Targeting Microtubule-associated Protein Tau (*MAPT*) Interactors for Novel Therapeutic Interventions for Rapidly Progressive Alzheimer's Disease

Fatimah
00000329808

Supervisor
Dr. Aneeqa Noor

DEPARTMENT OF BIOMEDICAL SCIENCES
SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING
NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY ISLAMABAD

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Author
Fatimah
00000329808

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Thesis Supervisor:

Dr. Aneeqa Noor

Thesis Supervisor Signature

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Dedicated to my parents, sisters and husband.

List of Abbreviations

AD	Alzheimer's disease
AGD	Argyrophilic Grain Disease
AGE	Advanced glycation end products
ALS	Amyotrophic Lateral Sclerosis
APLP1	Amyloid precursor like proteins 1
APLP2	Amyloid precursor like proteins 2
APP	Amyloid Precursor Protein
APPL	Amyloid precursor protein like
Aβ	Amyloid-βeta
BioGRID	Biological General Repository for Interaction Datasets
CBD	Corticobasal Degeneration
CBS	Corticobasal Syndrome
CJD	Creutzfeldt-Jakob disease
CTD	Comparative Toxicogenomic Database
CVD	Cardiovascular Disease
ER	Endoplasmic Reticulum
FTD	Frontotemporal Dementia
FTLD	Frontotemporal lobar degeneration
GGT	Globular Glial Tauopathy
HD	Huntington's disease
MAPT	Microtubule-associated Tau protein
MRI	Magnetic Resonance Imaging
MTBD	Microtubule binding domain
ND	Neurodegeneration
Neum	Neuromodulin
NFTs	Neurofibrillary tangles
PART	Primary age-related Tauopathy
PCD	Protein-conformational disease
PD	Parkinson's disease
PDB	Protein Data Bank

PET	Positron Emission Tomography
PSP	Progressive Supranuclear Palsy
PTM	Post Translational Modification
ROS	Reactive Oxygen Species
rpAD	Rapidly Progressive Alzheimer's Disease
sAD	Sporadic Alzheimer's Disease
SP	Senile Plaques
Syp	Synaptophysin
UniProt	Universal Protein Resource

Abstract

Rapidly Progressive Alzheimer's disease (rpAD) is a disease characterized by rapid cognitive loss. It progresses quickly over the course of weeks to months and sometimes may take up to two to three years. It’s rare and difficult to diagnose although accurate diagnosis is extremely crucial for its treatment. Recent evidence suggests Tau as a promising therapeutic agent for the development of disease-modifying drugs for rpAD due to the involvement of Tau abnormalities in rpAD neurodegeneration. To determine whether Tau is a suitable therapeutic target, *in silico* tools were used to analyse the interaction of proteins isolated by Tau IP. It is identified that proteins interact with *MAPT* during the pathology of disease and play a role in progression of rpAD. The specific interaction between the *MAPT* region belonging from the MTB domain 2MZ7 and its interactors Neuromodulin, Synaptophysin and RhoA were investigated using molecular docking and visualization tools like PyRx, PyMOL, Discovery Studio, and LigPlot+ and binding energies and bonding between the amino acids was observed and evaluated. Protein network analysis revealed an aberration towards apoptotic pathway and signaling pathway. Furthermore, binding of different drug compounds including Methamphetamine, Ascorbic acid and Doxorubicin were evaluated through docking *in silico*. Doxorubicin showed the highest binding energy of -6.0 kcal/mol with 2MZ7. These drug compounds and their interaction with 2MZ7 yielding high binding affinity shows that binding site of *MAPT* can be blocked using Doxorubicin to prevent the interaction of pathological proteins that are involved in the accelerated progression of rpAD.

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Chapter 1
Introduction

1.1 Tauopathies

The abnormal deposition of proteins around and inside the neurons is commonly termed as an important feature of neurodegenerative diseases. The research which points to dominant mutation in the Microtubule associated tau protein (*MAPT*) encoding tau supports that abnormal tau proteins are involved in tau-associated disease progression of neurodegenerative diseases commonly known as tauopathies (Wolfe, 2012). Tau pathology is normally characterized by hyperphosphorylated tau proteins and abnormal tau aggregates in the brain. Studies show that tauopathies differ in tau isoforms phosphorylation and molecular classification. Tau protein is crucial for maintaining and stabilizing microtubules and neuronal axons. A disturbance in Tau expression can cause protein degradation along with disturbances in cytoskeleton formation. It can impair the axonal growth, alter mitochondrial dysfunction, produce alterations in synaptic function of neurons (Tong Guo, 2017) and disrupt the formation of other tau structures present in the brains of dementia patients (Andreadis, 2005).

Disorders such as Alzheimer's Disease (AD) or Dementia are generally characterized with neurofibrillary tangles (NFTs) and are correlated with cognitive decline along with Amyloid-β (Aβ) overproduction and tau hyperphosphorylation (G.Amadoro, 2011) and in early years of study of dementia and AD, it was also characterized by the presence of plaques and tangles in the brain (Goedert, 2009). Tau phosphorylation, aggregation, cognitive impairment and finally neuronal cell death are all associated with tauopathies and neurodegenerative disorders (Luc Buée, et al., 2010). Loss of tau protein also contributes towards destabilization of microtubules which eventually leads to neuronal degeneration (Pascale Barbier, 2019). Tauopathies are different on the basis of isoforms mainly 3R and 4R. Mutations of the tau gene can cause diseases like Pick's Disease, Globular Glial Tauopathy (GGT), Corticobasal Degeneration (CBD), Argyrophilic Grain Disease (AGD), Progressive Supranuclear Palsy (PSP) and Frontotemporal Dementia (FTD) etc. (Kovacs, 2017). AD is also a tauopathy caused by numerous factors including aging, mutations, environmental factors such as exposure to aluminum, head injuries, infection, vascular diseases among others (Armstrong, 2019).

During AD, soluble neuronal proteins become misfolded and lose functionality. The pathology of AD is associated by the formation of neurofibrillary tangles (NFTs) which are associated

with hyperphosphorylated Tau protein in the brain and aggregation of amyloid β (Aβ) plaques (Selkoe, 1997). There is also evidence of synaptic degeneration and neuronal loss in relation to cognitive decline in AD (Natalia Louneva, 2008).

Rapidly Progressive Alzheimer's Disease (rpAD) is also a type of AD which is characterized by a faster, rapid disease progression and cognitive decline. Numerous patients with rapidly progressive dementia and fast cognitive decline along with additional neurological symptoms sometimes are consistent in relation to Creutzfeldt-Jakob disease (CJD) which often presents with a shorter time of survival (I, 2010). Even with enormous interest, clear and distinctive consensus regarding the characteristics of rpAD is still difficult (Jagan A. Pillai, 2018) and although AD and rpAD share similar features, studies have shown there is a characteristic molecular signature of Aβ aggregation and plaques which may distinguish rpAD from AD (Samir Abu-Rumeileh, 2018).

1.2 Epidemiology

Approximately, 47 million people in the world currently suffer from AD and the numbers are only expected to increase by 62% by 2030 (dos Santos Picanco, et al., 2018). It is estimated that at the age of 65 and above prevalence of AD doubles every 5 years and by 2025, almost 115 million people are thought to be affected by AD (Jana Povovaa, 2012). Studies show that almost 58% of the individuals suffering from dementia or AD are residing in developing, low and middle-income nations and this number is thought to increase to 68% by the years 2050 (McGill-Carter, 2020). In developing nations, 1 in 10 people show signs of dementia or related diseases, whereas in Europe and US the percentage of people over 65 suffering from AD is 4.4% and 9.7% respectively. While in regions like Africa and China, it's approximated to be 1.6% and 4% respectively (Chengxuan Qiu, 2022). The prevalence of AD in USA as compared to Europe, Africa or China is higher (Jana Povovaa, 2012).

1.3 Significance of Tauopathy Study

Tauopathies are still largely untreatable diseases and have varied symptoms, signs and pathologies. Apart from this, the mechanisms underlying different tauopathies and neurodegeneration are very delicate and intricate and mechanisms like tau aggregation, hyperphosphorylation and propagation play crucial roles. The risk of tauopathies is mainly linked with genetics but studies have also peeked into related genetic variants to help understand the disease mechanisms (Yi Zhang, 2022). AD, which is characterized by cognitive decline and loss of memory, has no cure up to date. Amyloid plaques are characterized as

hallmarks of AD pathology (Vassar, 2007) and studies have shown that targeted strategies towards reducing amyloid to reverse cognitive decline symptoms have not been very useful (Rebecca G. Canter, 2016). Currently, no disease modifying therapies have been approved majorly because of poor characterization and biological processes related to the pathology of tau which are unresolved still (Majedul Islam, 2022). During the past two decades, despite numerous and broad researches conducted on tau and on targeting the hallmarks of AD such as Aβ plaques, therapeutic findings which could be significant towards the treatment of this disease have not been found. Tau protein, being a significant pathological target in progression of AD can play a promising role as a target candidate for researching and developing therapeutic targets (Shibi Muralidar, 2020).

Therefore, it is important to identify other different biomarkers, studies, diagnosis and novel therapeutics along with model systems such as cell-based models (Aaron D. Gitler, 2017) to help understand the underlying mechanisms and pathology of tauopathies, to research new advances in neurodegenerative diseases and to achieve early diagnosis and disease modifying therapeutics (Yi Zhang, 2022).

1.4 Objectives of Study

The study involves *in sillico* analysis of Tau with its interactor proteins using online tools and software. The objectives of this study include identification of rpAD targets through Immunoprecipitation, *in-sillico* analysis of Tau interactors in rpAD, a protein-protein network and pathway analysis and drug screening. The study will establish the role of proteins involved in neurodegenerative diseases and aid in identifying novel therapeutic targets to prevent AD.

Chapter 2
Literature Review

2.1 Neurodegeneration:

Neurodegeneration refers to loss of structure or function in the neuron cells and in more comprehensive terms, neurodegeneration corresponds to any condition which affects neurons. Numerous pathological conditions come under the umbrella of neurodegeneration, and they arise and progress in unknown manners. They are mostly characterized by loss of neurons progressively and can be classified by their primary clinical features or molecular abnormality (Dickson, 2017). Neurodegenerative diseases can also cause neuronal impairment and cognitive decline in many older adults (Michael G. Erkkinen M.-O. K., 2018). There are among hundreds of different neurological disorders, but special attention has been given to diseases like Amyotrophic Lateral Sclerosis (ALS), AD, Parkinson's Disease (PD), Huntington Disease (HD), Frontotemporal Dementia (FTD) etc. Neurodegenerative disorders of the CNS can be divided into diseases of cerebral cortex, basal ganglia, brainstem, or the cerebellum. Within each group, different diseases are further grouped according to their clinical features, for example, dementing AD is mostly attributed to the cerebral cortex. The most intense discussion is around the cause of these diseases and takes under consideration different genetic or environmental factors which could contribute towards the initiation and progression of these diseases. However, genetic contribution is at a minimal and the better-known causes are the toxic environmental factors (Serge Przedborski, 2003). Neurodegenerative diseases are a growing cause of mortality in the elderly and can differ vastly in their physiologies. These can include symptoms like loss of cognitive functions, memory losses and a general loss of motor function including the ability to speak, move or breathe (Michael G. Erkkinen, 2018).

2.2 Tauopathies

Tauopathies are heterogeneous neurodegenerative diseases associated with the irregular deposition of abnormal *MAPT* protein or neuronal and glial inclusion of Tau in the nerve cells. Several neuropathological phenotypes are distinguished in Tauopathies based on characteristics like cell types, genetic variations, or presence of distinctive isoforms of Tau (Kovacs, 2017). Numerous other factors such as biochemical properties of the abnormal protein based upon its post translational modification, the prion like propagating ability of the protein, association and interaction with other co-existing proteins, and other vulnerabilities like

structure, genetics, epigenetics, environmental factors also play a role in this (Jacky Ganguly, 2020). A primary Tauopathy refers to a disorder where the predominant and an important feature is the deposition of Tau protein, but secondary Tauopathies have also been recognized based on several different Tau pathologies and other diverse and different driving forces. Tauopathies are separated from each other on the basis of 3R and 4R Tau isoform ratio or the 60, 64 and 68 kDa phospho-Tau bands. Several mutations in Tau gene can also play a role in causing hereditary FTD associated with Frontotemporal lobar degeneration (FTLD) and the nomenclature for primary tauopathies may sometimes overlap with the updated classification of FTLD. The FTLD primary Tauopathies are also dependent on the Tau isoform which predominates the morphology of the disease. On basis of these classifications, 3R isoform Tauopathies play a role in Pick's Disease whereas 4R Tauopathies comprise of PSP, CBD, GGT and AGD. Some form of Tauopathies, like NFT Predominant Senile Dementia (NFT Dementia), also comprise of mixed isoforms of 3R and 4R Tau. NFT Dementia is now also included under the heading of primary age-related Tauopathy (PART) (Kovacs, 2017).

2.2.1 Diagnosis:

The diagnosis on different Tauopathies is dependent upon factors like history, symptoms and their onset, progression and the overall course of the disease. Although, there are a number of neurodegenerative diseases, the most attention arguable is given to AD, PSP and Corticobasal Syndrome (CBS). Each syndrome presents with varying symptoms such as memory loss and motor impairment pathology in hippocampus and entorhinal cortex are a characteristic of AD, similarly impaired ocular movements and frequently falling is associated with PSP or CBS pathology. In the detection of early signs and symptoms of neurodegenerative diseases comprehensive neurophysiological evaluations hold major value. Different aspects of these evaluations include cognition, behavior, and movement. To distinguish clinical syndromes, cognitive profiles are interpreted in relation to histories and reported symptoms. A typical patient suffering from AD will show cognitive impairment, rapidly forgetting things and increased forgetfulness or some degree of anomia. Patients with PSP can show varying degrees of dysfunction and trouble with motorized sequences and CBS patients often present apraxia and motor dysfunction. In relation to neurophysiological evaluations, neurological exams provide further insights and information about diagnosis of the disease. Neuroimaging is also a useful method for identification of numerous changes in the brain which could be Tau mediated. Positron Emission Tomography (PET) scans and Magnetic Resonance Imaging (MRI) are some of the major neuroimaging techniques for acquiring insights on different brains

sections. AD patients on an MRI scan show hippocampal and parietal atrophy whereas PSP patients show midbrain atrophy and those with CBS show changes in basal ganglia and bilateral frontal lobes (Miranda E. Orr, 2017).

2.2.2 Clinical Pathology / Symptoms:

Clinical pathology of Tauopathies is characterized by dementia, movement disorders, loss of motor function or motor neuron diseases. These can be either in combinations or present in isolation depending upon the anatomical structures affected by the protein accumulation and aggregation (Jacky Ganguly, 2020). Progressive aphasia which is a clinical feature of FTD also presents in many forms of Tauopathies. Primary Tauopathies may rarely feature motor neuron disease, but as the disease progresses a plethora of new symptoms and clinical presentations apart from FTD or Progressive aphasia also appear. For example, Richardson syndrome is associated with postural instability, frontal lobe symptoms, vertical supranuclear gaze palsy. CBS is a presentation associated with CBD and characterized by asymmetric movement disorder which is progressive and can include different groupings of akinesia, ideomotor apraxia, dystonia, rigidity etc. Symptoms like cognitive decline, urinary incontinence, disturbance in formation of memories, changes in personality, aggression, ill temperament are reported to occur during AGD. FTD suspicion may also be raised due to symptoms like abnormal or aggressive behavior, whereas symptoms like paranoia, depression and disorientation may also be seen in individuals with PART or NFT Dementia. Pathology similar to CBD may also present in combination along with Richardson syndrome, cerebellar ataxia, or dementia which has similar features to AD (Kovacs, 2017).

2.3 Alzheimer's Disease:

AD is a neurodegenerative and a protein-conformational disease (PCD) which is caused as a result of polymerization of proteins. Due to factors like age, genetic mutations, or other external factors such as head injuries, vascular diseases, infections, or environmental factors, some of the soluble neuronal protein become misfolded and alter their conformations which leads to abnormality or loss of neuronal function. AD can also intensify due to other infectious agents such as HIV etc. Extracellular aggregation of Aβ plaques and intracellular NFTs are also associated with AD pathology, these structures are mostly composed of hyperphosphorylated Tau protein in human brain and are particularly found in limbic and cortical region (Selkoe, 1997). Aβ plaques formulate in the basal and temporal regions of the brain initially and later progress through other parts of the brain such as hippocampus, diencephalon, amygdala, and

basal ganglia etc. In some critical cases these plaques are also found in mesencephalon, cerebellar cortex, and lower brain stem. The Aβ plaques also cause the Tau tangle formation to accumulate in entorhinal and transentorhinal areas of the brain (Sneham Tiwari, 2019).

AD is a major factor in progression and induction of dementia and presents with memory loss and progressive decline of neurocognitive functions. The clinical phases of AD can be divided into pre symptomatic stage, the early or mild stage, moderate AD stage and severe AD stage. Pre symptomatic stage can be attributed towards slight memory loss or early pathological changes in the brain areas like cortex or hippocampus. Apart from this, at this stage no physical or functional impairment occurs and there are no particular AD symptoms. The early stage is where symptoms like loss of concentration, memory loss, mood changes, disorientation in focusing towards time or space, depression start to appear (Apostolova, 2016). Moderate stage is characterized by spreading of the disease towards cerebral cortex which in turn results in increased memory loss, difficulty in speaking, writing, reading etc. It can also lead to loss of impulse control and loss of facial and special recognition in the patient suffering from AD. The late stage or severe AD is where NFTs start forming in the brain and neuritic plaques accumulate in the brain resulting in progressive loss of function and cognitive dysfunction. Patient can lose all facial recognition and not recognize family members and friends. Late-stage AD also leads to difficulty in functions like breathing, swallowing, urinating and eventually leads to death (Karaman, 2020).

Rapidly Progressive Alzheimer's Disease (rpAD) is also a type of AD which is characterized by a faster, rapid disease progression and cognitive decline. Even with enormous interest, clear and distinctive consensus regarding the characteristics of rpAD is still difficult (Jagan A. Pillai, 2018) and although AD and rpAD share similar features, studies have shown there is a characteristic molecular signature of Aβ aggregation and plaques which may distinguish rpAD from AD (Samir Abu-Rumeileh, 2018).

2.3.1 Amyloid Precursor Protein (APP):

Amyloid Precursor Protein (APP) is an important plasma membrane protein and a part of associated proteins which include mammalian amyloid precursor like proteins (APLP1 and APLP2) and Amyloid precursor protein like (APPL) in *Drosophila.* It's a transmembrane protein of integral value and has extracellular domains. The physiological function of APP requires more understanding, but transfected cell line studies have shown APP role as a moderator in growth, motility, cell survival along with neurite growth and functions. APP importance is highlighted in studies including neuronal abnormalities where it showed

improved cognitive functioning and synaptic activities. APP consists of 770 amino acids where 28 residues are included in Aβ and 14 residues are from the transmembrane APP domain. One transmembrane protein encoded by APP is either cleaved in a normal state or in a diseased state leading to certain post translational modifications such as alternative splicing, glycation, proteolysis, and phosphorylation (Sneham Tiwari, 2019).

In diseased state, alternative splicing of APP occurs when cleaved by β-secretases (BACE1) and γ-secretases, produces insoluble Aβ fibrils. Oligomers are then formed from these fibrils which then start to diffuse in the synaptic cleft causing an interference in the synaptic signaling. These fibrils, subsequently, polymerize and form insoluble amyloid fibrils which then accumulate into plaques. This polymerization activates the kinases in the brain which leads to hyperphosphorylation *MAPT* protein, which then polymerize into NFTs. NFTs are protein filaments which are helically wound are present in pairs in the neuronal cytoplasm. The Tau protein which is known as microtubule binding and stabilizing protein, pairs with tubulin protein for the formation of mature and stable microtubules. These microtubules form interconnecting bridges and form a stable microtubule connected network. The interaction of Tau protein with the kinases causes the microtubules to get unstable, which cause dissociation of the tubule units and form into large aggregates of Tau filaments leading to the formation of NFTs. The NFTs are unstable, fibrillary, and insoluble in the neuronal cytoplasm The formation of NFTs results in loss of communication between signal processing and neurons. They also result in microglia to recruit and surround these plaques which stimulate the microglial activation and the inflammatory responses and cause neurotoxicity in the brain and finally lead to neuron apoptosis (Sneham Tiwari, 2019).

