EVALUATION OF NOVEL LIGAND FOR THE MAINTENANCE OF NATIVE $A\beta_{(1-42)}$ PEPTIDE CONFORMATION: RELEVANCE TO ALZHEIMER'S DISEASE

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ABSTRACT: Alzheimer's disease (AD) is a disorder of the central nervous system with progressive neurodegeneration, cognition, and memory loss. A major molecular hallmark of the disorder includes extracellular deposition of the Amyloid beta-peptide (Aβ) in senile plaques, the appearance of intracellular neurofibrillary tangles. The Aβ peptide is produced by sequential cleavage of the Amyloid precursor protein (APP) by α, β, and γ-secretase. The secretase β and γ generate a number of isoforms of peptides containing 36-43 amino acid residues in length. The mainly universal isoforms are $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$. $A\beta_{(1-42)}$ C-terminal domain (29-42 amino acid residues) adopts β conformation, and the N-terminal domain (10-24 amino acid residues) facilitates a dynamic equilibrium between α- helix and β-strand. Thus Aβ polymerization requires unfolding of the native α-helical structure of Aβ. Stabilization of the Aβ central α-helix is an efficient step to foil the Aβ polymerization. Here we report test compounds which to bind and stabilize the 11-30 amino acid regions of $A\beta_{(1-42)}$ in α-helical conformation. On docking, the ligands with Aβ peptide followed by Molecular dynamics simulation for 20ns favored the identification of test compound NIAID. That postulated to bind and stabilize the Aβ central α-helix. So stabilization of Aβ secondary structure with a ligand that maintains the α-helical conformation may provide clues in developing drugs to control the Aβ deposition observed in AD patients.

Keywords:

Alzheimer's disease, NIAID, Molecular Docking and Molecular dynamics simulation, $A\beta_{(1-42)}$ α-helix stabilization

INTRODUCTION: AD is caused by fibril and plaque formation of the Amyloid beta-peptide (Aβ) in the central nervous system [1, 2]. Aβ assemblies include intermediate to monomeric and fibrillary forms which are considered as the source of cytotoxicity [3]. Such Aβ assemblies include low-number oligomers and larger assemblies known as protofibrils, globulomers [4, 5].

The some of the Aβ peptides are an integral membrane protein, called the amyloid precursor protein (AβPP), cleaved predominantly into elongated 40-residue peptide ($A\beta_{1-40}$).

Also, a C-terminally elongated 42-residue version which also can be excised ($A\beta_{1-42}$) and this longer variant is the main constituent of parenchymal amyloid deposits [6, 7]. The link between Aβ aggregation and AD implies that any inhibitors of their aggregation should be able to slow down disease progression. Several low molecular mass Aβ aggregation inhibitors have been identified by screening library of the compound as well as rational design strategies. They include chemically diverse compounds such as Rifampicin, Curcumine, Inositol, Melatonin, D737, and Dobutamine [8, 9, 10]. These inhibitors are predicted to bind to Aβ in an elongated β-strand-like conformation and prevent its polymerization. A potential problem with this strategy is that blocking the later stages of fibril formation will not favor the formation of prefibrillar oligomeric forms that are cytotoxic [11, 12, 13]. No Aβ oligomers have been structurally determined, and thus these oligomers cannot yet be targeted by rational design. A further problem with some of the Aβ polymerization

inhibitors is that they can also act as aggregators [14]. Chemical aggregators can physically sequester proteins in a promiscuous and nonspecific manner which is typically made up of conjugated aromatics, hydrophobic and dye-like features [14, 15] and also a library of peptide inhibitor candidates have formed which systematically are mutating the RGTFEGKF amino acid residues [16]. In light of these circumstances, an alternative strategy to reduce Aβ aggregation and toxicity is warranted. One possible approach, explored herein, is to trap Aβ in a state similar to its perceived structure in membrane-embedded AβPP, by targeting the discordant α-helix region.

