In silico Characterization of Prion Proteins and interactive association with Amyloid beta as novel therapeutic trends for Alzheimer's Disease

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Abstract

Alzheimer's Disease (AD) is a neurodegenerative disorder and is a form of dementia. The plague formed due to amyloid beta fibrillation is one of the characteristics in causing the disease. In the disease condition Aβ interacts with number of proteins and one of them is Prion protein. This interaction causes the Long Term Potentiation (LTP) inhibition in brain hippocampus region and also early development of AD. The research investigates the in silico analysis of proteins such as Aβ42 and Prion protein interaction in AD. Much of the experimental work is done to study their interaction but the aim of this study was to study their interaction computationally. In this paper, the interaction study was conducted between two proteins i.e. Aβ42 and PrP involve in Alzheimer's disease using LigPlot+ software. For protein-protein docking GRAMM-X web server is used and for protein-ligand docking PyRx software is used. The LigPlot+ software was used to analyze their interaction. The proteins Post Translational Modifications (PTM) sites were also studied using NetPhos web server. The RNA binding sites were also predicted using KYG: RNA server. The result of LigPlot+ shows that Aβ42 interacts with PrP through three hydrogen bonds. The Aβ42 residues involve in hydrogen bonds are Leu34, Ser26, Ala30 and the PrP residues involve in hydrogen bonds are Ile138, Arg220, and Ser135. After this the Aβ42 is docked with anti-Alzheimer's drugs such as Aricept, Exelon, Namenda and Razadyne. The ligand docked Aβ42 was then interacted with PrP and it showed low affinity with Aβ42. All the four drugs showed same effect as in all the scenarios the Aβ42 showed hydrogen bond interaction at only one site i.e. Aβ42 residue His14 bonds with PrP residue Leu130. This is because all the four drugs have same mechanism of action. From this it is concluded that in Alzheimer's disease PrP showed affinity for Aβ42 but after the Aβ42 is treated with anti-Alzheimer drugs the affinity of PrP to Aβ42 became low.

Keywords: Alzheimer's Disease; Aβ42; PrP; docking; GRAMM-X; PyRx; LigPlot+; NetPhos; KYG: RNA

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Chapter 1

Introduction

Chapter 1: Introduction

Background of the Problem:

Neurodegenerative disorders refer to any type of disease that causes neuronal cell death which eventually causes death. The neurological conditions include Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). (Bourgognon et al., 2018)

AD is a type of dementia (Kong et al., 2019) and is characterized as chronic as well as progressive neurodegenerative disorder. The progression of AD is marked by an aggravating decline in the health of patients with years, disability which leads to inability to live independently and perform daily routine tasks and leads to death. (Tatarnikova et al., 2015) The characteristic symptoms of AD entail short-term memory loss, visual as well as spatial disorders, speech and cognitive dysfunction (Kong et al., 2019) and dearth of reasoning and judgment (Usman et al., 2010).

AD's pathological characteristics involve plaques formation due to amyloid beta (Aβ) proteins deposition and neurofibrillary tangles as a result of tau protein hyperphosphorylation (Kong et al., 2019). The reduction of cognitive function may be linked with substantial decline in brain volume in AD patient relative to healthy patient. The atrophy of brain is due to synapses degeneration and neuronal death in the hippocampus region of the brain, which deals with memory and spatial orientation. (Cheignon et al., 2018)

On the basis of its onset, AD is classified into two types; Early Onset AD (EOAD) and Late Onset AD (LOAD). EOAD cases are less than 0.1% of all the cases and onset is between the age of 30 and 65 years. The age of onset of LOAD patients is more than 65 years and is characterized as most common form of AD. (Kong et al., 2019)

As the life expectancy is increasing, the number of AD patients is also increasing. The actual AD patients and the resulting expenses are considered to be significantly greater as most of the AD patients have not been diagnosed as well as not registered and therefore unable to receive any treatment and care. (Elflein, 2019)

Processing of APP in AD:

Two pathways are involved in the processing of APP i.e. Non-amyloidogenic and Amyloidogenic pathway (Kong et al., 2019). Secretases are the enzymes that are involved in APP proteolysis (Tatarnikova et al., 2015). Non-Amyloidogenic pathway involves the cleavage of APP by α-secretase followed by γ-secretase cleavage (Kong et al., 2019). This is called non-amyloidogenic because cleavage occurs within Aβ sequence and it inhibits the formation of amyloid peptide molecule (Tatarnikova et al., 2015). In α- cleavage, APP is cleaved between 16 and 17 amino acid residues at the N-terminus and result into sAPPα and α- Carboxy terminal fragments (α-CTFs or CTF83). Then γ-secretase degrade α-CTFs and result into P3 and incomplete Aβ residues i.e. Aβ 17-40 and Aβ 17-42 and these fragments of Aβ are not involved in forming amyloid deposits. (Kong et al., 2019) The sAPPα cleave by β- to produce truncated Aβ 1-16 (Cheignon et al., 2018). Amyloidogenic pathway involves the cleavage of APP by β-secretase (aspartyl protease) and followed by γ-secretase cleavage (Kong et al., 2019). It is less common pathway. Through endocytosis the APP and β-secretase get into the cell and then β-secretase start cleavage process with the proteins such as PICALM, BIN1, and CD2AP. (Tatarnikova et al., 2015) The β-secretase (BACE) releases N-terminus Aβ to produce sAPPβ and β- Carboxy terminal fragments (β-CTFs or CTF99) by cleaving APP in extracellular region (Kong et al., 2019) i.e. 16 amino acid residues distance from α_secretase cleavage site (Tatarnikova et al., 2015). The γ-secretase than cleave β-CTF to produce P3 protein of 3kDa and Aβ40 (major) and Aβ42 (minor) (Kong et al., 2019).

The CTFs that result from α- and β- cleavage remain bound to the membrane and further subjected to γ-secretase. The products are than release into the extracellular and intracellular space (Tatarnikova et al., 2015) and both also produce APP intracellular domain (AICD). On the basis of γ-secretase cleavage site numbers of Aβ peptides are released ranging from Aβ38 to Aβ43. The Aβ42 are in less concentration in brain than Aβ40. (Cheignon et al., 2018)

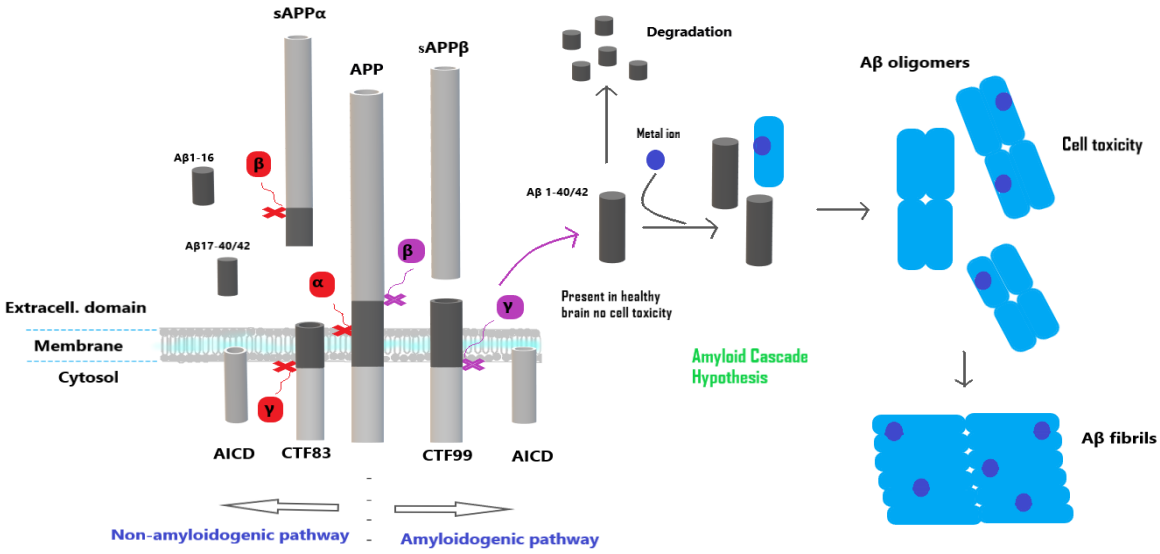

Figure 1: APP processing schematic diagram

In the non-amyloidogenic pathway the APP is first cleaved by α-secretase and produce sAPPα and CTF83 and then sAPPα cleaved by β-secretase to produce Aβ 1-16 and CTF83 cleaved by γ-secretase and release Aβ17-40 or Aβ 17-42 plus AICD. On the other hand in amyloidogenic pathway, APP is first cleaved by β-secretase and produces sAPPβ and CTF99 and then CTF99 undergoes γ-cleavage and produces Aβ 1-40 or Aβ 1-42 and they further converted into neurotoxic forms. (Cheignon et al., 2018)

Statement of Purpose:

The purpose of this study is to study the interaction between two important proteins that are involved in progression of AD. Although, amyloid beta plays a critical role in the disease progression but it require other proteins to interact with to induce its neurotoxicity. Here, in this study we will study the interaction between Aβ and prion protein computationally using bioinformatics tools.

The interaction can be used to study the bonds that are formed after the interaction and also what changes it causes after the interaction that leads to the AD.

Statistics of AD:

In 2018, about 50 million AD patients were reported and this number is expected to rise to 82 million by 2030 and 152 million by 2050. The figure 2 shows the graph of estimated number of people suffer from AD in years 2018, 2030 and 2050. (Elflein, 2019) The number of patients

doubles by every 20 years (Cheignon et al., 2018). In developing countries, the estimate is 58% and is expected to rise to 71% by 2050 [8]. The age is the highest risk variable for AD (Cheignon et al., 2018). The prevalence of AD is particularly high, about 10% of people above the age of 60 are reported to indicate symptoms of AD. The frequency is 50% for the people above the age of 85. (Kong et al., 2019) The people over age 65, 75 and 85 are affected by the disease by 5-8%, 15-20%, and 25-50% respectively. Due to higher life span of women as compare to men and due to drop in estrogen levels triggered by menopause, the women are more susceptible to AD. In Asia, with 22.9 million people the occurrence is high whereas in Europe it is 10.5 million and in America the number is 9.4 million. People with Down's syndrome condition are at more risk of EOAD as they bear an extra chromosome 21 where the gene encode for APP is located. (Cheignon et al., 2018)

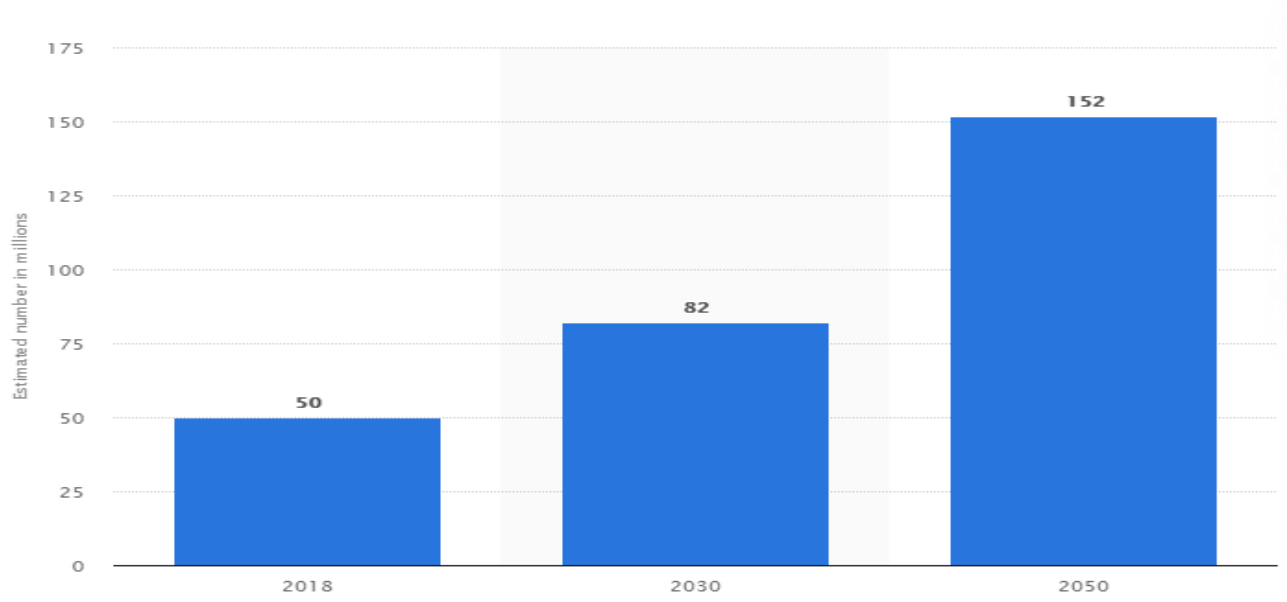

Figure 2: The graph shows the estimated number of people worldwide suffer from AD in 2018, 2030 and 2050.

Objective:

The main objective is to better understand conformational changes that occur after their interaction. Which will be helpful in understanding the AD pathology and also will provide the bases to identify how this disease can be treated? To identify the potential novel therapeutic interventions and targets for Alzheimer's disease.

