COMPUTER AIDED PROTEIN STRUCTURE PREDICTION OF THE GAMMA SECRETASE USING MODELLER

A major project report submitted in partial fulfilment of the requirement for the degree of

Master of Technology

In

Bioinformatics

Submitted by

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CERTIFICATE

This is to certify that the M. Tech. dissertation entitled **"Computer Aided Protein Structure Prediction Of The Gamma-secretase Using Modeller".**, submitted by **Zeetendra Singh (DTU/13/M.TECH/368)** in partial fulfilment of the requirement for the award of the degree of Master of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by him/her under my guidance.

The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

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LIST OF ABBREVIATIONS

1. APP: Amyloid Precursor Protein
2. APH-1: Anterior Pharynx-Defective-1
3. PEN-2: Presenilin Enhancer-2
4. PDB: Protein Databank
5. AD: Alzheimer's Disease
6. NICD: Notch1 Intracellular Domain
7. MDR: Multidrug Resistance
8. ER: Endoplasmic Reticulum
9. FDA: Food and Drug Administration
10. 4HNE: 4-Hydroxynonenal
11. OH: Hydroxyl Radical
12. GPUs: Graphics Processor Units
13. BLAST: Basic Local Alignment Search Tool
14. NCBI: National Centre For Biotechnology Information

MAJOR PROJECT

COMPUTER AIDED PROTEIN STRUCTURE PREDICTION OF THE GAMMA SECRETASE USING MODELLER

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ABSTRACT

Gamma-secretase is a multi-subunit protease complex, an integral membrane protein that cleaves single-pass transmembrane proteins at residues within the transmembrane domain. The most substrates of gamma-secretase are APP, a large integral membrane protein that, when cleaved by both gamma and beta-secretase, produces a short 39-42 amino acid peptide called amyloid-beta whose abnormally folded fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease (AD) patients.The gamma-secretase complex consists of four individual proteins: presenilin, PEN-2 (presenilin enhancer-2), APH-1 (anterior pharynx-defective-1), nicastrin. We know the structure of two subunits presenilin and nicastrin, but two other subunit's (APH-1 and PEN-2) structures are still unknown.

Our purpose is 'to predict the structure of sub units of gamma-secretase and use this structure to predict the complex structure of the gamma-secretase'.

The amino acid sequence of gamma-secretase two subunits; PEN-2 and APH-1 are searched in the NCBI database. Tertiary structures, as well as quaternary structures of proteins predicted by MODELLER and five models of PEN-2 and APH-1 subunits were generated. After the structure validation of those two subunits by Ramachandran plot, based on highest number of residue in the favoured region and allowed region, both subunits are selected for docking. Docking of all four subunits (presenilin, APH-1, PEN-2, nicastrin) are done and finally predicted the complex structure of all of the subunits of gamma secretase. The energy was found to be -560.86 KJ.

The tertiary structure of the gamma-secretase enzyme is being predicted with the most number of residues in the favoured region in Ramachandran plot. The residue in the favoured region and the allowed regions are found to be 91.40 % and 6.0 % of the total residues respectively. This indicates a good interaction of subunit structures to form the complex structure of gamma- secretase enzyme.

1. INTRODUCTION

Gamma-secretase is a multi-subunit protease complex, itself an integral membrane protein, that cleaves single-pass transmembrane proteins at residues within the transmembrane domain. The most substrate of gamma-secretase is amyloid precursor protein, a large integral membrane protein that, when cleaved by both gamma and beta secretase, produces a short 39-42 amino acid peptide called amyloid-beta whose abnormally folded fibrillar form is the primary component of amyloid plaques found in the brains of AD patients (De Strooper B et al., 1999).

The gamma-secretase complex consists of four individual proteins: presenilin, PEN-2, APH-1, nicastrin. The structure of presenilin and nicastrin are already present in PDB while structures of APH-1 and PEN-2 are still unknown. Presenilin, an aspartyl protease, is the catalytic subunit; mutations in the presenilin gene have been shown to be a major genetic risk factor for AD (Shirotani, K; Edbauer, D; Prokop. et al., 2004). In humans, two forms of presenilin and two forms of APH-1 have been identified in the genome; one of the APH homologs can also be expressed in two isoforms via alternative splicing, leading to at least six different possible gamma-secretase complexes that may have tissue or cell type specificity (Sobhanifar, S; Schneider, B; Löhr, F; Gottstein, D; Ikeya et al., 2010).

The proteins in the γ- secretase complex are heavily modified by proteolysis during assembly and maturation of the complex; a required activation step is in the autocatalytic cleavage of presenilin to N- and C-terminal fragments. Nicastrin's primary role is in maintaining the stability of the assembled complex and regulating intracellular protein trafficking (Zhang YW, Luo WJ. et al., 2005). PEN-2 associates with the complex via binding of a transmembrane domain of presenilin (Watanabe N, Tomita T. et al., 2005) and, among other possible roles, helps to stabilize the complex after presenilin proteolysis has generated the activated N-terminal and C- terminal fragments (Prokop S, Shirotani K, Edbauer D. et al., 2004). APH-1, which is required for proteolytic activity, binds to the complex via a conserved alpha helix interaction motif and aids in initiating assembly of premature components (Lee SF, Shah S, Yu C, Wigley WC, Li H. et al., 2005).

Recent research has shown that interaction of the γ-secretase complex with the gamma-secretase activating protein facilitates the gamma cleavage of amyloid precursor protein into beta-amyloid (He G, Luo W, Li P, Remmers C. et al., 2010).

The gamma-secretase complex is thought to assemble and mature via proteolysis in the early endoplasmic reticulum (Capell A, Beher D, Prokop S, Steiner H. et al., 2005). The complexes are then transported to the late Endoplasmic reticulum where they interact with and cleave their substrate proteins (Kim SH, Yin YI, Li YM. et al., 2004). Gamma-secretase complexes have also been observed localized to the mitochondria, where they may play a role in promoting apoptosis (Hansson CA, Frykman S, Farmery MR, et al., 2004).

Amyloid precursor protein secretase

Secretases are enzymes that "snip" pieces off a longer protein that is embedded in the cell membrane.

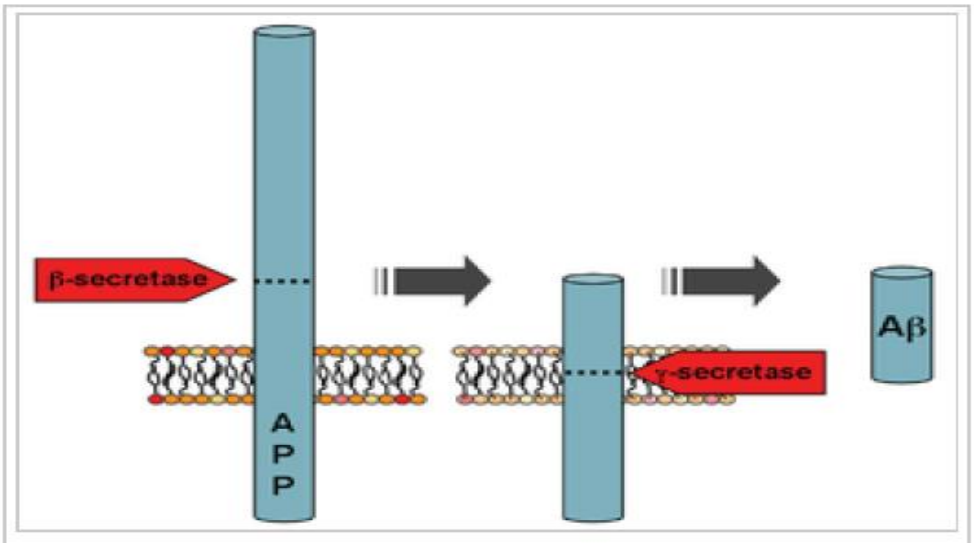


Fig.1: Processing of the amyloid precursor protein

secretases act on APP to cleave the protein into three fragments. Sequential cleavage by beta-secretase and gamma-secretase produces the beta peptide fragment that aggregates into clumps called "plaques" in the brains of AD patients. If alpha-secretase acts on amyloid precursor protein first instead of beta-secretase, no amyloid-β is formed because α-secretase recognizes a target protein sequence closer to the cell surface than beta-secretase.

The non-pathogenic middle fragment formed by an alpha/gamma cleavage sequence is called P3.

Beta-secretase is a transmembrane protein with an extracellular aspartic acid protease domain.

The alpha-secretase gene has not been conclusively identified but is believed to be a metalloproteinase

Gamma-secretase is actually a protein complex containing presenilin, presenilin enhancer-2, nicastrin, anterior pharynx-defective-1. Presenilin is believed to harbor the protease domain and represents an important example of an uncommon type of protease that cleaves targets within the cell membrane.

Besides their involvement in the pathogenesis of Alzheimer's, these proteins also have other functional roles in the cell.

Gamma-secretase plays a critical role in developmental signalling by the transmembrane receptor Notch, freeing the cytoplasmic tail of Notch to travel to the cell nucleus to act as a transcription factor.