2.3.2 Epidemiology

More than 35 million people suffer from dementia worldwide and almost 75% of them are affected by AD and the prevalence of AD doubles every 5 years after 65 years of age and by 2050, because of doubling of this ratio approximately 115 million people are believed to be affected by AD (Jana Povovaa, 2012). Among developed nations 1 in 10 people with ages over 65 show signs and symptoms for Dementia or Dementia related diseases. Among these AD accounts for 70% and Vascular Dementia accounts for 25% of all dementia cases. The population-based data and studies for Europe accounts for 4.4% of people over 65 years of age to suffer from AD. Whereas in US, this prevalence was 9.7% for people over the age of 70 years. The region-specific statistics for prevalence of AD are approximated to be 1.6% in Africa, 4% in China and Western Pacific Regions, 4.6% in Latin America, 6.4% in North

America (Chengxuan Qiu, 2009). In comparison with Africa, Asia or Europe, the prevalence of AD is higher in the United States. For some undefined reasons, African Americans and Hispanics living in US are more prone to development of AD as compared to Africans in their own land (Stern, 2012). The disease impacts majorly not only the patients but also their caretaker persons and entire society (Jana Povovaa, 2012).

2.3.3 Risk factors

Aging is one of the most significant risk factors in AD and is an irreversible and incurable system where complex cell systems and multiple important organs lose function, brain volume and weight is reduced, synaptic activities reduce, and ventricles enlarge in specific areas due to formation of Senile Plaques or Tau aggregation or NFTs. Several other disorders such as hypometabolism, cholesterol dyshomeostatis, mitochondrial dysfunction, loss of cognitive abilities, depression, anxiety, diabetes etc. also accompany aging, but in normal aging processes, the development of AD can still occur. Depending on the age, the onset of AD is divided into stages such as Early-onset AD, which is quite rare with 1-6% of the total cases, and Late-onset AD in ages above 60 or 65 years and covers remaining of AD cases (Karaman, 2020).

In recent years the role of nutrition in our diet and its effects on AD have been studied. Foods containing high amount of calories and rich in saturated fatty acids were reported to increase the AD risk whereas food containing supplements such as vitamins, polyphenols, antioxidants were associated with a reduce risk of development of AD. Heat sensitive micronutrients like vitamin C and folates when go through the process of food processing are subjected to heat sensitive degradation, loss of water and production of secondary toxic products due to the non-enzymatic glycation of lipids, proteins and nucleic acids present in these foods. These toxic secondary products, known as Advanced glycation end products (AGEs) induce inflammation and oxidative stress in cell surface receptors and body proteins causing an alteration to their structure and function. Studies demonstrate that elevated levels of AGEs in the body are associated with decline in cognitive abilities and progression of AD. Malnutrition and deficiency of nutrients like Folate, Vitamin D and Vitamin B12 may also be associated with a decrease in cognitive functions and thus also contribute towards AD risk factors (Karaman, 2020).

Diseases like Cardiovascular diseases (CVDs) also pose as an important risk factor for AD due to neural tissue loss which increases degeneration in the brain and enhances amyloid and Tau pathologies leading to development of AD. In cases of heart failure, insufficient supply to body

and brain may lead to hypoxia and neural damage. Hypertension, which is characterized as thickening of blood vessels and reduction of cerebral blood flow may sometimes cause a cerebral edema which identifies as a risk factor of AD as well. Health risk factors such as obesity and diabetes also play a role in development of AD. Obesity poses as a well-known risk factor for CVDs, diabetes and cancer which are in turn identified as risk factors for AD. Consumption of more calories than the calories burning leads to increase in body fat which can in turn result in hypertension, decreased blood supply to brain resulting in brain ischemia, memory loss or vascular dementia. A high fat diet paired with diabetes can result in hyperglycemia which affects blood vessels and peripheral tissues. Hyperglycemia for longer terms can cause a reduction in cognitive abilities and can cause an increase in mitochondrial dysfunction, oxidative stress in cells, and amyloid-beta accumulation in the brain, all leading to the development of AD (Jana Povovaa, 2012).

2.4 Role of Tau in Clinical Variants of AD:

Tau proteins are expressed in neurons and glial cells and belong to the family microtubule-associated protein (MAP) where they are primarily responsible for cytoskeleton stabilization and also play an important role in the formation of microtubules from tubulin monomers to formulate the neuronal microtubule network. Because of its ability to bind to microtubules, Tau performs an important role in modulating the microtubule dynamics in the brain and promote neurite growth, axonal transport and also synapsis. Tau also contributes towards axonal stability and consequently also help in neuronal development and neuronal polarity. Tau protein establish links within microtubules, which are important for axon formation and maintenance of cell shape, and within the cytoskeleton as well (L Buée, 2000). The role of Tau in microtubule assembly regulates the process of neuron morphology, but recent studies have shown interaction od Tau with cell membranes including plasma membrane, Golgi bodies and endoplasmic reticulum (ER). It may also normalize intracellular signaling facilitated by protein interaction with proteins Pin-1 and Fyn proposing a role in cell signaling. Primarily, Tau is characterized by being an axonal protein, but has also been found in neuronal nuclei where it plays a role in gene expression by binding with either DNA or RNA (Carolina Alquezar, 2021). Tau protein's expression, translated by a gene on chromosome number 17, is regulated by the alternative splicing forms six different isoforms in adult human brain (L Buée, 2000). The difference in the isoforms is due to the highly acidic amino acid inserts which is either 29 or 58 amino acids at the N-terminal and presence of three or four repeats at the C-terminal. These inserts at the C-terminal are responsible for the binding of Tau to the microtubules. All the six

isoforms of Tau have their particular role and physiology in different biological activities and stimulate the microtubule development with different efficiencies (K. Iqbal, 2008). Tau and its isoforms play a crucial role in the stabilization of the microtubules of neuronal axons, neurite's extension and stabilization and contribute to a decrease in their instability (Jesus Avila, 2016). Under certain conditions, Tau can lose its functionality of microtubule-binding and as a result accumulate in cytosol of cells forming cytosolic inclusions and aggregates of fibrillar forms of Tau known as NFTs (Carolina Alquezar, 2021).

Several neurodegenerative diseases such as FTD, Pick Disease and PD have shown various Tau isoforms in altered proportions and are linked to chromosome 17 (K. Iqbal, 2008). In several of these diseases, an important pathological event is the aggregation of Tau isoforms into intraneuronal filamentous inclusions. The major cause of this aggregation in the brain is phosphorylation of Tau, which make Tau proteins an important and reliable marker in neurodegenerative diseases. In AD, Tau protein is a major constituent of fibrillar lesions in glial and intraneuronal region and is important for the development of NFTs. Apart from NFTs, Senile Plaques (SP) are also observed during AD. Senile plaques are a result of extracellular accumulation of Aβ into amyloids which derive from their precursor protein APP. Both NFTs and SPs are observed in the hippocampus and entorhinal cortex and throughout cerebral cortex respectively in the brain. Other areas such as amygdala, dorsal raphe, and locus coeruleus are also affected by the formation of NFTs in the brain (L Buée, 2000).

2.4.1 Isoforms

There are 6 isoforms of Tau expressed in the human brain which are produced as a result of alternate mRNA splicing of the transcripts from the *MAPT* gene. The alternate splicing of 2, 3 and 10 exons of the gene contribute towards the isoform formation and their range starts from 352 to 441 amino acids The isoforms of Tau are characterized by the presence or absence of either 29 or 58 amino acids in the N-terminal (Kovacs, 2017). The isoforms either contain three or four microtubule-binding tandem repeat sequences of 31 or 32 amino acids at the C-terminal which form 3R or 4R Tau or goes through 58 or 29 amino acid insertions at the N-terminal which give rise to 0N, 01 or 02 N-terminal inserts. These N-terminals are highly acidic in nature. The alternate splicing of the exon 10 of *MAPT* gene particularly results in the formation of 3R and 4R isoforms of Tau. However, the isoform composition, physiology and their morphology can be different for different Tauopathies. Only the shortest, out of all the Tau isoform is expressed in the fetal brain and the rest are expressed in an adult brain. In AD, both 3R and 4R isoforms play a role in formation of neuronal inclusion (Ling Wu, 2022). Apart

from this, various Tau isoforms with altered proportions are also found in other neurodegenerative diseases like Pick's Disease, FTD or Parkinsonism (K. Iqbal, 2008).

2.4.2 Post Translational Modifications

Post-translational modifications (PTMs) are modifications in a protein which occur shortly after the translational process by ribosomes is done or after the folding and localization of a protein is completed. These modifications are catalyzed by different kinds of enzymes and include addition of sugars, proteins or other specific chemical groups to the residues of the targeted proteins. PTMs regulate nearly all cellular processes and are therefore an important component for increase in proteome complexity and in formation of proteoforms, which are different forms of proteins produced from the genome with a wide variation in sequences and have distinct functional roles. PTMs can also influence structural changes, protein functions, protein aggregations and protein-protein interactions. PTMs also regulate protein clearance with the help of degrons, which are peptide sequences that target protein degradations. All proteins in eukaryotes which have a tendency to aggregate in different neurodegenerative diseases are prone to PTMs. Unfolded proteins which lack a three dimensional and tertiary structure and have dynamically fluctuating conformations, unlike folded proteins, are also more susceptible to PTMs.

Tau is an unfolded protein and can be modified by several PTMs. Based on its association with microtubules and amino acid composition, the main function of Tau protein is modulation of microtubules further associated with numerous functions in the brain. Diseases which are a result of Tau inclusions in the brain are referred to as Tauopathies and consist of multiple neurodegenerative diseases with distinguished pathological characteristics. The immunopositive aggregates of Tau are a hallmark of Tauopathies. The cellular protein homeostasis or proteostasis network is important for the proper functioning of Tau and has a role in prevention of accumulation of Tau aggregates. PTMs are considered extremely important for Tau proteostasis and helps in regulating Tau aggregation, levels and functions. These PTMs also stimulate changes in the clearance, conformation and aggregation of Tau protein. But not all PTMs are pathological and also have normal roles in Tau functioning. These PTMs have also been identified in Tau from healthy brains and phosphorylation is the most studied PTM traditionally (Carolina Alquezar, 2021).

Chapter 3
Materials and Methods

3.1 Materials

Online softwares and tools listed in Table 1 and Table 2 were used to collect data and perform *in silico* analysis.

Table 1: Software and Tools. Softwares and tools used in the study are enlisted below along with their applications and developer.

Software/Tool	Application	Developer	References
PyRx	Molecular Docking	Source Forage	(Python Prescription, 2022)
Discovery Studio	3D analysis and visualization	Biovia	
PyMOL	3D analysis of Protein-ligand complex	Schrodinger, Inc	(Shuguang Yuan, 2017)
LigPlot+ v.2.2	2D analysis of Protein-ligand complex	Roman Laskowski	(Graphical User Interface for the LIGPLOT and DIMPLOT programs)
ChemDraw Pro 12.0	Drawing 2D structures	PerkinElmer	

Table 2: Online Sources. Online sources used in the study for research and collection of data are enlisted below along with their applications

Online Source	Applications	URL	References
Universal Protein Resource (UniProt)	Database for sequences of proteins and their functional information	https://www.uniprot.org	(Consortium, 2008)
STRING	Database for predicted protein-	https://String-db.org	(STRING Database — Content, 2022)

	protein interactions		
RCSB Protein Data Bank (PDB)	Database for 3D protein structures	https://www.rcsb.org	(consortium, 2019)
Biological General Repository for Interaction Datasets (BioGRID)	Database of known protein-protein interactions	https://thebiogrid.org	
Comparative Toxicogenomics Database (CTD)	Database for chemical compounds	https://ctdbase.org	(Allan Peter Davis, 2021)
PubChem	2-D Structures of drug compounds	https://pubchem.ncbi.nlm.nih.gov	(Sunghwan Kim, 2016)
Diagrams.net	Flowcharts, network diagrams	https://www.diagrams.net/	

3.2 Methodology

3.2.1 Selection of Proteins

Tau protein was selected after the literature review. Due to the presence of different Tau isoforms, three different protein structures were selected each belonging to a different domain in Tau protein. 2MZ7 (267 – 312) belonged to the region R1-R3 in the Microtubule Binding Domain (MTBD) region of the Tau protein. 6N4P (5-10) belonged to the N-terminal region and 7QKZ (305 – 379) belonged to the R3 – C terminal region (Figure 1).

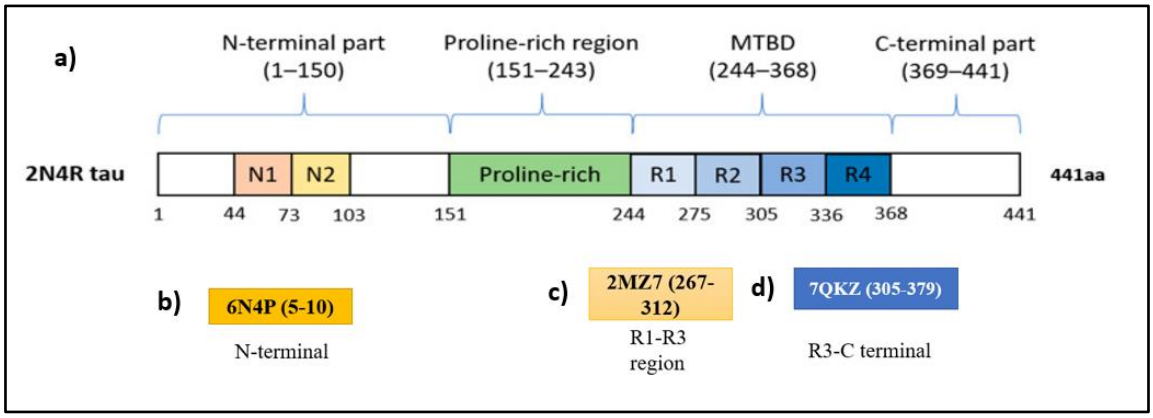


Figure 1. Structure of Tau and selected regions. a) The structure of Tau protein indicating N-terminal, C-terminal, MTBD and Proline rich region. **b)** The selected regions include 6N4P in the N-terminal consisting of amino acids from 5 – 10, **c)** 2MZ7 in R1-R3 region consisting of amino acids from 267 – 312 and **d)** 7QKZ in R3-C terminal consisting of amino acids from 305 – 379.

The protein structures were downloaded from PDB database in 3D PDB format and visualized in BIOVIA Discovery Studio and further cleaned using the software (Figure 2).

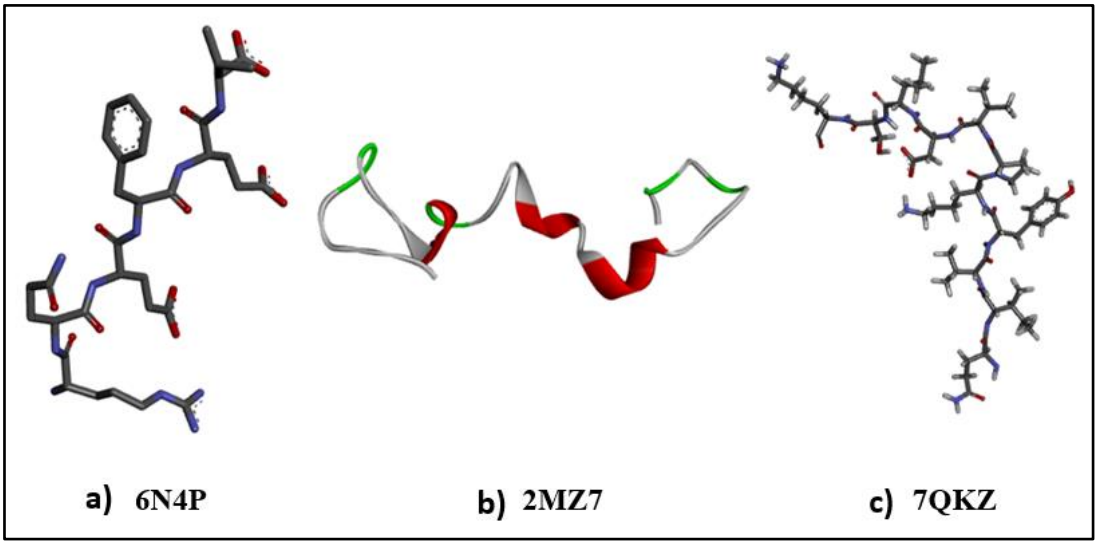


Figure 2. 3D Structures of Proteins. 3D structures of protein a) 6N4P, b) 2MZ7 and c) 7QKZ retrieved from PDB for docking in PyRx.

3.2.2 Preparation of Protein Molecules

The protein molecules (2MZ7, 6N4P, 7QKZ) were visualized in Discovery Studio and the structures were cleaned using Discovery Studio. Water molecules and Het atoms were also removed from the structures using the same process. The protein molecules after cleaning were uploaded in PyRx and were compressed and their energy was minimized by using Open Babel software that is already implanted in PyRx. The protein molecules were then auto docked into macromolecules in PyRx. This converts all the molecules in PDBQT format which is then used for macromolecule-ligand interaction.

3.2.3 Selection of Ligands

Ethics Statement:

The Department of Neurology, University Medical Center, Gottingen, provided the samples for Sporadic AD (sAD), rpAD and control brain samples after informed consent of the patients or their guardians. Gottingen's local ethical committee approved the study. (No 24/8/12).

Preparation:

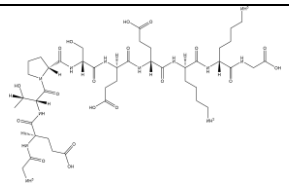
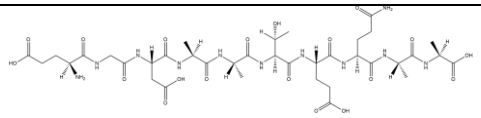
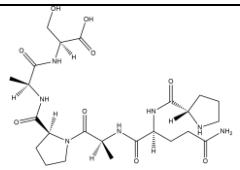
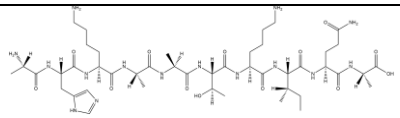
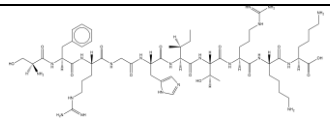
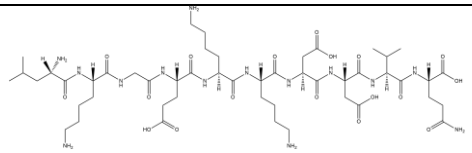
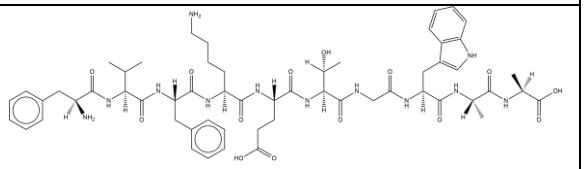
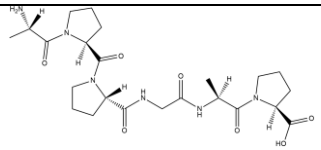
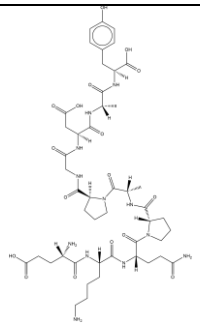
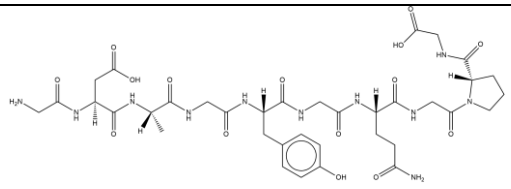
For all sAD, rpAD and control cases, CERAD system was used for the scoring of Tau pathology while the NFT scoring was done using the Braak and Braak. A tris-triton lysis buffer was used to homogenize brain tissue (10% w/v). Proteins extracted in Tris-Triton buffer were quantified using Bradford'sassay. The IP of Tau was performed using Dynabeads (1.5 mg/0.5 mg of protein sample). The beads were washed twice with 0.3% CHAPS and then incubated with 4 µl Tau antibody, Ab33324 (Abcam, UK) for 30 min at 4°C. The interactors were eluted in Laemmli buffer and stored at -20°C. For identification, Laemmli buffer was used to dilute the samples and then subjected to Liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI MS/MS).

