DOCKING STUDIES OF TAU PROTEIN FOR ALZHEIMER'S DISEASE

By

MANAS AGARWAL-021513

MAY-2006

Submitted in partial fulfillment of the Degree of Bachelor of Technology

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY-WAKNAGHAT
MAY-2006

CERTIFICATE

This is to certify that the following Project work entitled "**DOCKING STUDY OF TAU PROTEIN FOR ALZHEIMER'S DISEASE**" submitted by **Manas Agarwal (021513)** in partial fulfillment for the award of Degree of Bachelor of Technology in **Bioinformatics** is a *bona fide* record of the work carried out under my supervision. It is further stated that no part of this work has been published or submitted to any other University or Institute for any other degree or diploma.

Mr. Harkewal Singh
Lecturer
Department of Biotechnology & Bioinformatics
Jaypee University of Information technology

Date: 5/5/2008

Dr. Soma Marla
H.O.D.
Department of Biotechnology & Bioinformatics
Jaypee University of Information Technology

Date: 5/5/2008

ACKNOWLEDGEMENT

I take this opportunity to express my indebted gratitude to all those who helped me directly or indirectly while carrying out this project work. It is my pleasure to acknowledge the help of all individuals.

I am obliged to Mr. Harkewal Singh, Lecturer, Department of Biotechnology and Bioinformatics, JUIT, Waknaghat for the guidance, co-ordinance and inspiration that I have been receiving from him. I would like to express my gratitude and heartfelt thanks to Dr. Soma Marla, H.O.D. Department of Biotechnology and Bioinformatics, JUIT, Waknaghat for his constant support during the project.

I take this opportunity to thank our friends, teachers and all others who helped and support me for the completion of project.

Manas Agarwal

TABLE OF CONTENTS

CHAPTER 1

ABSTRACT

Alzheimer's disease (AD) is a fatal brain disorder and alone in United States approximately 4.5 millions Americans are suffering from this disease which is expected to range between 11.3 million to 16 million by 2050!

It 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 memory, language, orientation and judgment. 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 to 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. 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 and it can be as a lead drug 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 that bind to give the best biologically active conformation, were analyzed. This shall enable us to design a lead molecule that in turn can be useful to inhibit this fatal brain disorder.

CHAPTER 2

INTRODUCTION

Alzheimer's disease (AD) is a form of dementia (a serious deterioration in several mental functions, such as memory, language, orientation and judgement). Dementia is a major cause of ill-health, with approximately 6 million sufferers in the European Union and 800,000 in the UK. In United States approximately 4.5 millions Americans are suffering from this disease which is expected to range between 11.3 million to 16 million by 2050!

The symptoms of AD are due to 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, a toxic protein that comes from normal amyloid precursor protein (APP)) and Neurofibrillary Tangles.

Neurofibrillian 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 cells affected by AD the train track structures collapses, tau is changed chemically and can no longer hold the pieces together. 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 CSF (Cerebro-Spinal Fluid - A clear, colorless fluid that contains small quantities of glucose and protein. Cerebrospinal fluid fills the ventricles of the brain and the central canal of the spinal cord).

Currently avialbalbe drugs like tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®), or galantamine (Razadyne®, formerly known as Reminyl®) Alzheimer's Disease Medication Fact Sheet, Dec 2005; NIH Publication No. 03-3431) available today targets 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 (Adriana Ferreira, NUIN—2002/ "Tau is Essential to Beta-Amyloid-Induced Neurotoxicity" (vol. 99, pp. 6364-6369; www.pnas.gov/cgi/doi/10.1073/pnas.092136199)).

The abnormal functioning of tau proteins; and hence the formation of tangles should be controlled by blocking the active sites of the 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.
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 docking energy.
The Flexible alignment of the five best ligand molecules was done to check the similarity between the ligand molecules obtained, and to find the common portion of the ligand responsible for blocking the active site of the protein molecule.

CHAPTER 3

REQUIREMENTS

SYSTEM REQUIREMENTS

- ✓ Intel® Pentium® 4
- ✓ 1.80 GHz
- ✓ 256 MB RAM

OPERATING PLATFORM

a) Microsoft Windows XP Pro 2002
   Service Pack 2

b) Red Hat LINUX 9.0

SOFTWARE PACKAGES

- ✓ MOE (Molecular Operating Environment)
- ✓ AcSite (Active Site Prediction)
- ✓ Rastop, Spdbv (3D molecular viewer)

PROTEINS (Tau prteins)

- ✓ 1J1B.pdb
- ✓ 2BTP.pdb

CHAPTER 4

MOE

MOE stands for **Molecular Operating Environment.**
MOE is an interactive, windows-based chemical computing and molecular modeling tool with a broad base of scientific applications:

Bioinformatics	Structure and Family Databases, Fold/Homologue Identification, Alignment, Consensus
Cheminformatics	Descriptors, Similarity and Diversity, Conformation Databases, 3D Search, Pharmacophores
High Throughput Discovery	HTS Analysis, Binary QSAR, Combinatorial Library Design
Structure Based Design	Active Site Detection, Contact Potentials, Docking, Fragment Analysis
Protein Modeling	Homology Modeling, Mutation, Mechanics, Dynamics, Analysis
Molecular Modeling and Simulations	Forcefields, Electrostatics, Mechanics, Dynamics, Diffraction, 3D Molecule Builders, Molecular Surfaces
Methodology Development and Deployment	Prototyping, Integrating, Distributed Computing

MOE provides a comprehensive visualization interface that permits multiple views of a molecular system:

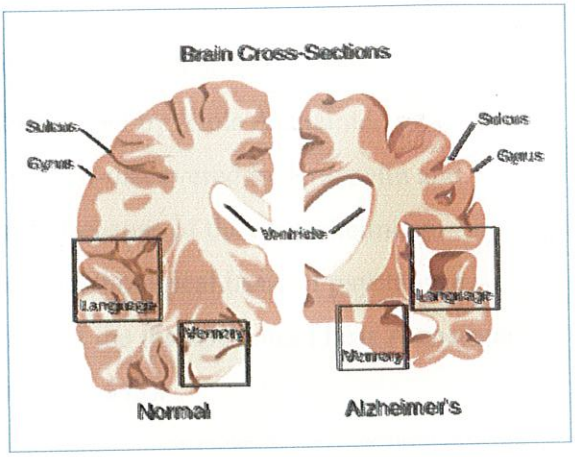
- ✓ MOE Window displays molecular systems in a variety of rendering styles
- ✓ Atom Manager displays properties of molecular objects loaded in MOE
- ✓ Sequence Editor displays a chain and residue level view ideal for protein modeling
- ✓ Database Viewers display and manage large collections of molecules or the many conformations of a single molecule

The interface can be either menu-driven or command-line based, or a combination of both. The **SVL Commands** window displays the output generated by SVL commands and MOE applications. MOE also includes an assortment of management windows that control such things as geometric constraints, debugging, module loading, and task control.

CHAPTER 5

ALZHEIMER'S DISEASE

Alzheimer's disease is a form of dementia, which means a serious deterioration in several mental functions, such as memory, language, orientation and judgement. Dementia is a major cause of ill-health, with approximately 6 million sufferers in the European Union and 800,000 in the UK.



Alzheimer's disease is the commonest cause of dementia, accounting for about 65% of dementia in the elderly. Dementia due to narrowing of the brain's blood vessels (vascular dementia) and Lewy body disease make up most of the remainder. Rarely, dementia can be reversed, for example if it is due to thyroid hormone deficiency or deficiency of vitamin B-12 or folic acid. It is therefore important that all patients with dementia are screened for these treatable causes.

In some cases of Alzheimer's disease, mental decline may occur relatively rapidly whilst in others the loss of cognitive ability may be more gradual. Alzheimer's disease shortens the life span and, although patients may live for as many as 15 years after diagnosis, the average period to death (usually from an infection) is about 8 years.

CHAPTER 6

HISTORY

The symptoms of the disease as a distinct entity were first identified by Emil Kraepelin, and the characteristic neuropathology was first observed by Alois Alzheimer, a German psychiatrist, in 1906. In this sense, the disease was co-discovered by Kraepelin and Alzheimer, who worked in Kraepelin's laboratory. Because of the overwhelming importance Kraepelin attached to finding the neuropathological basis of psychiatric disorders, Kraepelin made the generous decision that the disease would bear Alzheimer's name (*J. Psychiat. Res.,* 1997, Vol 31, No. 6, pp. 635-643).

For most of the twentieth century, the diagnosis of Alzheimer's disease was reserved for individuals between the ages of 45-65 who developed symptoms of presenile dementia, which was considered to be a more or less normal outcome of the aging process. In the 1970s and early 1980s, however, the name "Alzheimer's disease" began to be used, within and outside the medical profession, equally for individuals age 65 and older with senile dementia, and was eventually adopted formally for all individuals with the common symptom pattern and disease course in the psychiatric and neurological nomenclature.

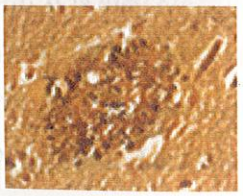
CHAPTER 7

POSSIBLE CAUSES

Alzheimer's disease is not an inevitable part of normal ageing, nor even an acceleration of ageing; it is a true disease. But it is a disease, like heart disease and cancer which is increasingly common as we age. The incidence of Alzheimer's disease approximately doubles for every 5 years of age over the age of 60, so that about 25% of those over 80 years old have the disease.

The symptoms of Alzheimer's disease are caused by a loss of nerve cells in certain regions of the brain, principally the cerebral cortex, and the part that controls our higher mental functions and which makes us unique as humans. 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; see diagram) that causes the first symptoms. The disease progresses and spreads throughout the cerebral cortex, gradually affecting those parts of the cortex that deal with almost all our other higher cognitive functions and our behaviour. Certain parts of the cortex are spared in the early stages: for example, the processing of vision and the control of normal movement are not affected until the later stages.

The neurons are involved in the travel of electric charges, resulting in the release of messages. Alzheimer's disease disrupts this intimate signaling system, resulting into formation of abnormal **Senile Plaque** and **Neurofibrillary Tangles.**



SENILE PLAQUES

NEUROFIBRILLARY TANGLES

Senile Plaques: Amyloid plaque consists principally of a polypeptide of 4 kDa refereed to as the amyloid protein. The amyloid protein is derived from the much larger molecule amyloid protein precursor, APP. During metabolism APP becomes included in the membrane of the nerve cell, some parts inside the cell and some parts outside. In this state proteases cleave APP to produce the amyloid protein. The sequence is cleaved out by proteases referred to as a beta and gamma secretases.

Neurofibrillary Tangles: Neurofibrillian tangles are abnormal collections of twisted threads found inside nerve cells. The main component of the tangels is one form of the protein tau. The tau protein has a 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 cells affected by AD the train track structures collapses, tau is changed chemically and can no longer hold the pieces together. A changed form of a protein kinase hyper phosphorylates tau and cause cytoskeleton collapse. The collapse of the transport system first may result in malfunctions in communication between nerve cells and later lead to neuron death.

Amyloids: Amyloids are various types of insoluble fibrous protein aggregations sharing specific traits when examined microscopically. The name *amyloid* comes from the early mistaken identification of the substance as starch (*amylum* in Latin), based on crude iodine-staining techniques. Amyloids are present in some neurodegenerative diseases but play a normal productive role in processes such as melanin formation.

Tau: It is also known as Beta 2 transferrin, desialated transferrin. Tau is a CSF (Cerebro-Spinal Fluid - A clear, colorless fluid that contains small quantities of glucose and protein. Cerebrospinal fluid fills the ventricles of the brain and the central canal of the spinal cord. Analysis of the cerebrospinal fluid can be accomplished using lumbar puncture. The presence of white blood cells or bacteria within the cerebrospinal fluid can indicate a bacterial infection).

CHAPTER 8

SYMPTOMS

AD begins slowly. At first, the only symptom may be mild forgetfulness, which can be confused with age-related memory change. Most people with mild forgetfulness do not have AD. In the early stage of AD, people may have trouble remembering recent events, activities, or the names of familiar people or things. They may not be able to solve simple math problems. Such difficulties may be a bother, but usually they are not serious enough to cause alarm.

Some of the symptoms of the disease are like loss of memory — first and most characteristic symptom; often, early childhood memories are preserved whereas recognition of a loved one, work colleague or sense of daily routine is lost. As the disease progresses, language difficulties, difficulties in co-ordination, depression, paranoia, aggressive outbursts, delusions and hallucinations may take hold. Self-neglect, incontinence, wandering and disorientation necessitate constant supervision.

In general, common early symptoms of Alzheimer's are:

- confusion
- disturbances in short-term memory
- problems with attention and spatial orientation
- personality changes
- language difficulties
- unexplained mood swings

CHAPTER 9

WHO'S AT RISK

AD is not fixed to a particular geographical area with no social or cultural boundaries. Very rarely it may occur across generations as an inherited disease affecting 50% of family members within which the age of onset is predictable, but more commonly it occurs without a strong family history of the disease.

Research has shown that the clinical symptoms may be caused by more than one factor. One of these factors involves a protein called beta-amyloid. The gene responsible for this amyloid protein is located on chromosome 21. Individuals suffering from Down's syndrome, who have more than the normal two copies of this amyloid gene, or people who have a mutation in one of their two amyloid genes are at high risk. Others at risk are those people who have mutations in the presenilin genes which appear to interact with amyloid. Two such genes have been identified so far - one located on chromosome 14 and another on chromosome 1.

Dominantly inherited forms of the disease have become the focus of research. However, they represent only about 1% of AD cases worldwide. The identification of genetic mutations in affected individuals means that the inheritance of the disease can be determined with accuracy across each generation. Genetic testing to determine who will suffer from the disease within these families is technically possible, but until a treatment is available this is being discouraged.

Another form of 'risk' can be likened to the everyday risks we take in our lives, such as traveling: we know that it is more risky to travel by bicycle than on foot, in other words, a cyclist has a higher susceptibility to injury than a pedestrian. In the same way, there are certain genes and certain environmental factors that increase our susceptibility to develop AD. Having these genes, or being exposed to these environmental factors, does not mean that we will inevitably get the disease any more than cycling inevitably means that we will get injured - it just increases the chances that we will.

CHAPTER 10

CURE FOR AD

There is currently no cure for Alzheimer's Disease. Because it is a progressive degenerative disease of the nervous system, it is unlikely we can expect a 'cure' in the sense that one can cure an infectious disease. The idea of transplanting healthy nerve cells into the brain to replace those that have died in order to 'cure' Alzheimer's is not realistic because many different types of nerve cell are affected in the disease, each with specific connections to other nerve cells.

Two approaches are being tried—

- First, treatments are being developed either to alleviate some of the symptoms or to slow down the process of nerve degeneration.
- Second, the search is on for measures that might prevent the disease from developing.

One group of nerve cells that degenerate in Alzheimer's disease makes the chemical acetylcholine, which acts as a transmitter of information between nerve cells. There are drugs in the UK which are designed to inhibit the enzyme that normally breaks down acetylcholine. The cholinesterase inhibitors increase the levels of acetylcholine in the cerebral cortex and give some improvement in the memory problems early on in Alzheimer's disease. However, cholinesterase inhibitors do not prevent nerve cells from dying and so they do not influence the development of the disease. Drugs under development include chemicals that inhibit the formation of the protein β-amyloid and it is hoped that these drugs, if given early enough, might slow down the disease process. **This class of drug is unlikely to be available for several years.**

A few clinical trials are in progress, or planned, to see whether modifying risk factors might slow the development of the disease. Large doses of vitamin E are being tested to see if the anti-oxidant properties of this vitamin might prevent some nerve cells from dying and slow down the decline in cognitive abilities.

Anti-inflammatory drugs are being tested because Alzheimer's disease appears to be less common in those who regularly take these drugs. Trials of blood pressure lowering drugs are in progress, since these might reduce the development of vascular dementia (due to narrowing of the arteries in the brain) but also possible Alzheimer's disease.

The unexpected finding that Alzheimer's is associated with several of the established risk factors of heart disease and stroke raises the possibility of preventing Alzheimer's disease by dietary modification and by changes in life-style, which have been so successful in the diseases of the vascular system. Much research needs to be done to test this and other hypotheses about risk factors, but the rewards are potentially great.

CHAPTER 11

AVAILABLE DRUGS & SIDE EFFECTS

Five prescription drugs (see figure) tacrine **(Cognex®)**, donepezil **(Aricept®)**, rivastigmine **(Exelon®)**, or galantamine **(Razadyne®, formerly known as Reminyl®)**, memantine **(Namenda®)**, currently are approved by the U.S. Food and Drug Administration to treat people who have been diagnosed with Alzheimer's disease (AD). Treating the symptoms of AD can provide patients with comfort, dignity, and independence for a longer period of time and can encourage and assist their care givers as well.

NOTE:

It is important to understand that none of these medications stops the disease itself.

CAUTION:

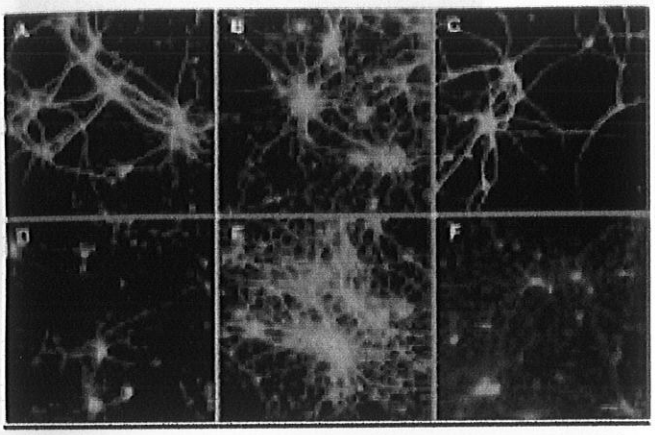
Use of Cholinesterase inhibitors can increase risk of stomach ulcers, and because long use of non-steroidal anti-inflammatory drugs (NSAIDs) such as Asprin or Ibuprofen can also cause stomach ulcers, NSAIDs should be used with caution in combination with these medications.

DRUG NAME	DRUG TYPE AND TREATMENT	MANUFACTURER'S RECOMMENDED DOSAGE	COMMON SIDE EFFECTS	POSSIBLE DRUG INTERACTIONS
Namenda® (memantine) Blocks the toxic effects associated with excess glutamate and regulates glutamate activation.	N-methyl D-aspartate (NMDA) antagonist prescribed to treat symptoms of moderate to severe AD	• 5 mg, once a day, available in tablet form • Increase to 10 mg/day (5 mg twice a day), 15 mg/day (5 mg and 10 mg as separate doses), and 20 mg/day (10 mg twice a day) at minimum of one week intervals if well tolerated.	Dizziness, headache, constipation, confusion	Other NMDA antagonist medications, including amantadine, an antiviral used to treat the flu, dextromethorphan, prescribed to relieve coughs due to colds or flu, and ketamine, sometimes used as an anesthetic, have not been systematically evaluated and should be used with caution in combination with this medication.
Razadyne® (galantamine, formerly known as Reminyl®) Prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine in the brain.	Cholinesterase inhibitor prescribed to treat symptoms of mild to moderate AD	• 4mg, twice a day (8mg/day), available in tablet or capsule form • Increase by 8mg/day after 4 weeks to 8mg, twice a day (16mg/day)if well tolerated. • After another 4 weeks, increase to 12mg, twice a day (24mg/day) if well tolerated.	Nausea, vomiting, diarrhea, weight loss	Some antidepressants such as paroxetine, amitriptyline, fluoxetine, fluvoxamine, and other drugs with anticholinergic action may cause retention of excess Reminyl in the body, leading to complications; NSAIDs should be used with caution in combination with this medication.*
Exelon® (rivastigmine) Prevents the breakdown of acetylcholine and butyrylcholine (a brain chemical similar to acetylcholine) in the brain.	Cholinesterase inhibitor prescribed to treat symptoms of mild to moderate AD	• 1.5mg, twice a day (3mg/day), available in capsule and liquid form • Increase by 3mg/day every 2 weeks to 6mg, twice a day (12mg/day) if well tolerated.	Nausea, vomiting, weight loss, upset stomach, muscle weakness	None observed in laboratory studies; NSAIDs should be used with caution in combination with this medication.*
Aricept® (donepezil) Prevents the breakdown of acetylcholine in the brain.	Cholinesterase inhibitor prescribed to treat symptoms of mild to moderate AD	• 5mg, once a day, available in tablet form • Increase after 4-6 weeks to 10mg, once a day if well tolerated.	Nausea, diarrhea, vomiting	None observed in laboratory studies; NSAIDs should be used with caution in combination with this medication.*
Cognex® (tacrine) Prevents the breakdown of acetylcholine in the brain. **Note:** Cognex is still available but no longer actively marketed by the manufacturer.	Cholinesterase inhibitor prescribed to treat symptoms of mild to moderate AD	• 10mg, four times a day (40mg/day), in capsule form • Increase by 40mg/day every 4 weeks to 40mg, four times a day (160mg/day), if liver enzyme functions remain normal and if well tolerated.	Nausea, diarrhea, possible liver damage	NSAIDs should be used with caution in combination with this medication.*

CHAPTER 12

NEED FOR NEW DRUG

The tau protein must be present to enable beta-amyloid plaques to cause the degeneration of brain cells found in Alzheimer's disease (Adriana Ferreira, NUIN—2002/ "Tau is Essential to Beta-Amyloid-Induced Neurotoxicity" (vol. 99, pp. 6364-6369; www.pnas.gov/cgi/doi/10.1073/pnas.092136199)).



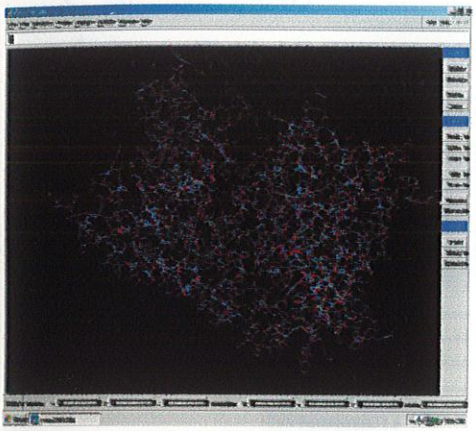
Cultured hippocampal neurons obtained from wild type (A & D), tau knockout (B & E) and human tau transgenic (C & F) mice were incubated in the absence(A-C) or presence (D-F) of fibrillar beta-amyloid. Tau-depleted neurons did not degenerate in the presence of fibrillar beta amyloid.

The present drugs targets the amyloid proteins, so new drugs are required which may block the abnormal functioning of Tau protein, thus preventing the formation of Neurofibrillary Tangles, and hence may be used for curing the Alzheimer's Disease.

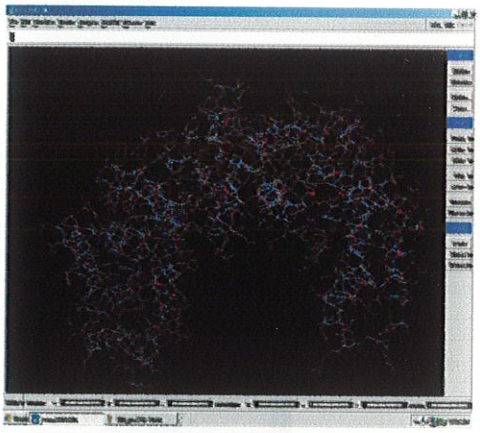
CHAPTER 13

PROCEDURE

The Docking of the protein was done using MOE, for two proteins—

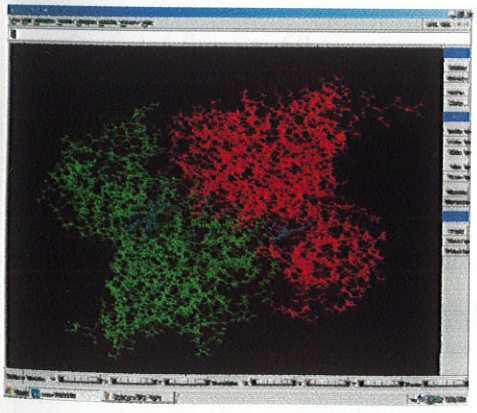


1J1B.pdb

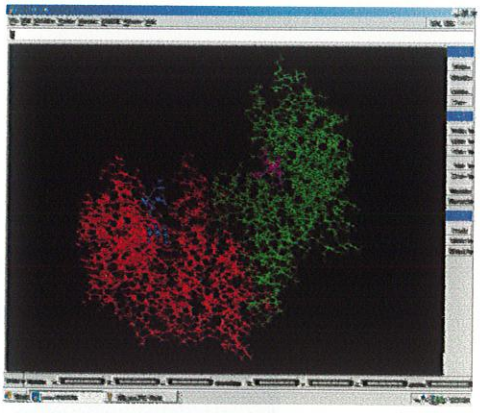


2BTP.pdb

Both the proteins consist of two ligand molecules, so the docking was performed separately for each of the ligands.



1J1B

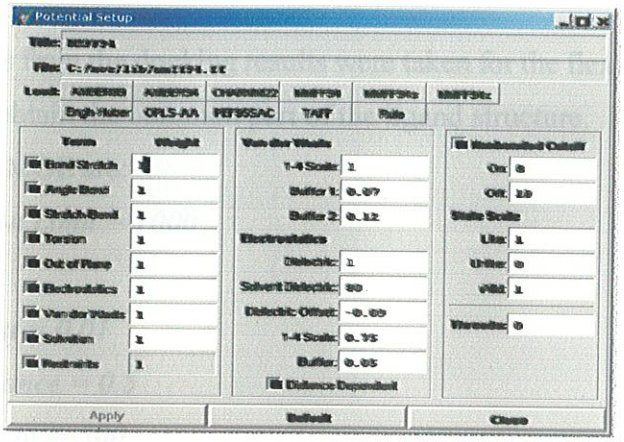


2BTP

Before performing the Docking process, the energy of the protein molecule was minimized using the Energy minimization algorithm of MOE tool. The heavy molecules were fixed before minimization and Hydrogen molecules were added to it. The minimized structures were saved as 1j1b_min.moe & 2btp_min.moe in the working directory.

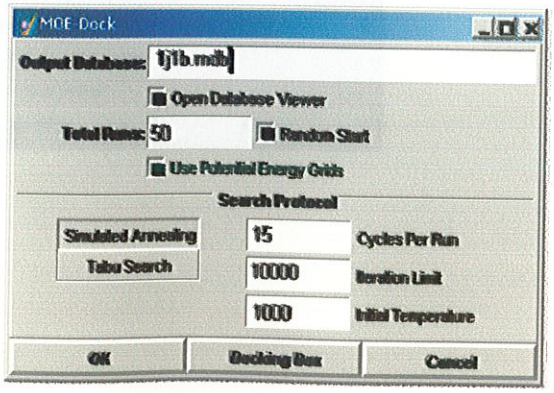
The following parameters were used for energy minimization:—

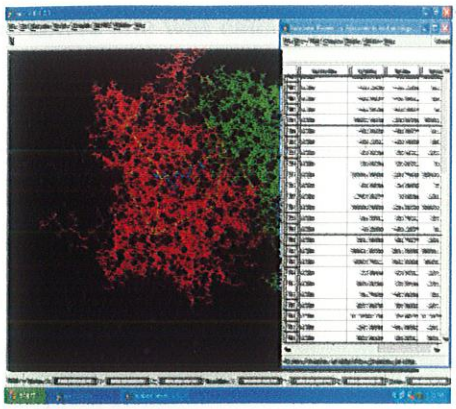
- ✓ *Gradient = 0.05*
- ✓ *Force Field: MMFF94X + Solvation*
- ✓ *Chiral Constraint: Current Geometry*



The minimized structure was used as the template for Docking. 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*



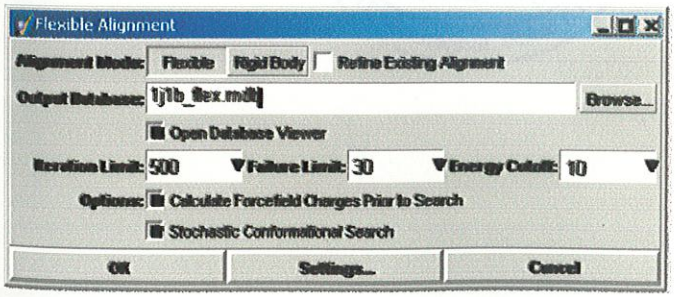


Docking in Progress

The 5 best structures from the docking results were taken for the flexible alignment of the ligand molecule to obtain the common part of the ligand structure.

Parameters used for MOE are: —

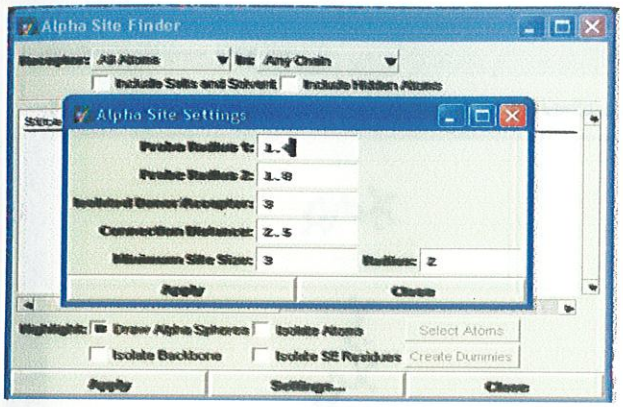
- ✓ *Configuration Limit = 1000*
- ✓ *Alpha = 2.5*
- ✓ *Gradient Test = 0.01*
- ✓ *RMSD Tolerance = 0.5*
- ✓ *Maximum Steps = 500*
- ✓ *Iteration Limit = 500*
- ✓ *Failure Limit = 30*
- ✓ *Energy Cut-Off = 10*
- ✓ *Rigid Body*



The pocket sequence of the active site was calculated by using active site finder tools of MOE & AcSite.

Parameters used in MOE: —

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



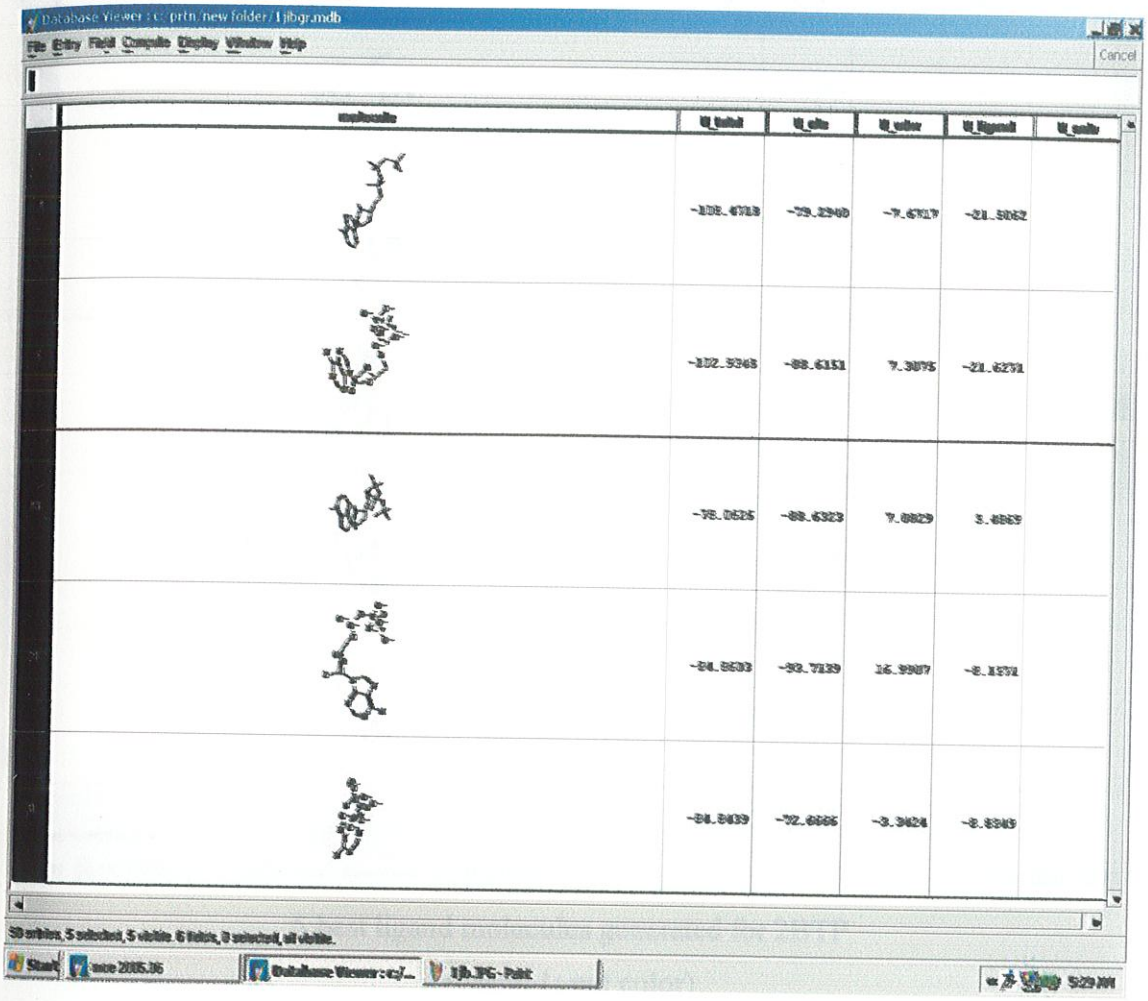
CHAPTER 14

RESULTS & DISCUSSIONS

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

5 best ligand molecules generated for 1J1B

(Chain 1; red color)

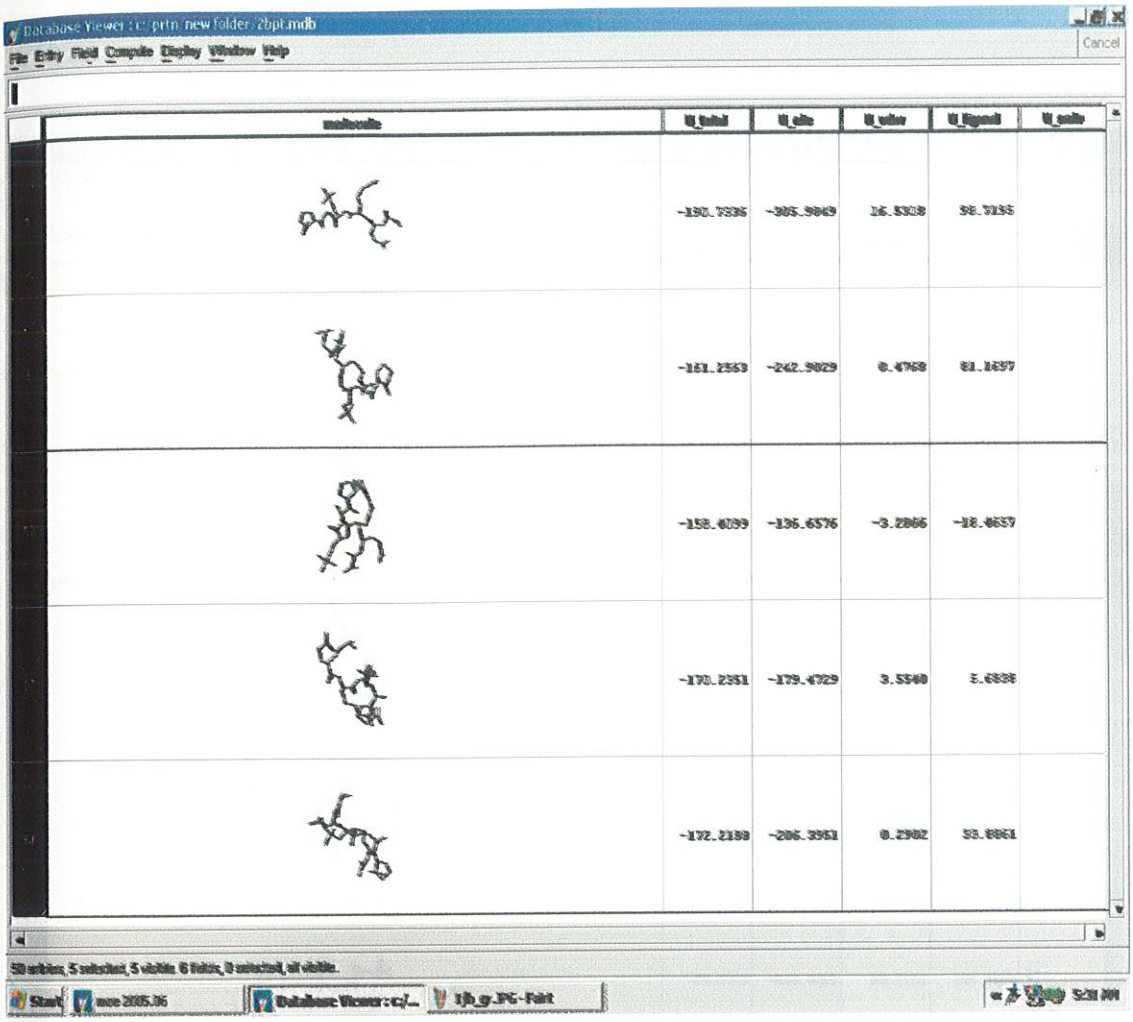


5 best ligand molecules generated for 1J1B

(Chain 2; green color)

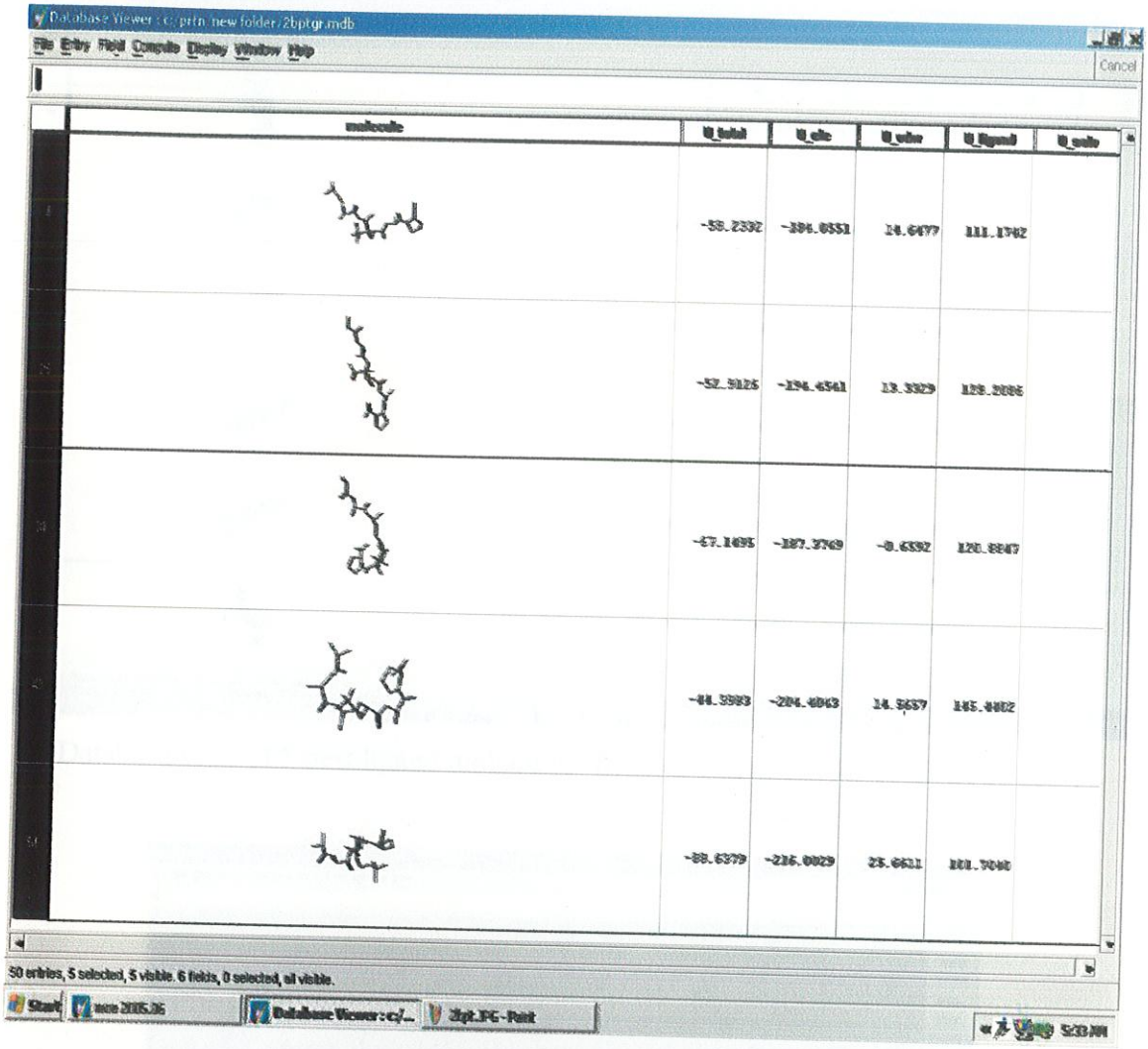
NOTE:

There were 50 structures generated for the red chain as well as green chain of 1J1B.



5 best ligand molecules generated for 2BTP

(Chain 1; red color)



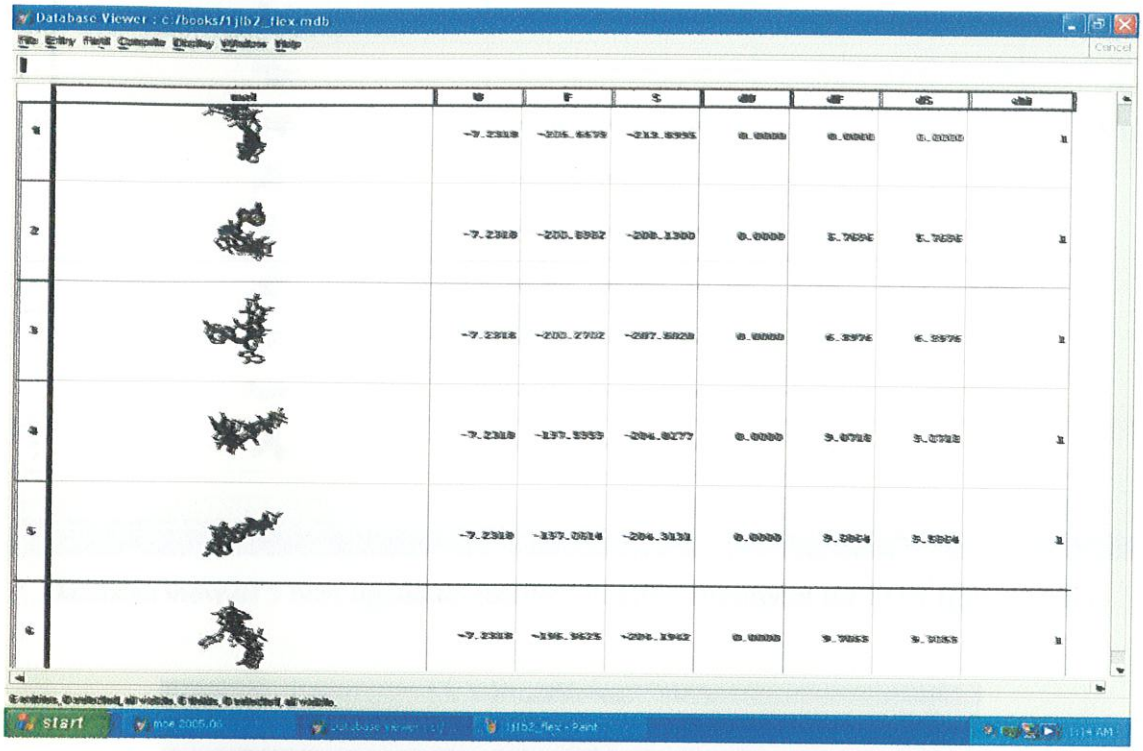
5 best ligand molecules generated for 2BTP

(Chain 2; green color)

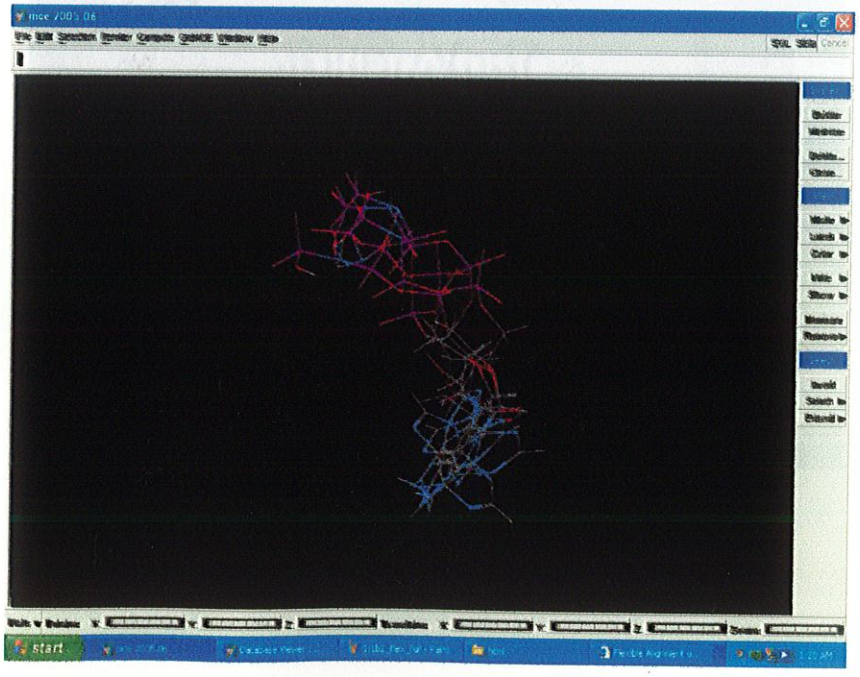
NOTE:

There were 50 structures generated for the green chain as well as red chain of 2BTP.

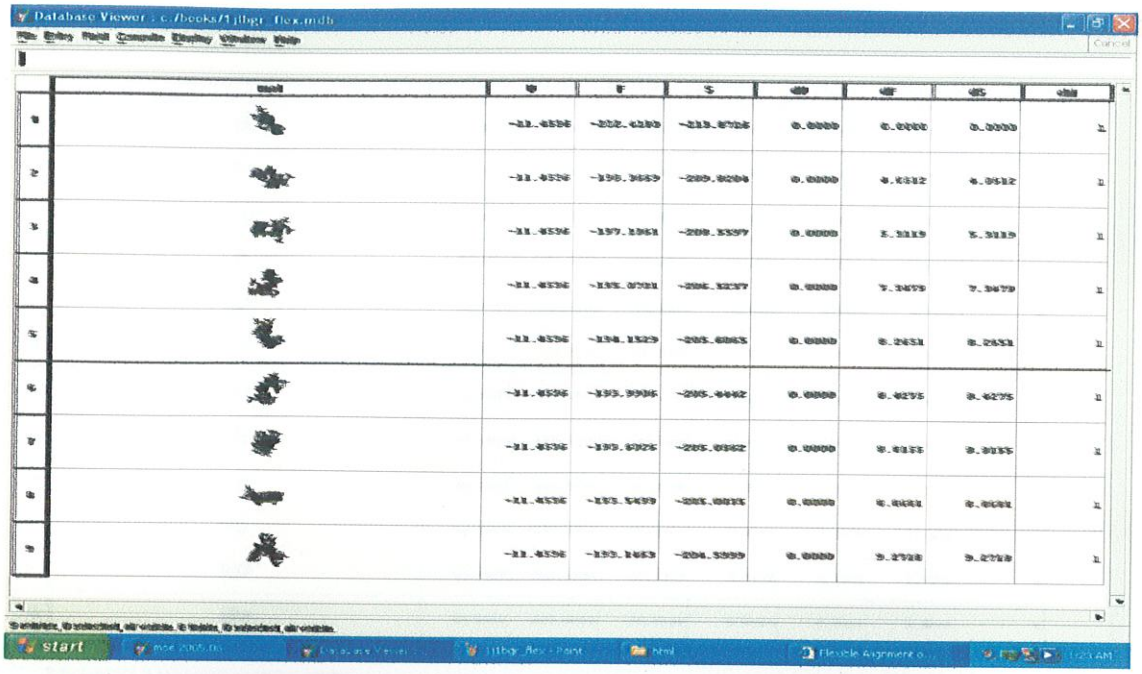
The flexible alignment algorithm produced the alignment results of the 5 considered ligand molecules, so that the similarity score and alignment score should be least.



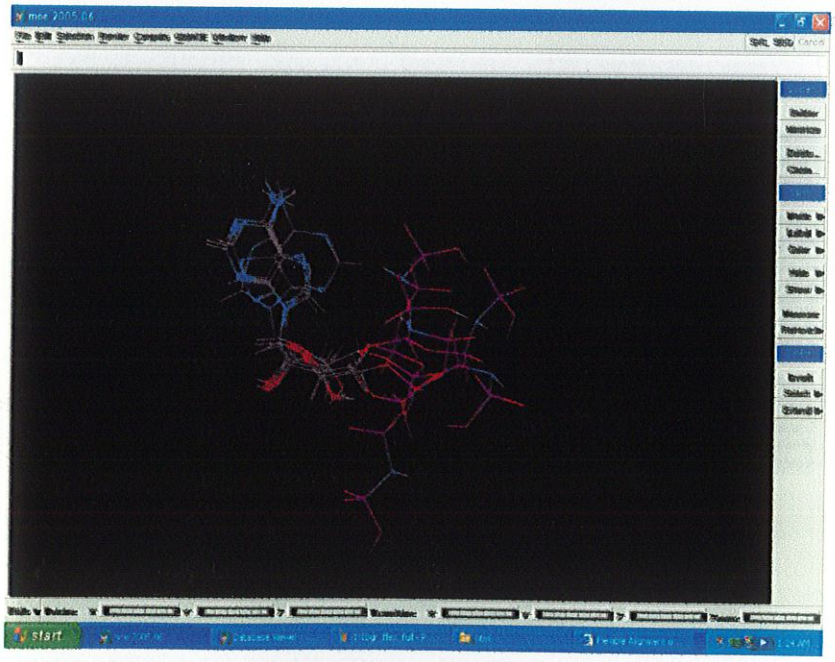
Database view of 5 best ligand molecules' flexible alignment for 1J1B (red chain)



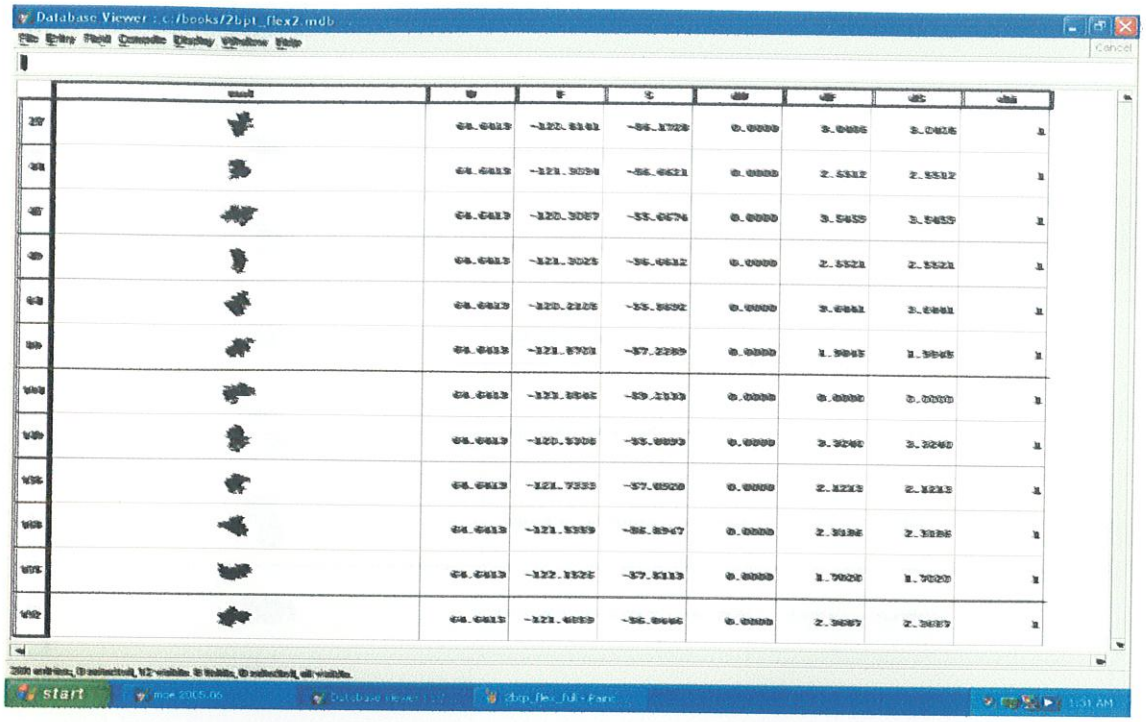
Best alignment of the 5 ligand molecules



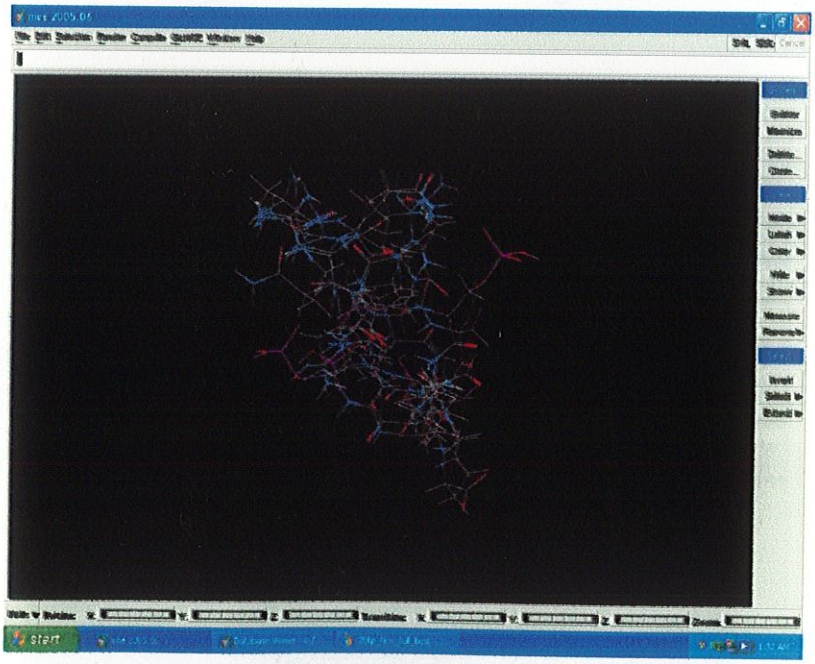
Database view of 5 best ligand molecules' flexible alignment for 1J1B (green chain)



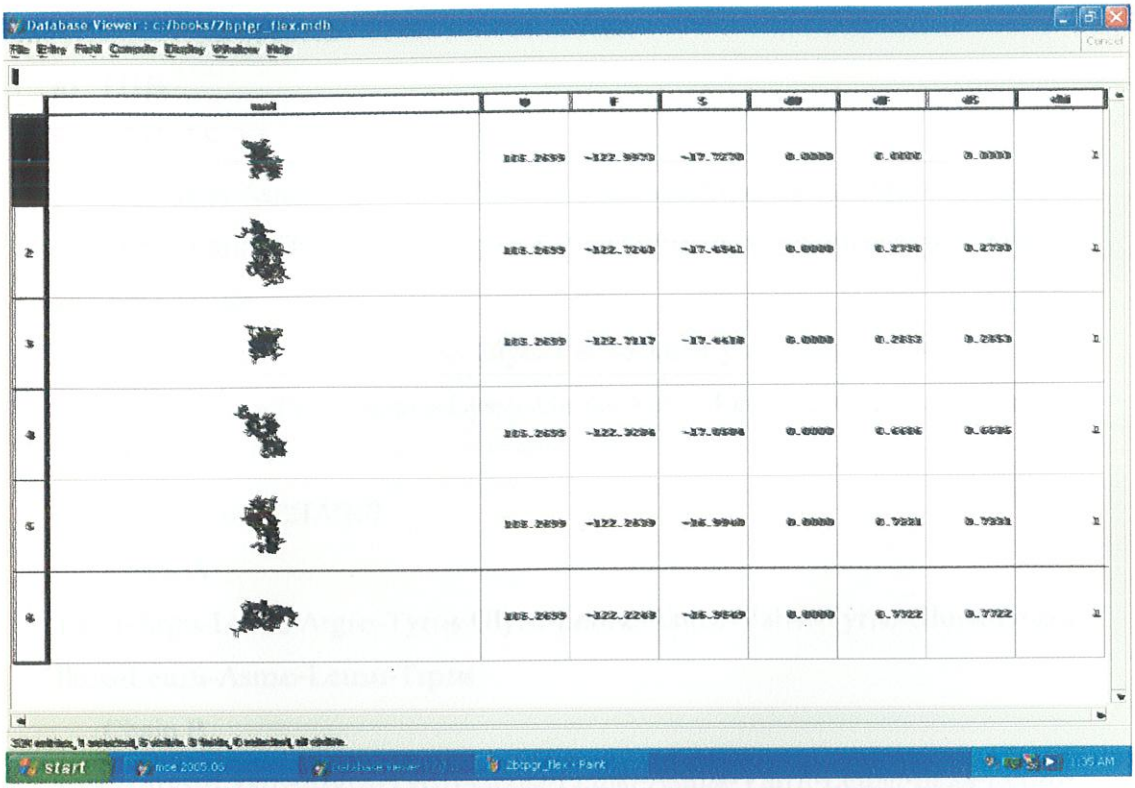
Best alignment of the 5 ligand molecules



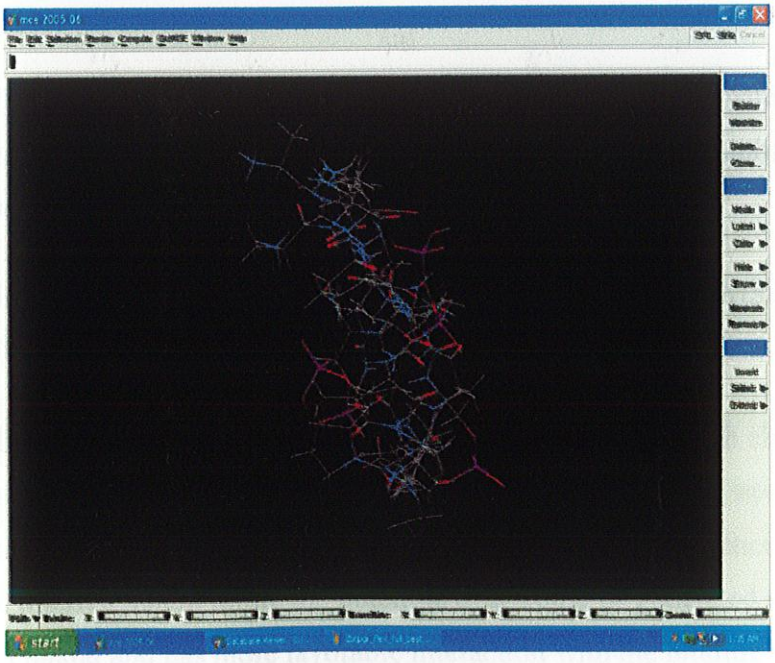
Database view of 5 best ligand molecules' flexible alignment for 2BTP (red chain)



Best alignment of the 5 ligand molecules



Database view of 5 best ligand molecules' flexible alignment for 2BTP (green chain)



Best alignment of the 5 ligand molecules

The Amino Acids sequence of the Pocket site for:

a) 1J1B

RED CHAIN

Ile_{28}-Gly_{29}-Asn_{30}-Gly_{31}-Ser_{32}-Phe_{33}-Gly_{34}-Val_{36}-Ala_{49}-Lys_{51}-Val_{76}-Leu_{98}-Asp_{99}-Tyr_{100}-Val_{101}-Thr_{104}-Arg_{107}-Asp_{147}-Lys_{149}-Gln_{151}-Asn_{152}-Leu_{154}-Asp_{166}-Asp_{242}

GREEN CHAIN

Ile_{40}-Gly_{41}-Asn_{42}-Gly_{43}-Phe_{45}-Gly_{46}-Val_{48}-Ala_{61}-Lys_{63}-Val_{88}-Leu_{110}-Asp_{111}-Tyr_{112}-Val_{113}-Thr_{116}-Asp_{159}-Lys_{161}-Gln_{163}-Asn_{164}-Leu_{166}-Cys_{177}-Asp_{178}-Asp_{230}

b) 2BTP

RED & GREEN CHAINS

Chain A

Lys_{71}-Arg_{78}-Lys_{138}-Arg_{145}-Tyr_{146}-Gly_{187}-Leu_{190}-Asn_{191}-Val_{194}-Tyr_{197}-Glu_{198}-Leu_{234}-Ile_{235}-Leu_{238}-Asn_{242}-Leu_{245}-Trp_{246}

Chain B

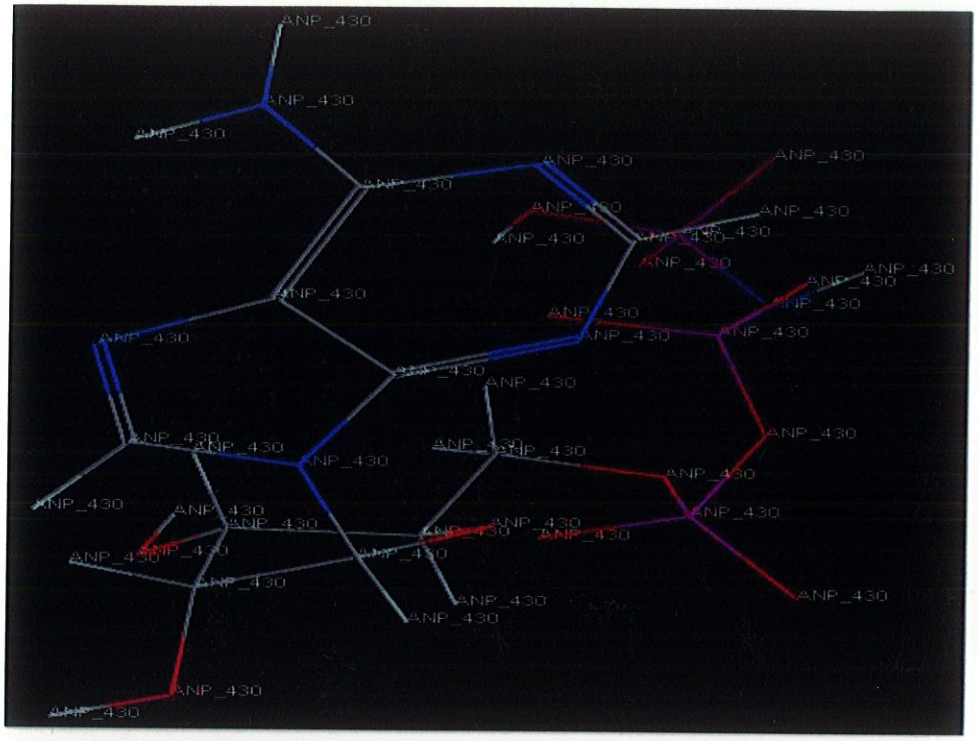
Lys_{48}-Arg_{55}-Lys_{115}-Arg_{122}-Tyr_{123}-Gly_{164}-Leu_{167}-Asn_{168}-Val_{171}-Leu_{207}-Ile_{208}-Leu_{211}-Asn_{215}-Leu_{218}

NOTE:

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 best ligands for 1JIB (green and red chains) and for 2BTP (both chains) (see figures below) have been reported with amino acid residues. Based upon the residue information of the active sites of Tau Protein and the ligand's sequence information, it could be inferred that in future the potential drug could be generated which replaces the current ligand and has more favorable interaction with the binding site. Further this should enable us to block the abnormal functioning of tau protein which may stop the fatal brain disorder i.e. AD.



(a)

(b)

(c)

Fig. (a)-Ligand for 1JIB (Red Chain), (b) - ligand for 1JIB (Green Chain) & (c) - ligand for both chains of 2BTP

In the following figures shown above:
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.

The same kind of bonding is between the following residues of 1st and 3rd chain of 1J1BO 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.

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.

Complete Description of the contacts is shown below (Table 1 & 2):-

Table 1 – Protein Contact Report of 2BTP.pdb

Contact types:

Ionic bonds
Hydrophobic contacts
Hydrogen bonds
Disulfide bonds

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
HB	1:2BTP.A	100	TYR82.OH	2:2BTP.B	17	ARG18.NE	3
HB	1:2BTP.A	107	GLU89.OE1	2:2BTP.B	17	ARG18.NH1	3
HB	1:2BTP.A	71	LYS49.NZ	3:2BTP.P	6	PRO7.O	9
HB	1:2BTP.A	78	ARG56.NH1	3:2BTP.P	4	SEP5.O3P	1
HB	1:2BTP.A	145	ARG127.NH1	3:2BTP.P	4	SEP5.O1P	1
HB	1:2BTP.A	146	TYR128.OH	3:2BTP.P	4	SEP5.O2P	1
HB	1:2BTP.A	191	ASN173.OD1	3:2BTP.P	5	ALA6.N	8
HB	1:2BTP.A	242	ASN224.ND2	3:2BTP.P	3	ARG4.O	10
HB	2:2BTP.B	48	LYS49.NZ	4:2BTP.Q	6	PRO7.O	9
HB	2:2BTP.B	55	ARG56.NH1	4:2BTP.Q	4	SEP5.O3P	1
HB	2:2BTP.B	122	ARG127.NH1	4:2BTP.Q	4	SEP5.O1P	1
HB	2:2BTP.B	123	TYR128.OH	4:2BTP.Q	4	SEP5.O2P	1
HB	2:2BTP.B	168	ASN173.OD1	4:2BTP.Q	5	ALA6.N	8
HB	2:2BTP.B	215	ASN224.ND2	4:2BTP.Q	3	ARG4.O	10
ION	1:2BTP.A	107	GLU89.OE1	2:2BTP.B	17	ARG18.NH1	12
ION	1:2BTP.A	71	LYS49.NZ	3:2BTP.P	6	PRO7.O	5
ION	1:2BTP.A	78	ARG56.NH1	3:2BTP.P	4	SEP5.O3P	2
ION	1:2BTP.A	145	ARG127.NH1	3:2BTP.P	4	SEP5.O1P	2
ION	2:2BTP.B	48	LYS49.NZ	4:2BTP.Q	6	PRO7.O	5
ION	2:2BTP.B	55	ARG56.NH1	4:2BTP.Q	4	SEP5.O3P	2
ION	2:2BTP.B	122	ARG127.NH1	4:2BTP.Q	4	SEP5.O1P	2

Table 2 – Protein Contact Report of 1JIB.pdb

Contact types:

Ionic bonds
Hydrophobic contacts
Hydrogen bonds
Disulfide bonds

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

CHAPTER 15

CONCLUSIONS

In this project, I studied the abnormal functioning of Tau protein as a causative agent of Alzheimer's disease. Tau protein is present in the brain of normal human being and its working is normal in healthy person. But in case of diseased person, Tau starts behaving abnormally and results in the formation of Neurofibrillary tangles. These tangles are responsible for AD. The formation of tangles can be blocked by altering the active site of the abnormal tau protein. The active site sequence of the target protein was found by using MOE and the ligand molecules against this active site were designed, which interacts with the residues present at the active site. Theses ligand molecules may be used as drug molecules against infected Tau protein to stop the cause of AD.

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.

This project will also be helpful for the researchers, who are involved in the drug designing against AD. This process of designing the drugs is less expensive then the present procedures in use and also they are less time consuming. In future the potential drug could be generated which replaces the current ligand and has more favorable interaction with the binding site.

CHAPTER 16

BIBLIOGRAPHY

Websites Used:

- ✓ **http://www.nia.nih.gov/Alzheimers/AlzheimersInformation/Treatment/**
- ✓ **http://alpha2.bmc.uu.se/Courses/Bke1/2000/Projects/Alzheimer/**
- ✓ **http://homepages.strath.ac.uk/~dfs99109/BB310/AD.html**
- ✓ **http://www.j-alz.com/index.html**
- ✓ **http://en.wikipedia.org/wiki/Alzheimer's_disease**

Publications:

- ✓ Alzheimer's Disease Fact sheet
  - ❖ NIH publication no. 03-3431 December 2005
- ✓ Tau protein needed for Alzheimer's disease
  - ❖ Adriana Ferreira (NUIN news – 2002)
- ✓ C-terminal inhibition of tau assembly in vitro and in Alzheimer's disease
  - ❖ Aida Abraha, Nupur Ghoshal, T. Chris Gamblin, Vincent Cryn, Robert W. Berry, Jeff Kuret and Lester I. Binder
- ✓ Experimental Alzheimer drugs targeting beta-amyloid and the "amyloid hypothesis
  - ❖ Fact Sheet: Nov 30, 2005
- ✓ Geometric methods in Molecular Docking
  - ❖ Saumyadipta Pyne, Gayathri Priyalakshmi (BII)
- ✓ Approaches to Molecular Docking
  - ❖ Aruna Ranganath K., Proff. K. C. Shet, Dr. N. Vidyavathi (BII)
- ✓ Alzheimer's disease
  - ❖ Chandra A. Reynolds, Michael Crowe (Nature)