Chapter 2

Literature Review

Chapter 2: Literature Review

Alzheimer disease and Aβ:

Amyloid beta results from proteolysis of Amyloid Precursor Protein (APP), which is a Type I transmembrane protein (Tatarnikova et al., 2015). The exact function of APP is still unknown. Using fatty membrane APP moved from inside to outside of membrane. APP is cleaved through various enzymes and resulted into small units and one of them is beta-amyloid. (www.alz.org) The amyloid beta (Aβ) is the useful proteins for the neurons (Ribaric, 2018).

The Aβ is one of the hallmarks of AD as it occurs in the specific portions of the brain in the form of plaques. The research shows that the intracellular build-up of Aβ is the initial event in the progression of AD. (LaFerla et al., 2007)

In the AD, the biochemical change alters the proteostasis to convert non-toxic and soluble Aβ to toxic Aβ peptides products such as misfolded soluble Aβ, Aβ oligomers and Aβ dimers (Ribaric, 2018). The role of Aβ is not limited to AD but Aβ plaques also play their role in PD dementia, vascular dementias and also in the aged people brain with no cognitive impairment. The studies have shown that Aβ sequence involve in the severity and progression of disease. (Tharp & Sarkar, 2013)

The metabolism of Aβ depends on external and internal factors. The internal factors include cellular, genetic and vascular factors whereas external factors include stress and hypoxia. These factors affect Aβ generation, accumulation, degeneration and also release. (Tatarnikova et al., 2015)

Aβ structure and function:

The Aβ exists in various forms such as monomer, insoluble fiber and soluble oligomer. The monomer and insoluble fiber form of Aβ does not involve in synaptic plasticity. Whereas soluble Aβ oligomer as well as Aβ dimer plays an important role in impairment of structure and also function of synapsis in the AD brain. (Kong et al., 2019)

In 1984, the Aβ primary sequence was discovered from amyloid plaques (Chen et al., 2017). It was done by George Glenner and Caine Wong as the isolated and did partial sequencing of Aβ (Masters & Selkoe, 2012). The monomer Aβ can assemble into various forms such as, oligomers, amyloid fibrils and protofibrils. The Aβ oligomer is soluble and spread in the brain whereas Amyloid fibrils are insoluble and larger and assembles further into amyloid plaques. The Aβ vary in size ranging from 37 to 49 amino acid residues. (Chen et al., 2017)

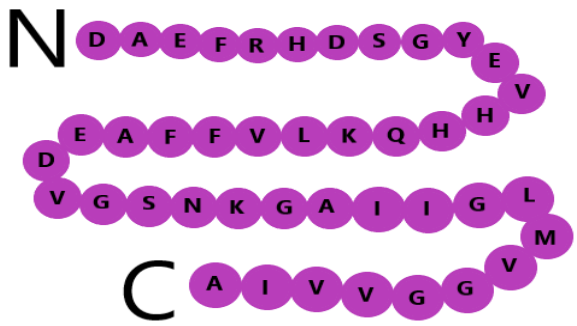

Figure 3: The Aβ monomer with 42 amino acid residues (modified). (Chen et al., 2017)

There are two Aβ peptides Aβ40 (1-40) and Aβ42 (1-42) have different conformations (Chen et al., 2017). The Aβ40 peptide occurs in the form of monomers, trimmers as well as tetramers whereas Aβ42 exists in dimers, tetramers, pentamer, hexamer and dodecamers forms (Nasica-Labouze et al., 2015). The Aβ42 has more ability to form plaques than Aβ40 as Aβ42 C-terminus is structured and 31-34 as well as 38-41 residues form β-hairpin that reduces the flexibility (Chen et al., 2017). This makes Aβ42 monomers and Aβ42 oligomers to be more neurotoxic (Ribaric, 2018). The sequence of Aβ42 wild type (WT) is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA which is generated by enzymatic cleavage of APP by β-secretase and γ –secretase (Cheignon et al., 2018).

The Aβ fibrils exist in parallel and antiparallel patterns. The Aβ peptides were cross linked by transglutaminase (tTg) which shows that it is a parallel β-sheet structure with hydrogen bonds. The Aβ fibrils that exist as β-sheets are Aβ40, Aβ35 and Aβ42. On the basis of molecular weight the Aβ oligomers exist from high-order assemblies to low molecular weight assemblies (dimers,

tetramers, and pentamers) to mid molecular weight (hexamers, nonamers, dodecamers) to fibrils. (Chen et al., 2017)

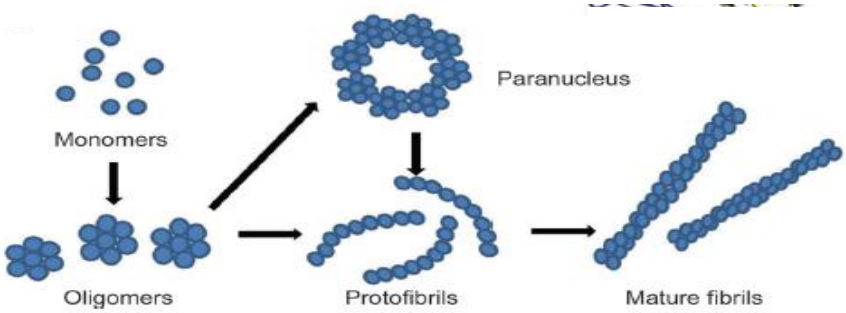

Figure 4: The conversion of Aβ monomer to mature fibrils. (Chen et al., 2017)

Prion Protein:

It is an infectious protein agent responsible for causing various neurodegenerative disorders in mammals known as Transmissible Spongiform Encephalopathies (TSEs). (Batlle et al., 2017) Prion protein is a 208 amino acids protein (Altmeppen et al., 2012) with a molecular weight of about 33 to 36 kDa and is anchored on extracellular membrane of neurons, peripheral cells and glial cells. The protein shows expression in both healthy as well as infected people. (Batlle et al., 2017)

The PrPC assumes its physiological form following translation and cotranslational when exudes into endoplasmic reticulum lumen. It has two parts, C-terminal known as globular domain and N-terminal flexible tail. The N-terminal part of protein is disordered and contains Octarepeat Region (OR), Neurotoxic Domain (ND), Hydrophobic Domain (HD) and two charge clusters i.e. CC1 and CC2 (Fig 1). (Wulf et al., 2017) This part comprises of residues 23 to 124 (Damberger et al., 2011). On the other hand, C-terminal part or globular domain is more structured and is consists of three alpha-helices, two beta strands, two N-glycosylation sites (the sites are highly conserved and are asparagine residues 181 and 197 (Zhang et al., 2019)) (Fig 1), disulfide bond (Altmeppen et al., 2012) and GPI-anchor at its upstream (Wulf et al., 2017) that allow it to attach itself onto the membrane (Altmeppen et al., 2012). The globular domain comprises of residues 125-228. The protein also contains short C-terminal tail of residues 229 to 231. (Zhang et al., 2019)

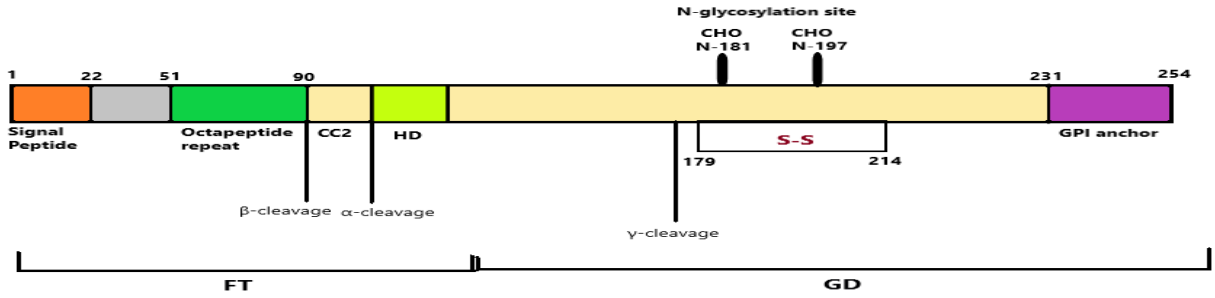

Figure 5: The diagram showing structure of PrPC (modified).

The N-terminus is important for the endocytosis of PrPC and the OR region allow to bind ions. (Zhang et al., 2019)

Today, the Protein Data Bank (PDB) has more than 70 PrP structures. The PDB has registered about 50 NMR structures and approximately 20 X-ray crystallography structures. These are all PrPC structures. The fig 2 below shows a human PrPC structure. (Zahn et al., 2000)

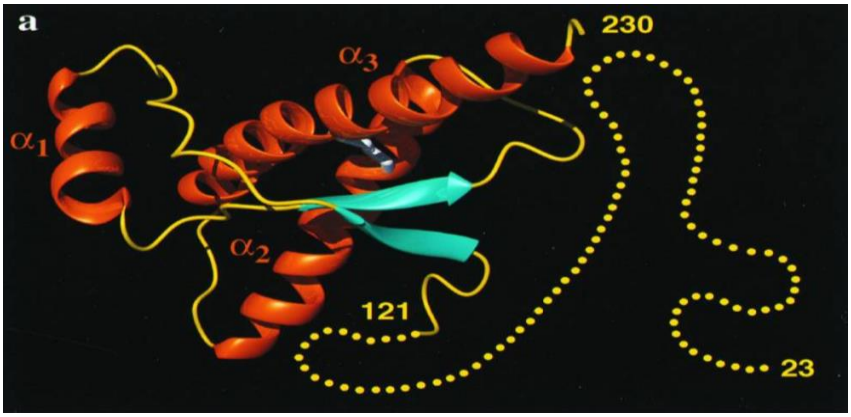

Figure 6: The three-dimensional structure of human prion protein.

In the structure the red color is showing alpha helices, cyan color showing β-strands, yellow color showing nonregular secondary structure within C-terminal and the yellow dots showing flexible disordered tail of residues 23 to 121. The figure was prepared by program MOLMOL. (Zahn et al., 2000)

PrPC perform several physiological functions such as neurogenesis, synaptogenesis as well as neuritogenesis and also involve in cell adhesion, neuroprotection, differentiation and copper homeostasis. It also involve in cellular signaling pathways either plays a central role or act as regulator. PrPC require a molecule to for transducing signal to the cytosol. (Altmeppen et al., 2012) Through its N-terminal cleavage products the PrPC sends signals. Eventually, PrPC also plays an important role to detect aggregates of Aβ. (Wulf et al., 2017)

Aβ and Prion protein interaction:

For the interaction to occur expression of PrPC is required. The studies have shown that recombinant PrPC binds with soluble Aβ42 oligomers through two motifs spanning 23-27 and 95-110 (Salazar et al., 2017) residues (Kong et al., 2019). Another research showed that the flexible N-terminal region spanning ~95- 110 and 23- 27 are the recognition site for the Aβ oligomer. The PrPC, α-helical domain, that is spanning 113-231 residues do not have any role in Aβ fiber growth whereas the N-terminal part of PrPC i.e. 23-126 residues fiber formation of Aβ. (Nasica-Labouze et al., 2015)

To determine early cause of AD pathophysiology is associated with the distinct roles of various PrPC domains. In unstructured N-terminal half of PrPC, the OR domain i.e. 60-95 residues involve in binding of Copper ion. The residues spanning 95-134 residues comprises charge cluster with residues 95-110 and Hydrophobic character segment having 112-134 residues. Both these regions play important role in neurodegenerative activity. The researchers have found that if residues 32-121 were to be deleted from PrPC than it will not allow the binding of Aβ42 oligomers showing globular domain do not play role in Aβ binding. In PrPC, researchers have indicated three Aβ42-specific regions such as 47-59, 53-65, and 87-99 and also three Aβ42-nonspecific regions i.e. 25-37, 37-49, and 99-111 in N-terminal flexible, unstructured domain. (Zhang et al., 2019)

In the hippocampus region of brain, the Aβ neurotoxicity can be reduced by adding PrPC synthetic peptide of residues 98-107. On the other hand, addition of PrPC peptides of residues 213-230 does not have any effect on neurotoxicity induced by Aβ. The N-terminal of PrPC spanning 23-27 and 92-110 regions, which are considered to be important for Aβ interaction, if deleted, will cause reduction in binding. (Zhang et al., 2019)

Table 1: The table shows PrPC regions characterization binding to the Aβ.

