COVID-19 (6LU7) predictive binding association with Aβ oligomers and possible link to Alzheimer's disease

Areej Sohail Khan
00000277267

Supervisor
Dr. Saima Zafar

DEPARTMENT OF BIOMEDICAL SCIENCES
SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING
NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY
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Author
Areej Sohail Khan
00000277267

A thesis submitted in partial fulfillment of the requirements for the degree of MS Biomedical Sciences

Thesis Supervisor:
Dr. Saima Zafar

Thesis Supervisor's Signature:
_____

DEPARTMENT OF BIOMEDICAL SCIENCES
SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING
NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY, ISLAMABAD
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Examination Committee Members

1. Name: **Dr. Nosheen Fatima** Signature:_____

2. Name: **Dr. Omer Gillani** Signature:_____

3. Name: **Dr. Asim Waris** Signature:_____

Supervisor's name: **Dr. Saima Zafar** Signature:_____
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Abstract

The high rise pandemic of Coronavirus Disease 2019 (COVID-19) makes the world face medical challenges associated with multifaceted nature of its pathology. SARS-CoV-2 affects several organs and systems as it enters the host's body one of which is the brain. Over 80 million humans around the globe, including those with neurodegenerative disease (NDD), have been diagnosed with coronavirus disease 2019 (COVID-19) to date. COVID-19 affects the brain in many ways including direct infection of neural cells with SARS-CoV-2, severe systemic inflammation that floods the brain with pro-inflammatory agents leading to damaging cells and leading to symptoms presenting cognitive impairment. COVID-19 positive patients showcase neurological symptoms leading to the belief that coronavirus disease plays a role in neurodegenerative diseases. The most common NDD, Alzheimer's disease (AD) is characterized by its multifactorial nature leading to research on risk factors that emphasizes on the inflammation of toxicity and mutual death of cells due to amyloid beta and its conformers, namely monomeric and oligomeric forms. Amyloid beta oligomers initiate toxicity and neural death of cells in AD. The main aim of this study is to decipher the interactive association between toxic forms of amyloid beta oligomer against COVID-19 main protease. We used PDB and Pubchem for library retrieval that was loaded in to discovery studio to extract the active binding site of main protease of SARS-CoV-2 and prepare ligands for docking. Furthermore, we utilized PyRx for docking to investigating binding energies of conformations attained, the best affinity ligands were formed into a complex by the use of Pymol that were than visualized using Discovery studio where 2D interactions were also observed that later were further analyzed using Ligplot+ to get an insight on bond length and strength along with bond types. Aβ oligomer 31-35 binds actively to the active site of M-pro of SARS-CoV-2 at a high affinity rate of -6.3kcal/mol. 6LU7 complex with amyloid 31-35 (Complex 1) when docked

with the receptor of apoptotic pathway showed enhanced predictive association. Bioinformatics tools in this research substantiated the important interactive partners amongst amyloid oligomers to COVID-19 highlighting that SARS-Cov-2 may play a role in apoptotic demise of cells ultimately leading to neurodegeneration.

Keywords: Alzheimer's Disease; Aβ oligomers; COVID-19; Dementia; Docking; Neurodegenerative Disorder ; PyRx; PyMol; LigPlot+

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Chapter 1

Introduction

Introduction

The pandemic of recent times as a result of a SARS-CoV-2 or coronavirus-2 infection which is a severe acute respiratory syndrome started spreading in 2019 has infected more than 570 million people leading to the hefty death toll of around 6.8 million people across the globe at present (World health organization., 2022). SARS-CoV-2 is of the beta-coronavirus genus which triggers the COVID-19 disease. COVID-19 simply coined is a respiratory illness leading mostly to dry cough, fever, and fatigue whereas in worst cases an acute respiratory distress syndrome. Most of the people who are infected with COVID-19 are asymptomatic or paucisyntomatic and recover. While around 4% of the people infected with COVID-19 develop severe symptoms which result in inflammatory response and low blood oxygen levels, which in worst case scenarios can lead to multi-organ failure resulting in death (Chiricosta, *et al.*, 2021). People infected by coronavirus-2 about 35% of them even after testing negative to the virus exhibit symptoms including headaches, fatigue, anosmia as well as joint pain after two or more weeks which is associated to what we now refer to as "long COVID-19" as summarized by the Yu *et al* (Figure 1). The symptoms of COVID-19 vary, and can be associated to lungs, digestive tract, heart, brain and other tissues as well (Lopez-Leon., *et al*, 2021). Most of the patients who died of COVID-19 were older people and had one or more pre-existing conditions raising questions as to why the particular age group. When it comes to neurological patients, individuals that suffer from dementia are reported to be above 55 million and have greater chances to be infected by coronavirus-2 (Politti *et al*., 2021).

A proven ordeal is that 60-80% of dementia cases that are present can easily be attributed to Alzheimer's disease (AD) yet there have been no published reports that cement the link of the development of severe COVID-19 with AD or discuss the depth of their relation. B-amyloid (Aβ) is a major component of the pathology of senile plaques found in brains effected by AD and is considered one of the two prime pathological markers of the disease second being Tau (Mattson *et al* ., 2004), it has also been the most used pharmaceutical target by organizations for immunotherapy treatment (Plotkin, & Cashman., 2020). Most mutations around the Aβ sequences end up as early onset AD or familial AD as a result of increase in accumulation or the expression of Aβ. The mutations in Aβ also impact the residue by changing physicochemical properties that are far from the site where the mutation occurred and are likely to be self-aggregated (Lin *et al*., 2012). Some studies reported anti-microbial Aβ activity, the infection caused by the virus herpes simplex was proposed to be involved in the plaque formation for Aβ (Saad *et al*., 2014). The links that have been reported between COVID-19 and Alzheimer's disease (AD), are mechanistic in nature and are focused so far by the recent research on either microvascular injury or neuro-inflammation (Zhou *et al.,* 2021) the neurodegeneration associated with AD is linked to the accumulation of amyloid plaques (van der Kant & Goldstein, 2015). The conditions that have been reported to increase the COVID-19 vulnerability including diabetes mellitus and obesity also increase the levels of circulating Aβ and have been proven to impact the AD progression (Kipshitzed, 2020; Meinhardt, 2021).

The various forms that Aβ exists in soluble oligomer, insoluble fibers and monomers. The Aβ monomer and the Aβ insoluble fiber doesn't involve synaptic plasticity. While the Aβ oligomer and Aβ dimer are very important players on the impairment structure as well as synaptic function in AD brain (Kong *et al.,* 2019).

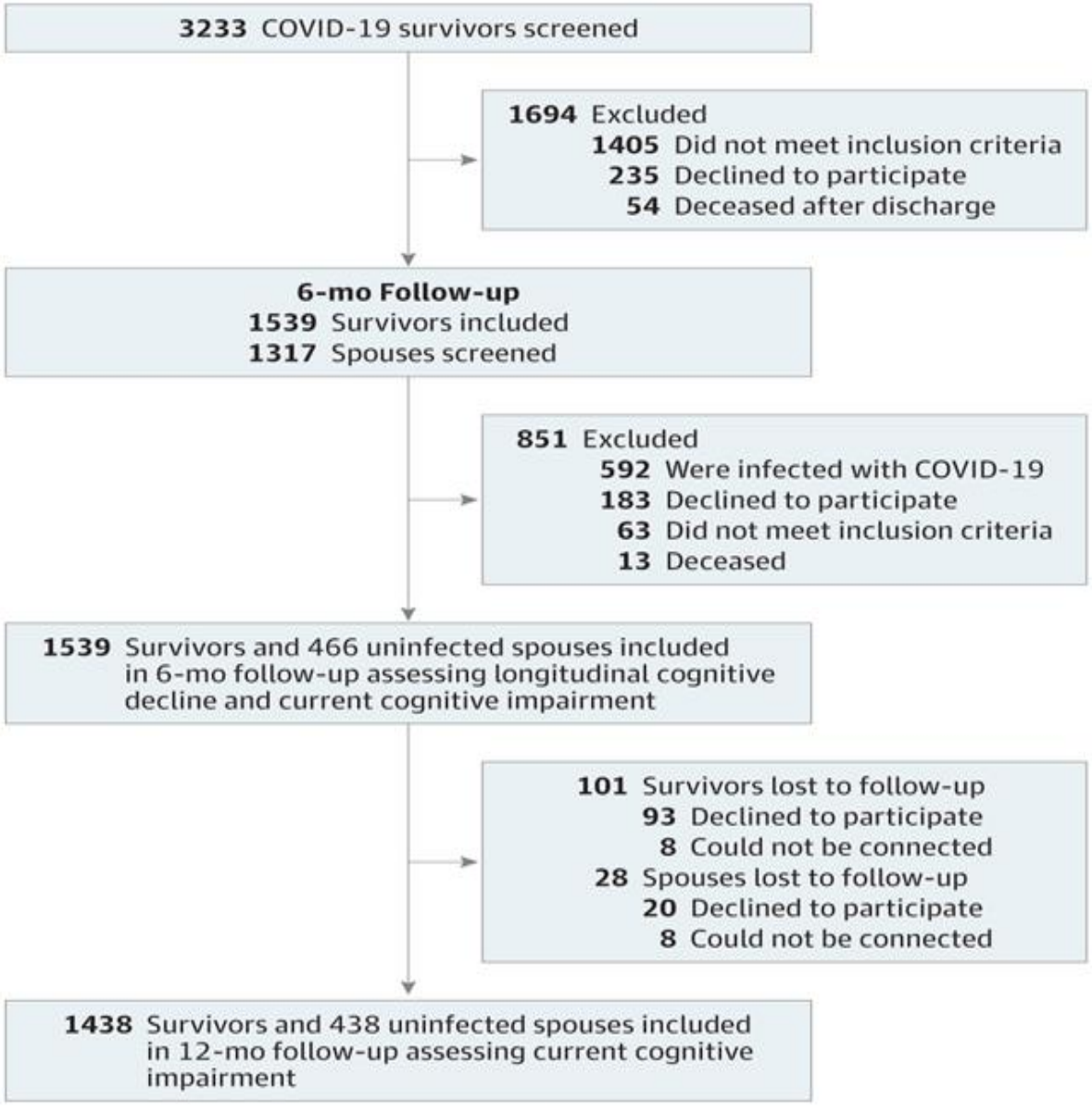


Figure 1: Screening Flowchart of Longitudinal Cognitive (Yu et al,. 2022).

On top of all of this the individuals suffering from AD rarely exhibit common symptoms like cough or fever, rather show symptoms including diarrhea, drowsiness and delirium which is triggered as a result of hypoxia. A number of studies focused on AD patients that are infected by the coronavirus-2 but it is still unclear how the clinical course is modified by the virus (Ferini-Stramb *et al*., 2021). It is highly likely that the coronavirus-2 infection triggers neurological conditions including AD. Some of the biochemical process are shared by both AD and COVID-19, both alter

the homoeostasis of the blood-brain barrier, trigger neuro-inflammation and hypoxia. Angiotensin-converting enzyme 2 (ACE2) receptor is used by coronavirus 2 as an entry point in the human cell while in AD patients it is correlated with oxidative stress levels (Rahman *et al*., 2021). Anosmia is one of the major symptoms of COVID-19, one of the possible pathways towards the brain for SARS-CoV-2 could be the olfactory epithelium as it is close to the frontal cortex (Chiricosta *et al*., 2021). In this context the frontal cortex is responsible for memory, reasoning as well as learning behavior and is damaged in advanced AD (Politti *et al*., 2021). Due to this reason, the frontal cortex in the brain should be the highest link between coronavirus-2 and AD patients. However, very small amounts of data points are focused on older patients with most of it focused on patients without dementia. People that suffer from dementia showed an increased mortality risk by 40% when compared to patients without dementia which does not give a complete explanation (Bianchetti *et al*., 2020). It is however still unclear if the dementia patient's pathology actually has any direct effects or plays any role in the development of severe COVID-19.