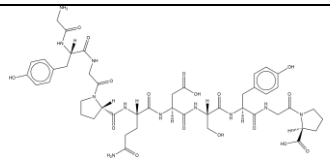
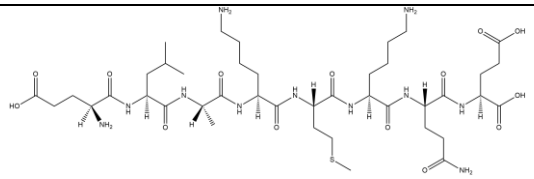
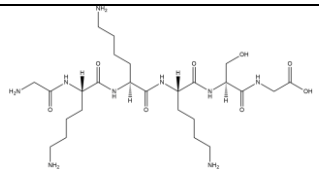
Total proteins which interacted with Tau were 53, out of which 7 proteins were found in AD, 8 proteins were found in control, 11 in rpAD, 4 in rpAD and AD, 13 in rpAD and control group, 2 in AD and control group, and 5 in rpAD, AD and control group. The rpAD and AD interactors were selected if they appeared in 2 out of 3 samples. The total number of rpAD samples conforming to this criteria were 11 out of which 3 sample proteins namely Neuromodulin, Synaptophysin and Transforming Protein RhoA were selected for *in silico* analysis and interactions.

3.2.4 Preparation of Ligands

The ligands were prepared using the software ChemDraw (Table 3) and the sequences and domains of the ligands were obtained through UniProt. The ligand was prepared and downloaded in 2D .sdf format which is then used in PyRx software for docking.

Table 3: Ligands and their structures. Targeted ligands Neuromodulin, Synaptophysin, Transforming protein RHOA and their 2D structures drawn using ChemDraw.

Ligand	Sequence	2D Structure
Neuromodulin	GETPSEEKKG	
	EGDAATEQAA	
	PQAPAS	
	AHKAATKIQA	
	SFRGHITRKK	
	LKGEKKDDVQ	
Synaptophysin	FVFKETGWAA	
	APPGAP	
	EKQPAPGDAY	
	GDAGYGQGPG	

	GYGPQDSYGP	
Transforming protein RhoA	ELAKMKQE	
	GKKKSG	

3.2.5 Molecular Docking

Molecular docking is a process which predicts noncovalent binding between a protein and a ligand to predict the binding energies, binding conformations and binding affinities. Vina Wizard module inbuilt in PyRx was used for the docking of ligands with macromolecules and the results were obtained for all the proteins and ligands presented in the table. AutoDock Vina improves the accuracy and specificity of the binding molecules and uses multithreading on multi-core machines. (Olson, 2010)

3.2.6 Protein-Ligand Interaction

Analysis of the interactions of Protein-Ligand complex was done by downloading the complex in PDB format from PyRx and visualizing in BIOVIA Discovery Studio for a 3D visualization and a 2D image depicting bonding between the protein and ligand. The complex was then submitted to PyMOL for producing a protein-ligand complex and then subjected to LigPlot+ for a 2D image and a detailed bonding interaction in the complex.

3.2.7 Protein-Protein Network Analysis

The interactive/ neighboring proteins for each of the four major proteins (*MAPT*, Neuromodulin, Synaptophysin, Transforming Protein RhoA) were obtained using databases like STRING and BioGRID and a network diagram of the common proteins was made using online tool Diagrams.net. The network diagram portrays specific pathways involved in different mechanisms such as apoptosis, signal transduction, cellular growth etc.

3.2.8 Drug Screening

Three drugs, Methamphetamine, Ascorbic Acid and Doxorubicin were selected using CTD and DrugBank and the structures were downloaded using PubChem in 2D .sdf format. The drug compounds were then docked with protein structure 2MZ7 using Autodocking Vina Wizard in PyRx and then visualized in 3D in Discovery Studio and a 2D image of the bonding between the complex was obtained. The Drug-protein complex was then visualized in PyMOL and the subjected to LigPlot+ for a detailed interactive image between the drug and protein.

The drugs were also docked with the Protein-Ligand complex for each of the three proteins using AutoDock Vina Wizard in PyRx and the 2D and 3D images of the Drug-Protein-Ligand complex and their bonding interactions were obtained using the same process in Discovery Studio, PyMOL and LigPlot+.

Chapter 4

Results

4.1 Molecular Docking Analysis

Tau Protein under the PDB ID: 2MZ7, 6N4P and 7QKZ was selected as a target protein and three proteins, Neuromodulin, Synaptophysin, Transforming Protein RhoA, were selected as ligands from an IP study performed in the University of Göttingen, Germany. Molecular docking was done in PyRx and binding energies were obtained enlisted in Table 4, Table 5 and Table 6. Visualizations in 2D and 3D were done using LigPlot+, Discovery Studio and PyMOL. LigPlot+ was used to analyze the interactions and bonding between the protein-ligand complex. The sequences with highest binding energies were selected for further 2D and 3D analysis, the rest of the sequences with their 2D analysis are mentioned in Appendix A.

Table 4: Ligands with 2MZ7 and their binding energies. Ligands Neuromodulin, Synaptophysin and RhoA are docked with 2MZ7, and binding energies are listed with highest binding energies highlighted in bold.

Ligand	Target	Binding Energy Kcal/mol
Neuromodulin 105 - 114	2MZ7	-4.2
Neuromodulin 115 - 124	**2MZ7**	**-5.2**
Neuromodulin 125 - 130	**2MZ7**	**-5.2**
Neuromodulin 31 - 40	2MZ7	-4.6
Neuromodulin 41 - 50	2MZ7	-5.1
Neuromodulin 51 - 60	2MZ7	-4.8
Synaptophysin 221 – 230	2MZ7	-5.9
Synaptophysin 235 – 240	**2MZ7**	**-6.1**
Synaptophysin 241 - 250	2MZ7	-5.3
Synaptophysin 251 - 260	2MZ7	-5.9
Synaptophysin 261 - 270	2MZ7	-5.6
RhoA 130 - 137	**2MZ7**	**-5**
RhoA 184 – 189	2MZ7	-4.6

PyRx Vina shows that Neuromodulin 115 – 124 and Neuromodulin 125 – 130 show the highest binding energy with the target protein. In case of Synaptophysin, 235 – 240 show the highest

binding energy. Transforming Protein RhoA 130 – 137 shows the highest binding energy with 2MZ7.

Table 5: Ligands with 6N4P and their binding energies. Ligands Neuromodulin, Synaptophysin and RhoA are docked with 6N4P, and binding energies are listed with highest binding energies highlighted in bold.

Ligands	Target	Binding Energy Kcal/mol
Neuromodulin 105 - 114	6N4P	-3.2
Neuromodulin 115 - 124	6N4P	-3.9
Neuromodulin 125 - 130	**6N4P**	**-4.7**
Neuromodulin 31 - 40	6N4P	-2.8
Neuromodulin 41 - 50	6N4P	-4
Neuromodulin 51 - 60	6N4P	-2.9
Synaptophysin 221 – 230	6N4P	-4
Synaptophysin 235 – 240	**6N4P**	**-3.9**
Synaptophysin 241 - 250	6N4P	-3.5
Synaptophysin 251 - 260	6N4P	-3.8
Synaptophysin 261 - 270	6N4P	-3.8
RhoA 130 - 137	6N4P	-2.7
RhoA 184 – 189	**6N4P**	**-2.9**

PyRx Vina shows that Neuromodulin 125 – 130 shows the highest binding energy with the target protein. In case of Synaptophysin, 235 – 240 show the highest binding energy. Transforming Protein RhoA 184 – 189 shows the highest binding energy with 6N4P.

Table 5: Ligands with 7QKZ and their binding energies. Ligands Neuromodulin, Synaptophysin and RhoA are docked with 7QKZ, and binding energies are listed with highest binding energies highlighted in bold.

Ligands	Target	Binding Energy Kcal/mol
Neuromodulin 105 - 114	7QKZ	-3
Neuromodulin 115 - 124	7QKZ	-3.6
Neuromodulin 125 - 130	**7QKZ**	**-4.5**
Neuromodulin 31 - 40	7QKZ	-4.1
Neuromodulin 41 - 50	7QKZ	-3.5
Neuromodulin 51 - 60	7QKZ	-3.2
Synaptophysin 221 – 230	7QKZ	-4.2
Synaptophysin 235 – 240	7QKZ	-4.2
Synaptophysin 241 - 250	**7QKZ**	**-4.3**
Synaptophysin 251 - 260	7QKZ	-4
Synaptophysin 261 - 270	7QKZ	-4.1
RhoA 130 - 137	7QKZ	-3
RhoA 184 – 189	**7QKZ**	**-3.6**

PyRx Vina shows that Neuromodulin 125 – 130 shows the highest binding energy with the target protein. In case of Synaptophysin, 241 – 250 shows the highest binding energy. Transforming Protein RhoA 184 – 189 shows the highest binding energy with 7QKZ.

4.2 Binding Energy Graph

The comparison of the ligands with all three target proteins shows that the binding affinities of all the ligands were highest with 2MZ7 protein molecule. Neuromodulin 115 – 124 and Neuromodulin 125 – 130 show a binding energy of -5.2 each. Synaptophysin, 235 – 240 shows a binding energy of -6.1 and Transforming Protein RhoA 130 – 137 shows a binding energy of -5.0 with 2MZ7 (Figure 3).

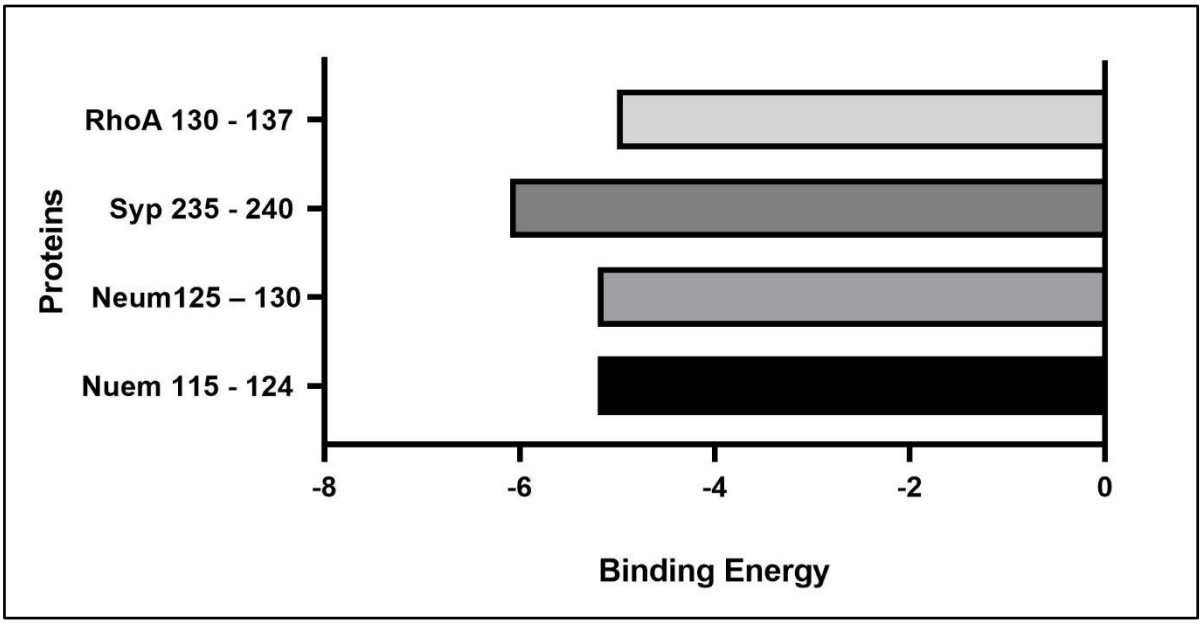


Figure 3. Binding Energy graph of Neuromodulin, Synaptophysin and RhoA. Binding energies evaluated by PyRx are shown in the graph. 2MZ7 had the highest binding energy with interactors among all three regions with Neum 115 – 124 and Neum 125 – 130 at a binding energy of -5.2 kcal/mol, RhoA 130 – 137 at binding energy of -5 kcal/mol and Syp 235 – 240 is shown having the highest binding energy at -6.1 kcal/mol.

4.3 Molecular Interaction Analysis

The protein-ligand complex was subjected to PyMOL for a 3D visualization and then submitted to Discovery studio and LigPlot+ for detailed 2D diagrams showing the bonds and interactions between the protein and the ligand.

4.3.1 Neuromodulin

The highest binding affinity for Neuromodulin was shown by two sequences, Neuromodulin 115 – 124 (EGDAATEQAA) and Neuromodulin 125 – 130 (PQAPAS) which was -5.2 for both.

The protein-ligand complex for Neuromodulin 115 – 124 (EGDAATEQAA) after being docked in Vina Wizard in PyRx was visualized in PyMOL and then in Discovery Studio (Figure 4) to obtain a 2D image of interactions and bonding. The binding site of 2MZ7 is surrounded by 15 amino acids such as ASP 283, LEU 282, LEU 284, ASN 279, GLN 269, GLN 268, CYS 291, SER 289, LYS 290, LYS 267, ILE 277, HIS 268, PRO 270, GLY 271 and ASN 286. Hydrogen bonds are the most visible interactions existing between 2MZ7 and Neuromodulin (115-124) in both Carbon and Conventional forms, along with Van der Waals and one Unfavorable Acceptor-Acceptor interaction (Figure 5).

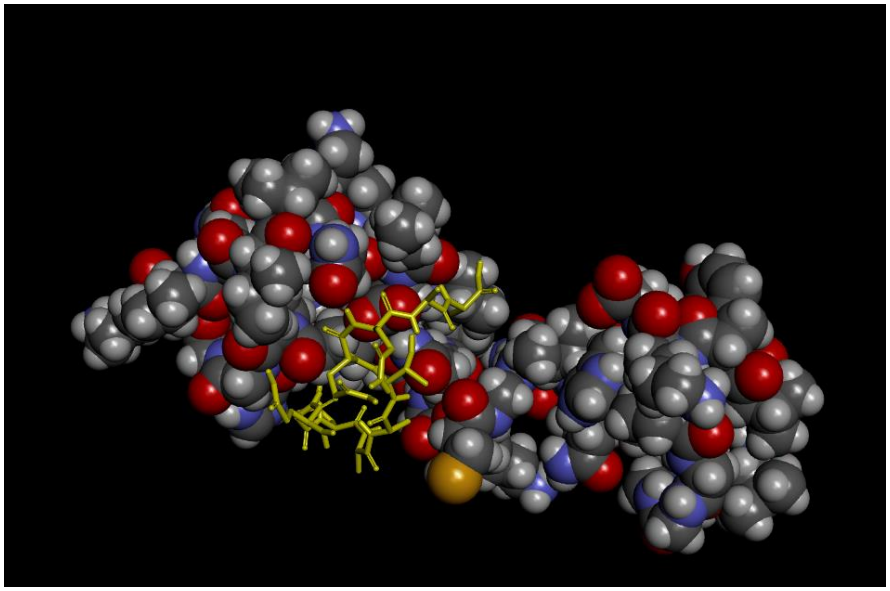


Figure 4. 2MZ7- Neuromodulin 115 – 124 surface representation. Representation of ball and socket 2MZ7 and Line structure Neuromodulin docked via PyRx and visualized by Discovery Studio.

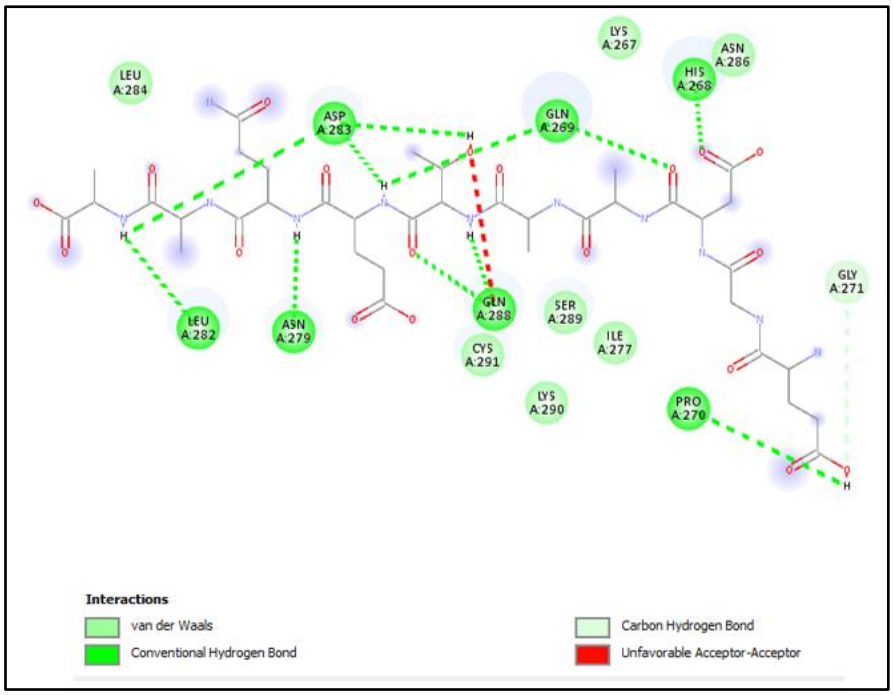


Figure 5. 2D Analysis of 2MZ7 and Neuromodulin 115 – 124. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green), hydrogen bond (shown in dark green), carbon hydrogen bonds (shown in blue) and Unfavorable acceptor-acceptor bond (shown in red).

The protein-ligand complex for Neuromodulin 125 – 130 (PQAPAS) after being docked in Vina Wizard in PyRx was visualized in PyMOL and then in Discovery Studio (Figure 6) to obtain a 2D image of interactions and bonding. The binding site of 2MZ7 is surrounded by 11 amino acids which are ASN 286, ASN 279, GLN 288, SER 289, GLY 271, GLN 269, ASP 283, HIS 268, PRO 270 and ILE 277. Hydrogen bonds are the most visible interactions existing between 2MZ7 and Neuromodulin (125-130) in both Carbon and Conventional forms, along with Van der Waals (Figure 7).

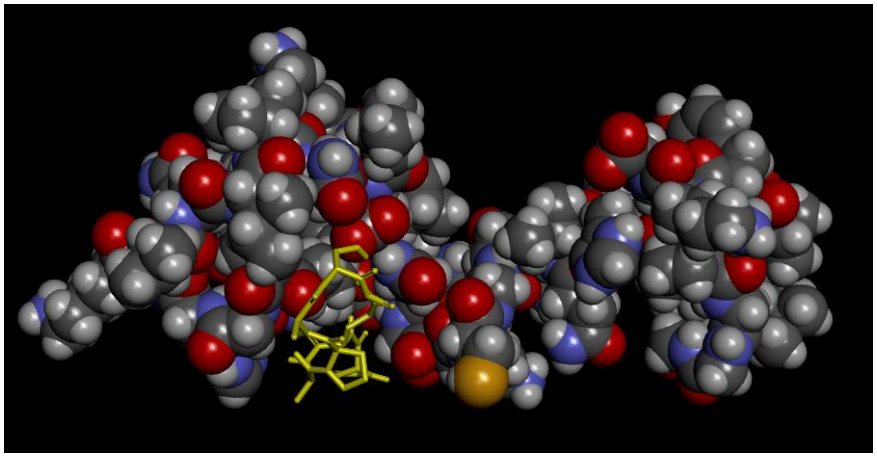


Figure 6. 2MZ7- Neuromodulin 125 – 130 surface representation. Representation of ball and socket 2MZ7 and Line structure Neuromodulin docked via PyRx and visualized by Discovery Studio.

