THE STUDY OF GPCRS AS THE POTENTIAL DRUG TARGETS AND APPLYING THE KNOWLEDGE OF GPCRS TO:

1. EXPLORE THE CAUSE OF ALZEHIMER'S DISEASE.
2. POSSIBLE WAYS TO PERVENT THIS DISEASE.
3. DESIGN THE POTENTIAL DRUG FOR AD.

By

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Submitted in partial fulfillment of the degree of Bachelor of Technology

DEPARTMENT OF BIO-INFORMATICS
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CERTIFICATE

This is to certify that the work entitled," The study of GPCRs as potential drug targets, and applying the knowledge of GPCRs to

1. Explore the causes of Alzheimer's disease.
2. Possible ways to prevent this disease.
3. Design the potential drugs for AD."

Submitted by ADITYA SINGH in partial fulfillment for the award of degree of Bachelor of Technology in Bio-Informatics of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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ACKNOWLEDGEMENT

I Aditya Singh is very happy to present this project to you. This Project has been done on MOE and we have worked very hard to extract utilities of MOE to provide all the sophistication and results true to my knowledge. This project could not have been possible without the help of my respected supervisor and my friends who supported me throughout the project.

The demand for bioinformaticians is very high and it's the responsibility of a bioinformaticians to study and help this world in every possible way by making the small islands of knowledge assessable to common and all.

I hope the knowledge I have gained by working on this project will help all in need.

"The possession of knowledge does not kill the sense of wonder and mystery. There is always more mystery"

ANAIS NIN

Aditya Singh

021509

TABLE OF CONTENTS

BASIC REQUIREMENTS

The package for the project will require

1) MOE software
2) IBM or compactable PC
3) Internet connection
4) 128 MB RAM
5) 256 MB Hard Disk

PROJECT DESIGN

Flow Chart:

The project "The study of GPCRs as potential drug targets, and applying the knowledge of GPCRs to

1. Explore the causes of Alzheimer's disease.
2. Possible ways to prevent this disease.
3. Design the potential drugs for AD"

Has been undertaken at Jaypee University of Information Technology Waknaghat.

The project is carried out in three phases

1. Data Collection
2. Input data compilation
3. Data analysis

LIST OF ABBREVITIONS

AD: Alzheimer's disease

APP: amyloid precursor protein

E.C number: Enzyme Classification number

FAD: familial Alzheimer's disease

GPCRs: G-protein-coupled receptors

GRKs: G-protein-coupled receptor kinases

HTS: High throughput screening

PS: presenilin

LOAD: late onset Alzheimer's disease

MOE: molecular operating environment

NSAIDs: non-steroidal anti-inflammatory drugs

PMID: PubMed Identifier

IU: International Unit

IDE: Insulin Degrading Enzyme

DHA: Docahexaenoic acid

SVL: Scientific Vector Language

ABSTRACT

The first most important step in this project is the collection of data. The data for the project was collected from various websites these websites were found with the help of Google search engine. The protein was chosen from pdb website and they were thus downloaded in pdb format. They were then saved on the disk and thus were used as input data for the MOE software. The first step in the MOE software is energy minimization. The stability of the protein depends on its energy level. Lower the energy more stable is the protein. The protein was then saved in moe format with minimum energy. The next step in the process is homology modeling. The homology modeling is done to get the structure of the protein. This also helps in saving the file in mdb format. The ligands were found for the protein and then the next step is docking. Docking is done to get the right orientation of the active site and the ligand. This helps in finding the right side of the binding and the activity. Though these steps seem simple from the point of view that they are small but the practical implementation of these steps is quite hard. As the implementation of these steps requires a lot amount of time and understanding. The steps are to be completed and studied .also one of the major step was the generation of pharmacophore database or ph files. The moe is a powerful tool and help in achieving these steps without any further delay.

INTRODUCTION

MOE stands for molecular operating environment. MOE is not a software package in the usual sense, but an integrated Methodology Development Platform; that is, a tool for chemical computing software development and deployment. MOE integrates visualization, simulation and application development in one package. Custom methodology modules can be developed with the built-in high-performance data-parallel programming language SVL, the Scientific Vector Language.

MOE delivers the following benefits:

Time Savings. SVL programs are concise and easy to write. Reductions in code size of 10 to 1 are routinely realized. Because SVL programs are small and portable (running on any platform that MOE runs on), maintenance costs are minimized as well.

Creative Throughput. New methodology can be prototyped, experimented with, modified and verified quickly. In almost all cases, no recoding to C or Fortran is necessary since SVL is a high-performance programming language.

Research Competitiveness. With C or Fortran, the incorporation of the latest theoretical ideas requires significant resources. Often, the costs are prohibitive and scientists are forced to wait until commercial versions become available. With MOE, the latest published methodology can be implemented and validated with minimal expenditure.

Cost Savings. A significant amount of commercial computational chemistry software can be implemented with MOE with extremely few lines of code. The MOE system provides the critical mass of tools needed to reduce the dependence on software that integrates poorly with in-house methodology.

The following is a schematic of the architecture of MOE.

1. SVL Methodology, Libraries, Applications

Using MOE's built-in high-performance programming language, SVL, new chemical computing methodology can be implemented quickly and easily. >From rapid methodology prototyping and validation to application deployment, MOE dramatically increases a computational chemist's productivity and research throughput.

The Scientific Vector Language (SVL), is a new high-performance data-parallel programming language built into the MOE Molecular Operating Environment. SVL is an embedded language; that is, its compiler and run-time environment are an integral part of MOE. SVL serves as the command, macro, scripting, and high-performance computing language of MOE. Standard and add-on SVL applications from Chemical Computing Group provide the starting point for as-is applications use or custom SVL methodology development. The base MOE system comes with the source code for many SVL programs.

2. Graphical User Interface Toolkit

The Graphical User Interface Toolkit facilities of SVL make interface design and implementation fast and painless. Powerful and automatic control panel layout algorithms, a rich set of high-level interface widgets, bubble help facilities and the multi-thread paradigm are the gateway to fast interface development.