Position of PrPC binding to Aβ	**Forms of Aβ42**	**Sources of Aβ42**
PrPC 95-105	Oligomers (~500 kDa)	Synthesized
PrPC 23-27, PrPC 92-110	Oligomers (> 75 kDa)	Synthesized
PrPC 96-104	Dimers (8 kDa)	Brain-derived
PrPC 95-105, helix-1 (PrPC 144-156)	ADDLs (8~56 kDa)	Synthesized/brain-derived
PrPC 47-59, PrPC 53-65, PrPC 87-99	Oligomers	Brain-derived
PrPC 98-107	ADDLs (trimer~24 mer, 108 kDa)	Synthesized
PrPC 95-105	Dimers (8~9 kDa)	Brain-derived
PrPC 95-110	Oligomers (> 25 kDa)	Synthesized
PrPC 23-111	Globulomer AβO (~200 kDa)	Brain-derived
PrPC 23-31, PrPC 95-105	ADDLs (Dimers 8-108 kDa)	Synthesized
PrPC 23-27, PrPC 95-110	Dimers to pentamers (8~21 kDa) 150~300 kDa	Synthesized Brain-derived

Aβ monomer and their fibrils do not bind effectively with PrPC (Kang et al., 2019). The affinity of Aβ to bind with PrPC is very little as compare to Aβ oligomers (Nasica-Labouze et al., 2015). The PrPC involves in the inhibition of Aβ fibril formation by stabilizing Aβ oligomer (Kang et al.,

2019). The PrPC involves in neurotoxicity of Aβ in AD as it concentrate and trap the Aβ oligomeric form (Nasica-Labouze et al., 2015). The interaction between larger Aβ42 oligomers and PrPC is more effective than small Aβ42 oligomers in the AD brain and also the binding requires integrity of lipid raft (Kang et al., 2019).

Role of Prion protein in aggregation of Aβ:

Aβ plays a critical role in pathogenesis of AD but to cause neurotoxicity it requires other molecules. PrPC, which is a GPI-anchored glycoprotein, is one of them. (Kang et al., 2019) Beside prion disease PrPC also plays an important role in other neurodegenerative disorders i.e. AD. (Altmeppen et al., 2012) The interaction provides insight into the pathophysiology of AD related neurodegenerative disorders. (Zhang et al., 2019) The research has found that high affinity binding of Aβ oligomer with PrPC plays a key role in early pathogenesis of AD disease. (Salazar et al., 2017) The binding also causes the inhibition of long term potentiation (LTP) in the hippocampus region of the brain. (Nasica-Labouze et al., 2015)

The research have found that interaction of Aβ with PrPC only occurs in AD brain not in nondememnted brain and also interactions only occurs at the N-terminal half of the PrPC. (Zhang et al., 2019) Recent studies show that PrPCs effect on the neurotoxicity of Aβ oligomeric species. The peptide involves the initiation and progression of AD and β-sheet conformation that is due to neurodegenerative disorder. It is currently in debate how this interaction causes neurotoxic effects in AD. The beta-site- APP-cleaving enzyme 1 (BACE1) is regulated negatively by PrPC and thus causes reduction in the Amyloidogenic processing of APP to form Aβ. [16] The Aβ oligomers that are trapped in PrPC bind with A11 antibody and SEC (Oligomer specific) shows that there is 12 and 24 size of Aβ monomers (Nasica-Labouze et al., 2015).

The recent studies showed that PrPC not only interact with Aβ oligomer but also with other Aβ forms such as Aβ-derived diffusible ligands (ADDLs), synthetic Aβ oligomers and AD brain soluble extracts. The interaction of PrPC with various amyloid forms causes neuronal degeneration and studies have showed that these are low molecular weight Aβ species. The high molecular weight Aβ i.e. ADDLs causes synaptic plasticity as well as memory dysfunction in the AD patient when binds with PrPC. This binding also causes LTP inhibition in AD brain. The studies have also revealed that there is strong binding affinity between high molecular mass Aβ assemblies i.e. 158-300 kDa and PrPC than small, synthetic Aβ42 oligomers. (Zhang et al., 2019)

The binding between proteins require lipid rafts as it involves in cell signaling regulation, so the binding activates the cellular signaling. By adding Aβ oligomers into the neuronal cells expressing PrPC, it activates the synaptic cytoplasmic phospholipase A (2) which translocate into lipid rafts and forms complex with Aβ oligomers and PrPC causing damage to synapse. The Fyn kinase colonizes with PrPC in the lipid rafts and PrPC aggregation causes Fyn kinase activation. Also the activation of Fyn kinase, due to binding of PrPC and Aβ oligomers, leads to N-methyl-D-aspartate (NMDA) subunit NR2B phosphorylation which causes its degradation. In the AD mouse, the Aβ induced toxicity is enhanced by Fyn overexpression as it causes tau hyperphosphorylation and dyshomeostasis of neuronal Ca (II). According to this finding, the Aβ induced toxicity can be reduced by reducing or inhibiting the Fyn activity. (Kang et al., 2019)

One other protein that takes part in binding of Aβ oligomer with PrPC is metabotropic glutamate receptor (mGluR5), which is a postsynaptic transmembrane protein. The mGluR5 plays an important role in linking Fyn to Aβ oligomer-PrPC. This whole activation of Fyn and binding of PrPC- Aβ oligomer causes eEF2 phosphorylation which leads to dendritic spine loss. (Kang et al., 2019)

Impact of Aβ42 monomer on AD progression:

Among the two important amyloid beta species the Aβ42 shows more aggregation tendency and neurotoxicity than Aβ40 due to presence of two additional amino acid residues that are hydrophobic in nature. The Aβ monomers play important role in physiological processes but their role in AD pathogenesis is still in debate. (Tamagno et al., 2018)

The structure of Aβ monomer is mostly α-helical and also has random coils. The Aβ42 monomer is very much susceptible to aggregation and in result they form soluble oligomers of variable morphology and sizes such as dimers, trimmers as well as large fibrils. The pure monomeric form of Aβ protects the neurons through excitotoxixity and also trophic deprivation by activating phosphatidyl-inositol-3-kinase pathway. More recent proof of Aβ42 monomers was also found to mediate neuronal absorption of glucose, stimulating superfamily insulin receptor IGF-IRs and allowing glucose transfer i.e. Glut3 from cytosol to plasma membrane. These findings indicate the positive important function of Aβ42 monomers in survival of neurons; hence the therapeutic approaches can take this neuroprotective function into account. (Tamagno et al., 2018)

Due to Aβ's central role in AD pathogenesis, it is believable that it can play an important role in linking the two mechanisms i.e. autophagy and apoptosis. It is known that autophagy impairment

leads to accumulation of Aβ in the vacuoles and causes cell death. The AD murine and cellular models show that the AβPP and Aβ are localized in the autophagosomes. In addition, the Aβ42 and p62 (autophagic flux marker) accumulation lead to autophagic clearance dysfunction and impair lysosome. Good evidence suggests that during autophagy Aβ is also generated. Physiologically, due to effective lysosomal degradation clearance the autophagy cannot influence Aβ production whereas autophagy is the site for AβPP processing and Aβ production in pathogenesis that's why in AD brain many autophagic vacuoles are found especially in perikarya of neurons and in dystrophic neurites contain tangles. (Tamagno et al., 2018)

Previous research shows that Aβ peptides can lead to aggregation of tau through three main mechanisms: 1) Aβ activate certain kinases and phosphorylate tau and this enables the tau ability to bind to tubulin. 2) Aβ disrupts Tau's proteasomal degradation, increasing protein's free state. 3) Tau is nucleated by Aβ aggregates. (Tamagno et al., 2018)

In AD pathogenesis, both Aβ species are considered relevant. The findings indicate that therapy of AD not only involve elimination of oligomers but also the monomers and allow novel clinical methods to treat disease. (Tamagno et al., 2018)

Chapter 3: Methodology

Software and Online Resources

UniProt:

The UniProt database (http://www.uniprot.org/) is the center of all the grouped activities. Some of the important databases such as Swiss-Prot, TrEMBL and PIR_PSD are consolidated in it. The purpose of this is to provide proteins with meaningful information and comments. Any information that is missing in Swiss-Prot and TrEMBL is present in this database. (Apweiler et al., 2004)

RCSB PDB:

The RCSB PDB (https://www.rcsb.org/pdb/home/sitemap.do) is a protein data bank. The site contains 3D shapes of proteins and nucleic acids. This is the single archive that contains all the structural information of all the biological macromolecules. The database contains structures determined from different techniques such as NMR, X-ray crystallography and cryoelectron microscopy. (Berman et al., 2000)

DRUGBANK:

The DrugBank is a bioinformatics database that contains all the information and structures of drugs and also their potential protein targets. The database include 4100 drug entries with 800 of them are FDA approved. (Wishart et al., 2006)

PyMol:

It is a visualizing tool and also model molecules and makes them presentable. The PDB structure is loaded and one can also change the color of the protein and label the residues of the protein. It is a tool for molecular graphics that is used mostly to visualize three dimensional structures of proteins, small molecules, nucleic acids, surfaces, trajectories and electron densities. This tool can also be used to edit the molecules and also make movies. (Yuan et al., 2017)

NetPhos 2.0:

The server uses Support Vector Machine (SVM) concept with sequence of protein profile and its coupling pattern. The predictive models are trained using library LIBSVM. (Wong et al., 2007) The phosphorylation of serine, tyrosine or threonine plays its role in the signaling pathway. This network is used to predict phosphorylation sites in the protein

sequence. (Blom et al., 1999) The server is available at http://www.cbs.dtu.dk/services/NetPhos/.

KYG: RNA

The KYG:RNA inputs protein structure and predicts binding sites of proteins for RNA. The server only predicts RNA binding sites of proteins whose structures are known. (Terribilini et al., 2007) The server is available at http://cib.cf.ocha.ac.jp/KYG/.

GRAMM-X:

The GRAMM-X server is available at http://vakser.bioinformatics.ku.edu/resources/gramm/grammx. The docking tool uses Fast Fourier Transformation method by employing knowledge based scoring. The input of the server is processed by 320 processor Linux cluster. (Tovchigrechko & Vakser, 2006)

PyRx:

The drugs or ligands are docked with protein using AutoDock Vina in PyRx, which is a virtual screening tool. The tool has docking wizard and has easy to use interface which plays an important role in Computer Aided Drug Design (CADD). For our work we used PyRx 0.8 version which can be downloaded from (http://pyrx.sourceforge.net.)

LigPlot+:

The LigPlot+ has two domains. LigPlot, which is for Protein-ligand interaction studies. Dimplot is used for Protein-Protein interaction studies. We used Dimplot for interaction studies. The Dimplot generates 2-D protein-protein interaction diagram from 3D structure. The diagram shows at which points hydrogen bonds and hydrophobic bonds are formed. (Laskowski & Swindells, 2011)

Methodology:

Aβ42-PrP Interaction analysis:

In this interaction analysis the 3D structures of proteins were downloaded from RCSB PDB in .pdf format. The structures are then visualized using PyMol software. After this the structures are input as receptor and ligand for interaction in Gramm-X tool. The docked

result is obtained after several hours and then the interaction is analyzed using LigPlot+ software.

UniProt Database:	FASTA sequence retrieval
RCSB PDB:	3D structure retrieval in .pdb
PyMol software:	Visualization of pdb proteins
GRAMM-X:	Aβ42-PrP Docking
LigPlot+ (DimPlot):	2D interaction analysis of docked proteins

RNA binding sites prediction of both Aβ42 and PrP:

The already downloaded PDB structures of proteins were input in KYG:RNA tool and this tool will predict the RNA binding sites on the proteins using scoring methods.

RCSB PDB:	3D structure retrieval in .pdb
KYG:RNA:	Prediction of RNA binding sites on Proteins
	Result analysis

Post-Translational Modification analysis of Aβ42 and PrP:

For post translational modification study FASTA sequence of both the proteins were obtained from UniProt database. Then these sequences were used as input in NetPhos 2.0 tool to predict phosphorylation site in the sequence and analyze the result.

UniProt Database:	FASTA sequence retrieval
NetPhos 2.0:	Phosphorylation study of Proteins site
Post Translational Modification:	Result analysis

Aβ42-drug-PrP analysis:

After Aβ42 and PrP docking, Aβ42 is docked with anti-Alzheimer drugs such as Aricept, Exelon, Namenda and Razadyne and then docked Aβ42 is interacted with PrP and interaction is analyzed. The drugs were obtained from DrugBank in the form of .sdf format. The proteins were already obtained from RCSB PDB. The protein and drug docking is performed using PyRx software and then Gramm-X is used to dock PrP with already docked Aβ42. Then results are analyzed using LigPlot+ software.