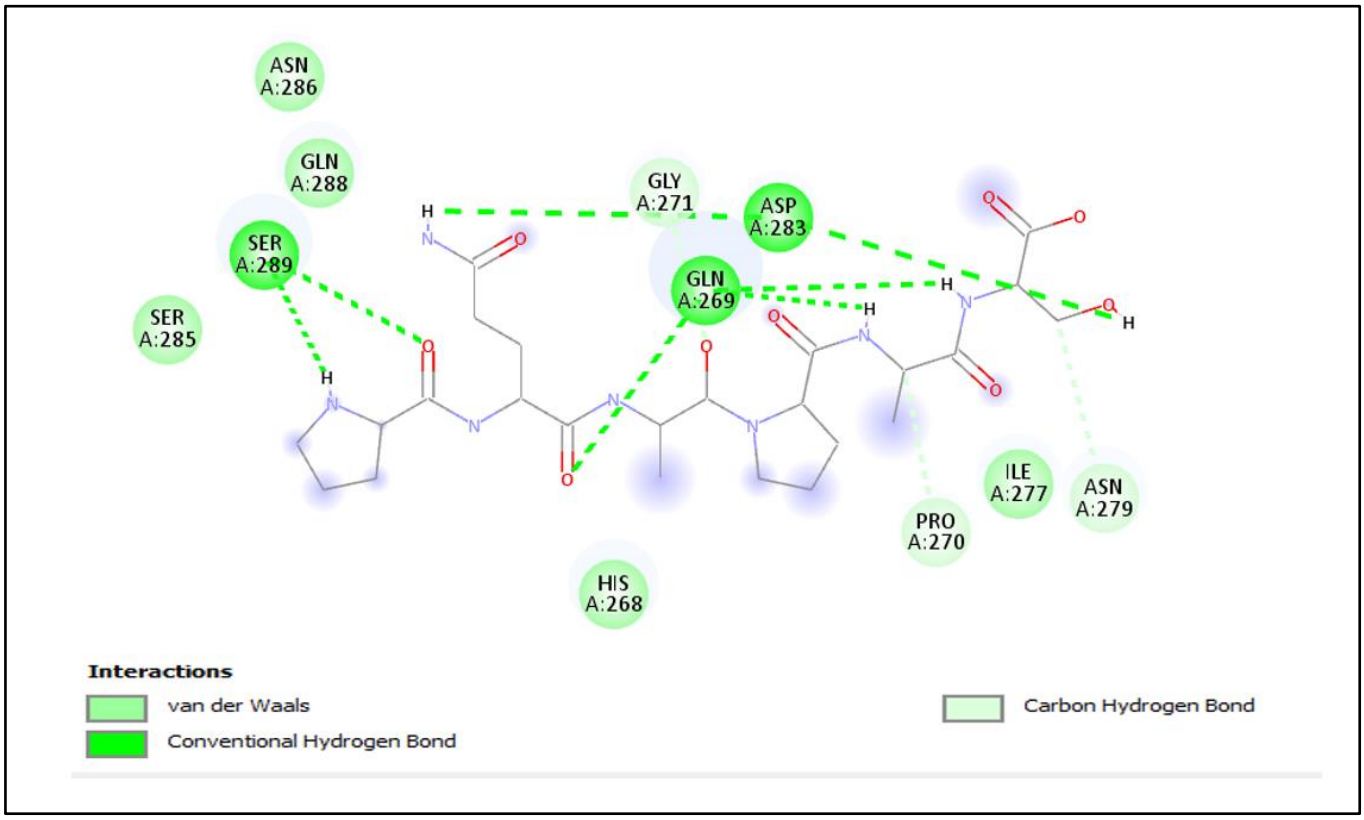


Figure 7. 2D Analysis of 2MZ7 and Neuromodulin 125 – 130. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green), hydrogen bond (shown in dark green) and carbon hydrogen bonds (shown in blue).

4.3.2 Synaptophysin

The highest binding affinity for Synaptophysin was shown by the sequence Synaptophysin 235 – 240 (APPGAP) which was -6.1 and the protein-ligand complex after being docked in Vina Wizard in PyRx was visualized in PyMOL and then in Discovery Studio (Figure 8) to obtain a 2D image of interactions and bonding. The binding site of 2MZ7 is surrounded by 9 amino acids which are GLN 269, GLN 288, ASP 283, LEU 282, LEU 284, SER 293, ILE 297, GLY 292 and PRO 312. Hydrogen bonds are the most visible interactions existing between 2MZ7 and Synaptophysin (235-240) in both Carbon and Conventional forms, along with Van der Waals, one Alkyl and one Unfavorable Donor-Donor interaction (Figure 9).

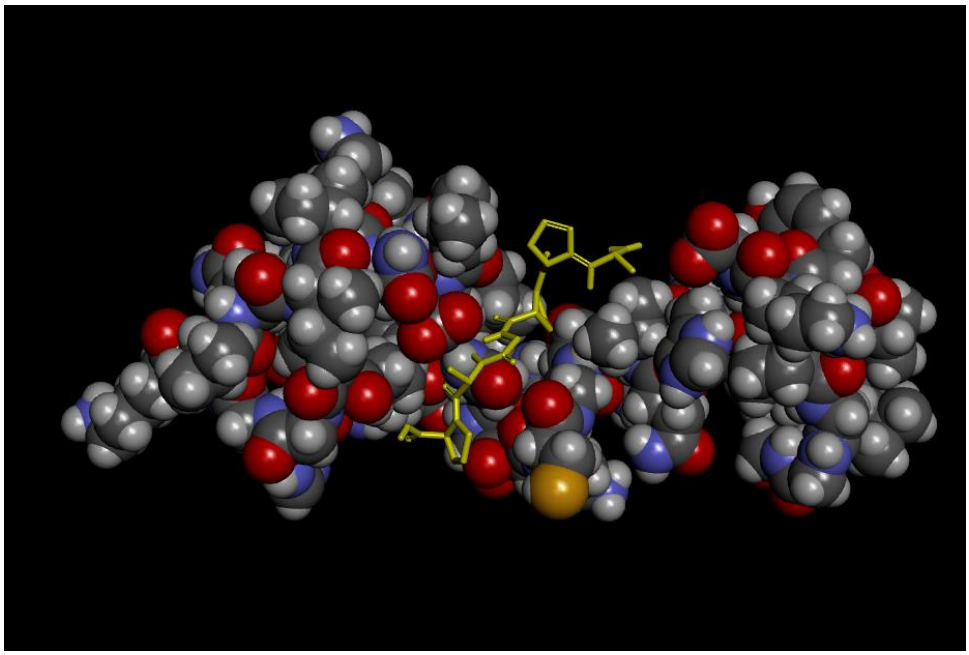


Figure 8. 2MZ7-Synaptophysin 235 - 240 surface representation. Representation of ball and socket 2MZ7 and Line structure Synaptophysin docked via PyRx and visualized by Discovery Studio.

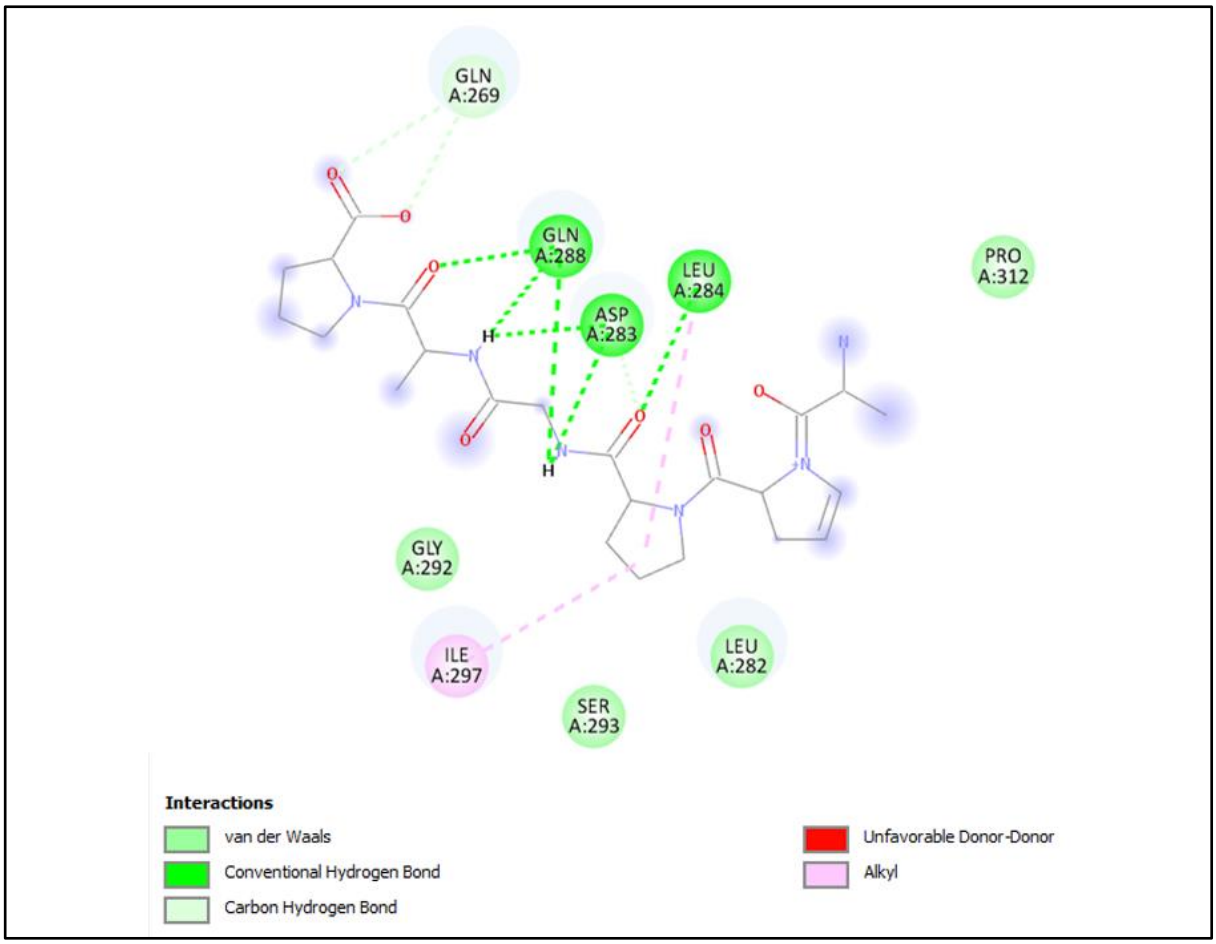


Figure 9. 2D analysis of 2MZ7 and Synaptophysin. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green), hydrogen bond (shown in dark green), carbon hydrogen bonds (shown in blue), alkyl bond (shown in purple) and Unfavorable donor-donor bond (shown in red).

4.3.3 RhoA

The highest binding affinity for Transforming Protein RhoA was shown by the sequence RhoA 130 – 137 (ELAKMKQE) which was -5 and the protein-ligand complex after being docked in Vina Wizard in PyRx was visualized in PyMOL and then in Discovery Studio (Figure 10) to obtain a 2D image of interactions and bonding. The binding site of 2MZ7 is surrounded by 15 amino acids which are ILE 297, GLY 292, GLY 271, LEU 289, LEU 282, ASP 283, CYS 291, ASN 279, ASN 286, GLN 269, GLN 288, SER 289, SER 293, SER 285 and HIS 268. Hydrogen bonds are the most visible interactions existing between 2MZ7 and Transforming protein RhoA (130-137) in both Carbon and Conventional forms, along with Van der Waals, one Alkyl, one Attractive charge and one Unfavorable Acceptor-Acceptor interaction (Figure 11).

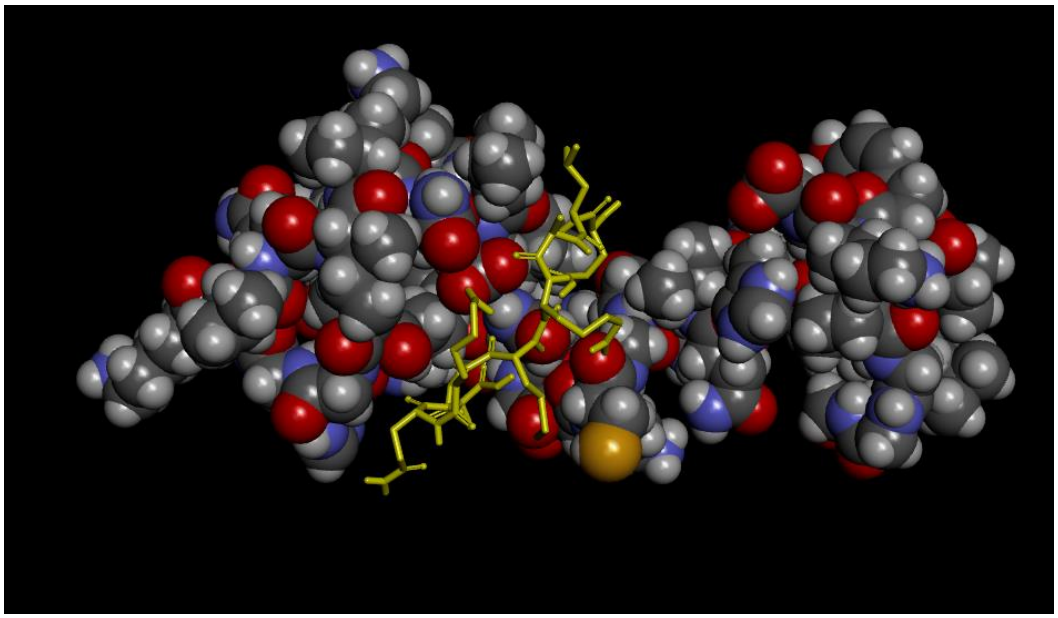


Figure 10. 2MZ7-RhoA surface representation. Representation of ball and socket 2MZ7 and Line structure RhoA docked via PyRx and visualized by Discovery Studio.

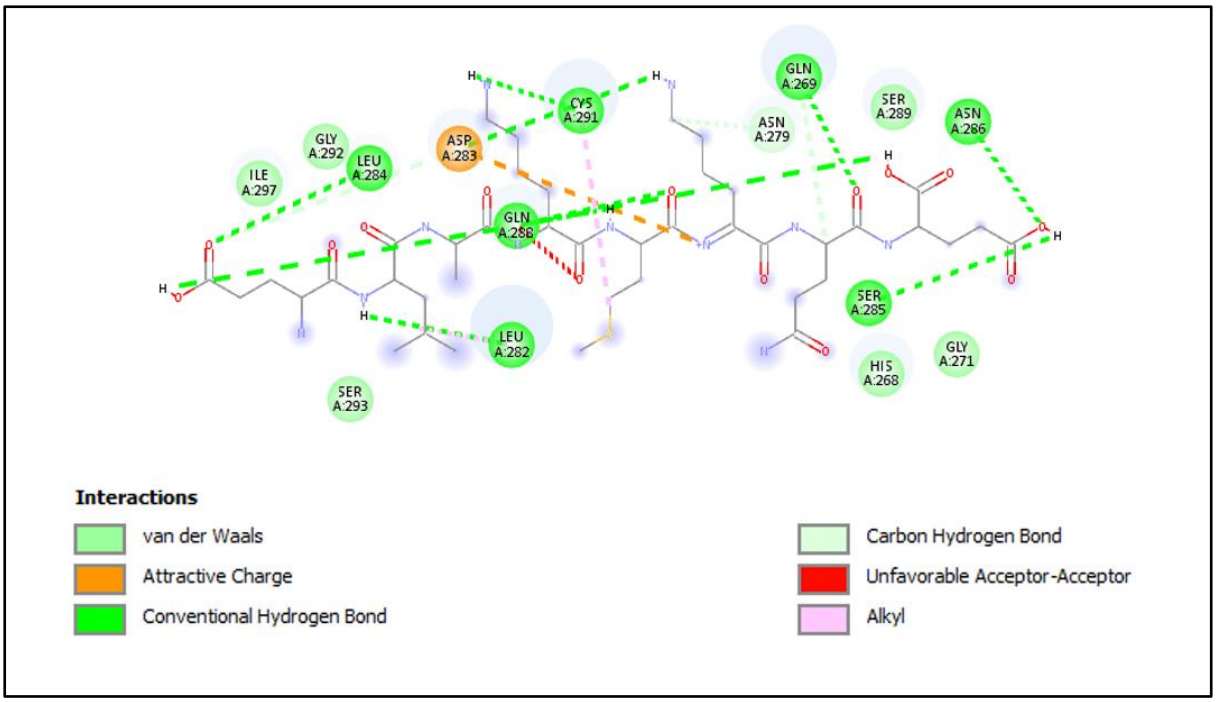


Figure 11. 2D Analysis of 2MZ7 and RhoA. 2D visualization in Discovery Studio showing bonding between the amino acids including Attractive charge (shown in orange), Van der Waals (shown in light green), hydrogen bond (shown in dark green), carbon hydrogen bonds (shown in blue), alkyl bond (shown in purple) and Unfavorable acceptor-acceptor bond (shown in red).

4.4 LigPlot+ Analysis

The protein-ligand complex after being visualized in Discovery Studio and PyMOL is then subjected to LigPlot+ and a 2D image with a detailed interactive analysis is obtained for each complex. The LigPlot+ analysis shows ligand bonds, non-ligand bonds, hydrogen bonds with their prescribed length, non-ligand residues which are involved in hydrophobic reactions and some corresponding atoms which are involved in hydrophobic interactions.

4.4.1 2MZ7 - Neuromodulin (125-130) and (115 – 124) Complex

The 2D interaction of Neuromodulin 125 – 130 and Neuromodulin 115 – 124 in LigPlot+ (Figure 13).

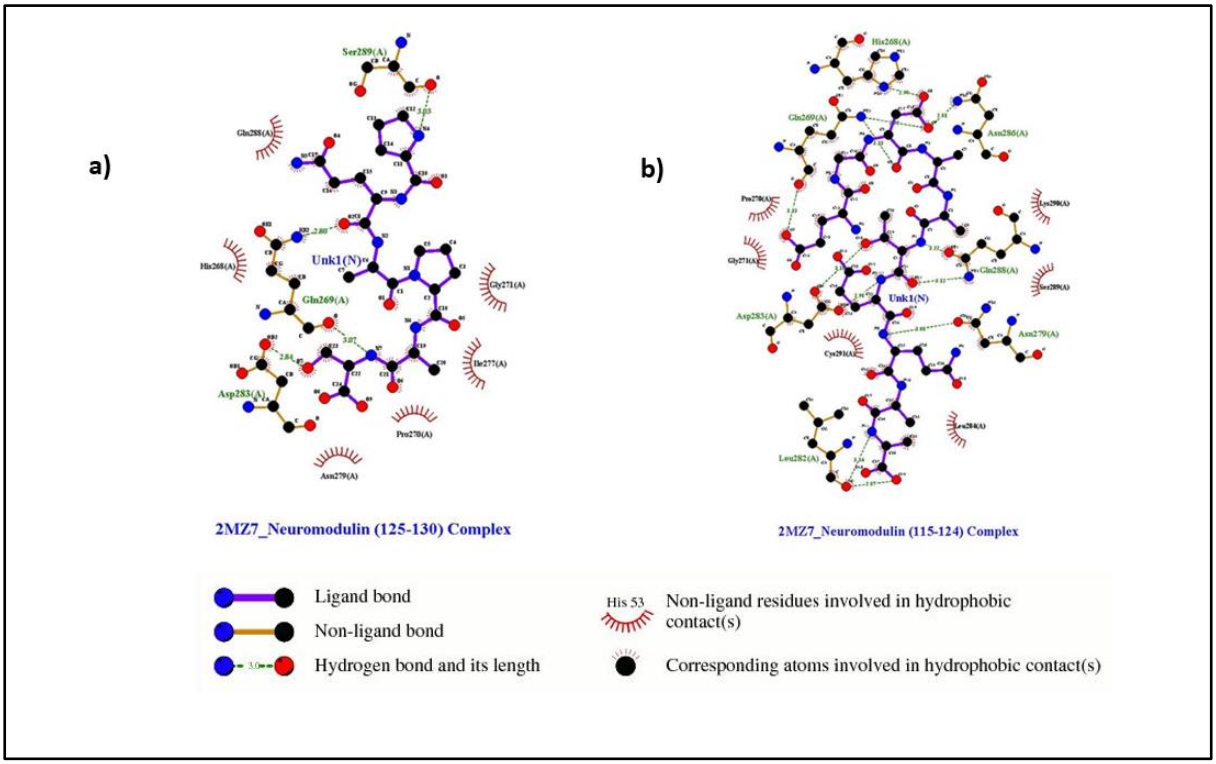


Figure 12. 2D Protein-Ligand complex evaluated by LigPlot of a) Neuromodulin 125 – 130 and b) Neuromodulin 115 – 124. 2D analysis of bonding evaluated by LigPlot showing Ligand bond (shown with a purple line), non-ligand bond (shown with a brown line), Hydrogen bond with their lengths (shown with a dotted line), Corresponding atoms (shown with black dots) and non-ligand residues involved in hydrophobic interactions (shown with red semi circles).

4.4.2 2MZ7 - Synaptophysin (235-240) Complex

The 2D interaction of Synaptophysin 235 – 240 in LigPlot+ (Figure 14).

