Docking studies of Tau Protein

Harkewal Singh, Dr. Soma S. Marla, Manas Agarwal, Jaypee University of Information Technology, Waknaghat, Solan (H.P.) India 173215

Abstract-**Alzheimer's disease (AD)[1] is a fatal brain disorder and alone in United States approximately 4.5 millions Americans are suffering from this disease which is expected by 2050 to range between 11.3 million to 16 million[2]**
Alzheimer's disease is a form of dementia, in which nerve cells in memory areas of brain and eventually other areas begin to die at accelerated rate resulting in serious deterioration in several mental functions, such as loss in memory, language, orientation and judgment [3]
AD is characterized by the formation of senile plaques (made of β amyloid, a toxic protein that comes from normal protein) and neurofibrillary tangles (followed by changes in tau protein) resulting in neuronal destructions. Currently available drugs against AD target the acetylcholine cycle thus stopping the abnormal breakdown of acetylcholine. The modern docking programs/software packages e.g. MOE, AcSite, Spdbv, and Rastop etc can be used to find the active site of the tau protein [4]. The ligand against this active binding site can be found by MOE. The exact confirmation and configuration of the ligand can be calculated to find the best molecule with minimum binding energy [5] and it can be used to develop potential drug molecules against the disease. This work is an attempt to find out the amino acid sequences responsible for biologically active structure which should enable us to design a lead molecule against the disease.
In this work we have carried out the docking analysis of Tau protein responsible for AD. 50 structures after the docking were saved in the form of a database. The best five structures in terms of energy were taken, and the amino acids residues of the ligand and receptor molecule which bind to give the best biologically active conformation, were analyzed.

Index Terms—**Alzheimer's disease, Docking, MOE Tau Protein**

I. INTRODUCTION

The symptoms of Alzheimer's disease appear due to the loss of nerve cells in certain regions of the brain, principally the cerebral cortex, and the part that controls our higher mental functions. The degeneration of these nerve cells leads to a loss of millions of the connections (synapses) between nerve cells; it is the loss of connections in the part of the brain dealing with memory (medial temporal lobe) that causes the first symptoms.
The neurons are involved in the travel of electric charges, resulting in the release of messages. AD disrupts this intimate signaling system, resulting into formation of abnormal Senile Plaque (made of β-amyloid**.** [6]
Neurofibrillary Tangles are abnormal collections of twisted threads found inside nerve cells. The main component of the tangles is one form of the protein tau. Tau protein has the ability to bind and stabilize the cells' internal skeleton called microtubule. In neuron, cells that are healthy microtubules form structures like train tracks, which guide nutrients and molecules from the centre bodies of the cells down to the end of the axons. Tau normally forms the connector pieces of the microtubule tracks. In the cells which are affected by AD, the train track structures collapses, tau is changed chemically and can no longer hold the pieces together [7]. A changed form of a protein kinase hyper phosphorylates tau and causes cytoskeleton to collapse. This collapse of the transport system first may result in malfunctions in communication between nerve cells and later lead to neuron death. Tau is also known as Beta 2 transferrin, desialated transferrin. Tau is a Cerebro-Spinal Fluid[8],[9].

Drugs like **tacrine** (Cognex®), **donepezil** (Aricept®), **rivastigmine** (Exelon®), **or galantamine** (Razadyne®, formerly known as Reminyl®) **memantine** (Namenda®) [10] available today target β-amyloid, as the possible drug receptor protein for Alzheimer's disease. But reports suggest that tau protein is also responsible for the occurrence of Alzheimer's disease by forming the neurofibrillary tangles [11].

II. MATERIALS AND METHODS

In this work we attempted to carry out the docking of Tau protein with the following infrastructure.
A. SYSTEM USED - Intel® Pentium® 4, 1.80 GHz**,** 256 MB RAM

B. OPERATING PLATFORM Microsoft Windows XP Pro 2002 Service Pack 2**,** Red Hat LINUX 9.0

C. SOFTWARE PACKAGES

MOE (Molecular Operating Environment)
Rastop, Spdbv (3D molecular viewer), Acsite