Nuclear magnetic resonance (NMR) data showed that $Aβ_{(1-40)}$ adopts a folded structure including two α-helical regions (residues 15-24 and 29-35) in water or sodium dodecyl sulphate (SDS) micelles which provide a water membrane interface mimicking environment and that $Aβ_{(1-42)}$ adopts an unfolded structure including two β-strands (residues 17-21 and 31-36) in aqueous solution [17, 18]. Using NMR, it has also been shown that an $Aβ_{(1-42)}$ fibril is a β-sheet composed of two β-strands (18-26 and 31-42) [19]. This structure of Aβ is the unfolded elongated β-strand that departs from the membrane structure, (α-helical) region like forms and that the β-strand of Aβ enable the formation of β-sheets of fibrils and prefibrillar aggregates [20, 21]. Recent experimental studies demonstrated that trapping Aβ in a state similar to its native structure by stabilizing the Aβ central helix (residues 15-24) is an effective strategy to reduce Aβ polymerization and Aβ toxicity [22, 23]. The 3D structure of a disease-relevant $Aβ_{(1-42)}$ fibril polymorph has also been deciphered [24]. The 3D structure is composed of two molecules per fibril layer, forming a double-horseshoe–like cross–a β-sheet entity with maximally buried hydrophobic side chains.

Residues 1–14 are partially ordered and in a β-strand conformation [24]. The actual secondary structure of $Aβ_{(1-42)}$ have been determined it is about, 45% in two α-helical forms (α1: residues from 11–24, α2: residues from 27–33) and are joined by a flexible hinge.

In the present study, the effect of the test compound NIAID screened from PubChem library on the unfolding process of the Aβ central helix (11-24) was investigated by Docking and Molecular Dynamic (MD) simulations. The difference between native-type Aβ with and without ligand has been studied during MD simulation, and here we demonstrated that the given ligand is effective in stabilizing the Aβ central helix. The NIAID compound was finally identified as potential lead molecule based on stabilizing the Aβ central helix.

This compounds can be easily synthesized and have structural novelty, which allows for further examination of their abilities to inhibit Aβ confirmation through *in-vitro* and *in-vivo* biological tests and will act as potential agents to treat Alzheimer's disease.

MATERIALS AND METHODS: The α-helical form of Aβ peptide (14–23 residues) provides a suitable target for stabilizing ligands. The Peptide $Aβ_{(1-42)}$ (1ZOQ) has retrieved from PDB, which has Resolution of 2.37 Å, R-Value of 0.214 (obs.), R-Free energy of 0.226. For possible interaction of human Aβ, we concentrated on 2 partial surfaces of this Aβ helix. One surface of the helix is largely hydrophobic and adjacent to Glu-22 and Asp-23. A compound conjugated with hydrocarbon chain could potentially interact with this surface. Also in Aβ helix adjacent to Glu-22 and Asp-23 is a surface containing Phe-20, which connects these residues with Lys-16 and His-14. To investigate the concept, we have evaluated possible chemical interactions of Aβ peptide by 350 test compounds that could be initial potential ligands for these surfaces, all compounds were retrieved from PubChem. The potential test compounds and the Aβ peptide were submitted to AutoDocking [25].

Finally, docked complex of each test compounds and Aβ peptide was used to do further analysis and molecular dynamics simulation. In AutoDock analysis, we have selected 30 test compounds, on the bases

of their lowest binding energy score and interaction with Aβ middle amino acids residues (E11VHHQKLVFFAEDVGS26). The selected test compounds are given in **Table 1**.

Molecular Dynamic Simulation: The entire MD simulation was performed using the Macro model package from the Schrodinger Program. The force-field parameters for the ligand and protein were picked from the AMBER force field. For $Aβ_{(1-42)}$ stochastic dynamic method is used. The SHAKE algorithm was applied to fix all covalent bonds containing a hydrogen atom allowing a 1.5fs time step to be used in the integration of Newton's equation. To observe the structural changes of Aβ quantitatively after MD simulation, the root means– square deviation (RMSD) and deviation of Torsion angle were calculated. The RMSD is calculated for backbone heavy atoms against the time coordinates. Therefore, the simulation for each system was performed at 300-330K to accelerate the dynamics of Aβ. RMSD analyses were carried out by using the trajectories obtained by MD simulations at 320K for native $Aβ_{(1-42)}$ and simulations analyses for $Aβ_{(1-42)}$ with the test compounds was carried out for the trajectories obtained up to 20ns at 320K. The data of every 2ns of the trajectories after the heating time of the MD simulations were used for the revelation of the structural changes of Aβ with and without test compounds during simulations was carried out by using the visual molecular dynamics (VMD) Software. The test compound NIAID (PubChem SID = 24721112) was the only ligand which retains the native α-helical conformation of $Aβ_{(1-42)}$ during MD simulation upto 20ns at 320K while as other test compounds have failed to retain the Aβ in alpha helical conformation. To discriminate the type or pattern in the Aβ structure, the number of α-helical backbone hydrogen bonds (αHB) in the middle region (11-24) was calculated, using the criterion acceptor hydrogen ≤ 2.4A˚ to define the existence of hydrogen.