DrugBank:	3D structure of drug in .sdf
RCSB PDB:	3D structure retrieval in .pdb
PyRx:	Protein-Drug docking
GRAMM-X:	Interacting protein with Protein-Drug complex
LigPlot+ (DimPlot):	2D interaction analysis of docked proteins

Chapter 4: Results and Discussion

Chapter 4: Results and Discussion

Aβ42:

- **FASTA sequence from UniProt:**

>sp|P05067|672-713
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

- **3D structure from PDB (PDB ID 1Z0Q):**

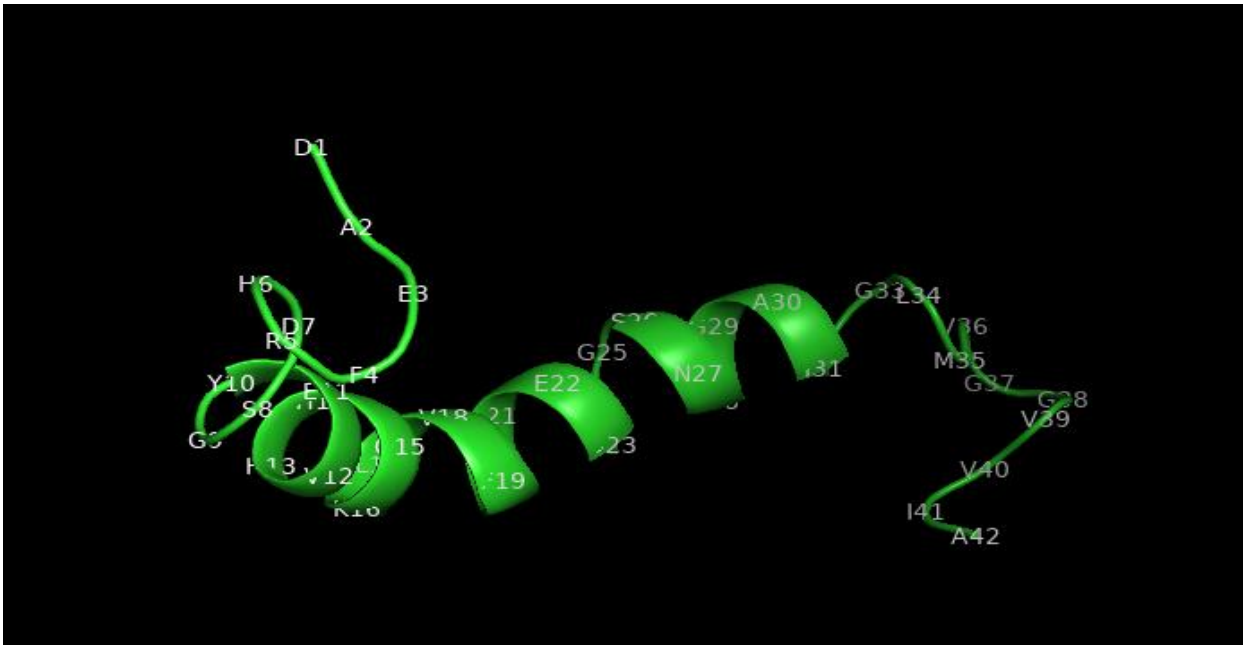

Figure 7: The 3D structure of Aβ42 retrieved from RCSB PDB (PDB Id: 1Z0Q) and visualized in PyMol software.

PrP:

- **FASTA sequence from UniProt:**

>sp|P04156|90-231
GQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDY
EDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDV
KMMERVVEQMCITQYERESQAYYQRGSS

- **3D structure from PDB (PDB ID 2LSB):**

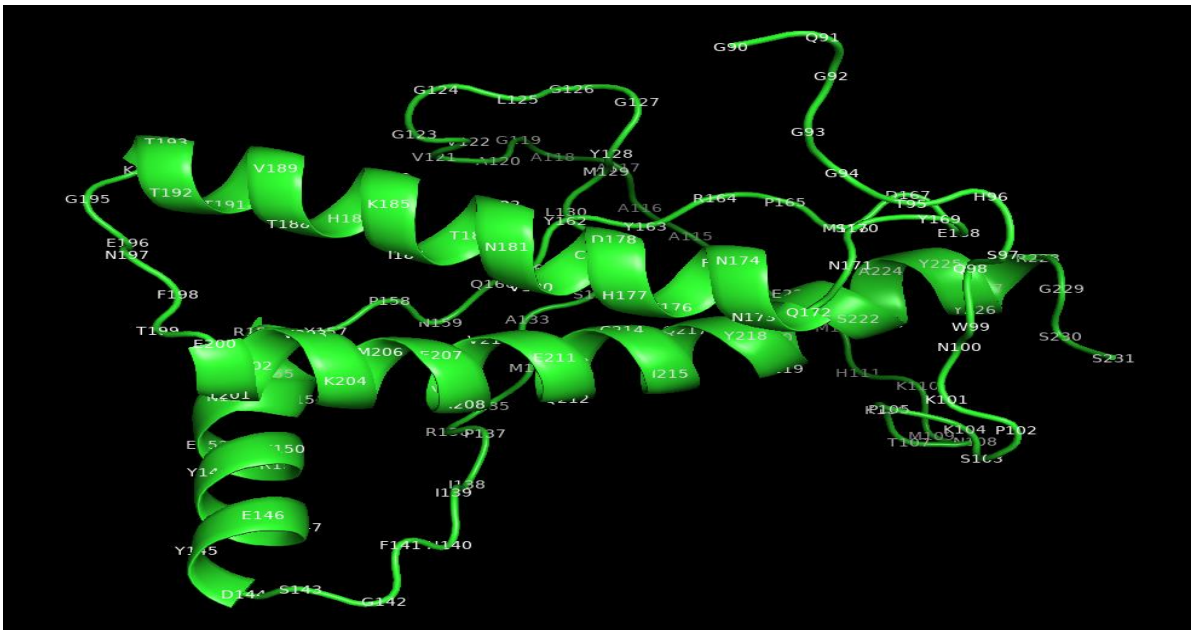

Figure 8: The 3D structure of PrP retrieved from RCSB PDB (PDB Id: 2LSB) and visualized in PyMol software.

Aβ42-PrP Interaction analysis:

Some proteins interact with other proteins and molecules to perform desire function. The interaction or docking of proteins was performed using Gramm-X and the information of bonds and the distance between them were obtained from DimPlot.

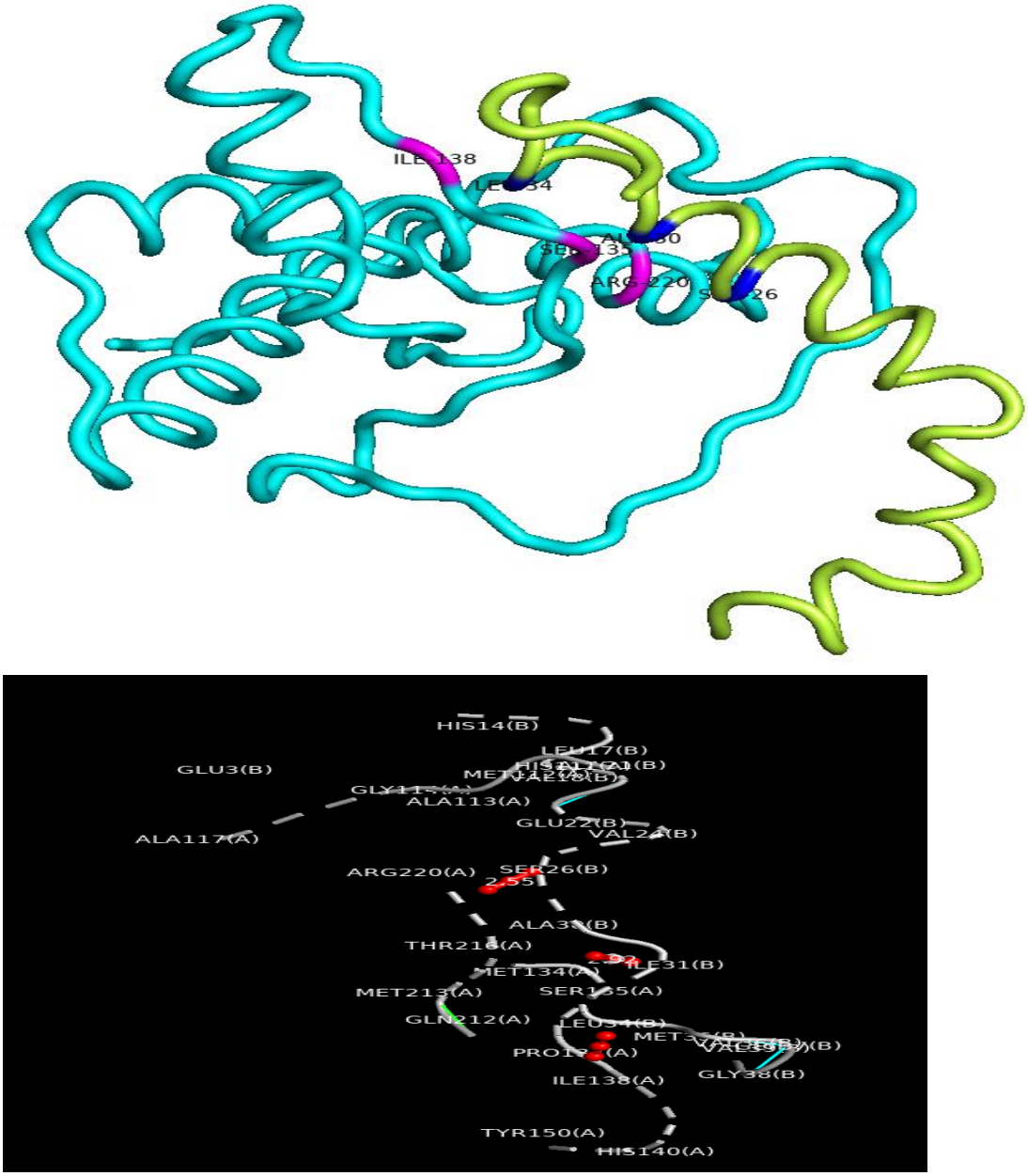

Figure 9: The docked Aβ42 and PrP complex from GRAMM-X is then visualized through PyMol software.

The figures show three points at which hydrogen bonds are formed between Aβ42-PrP complexes.

Interaction study by LIGPLOT+ (DimPlot):

The green dotted line shows hydrogen bond between amino acid residues. The red half circles show PrP amino acids and pink half circles shows Aβ42 amino acids. The number written on the dotted line is the distance between two residues in Angstrom.

The hydrophobic residues of Aβ42 that are involve in interaction with PrP are His111, His140, Pro137, Tyr150, Met213, Ala117, Gln212, Met134, Thr216, Met112, Ala113, Gly114 and hydrophobic residues of PrP involve in interaction are Val24, Gly38, Gly37, Val39, Glu3, Val36, Met35, His14, Leu17, Ile31, Ala21, Val18, Glu22.

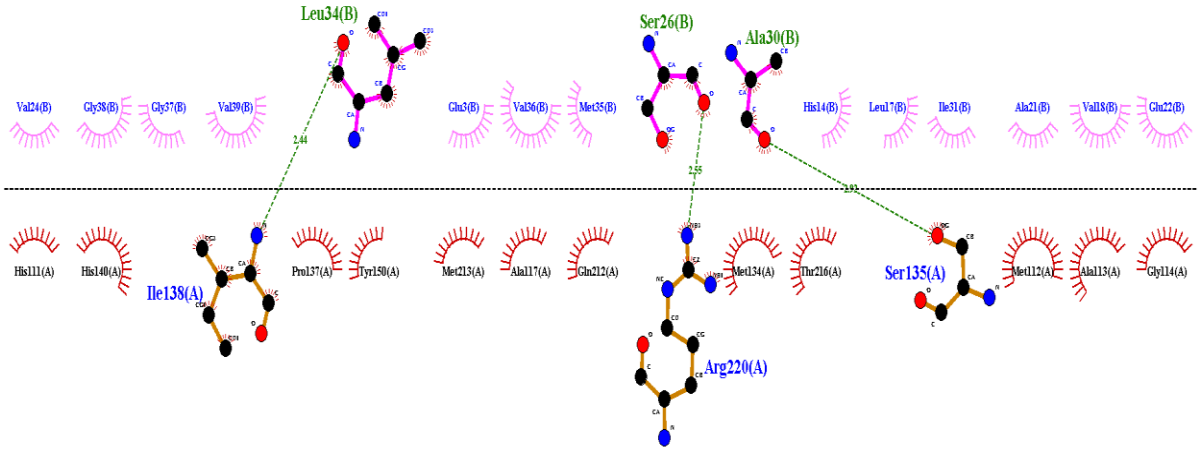

Figure 10: The DimPlot analysis of interaction of Aβ42 (Chain B) with PrP (Chain A)

Table 2: The table shows atoms involved and the distance between residues involved in Hydrogen bonding between the two proteins.

PrP residues	Atoms involved	Aβ42 residues	Atoms involved	Distance (Å)
Ile 138	N	Leu 34	O	2.44
Arg 220	NH2	Ser 26	O	2.55
Ser 135	OG	Ala 30	O	2.92

RNA binding sites prediction of both Aβ42 and PrP:

The identification RNA binding site on the protein is performed so that we can predict at which amino acids the RNA can bind to. For this purpose KYG:RNA is used which gives us graph and the colored structure showing RNA binding sites.

