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## EVALUATION OF NOVEL LIGAND FOR THE MAINTENANCE OF NATIVE A $\beta$ <sub>(1-42)</sub> 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 (A $\beta$ ) in senile plaques, the appearance of intracellular neurofibrillary tangles. The A $\beta$  peptide is produced by sequential cleavage of the Amyloid precursor protein (APP) by  $\alpha$ ,  $\beta$ , and  $\gamma$ -secretase. The secretase  $\beta$  and  $\gamma$  generate a number of isoforms of peptides containing 36-43 amino acid residues in length. The mainly universal isoforms are A $\beta$ <sub>(1-40)</sub> and A $\beta$ <sub>(1-42)</sub>. A $\beta$ <sub>(1-42)</sub> C-terminal domain (29-42 amino acid residues) adopts  $\beta$  conformation, and the N-terminal domain (10-24 amino acid residues) facilitates a dynamic equilibrium between  $\alpha$ -helix and  $\beta$ -strand. Thus A $\beta$  polymerization requires unfolding of the native  $\alpha$ -helical structure of A $\beta$ . Stabilization of the A $\beta$  central  $\alpha$ -helix is an efficient step to foil the A $\beta$  polymerization. Here we report test compounds which to bind and stabilize the 11-30 amino acid regions of A $\beta$ <sub>(1-42)</sub> in  $\alpha$ -helical conformation. On docking, the ligands with A $\beta$  peptide followed by Molecular dynamics simulation for 20ns favored the identification of test compound NIAID. That postulated to bind and stabilize the A $\beta$  central  $\alpha$ -helix. So stabilization of A $\beta$  secondary structure with a ligand that maintains the  $\alpha$ -helical conformation may provide clues in developing drugs to control the A $\beta$  deposition observed in AD patients.

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

The some of the A $\beta$  peptides are an integral membrane protein, called the amyloid precursor protein (A $\beta$ PP), cleaved predominantly into elongated 40-residue peptide (A $\beta$ <sub>1-40</sub>).

Also, a C-terminally elongated 42-residue version which also can be excised (A $\beta$ <sub>1-42</sub>) and this longer variant is the main constituent of parenchymal amyloid deposits<sup>6, 7</sup>. The link between A $\beta$  aggregation and AD implies that any inhibitors of their aggregation should be able to slow down disease progression. Several low molecular mass A $\beta$  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,

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<p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5489-98">http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5489-98</a></p>	

Curcumine, Inositol, Melatonin, D737, and Dobutamine<sup>8,9,10</sup>. These inhibitors are predicted to bind to A $\beta$  in an elongated  $\beta$ -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<sup>11, 12, 13</sup>. No A $\beta$  oligomers have been structurally determined, and thus these oligomers cannot yet be targeted by rational design. A further problem with some of the A $\beta$  polymerization inhibitors is that they can also act as aggregators<sup>14</sup>. 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<sup>14, 15</sup> and also a library of peptide inhibitor candidates have formed which systematically are mutating the RGTFFGKF amino acid residues<sup>16</sup>. In light of these circumstances, an alternative strategy to reduce A $\beta$  aggregation and toxicity is warranted. One possible approach, explored herein, is to trap A $\beta$  in a state similar to its perceived structure in membrane-embedded A $\beta$ PP, by targeting the discordant  $\alpha$ -helix region.

Nuclear magnetic resonance (NMR) data showed that A $\beta$ <sub>(1-40)</sub> adopts a folded structure including two  $\alpha$ -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 A $\beta$ <sub>(1-42)</sub> adopts an unfolded structure including two  $\beta$ -strands (residues 17-21 and 31-36) in aqueous solution<sup>17, 18</sup>. Using NMR, it has also been shown that an A $\beta$ <sub>(1-42)</sub> fibril is a  $\beta$ -sheet composed of two  $\beta$ -strands (18-26 and 31-42)<sup>19</sup>. This structure of A $\beta$  is the unfolded elongated  $\beta$ -strand that departs from the membrane structure, ( $\alpha$ -helical) region like forms and that the  $\beta$ -strand of A $\beta$  enable the formation of  $\beta$ -sheets of fibrils and prefibrillar aggregates<sup>20, 21</sup>. Recent experimental studies demonstrated that trapping A $\beta$  in a state similar to its native structure by stabilizing the A $\beta$  central helix (residues 15-24) is an effective strategy to reduce A $\beta$  polymerization and A $\beta$  toxicity<sup>22, 23</sup>. The 3D structure of a disease-relevant A $\beta$ <sub>(1-42)</sub> fibril polymorph has also been deciphered<sup>24</sup>. The 3D structure is composed of two molecules per fibril layer, forming a double-horseshoe-like cross- $\beta$ -sheet entity with maximally buried hydrophobic side chains.