Result: Targeting the Aβ central α- helix (15-24 residues) is an effective strategy to reduce Aβ polymerization and toxicity. In AutoDock analysis test compound with lowest binding energy and interaction with Aβ middle amino acids residues (E11VHHQKLVFFAEDVGS26) was criteria to predict the favored compounds for dynamic molecular studies. The Docking simulation interactions of $Aβ_{(1-42)}$ with test compounds are given in **Table 1**.

The docking studies of various test compounds to the fragments of Aβ reveal that the stretch of residues from K16, to F20 broadly interact with the compounds and interestingly they are the key coordinating residues that are proposed to be responsible for conversion of Aβ alpha-helix into beta-sheet events. Among the entire test compounds, NIAID exhibits a favored binding to $Aβ_{(1-42)}$ segment and have shown the highest docking-score ≥ -14 Kcal/mol. The docking interaction analysis showed NIAID is interacting with the middle amino acid residues of $Aβ_{(1-42)}$ (K16L17V18F19 F20D23). The average numbers of hydrophobic interaction with Aβ might have increased the binding affinity of NIAID test compound with Aβ interfaces.

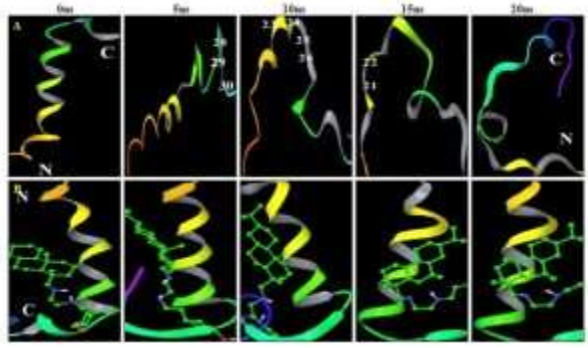

FIG. 1: STRUCTURAL DISTORTIONS IN THE Aβ(1-42) PROTEIN AT DIFFERENT TIME INTERVALS DURING THE MD SIMULATION: A) NATIVE AB(1-42), B) Aβ(1-42) WITH NIAID. FIGURE A SHOWS THE UNFOLDING OF HELICAL Aβ WHEREAS SUCH EVENT IS ABSENT IN FIGURE B

The further signify the docking interaction of diverse test compounds; these could be exploited as competitive inhibitors to facilitate the prevention of AD events. The AutoDock built complexes between Aβ (1-42), and the test compounds were submitted to MD simulations. The MD simulation of Aβ (1-42) with and without test compounds up to 20ns was analyzed to understand the structural distortion of Aβ (1-42) during the simulation.

From a thousand trajectories we have mentioned here five trajectories of different nano-seconds (0, 5, 10, 15, and 20). The MD simulations of Aβ(1-42) with and without test compound were performed to analyze the structural alteration of native Aβ(1-42) are shown in **Fig. 1A**. The structure of these trajectories showed the native Aβ central helix unfolded during the simulation at different nano-seconds.

Simulations structure at different periods showed that the central α-helix of Aβ(1-42) is completely unfolded without the ligand up to 20ns at 300K **Fig. 1A**. The amino acid K28G29A30 and E22D23V24G25S26 in the middle region of Aβ(1-42) are relatively disordered and converted into β-sheet at 5ns and 10ns respectively. MD simulation of Aβ(1-42) peptide with NIAID compounds was performed up to 20ns at 320K showed Aβ peptide retains its α-helix form The NIAID was only tested compound which retained the Aβ native confirmation **Fig. 1B**. During MD simulations, the secondary structures adopted or retained by Aβ with and without ligand are shown in **Fig. 2**.

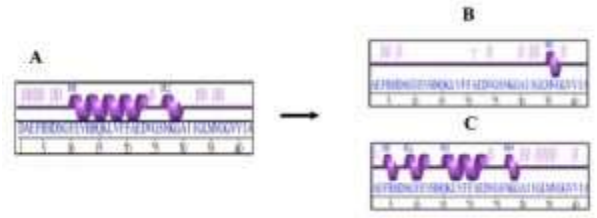

FIG. 2: SECONDARY STRUCTURES ADOPTED BY Aβ(1-42) PROTEIN INITIAL (A) AND FINAL (B-C) DURING A 20ns MD SIMULATION WITH AND WITHOUT THE LIGANDS A: NATIVE Aβ(1-42) at 0ns, B: NATIVE Aβ(1-42) at 20ns IN ABSENCE OF LIGAND AND C: Aβ(1-42) at 20ns IN PRESENCE OF NIAID

