 A << 943 CB PHE 62 B 3.82 |
| 27. 295 CB PHE 19 A << 1200 CG1 VAL 81 B 3.72 |
| 28. 299 CD1 PHE 19 A << 1157 SD MET 77 B 3.79 |
| 29. 319 CD1 PHE 20 A << 897 CD2 LEU 59 B 3.78 |
| 30. 321 CE1 PHE 20 A << 897 CD2 LEU 59 B 3.42 |
| 31. 325 CE2 PHE 20 A << 891 CG LEU 59 B 3.67 |
| 32. 323 CZ PHE 20 A << 891 CG LEU 59 B 3.85 |
| 33. 323 CZ PHE 20 A << 897 CD2 LEU 59 B 3.70 |
| 34. 335 CB ALA 21 A << 1002 CG1 VAL 66 B 3.73 |
| 35. 429 CE LYS 28 A << 979 CD GLU 64 B 3.58 |
| 36. 429 CE LYS 28 A << 981 OE2 LEU 64 B 3.51 |
| 37. 432 NZ LYS 28 A << 979 CD GLU 64 B 3.14 |
| 38. 432 NZ LYS 28 A << 980 OE1 GLU 64 B 3.25 |
| 39. 432 NZ LYS 28 A << 981 OE2 GLU 64 B 2.86 |
| 50. 530 CE MET 35 A << 844 O HIE 56 B 3.27 |
| 51. 554 CA GLY 37 A << 856 OE1 GLN 57 B 3.39 |
| 52. 557 C GLY 37 A << 847 CA GLN 57 B 3.85 |
| 53. 557 C GLY 37 A << 856 OE1 GLN 57 B 3.62 |
| 54. 558 O GLY 37 A << 844 O HIE 56 B 3.47 |
| 55. 559 N GLY 38 A << 847 CA GLN 57 B 3.77 |
| 56. 559 N GLY 38 A << 855 CD GLN 57 B 3.36 |
| 57. 559 N GLY 38 A << 856 OE1 GLN 57 B 3.28 |
| 58. 559 N GLY 38 A << 857 NE2 GLN 57 B 3.26 |
| 59. 561 CA GLY 38 A << 847 CA GLN 57 B 3.87 |
| 60. 561 CA GLY 38 A << 861 O GLN 57 B 3.82 |
| 61. 615 C ILE 41 A << 857 NE2 GLN 57 B 3.53 |
| 62. 616 O ILE 41 A << 855 CD GLN 57 B 3.65 |
| 63. 616 O ILE 41 A << 856 OE1 GLN 57 B 3.69 |
Table S4. Interactions of residues of CTerm of Human albumin with Aβ1-42 peptide obtained from Ligplot+ software.