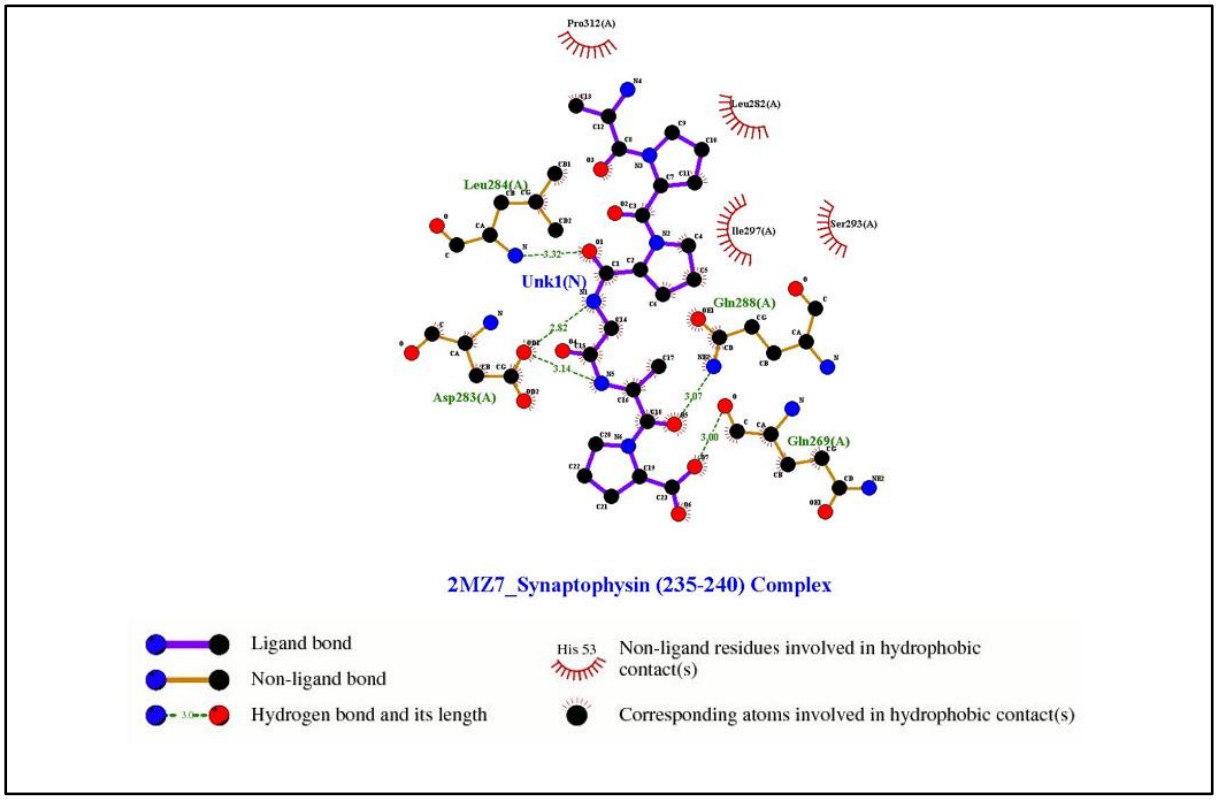


Figure 13. 2D Protein-Ligand complex of 2MZ7 and Synaptophysin evaluated by LigPlot. 2D analysis of bonding evaluated by LigPlot showing Ligand bond (shown with a purple line), non-ligand bond (shown with a brown line), Hydrogen bond with their lengths (shown with a dotted line), Corresponding atoms (shown with black dots) and non-ligand residues involved in hydrophobic interactions (shown with red semi circles).

4.4.3 2MZ7 – RhoA (130 – 137) Complex

The 2D interaction of RhoA 130 – 137 in LigPlot+ (Figure 15).

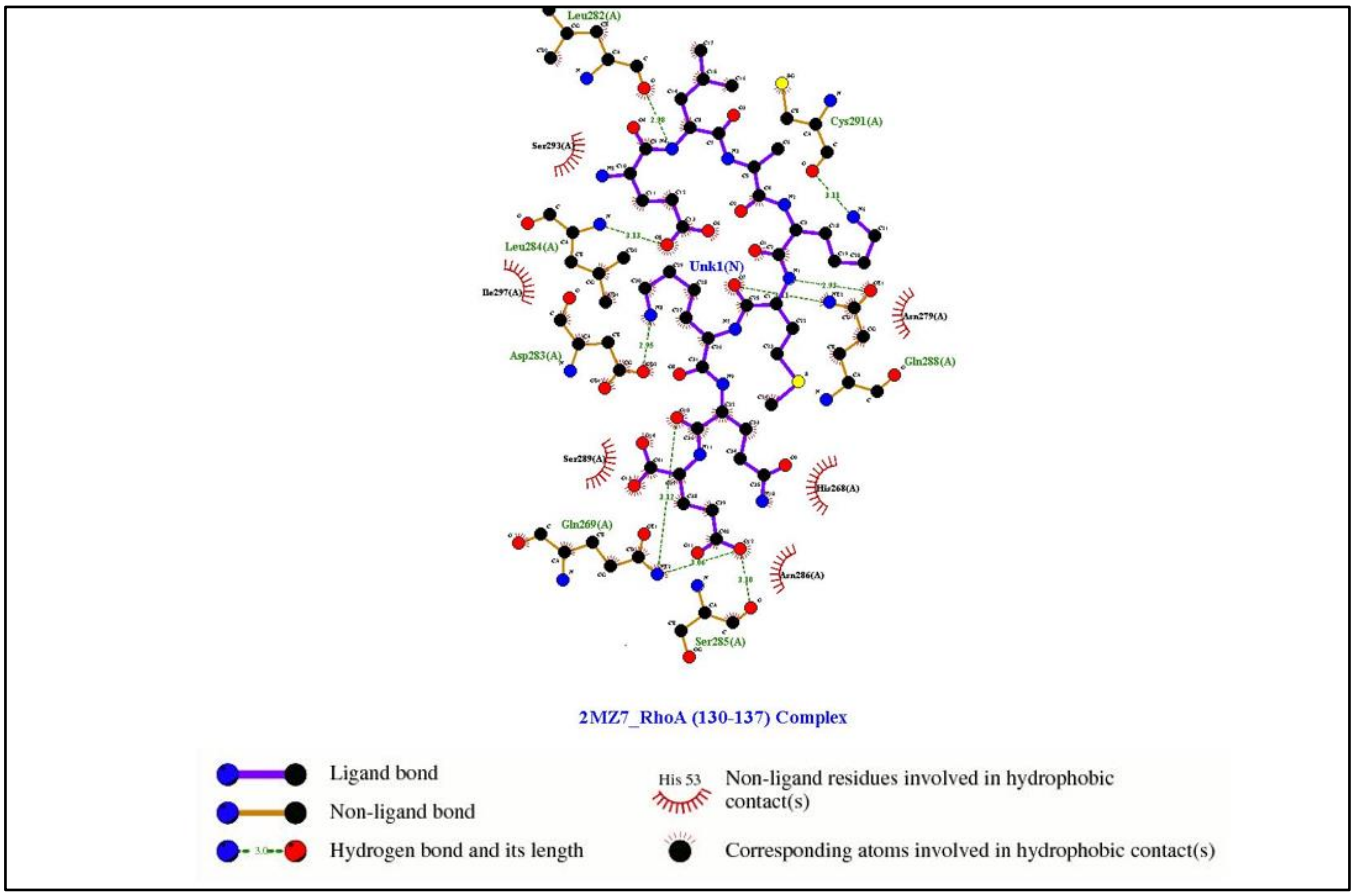


Figure 14. 2D Protein-Ligand complex of 2MZ7 and RhoA evaluated by LigPlot. 2D analysis of bonding evaluated by LigPlot showing Ligand bond (shown with a purple line), non-ligand bond (shown with a brown line), Hydrogen bond with their lengths (shown with a dotted line), Corresponding atoms (shown with black dots) and non-ligand residues involved in hydrophobic interactions (shown with red semi circles).

4.5 Protein-Protein Network Analysis

The interactive proteins for each of the four major proteins *MAPT*, Neuromodulin, Synaptophysin and RhoA were identified using databases such as STRING and BioGRID to identify a pathway and common interactive proteins between the four major ones (Figure 16). The major proteins identified were CASP1, CASP8, HSPB1, HSPA8, MARK2, ANAX5, GAB1, BIRC2, HAX1 which were identified to be involved in cell death, apoptosis, caspase regulation and signal transduction etc. (Figure 17).

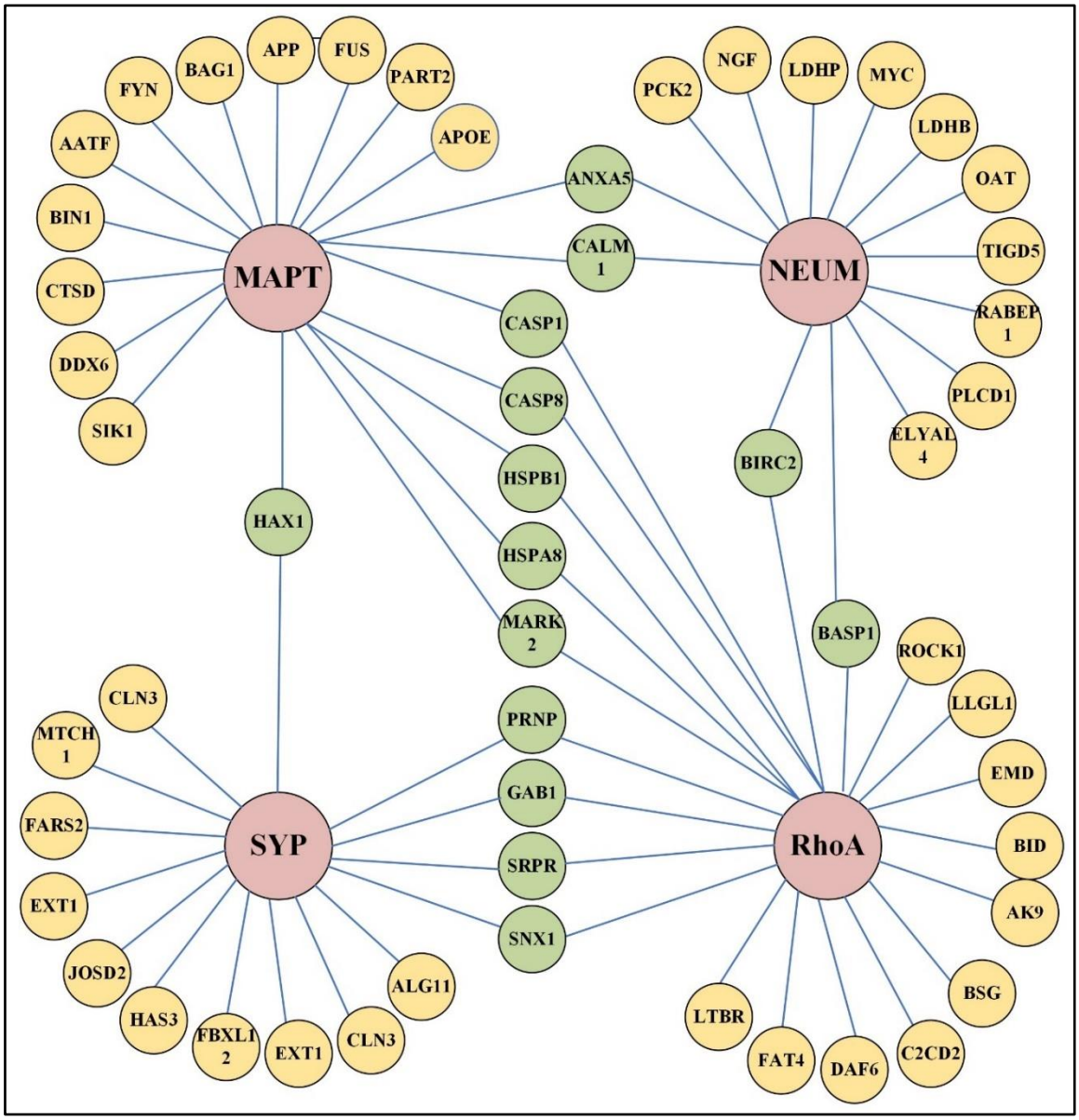


Figure 15. Protein-Protein Network Analysis. Protein analysis network shows four major proteins *MAPT*, Neum, RhoA and Syp (shown in pink), common proteins between them (shown in green) and uncommon proteins (shown in yellow).

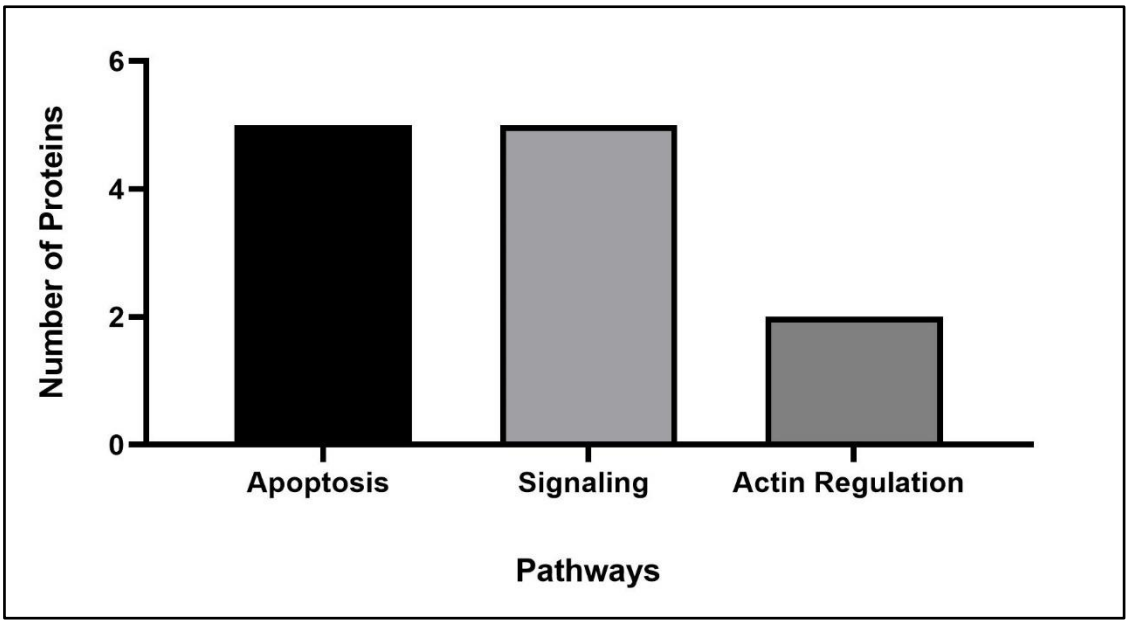


Figure 16. Protein-Protein Network Analysis Graph. Network analysis graph shows number of proteins involved in a particular pathway with 5 protein involved in Apoptosis, 5 in Signaling pathways and 2 in Actin Regulation.

4.6 Drug-Protein Interaction Analysis

Three drugs were selected for drug screening, Methamphetamine, Ascorbic Acid and Doxorubicin using CTD and DrugBank database and were docked with Tau protein structure 2MZ7 using Vina Wizard in PyRx. The binding energies were obtained (Table 7) and the drug-protein complex with the highest binding affinity was then subjected to Discovery Studio, PyMOL, LigPlot+ for 2D and 3D visualization and bonding interactions (Figure 18).

Table 6: Drugs docked with 2MZ7 and their binding energies. Drugs Methamphetamine, Doxorubicin and Ascorbic Acid are docked with 2MZ7, and their binding energies are listed with highest binding energies highlighted in bold.

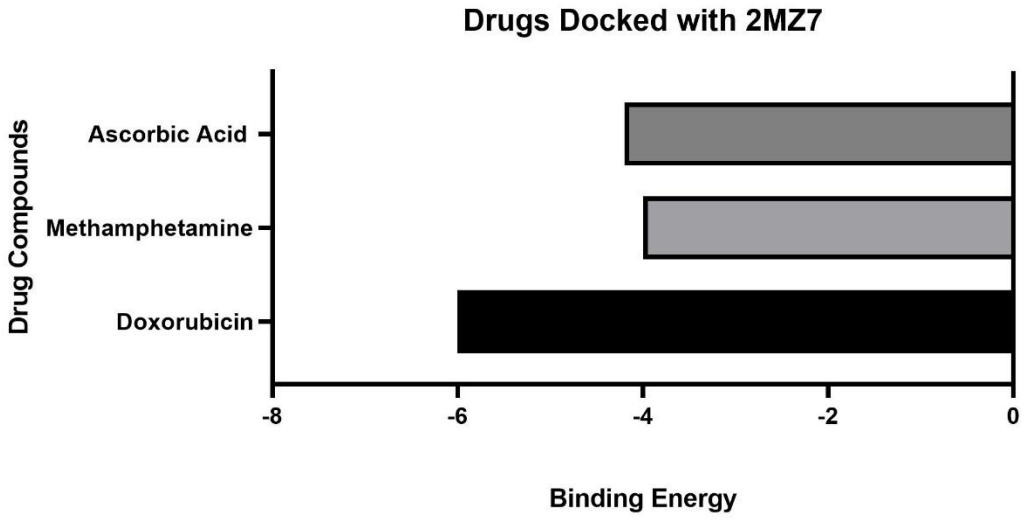
	**Target**	**Binding Energy**
Ascorbic Acid	2MZ7	-4.2
Methamphetamine	2MZ7	-4
Doxorubicin	**2MZ7**	**-6.0**

Figure 17. Drug-Protein Complex binding energy evaluated by PyRx. Drug compounds and protein 2MZ7 have been shown along with their binding energies with the highest binding energy of Doxorubicin highlighted in bold.

From the table, it is identified that Doxorubicin shows the most binding affinity with 2MZ7. This drug-protein complex of Doxorubicin and 2MZ7 is then used to obtain 2D and 3D visualizations through Discovery Studio, PyMOL and LigPlot+.

4.6.1 Doxorubicin-2MZ7 Complex

The 2MZ7- Doxorubicin complex is surrounded by 9 amino acids which are GLY 271, PRO 270, CYS 291, LYS 290, HIS 268, GLN 288, GLN 269, SER 285, SER 289 and ASN 286. The major bonds between the complex are Van der waal's forces, carbon hydrogen bond, alkyl bond, donor hydrogen bond and conventional hydrogen bonding (Figure 19).

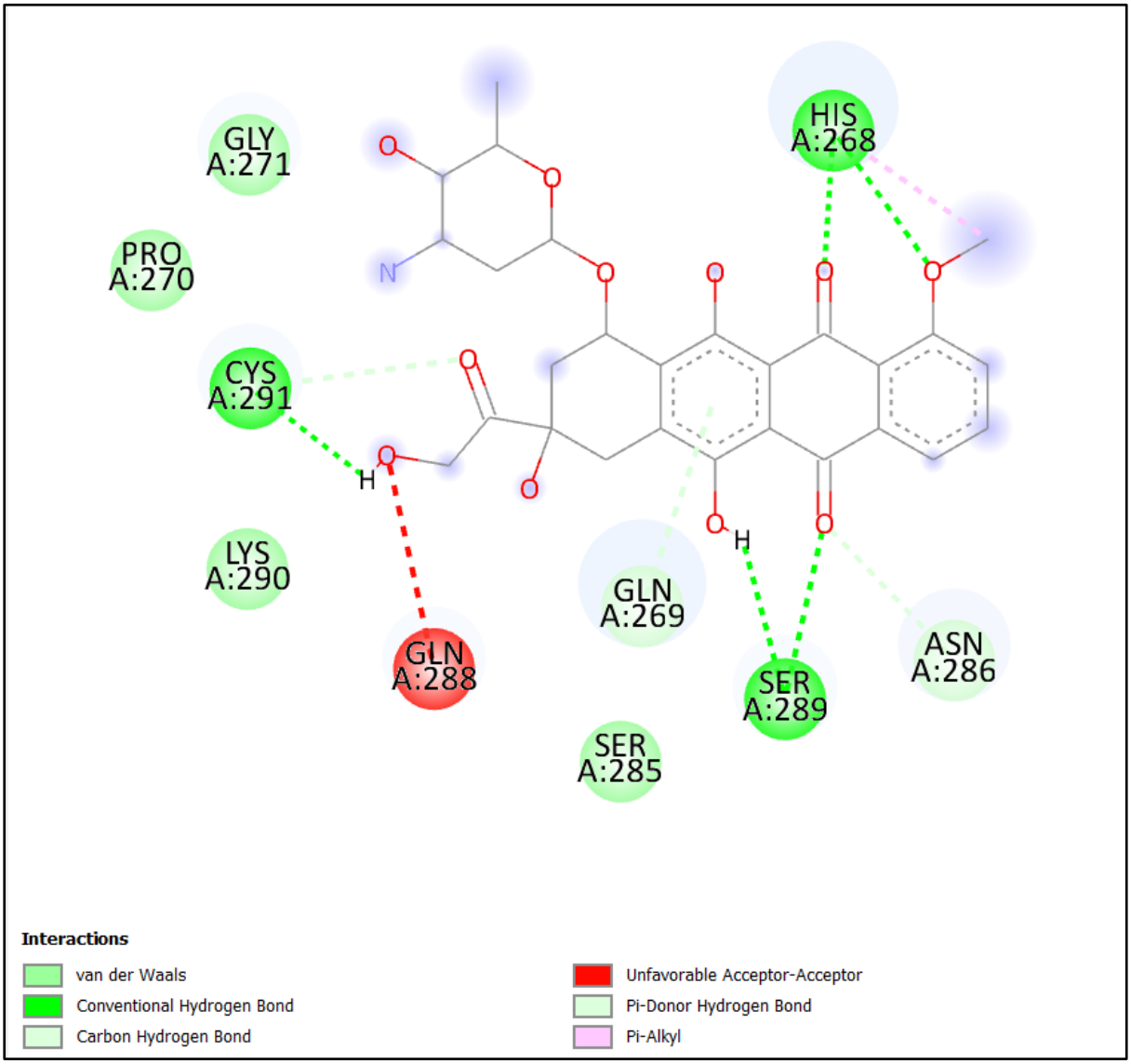


Figure 18. 2D analysis of Doxorubicin (Drug) with 2MZ7. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green) and hydrogen bond (shown in dark green), carbon hydrogen bond and donor hydrogen bond (shown in blue) and alkyl bond (shown in purple).

2MZ7-Doxorubicin Complex evaluated by LigPlot. 2D analysis of bonding evaluated by LigPlot showing Ligand bond, non-ligand bond, hydrogen bond with their lengths, corresponding atoms and non-ligand residues involved in hydrophobic interactions (Figure 20).

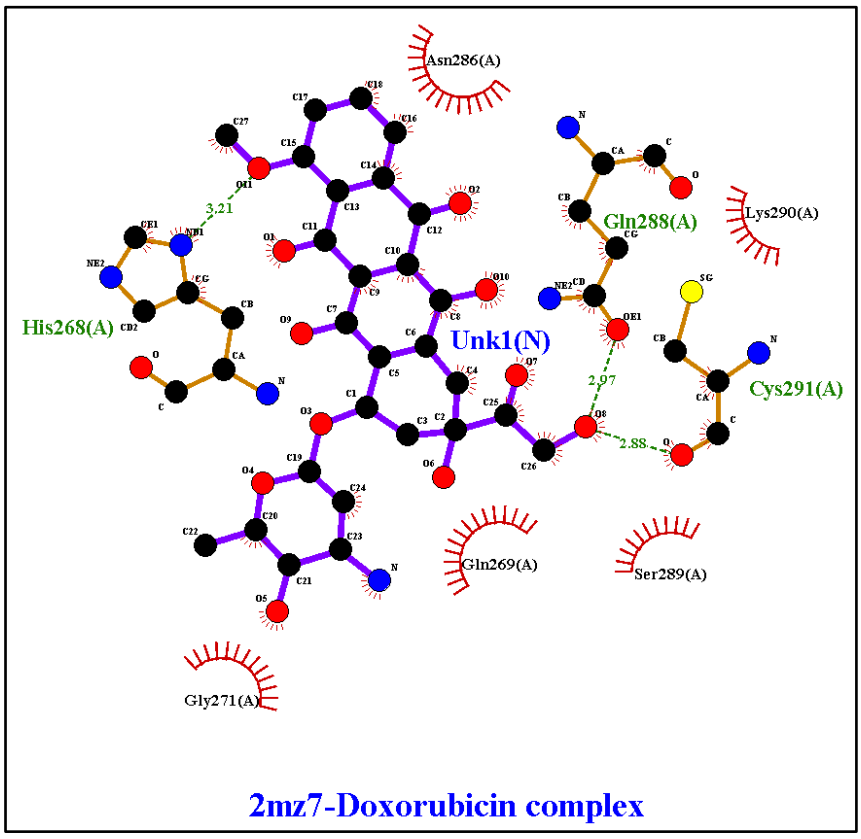


Figure 19. 2MZ7-Doxorubicin Complex evaluated by LigPlot. 2D analysis of bonding evaluated by LigPlot showing Ligand bond (shown with a purple line), non-ligand bond (shown with a brown line), Hydrogen bond with their lengths (shown with a dotted line), Corresponding atoms (shown with black dots) and non-ligand residues involved in hydrophobic interactions (shown with red semi circles).

4.7 Drug-Protein-Ligand Interaction Analysis

Drug-protein-ligand complex was docked via PyRx and binding energies were obtained (Table 8) and a graph was plotted (Figure 21). The complex was then visualized in 2D and 3D using Discovery Studio.

Table 7: Drug-protein-ligand complex binding energies. Drug-protein-ligand molecular docking is performed, and binding energies are obtained.

Ligands	Protein	Drug Compound	Binding Energy
Neuromodulin (115 – 124)	2MZ7	Methamphetamine	-4
		Ascorbic Acid	-4.2
		Doxorubicin	-6.1
Neuromodulin (125 – 130)	2MZ7	Methamphetamine	-4
		Ascorbic Acid	-4.3
		Doxorubicin	-6.0
Synaptophysin (235 – 240)	2MZ7	Methamphetamine	-4
		Ascorbic Acid	-4.2
		Doxorubicin	-6.3
RhoA (130 – 137)	2MZ7	Methamphetamine	-4
		Ascorbic Acid	-4.2
		Doxorubicin	-6.3

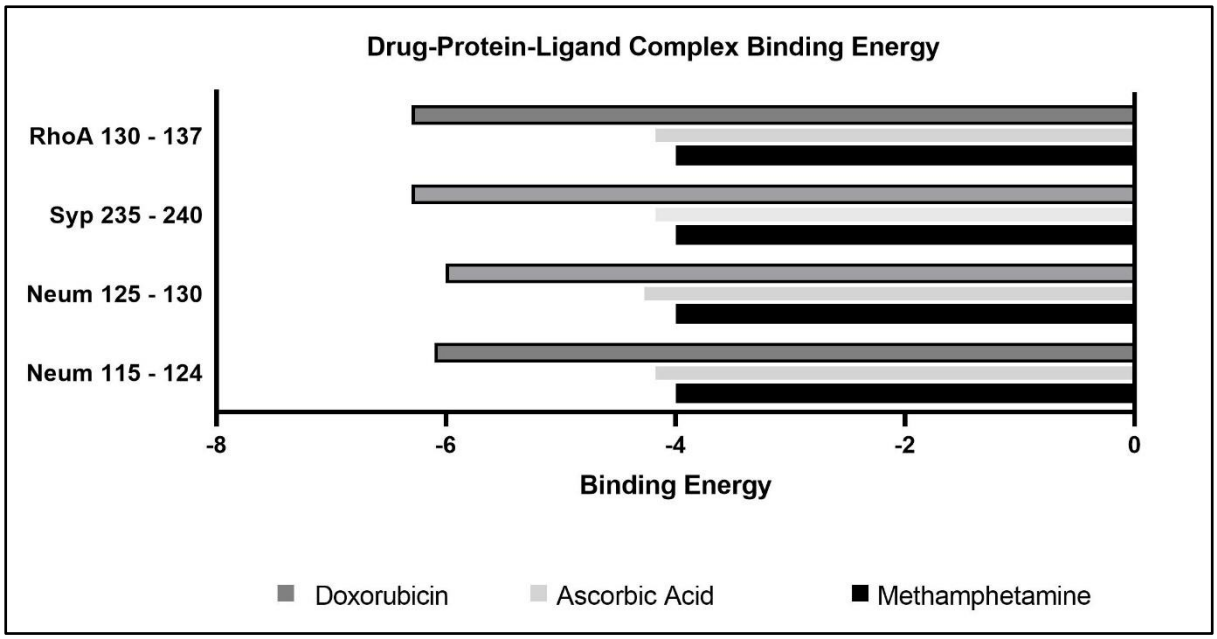


Figure 20. Drug-Protein-Ligand Complex Binding Energy evaluated by PyRx. Drug-protein-ligand complex with their respective binding energies for each of the drug compound.

From the above graph, Doxorubicin was identified as the drug with the highest binding affinity with the Protein-ligand complex. This complex was then subjected to Discovery Studio and PyMOL for 2D and 3D visualization and interaction between the amino acids.

4.7.1 Neuromodulin (115 – 124)-2MZ7-Doxorubicin Complex

3D visualization ((Figure 22) and 2D visualization (Figure 23) of Neuromodulin (115 – 124)-2MZ7- Doxorubicin complex in Discovery Studio

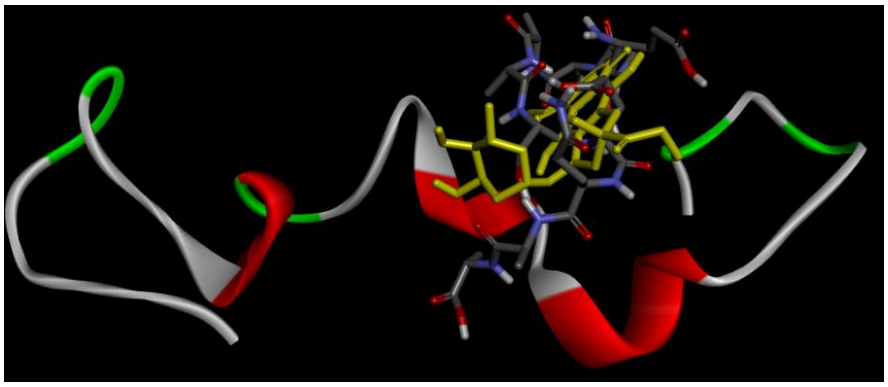


Figure 21. 3D visualization of 2MZ7-Neum (115-124)- Doxorubicin Complex. Representation of ribbon structure 2MZ7-Neum complex and line structure Doxorubicin docked via PyRx and visualized by Discovery Studio.

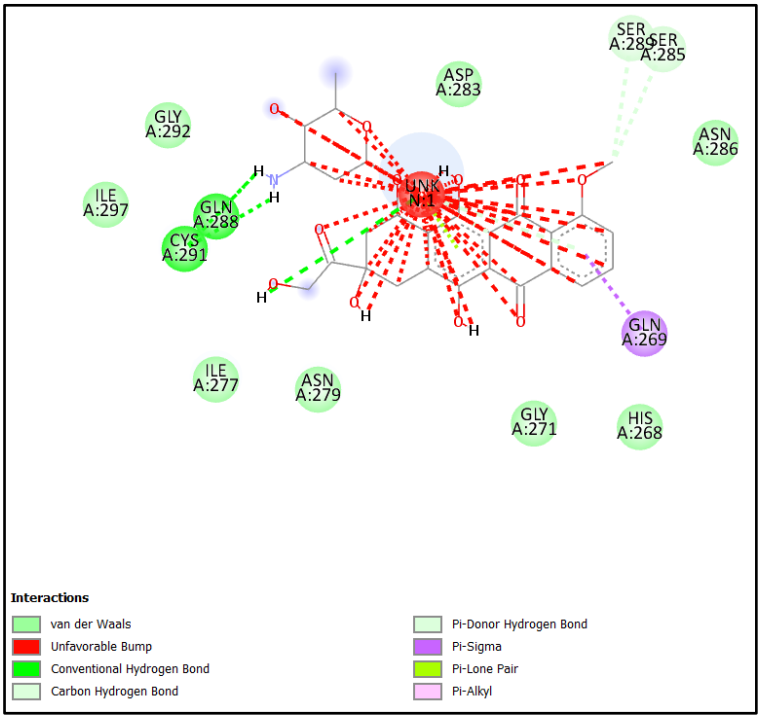


Figure 22. 2D interactions shown in 2MZ7-Neum-Doxorubicin Complex. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green), hydrogen bond (shown in dark green), carbon hydrogen bond and donor hydrogen bond (shown in blue), alkyl bond (shown in purple), Unfavorable interaction (shown in red), sigma bond (shown in dark purple) and a lone pair (shown in green).

4.7.2 Neuromodulin (125 – 130)-2MZ7- Doxorubicin Complex

3D visualization ((Figure 24) and 2D visualization (Figure 25) of Neuromodulin (125 – 130)-2MZ7- Doxorubicin complex in Discovery Studio.

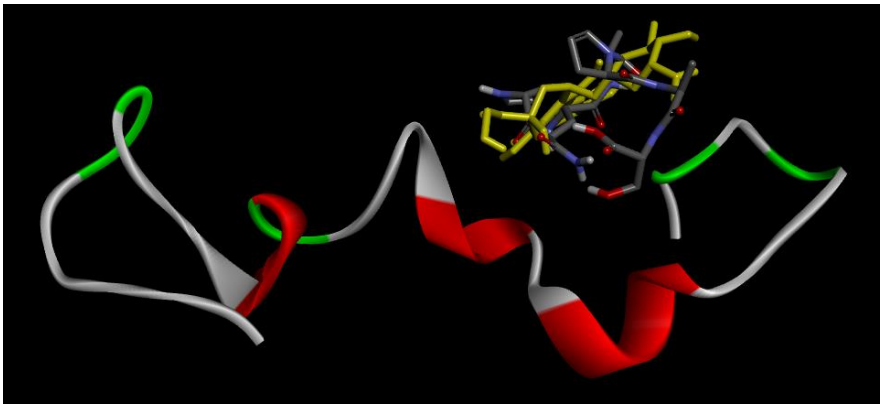


Figure 23. 3D visualization of 2MZ7-Neum (125-130)-Doxorubicin Complex. Representation of ribbon structure 2MZ7-Neum complex and line structure Doxorubicin docked via PyRx and visualized by Discovery Studio.

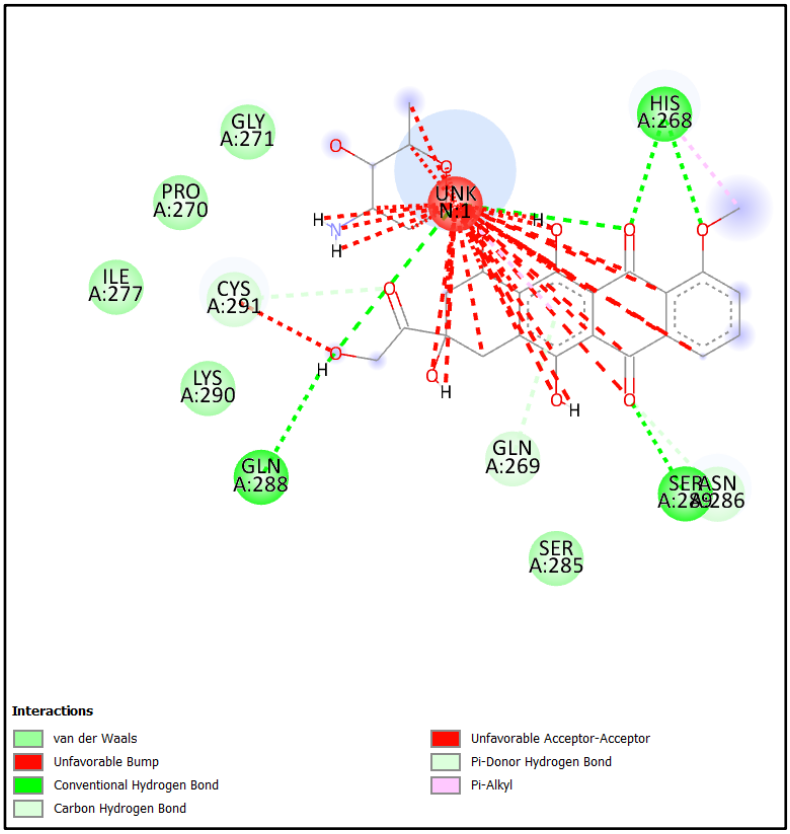


Figure 24. 2D interactions shown in 2MZ7-Neum-Doxorubicin Complex. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green), carbon hydrogen bond and donor hydrogen bond (shown in blue),

conventional hydrogen bond (shown in green), unfavorable bump (shown in red) and alkyl bond (shown in purple).

4.7.3 Synaptophysin (235 – 240)-2MZ7-Doxorubicin Complex

3D visualization ((Figure 26) and 2D visualization (Figure 27) of Synaptophysin (235 – 240)-2MZ7- Doxorubicin complex in Discovery Studio.

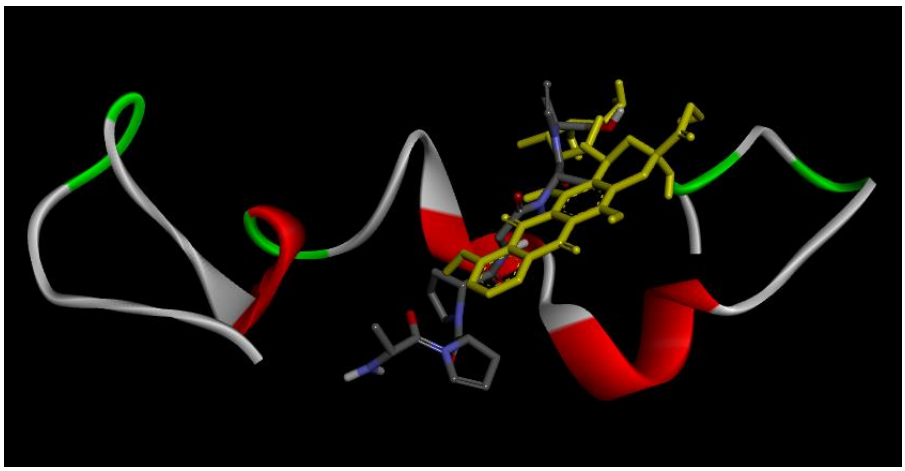


Figure 25. 3D visualization of 2MZ7-Syp (235-240)-Doxorubicin Complex. Representation of ribbon structure 2MZ7-Syp complex and line structure Doxorubicin docked via PyRx and visualized by Discovery Studio.

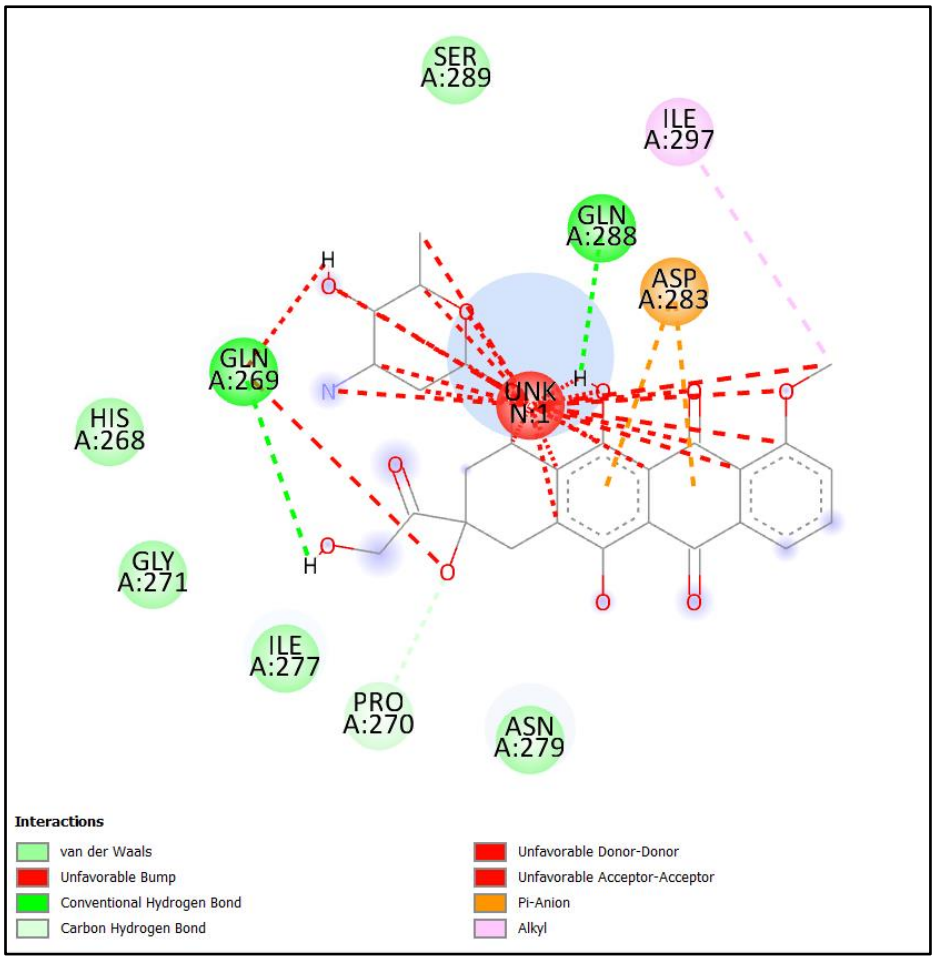


Figure 26. 2D interactions shown in 2MZ7-Syp-Doxorubicin Complex. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green), hydrogen bond (shown in dark green), carbon hydrogen bonds (shown in blue), alkyl bond (shown in purple), Pi anion (shown in orange) and an Unfavorable bump (shown in red).

4.7.4 RhoA (130 – 137)-2MZ7-Doxorubicin Complex

3D visualization ((Figure 28) and 2D visualization (Figure 29) of RhoA (130 – 137)-2MZ7-Doxorubicin complex in Discovery Studio

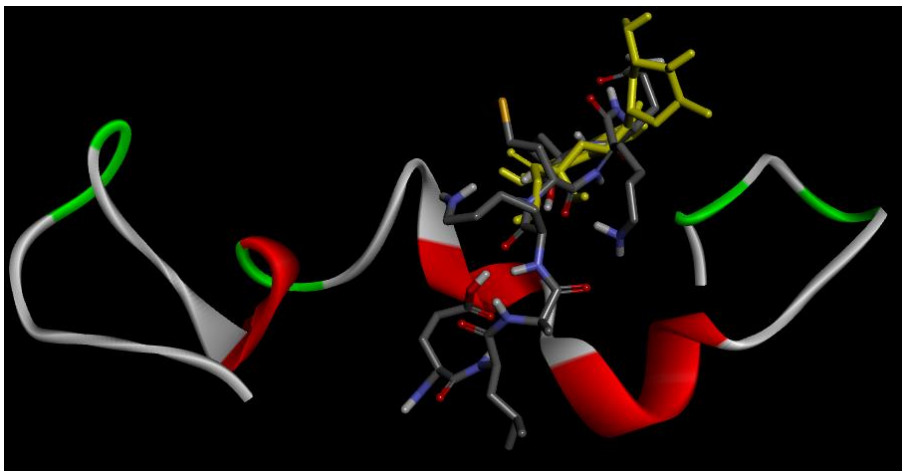


Figure 27. 3D visualization of 2MZ7-RhoA (130-137)-Doxorubicin Complex. Representation of ribbon structure 2MZ7-Neum complex and line structure Doxorubicin docked via PyRx and visualized by Discovery Studio.

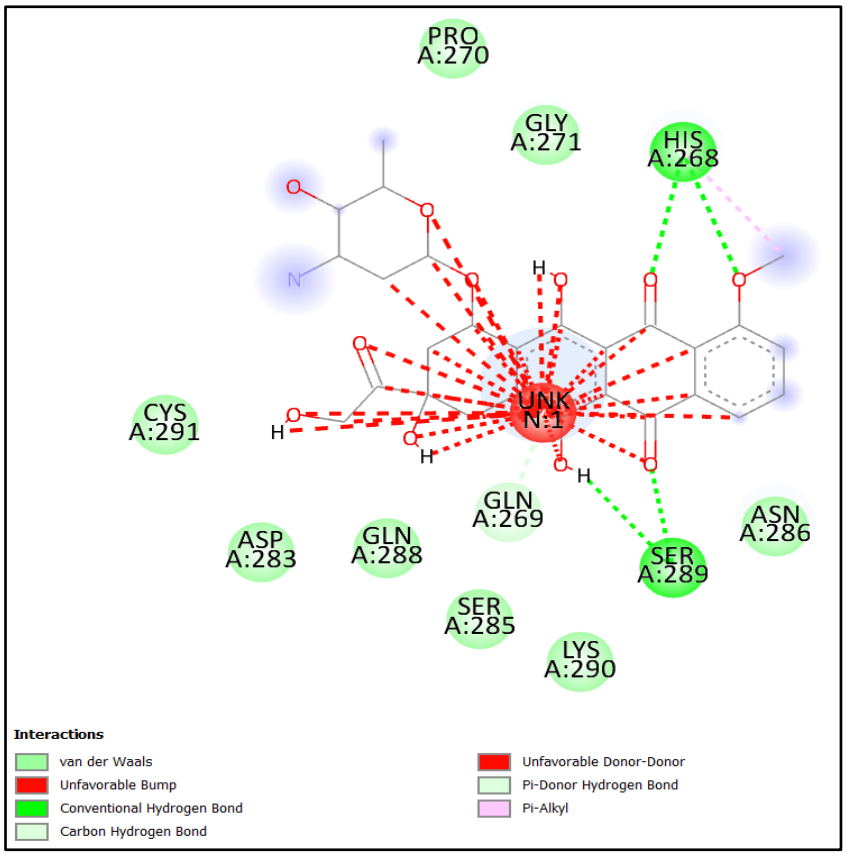


Figure 28. 2D interactions shown in 2MZ7-RhoA-Doxorubicin Complex. 2D visualization in Discovery Studio showing bonding between the amino acids including Van der Waals (shown in light green), carbon hydrogen bond and donor hydrogen bond (shown in blue), alkyl bond (shown in purple) and Unfavorable donor-donor bond (shown in red) and an unfavorable bump (shown in red).

Chapter 5

Discussion

In silico studies and computational approaches in the past decade have shown tremendous success in interactive studies, drug development and disease control. These studies have also been used to predict, explain and model biological molecules and can be used to utilize public and private databases. *In silico* studies can also be used for the discovery of biological markers and different prognostic and diagnostic tools for the identification of genes, diseases, expression profiles, drugs etc (Mark R Fielden, 2002). Due to affordability and access to technology, there has been significant amount of increase in generation of data. Thus, *in silico* analysis has presented a roadmap to scientists in the field of biomedical research and has paved the way for scientific communities to identify and overview molecular interactions and analysis which are helpful in disease management and drug development (David Murray, 2007).

Tau or *MAPT* protein is expressed in neurons and glial cells and belong to the family MAP where they are primarily responsible for cytoskeleton stabilization and also play a crucial role in the formation of microtubules from tubulin monomers to formulate the neuronal microtubule network. Tau also performs an important role in modulating the microtubule dynamics in the brain and promote neurite growth, axonal transport and also synapsis and contributes towards axonal stability, neuronal development and neuronal polarity (L Buée, 2000). The role of Tau in microtubule assembly regulates the process of neuron morphology, it may also normalize intracellular signaling facilitated by protein interaction with proteins Pin-1 and Fyn proposing a role in cell signaling. Primarily, Tau is characterized by being an axonal protein, but has also been found in neuronal nuclei where it plays a role in gene expression by binding with either DNA or RNA (Carolina Alquezar, 2021). A gene located on chromosome 17 is responsible for the translation of protein along with its regulation through alternative splicing which then results in six distinct isoforms of tau protein in the adult human brain (L Buée, 2000). The isoforms of Tau differ from each other in amino acid sequences at N-terminal and the presence of repeats at the C-terminal. All the distinct isoforms of Tau have their particular role and physiology in different biological activities and stimulate the microtubule development with different efficiencies (K. Iqbal, 2008).

In our study, 11 proteins were identified in playing a role in the progression of rpAD through experimentation out of which 3 proteins were selected namely, Neuromodulin, Synaptophysin and Transforming Protein RhoA. Neuromodulin (GAP43) is a synaptic growth protein majorly present in presynaptic terminal and is neural-specific. GAP43 binds to calmodulin protein

which is important for development and regeneration of neurons and is a protein kinase C substrate (Jean-Christophe Deloulme, 1990). GAP43 is also responsible for axonal growth and development by regulation actin and microtubules during spindle formation. In some studies, there has been an elevation of Neuromodulin in the CSF of AD patients thus indicating that protein levels fall in the frontal cortex and hippocampus and as a result CSF from brains of AD patients constitutes of Neuromodulin protein (Julia Remnestål, 2016).

Synaptophysin is the most abundant synaptic vesicle protein and despite being the most abundant, the functionality of synaptophysin remains somewhat enigmatic. Synaptophysin is believed to play a role in the vesicle availability after neuronal activity. (Chapman, 2011) During a study, it was observed that synaptic abnormalities in the hippocampus is linked with memory deficit in patients with AD and this might be some evidence of cognitive impairment in patients during early stages of AD (C I Sze, 1997).

Transforming protein RhoA is a GTPase from the Rho family and has crucial role in signal transduction and multiple signaling pathways. These are also involved in numerous other functions such as cell migration, apoptosis, cytoskeleton rearrangement etc. RhoA is highly expressed in nervous system and is involved in signaling in neuron and glial cells. Through evidence it is supported that RhoA signaling is involved in Aβ aggregation, Tau phosphorylation, synaptic damage and neuroinflammation during the progression of AD. It was also observed that RhoA levels were lower in the brain but higher in hyperphosphorylated Tau in NFTs in patients with AD (Sissel Ida Schmidt, 2022).

In our study, we aimed to evaluate the binding and stability of these proteins with the parent protein *MAPT* (Tau) and their corresponding significance in the progression of rpAD through *in silico* methods. The associative interactions between specific regions of *MAPT*, in this case, 2MZ7 (MTB domain) and 3 proteins were analyzed and studied using softwares Discovery Studio, PyRx, PyMOL and LigPlot+. The complexes were studied and analyzed on the basis of their binding energies and the formation of bonds between the molecules. It was observed that the binding sites of 2MZ7 are surrounded by 22 amino acids, and Neum (115 – 124) and Neum (124 – 130), Syp (235 – 240), RhoA (130 – 137) were able to produce interaction with amino acids within in the binding pocket of 2MZ7. Neuromodulin domain sequences are docked with 2MZ7 and sequences Neum (115 – 124) and Neum (125 – 130) produce the highest binding energy of -5.2 kcal/mol when docked with 2MZ7. Similarly, when Synaptophysin sequences are docked, SYP (235 – 240) yields the highest binding energy of -6.1 kcal/mol with 2MZ7. RhoA (130 – 137) shows the highest binding energy of -5.0 kcal/mol when docked with 2MZ7. The interaction of sequences of ligand proteins with 2MZ7 yielding

high binding affinity showcase how AD can be caused by different mechanisms such as signal transduction, apoptosis, synaptic transmission, of these interacting proteins. The results obtained from these interactions were also confirmed through reverse docking of 2MZ7 with each of the 3 proteins. During this analysis, 2MZ7 was broken down into 5 sequences and obtained in 2D .sdf format through ChemDraw and docked against the protein sequences Neum (115 – 124) and Neum (124 – 130), Syp (235 – 240) and RhoA (130 – 137). Reverse docking results showed highest binding energy of -4.4 kcal/mol of Neum (115 – 124) with Tau (267 – 276) and Tau (277 – 286). These results further made the binding and their binding energies of the 3 proteins with this particular region of Tau more evident.

Protein-Protein network analysis for the four major proteins including Tau, Neuromodulin, Synaptophysin and RhoA was also done. The data and research for the interactive proteins were obtained through online tools such as UniProt, BioGRID and STRING. All the interactive proteins were analyzed, and maximum number of common proteins were identified in between the proteins. Identified proteins were further analyzed for different pathways they were involved in such as apoptosis, signal transduction, actin regulation which may play a role in accelerated progression of AD.

The study also included the evaluation and docking of different drug compounds with Tau protein. The drugs chosen were Methamphetamine, Ascorbic Acid and Doxorubicin. Methamphetamine is known to be a potential nervous system stimulant and affects some neurochemical mechanisms which can be responsible for numerous bodily functions such as controlling heart rate, mood, body temperature, blood pressure, responses associated with hyperactivity, alertness and alarming conditions etc. some of the effects of Methamphetamine also resemble epinephrine associated fight or flight responses which can result in hypersensitivity, increased heart rate, vasoconstriction, bronchodilation, hyperglycemia. It can also sometimes result in decreased appetite, loss of focus and fatigue etc. Inside the brain, methamphetamine causes the release of multiple neurotransmitters like dopamine and serotonin. (Metamfetamine, 2007).

Ascorbic Acid, also known as Vitamin C, is a drug which can be used in healing medication, wound healing and sometimes also used as an antioxidant. It also works as a tissue repair drug and is an important component for the formation of collagen in the body. It can be reversibly oxidized to dehydroascorbic and plays a crucial role in numerous oxidation-reduction reactions (Ascorbic Acid , 2005). According to a study, pretreatment with Ascorbic acid has shown a significant effect in the decrease of Reactive oxygen species (ROS) production and has also shown an effective decrease in tau hyperphosphorylation (Guanmin Meng, 2015).

Doxorubicin also know as DOX is a chemotherapeutic drug which disrupts the DNS repair process in tumours or cancerous cells and is used as an anti-cancer drug. It also inhibits macromolecule biosynthesis and prevents DNA replication (Salvador et al., 2021). It is used as a treatment for numerous cancers including ovarian cancer, thyroid cancer, liver cancer, gastric cancer, oral cancer, lung cancer, various types of lymphomas, myelomas, and sarcomas. Inside the body, DOX oxidizes to an unstable compound which is then converted back, and free radicals are released, these free radicals in turn cause oxidative stress which leads to peroxidation in lipids causing a disruption in the DNA repair process along with membrane damage which then ultimately leads to an apoptotic pathway and eventually cell death (Singh & Kesharwani, 2021). Studies have shown improved survival of glioma patients treated with DOX and it has the potential to be used clinically against primary and metastatic brain tumours as a therapeutic drug (Treat et al., 2007). A study has also shown that these compounds are not only effective in human neuroblastoma and neuroglioma but have also led to a decrease in tau protein levels. These observations may suggest important insights into tau turnover mechanism and may also provide an evidence towards the use of FDA approved compounds for reducing tau levels (Chad A Dickey, 2006).

The drug compounds were docked with 2MZ7 region of Tau protein using PyRx and then evaluated for 2D and 3D analyzation and bonding in Discovery Studio, PyMOL and LigPlot+. The highest binding energy was observed to be of Doxorubicin of -6.0 kcal/mol. The drugs were also docked against a Protein-Ligand complex and evaluated similarly. The binding energies for a drug-protein-ligand complex were similar to the binding energies observed without the ligand. The interaction of these drug compounds with 2MZ7 yielding high binding affinity showcase that these compounds can be used to block the binding sites on *MAPT* (2MZ7 region) to prevent the interaction of pathological proteins with *MAPT* that are involved in the progression of rpAD.

During the 2D analysis in Discovery Studio some unfavorable bumps were also encountered with Drug-Protein-Ligand complexes. The unfavorable bumps sometimes depict a thrust that might destabilize the complex and are known for their low binding affinity, but these compounds are also expected to bind with higher affinity that what is obtained by *in sillico* docking during in-vitro models of study and experimentation (Yalçin-Özkat, 2022). Unfavorable bumps are also thought to have different meanings in different schools of thoughts. It may be believed by some this interaction may not depict a perfect interaction; it is also believed by others that this interaction might be caused by maximum tortional strain. It is also thought that unfavorable interactions might also not necessarily mean that the compounds

are not good inhibitors. Numerous factors need to be taken into account during molecular docking such as protein flexibility. Compounds which are docked might also have different or unexpected modes of binding or a different binding site. Other effects such as entropy, solvents, target interaction partners etc. also need to be taken into account which might cause unfavorable interactions during molecular docking processes (Okeke, 2022).

The study includes a novel approach towards intervention of novel therapeutics targeted for tauopathies specifically rpAD. The *in sillico* analysis, although has been used for numerous purposes, it is truly a novel approach to target interactors using IP studies and analyzing them. The specific binding energies of interactors Neuromodulin, Synaptophysin and RhoA with 2MZ7 region of Tau protein is a unique narrative and has not been found in literature before.

Despite controlled experimentation, a few limitations need to be considered for the project. Despite a thorough study of tauopathies, tau pathology, AD and rpAD variants it is essential for the study to be redefined and evaluated through animal models and *in vitro* and *in vivo* studies.

Chapter 6

Conclusion

The study evaluated that the identified interactor proteins interact with *MAPT* during the pathology of disease and play a role in progression of rpAD. The associative interactions in this study were analysed using the LigPlot+ software. Hydrogen bonding as well as hydrophobic interactions have been shown by the protein ligands. In this study, interaction between the *MAPT* region belonging from the MTB domain 2MZ7 and its interactors Neuromodulin, Synaptophysin and RhoA were investigated, and the results obtained were compared with existing results for AD through *in silico* methods using molecular docking and 2D and 3D visualisation tools like LigPlot++, Discovery Studio, PyRx and PyMOL. It was observed that the binding sites of 2MZ7 are surrounded by 22 amino acids, and Neum (115 – 124) and Neum (124 – 130), Syp (235 – 240), RhoA (130 – 137) were able to produce interaction with amino acids within in the binding pouch of 2MZ7. Neuromodulin domain sequences are docked with 2MZ7, Neum (115 – 124) and Neum (125 – 130) produce the highest binding energy of -5.2 kcal/mol when docked with 2MZ7. Similarly, when Synaptophysin sequences are docked, SYP (235 – 240) yields the highest binding energy of -6.1 kcal/mol with 2MZ7. RhoA (130 – 137) shows the highest binding energy of -5.0 kcal/mol. The ligand proteins and their interactions with 2MZ7 yielding high binding affinity showcase how AD can be caused by the apoptotic regulation of these interacting proteins. The protein-protein interaction analysis revealed the interaction of proteins contributing to a pathological pathway. These common interacting proteins between *MAPT*, Neum, Syp and RhoA have been found in different molecular pathways that are involved in apoptosis, cell death, signal transduction and caspase regulation etc. Many new drug compounds have been successfully developed using computational methods. In our study, we also evaluated the binding of different drug compounds such as methamphetamine, ascorbic acid, and doxorubicin after docking experiment *in silico*. The highest binding energy was showed by Doxorubicin which was at -6.0 kcal/mol when docked with 2MZ7. The drug compounds and their interactions with 2MZ7 yielding high binding affinity showcase that these compounds can be used to block the binding sites on *MAPT* (2MZ7 region) to prevent the interaction of pathological proteins with *MAPT* that are involved in the progression of rpAD.

Appendix A

Neuromodulin (105 – 114)

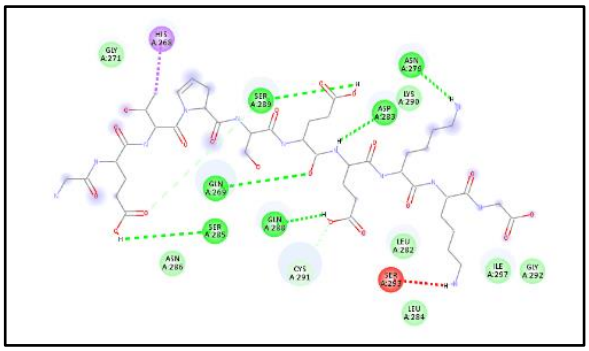


Figure 30. 2D visualization of Neuromodulin (105 – 114) in Discovery studio showing amino acids and bonds. 2D visualization of Neuromodulin (105 – 114) in Discovery Studio (Figure 30) showing the bonds between the amino acids. The binding energy of Neuromodulin (105 – 114) with 2MZ7 was -4.2 kcal/mol.

Neuromodulin (51 – 60)

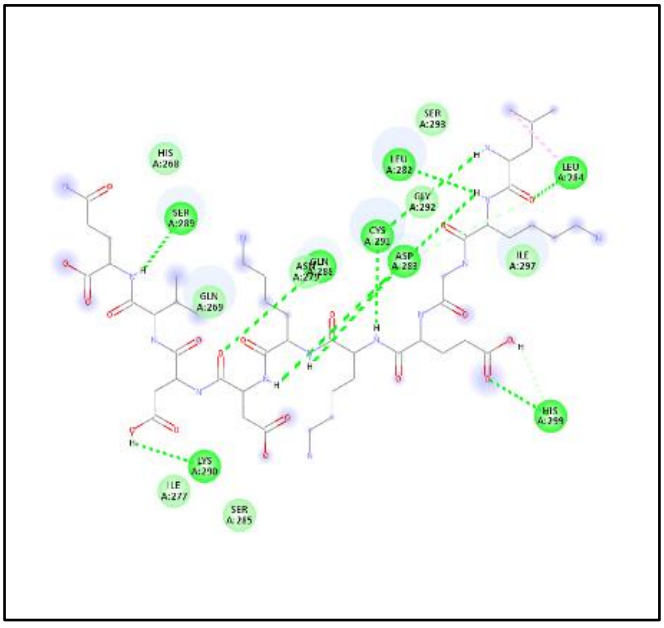


Figure 31. 2D visualization of Neuromodulin (51 – 60) in Discovery studio showing amino acids and bonds. 2D visualization of Neuromodulin (51 – 60) in Discovery Studio (Figure 31) showing the bonds between the amino acids. The binding energy of Neuromodulin (51 – 60) with 2MZ7 was -4.8 kcal/mol.

Synaptophysin (221 – 230)

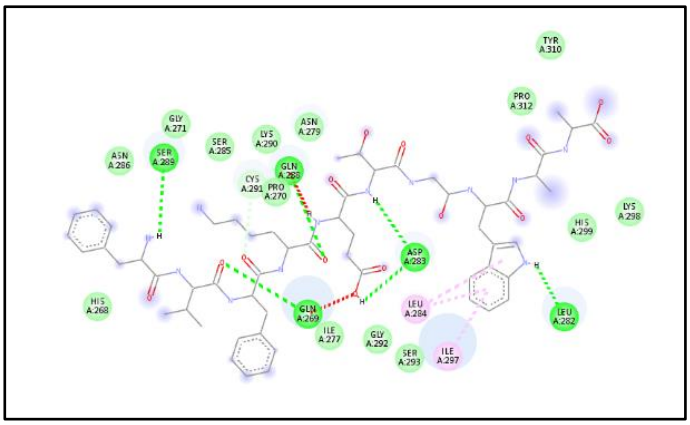


Figure 32. 2D visualization of Synaptophysin (221 – 230) in Discovery studio showing amino acids and bonds. 2D visualization of Synaptophysin (221 – 230) in Discovery Studio (Figure 32) showing the bonds between the amino acids. The binding energy of Synaptophysin (221 – 230) with 2MZ7 was -5.9 kcal/mol.

Synaptophysin (241 – 250)

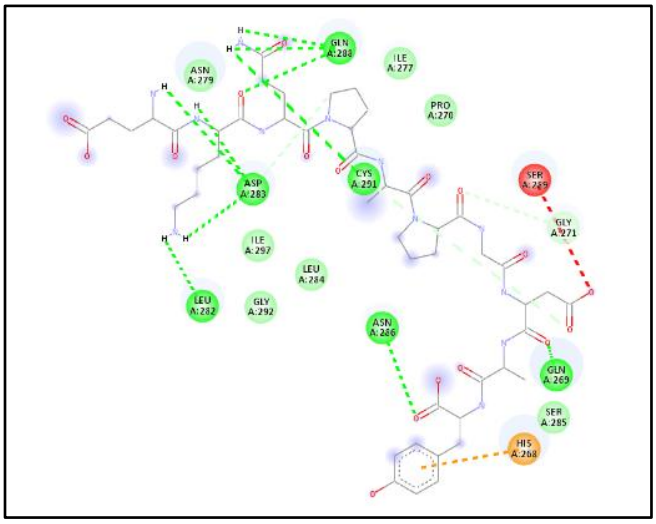


Figure 33. 2D visualization of Synaptophysin (241 – 250) in Discovery studio showing amino acids and bonds. 2D visualization of Synaptophysin (241 – 250) in Discovery Studio (Figure 33) showing the bonds between the amino acids. The binding energy of Synaptophysin (241 – 250) with 2MZ7 was -5.3 kcal/mol.

Synaptophysin (251 – 260)

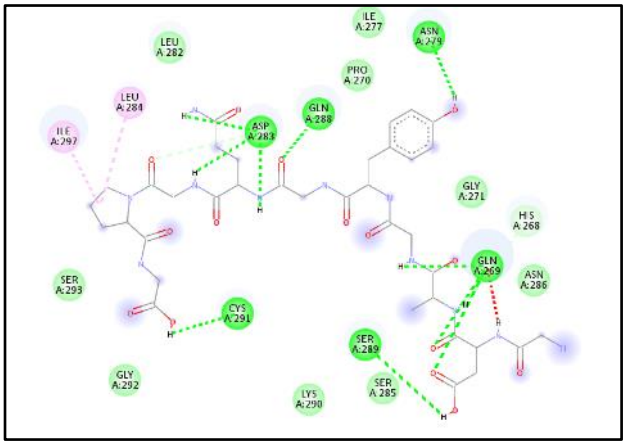


Figure 34. 2D visualization of Synaptophysin (251 – 260) in Discovery studio showing amino acids and bonds. 2D visualization of Synaptophysin (251 – 260) in Discovery Studio (Figure 34) showing the bonds between the amino acids. The binding energy of Synaptophysin (251 – 260) with 2MZ7 was -5.9 kcal/mol.

Synaptophysin (261 – 270)

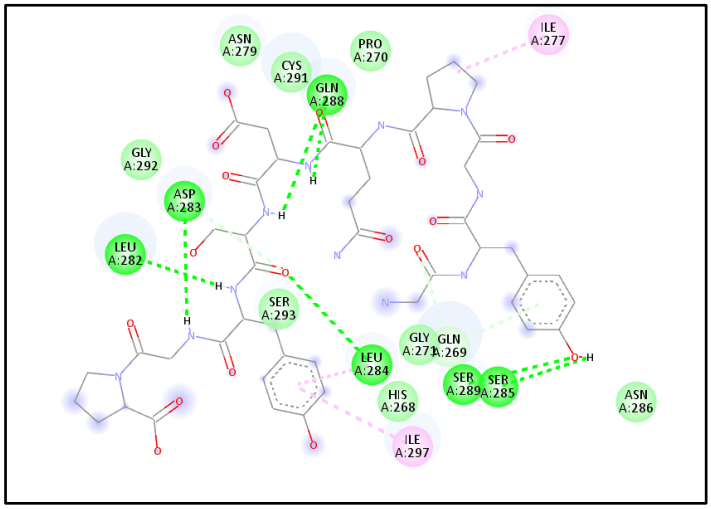


Figure 35. 2D visualization of Synaptophysin (261 – 270) in Discovery studio showing amino acids and bonds. 2D visualization of Synaptophysin (261 – 270) in Discovery Studio (Figure 35) showing the bonds between the amino acids. The binding energy of Synaptophysin (261 – 270) with 2MZ7 was -5.6 kcal/mol.

RhoA (184 – 189)

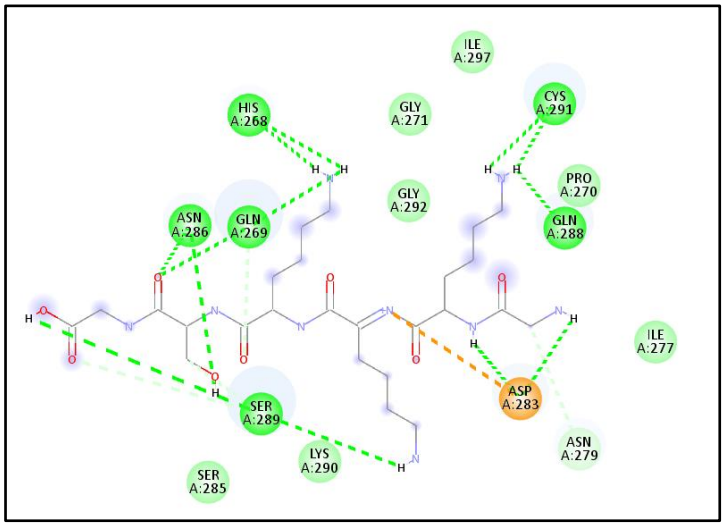


Figure 36. 2D visualization of RhoA (184 – 189) in Discovery studio showing amino acids and bonds. 2D visualization of RhoA (184 – 189) in Discovery Studio (Figure 36) showing the bonds between the amino acids. The binding energy of RhoA (184 – 189) with 2MZ7 was -4.6 kcal/mol.

Chapter 7

References

Abu-Rumeileh, S., Capellari, S., & Parchi, P. (2018). Rapidly progressive Alzheimer's disease: contributions to clinical-pathological definition and diagnosis. *Journal of Alzheimer's Disease*, *63*(3), 887-897.

Alquezar, C., Arya, S., & Kao, A. W. (2021). Tau post-translational modifications: dynamic transformers of tau function, degradation, and aggregation. *Frontiers in Neurology*, *11*, 595532.

Amadoro, G., Corsetti, V., Ciotti, M. T., Florenzano, F., Capsoni, S., Amato, G., & Calissano, P. (2011). Endogenous Aβ causes cell death via early tau hyperphosphorylation. *Neurobiology of aging*, *32*(6), 969-990.

Andreadis, A. (2005). Tau gene alternative splicing: expression patterns, regulation and modulation of function in normal brain and neurodegenerative diseases. *Biochimica Et Biophysica Acta (BBA)-Molecular Basis Of Disease*, *1739*(2-3), 91-103.

Apostolova, L. G. (2016). Alzheimer disease. *Continuum: Lifelong Learning in Neurology*, *22*(2 Dementia), 419.

Armstrong, R. A. (2019). Risk factors for Alzheimer's disease. *Folia neuropathologica*, *57*(2), 87-105.

Ascorbic Acid . (2005). Retrieved from DrugBank : https://go.drugbank.com/drugs/DB00126

Avila, J., Jiménez, J. S., Sayas, C. L., Bolós, M., Zabala, J. C., Rivas, G., & Hernández, F. (2016). Tau structures. *Frontiers in aging neuroscience*, *8*, 262.

Barbier, P., Zejneli, O., Martinho, M., Lasorsa, A., Belle, V., Smet-Nocca, C., ... & Landrieu, I. (2019). Role of tau as a microtubule-associated protein: structural and functional aspects. *Frontiers in aging neuroscience*, *11*, 204.

Breijyeh, Z., & Karaman, R. (2020). Comprehensive review on Alzheimer's disease: causes and treatment. *Molecules*, *25*(24), 5789.

Buée, L., Bussière, T., Buée-Scherrer, V., Delacourte, A., & Hof, P. R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Research Reviews*, *33*(1), 95-130.

Buée, L., Troquier, L., Burnouf, S., Belarbi, K., Van der Jeugd, A., Ahmed, T., ... & Sergeant, N. (2010). From tau phosphorylation to tau aggregation: what about neuronal death?. *Biochemical Society Transactions*, *38*(4), 967-972.

Canter, R. G., Penney, J., & Tsai, L. H. (2016). The road to restoring neural circuits for the treatment of Alzheimer's disease. *Nature*, *539*(7628), 187-196.

Cole, S. L., & Vassar, R. (2007). The Alzheimer's disease β-secretase enzyme, BACE1. *Molecular neurodegeneration*, *2*(1), 1-25.

Consortium, T. U. (2008). The Universal Protein Resource (UniProt). Nuclein Acids Research.

Consortium, w. (2019). Protein Data Bank: the single global archive for 3D macromolecular structure data. Nucleic Acids Research.

Davis, A. P., Grondin, C. J., Johnson, R. J., Sciaky, D., Wiegers, J., Wiegers, T. C., & Mattingly, C. J. (2021). Comparative toxicogenomics database (CTD): update 2021. *Nucleic acids research*, *49*(D1), D1138-D1143.

Deloulme, J. C., Janet, T., Au, D., Storm, D. R., Sensenbrenner, M., & Baudier, J. (1990). Neuromodulin (GAP43): a neuronal protein kinase C substrate is also present in 0-2A glial cell lineage. Characterization of neuromodulin in secondary cultures of oligodendrocytes and comparison with the neuronal antigen. *The Journal of cell biology*, *111*(4), 1559-1569.

Dickey, C. A., Ash, P., Klosak, N., Lee, W. C., Petrucelli, L., Hutton, M., & Eckman, C. B. (2006). Pharmacologic reductions of total tau levels; implications for the role of microtubule dynamics in regulating tau expression. *Molecular neurodegeneration*, *1*(1), 1-9.

dos Santos, P., Leide, C., Ozela, P. F., de Fatima de Brito Brito, M., Pinheiro, A. A., Padilha, E. C., ... & Izabel, L. (2018). Alzheimer's disease: a review from the pathophysiology to diagnosis, new perspectives for pharmacological treatment. *Current medicinal chemistry*, *25*(26), 3141-3159.

Dugger, B. N., & Dickson, D. W. (2017). Pathology of neurodegenerative diseases. *Cold Spring Harbor perspectives in biology*, *9*(7), a028035.

Erkkinen, M. G., Kim, M. O., & Geschwind, M. D. (2018). Clinical neurology and epidemiology of the major neurodegenerative diseases. *Cold Spring Harbor perspectives in biology*, *10*(4), a033118.

Fielden, M. R., Matthews, J. B., Fertuck, K. C., Halgren, R. G., & Zacharewski, T. R. (2002). In silico approaches to mechanistic and predictive toxicology: an introduction to bioinformatics for toxicologists. *Critical reviews in toxicology*, *32*(2), 67-112.

Ganguly, J., & Jog, M. (2020). Tauopathy and movement disorders—unveiling the chameleons and mimics. *Frontiers in Neurology*, *11*, 599384.

Gitler, A. D., Dhillon, P., & Shorter, J. (2017). Neurodegenerative disease: models, mechanisms, and a new hope. *Disease models & mechanisms*, *10*(5), 499-502.

Goedert, M. (2009). Oskar Fischer and the study of dementia. *Brain*, *132*(4), 1102-1111.

Gong, C. X., & Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Current medicinal chemistry*, *15*(23), 2321-2328.

Graphical User Interface for the LIGPLOT and DIMPLOT programs. (n.d.). Retrieved from LigPlot+ v.1.4: https://www.ebi.ac.uk/thornton-srv/software/LigPlus/manual/manual.html#:~:text=LigPlot%2B%20is%20a%20graphical%20front,conversion%20to%20various%20image%20formats.

Guo, T., Noble, W., & Hanger, D. P. (2017). Roles of tau protein in health and disease. *Acta neuropathologica*, *133*(5), 665-704.

Islam, M., Shen, F., Regmi, D., & Du, D. (2022). Therapeutic strategies for tauopathies and drug repurposing as a potential approach. *Biochemical Pharmacology*, 114979.

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., ... & Bryant, S. H. (2016). PubChem substance and compound databases. *Nucleic acids research*, *44*(D1), D1202-D1213.

Kovacs G. G. (2017). Tauopathies. *Handbook of clinical neurology*, *145*, 355–368. https://doi.org/10.1016/B978-0-12-802395-2.00025-0

Kwon, S. E., & Chapman, E. R. (2011). Synaptophysin regulates the kinetics of synaptic vesicle endocytosis in central neurons. *Neuron*, *70*(5), 847-854.

Louneva, N., Cohen, J. W., Han, L. Y., Talbot, K., Wilson, R. S., Bennett, D. A., ... & Arnold, S. E. (2008). Caspase-3 is enriched in postsynaptic densities and increased in Alzheimer's disease. *The American journal of pathology*, *173*(5), 1488-1495.

Mayeux, R., & Stern, Y. (2012). Epidemiology of Alzheimer disease. *Cold Spring Harbor perspectives in medicine*, *2*(8), a006239.

McGill-Carter, T. (2020). Market analysis Alzheimer's disease 2020. *J Psychiatry*, *22*, 21-22.

Meng, G., Liu, J., Lin, S., Guo, Z., & Xu, L. (2015). Microcystin-LR-Caused ROS generation involved in p38 activation and tau hyperphosphorylation in neuroendocrine (PC12) cells. *Environmental toxicology*, *30*(3), 366-374.

Metamfetamine. (2007). Retrieved from DrugBank : https://go.drugbank.com/drugs/DB01577

Muralidar, S., Ambi, S. V., Sekaran, S., Thirumalai, D., & Palaniappan, B. (2020). Role of tau protein in Alzheimer's disease: The prime pathological player. *International journal of biological macromolecules*, *163*, 1599-1617.

Murray, D., Doran, P., MacMathuna, P., & Moss, A. C. (2007). In silico gene expression analysis–an overview. *Molecular cancer*, *6*(1), 1-10.

Okeke, I., & Okeke, C. (2022). Molecular Docking and Analysis of In Silico Generated Ligands Against SARS-CoV-2 Spike and Replicase Proteins.

Orr, M. E., Sullivan, A. C., & Frost, B. (2017). A brief overview of tauopathy: causes, consequences, and therapeutic strategies. *Trends in pharmacological sciences*, *38*(7), 637-648.

Pillai, J. A., Appleby, B. S., Safar, J., & Leverenz, J. B. (2018). Rapidly progressive Alzheimer's disease in two distinct autopsy cohorts. *Journal of Alzheimer's Disease*, *64*(3), 973-980.

Povova, J., Ambroz, P., Bar, M., Pavukova, V., Sery, O., Tomaskova, H., & Janout, V. (2012). Epidemiological of and risk factors for Alzheimer's disease: a review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, *156*(2), 108-14.

Przedborski, S., Vila, M., & Jackson-Lewis, V. (2003). Series Introduction: Neurodegeneration: What is it and where are we?. *The Journal of clinical investigation*, *111*(1), 3-10.

Python Prescription. (2022). Retrieved from Pyrx Source Forge : https://pyrx.sourceforge.io/component/content/article/35-introduction/53-index

Qiu, C., Kivipelto, M., & Von Strauss, E. (2022). Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues in clinical neuroscience*.

Remnestål, J., Just, D., Mitsios, N., Fredolini, C., Mulder, J., Schwenk, J. M., ... & Häggmark-Månberg, A. (2016). CSF profiling of the human brain enriched proteome reveals associations of neuromodulin and neurogranin to Alzheimer's disease. *PROTEOMICS–Clinical Applications*, *10*(12), 1242-1253.

Salvador, D., Bastos, V., & Oliveira, H. (2021). Hyperthermia Enhances Doxorubicin Therapeutic Efficacy against A375 and MNT-1 Melanoma Cells. *International Journal of Molecular Sciences*, *23*(1), 35.

Schmidt, C., Redyk, K., Meissner, B., Krack, L., Von Ahsen, N., Roeber, S., ... & Zerr, I. (2010). Clinical features of rapidly progressive Alzheimer's disease. *Dementia and geriatric cognitive disorders*, *29*(4), 371-378.

Schmidt, S. I., Blaabjerg, M., Freude, K., & Meyer, M. (2022). RhoA Signaling in Neurodegenerative Diseases. *Cells*, *11*(9), 1520.

Selkoe, D. J. (1997). Alzheimer's Disease--Genotypes, Phenotype, and Treatments. *Science*, *275*(5300), 630-631.

Singh, V., & Kesharwani, P. (2021). Dendrimer as a promising nanocarrier for the delivery of doxorubicin as an anticancer therapeutics. *Journal of Biomaterials Science, Polymer Edition*, *32*(14), 1882-1909.

STRING Database — Content. (2022). Retrieved from STRING : https://string-db.org/cgi/about#:~:text=STRING%20is%20a%20database%20of,from%20other%20(primary)%20databases.

Sze, C. I., Troncoso, J. C., Kawas, C., Mouton, P., Price, D. L., & Martin, L. J. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *Journal of Neuropathology & Experimental Neurology*, *56*(8), 933-944.

Tiwari, S., Atluri, V., Kaushik, A., Yndart, A., & Nair, M. (2019). Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. *International journal of nanomedicine*, *14*, 5541–5554. https://doi.org/10.2147/IJN.S200490

Treat, L. H., McDannold, N., Vykhodtseva, N., Zhang, Y., Tam, K., & Hynynen, K. (2007). Targeted delivery of doxorubicin to the rat brain at therapeutic levels using MRI-guided focused ultrasound. *International journal of cancer*, *121*(4), 901-907.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, *31*(2), 455-461.

Wolfe, M. S. (2012). The role of tau in neurodegenerative diseases and its potential as a therapeutic target. *Scientifica*, *2012*.

Wu, L., Wang, Z., Lad, S., Gilyazova, N., Dougharty, D. T., Marcus, M., ... & Xu, B. (2022). Selective detection of misfolded tau from postmortem Alzheimer's disease brains. *Frontiers in Aging Neuroscience*, *14*.

Yalçin-Özkat, G. (2022). Computational studies with flavonoids and terpenoids as BRPF1 inhibitors: in silico biological activity prediction, molecular docking, molecular dynamics simulations, MM/PBSA calculations. *SAR and QSAR in Environmental Research*, *33*(7), 533-550.

Yuan, S., Chan, H. S., & Hu, Z. (2017). Using PyMOL as a platform for computational drug design. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, *7*(2), e1298.

Zhang, Y., Wu, K. M., Yang, L., Dong, Q., & Yu, J. T. (2022). Tauopathies: new perspectives and challenges. *Molecular Neurodegeneration*, *17*(1), 1-29.