It is proportionately illustrated that the stability of Aβ central α-helix is retained by NIAID. Starting with Aβ(1-42) in α-helical conformation, Aβ alone starts to unfold and lose its helical structure during the simulation, were in the presence of NIAID the α-helical conformation of Aβ is retained or refolded after partial unfold at the end of the simulations. This is postulated to roll and stabilize the Aβ central α- helix. MD simulation result analysis favored the identification of NIAID compounds as the best inhibitors for the conversion of α-helix of the Amyloid peptide into β-sheet conformation. The NIAID 2D chemical structure is shown in **Fig. 3**.

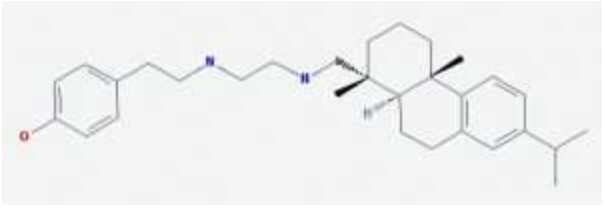

FIG. 3: THE 2D STRUCTURE OF THE TEST COMPOUND NIAID

To examine the structural change of Aβ quantitatively at different nano-seconds during simulation, the RMSD was calculated. The RMSD was calculated for heavy backbone atoms against the initial energy-minimized coordinates calculated for all atoms along the MD simulation time. During MD simulation of native Aβ (without ligand) we have analyzed that the central helix of Aβ is completely unfolded up to 20ns at 300K. The Mean RMSD value of native Aβ at 300K increased from 2ns to 10 ns and showed there is large instability in the structure of native Aβ α-helix after 5ns and changed completely into β-sheet up to 10ns. The Aβ without ligand showed central α-helix becomes fully extended and more flexible at the end of simulations. RMSD of native Aβ is shown in **Fig. 4A**. It has been analyzed that the average backbone RMSD of the middle region and the average number of alpha-helix hydrogen bonds (αHBs) showed fluctuation in the backbone. In the presence of NIAID backbone, RMSD of the Aβ up to 20ns was relatively stable during simulations, RMSD was small in every trajectory, and Aβ α-helix was relatively stable during simulation **Fig. 4B**.

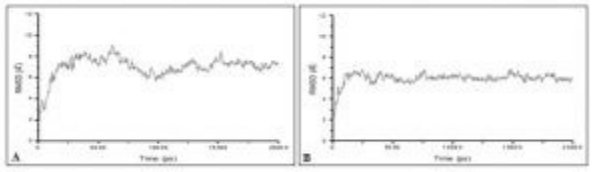

FIG. 4: RMSD PLOT SHOWING THE STRUCTURAL CHANGES IN Aβ DURING MD SIMULATION. A) NATIVE $Aβ_{42}$ IS HIGHLY UNSTABLE; B) $Aβ_{42}$ WITH NIAID BECOMES STABLE AFTER 5ns

To examine whether the Aβ central helix eventually unfolded by the end of the simulations, an average number of the αHBs of the Aβ middle region (residues 15-30) was calculated for the last 2ns of the 20ns simulation. The analysis showed the fluctuation of the Aβ backbone RMSD is relatively small in every trajectory. The trajectories were classified into three groups shown in **Table 2** group A (RMSD < 2.0A˚, 2 ≤ αHB ≤ 6), group B (2.0 A˚≤ RMSD < 4.0A˚, 1 ≤ αHB ≤ 4) group C (RMSD ≥2.0 A˚, αHB = 0). On diagram inspection, in group A trajectories it was ascertained that the Aβ central α-helix maintained its helical conformation during the whole simulation or refolded after partial unfolding by the end of the simulations, in group B trajectories partially unfolded at the end of the simulations, and in group C trajectories completely unfolded at the end of the simulations. The helical Aβ (group A) is observed in only two trajectories in the absence of a ligand up to 20ns, whereas it is observed in six trajectories in the presence of NIAID. In contrast, the completely unfolded Aβ (group C) is observed in three trajectories in the absence of a ligand, whereas it is not observed in any trajectory in the present compound. By analyzing the backbone RMSD of the whole simulation of each trajectory, it was found that Aβ helix was relatively stable during simulation in all trajectories in the presence of NIAID. This result indicates that the addition of NIAID compound is effective in stabilizing the Aβ central helix.