List of atom-atom interactions across protein-protein interface

| no. | name   | Res  | Chain | no. | name   | Res  | Chain |
|-----|--------|------|-------|-----|--------|------|-------|
| 1.  | N      | ALA  | A     | 2.  | 257    | OE1  | 17    |
| 3.  | 1      | N    | ALA  | 1.  | 953    | OD1  | 58    |
| 4.  | 5      | CA   | ALA  | 9.  | 668    | OD2  | 40    |
| 5.  | 12     | O    | ALA  | 6.  | 908    | CD1  | 55    |
| 6.  | 7      | CB   | ALA  | 7.  | 952    | CG   | 58    |
| 7.  | 7      | CB   | ALA  | 8.  | 953    | OD1  | 58    |
| 8.  | 7      | CB   | ALA  | 9.  | 954    | OD2  | 58    |
| 9.  | 27     | O    | GLU  | 10. | 907    | CG   | 55    |
| 11. | 27     | O    | GLU  | 11. | 916    | CD2  | 55    |
| 12. | 41     | O    | THR  | 12. | 854    | CD1  | 52    |
| 13. | 32     | CB   | THR  | 13. | 854    | CD1  | 52    |
| 14. | 34     | CG2  | THR  | 14. | 967    | CG2  | 59    |
| 15. | 75     | O    | THR  | 15. | 914    | CE2  | 55    |
| 16. | 66     | CB   | THR  | 16. | 914    | CE2  | 55    |
| 17. | 66     | CB   | THR  | 17. | 912    | CZ   | 55    |
| 18. | 68     | CG2  | THR  | 18. | 912    | CZ   | 55    |
| 19. | 141    | CG2  | ILE  | 19. | 780    | ND1  | 48    |
| 20. | 192    | CD2  | LEU  | 20. | 653    | CB   | 40    |
| 21. | 192    | CD2  | LEU  | 21. | 656    | CG   | 40    |
| 22. | 256    | CD   | GLU  | 22. | 662    | NE   | 40    |
| 23. | 256    | CD   | GLU  | 23. | 668    | NH2  | 40    |
| 24. | 257    | OE1  | GLU  | 24. | 656    | CG   | 40    |
| 25. | 257    | OE1  | GLU  | 25. | 662    | NE   | 40    |
| 26. | 257    | OE1  | GLU  | 26. | 664    | CZ   | 40    |
| 27. | 257    | OE1  | GLU  | 27. | 664    | CZ   | 40    |
| 28. | 257    | OE1  | GLU  | 28. | 662    | NH2  | 40    |
| 29. | 258    | OE2  | GLU  | 29. | 662    | NE   | 40    |
| 30. | 258    | OE2  | GLU  | 30. | 664    | CZ   | 40    |
| 31. | 258    | OE2  | GLU  | 31. | 668    | NH2  | 40    |
| 32. | 331    | CD   | LYS  | 32. | 600    | OD1  | 36    |
| 33. | 334    | CE   | LYS  | 33. | 600    | OD1  | 36    |
| 34. | 334    | CE   | LYS  | 34. | 601    | OD2  | 36    |
| 35. | 334    | CE   | LYS  | 35. | 779    | CG   | 48    |
| 36. | 334    | CE   | LYS  | 36. | 780    | ND1  | 48    |

Number of salt bridges: 1
Number of hydrogen bonds: 4
Number of non-bonded contacts: 56
Computational investigation on the role of C-Terminal of Human albumin on the dimerization of Aβ_{1-42} peptide

| Atom no. | Name  | Residue no. | Chain | Atom no. | Name  | Residue no. | Chain |
|----------|-------|-------------|-------|----------|-------|-------------|-------|
| 37       | NZ    | LYS         | A     | 599      | CG    | ASP         | B     |
| 38       | NZ    | LYS         | A     | 600      | OD1   | ASP         | B     |
| 39       | NZ    | LYS         | A     | 601      | OD2   | ASP         | B     |
| 40       | NZ    | LYS         | A     | 613      | O     | ALA         | B     |
| 41       | CD1   | LEU         | A     | 1069     | CG2   | ILE         | B     |
| 42       | CD1   | LEU         | A     | 1076     | CD1   | ILE         | B     |
| 43       | CB    | HIE         | A     | 1069     | CG2   | ILE         | B     |
| 44       | ND1   | HIE         | A     | 1033     | O     | GLY         | B     |
| 45       | CD2   | HIE         | A     | 1082     | N     | GLY         | B     |
| 46       | CD2   | HIE         | A     | 1084     | CA    | GLY         | B     |
| 47       | CB    | PRO         | A     | 1091     | CA    | LEU         | B     |
| 48       | CG    | PRO         | A     | 1088     | O     | GLY         | B     |
| 49       | CD    | PRO         | A     | 1087     | C     | GLY         | B     |
| 50       | CD    | PRO         | A     | 1088     | O     | GLY         | B     |

Salt bridges

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| Atom no. | Name  | Residue no. | Chain | Atom no. | Name  | Residue no. | Chain | Distance |
|----------|-------|-------------|-------|----------|-------|-------------|-------|-----------|
| 258      | OE2   | GLU         | A     | 668      | NH2   | ARG         | B     | 2.01      |
| 337      | NZ    | LYS         | A     | 600      | OD1   | ASP         | B     | 2.69      |

Number of salt bridges: 2
Number of hydrogen bonds: 4
Number of non-bonded contacts: 50