D. PROTEIN - (Tau protein)

1J1B.pdb, 2BTP.pdb

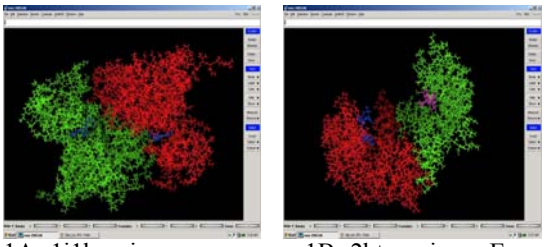
III. RESULTS AND DISCUSSIONS

The abnormal functioning of tau proteins; and hence the formation of tangles should be controlled by blocking the active sites of the target protein. The active sites were found using AcSite and MOE tools. The 3D structures of the active site were viewed by using tools like Rastop, Spdbv, etc.

The amino acid sequence was retrieved using MOE software and several ligand molecules were designed against the active site by targeting these amino acids.

A. Energy Minimization

The energy of the protein molecule was minimized using the Energy minimization algorithm of MOE tool. The minimized structures were saved as 1j1b_min.moe & 2btp_min.moe in the working directory.



1A- 1j1b_min.moe 1B- 2btp_min.moE

Fig. 1 - 1A and 1B Showing Minimized Structures in MOE window

The following parameters were used for energy minimization:—
Gradient = 0.05
Force Field: MMFF94X + Solvation
Chiral Constraint: Current Geometry

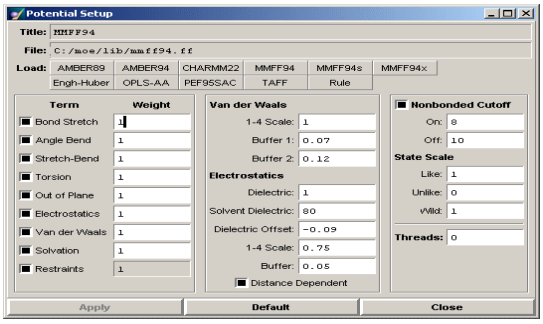


Fig. 2 – Various parameters employed in Energy Minimization of protein Molecule.

The minimized structure was used as the template for Docking.

B. Docking

The binding of the ligand molecule with the protein molecule was analyzed using MOE docking program to find the correct confirmation (with the rotation of bonds, structure of molecule is not rigid) and configuration (with the rotation of whole molecule, structure of the molecule remains rigid) of the ligand, so as to obtain minimum energy structure.J1B.pdb, 2BTP.pdb consists of two ligand molecules, so the docking was performed separately for each of the ligands.

The parameters used for the Docking are:—
Total Runs = 50
Cycle/Runs = 15
Iteration Limit = 10,000
Potential Energy Grid: ON
Annealing Algorithm: Simulated Annealing

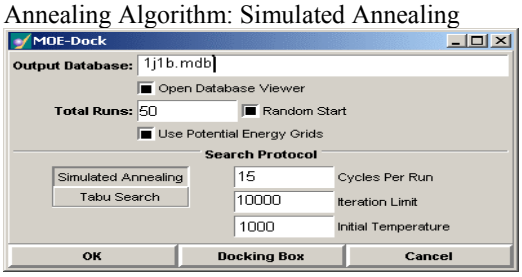


Fig. 3 – MOE window shown Docking Parameters employed

We saved a total 50 structures in the database. The active site of the receptor was found using MOE and docking was performed by selecting the active site.

C. Calculating the active Site Sequence

The pocket sequence of the active site was calculated by using active site finder tool of MOE.

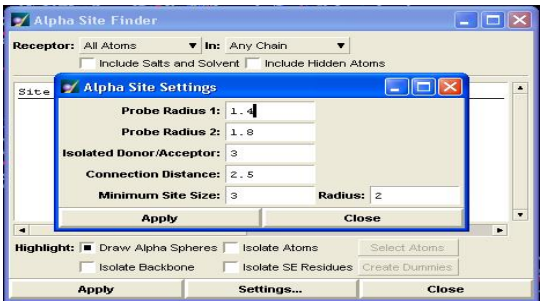


Fig. 4 - MOE window showing parameters used in Alpha Site Finder

Probe Radius 1 = 1.4, Probe Radius 2 = 1.8, Isolated Donor/Acceptor=3, Connection Distance = 2.5, Minimum Site Size = 3, Radius = 2

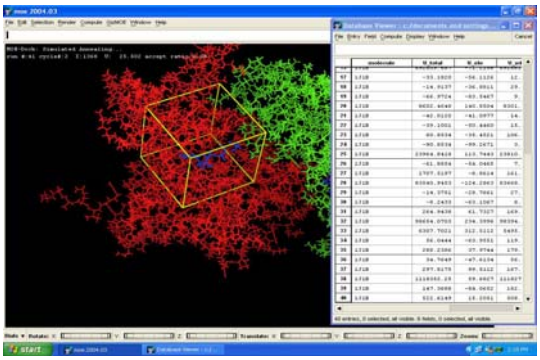


Fig. 5 –Dock box around the active site and the database generated.

D. Flexible Alignment

The best five of the ligand structures from the database were imported in MOE to analyze the common part of the ligand. This was achieved by the Flexible Alignment tool of MOE. Following parameters were used to calculate the flexible alignment (Fig. 6)

Many possible structures for the ligand were generated using MOE, and the best five (*having minimum energy*) were considered as the possible ligand molecules against the target proteins (*i.e. 1J1B & 2BTP*).

Each pdb has two chains which are shown in the figure as green and red respectively. Five best ligands against the two chains of 1JIB and 2BTP were generated after the docking. The amino acid sequence for the pocket site was found as following –

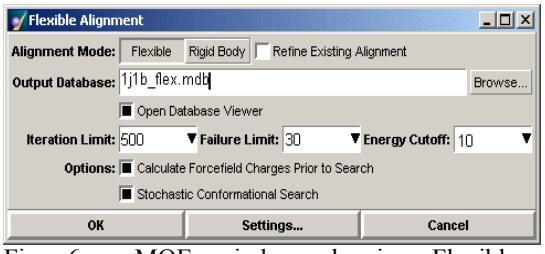


Fig. 6 - MOE window showing Flexible Alignment Parameters

Configuration Limit=1000, Alpha=2.5, Gradient Test=0.01, RMSD Tolerance=0.5, Maximum Steps=500, Iteration Limit=500, Failure Limit = 30, Energy Cut-Off = 10, Rigid Body

a)-1J1B

RED CHAIN-Chain A

Ile28-Gly29-Asn30-Gly31-**Ser32**-Phe33-Gly34-Val36-Ala49-Lys51-Val76-Leu98-Asp99-Tyr100-Val101-Thr104-Arg107-Asp147-Lys149-Gln151-Asn152-Leu154-Asp166-Asp242

GREEN CHAIN- Chain B

Ile40-Gly41-Asn42-Gly43-Phe45-Gly46-Val48-Ala61-Lys63-Val88-Leu110-Asp111-Tyr112-Val113-Thr116-Asp159-Lys161-Gln163-Asn164-Leu166-Cys177-Asp178-Asp230

b) - 2BTP

RED CHAIN -Chain A

Lys71-Arg78-Lys138-Arg145-Tyr146-Gly187-Leu190-Asn191-Val194-Tyr197-Glu198-Leu234-Ile235-Leu238-Asn242-Leu245-Trp246

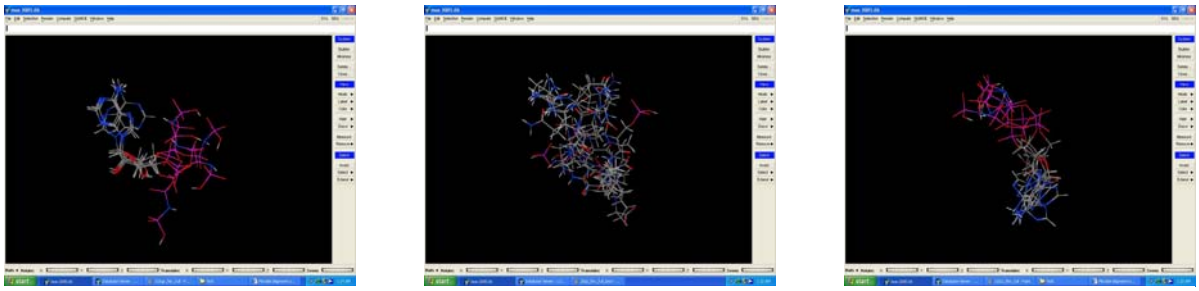
GREEN CHAIN-Chain B

Lys48-Arg55-Lys115-Arg122-Tyr123-Gly164-Leu167-Asn168-Val171-Leu207-Ile208-Leu211-Asn215-Leu218

IV. CONCLUSIONS

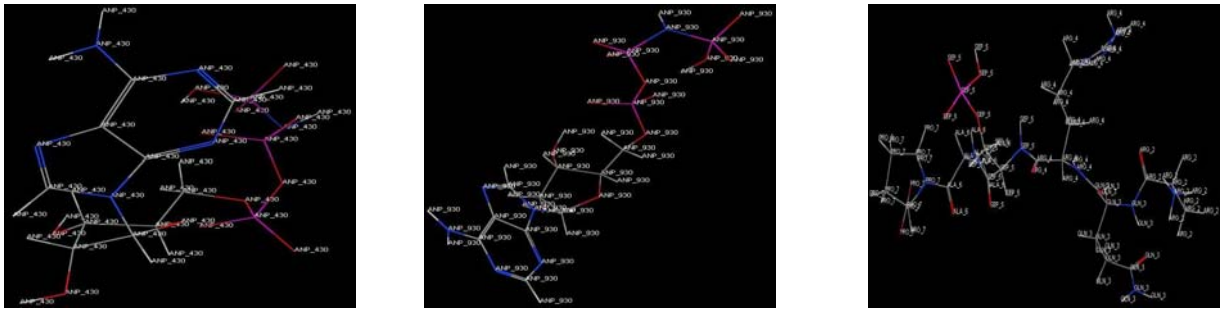
Ser32 is the differentiating residue between the two chains of 1JIB whereas in 2BTP both chains are similar to each other. This has been confirmed by Alpha Site finder of MOE as well as Acsite.

The docking results were confirmed with Patch Dock, (clustering RMSD 4.0) Version beta1.2. This works on shape complementarity principles. The contact analysis of Protein and Ligand has been performed using Sequence Editor and Protein Contact Modules of MOE. The Ligand of 1J1B has ANP_430 and ANP_930 residues while the ligand of 2BTP has PRO_7, ALA_6, SEP_5, ARG_4, GLN_3 AND ARG_2 residues. It suggests that NZ atom of LYS85 of 1J1B and O1A atom of ANP430 of 1st and 3rd chain of 1JIB are interacting with each other by hydrogen bonds.



(a) (b) (c)

Fig. 7 – MOE window showing the flexible alignment of best five ligands (a)-1JIB-green chain, (b)-1JIB-Red Chain & (c)- 2BTP-Red and green Chain; to find out the common region of ligands



(a) (b) (c)

Fig. 8 – MOE Window showing the Ligands of 1JIB (a)-green chain (b)-red chain and 2BTP both chains

The same kind of bonding is between the following residues of 1st and 3rd chain of 1J1B-
O atom of ASP133 and N6 of ANP430,
O atom of GLN185 and O3 of ANP430,
OD2 of ASP200 and N3B of ANP430
While there are **Ionic Interaction** between NZ atom of LYS85 and O1A atom of ANP430,
NZ atom of LYS183 and O3G of ANP430.
ANP 930 has again two kinds of Interactions viz. Hydrogen Bonding and Ionic.
Between following residues there are hydrogen bonding interactions –
NZ atom of LYS585 and O1A atom of ANP930
O atom of ASP633 and N6 atom of ANP930
NZ atom of LYS683 and O3G atom of ANP930
O atom of GLN685 and O3G atom of ANP930
OD2 atom of ASP700 and N3B atom of ANP930 **both of second and third chain of 1J1B respectively**.
Ionic Interactions for ANP930 are as following
NZ atom of LYS585 and O1A atom of ANP930,
NZ atom of LYS681 and O3G atom of ANP930,
of second and third chain of 1J1B respectively.
Complete Description is shown in the table 1 given in appendix.

Ligand for 2BTP has again two types of interactions viz. Hydrogen Bonding and Ionic Interactions. The complete information regarding the interactions is available in the table 2 of appendix For ANP we can get top 30 drugs information from RCSB. Most of the drugs have similarity with the structure of ANP. So it can be very well inferred that an analogous molecule from the database can be helpful to replace the existing ligand.