To inspect whether the ligand was in contact with $Aβ_{(1-42)}$ during MD simulation, the contact maps at different time scale during simulation were analyzed by visual assessment of the trajectories from 2ns to 20ns. All contact maps up to 20ns show ≥ 1 hydrogen bond interaction between $Aβ_{(1-42)}$ peptide and NIAID compound. We found that $Aβ_{(1-42)}$ and NIAID are quite flexible during simulation and forms hydrogen-bond interaction shown in **Fig. 5**. We have mentioned only three trajectories (10, 15, and 20 ns) among a thousand trajectories. The contact maps showed bonds between two basic functional groups (N1and N2) of NIAID compound with Aβ acidic amino acid residues (E22 and D23). We have analyzed the contacts between the acidic functional groups (O1) of given compounds and the basic residues (H14, K16, and K34)

of Aβ middle region and conjugate aromatic hydrocarbon region of NIAID compound contacts with Aβ middle non-polar part localized from L17 to V24. The yellow doted lines in **Fig. 5** are hydrogen-bonds confirm that ligand binds with Aβ during the simulation. The amino acid residues of $Aβ_{(1-42)}$ which takes part in hydrogen bonding are shown in **Table 3**. It is predicted that hydrogen bonds between hydroxyl group of amino acid K16, F19 and D23 bring the test compound and peptide closer. The hydrophobic properties of Aβ amino acids (residues K16L17V18F19F20A21 and V24) are responsible for its unfolding and aggregation. Analysis of simulation trajectories reveals that NIAID compounds forms the hydrogen bond with the respective amino acids (residue16-24) and prevents them to change the α-helix into β-sheet.

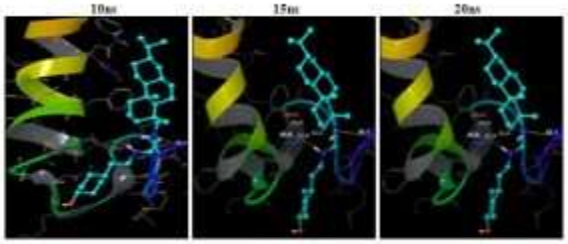

FIG. 5: THE RESIDUES INVOLVED IN THE INTRA-MOLECULAR HYDROGEN BOND FORMATION IN THE MIDDLE REGION OF $Aβ_{42}$ (10-30 RESIDUES) DURING A 20ns MD SIMULATIONS. HYDROGEN BONDING PATTERNS IN $Aβ_{42}$ WITH NIAID

Torsion angle value was also calculated to observe the alteration of Aβ structure quantitatively during the simulation. The Psi ψ) and Phi (φ) torsion angles as degree freedom provide an efficient way to study the conformations of simulated proteins. The online software ProCheck was used to plot the Ramachandran-graph of native $Aβ_{(1-42)}$ at 0ns **Fig. 6A** which showed maximum amino acids residues of Aβ are in α-helical conformation only a few of the amino acids residues are in β-sheet. After simulation of native Aβ up to 20ns, amino acids in β-sheet conformation has increased whereas amino acid in α-helical conformation has decreased.

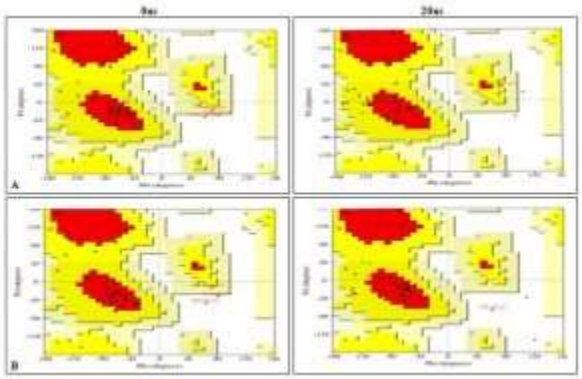

FIG. 6: RAMACHANDRAN PLOT OF THE ψ AND φ DISTRIBUTION PRODUCED BY PROCHECK AFTER MD SIMULATION [A, B, L] MOST FAVOURED REGION [a, b, l, p] ADDITIONAL ALLOWED REGION, [~a, b, ~l, ~ p] GENEROUSLY ALLOWED REGIONS; WHITE ARE DISALLOWED REGIONS. A) NATIVE $Aβ_{42}$ AT 0ns AND 20ns, B) $Aβ_{42}$ COMPLEXES WITH NIAID AT 0ns AND 20ns

The Ramachandran-plot of $Aβ_{(1-42)}$ in the presence of NIAID compound **Fig. 6B** showed after MD simulation only 5 amino acid of Aβ has changed into β-sheet while remaining amino acid of Aβ are in α-helical conformation. In native Aβ, there is a fundamental difference between initial and final torsion angles values after simulations. The difference in torsion angle values is conformation variation in the core structure of Aβ

during simulations. During dynamics, NIAID compound has prevented much deviation in Aβ angle values and not allowed the Aβ middle region to unfold and change into β –sheet.