Impairment in cognitive development has been linked to COVID-19 on several occasions as stated above, especially in hospitalized patients (Al-Aly, Xie, & Bowe, 2021; Beaud *et al*., 2021; Becker *et al*., 2021; Meppiel *et al*., 2021; Miners, Kehoe, & Love, 2020; Romero-Sánchez *et al*., 2020; Taquet, Geddes, Husain, Luciano, & Harrison, 2021; Zhou *et al*., 2020). That being said it is still unclear weither the neuropathology is a result of direct viral infection of CNS or as an indirect result of the immune response as well as the critical infection and hypercoagulability (Iadecola, Anrather, & Kamel, 2020; Varatharaj *et al*., 2020). The immune cells and vascular cells can be infected by SARS-CoV-2 as it can cross the barrier between blood-brain and cause damage to the CNS is a question in theory but still has to be answered by evidence (Cantuti-Castelvetri *et al*., 2020; Jacob *et*

al., 2020; Meinhardt *et al*., 2021; Neman & Chen, 2015; Solomon, 2021; Song *et al*., 2021; Yang *et al*., 2021). All the results and theories point to one direction and are in utter need of a cementing conclusion hence forth rising the question in picture that is what is the link between AD and COVID-19. Therefore in this research we try to tackle the relationship between Coronavirus and Alzheimer by using *Insilco* means and establishing a relation to one of the most popular hallmark of Alzheimer the Amyloid beta.

Objectives of the Study

1- Identification of Interactive association of amyloid-β oligomer (AβO) with COVID-19 M-pro.

2- Characterization of SARS-CoV-2 as a risk modulator for Alzheimer's disease.

Chapter 2

Literature Review

Literature Review

COVID 19 Pandemic

The cases of COVID-19 have been on rise since March 2020 across the world. The situation of viral spread turned into a pandemic is unprecedented and has aggravated psychological problems along with paving ways for new stressors be it economic (Chakraborty & Chatterjee, 2020; Cluver *et al*., 2020), academic (Cao *et al*., 2020; Anderson, 2020; Kecojevic *et al*., 2020), social (Balkhi *et al*., 2020; Dolan, 2020) or personal (Dubey *et al*., 2020; Shigemura, 2020).

SARS-CoV-2 infection is linked with an increased risk of impairments in cognitive functioning (Hampshire *et al*., 2021; Miskowiak *et al*., 2021). Severe COVID-19 is linked with having risk of early onset, late onset and gradual decline in cognitive functioning whereas non severe COVID-19 is linked with early onset, decline with age and co morbidities (Chang *et al*., 2014; Zlokovic *et al*., 2020) and cognitive decline. Also, it has been noticed that 21% individuals suffering from COVID-19 suffered from severe cognitive functioning decline. The findings of these studies highlight that COVID-19 may serve as an increasing cause of dementia in future.

Different mechanisms are involved in deterioration of cognitive functioning caused by COVID-19. The neurovascular elements are involved in development of post infection decline among the people who survived COVID-19 (Qin *et al*., 2021; Kas *et al*., 2021). Also, long lasting hypoxia contribute towards the decline in cognitive functioning (Solomon *et al*., 2021; Huang *et al*., 2021). Hypoxia releases the hypoxia-inducible factor (HIF)-1α, which causes an increase in the production

of β-amyloid. Hypoxia, on the other hand, also lower the rate at which the proteins break down which increases the level of proteins (Rhodes *et al*., 2022). Additionally, inflammatory factors do not settle down to their normal status even when months are passed after the recovery and it is more common individuals suffering from severe COVID-19 (Zhou *et al*., 2021).

Neurodegeneration is also caused as a result of this disease which contributes towards the cognitive functioning (Merkler *et al*., 2020). This is supported by the fact that individuals who are the survivors of COVID-19 have an increased number of neurodegenerative biomarkers (Prudencio *et al*., 2021; Virhammar *et al*., 2021; Song *et al*., 2021; Liu *et al*., 2022).

Bioinformatics: The main drive force for COVID-19

Bioinformatics application to research and the history of drugs has never been so important. COVID-19 urged the use of various bioinformatics tools in studying the molecular structure of the pathogen. A huge rise in the usage of these applications was observed which helped in developing affective vaccines, drug repurposing and discovery (Chukwudozie *et al*., 2021). For the validation of the experiments and further strengthening, different bioinformatics platforms and computational tools played their part (Chatterjee *et al*., 2021).

This virus belongs to the family Coronaviridae. It causes COVID-19, an infectious disease. Its biology has been under study since a long time but the bioinformatic tools designed especially to study this and for the treatment of COVID-19 have been developed recently (Hufsky *et al*., 2021). For the development of an effective and precise vaccine silico methos of bioinformatics, vaccino-genomics, immune-informatics, structural biology and molecular stimulations tend to play a major part (Ishack & Lipner, 2021).

The main COVID-19 protein under study is 6LU7. The protease of which consists of two chains A and C, chain A belongs to protease and chain C belongs to N3 inhibitor N-[(5methylisoxazol-3yl) carbonyl] alanyl-L-valyl-N-1- (1R,2Z)-4-(benzyloxy)-4-oxo-1–1- [(3R)-2-oxopyprolidin-3-3yl] methyl but-2-enyl)-L-lucinamide (Vijayakumar, 2022). (Figure 2)

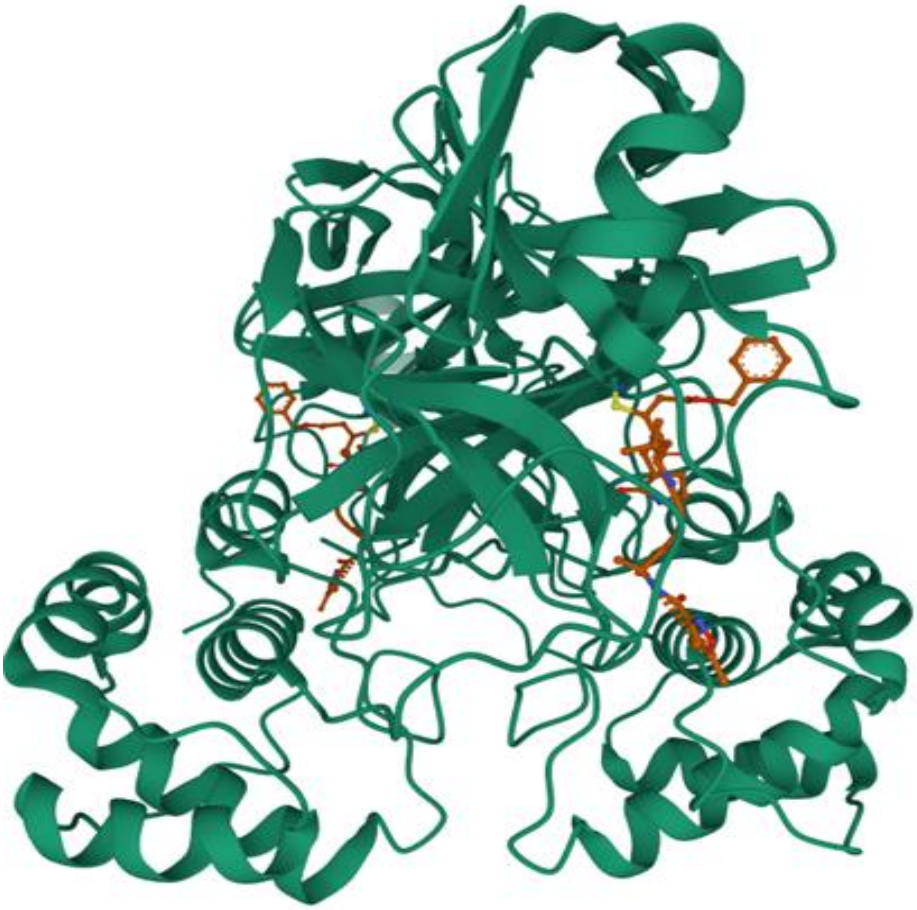


Figure 2: 3D Structure of 6LU7 Protein (PDB2021).

COVID 19 Pandemic and Brain

COVID-19: In the light of Inflammation

In COVID-19 brain autopsies, chronic neurological diseases and acute abnormalities are observed along with acute hypoxic injury, haemorrhage and minimal inflammation (Mukerji & Solomon, 2021). The blood of COVID-19 patients included high levels of inflammatory markers. Such people have an increased chance of having hyperinflammation and cytokine storm. It also indirectly induces stroke, toxic-metabolic encephalopathy, acute inflammatory demyelinating polyneuropathy/Guillain Barre syndrome, and other neuropsychiatric manifestations, such as psychosis, insomnia, and mood changes. (Wang *et al*., 2021). Post COVID-19 neuropsychiatric symptoms were found in 20 to 70 percent of patients in Germany and United Kingdom. SARS-CoV-2 can cause a damage on endothelia cells which causes brain damage and inflammation. Systematic inflammation leads an individual towards decreased monoamines and trophic factors and activation of microglia which causes increased glutamate and *N*-methyl-D-aspartate (NMDA)[3] and excitotoxicity. The brain damage caused by COVID-19 is similar to the damage to brain caused by brain injury. The neuropathology of COVID-19 plays an important part in understanding the neurodegenerative processes which are linked to neuroinflammation in diseases of the brain (Boldrini *et al*., 2021).

Effects of Dementia in COVID-19 patients

COVID-19 patients have displayed neurological dysfunctions ranging from mild ones including dizziness, headaches to severe ones involving encephalitis and ischemic stroke. Two basic hypotheses revolving around the brain inflammation and cognitive dysfunction caused by SARS-CoV-2 include that the cholinergic

neurotransmission plays an important part in different cognitive functions. This has been linked to neurodegenerative disorders including Alzheimer's disease (AD) (Fontana *et al*., 2020). Aged people suffering from COVID-19 are at higher risk of death and complications (Dix & Roy, 2022).

Patients suffering from COVID-19 also, have a loss of grey matter in the left para hippocampal gyrus, the left orbitofrontal cortex and left insula. Changes in the anterior cingulated cortex, supramarginal gyrus and temporal pole has also been observed. These changes are similar to those found in patients suffering from Alzheimer's disease and dementia (Douaud *et al*., 2021). Mortality rate of individuals having dementia and COVID-19 is much higher than individuals who do not suffer from dementia. Literature also highlighted the fact the dementia comorbid have a high risk of mortality from COVID-19 (Liu *et al*., 2020).

Monomeric and fibrillar Aβ

Monomeric types of Aβ have every now and again been proposed as harmful modulators in the improvement of AD. For instance, Taylor and partners (Taylor *et al*., 2003) detailed that most extreme cell harm saw in SH-EP1 cells and hippocampal neurons utilizing a SYTOX Green assay matches with the gathering of a monomeric Aβ animal varieties ready to multimerize into higher-n Aβ species, likewise called 'enacted monomer'. Similarly, mature Aβ fibrils have been proposed as intense neurotoxic AD-inducers, in spite of the fact that comparable conflicting discoveries concerning monomeric Aβ exist. The speculation that not all fibril morphologies are similarly poisonous, prompting variable outcomes concerning cytotoxicity, was effectively tested by Yoshiike and partners (Yoshiike *et al*., 2012) that's what they revealed, utilizing point changes and substance adjustment, both a β-sheet fibrillar structure as well as the surface physicochemical piece of the fibril characterize the harmful intensity of Aβ. One year earlier, Puzzo and Arancio (Puzzo and Aranico.,

2011) had shown that artificially inferred fibrillar Aβ can impede the late period of long haul potentiation. As it is truly challenging to guarantee the immaculateness of a monomeric or fibrillar Aβ arrangement, without pollution of either preseeds or protofibrillar material, it can't be barred that the poisonousness noticed for monomeric and fibrillar Aβ is really the consequence of defilement. Additionally, expanding proof proposes that the harmfulness of Aβ starts rather from oligomeric Aβ.