Aβ42 RNA binding site by KYG: RNA:

The server gives a graph and 3D structure. The scoring method that is used to predict RNA binding sites is A^2SPD scoring method. The scoring was done in such a way that those surface residues that are within 7.0 Å are given highest score and they are considered potential RNA binding site. The specificity of prediction done by A^2SPD is almost 80%. [32]

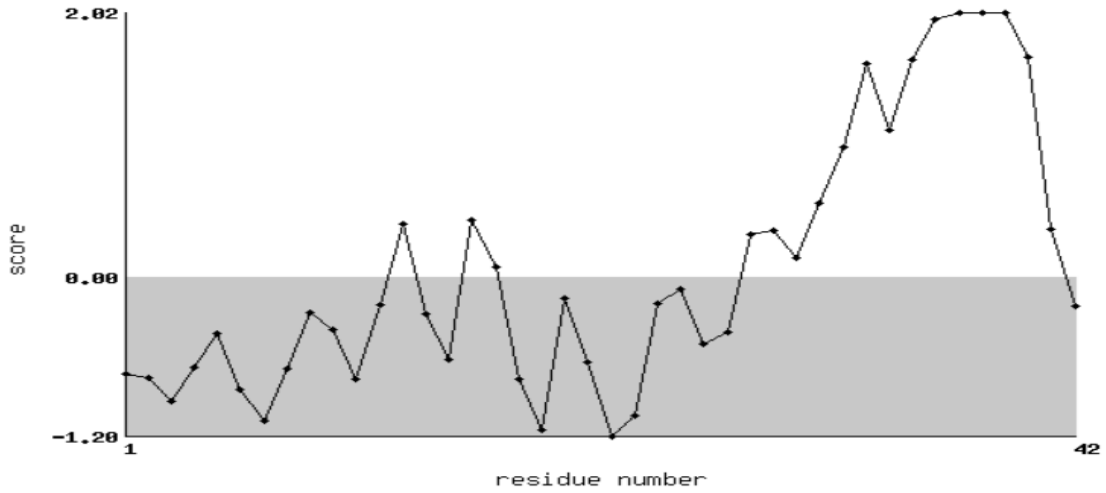

Figure 11: The black dots in the graph show Aβ42 amino acids and the height shows the score of amino acid.

In the graph the amino acids from 33 to 40 have high score so this region is the likely interface for RNA to bind. The amino acids at position 37, 38, and 39.

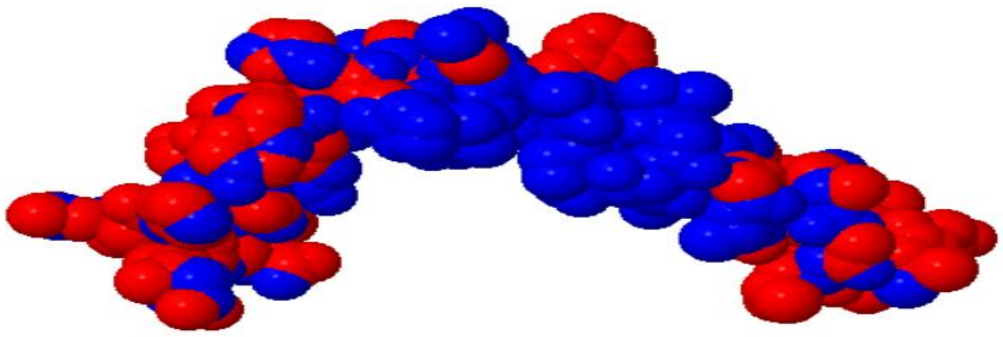

Figure 12: The Aβ42 protein is color coded residue-wise

In general, the colors are categorized into three types i.e. red, light blue and deep blue. The red shows highly likely to be an interface, the light blue shows unlikely to be an interface and the deep blue

PrP RNA binding site by KYG: RNA:

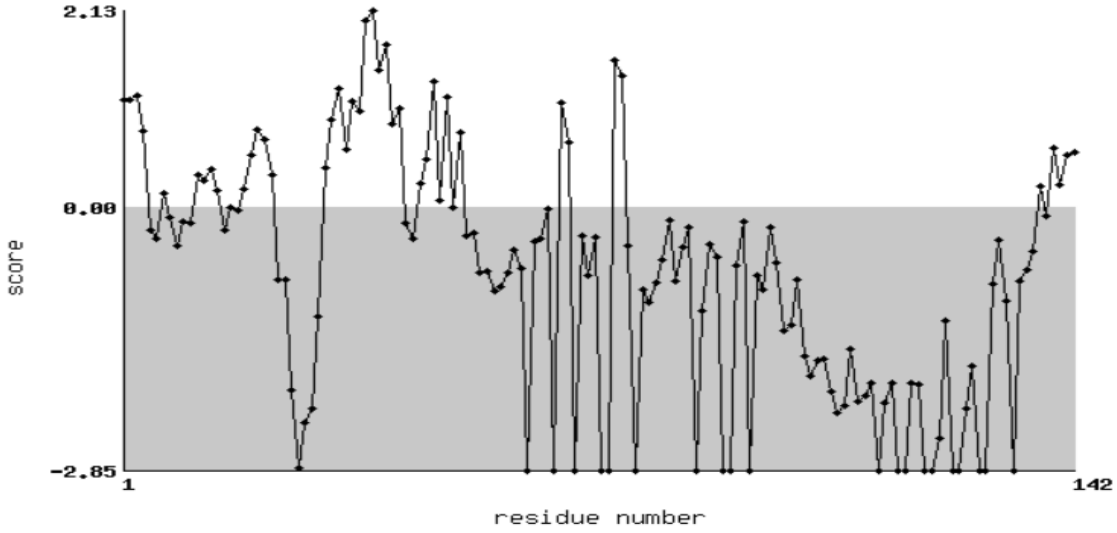

Figure 13: The black dots in the graph show PrP amino acids and the height shows the score of amino acid.

If the score is greater than the amino acid is the likely interface or the binding site for RNA. In the graph above the amino acids residues from 1-4, 12-15, and 31-42 shows highly likely to be RNA interface. The residue 38 has the highest score i.e. 2.13

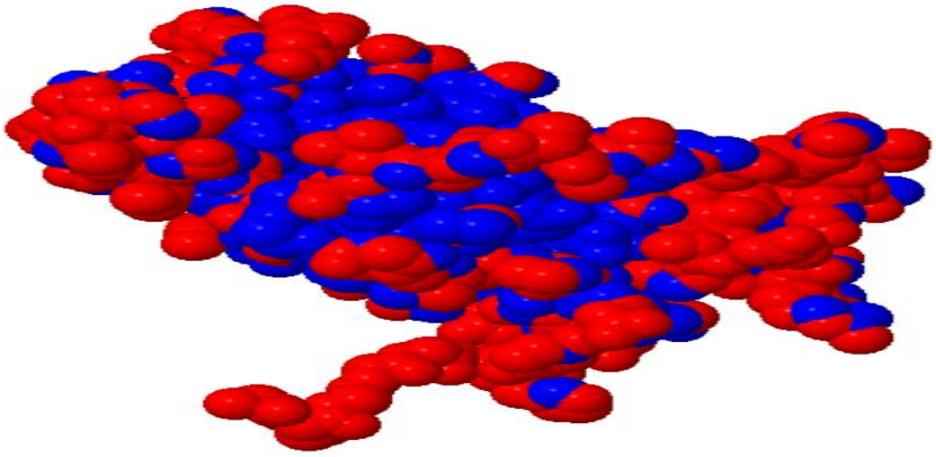

Figure 14: The PrP protein is color coded residue-wise.

In general, the colors are categorized into three types i.e. red, light blue and deep blue. The red shows highly likely to be an interface, the light blue shows unlikely to be an interface and the deep blue.

Post-Translational Modification analysis of Aβ42 and PrP:

The PTM of the proteins were performed to identify at which sites of the protein sequence the phosphorylation is done. As phosphorylation causes the protein to activate, deactivate or modify its function.

Post Translational Modification Study of Aβ42:

The phosphorylation sites on serine, tyrosine and threonine are predicted. The phosphorylation sites predicted in the sequence are 2 Serine and 1 Tyrosine at position 8, 10, and 26 of the Aβ42 sequence. The score of the prediction is between 0 and 1. The threshold to assign the residue to be phosphorylation site is 0.5. If the score is nearer to 1 than the position is likely phosphorylated site and if the score is slightly above 0.5 than the confidence of the site to be phosphorylated is very low. The high chances of phosphorylation may occur at 8, 10 and 26 position of sequence.

Table 3: The table shows position of sites, residues, and score of predicted phosphorylation sites.

Position	Context	Score	Prediction
8	FRHD**S**GYEV	0.963	*S*
10	HDSG**Y**EVHH	0.870	*Y*
26	EDVG**S**NKGA	0.787	*S*

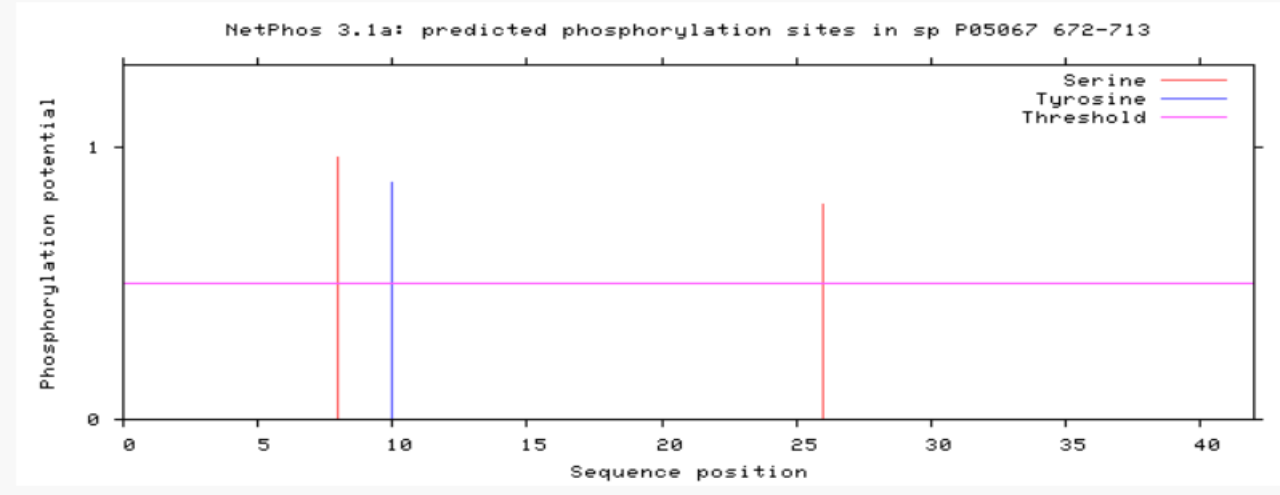

Figure 15: The graph shows which residues of Aβ42 have scores above the threshold i.e. 0.5. The red bars show serine residues and blue bar shows tyrosine residue.

Post Translational Modification Study of PrP:

For PrP, the phosphorylation sites predicted in the sequence are 9 Serine, 10 tyrosine, and 12 threonine. The score of the prediction is between 0 and 1. The threshold to assign the residue to be phosphorylation site is 0.5. If the score is nearer to 1 than the position is likely phosphorylated site and if the score is slightly above 0.5 than the confidence of the site to be phosphorylated is very low. The positions 54, 56, 60, 80, 94, 103, 104 and142 shows high phosphorylation chances as their scores are nearer to 1.

Table 4: The table shows position of sites, residues, and score of predicted phosphorylation sites.

Position	Context	Score	Prediction
6	QGGGTHSQW	0.455	*T*
8	GGTHSQWNK	0.640	*S*
14	WNKPSKPKT	0.562	*S*
18	SKPKTNMKH	0.606	*T*
39	GLGGYMLGS	0.407	*Y*
43	YMLGSAMSR	0.617	*S*
46	GSAMSRPII	0.506	*S*
54	IHFGSDYED	0.970	*S*
56	FGSDYEDRY	0.965	*Y*
60	YEDRYYREN	0.965	*Y*
61	EDRYYRENM	0.478	*Y*
68	NMHRYPNQV	0.417	*Y*
73	PNQVYYRPM	0.409	*Y*
74	NQVYYRPMD	0.419	*Y*
80	PMDEYSNQN	0.980	*Y*
81	MDEYSNQNN	0.439	*S*
94	CVNITIKQH	0.820	*T*
99	IKQHTVTTT	0.457	*T*
101	QHTVTTTTK	0.615	*T*
102	HTVTTTTKG	0.651	*T*
103	TVTTTTKGE	0.915	*T*
104	VTTTTKGEN	0.892	*T*
110	GENFTETDV	0.615	*T*
112	NFTETDVKM	0.508	*T*
127	QMCITQYER	0.519	*T*
129	CITQYERES	0.416	*T*
133	YERESQAYY	0.648	*S*
136	ESQAYYQRG	0.557	*Y*
137	SQAYYQRGS	0.395	*Y*

141	YQRGSS---	0.595	*S*
142	QRGSS----	0.823	*S*

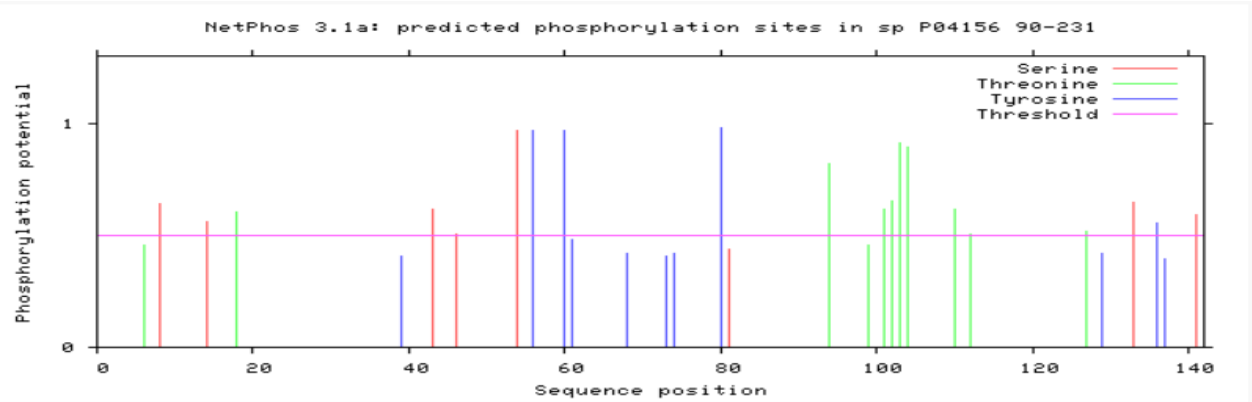

Figure 16: The graph shows which residues of PrP have scores above the threshold i.e. 0.5. The red bars show serine residues, blue bars show tyrosine residues and green bars show threonine residues.