DISCUSSION AND CONCLUSION: The amino acid sequence of $Aβ_{(11-24)}$ has a strong preference for β-strand structure. The α-helical form of this region of the Aβ peptide provides a suitable target for stabilizing by ligands. The residue of human Aβ E11VHHQKLVFFAEDV24, including the discordant regions is in α-helical conformation [26]. The evaluated test ligand NIAID shows hydrogen and hydrophobic chemical interactions with $Aβ_{(1-42)}$ amino acid residues which are responsible for the α-helical conversion into β-sheet. The α-helical propensity of the five amino acid residues (K16L17V18F19 and F20) is the dominating factor for the stability of the Aβ central helix during the molecular dynamics simulation.

The effect of the ligand on the stability of the $Aβ_{(1-42)}$ central helix (residue 11-24) was investigated by using the MD simulations. Detailed information on the structural changes upon loss of helicity in the presence and absence of the ligand was examined.

The Molecular dynamics results signify that the addition of given ligand is efficient in stabilizing the Aβ central helix. The investigation also showed that NIAID compounds bind to the highly elongated form of Aβ and was found to be proficient at forming parallel conformations with Aβ. Thus binding of the test compound to $Aβ_{(1-42)}$ was found to agitate the extension of β-sheet. The MD simulation in presence and absence of the test compounds up to 20ns showed how amino acid of native Aβ loses the helicity at different nano-seconds and also shows test compound NIAID retains the helicity of these amino acids during dynamics.

As indicated mainly by the Aβ backbone RMSD *vs.* initial structure and by the existence of αHB's of the Aβ. RMSD graph shows fluctuations of the native Aβ backbone, RMSD is relatively small in every trajectory, which infers protein is losing its conformation slowly. The Aβ central helix completely unfolded at the end of the simulation without test compound NIAID, whereas remains folded in the presence of given NIAID by the end of the simulation.

Compared to Aβ alone, the probability of the α -helical state for Aβ during simulations is higher in presence given test compound, and also stability of the Aβ central α-helix was retained. RMSD, αHBs and HB interaction between Aβ and test compound data thus indicate the ability of the compound to stabilize the Aβ central helix. Stabilization of the Aβ central α- helix is an effective step in preventing Aβ polymerization. It has been suggested that complete unfolding occurs *via* three steps mechanism: Sufficient loss of α-helical backbone hydrogen bonds (αHBs), strong interaction between non-polar side chains and strong interaction between polar side chains [27, 28, 29]. Thus, we suggest that test compound NIAID prevent the unfolding of Aβ helix by preventing the breakage of the αHBs and but also disturbing the interactions between polar side chains and between nonpolar side chains.

To understand the polar interactions, the existence of hydrogen bonding between NIAID and Aβ was analyzed. In overall ligand formed as a minimum as one HB with Aβ and the main reason for this is NIAID ligand has several acidic and basic functional groups which can interact to the acidic and basic residues of Aβ respectively. The intermolecular interactions between the Aβ polar residues and the NIAID polar functional groups are significant in stabilizing the Aβ central helix because they can prevent intra-molecular interactions between the Aβ polar residues that stimulate absolute unfolding of the Aβ central helix. Also, benzene-ring of NIAID straddles the Aβ middle non-polar part (residue 17-21). The inter-molecular interactions between the Aβ middle non-polar part and the ligand non-polar part are important in stabilizing the Aβ central helix since the Aβ middle non-polar part includes three non-polar residues (VFF) that have low α-helical propensity and high β-strand propensity [30, 31]. Therefore test compounds NIAID by establishing

hydrophobic interaction with Aβ stabilize its native formation. Thus could act as a lead molecule to be developed as a drug against AD in preventing fibril formation.

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CONFLICTS OF INTEREST: This article does not contain any studies about human participants or any other animals. The authors R. Boopathy and Dilawar Mir declare that they have no conflict of interest concerning Human and Animal Rights and Informed Consent.

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