Although beta-secretase cleaves the extracellular domains of several transmembrane proteins, its physiological function remains unknown.

Presenilin

Presenilins are a family of related multi-pass transmembrane proteins that functions as a part of the γ-secretase. There are two presenilin genes, called *PSEN1* that encodes presenilin 1 and *PSEN2* that codes for presenilin 2 (Smialowska A. et al., 2006). Both genes show conservation between species, with little difference between a rat and human presenilin.

Presenilin undergo cleavage in an α- helical region of one of the cytoplasmic loops to produce a larger smaller C-terminal and a N-terminal fragment that together form part of the functional protein (Spasic D, Tolia A, Dillen K, Baert V. et al., 2006). Cleavage of presenilin can be prevented by a mutation that causes the loss of exon 9, and results in loss of function. They play a key role in the modulation of intracellular Ca^{2+} involved in

presynaptic neurotransmitter release and long-term potentiation induction (Zhang C, Wu B, Beglopoulos V. et al., 2009).

Dominant mutations in the genes that encode presenilin proteins are the most common cause of familial early-onset Alzheimer's disease.

An important part of the disease process in AD is the accumulation of amyloid-beta protein. To form amyloid-beta, amyloid precursor protein must be cut by two enzymes, β secretases and γ- secretase. Presenilin is the sub-component of γ-secretase that is responsible for the cutting of amyloid precursor protein.

Gamma-secretase can cut amyloid precursor protein at several points within a small region of the protein, which results in an amyloid-beta of various lengths. The lengths associated with AD are 40 and 42 amino acids long. Amyloid-beta 42 is more likely to aggregate to form plaques in the brain than amyloid-beta 40. Presenilin mutations lead to an increase in the ratio of amyloid-beta 42 produced compared to amyloid-beta 40, although the total quantity of amyloid-beta produced remains constant. This can come about by various effects of the mutations upon γ - secretase (Bentahir M, Nyabi O, Verhamme J, Tolia A et al., 2006). Presenilins are also implicated in the processing of notch, an important developmental protein.

PSEN-1

γ -secretase is considered to play an important role in the generation of β-amyloid, accumulation of which is related to the onset of AD, from the β -AAP. Role in β-amyloid production Transgenic mice that over-expressed mutant PSEN-1 show an increase of β-amyloid-42 in the brain, which suggest PSEN-1 plays an important role in β-amyloid regulation and can be highly related to AD (Duff K, Eckman C, Zehr C, Yu X, Prada CM. et al., 1996). Further study conducted in neuronal cultures derived from PSEN-1deficient mouse embryos. They showed that cleavage by α and β-secretase was still normal without the presence of PSEN-1. Mean while, the cleavage by γ-cleavage of the transmembrane domain of amyloid precursor protein was abolished. A 5-fold drop of amyloid peptide was observed, suggesting that deficiency of PSEN-1can down regulate amyloid and inhibition of PSEN-1 can be a potential method for anti-amyloidogenic therapy in AD (Pitsi D. et al., 2006).

Role in Notch signaling pathway

In Notch signaling, critical proteolytic reactions take place during maturation and activation of Notch membrane receptor. Notch1 is cleaved extracellularlly at site-1 and two polypeptides are produced to form a heterodimer receptor on the cell surface. After the formation of the receptor, Notch1 is further cleaved in site-3 and release Notch1 intracellular domain form the membrane.

PSEN-1 has shown to play an important role in proteolytic process. In the PSEN-1 null mutant drosophila, Notch signaling is abolished and it displays a notch-like lethal phenotype. Moreover, in mammalian cells, deficiency of PSEN1 also causes the defect in the proteolytic release of Notch1 intracellular domain from a truncated Notch construct. The same step can be also blocked by several γ-secretase inhibitor.

Role in cancer

It has a role in AD, PSEN-1 also found to be important in cancer. A study of broad range gene expression was conducted on human malignant melanoma. Researchers classified the malignant melanoma cell lines into two types. The study showed that PSEN-1 is down regulated in cell type while it is over expressed in the other cell type. Another study on multidrug resistance cell line also reveals a role of PSEN-1 in cancer development. Because of the development to the resistance to chemical, multidrug resistance cells become a critical factor on the success of cancer chemotherapy. In the study, researchers tried to explore the molecular mechanism by looking into the expression of Notch1 intracellular domain and PSEN-1. They found that there is higher level expression of both proteins and a multidrug resistance-associated protein 1 was also found to be regulated by Notch1 intracellular, which suggest a mechanism of proteins and a multidrug resistance-associated protein 1 regulated by PSEN-1and notch signalling.

PSEN2

AD patients with an inherited form of the disease carry mutations in the presenilin proteins or APP. These disease-linked mutations result in increased production of the longer form of amyloid-β. Presenilins are postulated to regulate amyloid precursor protein processing through their effects on γ-secretase, an enzyme that cleaves amyloid precursor protein (Passer BJ, Pellegrini L, Vito P, Ganjei JK. et al., 1999). Also, it is thought that the presenilins are involved in the cleavage of the Notch receptor, such that they either directly

regulate γ-secretase activity or themselves are protease enzymes. Two alternative transcripts of presenilin-2 have been identified.

Nicastrin

Nicastrin is a protein that is part of the γ-secretase, which is one of the proteases involved in processing APP to the short AD-associated peptide amyloid β . The other proteins in the complex are presenilin, which is the catalytically active component of the complex, PEN-2 and APH-1 (Kaether C, Haass C. et al., 2006). Nicastrin itself is not catalytically active, but instead promotes the maturation and proper trafficking of the other proteins in the complex, all of which undergo significant post-translational modification before becoming active in the cell (Zhang YW, Luo WJ, Wang H, Lin P. et al., 2005). Nicastrin has also been identified as a regulator of neprilysin, an enzyme involved in the degradation of amyloid β fragment.

APH-1

APH-1 is a protein gene product originally identified in the Notch signaling pathway in *Caenorhabditis elegans* as a regulator of the cell-surface localization of nicastrin. APH-1 homologs in other organisms, including humans, have since been identified as components of the γ-secretase complex along with the catalytic subunit presenilin and the regulatory subunits nicastrin and presenilin enhancer 2. The γ-secretase complex is multimeric protease responsible for the intramembrane proteolysis of transmembrane proteins such as the Notch protein and APP. γ-secretase cleavage of amyloid precursor protein is one of two proteolytic steps required to generate the peptide known as amyloid-β , whose misfolded form is implicated in the causation of AD (Kaether C, Haass C. et al., 2006). All of the components of the γ-secretase complex undergo extensive post-translational modification, especially proteolytic activation; anterior pharynx-defective-1 and presenilin enhancer-2 are regarded as regulators of the maturation process of the catalytic component presenilin (Luo WJ, Wang H, Li. et al., 2003). Anterior pharynx-defective 1 contains a conserved α-helix interaction motif glycine-X-X-X-glycine that is essential to both assembly of the γ secretase complex and to the maturation of the components.

PEN-2

PEN-2 is a protein that is a regulatory component of the γ-secretase complex, a protease complex responsible for proteolysis of transmembrane proteins such as the Notch protein and APP. The γ-secretase complex consists of presenilin enhancer-2, anterior pharynx-defective-1, presenilin, nicastrin. presenilin enhancer-2 is a 101-amino acid integral membrane protein likely with a topology such that both the C-terminus and N-terminus face first the lumen of the endoplasmic reticulum and later the extracellular environment (Francis R, McGrath G, Zhang J et al., 2002). Biochemical studies have shown that a conserved sequence motif D-Y-L-S-F at the C-terminus, as well as the overall length of the C-terminal tail, is required for the formation of an active γ-secretase complex.

2. REVIEW OF LITERATURE

2.1 Alzheimer's Disease (AD)

Alzheimer's disease is one of the most common forms of dementia. The disease mainly affects the neuron cells in the brain. Accumulation of plaques and tangles are found in the Alzheimer's disease patience's brain (Burns A. et al., 2009). The plaques are extracellular deposition of fibrils and amorphous aggregation of the amyloid-beta-peptide in high amount. Neuro fibrillary tangles are an intracellular fibrillar aggregation of the microtubules associated tau proteins that are required for the neurons growth and maintenance. The plaques and tangles are mainly present in that region of the brain which are responsible for learning, memory and emotional behaviors such as hippocampus, entorhinal cortex, amygdala and basal forebrain (Burns A. et al., 2009). Brain regions with plaques and tangles exhibit reduced number of synapse and the neurons which use glutamate and acetylcholine as neurotransmitter are found to be mostly affected. Persons with Alzheimer's disease show mild forgetfulness and trouble remembering recent events at the beginning and as the disease progresses the person starts losing any sense, like unable to remember direction, names, and unable to recognize their own once at late stage. The most dangerous about this disease is that there is still no drug that can cure Alzheimer's disease. Although there are some drugs that can relatively decrease the progress the development of the disease as per U.S. Food and Drug Administration.

2.2 Cause Of The Disease

The cause of Alzheimer's disease is poorly understood (Burns A. et al., 2009). About 70% of the risk is believed to be genetic with many genes usually involved (Levy-Lahad. et al., 1995). Other risk factors include: a history of head injuries, depression, hypertension etc.