Aβ42-anti-Alzheimer drugs-PrP analysis:

The PyRx was used to dock Aβ42 with Aricept, Exelon, Namenda and Razadyne. The .sdf files of all the drugs was downloaded from DrugBank and then loaded into PyRx. Then these files are converted into PDBQT file to generate coordinates. Then the protein and drug is docked using AutoDock Vina in PyRx. The result with least binding energy was chose as it indicates successful docking is done. The process is done separately for all the drugs.

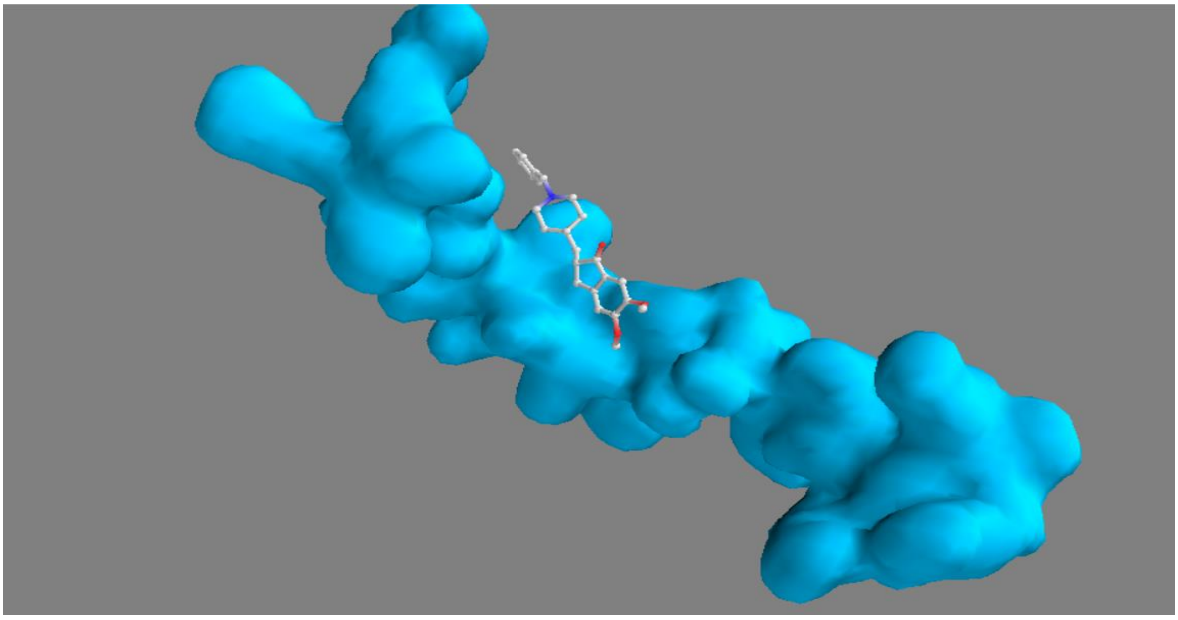

Figure 17: The figure shows the interaction of Aricept drug with Aβ42 protein using PyRx software.

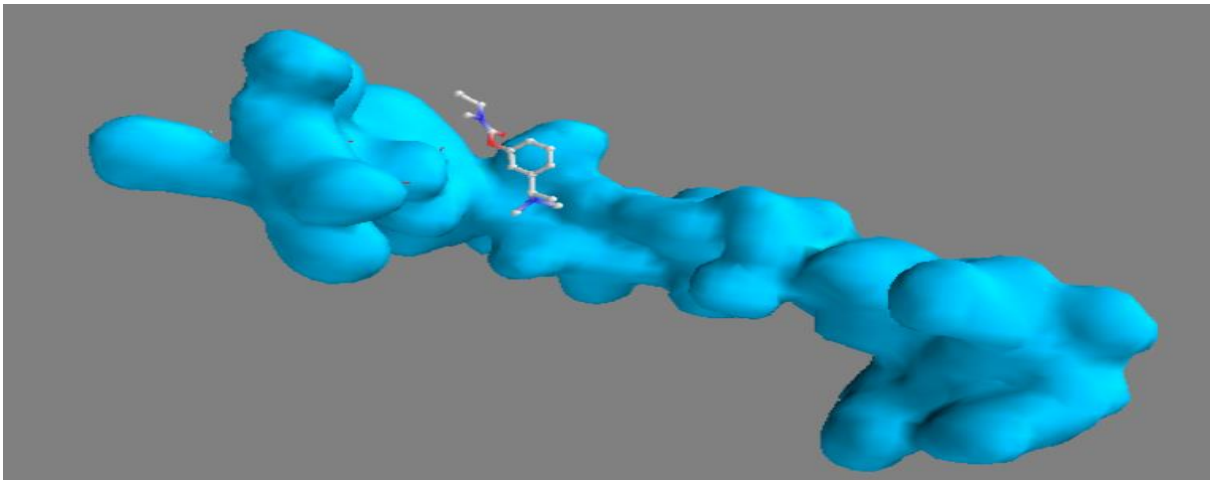

Figure 18: The figure shows the interaction of Exelon drug with Aβ42 protein using PyRx software.

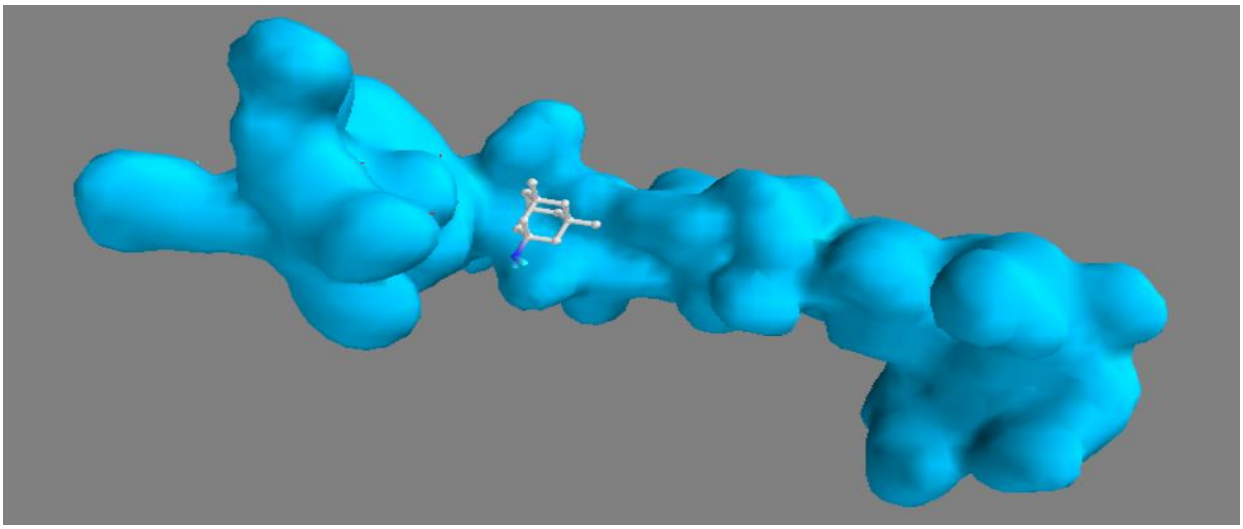

Figure 19: The figure shows the interaction of Namenda drug with Aβ42 protein using PyRx software.

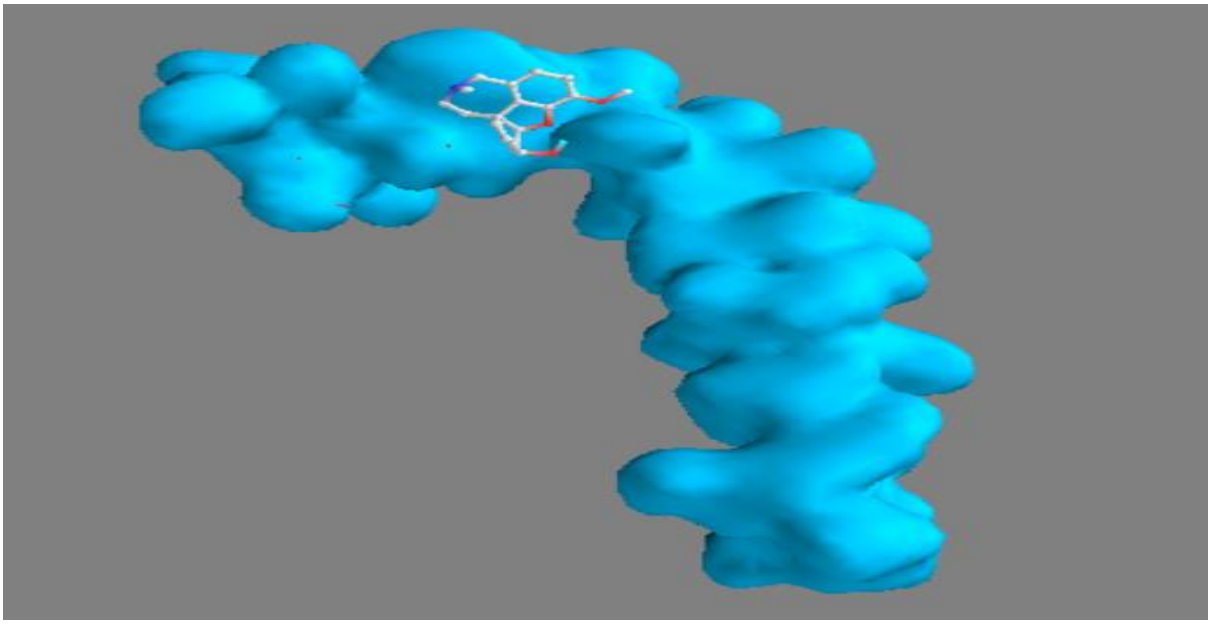

Figure 20: The figure shows the interaction of Razadyne drug with Aβ42 protein using PyRx software.

After the interactions the complexes were docked with PrP protein using GRAMM-X and then the interactions were studied using LigPlot+ software. The interaction analysis shows that docking of drug with Aβ42 changes the interaction of Aβ42 with PrP. The results show that the PrP interacts with Aβ42 in same manner in all cases as all the drugs have same mechanism of action.

The hydrophobic residues of Aβ42 that interacts with PrP are Gln15, Val12, Asp7, Glu11, Tyr10, His13, Leu17, Lys16, Val18, Glu22 and hydrophobic residues of PrP that are involve in interaction

are Ala116, Ala117, Ala118, Gly119, Val122, Ala115, Pro165, Met129, Tyr163, Ala133, Gln160, Gly131, Val121, Ala120, Ser132.

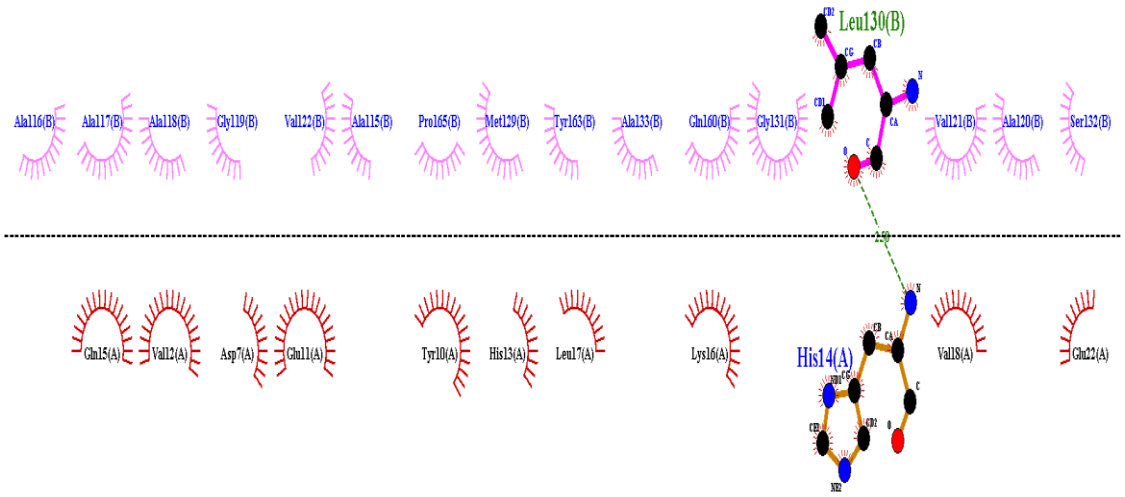

Figure 21: The Dimplot analysis of interaction of drugs such as Aricept, Exelon, Namenda and Razadyne docked with Aβ42 with PrP.