For example, a Molecular Mechanics energy minimization interface that looks like: complete with Bubble Help (or Tool Tips) and data value validation requires roughly fifty lines of SVL code.

3. Molecular Tools

The heart of MOE's chemical awareness is the collection of flexible and powerful molecular data structures and algorithms. These facilities can be exploited through the use of SVL, the built-in programming language of MOE. The generic molecular tools of MOE contain:

Hierachical object-oriented molecular data structures.

Flexible and extensible energy models.

Substructure search facilities.

Random-access read/write molecular databases.

Automatic bond order and RS chirality detection.

Protein construction and analysis facilities.

4. Portability Toolbox

The MOE system delivers two layers of portability.

Firstly, SVL, the built-in programming language of MOE, is a byte-code layer of portability in that SVL programs will run on all machines to which MOE has been ported.

Secondly, the internal MOE architecture has a portability layer that isolates all operating and window system specific code. With only 4000 lines of machine specific code, MOE is truly portable.

Our goal is to have every SVL program automatically parallelized. This means a single SVL source program would be used for both sequential and parallel computers.

MOE for Academics

Chemical Computing Group Inc. is pleased to make its chemical computing software, MOE - the Molecular Operating Environment, more available to members of the academic community. We will be providing academic researchers MOE's full range of capabilities and functionality, but at a greatly discounted pricing schedule.

MOE delivers academic researchers:

A customizable environment allowing for quick prototyping of new ideas and the ability to create your own end applications;

A unique teaching tool that allows your students to "look under the hood" at the underlying algorithms;

Out-of-the-box functionality covering the spectrum of drug research delivering powerful ready-to-use applications in the areas of: HTS, Combichem, protein/homology modeling, modeling & simulations and methods development.

MOE in Academia

Chemical Computing Group is pleased to announce that the latest version of its web page contains a "guest feature" by Dr. Jeffry Madura from Dusquesne University entitled: "Experiences Using MOE in Academia."

This feature explores how one university is using MOE to further research and education in 5 different areas: Docking, Poisson-Boltzmann Electrostatics, Gaussian Molecular Orbitals, Dynamics Animation, and in the Classroom. Samples of Dr. Madura's uses and experiences in each area are included in the article.

MOE can aid in your research through its:

Built in applications, source code for easy customization and an embedded programming language for quick prototyping of new ideas.

Full integration of visualization and simulation of proteins and small molecules.

Platform independence: running on WIN 95-98, NT, SGI UNIX/NT, SUN, DEC Alpha NT, and HP-UX.

Variety of Access Modes from graphical to batch and new to MOE WEB MODE ACCESS!

Turn key package that includes applications in the areas of HTS, Combichem, Protein Modeling and 3D Bioinformatics, Molecular Modeling and Simulation and Methods Development.

approximately 7-10 years, although cases are known where reaching the final stage occurs within 4-5 years, or up to 15 years.

Diagnosis

The diagnosis is made primarily by clinical observation and tests of memory and intellectual functioning over a series of weeks or months, with various physical tests (blood tests and neuroimaging) being performed to rule out alternative diagnoses. No medical tests are available to conclusively diagnose Alzheimer's disease pre-mortem, however.Interviews with family members and/or caregivers can be extremely important in the early phases as well, as the sufferer him/herself may tend to minimize his symptomatology or may be being observed on a day when his/her symptoms are in temporary dormancy.

Initial suspicion of dementia may be strengthened by performing the mini mental state examination, after excluding clinical depression. Psychological testing generally focuses on memory, attention, abstract thinking, the ability to name objects, and other cognitive functions. Results of psychological tests do not easily distinguish between Alzheimer's disease and other types of dementia but can be helpful in establishing the presence of and severity of dementia. They can also be useful in distinguishing true dementia from temporary (and more treatable) cognitive impairment due to depression or psychosis, which has sometimes been termed "pseudodementia".While expert clinicians who specialize in memory disorders can now diagnose AD with an accuracy of 85-90%, a definitive diagnosis of Alzheimer's disease must await the autopsy.

Pathology

Microscopy

There are several changes found in the brain in AD (in order of appearance):

- The deposition of an abnormal protein (amyloid beta) outside nerve cells in the form of amyloid. These are called diffuse plaques and amyloid also forms the core of more organized plaques called senile or neuritic plaques. Recently evidence has begun to accumulate implicating simpler, soluble forms of amyloid (oligomers) in the pathological process, and the presence of plaque amyloid does not correlate well with the degree of dementia. Amyloid also accumulates in the walls of small blood vessels in the brain. This is termed amyloid angiopathy (also called congophilic angiopathy). Another pathological feature of AD is the accumulation of abnormal protein filaments inside nerve cells in the brain, formed from aggregation of tau protein, which is normally present to stabilise microtubules.
- In AD, an abnormally phosphorylated form of tau protein accumulates as paired helical filaments. Tau accumulates in various forms:
    - As masses of filaments inside nerve cell body termed neurofibrillary tangles
    - Inside nerve cell processes in the brain termed neuropil threads

- Inside nerve cell processes that surround amyloid plaques - termed dystrophic neurites or plaque neurites.

General non-specific findings include:

- Diffuse neuropathology, nerve cells, their processes, and synapses are lost from key brain regions. This results in atrophy of the affected areas and enlargement of the ventricles.
- Loss of synaptic contacts between neurons may be related to disruption of axonal transport and to the dysregulation of cell adhesion proteins by presenilins. The presenilins have been identified as part of the processing pathways that produce the amyloid beta protein.

Neurochemistry

The neurotransmitters serotonin, acetylcholine, norepinephrine, and somatostatin are at decreased levels. Glutamate levels are usually elevated.

Disease mechanism

Three major competing hypotheses exist to explain the cause of the disease.