Residues 1–14 are partially ordered and in a  $\beta$ -strand conformation<sup>24</sup>. The actual secondary structure of A $\beta$ <sub>(1-42)</sub> have been determined it is about, 45% in two  $\alpha$ -helical forms ( $\alpha$ 1: residues from 11–24,  $\alpha$ 2: 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 A $\beta$  central helix (11-24) was investigated by Docking and Molecular Dynamic (MD) simulations. The difference between native-type A $\beta$  with and without ligand has been studied during MD simulation, and here we demonstrated that the given ligand is effective in stabilizing the A $\beta$  central helix. The NIAID compound was finally identified as potential lead molecule based on stabilizing the A $\beta$  central helix.

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

**MATERIALS AND METHODS:** The  $\alpha$ -helical form of A $\beta$  peptide (14–23 residues) provides a suitable target for stabilizing ligands. The Peptide A $\beta$ <sub>(1-42)</sub> (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 A $\beta$ , we concentrated on 2 partial surfaces of this A $\beta$  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 A $\beta$  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 A $\beta$  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 A $\beta$  peptide were submitted to AutoDocking<sup>25</sup>.

Finally, docked complex of each test compounds and A $\beta$  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 A $\beta$  middle amino acids residues (E11VHHQKLVFFAEDVGS26). The selected test compounds are given in **Table 1**.

**TABLE 1: INTERACTION ANALYSIS OF TEST COMPOUNDS WITH A $\beta$ 1-42. THE COMPOUNDS WERE INDEPENDENTLY DOCKED ONTO A $\beta$ 1-42 USING AUTODOCK. THE 2D STRUCTURE OF THE TEST COMPOUNDS ALONG WITH THEIR BINDING ENERGY AND RESIDUES OF A $\beta$ 1-42 PARTICIPATING IN THE H-BONDING AND HYDROPHOBIC INTERACTION ARE SHOWN IN TABLE. THE COMPOUNDS WERE RETRIEVED FROM PUBCHEM. RESIDUES OF A $\beta$ 1-42 INVOLVED IN THE BINDING OF THESE TEST COMPOUNDS ARE GIVEN ACCORDING TO THE ASCENDING ORDER OF BINDING ENERGY**

Name of Compounds	Residues of A $\beta$ <sub>(1-42)</sub> involved in the interaction with test compounds		Binding Energy (Kcal/mol)
	Hydrogen Bonds	Hydrophobic interactions	
24721112	K16, F19, D23	V12, Q15, F20, V24, N27, A30, I32, G33, V36	-14.4
11313	H14, F20	V12, H13, Q15, K16, F19, D23, V24, N27, A30, I32	-10
688035	K16, G33	Q15, F19, F20, V24	-8.7
3708931	D23, I32	Q15, F19, E22, G29, A30, G33, L34	-8.1
8786203	H14, F19	D23, N27, G29, A30, I31, G33,	-7.5
552906	K16, F20	Q15, F19, D23, V24, N27, G29, A30	-7.2
62770	F19	G15, K16, D23, N27, G29, A30I32, G33, L34	-7.0
4534	V12, D23	H13, Q15, K16, F19, F20	-7.1
91895552	F19, D23	V12, Q15, K16, V24, N27, A30, I32, G33	-7.0
2585	G15	K16, F19, F20, G22, D23, A30, I32, G33	-6.9
5757	D23	L17, F19, V24, A30, G33	-6.7
4534	V12, H13, D23	G15, K16, F19, F20	-6.1
9990	V12, K16	Q15, L17, F19, F20, D23, A30	-6.0
15818601	D23, G29	Q15, K16, F19, F20	-6.7
896	Q15, K16, F19, F20, D23	H14, L17, A30, I32, G33	-6.1
181139	H14, D23	G29, A30, I32, G33	-5.8
128419	K16, F20, D23, I32	F19, D23, V24, A30	-5.5
3018172	K16, F19	H13, G15, A30	-5.3
8786203	H13, H14, L17	Q15, F19, F20, V24, G29, A30, G33	-5.2
3853025	H13, H14, D23, I32	Y10, K16	-5.0
681	V12, H13, L16, D23	Q15, L17, F19, F20, A30, I32, G38	-5.0
23043846	H14, F19	Q15, V24, A30, G33	-5.0
5381226	H13, D23	H14, L17, A30, I32, G33	-5.0
638015	G15, D23	V12, K16, F20, L17, F19, V24	-5.0
5281672	Q15, K16, F20, D23	F19, F20, V24, N27, A30, I32	-5.0
55290627	Q15, D23, I32	K16, F19, F20	-5.0
54267694	V12, D23	F19, F20	-5.0
46856272	L16, D23	F19, V24, A30	-5.0
18347908	V12, K16	V24, DN27, A30	-4.9