V. OBSERVATIONS

The most important interactions which involve ligand and receptor's active site are hydrogen bonding and ionic. These suggest that new ligand should be generated keeping in view that it should be able to have stronger hydrogen and ionic interaction with the amino acid moieties of the binding site. Also there is no disulphide linkage between the two so the ligand should not have groups which can avail these interactions easily. The smiles string of the ligands is achieved from MOE which can give the actual structure of the ligand. The Chemical formula of ANP can be found from RCSB which shall help

us to build new ligand(s) to block the abnormal functionality of the Tau protein imparted due to the existing ligand.

APPENDIX

Table 1 describes about the protein contact analysis report of 1JIB and Table 2 about 2BTP.

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[11].Adriana Ferreira, Tau protein needed for Alzheimer's disease, NUIN news, 2002

Table 1 – Protein Contact Report of 1JIB.pdb

Protein Contacts Report
Fri Apr 21 09:34:03 2006
Contact types:

Ionic bonds
Hydrophobic contacts
Hydrogen bonds
Disulfide bonds

Do not report contacts within chains
Report contacts between different chains of like tags
Report inferred contacts of sequence-only data

Options:

Conservation : 1
Sequence separation : 4
Network separation : 0
Ionic cutoff : 4.5
Hydrophobic cutoff : 4.5
Disulfide cutoff : 2.5
HIS is Basic : TRUE
MET is Hydrophobic : TRUE
H bond between main and sidechain : TRUE

Chains:
---
1 1J1B.A TRANSFERASE
2 1J1B.B TRANSFERASE
3 1J1B TRANSFERASE

Contacts:

	Type	Chain	Pos	Residue	Chain	Pos	Residue	Net
1	HB	1:1J1B.A	32	SER66.OG	2:1J1B.B	242	ASP764.OD2	14
2	HB	1:1J1B.A	181	SER215.OG	2:1J1B.B	266	TYR788.OH	12
3	HB	1:1J1B.A	182	TYR216.OH	2:1J1B.B	268	GLU790.OE1	11
4	HB	1:1J1B.A	230	ASP264.OD2	2:1J1B.B	44	SER566.OG	13
5	HB	1:1J1B.A	254	TYR288.OH	2:1J1B.B	193	SER715.OG	10
6	HB	1:1J1B.A	51	LYS85.NZ	3:1J1B	1	ANP430.O1A	2
7	HB	1:1J1B.A	99	ASP133.O	3:1J1B	1	ANP430.N6	2
8	HB	1:1J1B.A	151	GLN185.O	3:1J1B	1	ANP430.O3*	2
9	HB	1:1J1B.A	166	ASP200.OD2	3:1J1B	1	ANP430.N3B	2
10	HB	2:1J1B.B	63	LYS585.NZ	3:1J1B	2	ANP930.O1A	1
11	HB	2:1J1B.B	111	ASP633.O	3:1J1B	2	ANP930.N6	1
12	HB	2:1J1B.B	161	LYS683.NZ	3:1J1B	2	ANP930.O3G	1
13	HB	2:1J1B.B	163	GLN685.O	3:1J1B	2	ANP930.O3*	1
14	HB	2:1J1B.B	178	ASP700.OD2	3:1J1B	2	ANP930.N3B	1
15	HYD	1:1J1B.A	33	PHE67.CE2	2:1J1B.B	245	VAL767.CG2	8
16	HYD	1:1J1B.A	183	ILE217.CG2	2:1J1B.B	241	VAL763.CG1	9
17	HYD	1:1J1B.A	229	VAL263.CG1	2:1J1B.B	45	PHE567.CZ	3
18	HYD	1:1J1B.A	229	VAL263.CG2	2:1J1B.B	195	ILE717.CG2	3
19	HYD	1:1J1B.A	233	VAL267.CG2	2:1J1B.B	45	PHE567.CE2	3
20	ION	1:1J1B.A	226	ASP260.OD1	2:1J1B.B	198	ARG720.NE	7
21	ION	1:1J1B.A	256	GLU290.OE2	2:1J1B.B	74	ARG596.NH1	6
22	ION	1:1J1B.A	256	GLU290.OE2	2:1J1B.B	158	ARG680.NH2	6
23	ION	1:1J1B.A	51	LYS85.NZ	3:1J1B	1	ANP430.O1A	5
24	ION	1:1J1B.A	149	LYS183.NZ	3:1J1B	1	ANP430.O3G	5
25	ION	2:1J1B.B	63	LYS585.NZ	3:1J1B	2	ANP930.O1A	4
26	ION	2:1J1B.B	161	LYS683.NZ	3:1J1B	2	ANP930.O3G	4

Table 2 – Protein Contact Report of 2BTP.pdb

Protein Contacts Report
Fri Apr 21 09:39:38 2006

Contact types:

Ionic bonds
Hydrophobic contacts
Hydrogen bonds
Disulfide bonds
Do not report contacts within chains
Report contacts between different chains of like tags
Report inferred contacts of sequence-only data

Options:

Conservation : 1
Sequence separation : 4
Network separation : 0
Ionic cutoff : 4.5
Hydrophobic cutoff : 4.5
Disulfide cutoff : 2.5
HIS is Basic : TRUE
MET is Hydrophobic : TRUE
H bond between main and sidechain : TRUE

Chains:
---
1 2BTP.A COMPLEX (SIGNAL TRANSDUCTION/PEPTIDE)
2 2BTP.B COMPLEX (SIGNAL TRANSDUCTION/PEPTIDE)
3 2BTP.P COMPLEX (SIGNAL TRANSDUCTION/PEPTIDE)
4 2BTP.Q COMPLEX (SIGNAL TRANSDUCTION/PEPTIDE)

Contacts:

	Type	Chain	Pos	Residue	Chain	Pos	Residue	Net
4	HB	1:2BTP.A	100	TYR82.OH	2:2BTP.B	17	ARG18.NE	3
6	HB	1:2BTP.A	107	GLU89.OE1	2:2BTP.B	17	ARG18.NH1	3
7	HB	1:2BTP.A	71	LYS49.NZ	3:2BTP.P	6	PRO7.O	9
8	HB	1:2BTP.A	78	ARG56.NH1	3:2BTP.P	4	SEP5.O3P	1
9	HB	1:2BTP.A	145	ARG127.NH1	3:2BTP.P	4	SEP5.O1P	1
10	HB	1:2BTP.A	146	TYR128.OH	3:2BTP.P	4	SEP5.O2P	1
11	HB	1:2BTP.A	191	ASN173.OD1	3:2BTP.P	5	ALA6.N	8
12	HB	1:2BTP.A	242	ASN224.ND2	3:2BTP.P	3	ARG4.O	10
13	HB	2:2BTP.B	48	LYS49.NZ	4:2BTP.Q	6	PRO7.O	9
14	HB	2:2BTP.B	55	ARG56.NH1	4:2BTP.Q	4	SEP5.O3P	1
15	HB	2:2BTP.B	122	ARG127.NH1	4:2BTP.Q	4	SEP5.O1P	1
16	HB	2:2BTP.B	123	TYR128.OH	4:2BTP.Q	4	SEP5.O2P	1
17	HB	2:2BTP.B	168	ASN173.OD1	4:2BTP.Q	5	ALA6.N	8
18	HB	2:2BTP.B	215	ASN224.ND2	4:2BTP.Q	3	ARG4.O	10
25	ION	1:2BTP.A	107	GLU89.OE1	2:2BTP.B	17	ARG18.NH1	12
26	ION	1:2BTP.A	71	LYS49.NZ	3:2BTP.P	6	PRO7.O	5
27	ION	1:2BTP.A	78	ARG56.NH1	3:2BTP.P	4	SEP5.O3P	2
28	ION	1:2BTP.A	145	ARG127.NH1	3:2BTP.P	4	SEP5.O1P	2
29	ION	2:2BTP.B	48	LYS49.NZ	4:2BTP.Q	6	PRO7.O	5
30	ION	2:2BTP.B	55	ARG56.NH1	4:2BTP.Q	4	SEP5.O3P	2
31	ION	2:2BTP.B	122	ARG127.NH1	4:2BTP.Q	4	SEP5.O1P	2