The main cause of this disease is the accumulation of amyloid-beta peptide protein in some part of the brain. Amyloid-beta peptide fragments aggregate leading to form fibril like structure which has an irreversible cross beta structure. Amyloid-beta peptides are form from the breakdown of APP by gamma-secretase, alpha-secretase, beta-secretase enzyme. But involvement gamma-secretase remains unclear. (APP is a transmembrane protein which has many isoforms ranging in size from 695 to 700 amino acids. The most abundant

form of isoform amyloid precursor protein 695 found in the brain in produced by neuron cell (Dickson,D.W.. et al., 1997).

Cleavage of amyloid precursor protein by alpha-secretase releases amyloid precursor protein alpha from the cell surface and leaves an 83 amino acid C-terminal amyloid precursor protein fragment. Amyloidogenic processing of amyloid precursor protein involves sequential cleavages by beta-secretase and gamma-secretase at the N and C terminal of amyloid-beta respectively. The 99 amino acid C-terminal fragment of amyloid precursor protein is cleaved by beta-secretase.

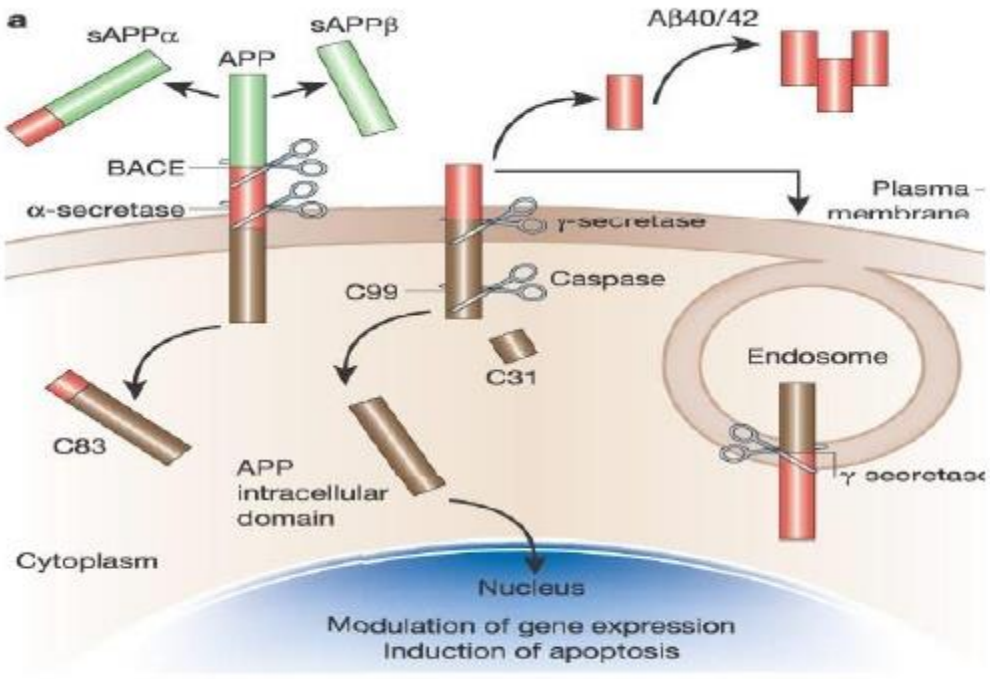


Fig.2: Breakdown of amyloid precursor protein by beta-secretase and gamma-secretase and formation of amyloid-beta 40/42, key fragment to the amyloid aggregation.

Cleavage can be internalized and further processed by gamma-secretase to produce amyloid-beta 40/42 in extracellular. Cleavage of C99 by gamma-secretase liberates an amyloid precursor protein intracellular domain that can translocate to the nucleus where it may regulate gene expression, including the induction of apoptotic genes. Cleavage of amyloid precursor protein /C99 by caspases produces a neurotoxic peptide . amyloid-beta 40/42 fragment forms aggregate and in later stage an irreversible fibril like structure which has a cross beta-sheet structure. This irreversible fibril like structure accumulates in some

part of the brain mainly in synaptic junctions where the presence of amyloid precursor protein is more. Alzheimer's disease tough is not inherited from parents but through molecular genetics it is found that the individual that produces more apoliprotein E4 has higher risk of getting Alzheimer's disease. The mechanism whereby E4 promotes Alzheimer's disease is not established, but there is evidence that E4 enhances amyloid-beta aggregation and reduces amyloid-beta clearance. In addition, data suggest that E4 might increase the risk of Alzheimer's disease by enhancing amyloidogenic processing of amyloid precursor protein, increasing oxidative stress and impairing neuronal plasticity (Roses, A. D. et al., 1997).

2.3 Harmful Effects

Amyloid precursor protein is required for the growth and maintenance of the neuron cells. Perturbed processing of amyloid precursor protein resulting in increased production of amyloid-beta at synapses may be an early event in Alzheimer's disease. Amyloid precursor protein is axonally transported and amyloid-beta therefore likely accumulates at synapses in high amounts in Alzheimer's disease. Amyloid-beta can have multiple adverse effects on the functions and integrity of both pre- and postsynaptic terminals including inducing oxidative stress, impairing calcium homeostasis and perturbing the functions of mitochondria and the endoplasmic reticulum. The increased amyloid-beta deposition that occurs in Alzheimer's disease most probably contributes to the demise of neurons because amyloid-beta can be directly toxic to neurons and also greatly increases their vulnerability to oxidative and metabolic stress, and toxicity. Another effect of accumulation of amyloid-beta peptide extracellular is the induced production of highly oxidative free-radicals. The neurotoxic action of amyloid-beta involves generation of reactive oxygen species and disruption of cellular calcium homeostasis. Interactions of amyloid-beta oligomers and Fe^{+2} or Cu+ generate hydrogen peroxide. When amyloid-beta aggregation occurs at the cell membrane, membrane-associated oxidative stress results in lipid peroxidation and the consequent generation of 4-hydroxynonenal, a neurotoxic aldehyde that covalently modifies proteins on cysteine, lysine and histidine residues. Oxidative modifications of tau protein by 4-hydroxynonenal and other reactive oxygen species can promote its aggregation and may thereby induce the formation of neurofibrillary tangles. Amyloid-beta can also cause mitochondrial oxidative stress and deregulations of Calcium homeostasis, resulting in impairment of the electron transport chain, increased production of superoxide anion

radical and decreased production of ATP. Fe^{+2} or Cu^{+} generates the hydroxyl radical, a highly reactive oxyradical and potent inducer of membrane-associated oxidative stress that contributes to the dysfunction of the endoplasmic reticulum.

2.4 Therapeutic Approach

The gamma-secretase, which cleaves amyloid precursor protein within a transmembrane region, involves four different proteins, anterior pharynx-defective-1, presenilin, nicastrin , presenilin enhancer-2. The active site of gamma-secretase requires the aspartyl protease activity of PS1 conferred by aspartate residues in adjacent transmembrane domains of the C- and N-terminal cleavage fragments of PS1 (red star). Anterior pharynx-defective-1, presenilin enhancer, nicastrin, are each critical components of gamma-secretase and each may modify enzyme activity in specific

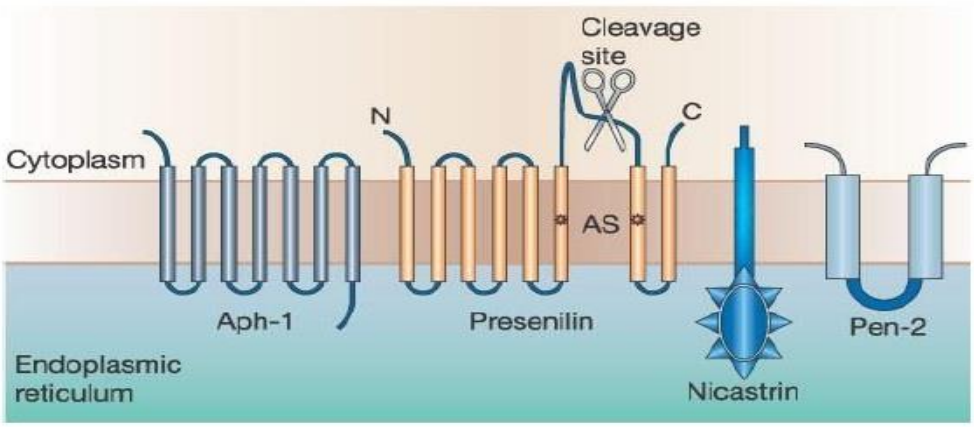


Fig.3: Four subunits of gamma-secretase, such as APH-1, Presenilin, Nicastrin and PEN-2.

way and in response to physiological stimuli. Amyloid precursor protein is only one of several proteins that are cleaved by gamma-secretase. The best way to stop this Alzheimer's disease is either we inhibit the gamma-secretase and beta-secretase enzyme or we inhibit the aggregation of amyloid β-peptide or reverse the fibril structure. But to inhibit the gamma-secretase and beta-secretase, we must know the structure of the enzyme.

2.5 Protein Structure Prediction

One of the major goals of bioinformatics is to understand the relationship between amino acid sequence and the 3D structure in protein. If these relationships are known then the structure of a protein could be reliably predicted from the amino acid sequence. Although experimental structure determination methods are providing high resolution structure information for a subset of the proteins, computational structure prediction methods will provide valuable information for the large fraction of sequences whose structures cannot be determined experimentally (Pirovano, W. et al., 2010).

Methods for the prediction of structure prediction from amino acid sequence includes-

- Attempts to predict secondary structure without attempting to assemble these regions in three dimensions.
- Homology modelling prediction of the three dimensions structure of protein from the known structures of one or more related proteins.
- Fold recognition, from a library of known structure, determine which of them shares a folding pattern with a query protein of known sequence but unknown structure.

α-Helix

The α-helix is a rigid, rod like structure that forms when a polypeptide chain twists into a helical conformational. The screw sense of alpha helix can be right handed or left handed. However right handed helices are energetically more favourable. In almost of proteins, the helical twist of the alpha helix is right handed. There are 3.6 amino acid residues is related to the next one by a rise of 1.5 angstrom along the helix axis. A single turn of alpha helix involve 13 atoms from O to the H bond, for the alpha helix is referred to as 3.6_{13} helix. Length of alpha helix is usually 10-15 amino acid residues.

β-Pleated sheet

β- Pleated sheets from when two or more polypeptide chain segment line up side by by side. Each individual segment is referred to as beta strand. Rather than being coiled, each beta strand is fully extended. The distance between adjacent amino acid along beta strand is approximately 3.5 A, in contrast with distance of 1.5 angstrom along an alpha helix and beta pleated sheets are stabilized by inter chain hydrogen bond that from between the peptide backbone N-H and carbonyl group of adjacent strands. Adjacent strands can be either parallel or anti parallel.

Turn

Most proteins have compact, globular shapes, requiring reversals in the direction of their polypeptide chains. Many of these reversible are accomplished by a common structure element called the turn. Turns composed three or four residues, are classified as third types of secondary structure. Those short, U- shaped secondary structures are stabilized by a hydrogen bond between their end residues. Glycine and proline are commonly present in turn. A beta turn is characterized by hydrogen bond in which the donor and receptor residues are separated by three residues. The turn and loops invariably lie on the surface of protein and thus participate in interactions between protein and other molecules.

Secondary structure prediction

Secondary structure prediction is a set of techniques that aim to predict the local secondary structure of proteins based only on knowledge of their primary structure – amino acid sequence. For protein, a prediction consists of assigning of amino acid sequences as likely alpha helix, beta sheets, turn.

Three widely used methods for secondary structure prediction are Chau & Fashman and GOR method, neural network models, nearest neighbour methods.

Tertiary structure

Tertiary structure refers to the unique 3D conformationals that globular proteins assume as a consequence of the interactions between side chains in their primary structure. All information need to fold protein into its native tertiary structure is contained within the primary structure of the peptide chain itself. The following types of covalent and non covalent interaction stabilize the tertiary structure (Zhang Y. et al., 2008).

The protein structure prediction are two main problems are the calculation of protein free energy and finding the global minimum of this energy. A protein structure prediction method must explore the space of possible protein structures which is astronomically large. These problems can be partially bypassed in "comparative" or homology modelling and fold recognition methods, in which the search space is pruned by the assumption that the protein in question adopts a structure that is close to the experimentally determined structure of another homologous protein.

2.6 Homology Modelling

Homology means having a common evolutionary origin, but does not necessary mean similarity. It is a qualitative description of the nature of relationship between two or more things, and it cannot be partial. Either there is an evolutionary relationship or there is not.

A major goal of structural biology is to predict 3 D structure of protein from the sequence of amino acids. Techniques such as x-ray diffraction or NMR are being used to develop 3 D structure of the protein. My proteins are simply too large for NMR analysis and cannot be crystallized for X-ray diffraction. Alternative strategies are being applied to develop models of protein structure. One method that can be applied to generate reasonable models of protein structure is homology modelling. It is based on the reasonable assumption that two homologous proteins will share very similar structures. This procedure called comparative modelling develops a 3D model from a protein sequence based on the structure of homologous proteins (Bowie JU, Luthy R, Eisenberg D. . et al., 1991).

Homology modelling is based on the reasonable assumption that two homologous proteins will share very similar structures. Because a protein's fold is more evolutionarily conserved than its amino acid sequence, a target sequence can be modelled with reasonable accuracy on a very distantly related template, provided that the relationship between target and template can be discerned through sequence alignment. It has been suggested that the primary bottleneck in comparative modelling arises from difficulties in alignment rather than from errors in structure prediction given a known-good alignment. Unsurprisingly, homology modelling is most accurate when the target and template have similar sequences.

It is based on two major observations

1. The structure of a protein is uniquely by its amino acid sequence.
2. During evolution, structure is more stable and changes much slower than the associated sequence so that similar sequences adopted practically identical structures, distantly related sequences still fold into similar sequences.

General procedures

In practice, homology modelling is a multi-step process that can be summarized in seven steps:

- Template recognition and initial alignment: Identify homologous proteins and determine extent of their sequence similarity with one another and unknown
- Alignment corrections
- Backbone generation
- Loop modelling
- Model optimization
- Model validation

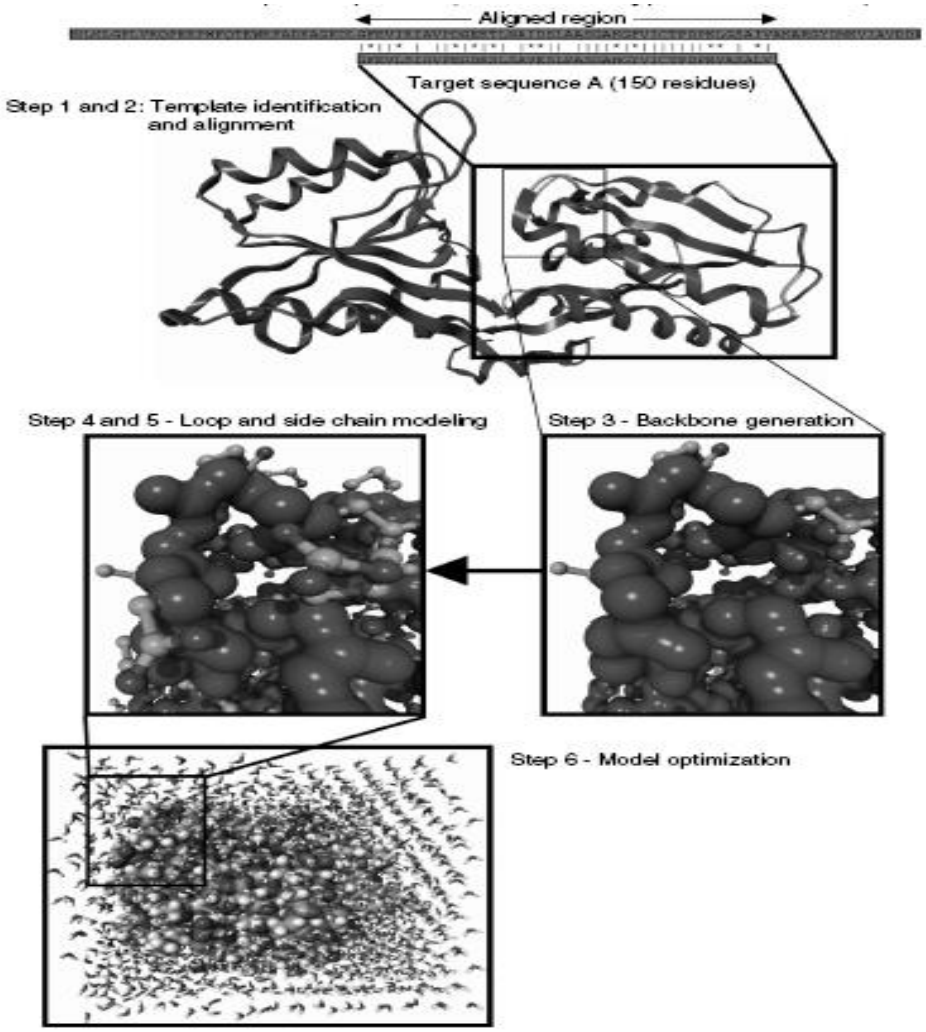


Fig.4: The steps to homology modelling. The fragment of the template corresponding to the region aligned with the target sequence forms the basis of the model. Loops and missing side chains are predicted, than the model is optimized.

Quaternary structures

In the case of complexes of two or more proteins, where the structures of the proteins are known or can be predicted with high accuracy, protein–protein docking methods can be used to predict the structure of the complex. Information of the effect of mutations at specific sites on the affinity of the complex helps to understand the complex structure and to guide docking methods (Chou KC Chou KC, Zhang C.T. et al., 1995).

2.7 Objective

The objective of this project is ‘to predict the structure of sub units of gamma-secretase and use this structure to predict the complex structure of the gamma-secretase’.

3. METHODOLOGY

3.1 Web Tools and Databases

National Centre for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov)

NCBI has been assigned with creating automated system for sorting and analyzing information about molecular biology, genetics, biochemistry etc. Facilitating use of like database and software by the research and medical community, coordinating effort together biotechnology information, performing research into advanced methods of computer based data processing for analysing the structure and function of biologically significant molecules.

At NCBI, database are connect by a unique search and retrieval system, referred as Entrez. Entrez permit a user to not only access and retrieve specific data from a single database however, access integrated data many NCBI database.

RCSB (www.rcsb.org)

The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids, and complex assemblies that help student and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease. The RCSB PDB builds upon the data by creating tools and resources for research and education in molecular biology, structural biology, computational biology, and beyond.

Use this website to access curated and integrated biological macromolecular information in the context of function, sequence, biological processes, evolution, pathways, and disease states. www.rcsb.org of biological macromolecules structure files download (PDB format) and use of PDB ID.

BLAST (www.blast.ncbi.nlm.nih.gov/Blast.cgi)

BLAST (Basic Local Alignment Search Tool) is an algorithm for comparing primary

Biological sequence information, such as the amino-acid sequences of different proteins or nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query

sequence with a library or database of sequences, identify library sequences that resemble the query sequence above a certain threshold.

BLAST is one of the most widely used bioinformatics programs for sequence searching. The heuristic algorithm it uses is much faster than other approaches, such as calculating an optimal alignment. Input sequences in FASTA format.

Protein-protein BLAST (blastp): Given a protein query, returns the most similar protein sequences from the protein database that the user specifies.

Chou & Fasman method (www.biogem.org/tool/chou-fasman)

The Chou–Fasman method for secondary structures prediction of proteins, the basic idea is that each amino acid residues is assigned three numbers that describes its propensity to be the part of alpha helix, beta sheets, turn respectively. A large number corresponds to a propensity for that kind of structure. This parameter may be determined from the occurrence of different amino acids in different types of secondary structure is known protein structures.

RAMPAGE (www.mordred.bioc.cam.ac.uk/~rapper/rampage.php)

RAPPER is an *ab initio* conformational search algorithm for restraint based protein modelling. It has been used for all atom loop modelling , whole protein modelling under limited restraints, comparative modelling, *ab initio* structure prediction, structure validation, and experimental structure determination with X-ray and nuclear magnetic resonance spectroscopy. Use Ramachandran plot for analysis.

A Ramachandran plot ([φ,ψ] plot), is a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure. The ω angle at the peptide bond is normally 180°, since the partial double bond character keeps the peptide planar. The figure at top right shows the allowed φ, ψ backbone conformational regions from the Ramachandran plot. Calculations: full radius in solid outline, reduced radius in dashed, and relaxed tau angle in dotted lines. Because dihedral angle values are circular and 0° is the same as 360°, the edges of the Ramachandran plot "wrap" right left and bottom to top. For instance, the small strips of allowed values along the lower left edge of the plot are a continuation of the large, extended chain region at upper left.

3.2 SOFTWARE

MODELLER

MODELLER is used for homology or comparative modelling of protein three dimensional structure. MODELLER is a computer program used in producing homology models of protein tertiary structures as well as quaternary structures (rarer). It implements a technique inspired by nuclear magnetic resonance known as *satisfaction of spatial restraints*, by which a set of geometrical criteria are used to create a probability density function for the location of each atom in the protein. The user provides an alignment of a sequence to be modelled with known related structures and MODELLER automatically calculates a model containing all non hydrogen atoms.
There are many steps such as:

- Searching for structures related to target protein
- Selecting a template
- Aligning target protein with the template
- Model building
- Model validation

Swiss PDB Viewer 4.1.0

Swiss PDB Viewer is an application that provides a user friendly interface for analyzing several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface. Working with these two programs greatly reduces the amount of work necessary to generate models, as it is possible to thread a protein primary sequence onto a 3D template and get an immediate feedback of how well the threaded protein will be accepted by the reference structure before submitting a request to build missing loops and refine side chain packing. Use energy minimization.

RasMol v 2.7.5.2

RasMol is visualization software and used primarily for the depiction and exploration of biological macromolecule structures, such as those found in the PDB. RasMol has become

an important educational tool as well as continuing to be an important tool for research in structural biology. RasMol has a complex version history. RasMol includes a language (for selecting certain protein chains, or changing colours etc.). PDB files can be downloaded for visualization from members of the Worldwide Protein Data Bank. These have been uploaded by researchers who have characterized the structure of molecules usually by X-ray crystallography, NMR spectroscopy and electron microscopy.

Hex 8.0.0

Hex 8.0.0 is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex 8.0.0 can also calculate protein- ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. It is the first protein docking program to be able to use modern graphics processor units to accelerate the calculations.

Open Babel

Open Babel is a chemical expert system mainly used for converting chemical file formats. Due to the strong relationship to informatics this program belongs more to the category chemoinformatics than to molecular modelling. Inter conversion of many chemical file formats.

3.3 Amino Acid Sequence Search

The amino acid sequence of two sub units of gamma-secretase; pen2 and aph1 are searched in the NCBI database. NCBI (http://www.ncbi.nlm.nih.gov/)
Amino acid Sequence of APH-1 subunit in FASTA format:

>gi|344313186|ref|NP_001230701.1| gamma-secretase subunit APH-1A isoform 4 [Homo sapiens]

MGAAVFFGCTFVAFGPAFALFLITVAGDPLRVIILVAGRCSALPTTSCLISGLSFGIIS
GVFSVINILADALGPGVVGIHGDSPYYFLTSAFLTAAIILLHTFWGVVFFDACERRR
YWALGLVVGSHLLTSGLTFLNPWYEASLLPIYAVTVSMGLWAFITAGGSLRSIQRS
LLCRRQEDSRVMVYSALRIPPED

Amino acid Sequence of PEN-2 subunit in FASTA format:

>gi|528281416|ref|NP_001268461.1| gamma-secretase subunit PEN-2 [Homo sapiens]
MNLERVSNEEKLNLCRKYYLGGFAFLPFLWLVNIFWFFREAFLVPAYTEQSQIKGY
VWRSAVGFLFWVIVLTSWITIFQIYRPRWGALGDYLSFTIPLGTP

3.4 BLAST

Protein-protein BLAST is done for both sub units. But the similarity with another subunit is found to be less than 80%. E-value in BLAST searches measures the probability of the search result in non-random. These are found similar protein sequence in PDB file.

3.5 Secondary Structure Prediction

Secondary structure is predicted using Chou & Fasman online server. This method works on the principle of probability parameters determined from relative frequencies of each amino acids appearance in each type of secondary structure. These are found value of α helix, β sheet and β turns and calculate on the basis of propensity of the structure among a certain number of residues.

Subunit	Secondary Structure	Total Residue	Percentage of the total Residue
Aph-1 (195 amino acids)	α- helix(H)	142	72.8
	β- sheet(E)	124	63.6
	β-turn(T)	17	8.7
PEN-2 (101 amino acids)	α- helix(H)	74	73.3
	β- sheet(E)	74	73.3
	β-turn(T)	10	9.9

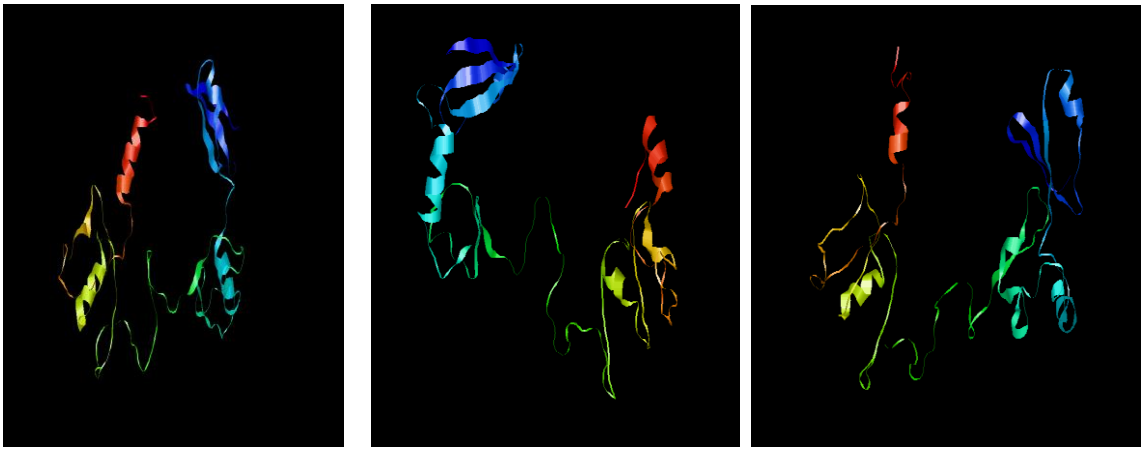
Table1: Secondary structure prediction of two subunits of gamma-secretase by Chou & Fasman method.

3.6 MODELLER Prediction Method

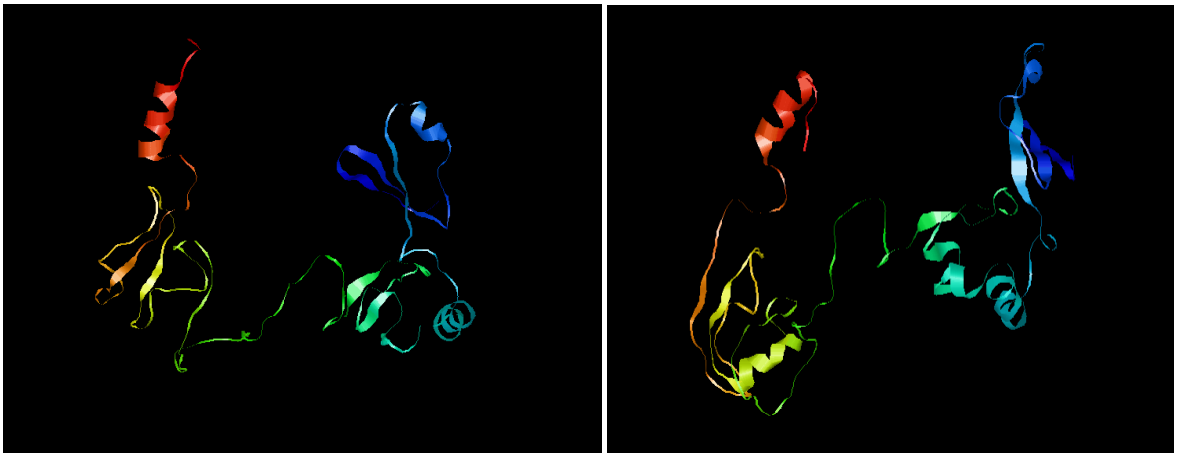
MODELLER predicts tertiary structures of protein as well as quaternary structures (rarer). The steps include - Searching for structures related to target protein, Selecting a template, Aligning target protein with the template, Model building, Model validation. Modeller generates five models of two proteins such as APH 1 and PEN 2.

Aph1(Number of groups-195, number of atoms-1482 & number of bonds-1522)	Number of Helix	Number of Turns	Number of strands	Number of H-Bonds
Model1	6	26	6	79
Model2	5	31	7	70
Model3	6	32	7	57
Model4	3	29	8	69
Model5	6	28	8	72

Table 2: Comparison of different models of APH-1 subunit generated by Modeller.



Model1 Model2 Model3

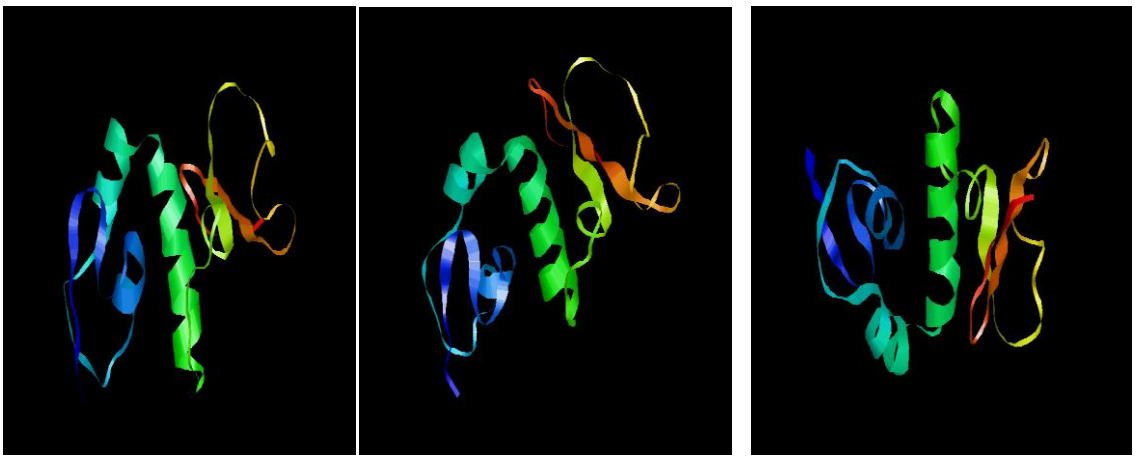


Model 4 model 5

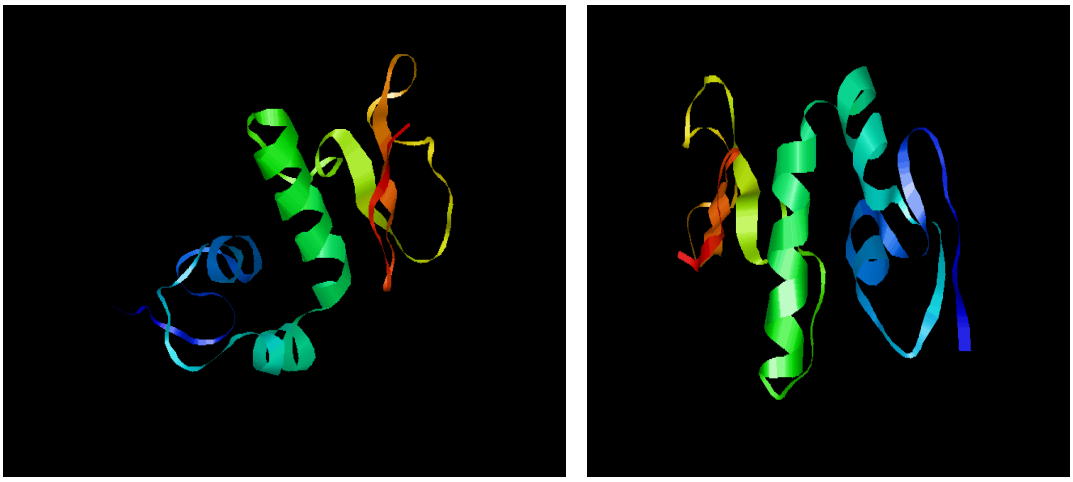
Fig.5. MODELLER prediction models for APH-1 subunit.

Pen-2 (Number of groups-101, number of atoms-859 & number of bonds-892)	Number of Helix	Number of Turns	Number of strands	Number of H-Bonds
Model1	3	12	6	50
Model2	3	12	6	49
Model3	3	10	7	50
Model4	3	10	3	48
Model5	3	12	7	50

Table 3: Comparison of different models of PEN-2 subunit generated by Modeller.



Model1 Model 2 Model 3



Model 4 Model5

Fig.6. MODELLER prediction models for PEN-2 sub unit.

3.7 Structure Validation

Ramachandran plot

A Ramachandran plot is a way to visualize backbone dihedral angle ψ (psi) against φ (pi) of amino acid residues in protein structure. There are three regions such as favoured region, allowed region and Outlier region.

We can find out which model has the highest number of residues in the favoured region and allowed region. Based on the number of residues in the favoured region and allowed region, the models are being selected- Model 5 (APH- 1) and Model 5 (PEN-2). RAMPAGE online server was used of Ramachandran plot analysis.

Subunit	Models	Number of residues in favoured region	Number of residues in allowed region	Number of residues in outlier region
APH-1	Model-one	177 (91.7 %)	10 (5.2 %)	6 (3.1 %)
	Model-two	170 (88.1 %)	13 (6.7 %)	10 (5.2 %)
	Model-three	177 (91.7 %)	10 (5.2 %)	6 (3.1 %)
	Model-four	183 (93 8 %)	9 (4.7 %)	3 (1.6 %)
	Model-five	184 (95.3 %)	9 (4.7 %)	0 (0.0 %)
PEN-2	Model-one	87 (87.9 %)	8 (8.1 %)	4 (4.0 %)
	Model-two	86 (86.9 %)	10 (10.1 %)	3 (3.0 %)
	Model-three	86 (86.9 %)	6 (6.1 %)	7 (7.1 %)
	Model-four	83 (83.4 %)	14 (14.1 %)	2 (2.0%)
	Model-five	87 (87.9 %)	10 (10.1 %)	2 (2.0%)

Table 4: Ramachandran analysis of different models.

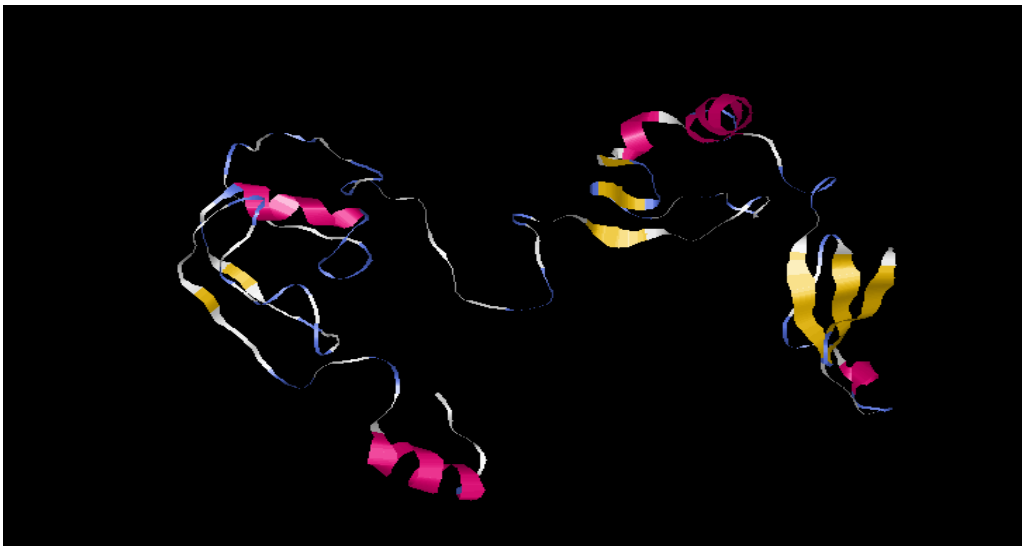


Fig.7: APH-1 subunit of the model-5 structure by RasMol software.

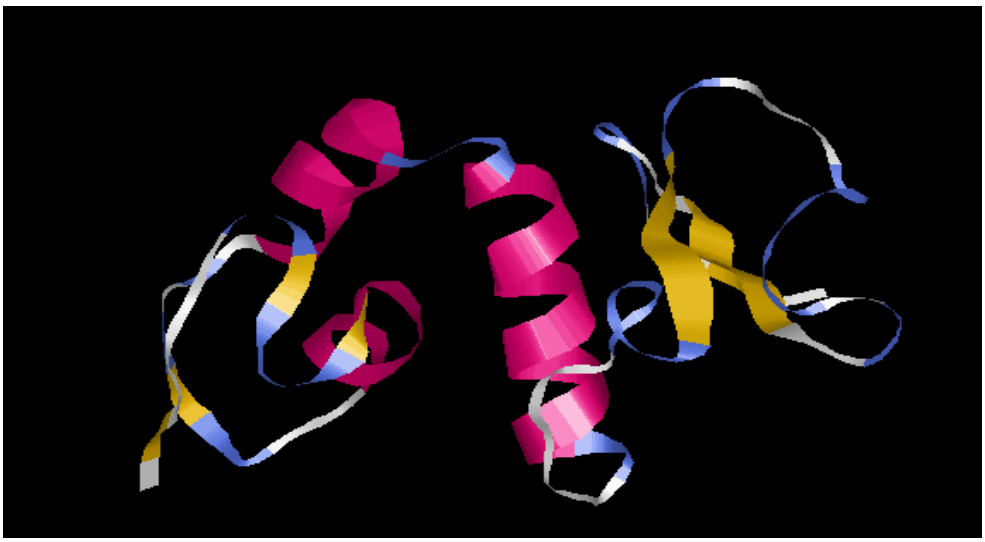


Fig.8: PEN-2 subunit of the model-5 structure by RasMol software.

3.8 Energy Minimization

Stable states of molecular systems correspond to local and global minima on their potential energy surface. Starting from a non-equilibrium molecular geometry, energy minimization employs the mathematical procedure of optimization to move atoms so as to reduce the net forces, the gradients of potential energy on the atoms until they become negligible. Energy minimization is usually carried out to determine a stable conformer. This process also is called geometry optimization. Energy minimization value depends on bonds angles, torsion, improper, non-bonded, electrostatic atoms/residues.

Energy minimization using Swiss PDB Viewer 4.1.0. Energy Minimization value of APH-1 (-2222.904 KJ/Mol) and PEN-2 (-2551.250 KJ/Mol).

Total residue	Bonds	Angles	Torsion	Improper	Non-bonded	Electrostatic	Total
APH1-(195)	283.898	814.529	852.829	372.373	-2661.47	-1885.06	-2222.904 KJ/Mol
PEN-2 (101)	133.604	594.654	551.978	250.936	-1744.00	-2339.42	-2552.250 KJ/Mol

Table 5: Energy minimization of two subunits APH-1 and PEN-2.

3.9 DOCKING

Docking is the method to determine the interaction between different molecules. We can predict the active site of the molecules where another molecule can come and bind to it. Use hex 8.0.0 software for protein- protein docking. We have to find the molecules which have better a affinity.

Aph-1 subunit is docked with Pen2 subunit. Then the resultant structure was docked with the presenilin subunit. Then the resultant of these three subunits was docked with Presenilin subunit. Finally, the complex structure of four subunits of Gamma-secretase is predicted.

1st Docking step: APH-1 subunit with PEN2 subunit.

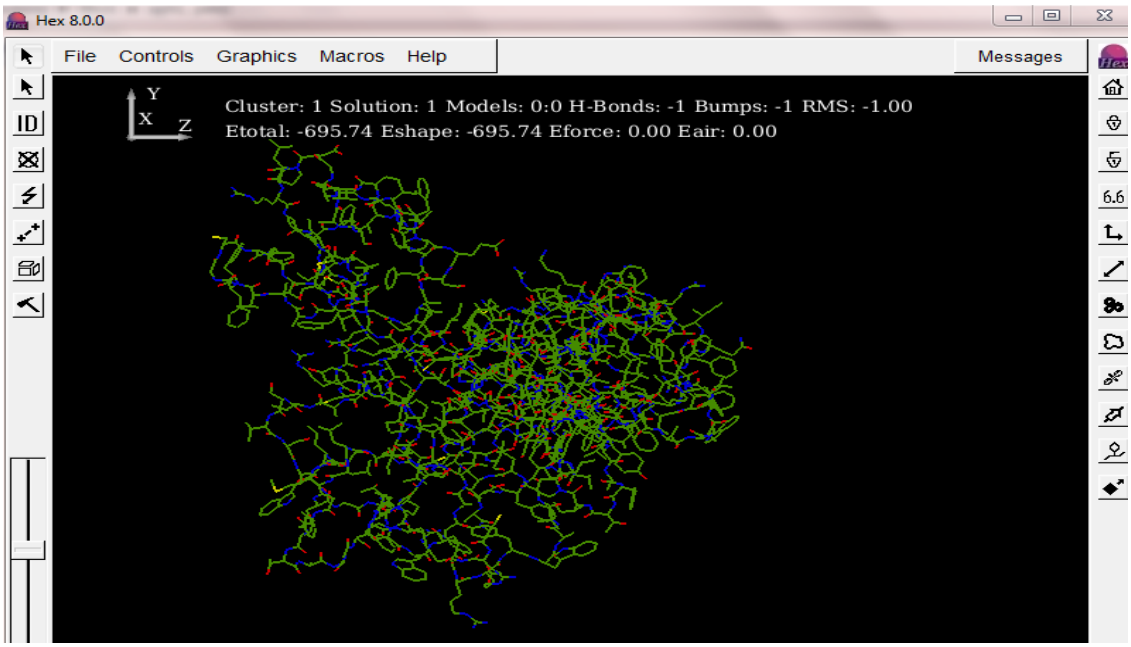


Fig.9: Docking result between APH-1 subunit with PEN2 subunit. The energy was found to be -695.74 kJ.

2nd docking step: The resultant structure from APH-1 and PEN 2 with presenilin subunit.

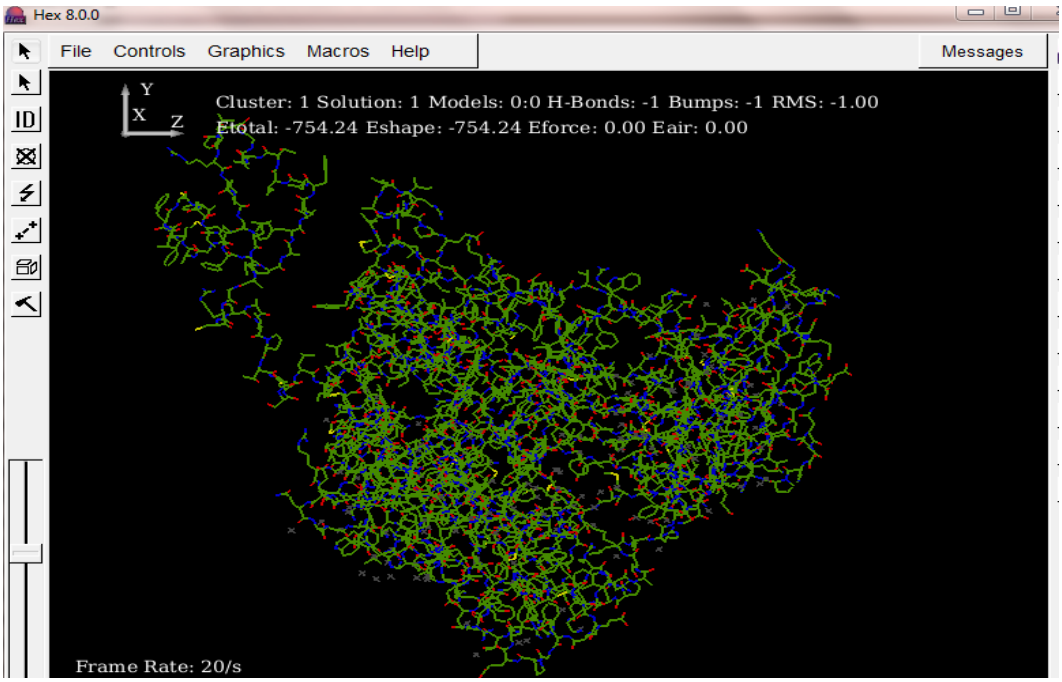


Fig.10: Docking result of APH-1 and PEN-2 with presenilin subunit. The energy was found to be -754.24 kJ.

3rd Docking step: The resultant structure of APH-1, PEN-2 and presenilin with nicastrin subunit.

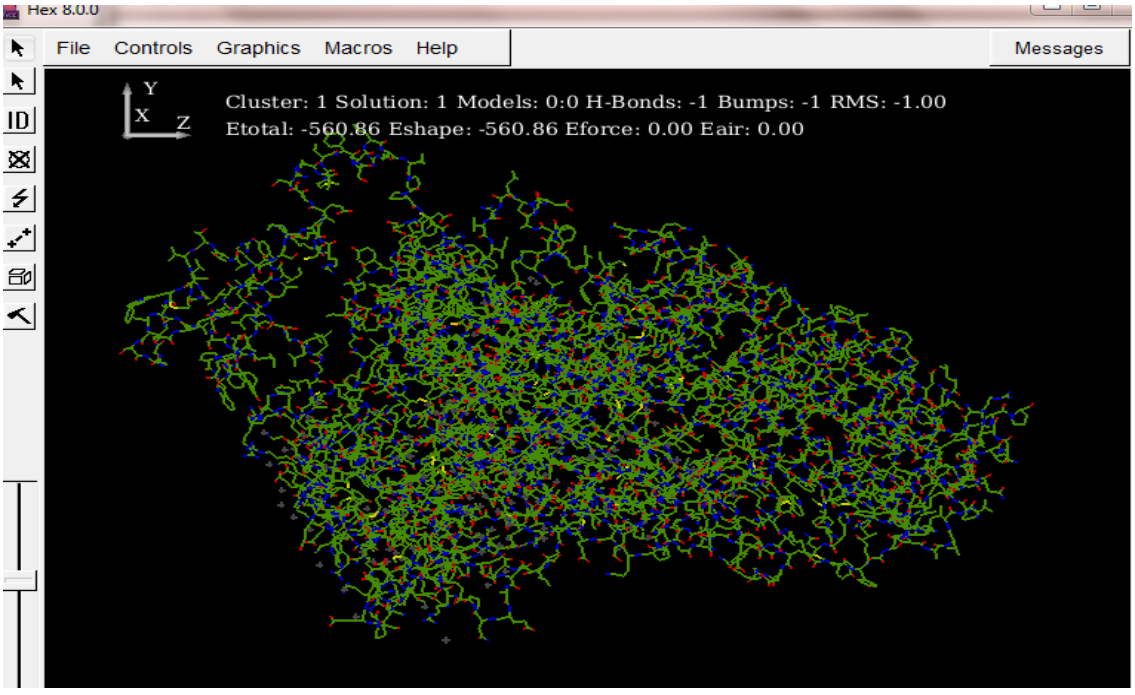


Fig.11: Docking result between APH-1, PEN-2 and presenilin with nicastrin subunit. The energy was found to be -560.86kJ.

4. RESULT AND DISCUSSIONS

The final docking result of complex structure of four subunits of gamma-secretase. The energy was found to be -560.86 KJ.

Fig.12: The complex structure of all the subunits of gamma-secretase.

The Ramachandran plot analysis of the gamma protein structure is done for the favoured region, allowed region and the outlier regions.

The tertiary structure of the gamma-secretase is being predicted with the most number of residues in the favoured region in Ramachandran plot. The residue in the favoured region and the allowed regions are found to be 91.40 % and 6.0 % of the total residues respectively. This indicates a good interaction of subunit structures to form the complex structure of γ-secretase.

Docked result of all the sub units (1044residues)	Number of Residue	Percentage of the total
Favoured Region	954	91.4
Allowed Region	63	6.0
Outlier Region	27	2.6

Table 6: Ramachadran plot analysis of the tertiary structure generated using all the subunits of the gamma-secretase.

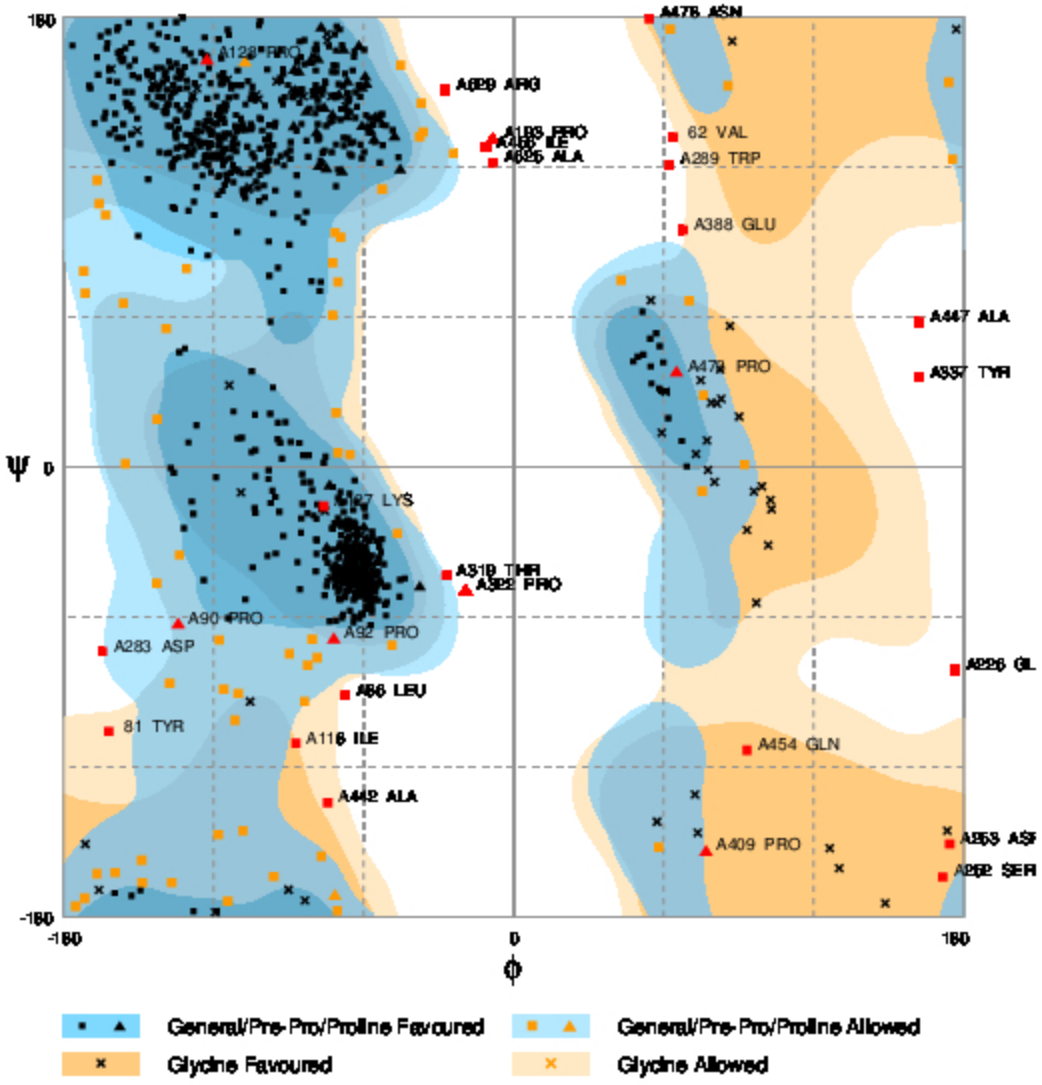


Fig.13: Ramachandran plot of the tertiary structure generated from all the subunits of gamma-secretase.

CONCLUSION AND FUTURE PERSPECTIVE

We predict the structure of subunits of gamma-secretase (APH-1 and PEN-2) using modeller software for homology modelling. Modeller software generates five models of each sub units of gamma-secretase. After that, we have done the structure validation of models by RAMPAGE online server. The model5 was selected based on highest number of favoured region and allowed region in Ramachandran plot. Then we used this structure to predict the structure of the complex gamma-secretase enzyme using of docking software Hex 8.0.0. The energy was found to be -560.86kJ.

The tertiary structure of the gamma-secretase enzyme was predicted with the most number of residues in the favoured region in Ramachandran plot. The residue in favoured region and the allowed regions were found to be 91.40 % and 6.0 % of the total residue respectively. This indicates a good interaction of subunit structures to form the complex structure of gamma-secretase enzyme. But the structure we have generated is not the perfect structure of the gamma-secretae enzyme. The dynamics of each atom present in the structure needs to be studied and the other stimulations are also to be considered. The orientation of each bond in the structure can also be determined by the flexible docking.

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