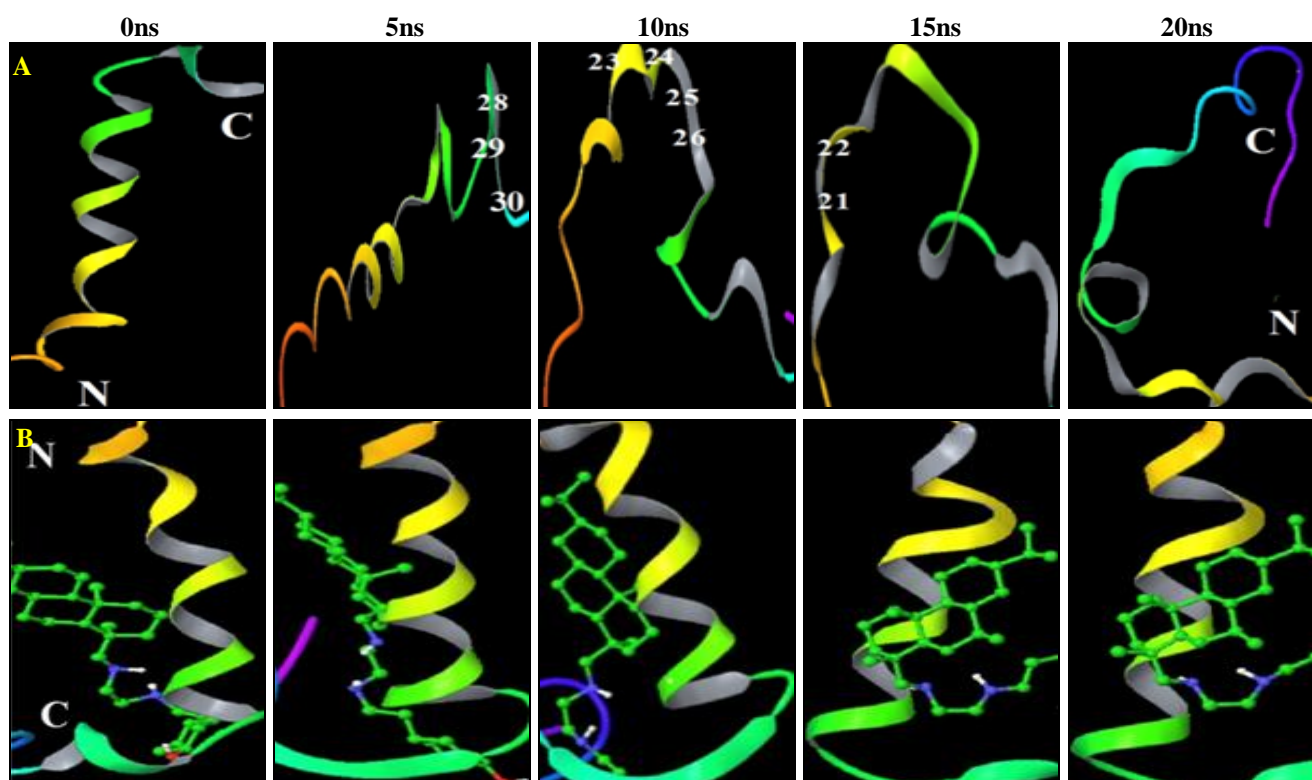
**Molecular Dynamic Simulation:** The entire MD simulation was performed using the Macro model package from the Schrodinger Program. The force-field parameters for the ligand and protein were picked from the AMBER force field. For A $\beta$ <sub>(1-42)</sub> stochastic dynamic method is used. The SHAKE 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 A $\beta$  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 A $\beta$ . RMSD analyses were carried out by using the trajectories obtained by MD simulations at 320K for native A $\beta$ <sub>(1-42)</sub> and simulations analyses for A $\beta$ <sub>(1-42)</sub> 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 A $\beta$  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  $\alpha$ -helical conformation of A $\beta$ <sub>(1-42)</sub> during MD simulation upto 20ns at 320K while as other test compounds have failed to retain the A $\beta$  in

alpha helical conformation. To discriminate the type or pattern in the A $\beta$  structure, the number of  $\alpha$ -helical backbone hydrogen bonds ( $\alpha$ HB) in the middle region (11-24) was calculated, using the criterion acceptor hydrogen  $\leq 2.4\text{\AA}$  to define the existence of hydrogen.

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

The docking studies of various test compounds to the fragments of A $\beta$  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 A $\beta$  alpha-helix into beta-sheet events. Among the entire test compounds, NIAID exhibits a favored binding to A $\beta_{(1-42)}$  segment and have shown the highest docking-score  $\geq -14$  Kcal/mol. The docking interaction analysis showed NIAID is interacting with the middle amino acid residues of A $\beta_{(1-42)}$  (K16L17V18F19 F20D23). The average numbers of hydrophobic interaction with A $\beta$  might have increased the binding affinity of NIAID test compound with A $\beta$  interfaces.

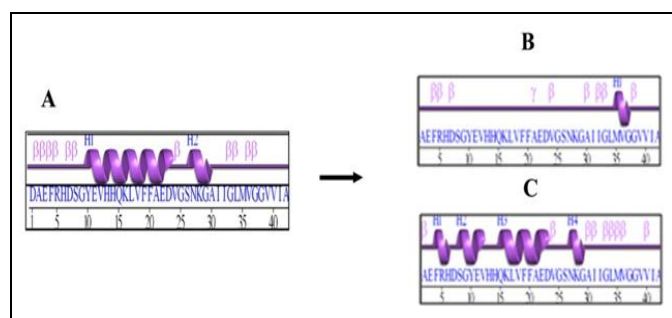


**FIG. 1: STRUCTURAL DISTORTIONS IN THE A $\beta_{(1-42)}$  PROTEIN AT DIFFERENT TIME INTERVALS DURING THE MD SIMULATION: A) NATIVE A $\beta_{(1-42)}$ , B) A $\beta_{(1-42)}$  WITH NIAID. FIGURE A SHOWS THE UNFOLDING OF HELICAL A $\beta$  WHEREAS SUCH EVENT IS ABSENT IN FIGURE B**

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 A $\beta_{(1-42)}$ , and the test compounds were submitted to MD simulations. The MD simulation of A $\beta_{(1-42)}$  with and without test compounds up to 20ns was analyzed to understand the structural distortion of A $\beta_{(1-42)}$  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 A $\beta_{(1-42)}$  with and without test compound were performed to analyze the structural alteration of native A $\beta_{(1-42)}$  are shown in **Fig. 1A**. The structure of these trajectories showed the native A $\beta$  central helix unfolded during the simulation at different nano-seconds.

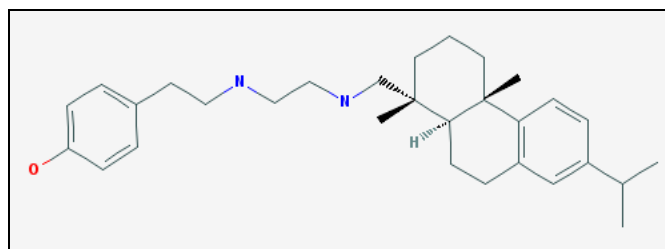
Simulations structure at different periods showed that the central  $\alpha$ -helix of  $A\beta_{(1-42)}$  is completely unfolded without the ligand up to 20ns at 300K **Fig. 1A**. The amino acid K28G29A30 and E22D23V24G25S26 in the middle region of  $A\beta_{(1-42)}$  are relatively disordered and converted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



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

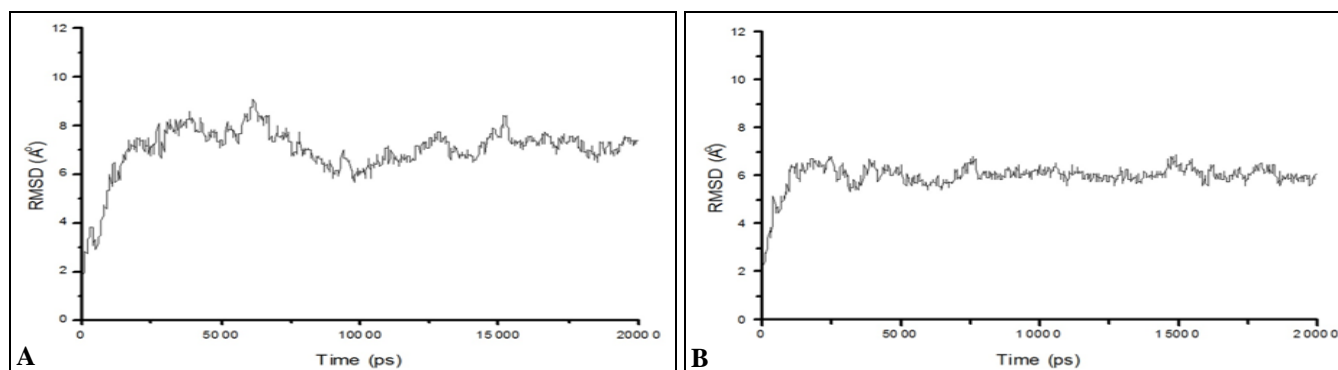
It is proportionately illustrated that the stability of  $A\beta$  central  $\alpha$ -helix is retained by NIAID. Starting with  $A\beta_{(1-42)}$  in  $\alpha$ -helical conformation,  $A\beta$  alone starts to unfold and lose its helical structure during the simulation, were in the presence of NIAID the  $\alpha$ -helical conformation of  $A\beta$  is retained or refolded after partial unfold at the end of the simulations. This is postulated to roll and stabilize the  $A\beta$  central  $\alpha$ -helix. MD simulation result analysis favored the identification of NIAID compounds as

the best inhibitors for the conversion of  $\alpha$ -helix of the Amyloid peptide into  $\beta$ -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  $A\beta$  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  $A\beta$  (without ligand) we have analyzed that the central helix of  $A\beta$  is completely unfolded up to 20ns at 300K. The Mean RMSD value of native  $A\beta$  at 300K increased from 2ns to 10 ns and showed there is large instability in the structure of native  $A\beta$   $\alpha$ -helix after 5ns and changed completely into  $\beta$ -sheet up to 10ns. The  $A\beta$  without ligand showed central  $\alpha$ -helix becomes fully extended and more flexible at the end of simulations. RMSD of native  $A\beta$  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 ( $\alpha$ HBs) showed fluctuation in the backbone. In the presence of NIAID backbone, RMSD of the  $A\beta$  up to 20ns was relatively stable during simulations, RMSD was small in every trajectory, and  $A\beta$   $\alpha$ -helix was relatively stable during simulation **Fig. 4B**.



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

To examine whether the A $\beta$  central helix eventually unfolded by the end of the simulations, an average number of the  $\alpha$ HBs of the A $\beta$  middle region (residues 15-30) was calculated for the last 2ns of the 20ns simulation. The analysis showed the fluctuation of the A $\beta$  backbone RMSD is relatively small in every trajectory. The trajectories were classified into three groups shown in **Table 2** group A (RMSD < 2.0Å, 2  $\leq$   $\alpha$ HB  $\leq$  6), group B (2.0 Å  $\leq$  RMSD < 4.0Å, 1  $\leq$   $\alpha$ HB  $\leq$  4) group C (RMSD  $\geq$  2.0 Å,  $\alpha$ HB = 0). On diagram inspection, in group A trajectories it was ascertained that the A $\beta$  central  $\alpha$ -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

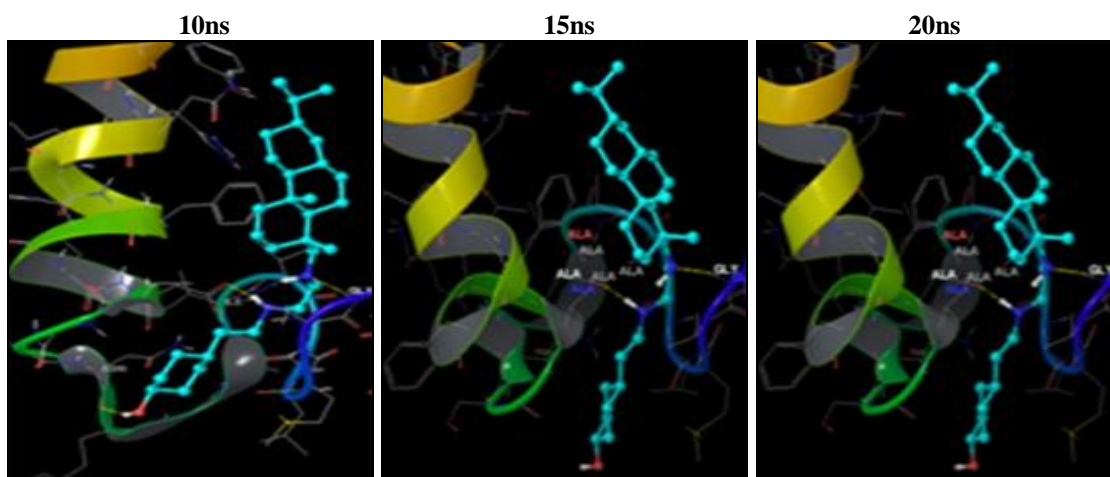
unfolded at the end of the simulations, and in group C trajectories completely unfolded at the end of the simulations. The helical A $\beta$  (group A) is observed in only two trajectories in the absence of a ligand up to 20ns, whereas it is observed in six trajectories in the presence of NIAID. In contrast, the completely unfolded A $\beta$  (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 A $\beta$  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 A $\beta$  central helix.

**TABLE 2: AVERAGE RMSD (Å) AND AVERAGE NUMBER OF INTRA-MOLECULAR HYDROGEN BONDS DURING LAST 2ns OF 20ns MD SIMULATIONS WAS CALCULATED FOR THE AB MIDDLE REGION (RESIDUES 15-30) IN THE ABSENCE AND PRESENCE OF THE NIAID COMPOUND. THE TRAJECTORIES ARE CLASSIFIED INTO THREE GROUPS: GROUP A (RMSD < 2.0 Å, 2  $\leq$   $\alpha$ HB  $\leq$  6), GROUP B (2.0 Å  $\leq$  RMSD < 4.0 Å, 1  $\leq$   $\alpha$ HB  $\leq$  4) GROUP C (RMSD  $\geq$  2.0 Å,  $\alpha$ HB = 0)**

Time (ns)	Average RMSD ( Å )		Average number of hydrogen bonds in the $\alpha$ -helix of A $\beta$		Group	
	A $\beta$	A $\beta$ + NIAID	A $\beta$	A $\beta$ + NIAID	A $\beta$	A $\beta$ + NIAID
18.2	2.30	0.95	2.4	4.2	B	A
18.4	2.69	1.53	2.8	3.9	B	A
18.6	2.75	0.81	0.6	3.5	C	A
18.8	1.40	1.85	1.1	2.7	A	B
19.0	1.80	1.46	1.2	4.2	A	A
19.2	5.30	2.21	2.2	4.8	B	B
19.4	5.10	1.36	0.5	3.7	C	A
19.6	3.13	2.12	3.75	4.1	B	B
19.8	4.82	3.15	1.46	3.4	B	B
20.0	5.30	1.49	0.5	3.8	C	A
Mean Value	3.23	1.7	1.65	3.83	-	-
S.D	1.25	0.68	1.09	0.56	-	-

To inspect whether the ligand was in contact with A $\beta$ <sub>(1-42)</sub> 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  $\geq$  1 hydrogen bond interaction between A $\beta$ <sub>(1-42)</sub> peptide and NIAID compound. We found that A $\beta$ <sub>(1-42)</sub> 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 (N1 and N2) of NIAID compound with A $\beta$  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,

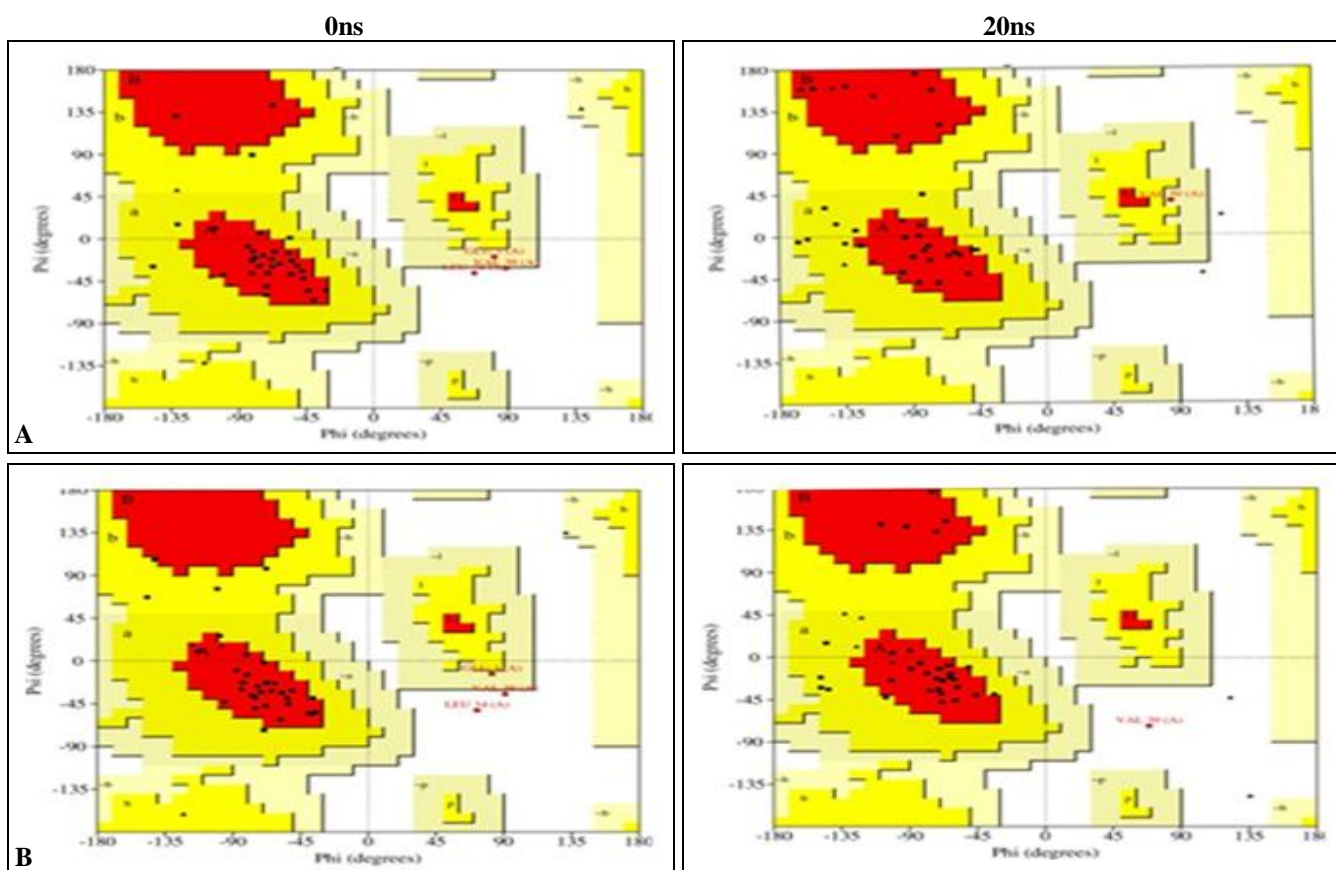
K16, and K34) of A $\beta$  middle region and conjugate aromatic hydrocarbon region of NIAID compound contacts with A $\beta$  middle non-polar part localized from L17 to V24. The yellow dotted lines in **Fig. 5** are hydrogen-bonds confirm that ligand binds with A $\beta$  during the simulation. The amino acid residues of A $\beta$ <sub>(1-42)</sub> 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 A $\beta$  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  $\alpha$ -helix into  $\beta$ -sheet.



**FIG. 5: THE RESIDUES INVOLVED IN THE INTRA-MOLECULAR HYDROGEN BOND FORMATION IN THE MIDDLE REGION OF Aβ<sub>42</sub> (10-30 RESIDUES) DURING A 20ns MD SIMULATIONS. HYDROGEN BONDING PATTERNS IN Aβ<sub>42</sub> WITH NIAID**

Torsion angle value was also calculated to observe the alteration of Aβ structure quantitatively during the simulation. The Psi ( $\psi$ ) and Phi ( $\phi$ ) 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<sub>(1-42)</sub> 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  $\psi$  AND  $\phi$  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<sub>42</sub> AT 0ns AND 20ns, B) Aβ<sub>42</sub> COMPLEXES WITH NIAID AT 0ns AND 20ns**

The Ramachandran-plot of A $\beta$ <sub>(1-42)</sub> in the presence of NIAID compound **Fig. 6B** showed after MD simulation only 5 amino acid of A $\beta$  has changed into  $\beta$ -sheet while remaining amino acid of A $\beta$  are in  $\alpha$ -helical conformation. In native A $\beta$ , 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 A $\beta$  during simulations. During dynamics, NIAID compound has prevented much deviation in A $\beta$  angle values and not allowed the A $\beta$  middle region to unfold and change into  $\beta$ -sheet.

**TABLE 3: TABLE SHOWING THE RESIDUES OF A $\beta$ <sub>42</sub> INVOLVED IN HYDROGEN BONDING WITH A NIAID TEST COMPOUND. THE HYDROGEN BONDING ANALYSIS WAS PERFORMED FOR 10 STRUCTURES OF A $\beta$ <sub>42</sub>-LIGAND COMPLEX OBTAINED AT DIFFERENT TIME INTERVALS OF A 20ns MD SIMULATION. CONTRIBUTING SIGNIFICANTLY TO STABILIZE THE A $\beta$   $\alpha$ -HELIX. MOSTLY THE AMINO ACID RESIDUES (K16, F19, F20, AND D23) RESPONSIBLE FOR TO ALLOW A $\beta$  BACKBONE TO UNWIND BINDS WITH TEST COMPOUND WITH HYDROGEN BONDS USING THE CRITERION DISTANCE ( $\leq 3.0 \text{ \AA}$ )**

Time (ns)	During dynamics residues in A $\beta$ <sub>42</sub> involved in hydrogen bonding with NIAID
0	K16, D23
2	D23, L34
4	V12, K16, L34
6	D23, G25
8	H14, D23, L34
10	D23, G37
12	F20, D23, G37
14	L17, F19, L34
16	D23, I32, G37
18	D23, I32, G37
20	D23, G37

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

The effect of the ligand on the stability of the A $\beta$ <sub>(1-42)</sub> 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 A $\beta$  central helix. The investigation also showed that NIAID compounds bind to the highly elongated form of A $\beta$  and was found to be proficient at forming parallel conformations with A $\beta$ . Thus binding of the test compound to A $\beta$ <sub>(1-42)</sub> was found to agitate the extension of  $\beta$ -sheet. The MD simulation in presence and absence of the test compounds up to 20ns showed how amino acid of native A $\beta$  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 A $\beta$  backbone RMSD vs. initial structure and by the existence of  $\alpha$ HB's of the A $\beta$ . RMSD graph shows fluctuations of the native A $\beta$  backbone, RMSD is relatively small in every trajectory, which infers protein is losing its conformation slowly. The A $\beta$  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 A $\beta$  alone, the probability of the  $\alpha$ -helical state for A $\beta$  during simulations is higher in presence given test compound, and also stability of the A $\beta$  central  $\alpha$ -helix was retained. RMSD,  $\alpha$ HBs and HB interaction between A $\beta$  and test compound data thus indicate the ability of the compound to stabilize the A $\beta$  central helix. Stabilization of the A $\beta$  central  $\alpha$ -helix is an effective step in preventing A $\beta$  polymerization. It has been suggested that complete unfolding occurs *via* three steps mechanism: Sufficient loss of  $\alpha$ -helical backbone hydrogen bonds ( $\alpha$ HBs), strong interaction between non-polar side chains and strong interaction between polar side chains<sup>27, 28, 29</sup>. Thus, we suggest that test compound NIAID prevent the unfolding of A $\beta$  helix by preventing the breakage of the  $\alpha$ HBs 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 A $\beta$  was analyzed. In overall ligand formed as a minimum as one HB with A $\beta$  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 A $\beta$  respectively. The intermolecular interactions between the A $\beta$  polar residues and the NIAID polar functional groups are significant in stabilizing the A $\beta$  central helix because they can prevent intra-molecular interactions between the A $\beta$  polar residues that stimulate absolute unfolding of the A $\beta$  central helix. Also, benzene-ring of NIAID straddles the A $\beta$  middle non-polar part (residue 17-21). The inter-molecular interactions between the A $\beta$  middle non-polar part and the ligand non-polar part are important in stabilizing the A $\beta$  central helix since the A $\beta$  middle non-polar part includes three non-polar residues (VFF) that have low  $\alpha$ -helical propensity and high  $\beta$ -strand propensity<sup>30, 31</sup>. Therefore test compounds NIAID by establishing hydrophobic interaction with A $\beta$  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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