The Aβos hypothesis

The “amyloid cascade hypothesis”, proposed in 1992, recommends that Aβ testimony is the most vital phase in the obsessive improvement of AD, which then, at that point, prompts tau pathologies, synaptic brokenness, neuron misfortune and dementia (Hardy *et al*., 1992). In this way, because of the huge harmfulness of Aβos, and the cozy connection among oligomers and discernment and memory, the speculation was overhauled to "Aβos cascade theory" (Hardy *et al*., 2002), which recommends that dissolvable Aβos straightforwardly cause brain signal brokenness, actuate neuronal apoptosis, and repress synaptic long haul potentiation (LTP). Numerous investigations have shown that Aβos apply neurotoxic impacts through various instruments, for example, receptor restricting, mitochondrial brokenness, Ca2+ homeostasis dysregulation and tau pathologies (Figure 3).

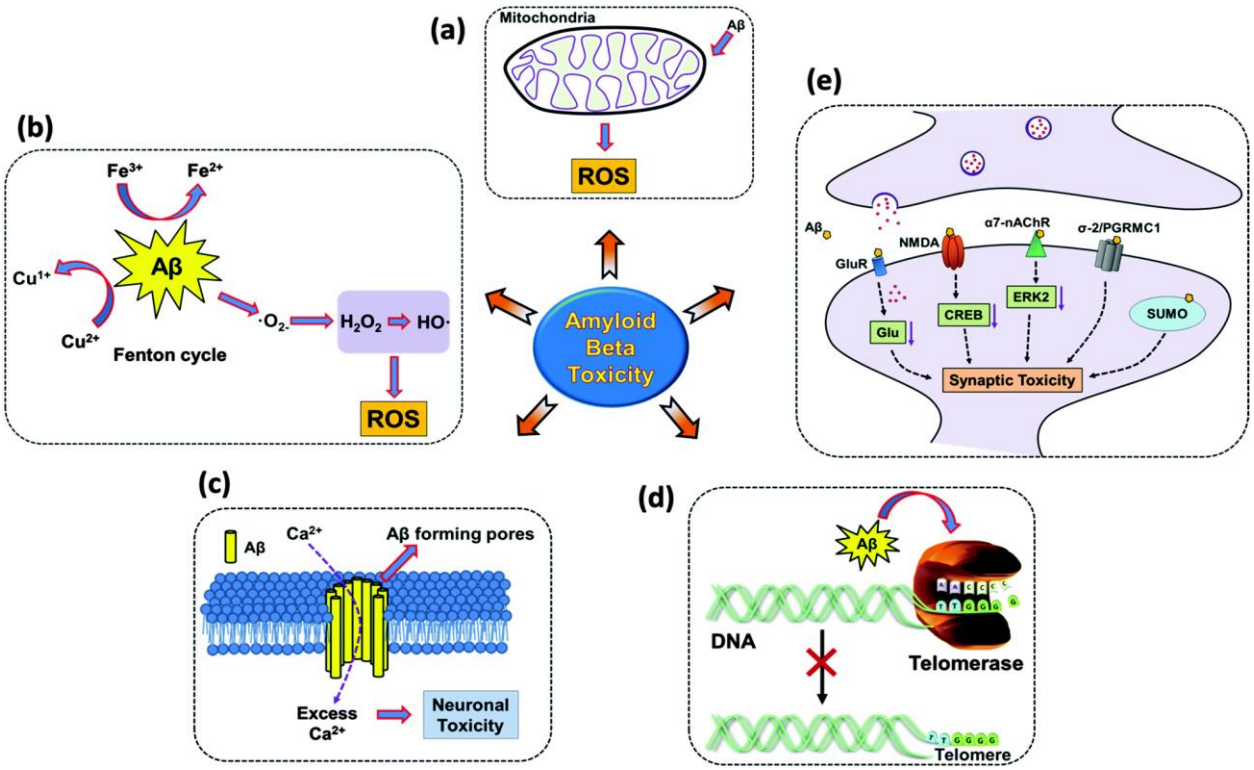


Figure 3: Amyloid beta toxicity (a) Ab cause mitochondrial dysfunction leading to ROS generation. (b) Oxidative stress caused by Ab oligomers. (c) Cell membrane disruption by Ab aggregates. (d) Telomerase inhibition. (e) Ab interfere with signalling pathways causing synaptic toxicity (Rajeshkar et al 2016)

Receptor-Mediated Neurotoxicity of Aβos

Aβos can bind to a variety of receptors on the surface of neurons, altering downstream signaling pathways, and sourcing cell death ultimately. There are a wide range of receptors (Figure 4), over 20 types that are recognized by Aβos such as cellular prion protein (PrPc), glutamate receptors, β2-adrenergic receptor (β2-AR), p75 neurotrophin receptor (p75NTR) and cyclin dependant kinase 5 (CDK5) and death receptor (Dr4) (Morazko *et al*., 2018).

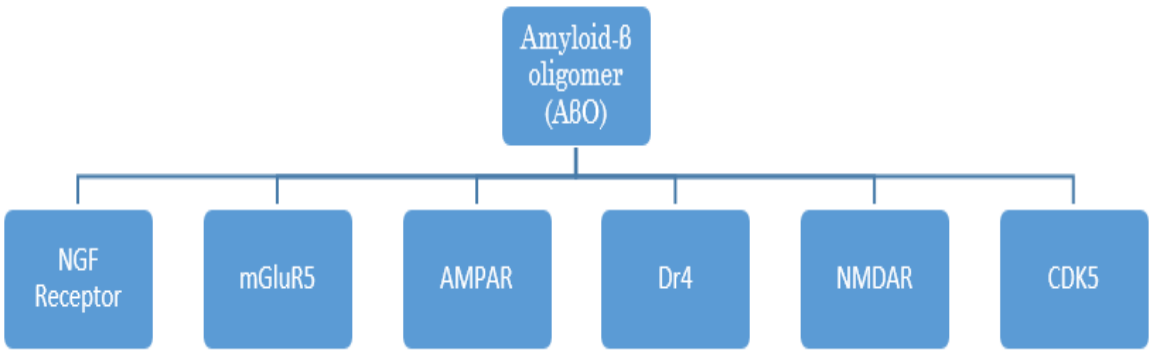


Figure 4: Amyloid Beta Oligomer Receptors (Rodriguiz et al; 2021, Martin et al; 2020, Fernando et al; 2016, Raina et al; 2012)

Aβos can hinder the action of glutamate receptors to disrupt synaptic pliancy. NMDAR is a glutamate receptor, which has particle channel action, and assumes a basic part in directing neurotransmitter development and synaptic versatility (Um *et al*., 2012). A few investigations have shown that Aβos upset NMDAR movement and hindered NMDAR-subordinate flagging pathway, prompting synaptic misfortune and the lessening of spinal thickness. What's more, Aβos upset the NMDAR-intervened postsynaptic Ca2+ announcing influencing the capability of NMDAR and modifying the accessibility of extracellular glutamate, which causes LTP debilitation (Liang *et al*., 2017) α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic corrosive receptor (AMPAR) is another glutamate receptor containing four subunits GluA1-A4, which takes part in tweak of synaptic versatility and homeostasis. Aβos prompt the decrease of AMPAR level by speeding up the debasement, and increment AMPAR

ubiquitination, bringing about the deficiency of AMPAR action and concealment of synaptic transmission (Diering *et al*., 2018). Besides, Aβos cause tau hyperphosphorylation and unusual gathering in dendritic spines, which thus shortfalls the Aβos-interceded AMPAR flagging pathway, framing an endless loop (Miller *et al*., 2014)

The Polymorphism of Aβos

Aβos are vastly heterogeneous and show varieties in size, adaptation, conglomeration mode, and harmfulness and appearance time in the cerebrum. To precisely investigate the attributes of various Aβos, the initial step is to isolate oligomers with various sizes or compliances. As of now, a few methodologies like centrifugation and size rejection chromatography (SEC) can isolate oligomers as indicated by sub-atomic size yet can't recognize conformities. Numerous Aβos-explicit antibodies like A11, OC, as well as W20, NAbs-Aβo, ready in our research facility, have been affirmed to perceive the high level conformity of Aβos (Liu *et al*., 2014; Zhou *et al*., 2014) which can be utilized to separate Aβos with various conformations.

Aβ Dimers

Aβ dimers are the smallest of Aβ aggregates, and they have three main characteristics namely being high levels in the brains of AD patients and transgenic mice, stability in SDS and strong denaturants (McDonald *et al*., 2015) and noteworthy neurotoxicity (Brinkmalm *et al*., 2019). It is projected that Aβ dimers are modelled by cross-link via tyrosine acid residue phenol coupling (DiY) and enzymatic coupling (QK) among glutamine and tyrosine buildups, bringing about the obstruction of dimers to the solid denaturants (O'Malley *et al*., 2018). The most recent review showed that Aβ dimers got from AD human cerebrums were made out of numerous Aβ monomers with various lengths, and these various monomers were covalently coupled at the ASP1 and Glu22 destinations to frame stable dimers neurotoxicity (Brinkmalm *et al*., 2019).

Nonetheless, it is indistinct whether the poison levels of the dimers framed through various cross-connecting destinations are unique.

It is for the most part viewed as that Aβ dimers might be the essential constituent unit of filaments or oligomers. In Tg2576 and J20 model mice, Aβ dimers were recognized after the age of 10 months, in which an enormous number of plaque stores showed up in the cortex, demonstrating that there might be a few associations between Aβ dimers and filaments (Jin *et al*., 2011). Also, Aβ dimers are for the most part seen in AD patients more than 60 years of age. The degree of Aβ dimers hence increments strongly, which shows the positive relationship among's dimers and plaque in the cerebrums (Lesne *et al*., 2013). Adding cerebrum determined Aβ dimers to essential neurons can diminish the length of neurons neurotoxicity (Brinkmalm *et al*., 2019), trigger synaptic harm and neuron passing, hinder LTP reaction and prompt tau hyperphosphorylation (Jin *et al*., 2011).

Aβ Trimers

Although there is no evident correlation amongst the trimmers of amyloid and plaque deposits, Aβ trimers are accountable as an aggregation unit of multiple Aβos namely the forms, hexamers and dodecamers (Jana *et al.,* 2016) Studies have shown that Aβ*56 is a kind of Aβos collected by trimers (Lesne *et al*., 2013) and the initial step of amylospheroids (ASPD) development is to from trimers (Matsumura *et al*., 2011). Also, Aβ trimers are the earliest Aβ totals in the cerebrums of AD human and transgenic mice. In the essential cortical neurons of Tg2576 mice, monomers and trimers can be distinguished before dimers, showing that trimers might be created at the beginning phase. Further examination recommended that Aβ trimers originally showed up in the undeveloped phase of Tg2576 mice, then, at that point, expanded consistently with maturing, and existed over the course of life (Lesne *et al*., 2006). Steady with AD creature models, in human cerebrum, the trimers exist since youth,

and their levels step by step increment with age, however there is no huge connection among's trimers and plaque stores (Lesne *et al*., 2006).

The harmfulness of Aβ trimers is at present questionable. It was found that trimers and tetramers arranged by PICUP collection had noticeable harmfulness to neurons, yet the poisonousness of dimers was extremely feeble. Besides, decreasing how much Aβ trimers in the mind of APP/PS1 mice essentially mitigated the mental hindrance (Lesne *et al*., 2013). At the point when the refined essential neurons were treated with Aβ trimers segregated from mind tissues of AD patients, trimers actuated the difference in tau compliance and caused the disturbance of axonal vehicle, the two of which were related with decrease of the kinesin-1 light chain (KLC1) (Jana *et al*., 2016). Notwithstanding, a few examinations contended that Aβ trimers acted exclusively as a central gathering unit for poisonous congregations as opposed to a harmful component. They found that the trimers secluded from 7PA2 cells or AD mouse cerebrums essentially restrained the LTP reaction, yet the harmfulness was more modest than homodimers and Aβ*56 (Lin *et al*., 2011). Moreover, there is no reasonable connection between Aβ trimers and the mental capacity of AD mice (Lesne *et al*., 2006), and the association among trimers and hyperphosphorylation of tau protein in the cerebrum of AD patients is likewise vague (Lesne *et al*., 2013).

Aβ oligomers

Most amyloids which represent toxic species of amyloids contain soluble oligomers. All soluble oligomers have the conformation dependent structure which is unique. The in vitro toxicity of such oligomers is under inhibition of oligomer specific antibody. They are different from fibrillar amyloid. These both share a common structure of formation and have the same mechanism in terms of toxicity (Kayed *et al*., 2003).

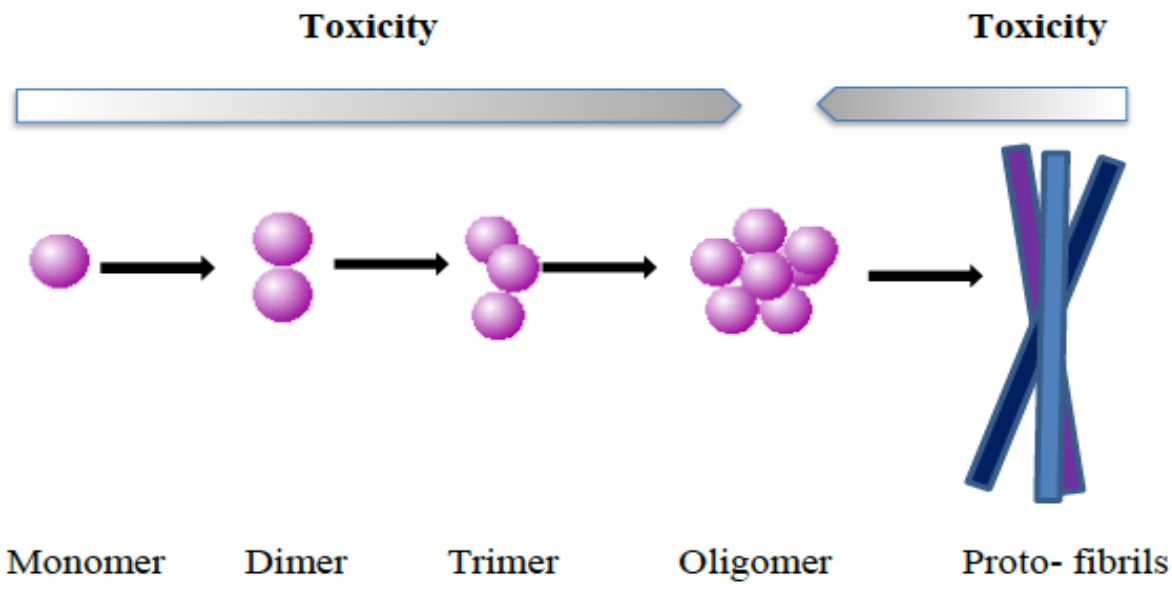


Figure 5: Oligomerization process of amyloid beta peptides (Elahi et al., 2016).

The soluble amyloid oligomers are representatives of primary pathologic species in degenerative diseases. A common group of pathologic processes is generated from permeabilization process and this includes intracellular calcium dyshomeostasis, production of reactive oxygen species, altered signalling pathways, and mitochondrial dysfunction that represent key effectors of cellular dysfunction and cell death in amyloid-associated degenerative disease (Glabe & Kayed, 2005). A relationship might occur between the toxicity induced by oligomer and inflammation. A negative correlation exists between the size of oligomer and toxicity caused after reaching a critical mass (Figure 5) (Sengupta *et al*., 2016).

Amyloid beta and it's relation to Alzheimer's

One of the most common diseases causing the most damages to the human brain in elderly humans is Alzheimer's disease. It is characterized by cognitive dysfunction among the patients (Schachter & Davis, 2000). A major pathological disturbance identified in people suffering from Alzheimer's disease is the abnormal neuroinflammation induced by Aβo. This results in impairment in memory. In

response to infections or damage to tissues, nucleotide-binding domain and leucine-rich repeat (NLR) pyrin domain-containing 3 (NLRP3) inflammasome is mostly activated (Sita *et al*., 2021).

The changes in genetics also lead towards the onset of Alzheimer's disease. The metabolism of small beta amyloid (Aβ) peptide is affected mainly during this disease which is one of two major components of the disease (Hyman, 2011). The accumulation of soluble Aβo starts almost two decades earlier than the clinical onset of the disease Death receptors DR4 and DR5 specifically get activated by oligomeric Aβ induction of extrinsic apoptotic pathways in human microvascular cerebral endothelial cells thus leading to activation of both caspase-8 and caspase-9 (Hector & Brouillette, 2021).

One of the key molecules in AD is amyloid beta peptide. Initially it was thought the accumulation of Aβo serves as a contributing factor of onset of AD as it causes formation of senile plaques and neurofibrillary tangles, neuronal loss, and dementia. Soluble Aβo play a crucial role in the aetiology of AD. Currently, Alzheimer's is believed to be caused by the synaptic dysfunction as a result of soluble Aβo. This is known commonly as oligomers hypothesis. Brains with AD have a higher level of Aβo. In addition to this, brains with AD also have a mutation in E693 delta which caused an increase in the production of Aβo (Tomiyama, 2010). Even if there is an elevated level of Aβo among people with AD, it is not necessarily related to the severity level of the disease (Sephton & Yu, 2008; Bloom, 2014).

COVID-19 and the A-beta

The links that have been reported between COVID-19 and Alzheimer's disease (AD), are mechanistic in nature and are focused so far by the recent research on either microvascular injury or neuro-inflammation (Zhou *et al*., 2021) the neurodegeneration associated with AD is linked to the accumulation of amyloid

plaques (van der Kant & Goldstein, 2015). Researches indicate that the neuroinflammation is highly involved in the pathophysiology of neurodegenerative diseases such as Alzheimer's. A very important mediator in the development of Alzheimer's disease is immunological sensor NLRP3 inflammasome. Researches indicate that the SARS-CoV-2 produces a systematic inflammatory response which mediates by the overstimulation in the pathway of NLRP3 inflammasome pathway. NLRP3 also helps in the activation of AD by causing an impairment in amyloid-beta peptide clearance. The conditions that have been reported to increase the COVID-19 vulnerability including diabetes mellitus and obesity also increase the levels of circulating Aβ and have been proven to impact the AD progression (Kipshitzed, 2020; Meinhardt, 2021).

The development of Alzheimer's disease in patients suffering from COVID-19 is also caused by the presence of pro-inflammatory cytokinesis which includes such as interleukin (IL)-1β which releases during the time when NLRP3 inflammasome is activated. It can also happen during IL-17 OR IL-16 and the tumour necrosis factor -α (TNF-α) which is the result of production of immune cells activate as a response to an infection (Wang *et al*., 2021).

Different changes have been linked with SARS-CoV-2 infection. It has been found out that people who have survived coronavirus disease may suffer from long term abnormalities which causes cognitive deficits and changes in the mood. One of the prominent features of COVID-19 is the central and systematic inflammation. The same neuroinflammations are found to be a feature of AD by triggering amyloid pathways and Major Depressive Disorder. COVID-19 is also linked to acute neurological affects. Such people are at a high risk of developing neuropsychiatric disorders (Silve *et al*., 2022).

Alzheimer's disease and COVID-19 *relation*

Neuroinflammation takes place in AD which affects the memory and cognition of the individual (Islam *et al*., 2020). Alzheimer's disease is highly associated with the neuropsychiatric symptoms and more than 80 percent of patients suffering from AD exhibit at least one of these symptoms over the period of time during the period of disease (Boutoleau-Bretonnière *et al*., 2020).

COVID-19 shares the same aetiology as many other diseases, including Alzheimer's disease too. For the development of affective medicine of COVID-29, its link with the AD is also reviewed. Both COVID-19 and AD share the same angiotensin-converting enzyme 2 (ACE2) receptors and pro-inflammatory markers The cholinergic system is affected by the Aβo which cause behavioural changes in the patients suffering from AD causing upto 75 percent changes in the brain (Rahman *et al*., 2021).

In the severe cases of COVID-19, the blood brain barrier is disrupted and is caused by the cytokine storms which is a systematic inflammation which is pathological mechanism of AD and damages the neural and glial cells. The pro inflammatory cytokines change the capacity of microglial cells and coverts them to phagocyte beta amyloids which causes the amyloid plaques to be accumulated. This in turns makes it easier for the brain to access central nervous system due to the high permeability of blood brain barrier. This increases the process of neuroinflammation and ultimately leads towards the neurodegenerative process (Ciaccio *et al*., 2021). One of the most highly observed cytokinesis is IL-6 and its increased levels are linked with a high probability of development of COVID-19 and mortality. It also serves as a marker for AD and COVID-19 both and its presence at higher levels causes cognitive dysfunction and progresses AD (Chen *et al*., 2020).

Studies have revealed that there are chances that AD might originate from COVID-19. Neurodegeneration caused by neuroinflammation is a highlighted

feature of Alzheimer's disease which speeds up the process of brain inflammation which is more common among elder individuals (Naughton *et al*., 2020). The neurodegenerative diseases are also caused by neuroinvasive potential (Abate *et al*., 2020). The various pathological changes in AD patients might include excessive expression of viral receptor angiotensin converting enzyme 2 and pro-inflammatory molecules, various AD complications including diabetes, lifestyle alterations in AD, and drug-drug interactions. COVID-19 also causes cognitive impairments after SARS CoV-2 invades the central nervous system which lead towards the development of AD. Furthermore, it also makes the behavioural symptoms worse in patients who are not affected by AD thus providing new challenges for the prevention of AD (Xia *et al*., 2021). Beta amyloid has also been found to be associated with COVID-19 pandemics, as shown in PET/CT scans (Laudicella *et al*., 2021). The neuropathological pathways causing Amyloid plaque ruminants typically associated with AD have been found to be activated in COVID-19 patients (Reiken *et al*., 2022). In conclusion, SAR-CoV-2 has substantial neurological symptom manifestations along with amyloid beta conformer potential role in dementia manifestation leading to the hypothetical conjecture that COVID-19 has a high potential to be a risk modulator for Alzheimer's disease

Chapter 3

Material and Methods

Materials and Methods

Software's and Tools Used

Different *Insilco* based software utilized in the study are enlisted in the Table 0.1.

Table 0.1: List of Software used in Study.

Software	Application	Developer
PyRx	Molecular docking	Source Forge
PyMOL	3-D analysis of ligand – protein complex	Schrödinger, Inc
Discovery Studio	Visualization and 2D analysis	Biovia
Ligplot+	2-D representation of ligand protein complex	Roman Laskowski

Software and Online Resources

RCSB PDB

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

PubChem

The DrugBank is a bioinformatics database that contains all the information and structures of drugs and also their potential protein targets. The database include 5K+ entries with 1000 of them are FDA approved. (Wishart *et al*., 2006)

Discovery Studio

BIOVIA Dicovery Studio modeling and simulation software allows scientists to perform computations of chemical, biological and materials properties; to simulate, visualize and analyze chemical and biological systems; and to communicate the results to other scientists (Haque *et al*., 2022)

PYMOL

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

PDBsum

For the evaluation of in depth structural analysis of the selected protein PDBsum tool was used available at (http://www.ebi.ac.uk/pdbsum) PDBsum enables one to get structural features of peptides, proteins and their ligands (Laskowski *et al*., 2018).

PyRx

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

LigPlot+

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

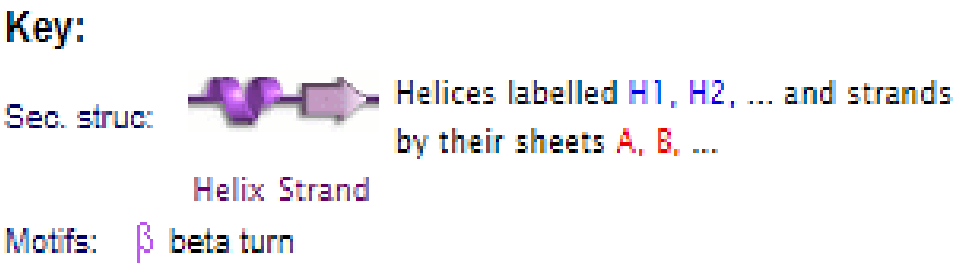
Methodology

Selection of Ligand and Protein

Covid-19 (PDB ID: 6LU7) was selected after literature review. The main COVID-19 protein under study is 6LU7. The protease of which consists of two chains A and C, chain A belongs to protease and chain C belongs to N3 inhibitor N-[(5methylisoxazol-3yl) carbonyl] alanyl-L-valyl-N-1- (1R,2Z)-4- (benzyloxy)-4-oxo-1–1- [(3R)-2-oxopyprolidin-3-3yl] methyl but-2-enyl)-L-lucinamide (Vijayakumar, 2022). Chain A was used in this study. In order to retrieve the ligand literatures were studied, PubChem, Science Direct, PubMed Scopus, Google Scholar etc were used by inserting searching terms such as “Amyloid Beta” or “Amyloid Beta Oligomers” or “Amyloid Beta (Random Proteomic Sequences)” (Yimer *et al*., 2019).

Secondary Structure Prediction

For the evaluation of in depth structural analysis of the selected protein PDBsum tool was used available at (http://www.ebi.ac.uk/pdbsum) PDBsum enables one to get structural features of peptides, proteins and their ligands (Laskowski *et al*., 2018). Enlarged secondary structure is given in Figure 4.



Ligand and Protein Preparation

3- Dimensional structure of covid-19 was downloaded from Protein data bank under PDB ID: 6LU7 (Liu *et al*., 2020) 2- Dimensional structure of 6LU7 is given in figure 6 and properties of 6LU7 is reported in table 1. Amyloid Beta Oligomers were taken from PUBCHEM (https://pubchem.ncbi.nlm.nih.gov/, accessed on 2 May 2022) in .sdf format. The structure of 6LU7 was further purified for docking by

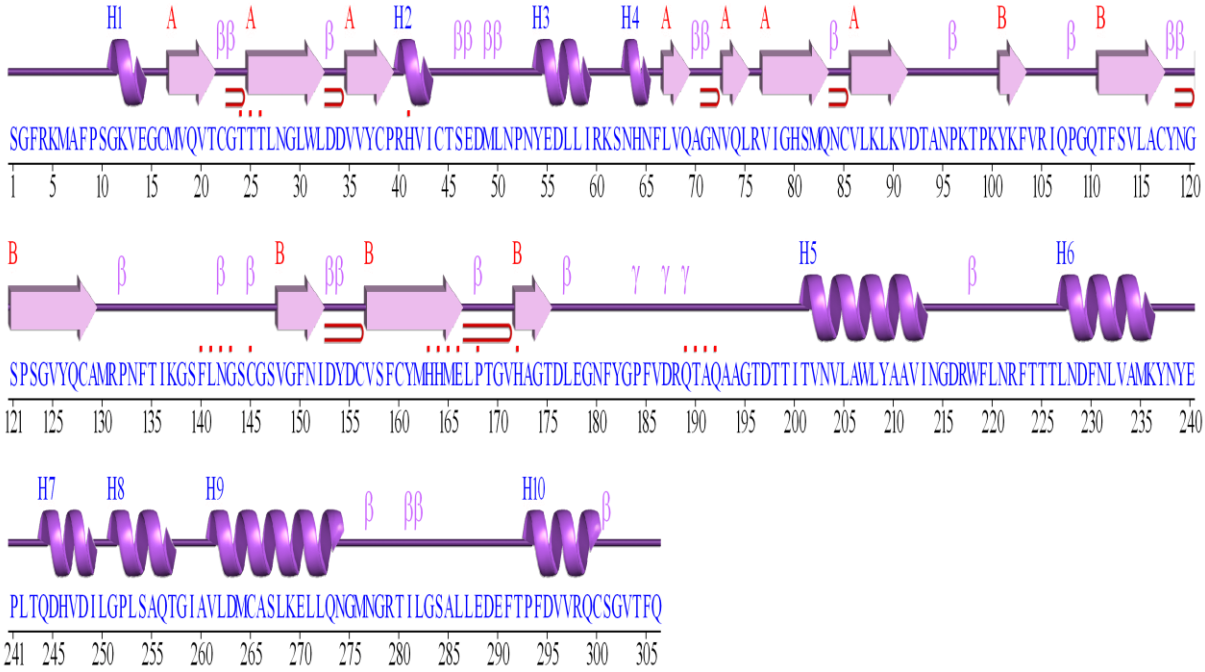


Figure 6: Secondary Structure of the Crystalized Covid-19 main Protease with Inhibiter N3 6LU7: Secondary Structure assessment reflects ten Helices.

removal of the attached ligand and water molecules to bear the active site open using Discovery Studio and saved in a PDB format for further analysis. Chain A (Figure 7) is used in this study (https://discover.3ds.com/discovery-studio-visualizer-download (accessed on 2 May 2022).

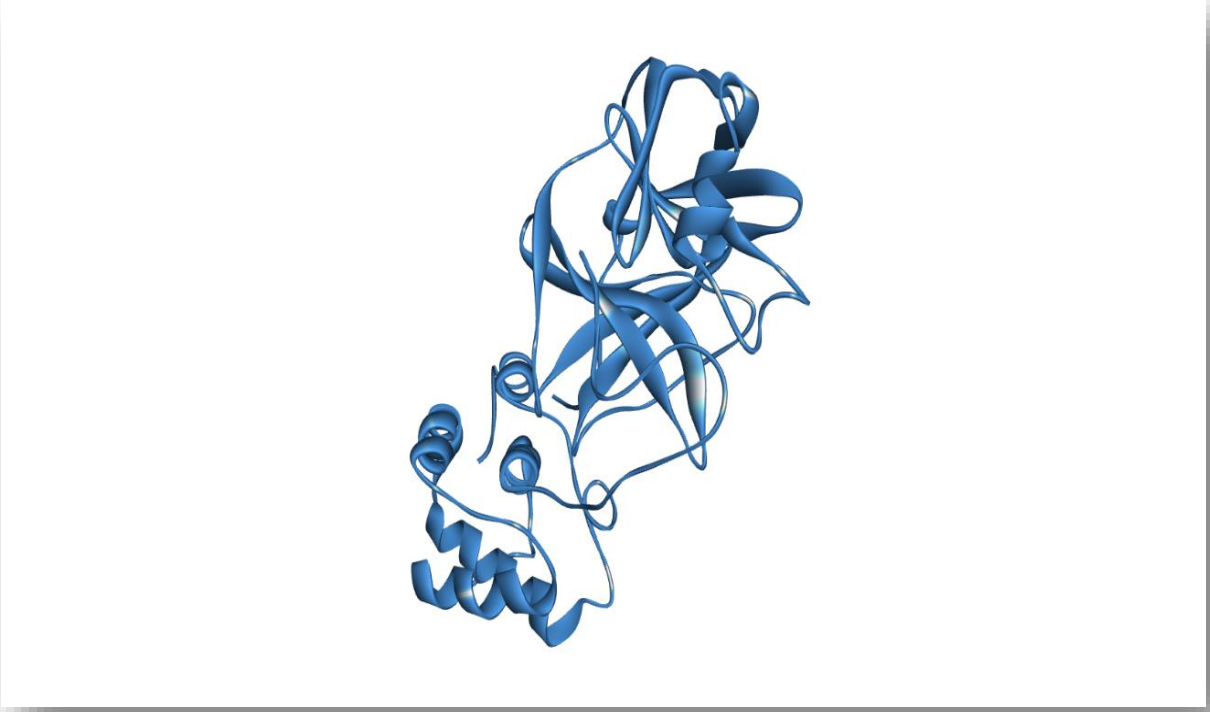


Figure 7: Prepared Receptor Molecule (6LU7 Chain A) using Discovery Studio ***(https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/).***

Table 1: List of Chemical Properties of 6LU7 (Liu et al, 2020).

Protein	The crystal structure of COVID-19 main protease in complex with an inhibitor N3
PDB ID	6LU7
Classification	Protein binding

Organism	Homo Sapien
Total formula weight	33825.5
Method	X-ray Diffraction
Sequence length	306
Chain	A

Molecular Docking through PyRx

PyRx

Pyrx is an open-source computer aided drug discovery software. This is used to dock compounds and study their binding energies to analyse binding affinity and interaction presence.

Docking

For docking through PyRx protocol proposed by Sargis Dallakyan and Arthur J. Olson was followed (Dallakyan, Sargis; Olson, 2015). Ligand and protein format was .sdf and .pdb, respectively. First receptor molecule was converted into PDBQT form by the inbuilt program.

Energy Minimization

Energy of ligand molecules using field strength in the MMFF94 (Merck molecular force field) (Tosco *et al*., 2014) was minimized by tool in Open Babel software package that is already implanted in PyRx (O'Boyle *et al*., 2011) and converts all the molecules in PDBQT format step wise.

Docking

Vina wizard module in PyRx was used for docking and the results obtain for both drugs and natural compounds are presented in table. Vina significantly improves the accuracy of the binding mode predictions by speeding up that is achieved from parallelism, by using multithreading on multi-core machines. AutoDock Vina automatically calculates the grid maps and clusters the results in a way transparent to the user with the highest of accuracy (Trot & Olson., 2010)

Ligand and receptor Interaction

In order to analyse the interaction between ligand and receptor, the docked molecules were submitted to pyMOL (Siam *et al*., 2017) to produce a ligand – receptor complex. pyMOL is an open-source tool use for Molecular Visualization. Ligand-receptor complex was then analysed through bioinformatic tool BIOVIA Dicovery Studio by modeling and simulation of a 2D interactive picture (Haque *et al*., 2022) after which Ligplot+ that is used for 2-D representation of the Protein-Ligand Complex was used (Laskowski & Swindells, 2011).

Chapter 4

Results and Discussions

Results and Discussion

SAR-CoV-2 under PDB ID: 6LU7 was selected as a target protein and different ranging Amyloid beta oligomers were used as candidate ligand. PyRx are was used for the molecular docking, PyMOL was used for ligand-protein complexes visualization along with Discovery studio to study and visualize in 2D and Ligplot for the analysis of the ligand-protein interaction.

PyRx Vina Analysis

PyRx tool was also used for the evaluation of Amyloid beta oligomeric ligands and to compare their binding affinity to the select the top binding target. Table 2 represents the binding energy of the compounds with 6LU7.

Table 2: Docking Score of 6LU7 with Amyloid Beta Oligomers using PyRx.

Ligand	Target	Binding Energy
Amyloid Beta 22-35	6LU7	-5.8
Amyloid Beta 17-42	6LU7	-5.2
Amyloid Beta 1-16	6LU7	-5.7
Amyloid Beta 29-40	6LU7	-5.3
Amyloid Beta 15-21	6LU7	-3.7
Amyloid Beta 25-35	6LU7	-5.2
Amyloid Beta 29-40	6LU7	-5.6

Amyloid Beta 31-35	6LU7	-6.3
Amyloid Beta 36-38	6LU7	-5.5
Amyloid Beta 1-7	6LU7	-5.3
Amyloid Beta 1-42	6LU7	-6.5

PyRx vina shows Aβ 1-42 showcases a -6.5 Kcal/mol binding energy but when focused the Aβ 31-35 (PubChem ID: 4423247) gives the best result (-6.3 Kcal/mol) which shows the high affinity of the two compounds. Aβ 22-35 give the binding energy (-5.8 Kcal/mol) and Aβ 29-40 showcases the energy (-56 Kcal/mol). Aβ 31-35 is the site of aptotic induction and an interaction with it at a high affinity showcases how dementia can be caused directly by Coronavirus infection. Ligplot analysis of Amyloid beta 31-35 and 6LU7 shows that Leu 141 and Tyr 237 make a Hydrogen bond with the ligand with the distance of 3.80Å and 3.26Å, respectively. Other amino acids show hydrophobic interaction with ligand.

Binding Energy Evaluation

Listed in the Chart below (Figure 8) is a comparative view of the binding energy in kcal/mol obtained from docking of ligands od amyloid beta oligomers with COVID-19 (PDB ID: 6LU7) representing the affinity of each compound with the suggested target.

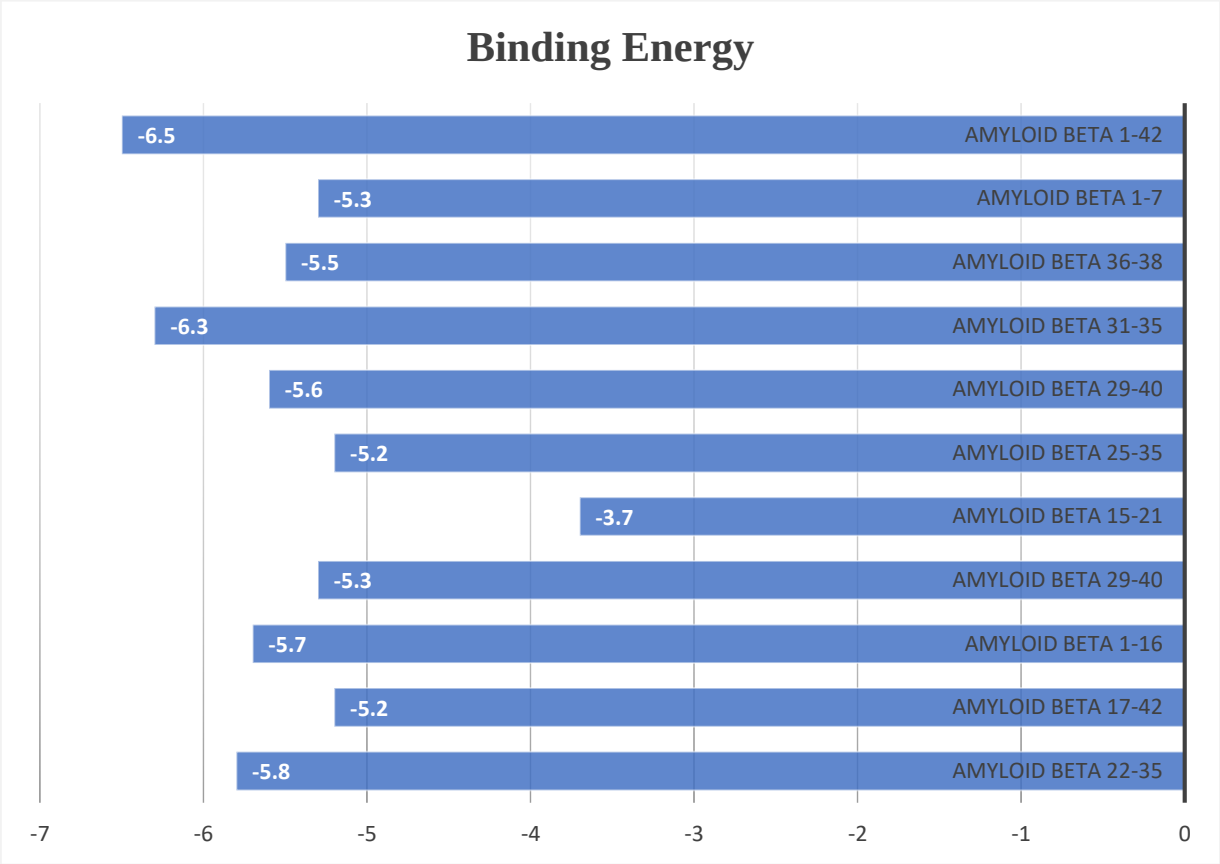


Figure 8: Binding Energy Chart of Docking Results Obtained from PyRx.

Sequence Analysis

Sequence of Amyloid beta 1-42 was analyzed in comparison to the amyloid oligomers attained which showcased the main high affinity sequence of the high affinity region. Figure 9 displays the sequence comparison done along with the highlighted region of Ile-Ile-Gly-Leu-Met that has the binding energy of -6.3.

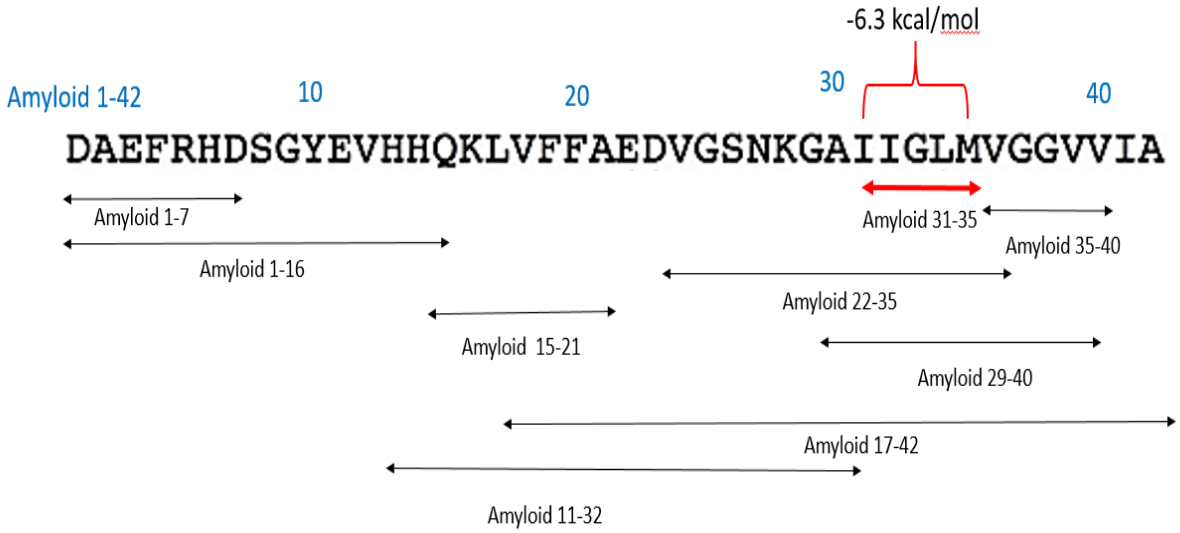


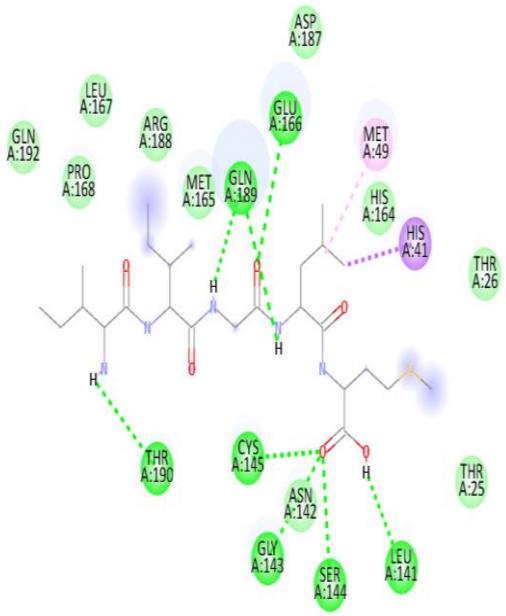
Figure 9: Sequence study for comparison of amino acid involvement in bonding.

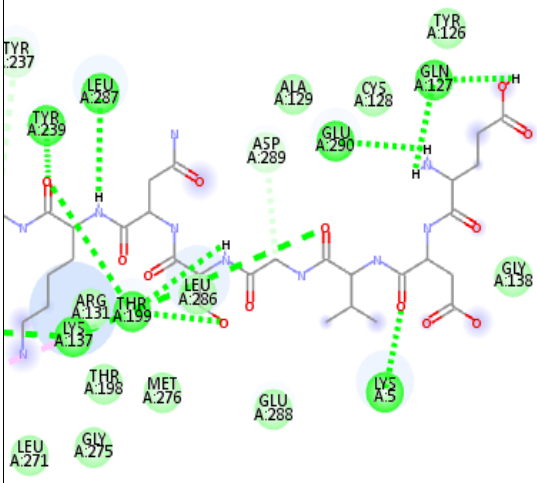
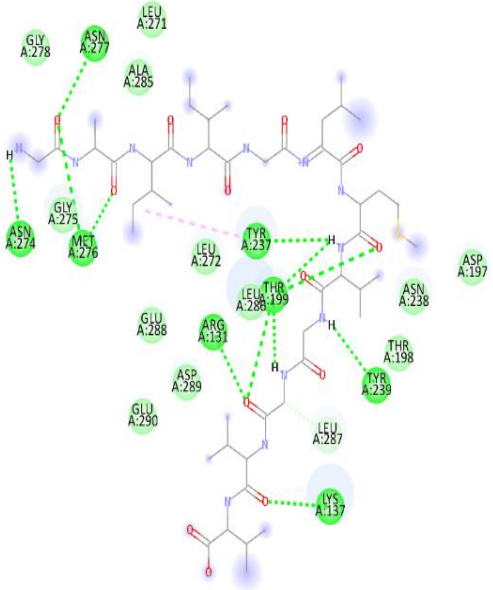
The sequence analysis pin pointed the main amino acid residues that imparted the affinity of highest attachment when in interactive association with 6LU7 main active site. The main amino acid as stated before all play vital roles in translation, permeability and receptor attachment.

Detailed Analysis of the Ligands and Protein Interaction

Active residues of 6LU7 that interacts with ligands using Discovery Studio are Thr 190, Leu 141, Gln 189 and Glu 166, Met 49, Gln 192, Leu 167 and Thr 26**.** Detailed analysis of the ligands and protein interaction are presented in the table 3, where it is describe how the and which amino acid residue is in the contact with the ligand, bonding type, the energy and bonding distance between are briefly explained.

Table 3: Detail Analysis of Top 3 Amyloid Oligomers interaction with 6LU7 obtained from Discovery Studio demonstrating the type of interaction, residue and binding energy.

Ligand	Structure interactions	Type of interactions
31-35		Vander walls are the most visible of interactions existing along with a few Pi-sigma, Alkyl and Hydrogen Bond (LEU 141, GLN 189).

29-40		Hydrogen bonds at three points in light greens can be witnessed in both carbon-hydrogen (Thr 269, Leu 141) and conventional forms, Along with wander walls interactions
22-35		Hydrogen bond with amino acid Tyr 237 and 239 along with Thr 199 and Leu 288 respectively is a dominate sight showing the stability of the given structure.

PyMol & Discovery Studio Visualization Studios

The PyRx was used to dock 6LU7 with Aβ (29-40, 31-35, 22-35). The PDBQT file generated by PyRx were opened in Pymol to generate coordinates and be used to visualize in Discovery studio. The result with least binding energy as stated above were chosen as it indicates successful docking is done. The process is done separately for all of the oligomers and then after all this the interactions were studied using LigPlot+ software.

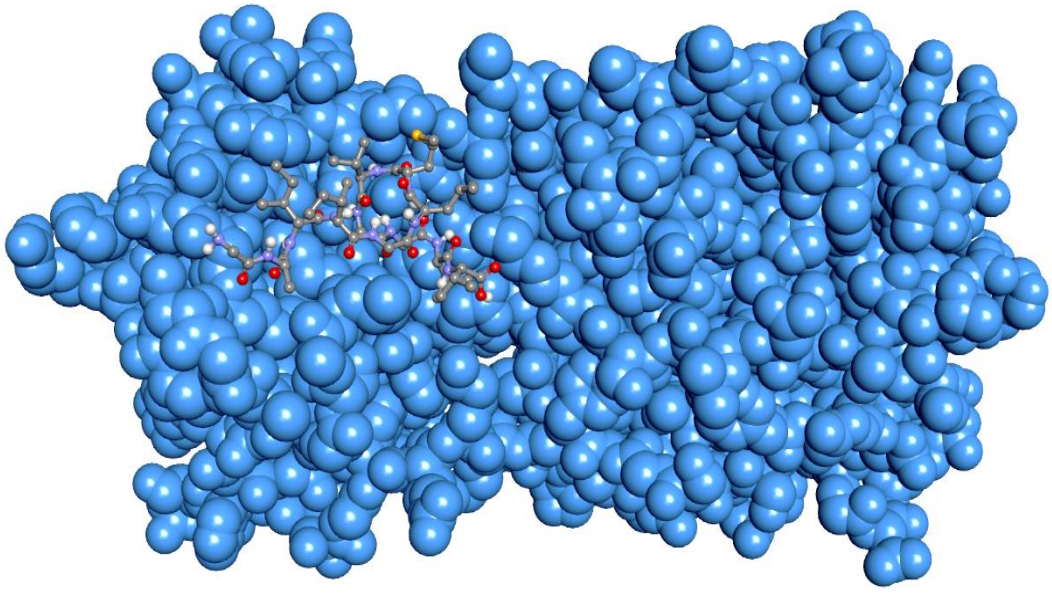


Figure 10: Interaction of Aβ 22-35 (ball and socket) with 6LU7 (blue) Protein using Discovery Studio Software.

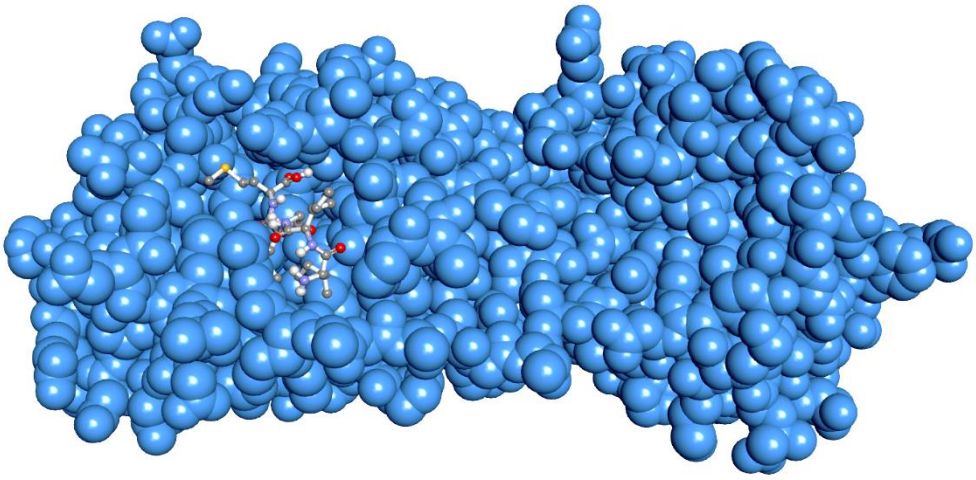


Figure 11: Interaction of Aβ 31-35 (Ball and Socket) with 6LU7 (Blue) Protein using Discovery Studio Software.

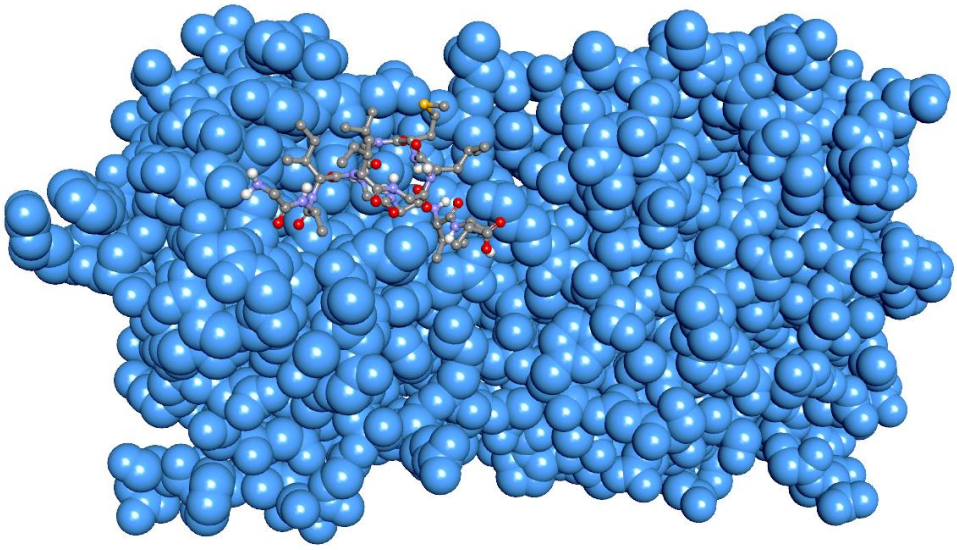


Figure 12: Interaction of Aβ 29-40 (ball and socket) with 6LU7 (blue) protein using Discovery studio software.

LigPlot+ Analysis

Ligplot software was used for the evaluation of the protein-ligand complex interaction. PDB file complexes made and retrieved from PyMOL were analyzed by using Ligplot. 2-D representation of protein-ligand complexes are presented in table 4 which showcases the bonding types between the amino acids and ligands and their binding affinity.

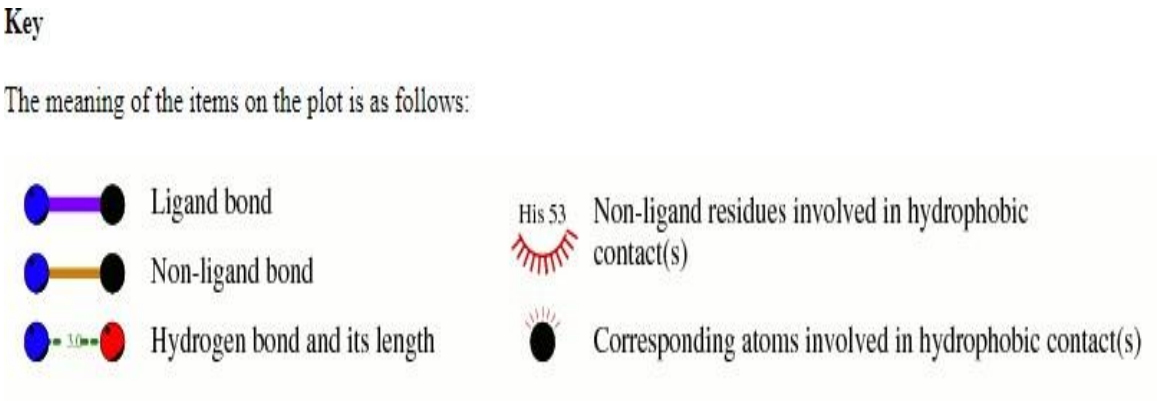
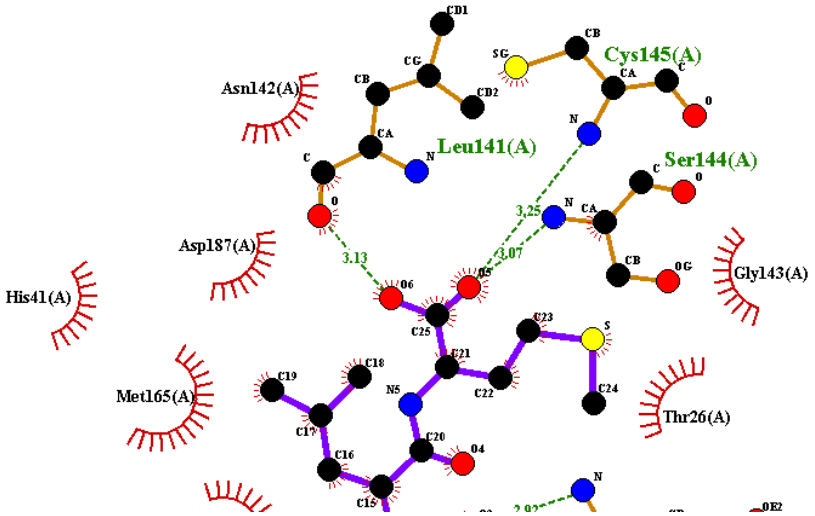
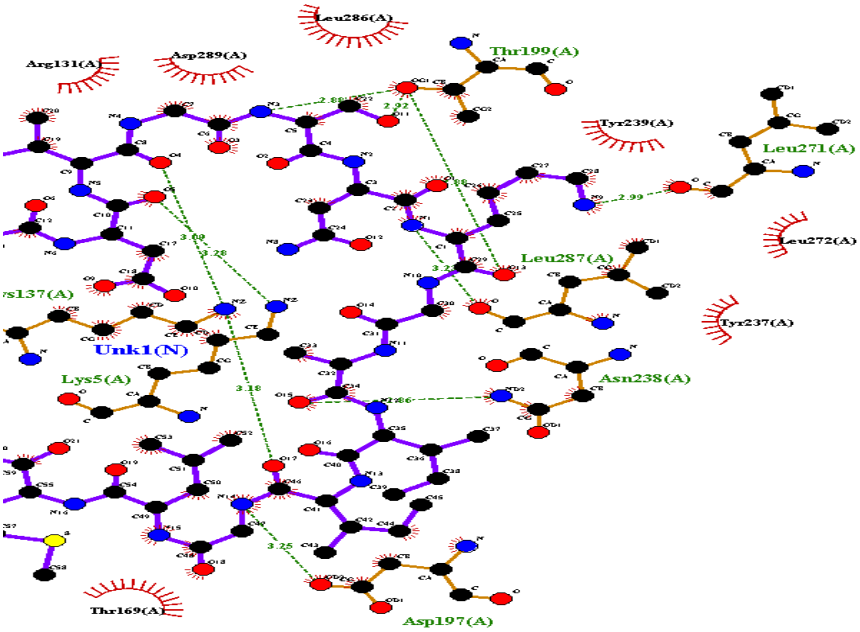
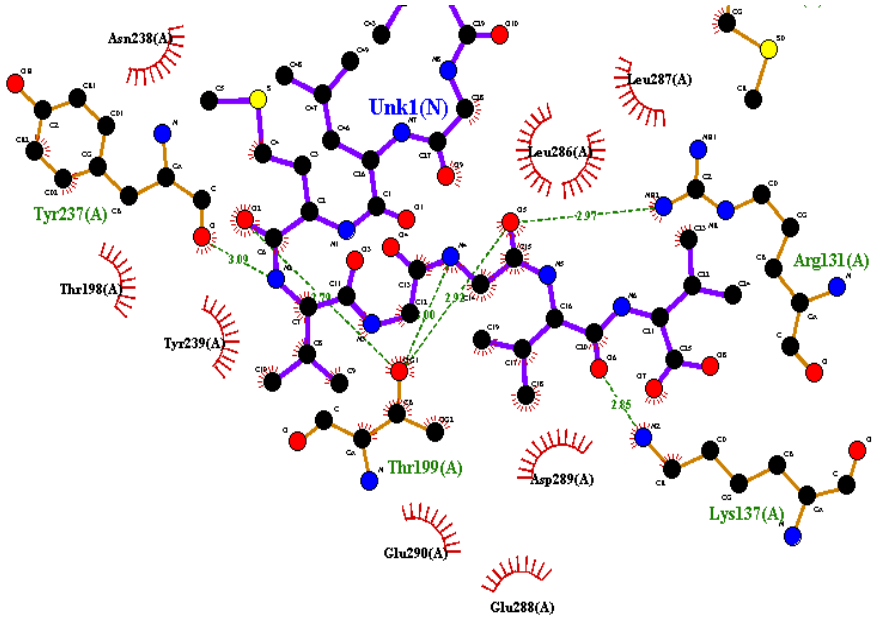


Table 4: 2-D Representation of Amyloid beta Oligomers interaction with 6LU7 using LigPlot.

Amyloid Beta	2-D Interaction
31-35	
29-40	

22-35	

In Amyloid beta 22-35, Leu 287 makes a Hydrogen bond with the distance of 3.18Å while in Amyloid beta 31-35, Cys 145 and Gln 149 makes a Hydrogen bond with the ligand with the distance of 3.35Å and 1.71Å, respectively. Other amino acids display hydrophobic interaction with ligand molecule. The top interative association in complex with 6LU7 are presented in Figure 13 for 2D interactive studies and analyzing bond lengths and there natures.

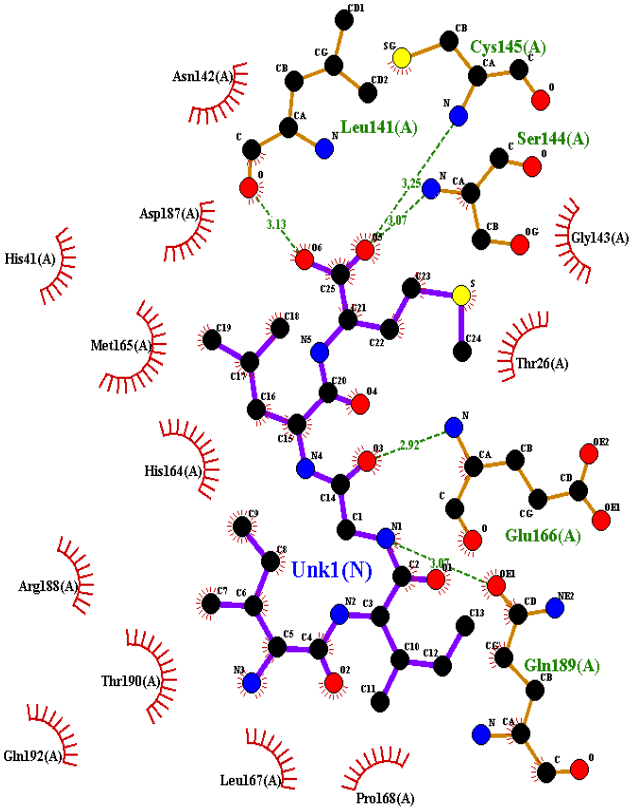


Figure 13: The figure shows interactive association of Aβ 31-35 with 6LU7 protein using Ligplot software.

The bond length of the amyloid beta 31-35 structure as displayed (Figure 13) showcases a favorable high energy bond at a viable bond length, this mean the stability of the complex is in high tier making it a stable complex that requires a high amount of energy to be separated.

6LU7/AMYLOID 31-35 complex

Amyloid collection around cerebral vessels is known to incite degeneration of the whole neurovascular unit (Richard *et al*., 2012). In addition to the fact that insoluble amyloid species collecting at the vascular walls cause changes of the smooth muscle and endothelial cell layers however amyloid testimony and associative micro

hemorrhages likewise happen in little slim vessels without the smooth muscle layer, underscoring the significance of CAA-subordinate components in mind endothelial cells. Expanding proof proposes that apoptotic biochemical fountains assume crucial parts in the neuronal brokenness and demise saw in AD (Lin *et al* 2010). Ongoing discoveries exhibit the enlistment of closely resembling Aβ-intervened cell demise systems in vascular cells as those portrayed in neurons in which mitochondrial brokenness and commitment of apoptotic pathways including cell passing receptors have been hypothesized (Ghiso *et al*., 2014).

DR4	Amyloid beta 31-35	6lu7	Binding Energy
☑	☑		-4.1
☑		☑	-2.8
☑	☑	☑	-6.0

Figure 14: Complex interactive association shows an aggressive approach

Death receptors are portrayed by a cytoplasmic district known as the "death space" that empowers the receptors to start cytotoxic signal exchange when connected by related ligands. DR4 interaction with Amyloid beta 31-35 has a binding energy of -4.1kcal/mol and activates the cascade of DISC whereas DR4 with 6LU7 and Amyloid beta 31-35 complex has a binding energy of -6.0kcal/mol thus showcasing a high binding affinity then either alone showcasing an aggressive approach.

Discussion

During the past decade, computational approaches have shown their success and power in assisting interactive studies, drug development and disease control. Keeping the increasing number of COVID-19 as well as Alzheimer disease patients in mind, searching for potential links and solutions to combat the disease chimera in efficient time, and in a cost-effective manner has become even more significant. Taking advantage of all available information on amyloid beta conformers and their role in neurodegeneration this study investigates the potential risk that COVID-19 may have on Alzheimer disease. The amyloid beta oligomers, an AD hallmark of current time were used to target the COVID-19 main protease alpha chain active sites. An amalgamation of computational tools; including molecular docking, virtual screening, molecular dynamics simulations and Ligplot analysis were used to identify an interactive association ground for Alzheimer disease and COVID-19. The insights into the Amyloid beta oligomers and their complexes, as well as their binding energies, were explained in prediction of 6LU7 dynamics. Our findings provide a foundation which would be helpful for further research for predictive association analysis in depth.

With the goal to investigate SARS-CoV19 as a risk factor for Alzheimer disease this study utilizes the available X-ray structure of COVID-19 main protein (PDB id 6LU7) as a starting point. Initially, interactive indicated a successful and robust docking protocol with Vina, PyRx. In this study Amyloid beta oligomer 31-35 interacted significantly with 6LU7 proving through pre-existing data and available literature that COVID-19 may lead to the apoptotic death of neural cells and eventually cause dementia. Amyloid beta oligomer 22-35 also showed predictive interactive association in a considerable capacity with 6LU7 pointing to the view

that COVID-19 may also play a role in negative inotropic effect.

The Aβ oligomeric residues involved in hydrogen bonds are Leu 34, and Met 35, Gln 189 and the COVID-19 residues involved in hydrogen bonds are Leu 141, Tyr 237 and Thr 199. From this it is concluded that Amyloid beta oligomers displayed predictive association for COVID-19 M-pro in regards to its active site.

Ligplot analysis further analyzed the ligand-protein complex interactions. All gathered intel of this research signals that Aβ interacts with 6LU7 in determinant surety and Aβ 31-35 is the best interacted ligand which in form of Complex 1 has increased binding affinity to receptor of apoptotic pathway. The study also gives a visual perspective of the interactions with displays of docking complexes visualization justifying the overall docking results. All these findings communally indicate that Aβ oligomeric protein reaction to COVID-19 may lead to determent of the brain transitioning into cognitive impairment ultimately leading to neurodegeneration. Our research not only demonstrated 6LU7 as a potential risk factor of Alzheimer's disease with the help of predictive association and *Insilco* study but also showcased how the interactions may effect apoptotic demise. Aside from interaction analysis different bioinformatics tools were employed to study protein-ligand complex interactions.

The prime Aβ oligomer interaction of 31-35 oligomer with SARS-CoV-19 suggests that COVID-19 likely plays some role in induction of cellular death through apoptotic signals leading to neurodegeneration which is a conclusion deducted by increased affinity of the complex with DR4 receptor of the apoptotic pathway. The projected protein-ligand links through Ligplot+ study and literature review conducted in the following research shows that in a capacity COVID-19 and AD might share a link.

Chapter 4

Conclusion

Conclusion

From this Predictive binding association study, it is concluded that Aβ oligomers interacts with Covid-19 during infection time of the disease. The associative interactions in this study were analyzed using the LigPlot+ software. The protein ligands in question all show both hydrogen bonding as well as hydrophobic interactions. In this study, specific interaction between the amyloid beta protein and the main protease from COVID-19 were investigated and later compared with obtained results and existing data for AD through bioinformatics approach using molecular simulation and visualization tools like Discovery studio, PyRx, PyMOL, Ligplot. We evaluated 12 amyloid beta oligomeric proteins and *Aβ 22-35, Aβ 29-40, Aβ 31-35* stood out among all-oligomeric protein interaction wise with 6LU7 in detail analysis, sidewise comparison revealed that *Aβ 31-35* has the lowest binding energy among all the amyloid beta oligomers that is **-6.3kcal/mol** energy obtained from PyRx thus making it the most affinitive ligand for 6LU7 (COVID-19 M-pro), the Ligplot+ tool than was appointed and it revealed that Leu 141, and Gln 189 are the common amino acid residues that interacts with the top three amyloid beta (*Aβ 22-35, Aβ 29-40, Aβ 31-35)* oligomer results. However the main bonds: hydrogen bonds at Thr 190, Leu 141, Gln 189, Glu 166 along with alkyl bonds at Met 49 and van der wall at Gln 192, Leu 167, Thr 26 formed by amyloid beta 31-15 with SARS-CoV-2 are the most vital for this study due to it being the highest affinity conformer. Also, 6LU7 and Aβ 31-35 complex showed an increased interactive association with

an active receptor for apoptotic pathway in charge of neural apoptosis showing an increased binding approach unlike either alone. COVID-19 hence can be considered a potential risk modulator for Alzheimer based on predictive binding associations studies.

Future prospects

The purpose of the study was to use bioinformatics approaches to predict the relation between AD and COVID-19 through the interaction of amyloid conformers with SARS-CoV-2 highlighting the sites of interaction between both to determine in restricted lengths the possibility of COVID-19 as a risk factor for Alzheimer's disease. *Insilco* analysis revealed that Amyloid beta 31-35 has high affinity for COVID-19 giving us an insight to what maybe the link to the dementia properties of COVID-19 patient in the long haul, thus in the same light suggesting how AD pathology questions for brain fog related COVID-19 queries can be tackled. This research can be used to study further how Alzheimer's disease etiology might be effected by the pandemic and how much impact COVID-19 has on cognitive impairment. These results point at interactive associations of Amyloid oligomers, and its conformers as potential interacts of COVID-19 and could also possibly be studied as novel therapeutic approach against Alzheimer's disease keeping in mind how COVID-19 acts as a catalytic agent to AD in receptor binding making it a possible risk factor. Also, how we can tackle COVID-19 in the light of AD and vice versa.

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