The oldest hypothesis is the "cholinergic hypothesis". It states that Alzheimer's begins as a deficiency in the production of acetylcholine, a vital neurotransmitter. Much early therapeutic research was based on this hypothesis, including restoration of the "cholinergic nuclei". The possibility of cell-replacement therapy was investigated on the basis of this hypothesis. All of the first-generation anti-Alzheimer's medications are based on this hypothesis and work to preserve acetylcholine by inhibiting acetylcholinesterases (enzymes that break down acetylcholine). These medications, though sometimes beneficial, have not led to a cure. In all cases, they have served to only treat symptoms of the disease and have neither halted nor reversed it. These results and other research have led to the conclusion that acetylcholine deficiencies may not be directly causal, but are a result of widespread brain tissue damage, damage so widespread that cell-replacement therapies are likely to be impractical.

The other two hypotheses each have their advocates, and have often been described (lightheartedly) as the "tau-ist" and "ba-ptist" viewpoints in scientific publications by Alzheimer's disease researchers. "Tau-ists" believe that the tau protein abnormalities come first and lead to a full disease cascade. "bA-ptists" believe that beta amyloid deposits are the causative factor in the disease. For example, the presence of the APP gene on chromosome 21 is believed to explain the high incidence of early-onset AD pathology in patients with Down syndrome, who carry three copies of chromosome 21 and thus APP itself. The "ba-ptist" theory is finding new supporters due to recent discoveries of impaired vascular and cerebrospinal fluid transport of beta amyloid out of the brain tissues, resulting in a greater risk for plaque formation. A third protein, alpha synuclein, which has already been shown to be important in Parkinson's disease, has also

Environmental Conditions

Studies have not shown strong link with toxins, vitamins, metals or diet, although rabbits fed a high-cholesterol diet in the presence of copper ions in their water did develop amyloid brain lesions and cognitive deficiencies. Likewise, linkage has been found between zinc or copper and reactive oxidative stress contributing to Alzheimer's pathology and the amyloid precursor protein has been shown to alter expression in response to metal supplementation and chelation. Therefore, it is hasty and premature to dismiss any and all environmental effects out of hand. There have been studies that link aluminium to the progression of Alzheimer's, but the results from these studies have not been confirmed and are not widely accepted by Alzheimer's experts.

Genetic linkage

Alzheimer's disease is linked to the 1st, 10th, 14th, 9th, 19th, and/or 21st chromosomes, among others. While some genes predisposing to AD have been identified, most cases are sporadic. However, sporadic AD most often involves some form of genetic susceptibility.

Epidemiology

Alzheimer's disease is the most frequent type of dementia in the elderly and affects almost half of all patients with dementia.

2-3% of persons aged 65 show signs of the disease, while 25 - 50% of persons aged 85 have symptoms of Alzheimer's and an even greater number have some of the pathological hallmarks of the disease without the characteristic symptoms. The proportion of persons with Alzheimer's begins to decrease after age 85 because of the increased mortality due to the disease, and relatively few people over the age of 100 have the disease.

Prevention

Efforts to find effective treatments for Alzheimer's after-the-fact have so far been disappointing. Age is the primary risk factor for Alzheimer's. The baby boom is approaching its golden years. Indeed, much of the concern about the solvency of governmental social safety nets is founded on estimates of the costs of caring for baby boomers, assuming that they develop Alzheimer's in the same proportions as earlier generations.

One study ("Leisure Activities and the Risk of Dementia in the Elderly," New England Journal of Medicine found that people who played chess on a regular basis went on to get Alzheimer's at a substantially lower rate than the general population. The chess relationship was stronger than any other factor, including dancing and solving crossword puzzles, both of which were also shown to be inversely proportional to getting Alzheimer's disease.

In a number of retrospective studies, regular physical exercise has appeared to be inversely related to the development of Alzheimer's. The Alzheimer's risk of those exercising regularly was half that of the least active. This research is consistent with the observation that virtually all measures designed to promote cardiac fitness and reduce stroke risk also seem to reduce Alzheimer's risk. However in one study, dance appeared to be the only exercise effective in reducing risk. One explanation is that dancing requires the use of complex mental skills such as performing correct steps while at the same time keeping track of the music. The presence of cardiovascular risk factors -- diabetes, hypertension, high cholesterol and smoking -- in middle age (ages 40 to 44) was found very strongly associated with late-life dementia (Neurology 2005;64:277-281. PMID 15668425).

Some studies have indicated that non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and aspirin may delay the onset, and lower the ultimate risk, of Alzheimer's disease. According to population studies, low but consistent daily NSAID used over a period of years such as ibuprofen (Advil, Motrin) seems to slow the progress of Alzheimer's. It seems that NSAIDs may affect the onset of the disease but is of little use for treating it once it has progressed to early or full-blown Alzheimer's.

It should be noted that some drugs such as acetaminophen, naproxen, and COX-2 inhibitors, such as celebrex and vioxx, were found to have no demonstrated benefit (and some evidence of cardiac harm). This ineffectiveness and the increase in adverse cardiac events associated with these agents was reported in various studies in 2004, and highlights the key role of ibuprofen in the original studies showing moderated risk associated with NSAID use (PMID 15720180).

A study (Archives of Neurology 2004; 61:82-88. PMID 14732624) has reported that the combination of vitamins E and C might, over time, sharply reduce the risk of Alzheimer's disease. Marked reduction (up to 80% risk reduction) was achieved after a period of more than five years, but only if dosage was 400 IU per day of vitamin E plus 500 mg or more per day of vitamin C. Lesser amounts, such as those found in multivitamin pills, appeared markedly less effective. Large doses of vitamin E without vitamin C had only a mild effect, while large doses of vitamin C without vitamin E had no benefit. However in one small study, 2000 IU per day of vitamin E did appear to delay the progression of early Alzheimer's by several months. Other evidence suggests that vitamin E becomes a damaging pro-oxidant if given in isolation (without other antioxidants). Vitamin E can be recharged after absorbing a free radical by another antioxidant such as vitamin C or Alpha-lipoic acid. Some studies suggest that a ratio of at least 1000 mg of vitamin C to 400 IU of vitamin E is ideal. Recent studies suggest that the most common forms of E sold in supplements, the dl-alpha or d-alpha tocopherol form, are of little value, and that the gamma form of vitamin E, or a mixture of all the tocopherols and tocotrienols that collectively make up vitamin E from food, provide the most benefit. Vitamin E is markedly less effective unless taken with oil.

Improved nutritional status of the B vitamin folic acid was found to reduce Alzheimer's incidence in a study of an order of nuns, many of whom volunteered to have their mental

status assessed and donated their brains for study after death. The "Nun's study" also revealed nuns who, in life, showed little or no dementia, but upon autopsy were found to have extensive Alzheimer's plaques. The unimpaired nuns' brains were free of evidence of stroke, including micro-strokes. Nuns whose brains revealed both plaques and stroke damage, however, were severely impaired in functioning while alive. Thus avoidance of risk factors for stroke may be a key element in preventing final progression to being disabled by Alzheimer's dementia. The discovery of the co-founding role of stroke supports other research showing that quitting smoking, weight reduction, and avoidance of diabetes all reduce Alzheimer's risk. Diabetes greatly increases Alzheimer's risk, and one factor at work may be that the enzyme charged with removing excess insulin from the blood, the Insulin Degrading Enzyme (IDE), also has the responsibility for removing Beta-amyloid plaques from the brain. Perhaps the excess insulin involved in the pre-diabetic metabolic syndrome, as well as insulin used to treat existing diabetes, may demand more IDE than the body is able to produce, leaving none to remove accumulating beta amyloid plaques from the brain.

Some evidence suggests that Alzheimer's risk may also be reduced by inclusion of certain kinds of fish in the weekly diet. Those that contain Omega-3 fatty acids are thought to most effective.

The natural chemical curcumin, found at 5% concentration in the spice turmeric, reduces Alzheimer's incidence in a mouse model and actually dissolves human senile plaques (beta amyloid) in the test tube (PMID 15590663). These factors suggest that inclusion of a bit of turmeric or curry spice in the diet may provide preventive value. Near 100% curcumin extract capsules are also available. Curcumin is a powerful antioxidant and a powerful anti-inflammatory. In India, where turmeric is commonly consumed in curry spices, Alzheimer's disease afflicts only approximately 1% of the elderly, whereas in the U.S. a much larger percentage is afflicted.

There may be a connection between the cholesterol level inside the brain cells and the deposition of the toxic amyloid plaques which make the brain cells die. In addition to lowering cholesterol, the so-called statins (drugs such as lovastatin, simvastatin, etc.) may have a beneficial role in reducing inflammation. However, retrospective studies into possible protective effects of statin drugs as a means of preventing or delaying Alzheimer's have been inconclusive; no protective effect was found in one large prospective observational study (Arch Neurol. 2005;62:1047-1051. PMID 16009757).

Prospective studies and well-analyzed retrospective studies show that smoking increases the risk of developing Alzheimer's (Biomed Pharmacother. 2004 Mar;58(2):95-9. PMID 14992790). The increased risk may be substantial (J Neurol Neurosurg Psychiatry 2000;68:622-626 (May). PMID 10766894). Cigarettes contain many substances in addition to nicotine, and the increased risks incurred by smokers are not to be confused with the controversial possible slowing of the progression of established Alzheimer's disease by administration of pure medical nicotine.

Nutrition and Alzheimer's

Some work is being done to investigate the role of raised levels of homocysteine, and possible nutritional prevention or treatment through taking of foods high in B vitamins and antioxidants to control the levels of homocysteine.

A deficiency of DHA, an omega-3 fatty acid, has also been implicated in Alzheimer's.

Insulin resistance has also been associated with Alzheimer's. Remarkably, genetic epidemiology has revealed that the ApoE4 allele is found at the highest rates in populations that are current or recently were hunter-gatherers, and at the lowest rates in populations that have long been adapted to agriculture. Some have suggested that the ApoE4 gene only contributes to Alzheimer's when it is found in conjunction with a high-carbohydrate diet.

Treatment

There is currently no cure for Alzheimer's disease, although there are drugs which temporarily reduce the degradation of neuronal signaling, and slow the worsening of some symptoms, such as dementia.

Acetylcholinesterase inhibitors

Acetylcholinesterase (AChE) inhibition was thought to be important because there is selective loss of forebrain cholinergic neurons as a result of Alzheimer's. AChE-inhibitors reduce the rate at which acetylcholine (ACh) is broken down and hence increase the concentration of ACh in the brain (combatting the loss of ACh caused by the death of the cholinergin neurons). Acetylcholinesterase-inhibitors seemed to modestly moderate symptoms but do not prevent disease progression including cell death.

Examples include:

- tacrine - no longer clinically used
- donepezil (marketed as Aricept)
- galantamine (marketed as Razadyne, formerly Reminyl)
- rivastigmine (marketed as Exelon)

Recently, a controversy has erupted about cholinesterase inhibitors because a study by Courtney (2004) in the respected medical journal The Lancet has suggested they are ineffective. The pharmaceutical companies, but also many independent clinicians, dispute the findings of the study, based on methodologic grounds.

NMDA antagonists

Recent evidence of the involvement of glutamatergic neuronal excitotoxicity in the aetiology of Alzheimer's disease led to the development and introduction of memantine.

Memantine is a novel NMDA receptor antagonist, and has been shown to be moderately clinically efficacious. (Areosa et al., 2004)

Vaccine

There are ongoing tests of an Alzheimer's disease vaccine. This was based on the idea that if you could train the immune system to recognize and attack beta-amyloid plaque, the immune system might reverse deposition of amyloid and thus stop the disease. Initial results in animals were promising. However, when the first vaccines were used in humans, brain inflammation occurred in a small fraction of participants, and the trials were stopped. Participants in the halted trials continued to be followed, and some showed lingering benefits in the form of slower progression of the disease. Recent studies in mice continue to show promise that an approach may be found to avoid the inflammation issue. It is hoped that research will provide a better formulation and that in the future it can be of use in families with history of Alzheimer's disease.

Pure Medical Nicotine

One study indicated that intake of pure medical nicotine might help delay progression of Alzheimer's disease in carriers, but not non-carriers, of the ApoE4 gene. The issue of whether medical nicotine intake may delay Alzheimer's progression among some sub-populations of patients remains a focus of debate. But no one is advocating smoking, as distinct from prescription nicotine, for the treatment or prevention of Alzheimer's. In prospective studies and well-analyzed retrospective studies, smoking is shown to increase the risk of developing Alzheimer's. Biomed Pharmacother. 2004 Mar; 58(2):95-9.

Ginkgo Biloba

Some studies, summarised in a 2004 conference paper, have suggested that ginkgo biloba shows promise for alleviating the effects of Alzheimer's, however the paper concedes that further research is required, as consumption of ginkgo biloba can have undesirable side-effects, especially for those with blood circulation disorders and those taking certain medications. Ginkgo should not be used by anyone taking anti-coagulants, pregnant women, or anyone using the anti-depressant drugs known as monoamine oxidase inhibitors (MAOI).

Social issues

Alzheimer's is considered to be a major public health challenge since the median age of the industrialized world's population is increasing gradually. For this reason, money spent informing the public of available effective prevention methods may yield disproportionate benefits. The role of family caregivers has also become more prominent, as care in the familiar surroundings of home may delay onset of some symptoms and delay or eliminate the need for more professional and costly levels of care

G-protein-coupled receptors (GPCRs), also known as *seven transmembrane receptors*, *heptahelical receptors*, or *7TM receptors*, are a protein family of transmembrane receptors that transduce an extracellular signal (ligand binding) into an intracellular signal (G protein activation). The GPCRs are the largest protein family known, members of which are involved in all types of stimulus-response pathways, from intercellular communication to physiological senses. The diversity of functions is matched by the wide range of ligands recognized by members of the family, from photons (rhodopsin, the archetypal GPCR) to small molecules (in the case of the histamine receptors) to proteins (for example, chemokine receptors). This pervasive involvement in normal biological processes has the consequence of involving GPCRs in many pathological conditions, which has led to GPCRs being the target of 40 to 50% of modern medicinal drugs

Physiological roles

GPCRs are present in a wide variety of physiological processes. Some examples include:

1. The visual sense: the opsins use a photoisomerization reaction to translate electromagnetic radiation into cellular signals. Rhodopsin, for example, uses the conversion of *11-cis*-retinal to *all-trans*-retinal for this purpose.
2. the sense of smell: receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors)
3. behavioral and mood regulation: receptors in the mammalian brain bind several different neurotransmitters, including serotonin and dopamine
4. regulation of immune system activity and inflammation: chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response
5. Autonomic nervous system transmission: both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways. These systems are responsible for control of many automatic functions of the body such as blood pressure, heart rate and digestive processes.

There are two types GPCRs viz chemosensory and endo GPCRs.

Receptor structure

GPCRs are integral membrane proteins that possess seven membrane-spanning domains or transmembrane helices. The extracellular parts of the receptor can be glycosylated. These extracellular loops also contain two highly conserved cysteine residues which build disulfide bonds to stabilize the receptor structure.

Early structural models for GPCRs were based on their weak analogy to bacteriorhodopsin for which a structure had been determined by both electron and X ray-based crystallography. In 2000, the first crystal structure of a mammalian GPCR, that of bovine rhodopsin, was solved. While the main feature, the seven transmembrane helices,

is conserved, the structure differs significantly from that of bacteriorhodopsin. Some seven transmembrane helix proteins (such as channelrhodopsin) that resemble GPCRs may contain different functional groups, such as entire ion channels, within their protein.

Ligand binding and signal transduction

While in other types of receptors that have been studied ligands bind externally to the membrane, the ligands of GPCRs typically bind within the transmembrane domain.

The transduction of the signal through the membrane by the receptor is not completely understood. It is known that the inactive G protein is bound to the receptor in its inactive state. Once the ligand is recognized, the receptor shifts conformation and thus mechanically activates the G protein, which detaches from the receptor. The receptor can now either activate another G protein, or switch back to its inactive state. This is an overly simplistic explanation, but suffices to convey the overall set of events.

It is believed that a receptor molecule exists in a conformational equilibrium between active and inactive states. The binding of ligands to the receptor may shift the equilibrium. Three types of ligands exist: agonists are ligands which shift the equilibrium in favour of active states; inverse agonists are ligands which shift the equilibrium in favour of inactive states; and neutral antagonists are ligands which do not affect the equilibrium. It is not yet known how exactly the active and inactive states differ from each other.

If a receptor in an active state encounters a G protein, it may activate it. Some evidence suggests that receptors and G-proteins are actually pre-coupled. For example, binding of G-proteins to receptors affects the receptor's affinity for ligands.

GPCR signaling without G-proteins

In the late 1990s, evidence began accumulating that some GPCRs are able to signal without G-proteins. The ERK2 mitogen-activated protein kinase, a key signal transduction mediator downstream of receptor activation in many pathways, has been shown to be activated in response to cAMP-mediated receptor activation in the slime mold *D. discoideum* despite the absence of the associated G-protein α- and β-subunits.

In mammalian cells the well-studied β2-adrenoceptor has been demonstrated to activate the ERK2 pathway after arrestin-mediated uncoupling of G-protein mediated signalling. It therefore seems likely that some mechanisms previously believed to be purely related to receptor desensitisation are actually examples of receptors switching their signalling pathway rather than simply being switched off.

Receptor regulation

GPCRs are known to become less sensitive to their ligand when they are exposed to it for a prolonged period of time. The key reaction of this downregulation is the phosphorylation of the intracellular (or cytoplasmic) receptor domain by protein kinases.

Phosphorylation by cAMP-dependent protein kinases

Cyclic_AMP-dependent_protein_kinases (protein kinase A) are activated by the signal chain coming from the G protein (that was activated by the receptor) via adenylate cyclase and cyclic AMP (cAMP). In a *feedback mechanism*, these activated kinases phosphorylate the receptor. The longer the receptor remains active, the more kinases are activated, the more receptors are phosphorylated.

Phosphorylation by GRKs

The G-protein-coupled receptor kinases (GRKs) are protein kinases that phosphorylate only active GPCRs.

Phosphorylation of the receptor can have two consequences:

1. *Translocation*. The receptor is, along with the part of the membrane it is embedded in, brought to the inside of the cell, where it is dephosphorylated and then brought back. This mechanism is used to regulate long-term exposure, for example, to a hormone.
2. *Arrestin linking*. The phosphorylated receptor can be linked to *arrestin* molecules that prevent it from binding (and activating) G proteins, effectively switching it off for a short period of time. This mechanism is used, for example, with rhodopsin in retina cells to compensate for exposure to bright light. In many cases, arrestin binding to the receptor is a prerequisite for translocation.

Receptor oligomerization

It is generally accepted that that G-protein-coupled receptors can form homo- and/or hetero-dimers and possibly more complex oligomeric structures. However, it is presently unproven that true hetero-dimers exist. Present bio-chemical and physical techniques lack the resolution to differentiate between distinct homo-dimers assembled into an oligomer or true 1:1 hetero-dimers. It is also unclear what the functional significance of oligomerization is. This is an actively studied area in GPCR research.

FUTURE SCOPE OF PROJECT

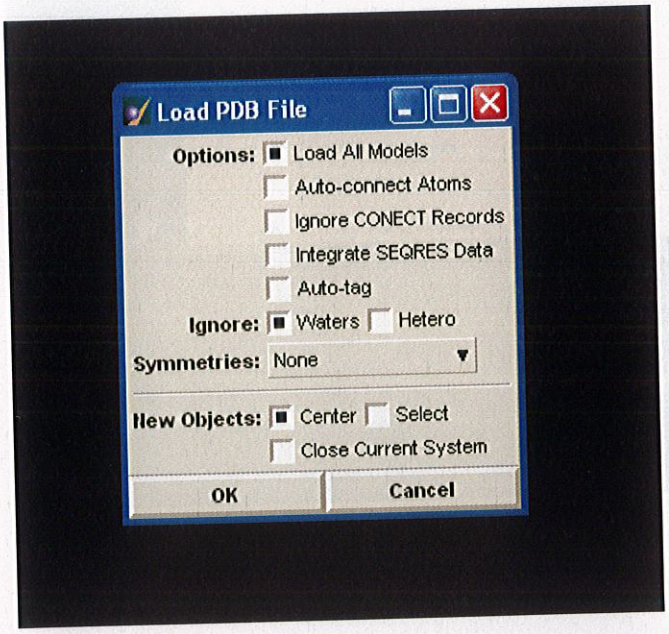
The best thing about this project is the enormous range of the resources on it. We have this project to help in the analysis of the AD in different manner. It is the project that can most be most useful for the bioinformatics student and researchers. With the help of this project our time& resources can be saved. It is a kind of project that can be continued in the future by incorporating new techniques and methods. The information given by this project help in the understanding of the disease at a new dimension.

Thus by understanding the disease its cause and the possible areas of research the treatment of the disease can be found and designed for safer use

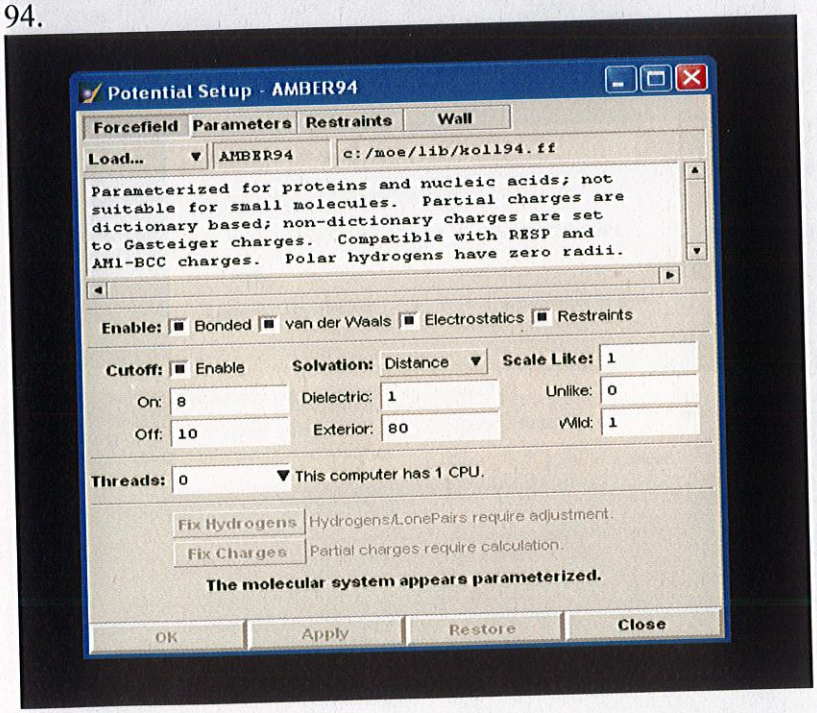
PARAMETERS USED

The following parameters were used for the whole method

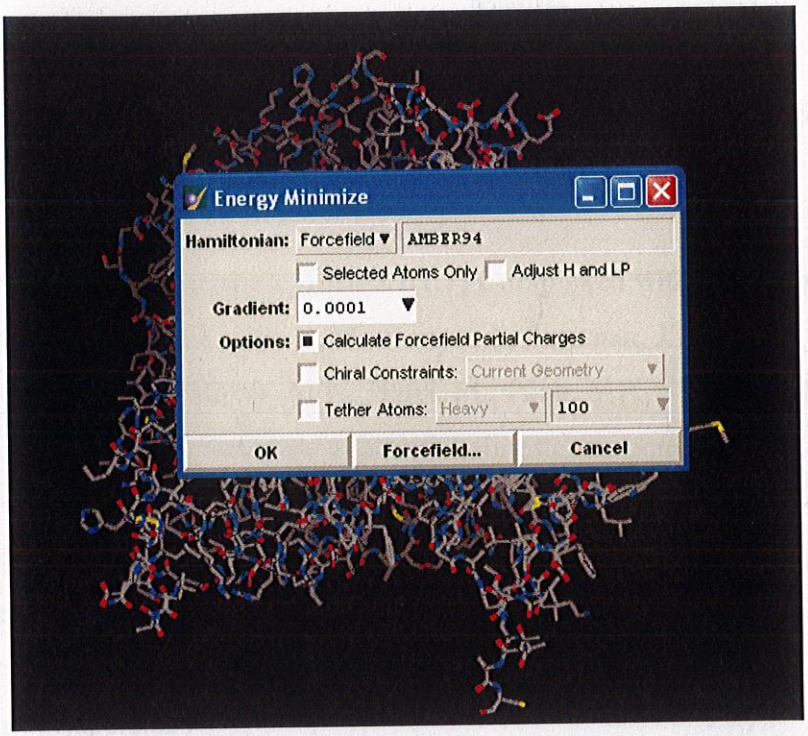
1.The protein was loaded in the MOE window and following parameters were set for the loading the protein loaded was 1VOT.



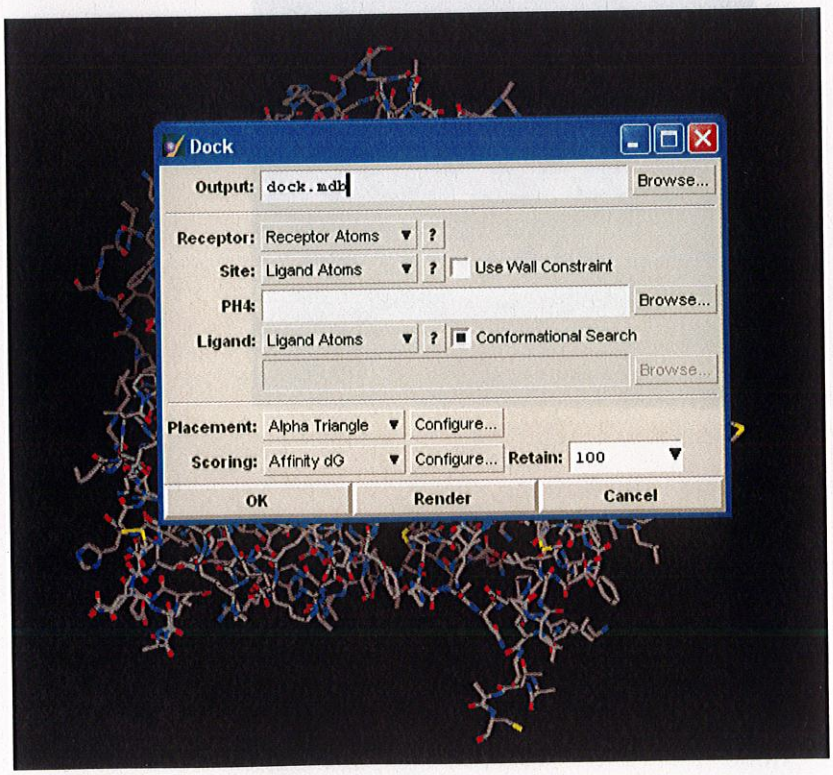
After loading of the protein the energy was minimized the force field used was Amber 94.



3.these were the parameters for energy minimization.



4. The third step after energy minimization is docking for docking the parameters set were.



Results

Two proteins were taken for the study of these proteins in the AD. The two proteins that were taken are as following:-

Title: Acetylcholinesterase (E.C. 3.1.1.7) Complexed with Huperzine A

Authors: Raves, M.L., Harel, M., Silman, I., Sussman, J.L.

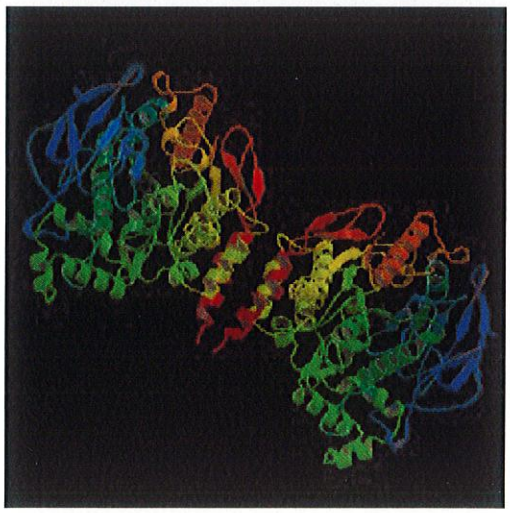
Primary Citation: Raves, M.L., Harel, M., Pang, Y.P., Silman, I., Kozikowski, A.P., Sussman, J.L. Structure of acetylcholinesterase complexed with the nootropic alkaloid, (-)-huperzine A. Nat.Struct.*Biol. V4 pp.57*-63, *1997*

History: Deposition 1996-06-23 Release 1997-06-16

Experimental method: X-ray Diffraction

Functional class: Hydrolase's

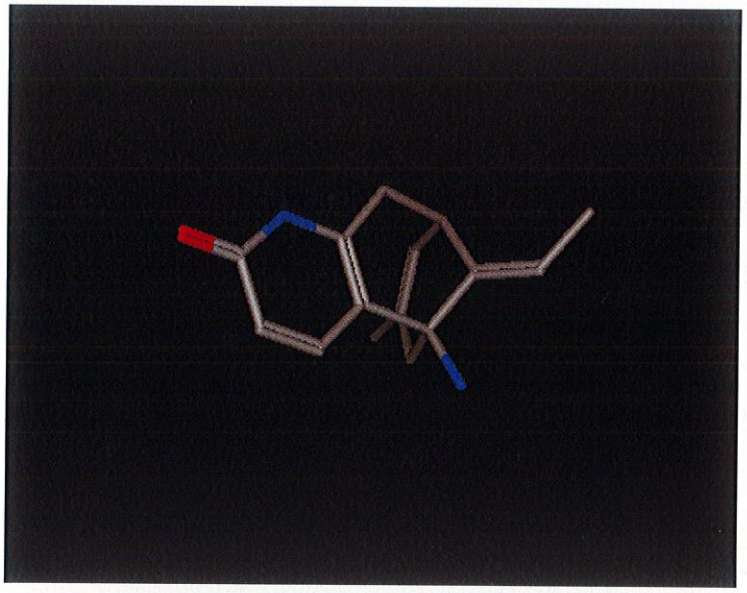
Ligand: Huperaine A



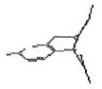
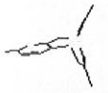
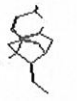
Docking results:

The docking is done to find the best possible fit for the ligand to the protein.

Ligand



Docking results:

	mol	mseq	S	ASE	E
1		1	-7.0414	-7.1014	-7.0594
2		1	-6.3280	-8.1092	-6.8623
3		1	-6.0575	-8.2033	-6.7012
4		1	-6.0128	-8.2267	-6.6770
5		1	-6.3173	-7.4583	-6.6596
6		1	-5.9154	-7.8903	-6.5079
7		1	-5.2496	-9.0349	-6.3852
8		1	-5.8996	-7.3313	-6.3291
9		1	-5.8740	-7.3435	-6.3148
10		1	-5.4408	-8.1799	-6.2625

The best fit would be the ligand with the minimum energy:
These are the 10 of many ligand configurations which are with minimum energy one of them is the best fit.

Title: Structure of Acetylcholinesterase Complexed with Huprine X at 2.1 A Resolution

Authors: Dvir, H., Harel, M., Silman, I., Sussman, J.L.

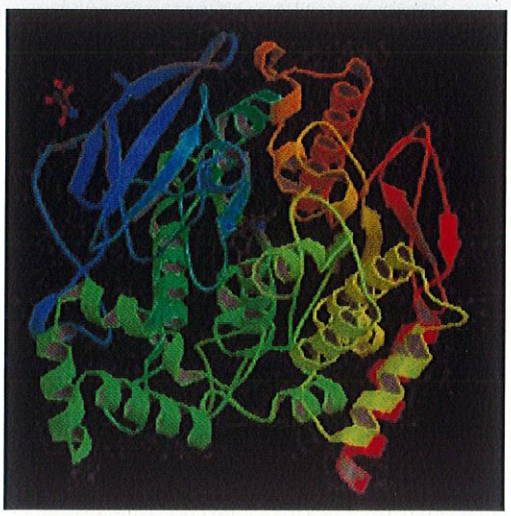
Primary Citation: Dvir, H., Wong, D.M., Harel, M., Barril, X., Orozco, M., Luque, F.J., Munoz-Torrero, D., Camps, P., Rosenberry, T.L., Silman, I., Sussman, J.L. 3D structure of Torpedo californica acetylcholinesterase complexed with huprine X at 2.1 A resolution: kinetic and molecular dynamic correlates. *Biochemistry v41 pp.2970-2981 , 2002*

History: Deposition 2000-08-08 Release 2001-08-02

Experimental method: X-ray Diffraction

Functional class: Cholinesterase

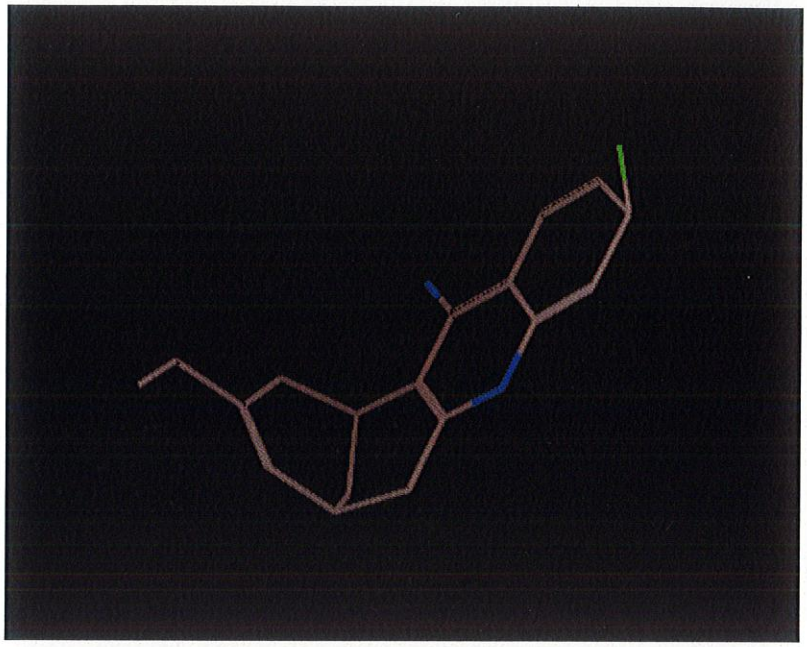
Ligand: NAG , HUX



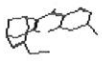
Docking results:

The docking is done to find the best possible fit for the ligand to the protein.

Ligand



Docking results:

	mol	mseq	S	ASE	E
1		1	-6.4591	-11.2460	-7.4952
2		1	-6.5554	-11.0163	-7.4937
3		1	-5.9311	-10.8389	-7.4034
4		1	-5.9697	-10.2759	-7.2615
5		1	-5.9821	-10.0620	-7.2061
6		1	-6.2869	-10.4227	-7.1277
7		1	-6.1500	-10.7341	-7.1252
8		1	-5.4551	-10.2440	-6.8918
9		1	-4.7091	-11.6373	-6.7876
10		1	-5.0671	-10.6450	-6.7405

The best fit would be the ligand with the minimum energy:
These are the 10 of many ligand configurations which are with minimum energy one of them is the best fit.

Discussions

The docking between Acetylcholinesterase (E.C.3.1.1.7) and Huprine A was successfully done and the best conformational structure was the one with lowest E (self energy) value. In this case lowest energy was -7.0594 kcal/mol. Similarly docking was successfully done between Acetylcholinesterase and Huprine X at 2.1 A resolution .The lowest E value obtained in this case was -7.4952 kcal/mol. The results obtained in this study were close to the experimentally proven results. Therefore docking with the MOE helps in analyzing the binding of ligands with the protein.

It was found that the binding of the protein Acetylcholinesterase with Huprine helps in reducing the degradation of acetylcholine protein which is responsible for the transfer of the data in the brain. It was further noted that the binding of Huprine X which is derivative of Huparine A results in 10-20 times more binding of the ligand with protein. This further helps in stopping the action of Enzyme Acetylcholinesterase

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