Table 5: The table shows atoms involved and the distance between residues involved in Hydrogen bonding between the two proteins.

PrP residues	Atoms involved	Aβ42 residues	Atoms involved	Distance (Å)
Leu 130	O	His 14	N	2.50

Discussion:

Aβ42-PrP Interaction analysis:

The Aβ42 and PrP interaction is performed to understand how these two proteins interact in the AD. Firstly, the proteins of interest were obtained from RCSB PDB and then was docked using online tool i.e. Gramm-X. After docking the docked proteins were input in the DimPlot, the segment in LigPlot, to analyze the interaction. The interaction shows hydrophobic interactions as well as three hydrogen bonds formed and also the distance between the two amino acids forming hydrogen bond. The result also shows the atoms in the amino acids that are involve in the bonds. The PrP's Ile138, Arg220 and Ser135 form hydrogen bonds with Aβ42's Leu34, Ser26 and Ala30 respectively.

RNA binding sites prediction of both Aβ42 and PrP:

The identification RNA binding site on the protein is performed so that we can predict at which amino acids the RNA can bind to. The server gives a graph and 3D structure. The scoring method that is used to predict RNA binding sites is A^2SPD scoring method. The scoring was done in such a way that those surface residues that are within 7.0 $\mathring{A}$ are given highest score and they are considered potential RNA binding site. The residues of both the proteins that have high score show high chances that RNA binds to those sites.

Post-Translational Modification analysis of Aβ42 and PrP:

The PTM of the proteins were performed to identify at which sites of the protein sequence the phosphorylation is done. As phosphorylation causes the protein to activate, deactivate or modify its function. The phosphorylation sites of Aβ42 on serine, tyrosine and threonine are predicted. The phosphorylation sites predicted in the sequence are 2 Serine and 1 Tyrosine at position 8, 10, and 26 of the Aβ42 sequence. For PrP, the phosphorylation sites predicted in the sequence are 9 Serine, 10 tyrosine, and 12 threonine. All the sites have score greater than 0.5 (threshold) and closer to 1, which shows high chances of phosphorylation at these sites.

Aβ42-anti-Alzheimer drugs-PrP analysis:

In the second part, the Aβ42 is first interacted with anti-Alzheimer drugs and then the Aβ42 is docked with PrP to analyze if the drugs cause any change in the interaction. For this, we used four FDA approved anti-Alzheimer drugs i.e. Aricept, Exelon, Namenda and Razadyne. These drugs were docked with Aβ42 one at a time using PyRx software. Then Aβ42-drug complex is then docked with PrP using Gramm-X tool and interaction result is then analyzed using Dimplot. There is a change in interaction before and after using the drugs. The Aβ42 and PrP interact with each other through single hydrogen bond i.e. Leu130 of PrP with His14 of Aβ42.

Chapter 5

Conclusion

Conclusion:

From this in silico interaction study, it is concluded that Aβ42 interacts with PrP during Alzheimer's disease. The interaction study was analyzed using the LigPlot+ software. The proteins show both hydrogen bonding as well as hydrophobic interactions. We also found the effect of anti-Alzheimer drugs on their interaction. For this, first we docked Anti-Alzheimer drug with Aβ42 and then interact this complex with PrP and study their interaction. The interaction between Aβ42 and PrP changed but all the four drugs caused similar effect. Although the PrP interacts more effectively with oligomeric structure of Aβ42 but till now its oligomeric structure is not discovered yet due to its unstable nature. We also analyzed the post translational modifications of both the proteins, which showed phosphorylation sites. The RNA binding sites of proteins were also studied using KYG: RNA server.

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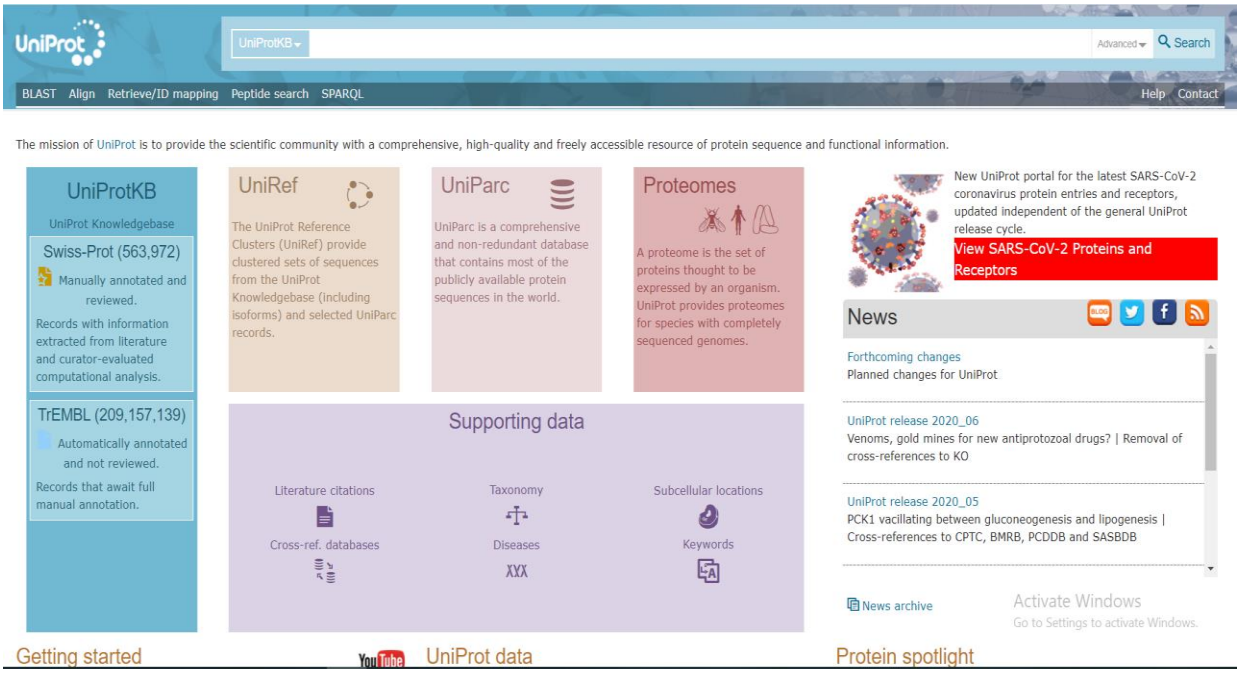
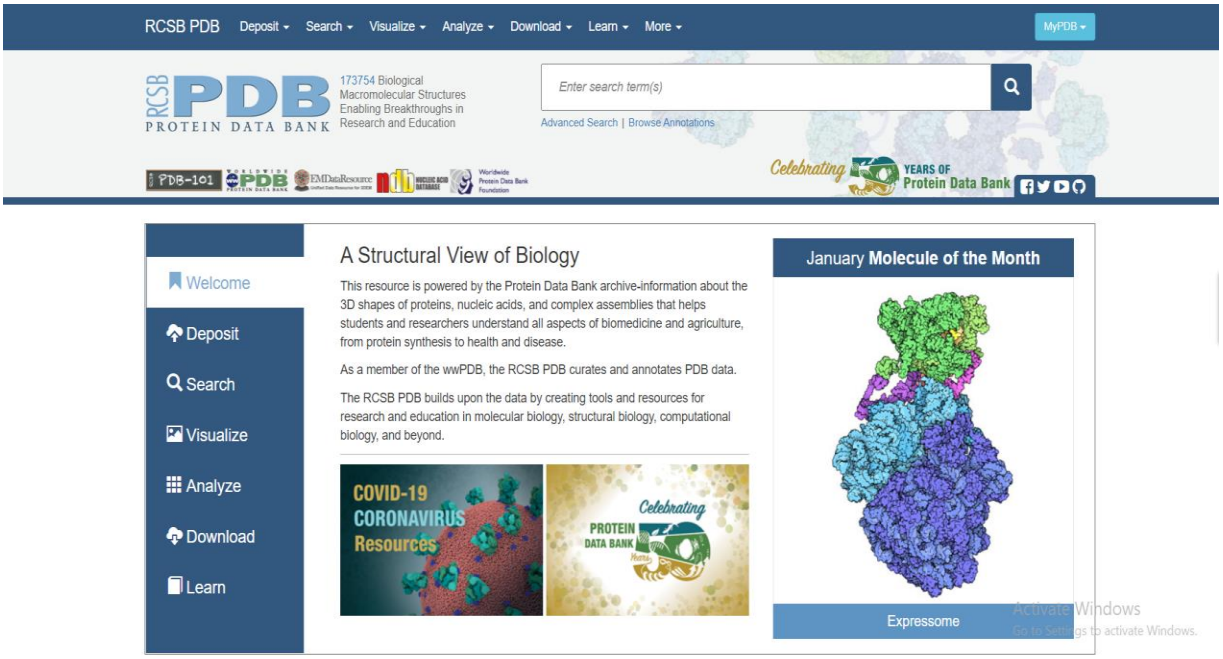
Supporting Data

Following are the home pages of the software and online resources used;

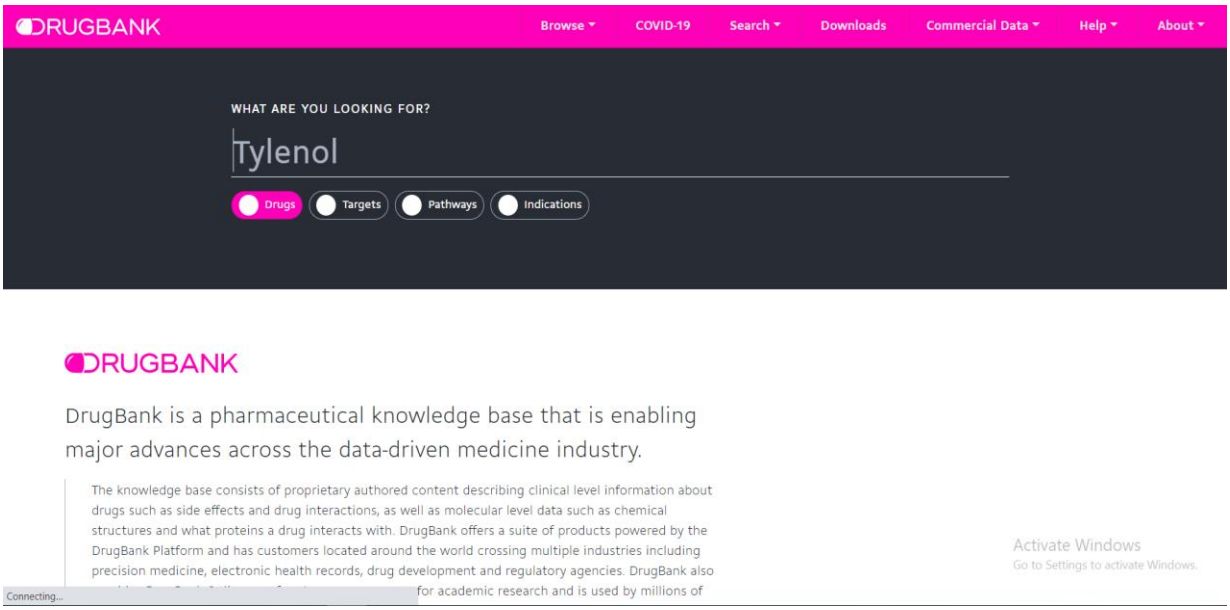
[1] UniProt:



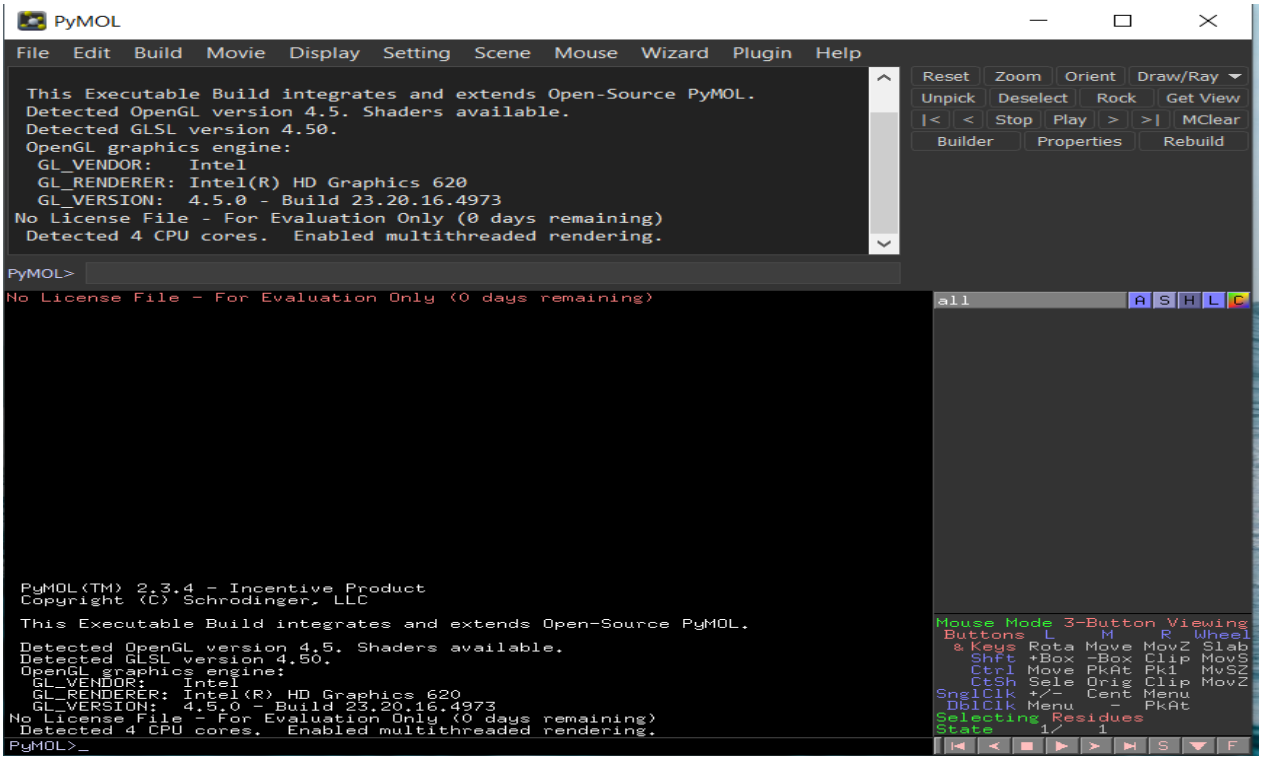
[2] RCSB PDB



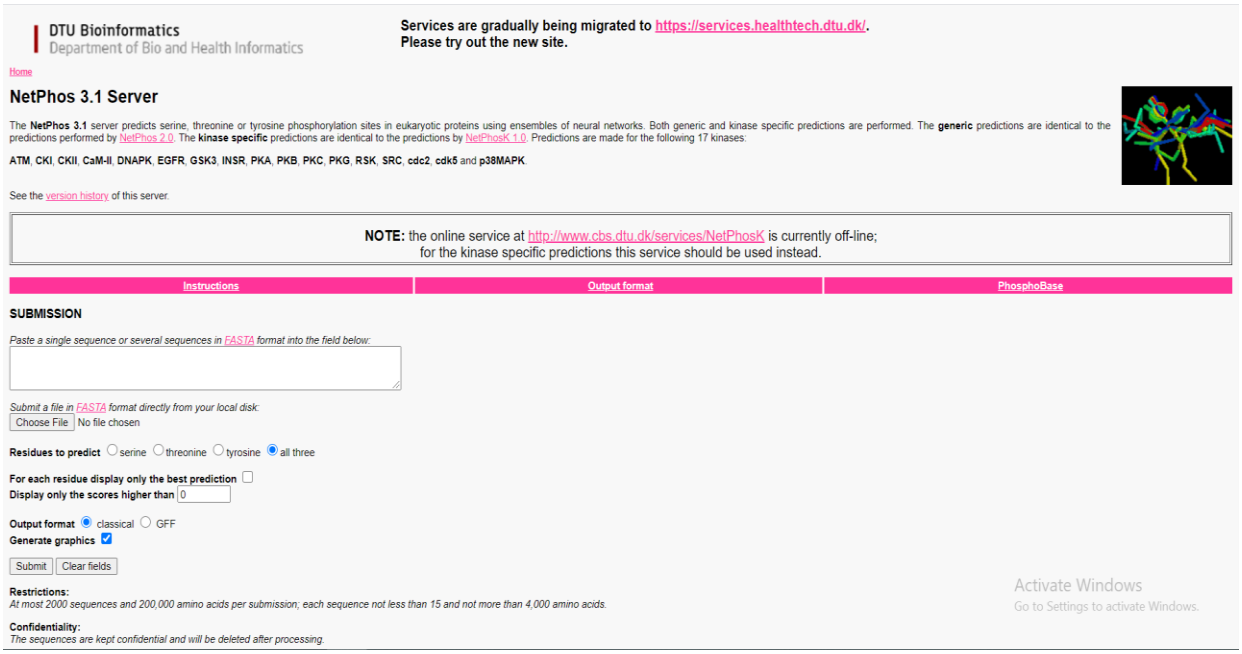
[3] DrugBank:



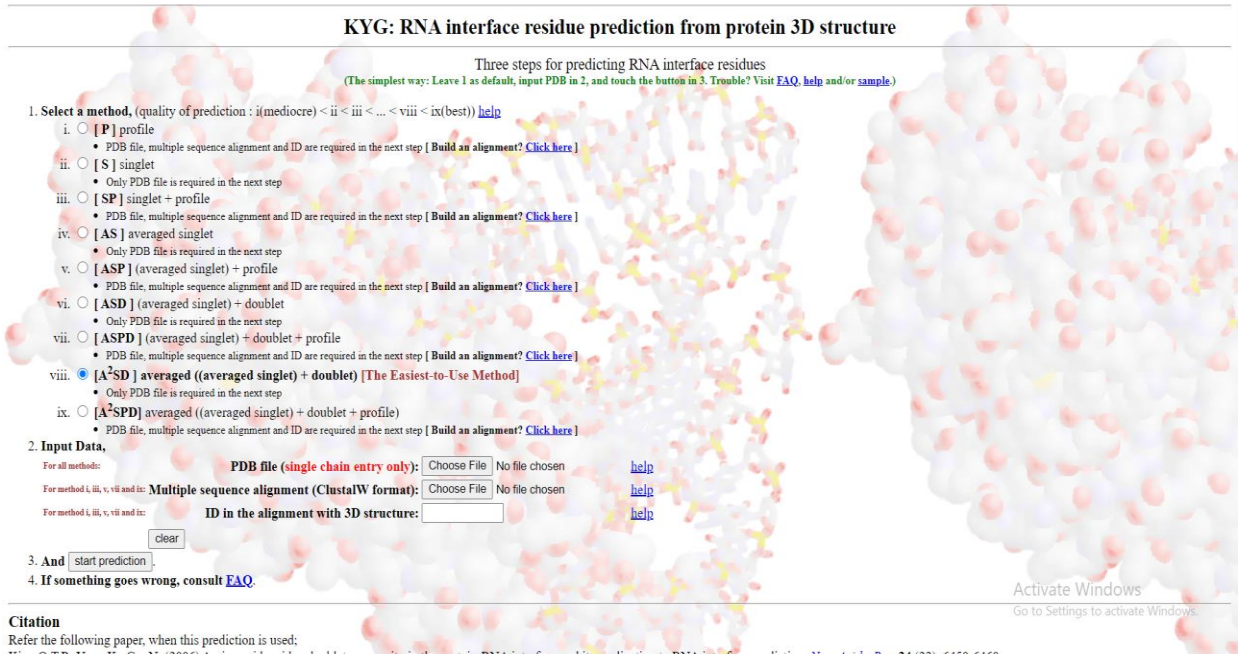
[4] PyMol:


PyMOL
File Edit Build Movie Display Setting Scene Mouse Wizard Plugin Help
This Executable Build integrates and extends Open-Source PyMOL.
Detected OpenGL version 4.5. Shaders available.
Detected GLSL version 4.50.
OpenGL graphics engine:
GL_VENDOR: Intel
GL_RENDERER: Intel(R) HD Graphics 620
GL_VERSION: 4.5.0 - Build 23.20.16.4973
No License File - For Evaluation Only (0 days remaining)
Detected 4 CPU cores. Enabled multithreaded rendering.
Reset Zoom Orient Draw/Ray
Unpick Deselect Rock Get View
Stop Play MClear
Builder Properties Rebuild
PyMOL>
No License File - For Evaluation Only (0 days remaining)
all
PyMOL(TM) 2.3.4 - Incentive Product
Copyright (C) Schrodinger, LLC
This Executable Build integrates and extends Open-Source PyMOL.
Detected OpenGL version 4.5. Shaders available.
Detected GLSL version 4.50.
OpenGL graphics engine:
GL_VENDOR: Intel
GL_RENDERER: Intel(R) HD Graphics 620
GL_VERSION: 4.5.0 - Build 23.20.16.4973
No License File - For Evaluation Only (0 days remaining)
Detected 4 CPU cores. Enabled multithreaded rendering.
PyMOL>_
Mouse Mode 3-Button Viewing
Selecting Residues
State 1/ 1

[5] NetPhos 3.1:

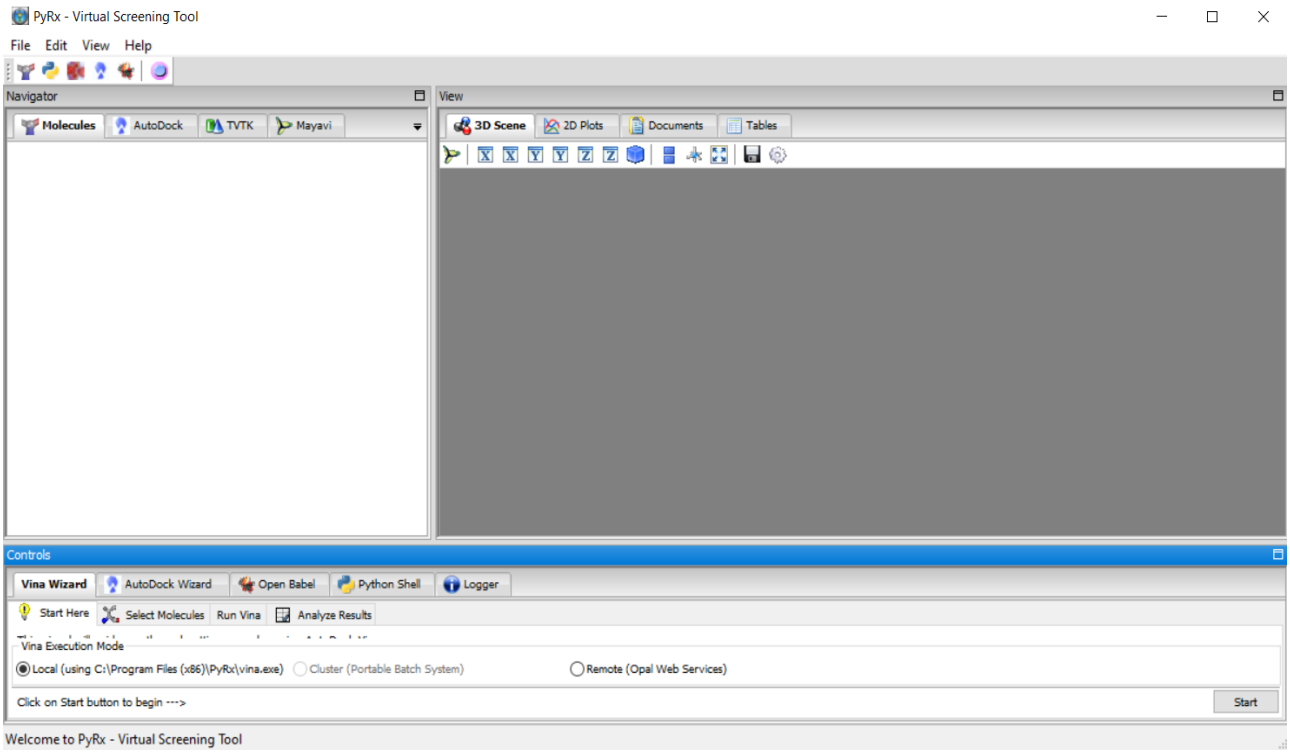

DTU Bioinformatics
Department of Bio and Health Informatics
Services are gradually being migrated to https://services.healthtech.dtu.dk/.
Please try out the new site.
Home
NetPhos 3.1 Server
The NetPhos 3.1 server predicts serine, threonine or tyrosine phosphorylation sites in eukaryotic proteins using ensembles of neural networks. Both generic and kinase specific predictions are performed. The generic predictions are identical to the predictions performed by NetPhos 2.0. The kinase specific predictions are identical to the predictions by NetPhosK 1.0. Predictions are made for the following 17 kinases:
ATM, CKI, CKII, CaM-II, DNAPK, EGFR, GSK3, INSR, PKA, PKB, PKC, PKG, RSK, SRC, cdc2, cdk5 and p38MAPK.
See the version history of this server.
NOTE: the online service at http://www.cbs.dtu.dk/services/NetPhosK is currently off-line;
for the kinase specific predictions this service should be used instead.
Instructions
Output format
PhosphoBase
SUBMISSION
Paste a single sequence or several sequences in FASTA format into the field below:
Submit a file in FASTA format directly from your local disk:
Choose File No file chosen
Residues to predict serine threonine tyrosine all three
For each residue display only the best prediction
Display only the scores higher than 0
Output format classical GFF
Generate graphics
Submit Clear fields
Restrictions:
At most 2000 sequences and 200,000 amino acids per submission; each sequence not less than 15 and not more than 4,000 amino acids.
Confidentiality:
The sequences are kept confidential and will be deleted after processing.
Activate Windows
Go to Settings to activate Windows.

[6] KYG:RNA:



[7] Gramm-X:

[8] PyRx:



[9] LigPlot+: