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INSILICO APPROACH TO PREDICT THE STRUCTURE OF AMYLOID PRECURSOR PROTEIN RESPONSIBLE FOR ALZHEIMER'S DISEASE

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ABSTRACT
Alzheimer's disease (AD) is one form of dementia that gradually gets worse over time. It affects memory, thinking, and behavior. Amyloid beta (A4) precursor protein (APP gene) is responsible for Alzheimer's disease and is coded by Amyloid Precursor Protein. APP is best known and most commonly studied as the precursor molecule whose proteolysis generates beta amyloid (Aβ), a 39- to 42-amino acid peptide whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients. Since the precise structure of amyloid precursor protein is not yet available. Therefore, a theoretical model of amyloid precursor protein was generated using homology Modeling. A search for templates revealed that PDB ID: 3MOQ shares query coverage 78% and maximum identity 96% to a hypothetical protein of Alzheimer, indicating this protein is evolutionary conserved. Validation of structure was done by using PROCHECK available at SAVES server. The validated model is submitted in PMDB (i.e ID: PM0078182). The predicted model of APP can be further used for drug target identification.

Keywords: Alzheimer's disease, APP, Homology Modelling, PMDB, SAVES

INTRODUCTION
Alzheimer's disease is the most common cause of dementia, affecting around 496,000 people in the UK. The term 'dementia' describes a set of symptoms which can include loss of memory, mood changes, and problems with communication and reasoning. These symptoms occur when the brain is damaged by certain diseases and conditions, including Alzheimer's disease [1]. This factsheet outlines the symptoms and risk factors for Alzheimer's disease, and describes what treatments are currently available. It was named after a German physician, Alois Alzheimer, who first described it in 1906 [2]. Alzheimer's disease is a progressive and irreversible brain disease that destroys mental and physical functioning in human beings, and invariably leads to death. It is said to be one of the leading causes of adult death in the United States. The disease can create emotional and

financial catastrophe for any families that has had a loved one inflicted with this horrible disease. Alzheimer's disease deteriorates the inflicted person's memory to the point that they cannot even remember who their children are. In a recent study done by scientist, they found that "based on recent estimates, about five percent of the American population over 65 suffers from this incurable disease. In the mid-1980's, at least 2 million people were condemned to live the last years of their lives in helplessness and mental disability. These results can be devastating to anyone who has a history of family members who have been inflicted by this disease. The course of Alzheimer's disease is not the same in every person, but symptoms seem to develop over the same general stages. In most people with Alzheimer's, symptoms first appear after age 60[3].

The early stages of Alzheimer's disease are difficult to diagnose. A definitive diagnosis is usually made once cognitive impairment compromises daily living activities, although the person may still be living independently. The symptoms will progress from mild cognitive problems, such as memory loss through increasing stages of cognitive and non-cognitive disturbances, eliminating any possibility of independent living, especially in the late stages of the disease[4].

A LOCUS segregating with familial Alzheimer's disease (AD) has been mapped to chromosome 21, close to the amyloid precursor protein (APP) gene. Recombinants between the APP gene and the AD locus have been reported which seemed to exclude it as the site of the mutation causing familial AD. [5] The APP gene provides instructions for making a protein called amyloid precursor protein. This protein is found in many tissues and organs, including the brain and spinal cord (central nervous system). Little is known about the function of amyloid precursor protein.

In 1991, the amyloid hypothesis postulated that amyloid beta (Aβ) deposits are the fundamental cause of the disease.[6] Support for this postulate comes from the location of the gene for the amyloid beta precursor protein (APP) on chromosome 21, together with the fact that people with trisomy 21 (Down Syndrome) who have an extra gene copy almost universally exhibit AD by 40 years of age.[7] Also APOE4, the major genetic risk factor for AD, leads to excess amyloid buildup in the brain.[8] Further evidence comes from the finding that transgenic mice that express a mutant form of the human APP gene develop fibrillar amyloid plaques and Alzheimer's-like brain pathology with spatial learning deficits.[9] Formation of senile plaques containing the β-amyloid peptide (Aβ) derived from the amyloid precursor protein (APP) is an invariant feature of Alzheimer's disease (AD). APP is cleaved either by β-secretase or by α-secretase to initiate amyloidogenic (release of Aβ) or nonamyloidogenic processing of APP, respectively. [10] The amyloid precursor protein (APP) is a transmembrane protein expressed in several cell types. In the nervous system, APP is expressed by glial and neuronal cells, and several lines of evidence suggest that it plays a role in normal and pathological phenomena.[11]

The most common APP mutation changes one of the protein building blocks (amino acids) in the amyloid precursor protein. This mutation replaces the amino acid valine with the amino acid isoleucine at protein position 717 (written as Val717Ile or V717I). Mutations in the APP gene can lead to an increased amount of the amyloid β peptide or to the production of a slightly longer and stickier form of the peptide. When these protein fragments are released from the cell, they can accumulate in the brain and form clumps called amyloid plaques. These plaques are characteristic of Alzheimer disease. A buildup of toxic amyloid β peptide and amyloid plaques may lead to the death of neurons and the progressive signs and symptoms of this disorder [12].

METHODOLOGY

Sequence alignment: The FASTA sequence of query protein (APP) was retrieved from NCBI Entrez sequence search (http://www.ncbi.nlm.nih.gov). Following BLASTp run (http://blast.ncbi.nlm.nih.gov/Blast.cgi), a Chain A, Amyloid Beta(18-41) Peptide Fusion With New Antigen Receptor Variable Domain by directed evolution (PDB ID: 3MOQ) was selected as template sequence (http://www.rcsb.org/pdb) (Figure:1).

Structure prediction: The 3D-structure of query protein was predicted by automated homology modeling program, Modeller9.10 [13]. For Modeller, the template and query sequences were carefully aligned to remove potential alignment errors. The default modeling process did end up with a loop (Figure:2).

Validation of Predicted Model: The model obtained was further submitted to modbase server (http://modbase.compbio.ucsf.edu/modloop/server) to rebuild the loop into its secondary structure. Validation of the model was done by Ramachandran plot analysis [14]. Structural models were visualized by Rasmol (2.6).

Active Site Identification: The active site of modeled structure of APP protein was predicted by using CastP database [15].

RESULT AND DISCUSSION

In this work, we describe homology modeling of the APP based on the X-ray structure of Amyloid beta(18-41) peptide. An Amyloid beta (18-41) peptide crystal structure 3MOQ was specifically selected on the basis of BLAST result and was utilized as a template for APP structure modeling. Structural models for App were built by MODELLER program [16] based on atomic coordinates of 3MOQ and were then energy minimized. The model with the lowest objective function (-2227.07446), which was considered as the best one, was selected and subjected to quality evaluation. The PROCHECK Ramachandran plot analysis shows that the main-chain conformations for 96.00% of amino acid residues are within the most favored or allowed regions (Figure 3). Loop modeling using MODLOOP analyzed by PROCHECK 3.0 showed 100% residues in the most favorable region and 0.0% in both generously allowed and disallowed regions of a Ramachandran plot (Figure 4). The validated structure was then submitted in PMDB (i.e ID: PM0078182). [17] (http://www.caspur.it/PMDB/) (Figure 5). The active site of APP protein shows structural pocket of area 61.6 with volume 52.1 and radius 1.4 (Figure 6). It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules.[7] The amino acids present in the pocket are labeled by green (Figure 7).

CONCLUSION:

We can conclude that the validated model of APP is now stable in all the aspects. So, this model can be further used for drug target identification.

ACKNOWLEDGMENT

I bow to almighty for always being on my side and bestowing me with blessing that have helped me sail through good and bad times. While doing this research I have nourished so many memories in my brain and so good feelings in my heart that is tough to decide how much to pen down. Still I want to write about all those whose guidance, love and care made me to feel like a part of this institute.

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16) A. Sali et al., Proteins, 23: 318 (1995) [PMID: 8710825]
17) Castrignanò T., D'Onorio De Meo P., Cozzetto D., Talamo I. G. and Tramontano, A PMDB - Protein Model Database

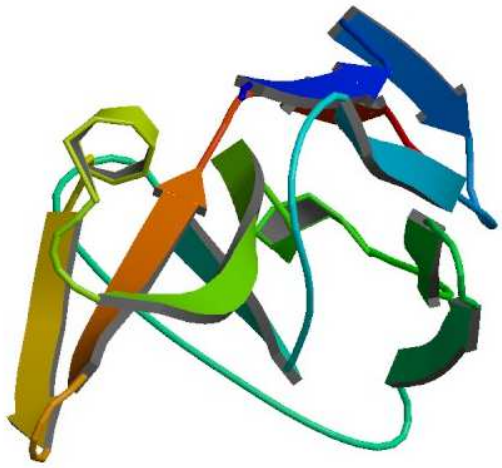

Figure 1: 3D structure of template (PDB:3MOQ)

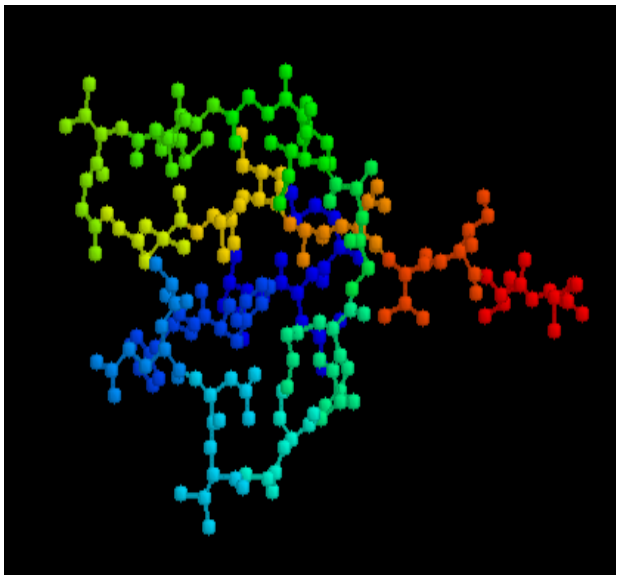

Figure 2: Modeled Structure of APP protein

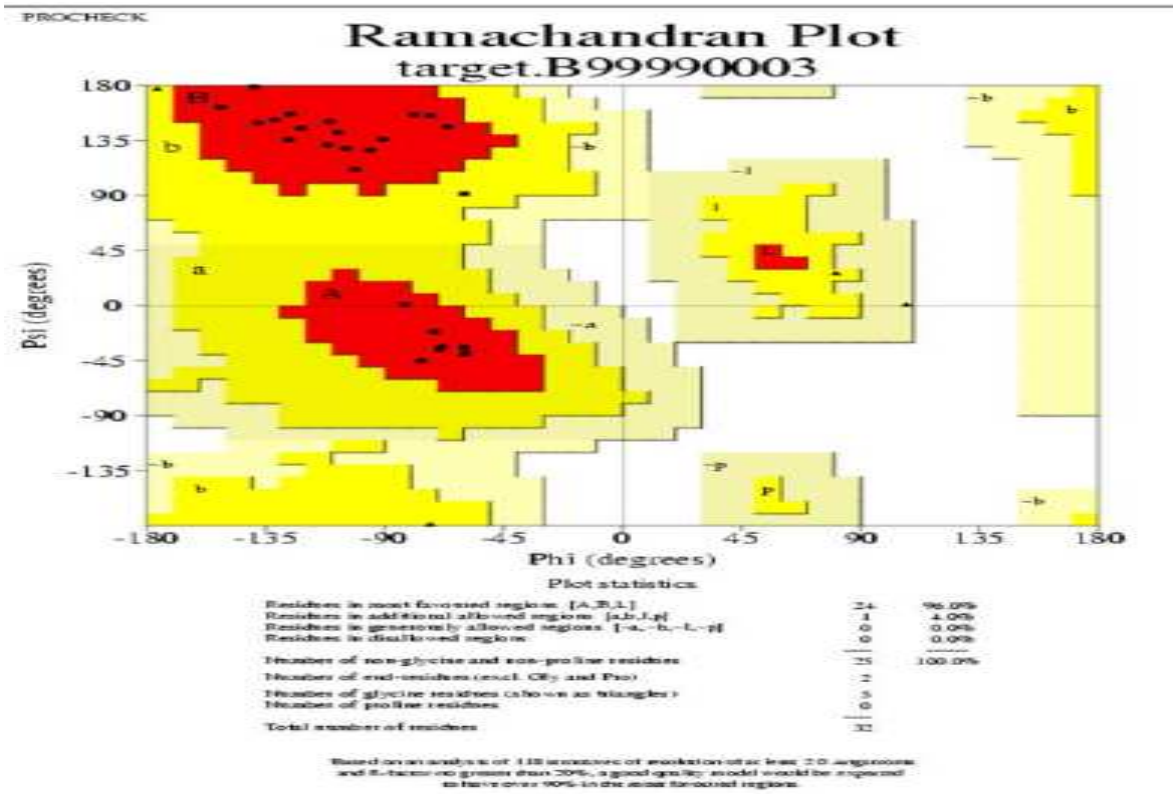

Figure 3: PROCHECK Ramachandran Plot Analysing 96.00% of amino acid residues are within the most favored or allowed regions.

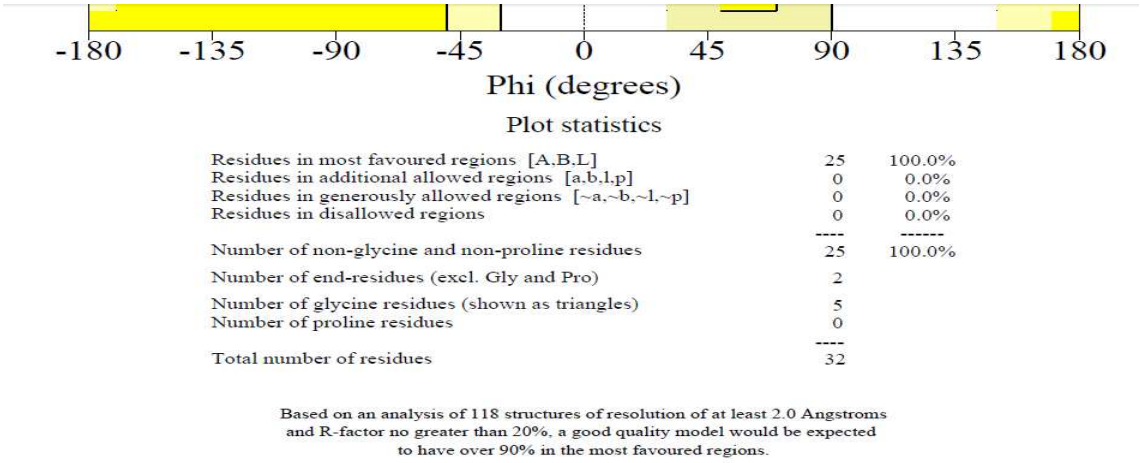

Figure 4: PROCHECK 3.0 showed 100% residues in favorable region.

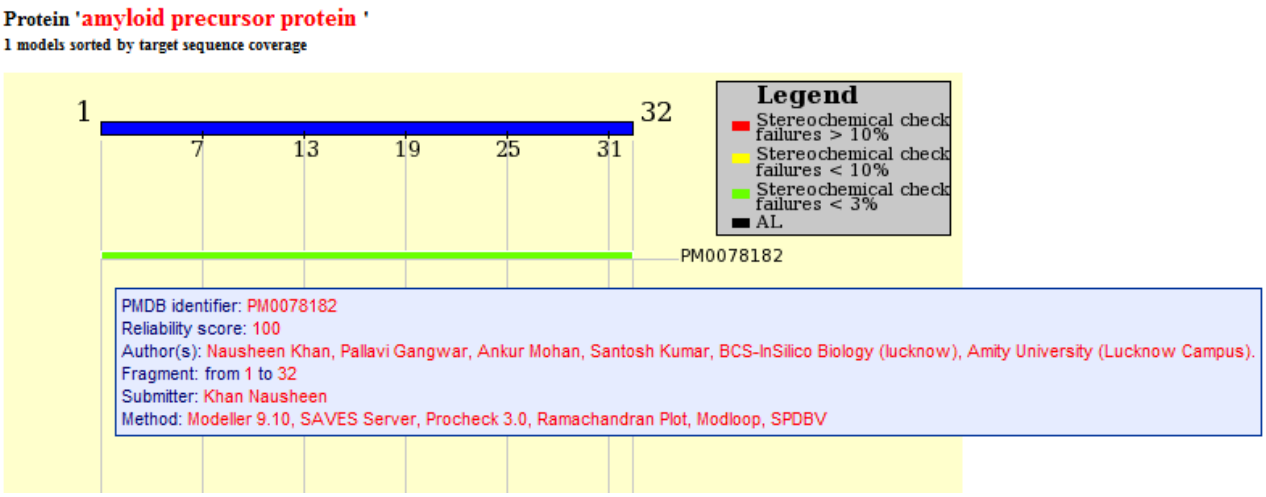

Fig 5: Submitted structure in PMDB

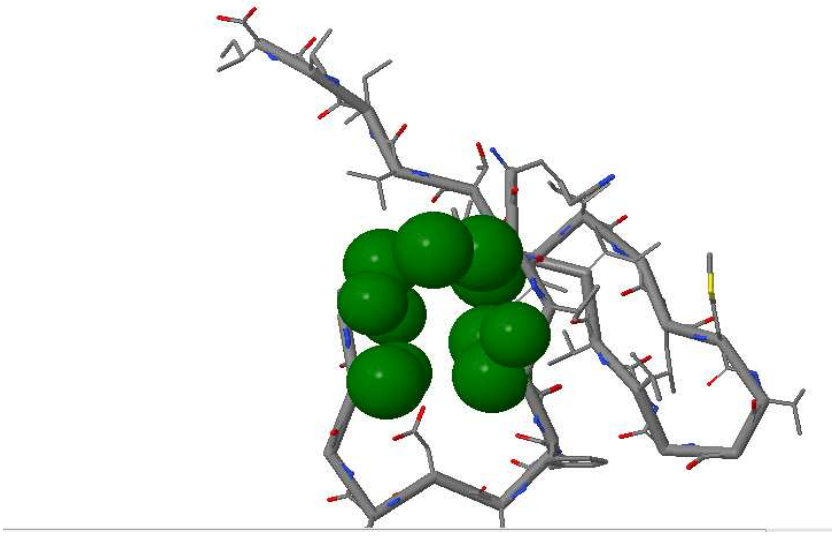

Figure 6: Active site of validated structure of APP protein.

Figure 7: Amino acid present in active site are labeled by green.