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ABSTRACT

Alzheimer's Disease is one of the most leading and progressively debilitating neurodegenerative diseases characterized due to the accumulation of beta-amyloid plaques, and presence of tau tangles, and neuroinflammation. While years of research have been dedicated to determining what causes the disease, and ultimately, a cure, there has been little to no success in finding one, and new targets must be discovered.

Receptor present on Myeloid Cells-2 (TREM2) is an important immunoreceptor that is mainly expressed on microglial innate immune cells, which are the first to respond to Central Nervous System (CNS). TREM2 was considered an important receptor that regulates microglial homeostasis by regulating many neuroprotective mechanisms including phagocytosis of Amyloid-Beta deposits, regulation of neuro-inflammatory processes, microglial survival/proliferation and preservation of synapses. A number of significant evidence both from the genetic and functional analyses indicate that failure to acquire function mutations or impaired TREM2 signaling pathways leads to impaired phagocytosis, chronic neuroinflammation, and accelerated Alzheimer's Disease (AD) progression. Contrary to this, it also demonstrates that stimulating TREM2 activity leads to better microglial neuroprotective responses, making TREM2 a promising target for enhancing its activity to develop disease-modifying therapies for AD via pharmacologic interventions.

Considering the high profile interest in natural bioactive molecules as novel neurotherapeutic agents, the capacity of naturally occurring plant polyphenols to be potential regulators of TREM2 is addressed in this study. Epigallocatechin Gallate (EGCG), apigenin, curcumin and resveratrol are known to have important properties for eg - potent antioxidant, anti-inflammatory properties. Also have the ability to cross the Blood Brain Barrier, and possess neuroprotective properties.

In the present study, a computational molecular docking approach was used to explore the molecular interactions between TREM2 and these polyphenolic compounds. The target, a macromolecule, was obtained from the Protein Data Bank, and it was the three-dimensional crystal structure of TREM2 (PDB ID: 5ELI). The binding affinities, interaction profiles, and structural complementarities of EGCG, apigenin, curcumin, and resveratrol were systematically evaluated in the TREM2 binding site using molecular docking simulations.

Based on the docking analysis, the four polyphenols showed a definite order of binding affinities. Apigenin showed binding affinity of −8.6 kcal/mol, and EGCG with an affinity of −8.5 kcal/mol, suggesting high specificity, stability, and thermodynamic favorability of their interactions with TREM2. The high binding energy of apigenin and EGCG implies that they can make multiple non-covalent interactions with the receptor, which help to stabilize the receptor-ligand complex. Curcumin showed moderate binding affinity (−7.1 kcal/mol), which is comparatively less, while resveratrol was found with the lowest binding affinity (−6.3 kcal/mol) in comparison to the other tested compounds with TREM2.

These superior binding affinities of apigenin and EGCG suggest that they possess a high potential for modulating the activity of the TREM2 receptor and, therefore, the functional responses of microglia that are central to the pathology of AD such as the regulation of inflammation and the clearance of amyloid-β. Both apigenin and EGCG could present promising neuroprotective therapeutic leads based on their ability to target TREM2.

Though this in silico molecular docking study gives preliminary results, it serves as a solid computational groundwork and a mechanistic explanation for the further in vivo preclinical and in vitro binding studies of these polyphenolic candidates in microglial models and in vivo

14 studies. In summary, this study provides valuable insights into the therapeutic regulation of TREM2 signaling by natural polyphenols and suggests new drug development approaches using plant-derived polyphenols in the context of AD.

The keywords used are TREM2, Alzheimer's disease, neuroinflammation, microglia, molecular docking, polyphenols, apigenin, EGCG, curcumin, resveratrol, amyloid-β, neuroprotection and in silico.

INTRODUCTION

1.1 BACKGROUND AND OVERVIEW OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is considered to be one of the major public health challenges of the 21st century. Alzheimer's is an irreversible, progressive, neurodegenerative disease in which the region of brain, responsible for memory, is affected, which causes irreversible loss of cognitive function. The disease causes memory loss, failure of comprehension, thinking and learning, leading to individuals being unable to carry out daily activities that require independence. The disease was first discovered in 1906 by a German psychiatrist, Alois Alzheimer. It was believed that the disease was only seen in middle aged people for decades, but now it is known that it affects older people, and is the most prevalent cause of age-related cognitive decline globally [1].

Al International has said that people suffering from this disease in the world is around 55 million. Approximately 60 to zheimer's disease (AD) has turned into an epidemic throughout the world. In India, the Dementia India Report predicts that the dementia burden may increase to 5.3 million by 2050, which will significantly strain the nation's healthcare systems, patients and their families.

There are several stages to the clinical presentation of Alzheimer. [13]. During the first stage of the disease (the pre-clinical or pre-symptomatic stage), there are changes in the brain of people which typically occur 10 years or more before they have symptoms. These changes include the appearance of amyloid-beta plaques and tau tangles. When the disease changes to the mild cognitive impairment (MCI) stage, the person with MCI loses memory and ability to think that is somewhat noticeable. In the late or end stage of dementia, patients experience severe memory loss, disorientation, loss of language, changes in personality and behaviour and complete loss of independent living abilities [1],[3].

Despite more than 100 years of research, Alzheimer's disease have no cure, and drugs that are available to date have symptomatic effect. The acetylcholinesterase inhibitors like donepezil, rivastigmine, galantamine and memantine approved for use against Alzheimer's disease [16] provide only short-term and modest improvements in cognitive symptoms. Newer amyloid-targeting drugs (e.g., aducanumab and lecanemab) have demonstrated some efficacy at lowering amyloid, but it is not known if they are effective or safe in clinical practice.

1.2 MOLECULAR PATHOLOGY OF ALZHEIMER'S DISEASE

Two major pathological features have been identified in Alzheimer's: extracellular plaques has primarily aggregates of AB peptides and neurofibrillary tangles (NFTs) containing tau protein. These are the classical elements, but recent studies have uncovered the appreciation that the disease pathogenesis is a highly intertwined and self-sustaining process that includes neuroinflammation, synaptic dysfunction, mitochondrial dysfunction, oxidative stress, and disrupted proteostasis.

Breakdown of Amyloid precursor protein by two enzymes, beta-secretase (BACE1) and gamma-secretase enzyme complexes to generate amyloid-beta peptides. In healthy brain, soluble Aβ monomers are rapidly cleared from the brain ,including enzymatic degradation, CSF drainage and receptor-mediated transcytosis across the BBB.

In normal biology, tau functions as a stabilising agent of the transportation machinery and microtubules of the axons. In the pathological condition of AD, the tau protein is phosphorylated abnormally, which results in the dissociation of the microtubules and finally leads to accumulation of the tau protein into NFTs by the formation of paired helical filaments [15]. In the human brain, pathological up-accumulation of the tau protein occurs in a consistent order: the entorhinal cortex, the hippocampus, then the limbic and neocortex. [1,2]

Neuroinflammation has come to be recognized as a third central pathological feature of Alzheimer's disease, instead of being a minor epiphenomenon. Microglia are innate immune cells constantly patrolling the neural parenchyma. Microglia can also become activated in response to Aβ deposition and neuronal damage, and initiate inflammatory responses that can be beneficial and protective in the acute phase by promoting the clearance of amyloid and tissue repair. However, prolonged and chronic activation leads to a pro-inflammatory dysfunctional state of microglia, with the excessive production of cytokines ,reactive oxygen species (ROS) and reactive nitrogen species, which further contributes to disease progression [5],[6].

1.3 TREM2: A CENTRAL REGULATOR OF MICROGLIAL FUNCTION IN ALZHEIMER'S DISEASE

Receptor present on Myeloid Cells-2 (TREM2) is one of the most important and promising targets discovered over the last ten years among the molecular pathways involved in microglial function in Alzheimer. TREM2 belongs to superfamily type 1 transmembrane receptor family and is predominantly expressed by microglial cells, as well as by peripheral myeloid cells like macrophages, osteoclasts and dendritic cells. [6],[7]

Two to four-fold risk variants in the TREM2 gene were identified and in particular the missense variant R47H in TREM2 brought TREM2 to the forefront of genetic research of AD [18]. This risk size is comparable to known genetic risk factor for sporadic Alzheimer, the APOE4 allele, which suggests that immune signaling by microglial cells is important in sporadic disease [7],[8].

TREM2 transmits signals via the transmembrane adaptor protein, which includes an immunoreceptor tyrosine-based activation motif (ITAM). Activated by ligand binding to the extracellular immunoglobulin-like domain of TREM2, activated TREM2 recruits and activates Syk kinase which initiates signaling pathways. All of these pathways regulate extensive aspects of microglial functions, including their chemotactic movement, phagocytosis, inflammatory cytokine production, metabolism, survival and proliferation [7],[8],[9].

TREM2 plays a role in several key protective functions relevant to AD. It promotes clearance of amyloid-beta aggregates, apoptotic neurons and cellular debris via phagocytes and suppresses the propagation of amyloid-beta toxic protein aggregates. In addition, TREM2 also confers metabolic fitness to transcriptionally distinct microglia (DAM) that are expressed near amyloid and involved in phagocytosis, lipid metabolism and function of lysosomes, which is a proposed adaptive protective response to the deposition of Aβ. The absence of TREM2 impairs this DAM state, contributes to amyloid clearance and leads to ongoing, persistent, and chronic neuroinflammation to drive neurodegeneration [8],[9],[19].

For these complex protective functions, TREM2 has been a target of interest in the field of disease, and strategies to boost TREM2 signaling, to block the shedding of the TREM2 ectodomain (to generate soluble sTREM2) and to stabilize the TREM2-DAP12 receptor complex are currently being explored. Nevertheless, small molecule modulators of TREM2,

especially of natural origin, are under-studied and hold great potential for computational and experimental drug discovery efforts [9].

1.4 NATURAL POLYPHENOLS AS NEUROPROTECTIVE AGENTS

Natural polyphenols are a very large groups. So far more than 8000 polyphenolic compounds have been discovered in the plant world and most of them are flavonoids, stilbenes, phenolic acids, lignans, tannins, etc. The polyphenols are abundant in food and, tea and coffee, red wine and spices, and they have been associated with a multitude of health benefits across numerous epidemiological and experimental studies [3],[4],[10].

Polyphenols have been investigated in numerous studies with regards to neurodegenerative diseases because of their activity on multiple pathogenic mechanisms related to Disease. These include antioxidant activity and induction of endogeneous antioxidant defense enzymes, anti-inflammatory activity by modulation of signaling pathways, inhibition of Aβ aggregation and promotion of disaggregation of preformed fibrils, inhibition of BACE1 and gamma-secretase activities, inhibition of tau hyperphosphorylation, modulation of autophagy and mitophagy, and the protection of synaptic structure and function [3],[4],[5].

Epigallocatechin-3-gallate (EGCG), apigenin, curcumin and resveratrol are some of the most widely studied phytochemicals with neuroprotective properties in various chemical classes.. Apigenin is a flavone that is present in edible plants like chamomile, parsley, celery, it is a blood brain barrier permeable compound and regulates neuroinflammatory signaling, and is a neurogenic stimulator in the hippocampus [4]. The rhizome of (turmeric) has been shown to contain curcumin, which has a number of properties that have been shown to reduce Aβ aggregation, disaggregate preformed fibrils, chelate metal ions that are thought to mediate Aβ toxicity and attenuate neuroinflammation [4],[5].

In spite of their promising multi-target neuroprotective activity, the clinical translation of these polyphenols in Alzheimer's disease has been limited by their pharmacokinetic drawbacks such as low aqueous solubility, low oral and inadequate blood brain barrier penetration. They do however have a wide variety of biological functions, and have been shown to be safe to use, making them attractive targets for structure-activity relationship studies and computational screening for novel molecular interactions with known AD targets such as TREM2 [3],[4],[10].

1.5 MOLECULAR DOCKING AS A COMPUTATIONAL DRUG DISCOVERY TOOL

Docking algorithms proceed by systematically sampling the conformational space of a flexible ligand in a specified search space on the receptor, scoring the geometric and energetic fit between each pose of the ligand and the receptor, and ranking the poses based on the calculated binding affinity. The most common docking programs are AutoDock, AutoDock Vina, DOCK, Glide, GOLD, and FRED which use different algorithms for searching for binding sites and different scoring functions to determine the binding affinity. The platform used in the present study is AutoDock Vina, which is faster and more accurate than its predecessor AutoDock 4 [11] and uses a gradient optimization method to determine a docking pose and a sophisticated empirical scoring function that considers steric, hydrophobic and hydrogen-bonding interactions.

Molecular docking has several promising benefits in drug discovery for neurodegenerative diseases. It allows for screening of large compound libraries against a target of interest quickly and cost effectively and is used to prioritise the most promising candidates for experimental

testing, thereby saving time and resources from traditional high throughput experimental screening. The information it provides is also invaluable in understanding structure-activity relationships and hit-to-lead optimization, giving atomic-level understanding and insights into the key non-covalent interactions that mediate ligand-receptor recognition[11],[12].

In the particular field of disease and neuroinflammation studies, molecular docking has yielded the potential interactions between natural compounds and known targets like acetylcholinesterase, BACE1, gamma-secretase, GSK-3β, HDAC, and, more recently, immune receptors such as TREM2. These computational investigations have shown the usefulness of in silico screening in the identification of polyphenols and other phytochemicals with favourable predicted binding characteristics, rational mechanism of action for their observed biological activity and the prioritisation of candidates for experimental follow-up [12],[21].

1.6 RESEARCH OBJECTIVES AND SCOPE OF THE PRESENT STUDY

The overall goal of this dissertation is to systematically evaluate the binding properties of selected natural polyphenols to TREM2 in silico by molecular docking to find candidate natural polyphenols that may modulate TREM2 activity and thus potentially augment microglial mediated neuroprotection in disease.

Study objective:

(i) To download and structure the three-dimensional crystal structure of TREM2 (PDB ID: 5ELI) for molecular docking.

(iii) To select, retrieve and prepare the three-dimensional structures of four representative natural polyphenols (EGCG, apigenin, curcumin and resveratrol) as candidate ligands.

(iii) To conduct molecular docking studies on each polyphenol compound in the TREM2 using AutoDock Vina within PyRx screening software to obtain the binding affinities and binding pose of each polyphenol compound in the binding site of TREM2.

(iv) To analyse and visualise the obtained protein-ligand complexes in BIOVIA Discovery Studio Visualizer to find out the important non-covalent interactions and binding residues.

(v) To discuss the docking results in the light of the TREM2 biology and disease pathology and suggest a computational rationale for selecting certain polyphenols for experimental testing as TREM2 modulators.

This study is limited to computational in silico methods, namely the molecular docking and does not include experimental laboratory validation. The findings are to be used to develop mechanistic hypotheses and prioritization frameworks that will inform future in experimental studies. The study only involves the four polyphenols with already established neuroprotective and anti-neuroinflammatory effects, and the TREM2 crystal structure with PDB ID 5ELI, which represents the extracellular immunoglobulin-like domain of human TREM2 in a conformation appropriate for the docking analysis of the ligands.

CHAPTER 2 : LITERATURE REVIEW

2.1 ALZHEIMER'S DISEASE: EPIDEMIOLOGY, ETIOLOGY, AND PATHOPHYSIOLOGICAL MECHANISMS

The impact of the disease on the population is enormous and will keep increasing with the ageing of the global population. Worldwide, it is estimated there are approximately 55 million people, this figure will rise to 78 million and 139 million by 2050. The worldwide is estimated to be over one trillion USD dollars per year, including direct health care expenses, indirect costs due to informal care and lost productivity [1],[2].

There are two broad types of disease that is etiological. The familial, early onset type (less than five percent of all cases) is due to autosomal dominant mutations all of which lead to elevated levels of the amyloidogenic Aβ42 peptide. The sporadic and late onset type (the great majority of cases) is the result of complex interaction of a number of genetic susceptibility loci, as well as environmental factors, lifestyle and age-related biological changes [1],[2],[3],[17].

The neuropathological hallmark is the progressive deposition of Aβ plaques and neuroinflammatory tau in specific areas of the brain, including the hippocampus, entorhinal cortex and association cortices, causing loss of synapses and neuroinflammation and ultimately leading to neuronal death throughout the brain. The amyloid cascade hypothesis (Hardy and Higgins 1992, Hardy and Selkoe 1999) suggests that an abnormal accumulation of Aβ is the initial event in the pathogenesis of AD, leading to a cascade of events including tau pathology, synaptic dysfunction, neuroinflammation and neurodegeneration [14]. While most therapeutic strategies over the past 30 years have focused on the anti-Aβ approach, the lack of success of multiple anti-Aβ therapeutic strategies in recent clinical trials has shifted the focus to a more general consensus that all four factor – neuroinflammation, tau pathology, synaptic dysfunction and metabolic abnormalities – must be targeted [3],[5],[22].

And astrocytes and peripheral immune cells, the primary cells implicated in neuroinflammation in Alzheimer's disease are the innate immune cells, called microglia. The last ten years of (GWAS) have identified a remarkable number of AD risk-loci within genes that are primarily or entirely expressed in the microglial immune system, such as TREM2, CR1, CLU, CD33, INPP5D, MEF2C and many others, confirming the idea that microglial immune signaling is a primary driver of AD risk and progression, not a mere secondary consequence of neurodegeneration [5,6,7].

2.2 TREM2 IN NEURODEGENERATION: STRUCTURE, SIGNALING, AND DISEASE RELEVANCE

The TREM2 gene is a glycoprotein of the immunoglobulin superfamily found on chromosome 6p21.1. The protein contains an extracellular immunoglobulin variable (IgV) domain for binding to the ligand, a short stalk domain, one transmembrane (TM) domain with a critical Lys that binds to the negatively charged adaptor DAP12 and a short cytoplasmic tail [7],[8].

The structure of TREM2 has been determined by X-ray crystallography and cryo-electron microscopy, which has significantly aided in understanding TREM2 function. The crystal structure of the human TREM2 extracellular domain (PDB ID: 5ELI) shows that the structure is a classical IgV-fold with a conserved disulfide bond with a positively charged surface that is expected to bind negatively charged lipid ligands and phospholipid-associated proteins that

aggregate during neurodegeneration. Structural studies have revealed that the key structural binding surface is a hydrophobic groove surrounded by charged patches, and it is this structural binding surface that has been a key informational basis for computational docking studies [7,8,9].

The ligands that have been identified to date are diverse compounds that reflect the receptor's role in the recognition and response to cellular damage and lipid dysregulations in neurodegeneration. They include soluble oligomeric forms of Aβ, apolipoprotein E, clusterin, sulfated glycosaminoglycans, (VLDL), and phosphatidylserine and phosphatidylethanolamine exposed on apoptotic cell membranes [8],[9]. The diversity of these ligands suggests that TREM2 can sense the lipid micro-environment and lipid status of the brain parenchyma.

The cellular changes induced by the TREM2 signaling are well documented in disease. During trophozoid deprivation conditions, TREM2 signaling is able to protect microglial cells from death by activating the PI3K-Akt-mTOR pathway and blocking apoptosis. It promotes the phagocytosis of microglia resulting in improved internalization and degradation of apoptotic neurons and Aβ aggregates in the lysosomes. It aids in the conversion of the metabolic pathway of the microglial cells from glycolysis to oxidation phosphorylation and fatty acid oxidation that is required to maintain the energy-consuming disease-associated microglial state. Moreover, TREM2 signaling regulates inflammatory cytokine release that normally results in a more anti-inflammatory microglial phenotype that prevents collateral neuronal damage [8],[9].

In contrast, loss-of-function mutations of TREM2, ectodomain shedding by the metalloprotease ADAM10 and a lack of engagement with ligands have all been shown to have a spectrum of negative effects which are variously manifested as decreased clustering of microglia around amyloid plaques, decreased amyloid compaction or clearance of Aβ, increased neuritic dystrophy around plaques, increased inflammatory signaling, and increased neurodegeneration. In parallel, the genetic deletion of Trem2 in mice is associated with an increased amyloid pathology, loss of disease-associated microglia, and impaired cognitive function [7],[8],[9].

Numerous lines of evidence support the translational relevance of TREM2 in human AD. In addition to the genetic risk associated with R47H, R62H, and other coding variants, soluble TREM2 (sTREM2) in the CSF has been shown to be a marker of microglial activation and disease progression, and to increase with tau pathology and be correlated with clinical deterioration. In a detailed systematic review of both TREM2 and sTREM2 in Alzheimer's disease, Zhang et al. (2025) identified that sTREM2 is a dynamic marker of microglial reactivity to amyloid and tau pathology and that there is a potential for TREM2 signaling enhancing drugs to be disease modifying drugs. Yin et al. (2024) conducted a second review, which again affirmed the role of TREM2 in regulating neuroinflammation, phagocytosis and microglial survival, and provided genetic, transcriptomic and proteomic evidence that TREM2 is involved in AD disease progression [2].

2.3 NATURAL POLYPHENOLS IN ALZHEIMER'S DISEASE: MECHANISMS AND EVIDENCE

In the past 20 years, there has been a compelling epidemiological, preclinical and, increasingly, clinical evidence that indicated that dietary and plant-derived polyphenols may afford to some extent neuroprotection against Alzheimer's disease. Some epidemiological studies have found inverse associations between dietary polyphenol patterns (e.g. Mediterranean and

Mediterranean-like diets) and cognitive decline and dementia in older individuals. These resulted in detailed mechanistic studies of particular polyphenolic compounds and their molecular targets and biological activities in AD models [3],[4],[10],[20].

2.3.1 Epigallocatechin-3-Gallate (EGCG)

EGCG is the most common polyphenol in green tea, and the most studied polyphenol anti-Alzheimer drug. C22H18O11, molecular weight 458.37 g/mol, chemically it is a flavan-3-ol that is esterified with gallic acid and has several catechol and galloyl hydroxyl groups contributing to its high antioxidant and metal-chelating properties. EGCG has been shown to act as a beta-sheet breaker in the secondary structure of Aβ; to disaggregate preformed Aβ fibrils; to chelate transition metal ions (copper, zinc, iron), which have Aβ oligomerization; and to inhibit the activity of BACE1 [3],[4].

EGCG inhibit activation of NF-κB, and reduce cyclooxygenase-2 (COX-2) expression and inducible nitric oxide synthase (iNOS), and suppress the production of tumor necrosis factor. Conducted a review on polyphenols and their neuroprotective effects, highlighting the multi-targeted profile of EGCG: anti-amyloid, antioxidant and anti-neuro-inflammatory action [3]. In several studies of APP/PS1 and 3xTg-AD transgenic mouse models, dietary supplementation with EGCG has been found to reduce amyloid plaque burden, reduce levels of neuroinflammatory markers and improve cognitive deficits in behavioral paradigms [3, 4].

2.3.2 Apigenin

One of the flavones found in high quantities in chamomile flowers is apigenin which is also found in large amounts in the flowers of parsley, celery and other edible plants. Apigenin has been the subject of much research in the field of disease because of its unique pharmacokinetic characteristics compared to other polyphenols, such as its metabolic stability and its low toxicity profile [4].

The anti-neuroinflammatory activity of apigenin can be achieved through multiple mechanisms, including the modulation signaling pathways, repression of microglial activation markers and cytokines. A review by Özge Şahin et al. (2025) reported that apigenin has been shown to have neuroprotective properties in diseases, suggesting that its multi-target activity and anti-neuroinflammatory and anti-amyloidogenic effects render it a promising therapeutic agent [4]. A particular interest of apigenin is its ability to modulate microglial function and inflammatory signaling pathways, which makes it an interesting candidate for further studies as a potential TREM2 modulator.

2.3.3 Curcumin

Turmeric (Curcuma longa) is a spice used for centuries as a potent medicinal herb in Ayurvedic and Curcumin is the major polyphenolic pigment present in turmeric. Curcumin has received extensive preclinical studies and a few clinical trials and has been extensively studied natural in Alzheimer's studies [4],[5].

Curcumin's anti-Alzheimer mechanisms are very wide-ranging. It directly binds to and inhibits the aggregation of both Aβ and tau, disaggregates preformed Aβ aggregates, chelates metal ions, inhibits BACE1 and gamma secretase activity and activates Nrf2-mediated antioxidant responses, and has potent inflamation stopper and STAT3 signaling pathways. Specifically, Chen et al. (2025) carried out a detailed review on polyphenols, namely curcumin for AD, and conducted an extensive analysis of their molecular and therapeutic mechanisms, based on in silico data [5]. Even with these promising preclinical characteristics, the clinical translation of curcumin has been limited by very low aqueous solubility, low oral bioavailability and rapid

metabolic inactivation that has led to the development of interest in the formulation of curcumin into nanoparticles and the development of structural analogues that have better pharmacokinetic properties [4],[5].

2.3.4 Resveratrol

A stilbene polyphenol, produced by a variety of plant species injury and pathogen attack, and is particularly abundant in the skin of red grapes, red wine, berries and peanuts. The involvement of resveratrol in the activation of the NAD, a central control point for cellular metabolism, stress response and longevity pathways, is the primary reason for the extensive research into its role in aging.

In disease models, resveratrol has neuroprotective effects, activating SIRT1, which results in the removal of misfolded tau and the clearance of Aβ oligomers by autophagy. It also has an effect on AMPK signaling, inhibits the activation of NF-κB and NLRP3 inflammasome, prevents mitochondrial dysfunction and oxidative stress, and regulates APP processing to decrease Aβ production. Zhang et al. (2021) summarized the role of polyphenols, such as resveratrol, as modulators of neuroinflammation and neurodegeneration in Alzheimer's disease and its capacity to regulate microglial activation states and inflammatory signaling networks [10]. For mild to moderate AD, the clinical trials with resveratrol have been found to be generally safe and well-tolerated, with some evidence of its effects on CSF Aβ40 levels and on neuroinflammatory biomarkers, however, efficacy outcomes have been inconsistent [4],[10].

2.4 MOLECULAR DOCKING IN NEUROPHARMACOLOGY: METHODS AND APPLICATIONS

The molecular docking technique is an essential instrument in drug discovery when dealing with neurodegenerative diseases since it is able to identify lead compounds and to explain the interaction mechanism between these compounds and disease-related protein targets, in a fast and economical manner. The molecular docking problem can be divided into three related computational problems: (1) the conformational and positional search algorithm for samples; (2) the scoring function that evaluates and ranks samples; (3) the protein representation to capture conformation features relevant to the binding of the ligand [11],[12].

Developed at the Scripps Research Institute by Trott and Olson and published in 2010, AutoDock Vina is based on an iterated local search global optimizer, a gradient optimizer for pose refinement, and an empirical scoring function that is calibrated using experimental protein-ligand co-crystal structures and binding affinity data. The prediction of binding is based on a scoring function that combines the steric (repulsive/dispersive) and hydrophobic/hydrogen bonding components, and a score below 0 kcal/mol indicates that the molecule will bind better [11]. In this present study, the program PyRx was used as a graphical interface of AutoDock Vina which is a platform for 3D protein preparation, docking and analysis of docking results, thus significantly decreasing technical barriers to HTS-VS [11].

A major question is whether the molecular docking predictions are correct or not, which is highly dependent on the accuracy of the input protein structure, the completeness and accuracy of the ligand preparation, including the correct protonation state and definition of the torsions, and the selection of an appropriate search space (grid box) that covers the biologically relevant binding site sufficiently. If binding site is not well defined from the experimental data, blind docking over the entire protein surface (as employed here) is an unbiased approach for identifying the preferred binding site [11],[12].

18 Once the top-ranked docked poses are obtained, it's straightforward to characterize the non-covalent interactions between the ligand and receptor in the visualization software, such as BIOVIA Discovery Studio Visualizer. All these interactions contribute to binding affinity and selectivity in different ways, and includes conventional hydrogen bonds (with donor-acceptor distances below 3.5Å), pi-cation (aromatic rings with positively charged residues) interactions, pi-pi stacking (face-to-face and T-shaped) interactions and halogen bonds [12].

In the field of TREM2 and neuroinflammation research, Ulland and Colonna (2020) reviewed the mechanism of microglial activation and the involvement of TREM2 in Alzheimer, detailing TREM2's its potential as a drug target, and examining strategies to modulate TREM2 function in Alzheimer's disease [7]. Deczkowska comprehensively studied the physiology, pathology and therapeutic potential of the TREM2 signaling pathway, providing mechanistic rationale for targeting TREM2 in neurodegeneration [8]. Sudom et al. (2023) demonstrated that pharmacological modulation of TREM2 activity can influence microglial responses and amyloid pathology in AD mouse models, which suggests that TREM2 is a potential therapeutic target [9].

Previous computational docking performed to understand the interactions of natural compounds with TREM2; this is still an emerging area. Vicente-Zurdo et al. (2024) and Özge Şahin et al. (2025) both investigated the molecular mechanisms and in silico data for polyphenols as neuroprotective agents in disease, providing insights into the role of docking in understanding protein-ligand interactions and predicting bioactivity [3],[4]. The current study is linked to the in silico use of computational methods by investigating polyphenolic compounds with the help of in silico methods in the context of Alzheimer's disease specifically [5]. These publications provide the scientific rationale and methodological precedent for the in-silico evaluation of natural polyphenols as TREM2 modulators which took place in this dissertation.

2.5 GAP ANALYSIS AND RATIONALE FOR THE PRESENT STUDY.

Although the pathological importance of TREM2 dysfunction in disease has been well established, and the neuroprotective role of polyphenolic compounds has been corroborated by numerous publications, polyphenolic compounds and the TREM2 protein have never been systematically characterized by computational docking analysis. Previous molecular docking investigations of polyphenols for AD have been mostly directed toward the classical enzymatic targets (acetylcholinesterase, BACE1, and GSK-3β), and with only a few studies targeting immune receptor targets (TREM2) [3],[4],[5].

In addition, although the genetic and functional evidence for TREM2 as a primary regulator of risk and progression of disease is undeniable, and several biopharmaceutical strategies to enhance TREM2 signaling are in the preclinical and early clinical development, the possibility of naturally occurring small molecules that can modulate TREM2 function remains unexplored. Given the multi-target neuroprotective properties, the excellent safety profiles, brain permeability, and anti-neuroinflammatory effects of natural polyphenols, this class of compounds is an interesting group of potential TREM2 modulators that should be explored systematically using computational tools [7],[8],[9].

To fill this gap, the present study marks to systematically analyze the molecular docking of four representative polyphenols (EGCG, apigenin, curcumin, and resveratrol) with the TREM2 crystal structure using both binding affinity and binding pose as criteria, as well as to identify major interacting residues and to interpret the results TREM2 biology and disease pathogenesis. The results will be used to offer a mechanistic computational reason for

prioritizing candidate polyphenols to be further validated through experimental testing in cell-based and animal models of Alzheimer.

CHAPTER 3 : METHODOLOGY

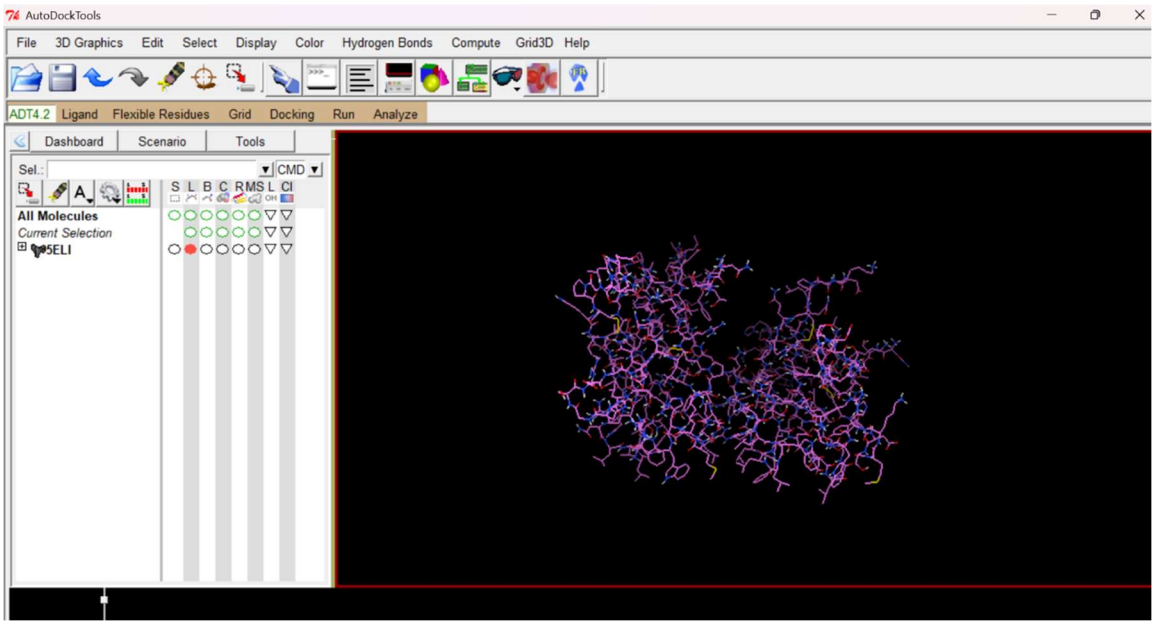
Molecular docking helps to find out the binding interactions between TREM 2 and a panel of commonly used Natural plant-derived polyphenols for example epigallocatechin gallate (EGCG), apigenin, curcumin, and resveratrol to predict their potential to stabilize IL-11 through favorable interactions. Here, we used PyRx for virtual screening of multiple excipients (ligands) targeting TREM 2. The following paragraphs detail the approach utilized for ligand selection, protein preparation, molecular docking, and validation procedures.

3.1 PROTEIN STRUCTURE RETRIEVAL AND PREPARATION

• The 3D structure of (TREM2) was retrieved From the AlphaFold Protein structure Database. The predicted structure was produced from the AlphaFold2 deep learning model created by DeepMind and EMBL-EBI.

• The prediction confidence for every residue was eliminated using the pLDDT scores provided. Regions with low confidence were visually checked and, if necessary, were removed from docking studies to maintain the reliability of interaction predictions.

• All water molecules and heteroatoms were removed using Auto Dock Tools. Polar hydrogens and Gasteiger charges were assigned. The protein was stored in PDBQT format for docking.



Screenshot showing loading of protein in Auto Dock 4.0

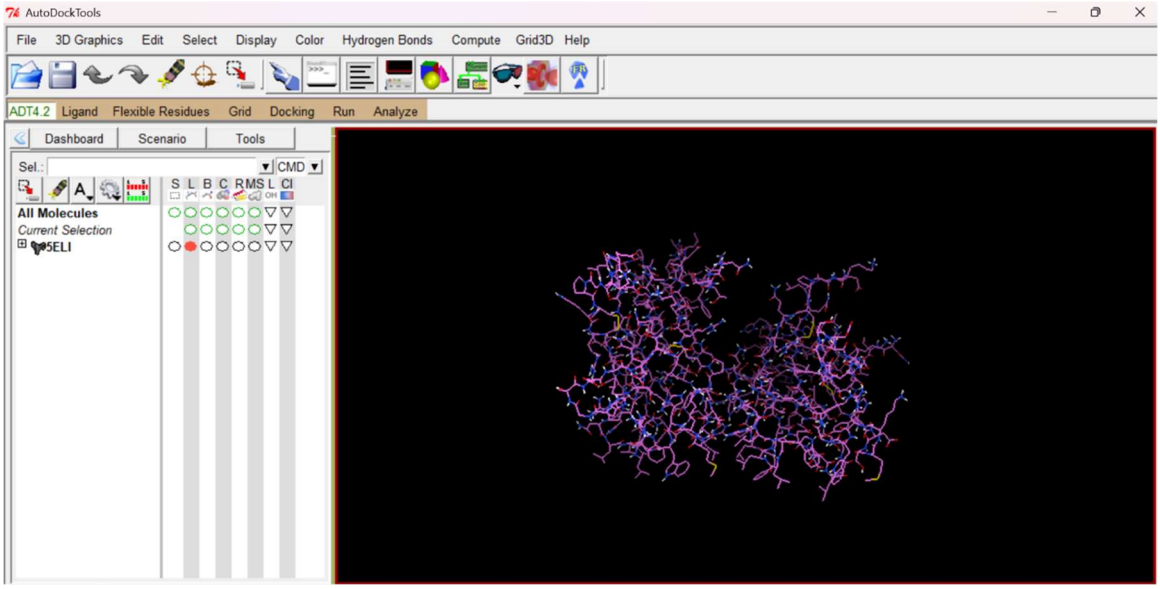


Fig 3.2: Screenshot showing protein after modification

1. Download the ligand

Visit PubChem at www.pubchem.ncbi.nlm.nih.gov.

• 3D Structures of EGCG, Apigenin, Curcumin, Resveratrol, were fetched from PubChem (https://pubchem.ncbi.nim. nih.gov/in) and were converted into PDBQT format using Auto Dock Tools.

• Geometry optimization and torsion tree definitions were applied to prepare flexible ligand

2.Performing docking using pyrx

Auto dock Vina will be the docking tool that we use. We use the Vina algorithm to dock it in Pyrx. Launch Pyrx GUI and followed the steps given below:

2.1Protein Loading

• Select "File" -> "Load Molecule" or simply click the first icon in the upper left corner. Choose the protein structure that you downloaded. referred to here as "TREM 2"

• Convert pdb format of protein to pdbqt by right clicking on TREM 2 then on display and now select macromolecule.

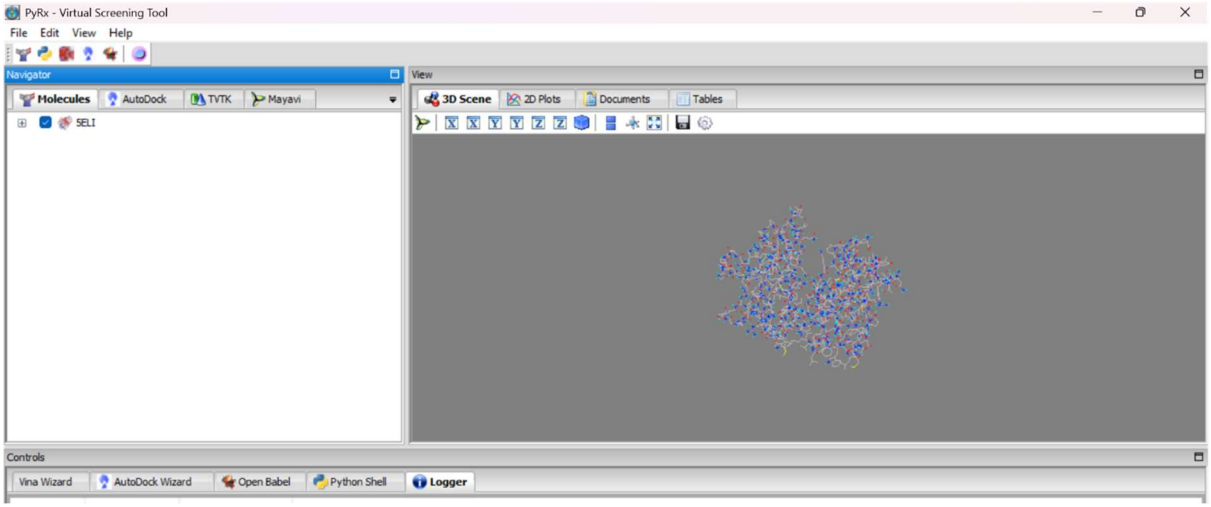


Fig 3.3 Screenshot showing conversion of protein file pdb to pdbqt

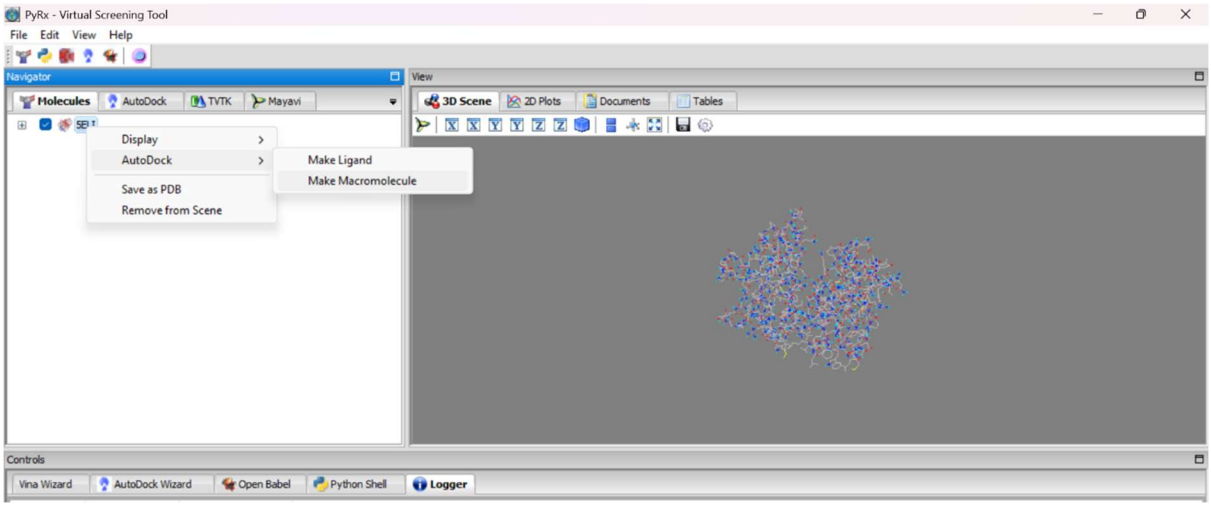


Fig 3.4 Conversion of protein file pdb to pdbqt

2.2 LIGAND LOADING

• In PyRx, click on OpenBabel and select on insert new item present on bottom right corner.

• Now select each ligand from folder one by one and upload it.

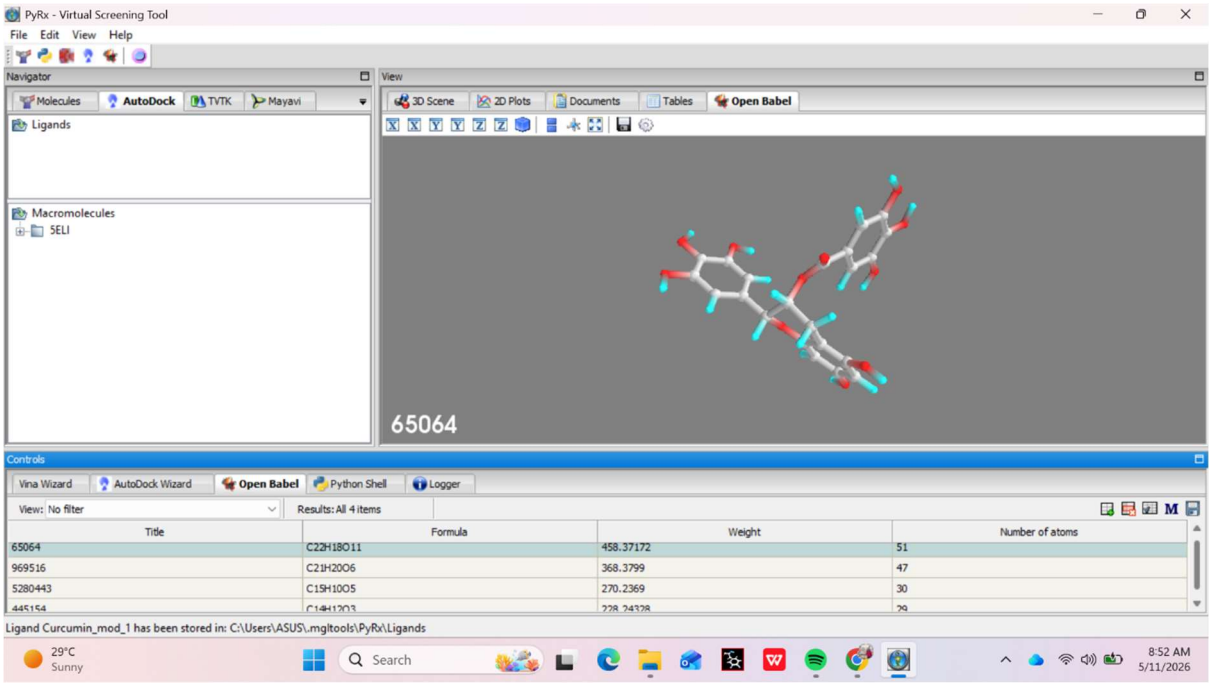


Fig 3.5: Screenshot showing loading of ligands

• After uploading all ligands, right click on ligand and select minimize all to decrease the energy.

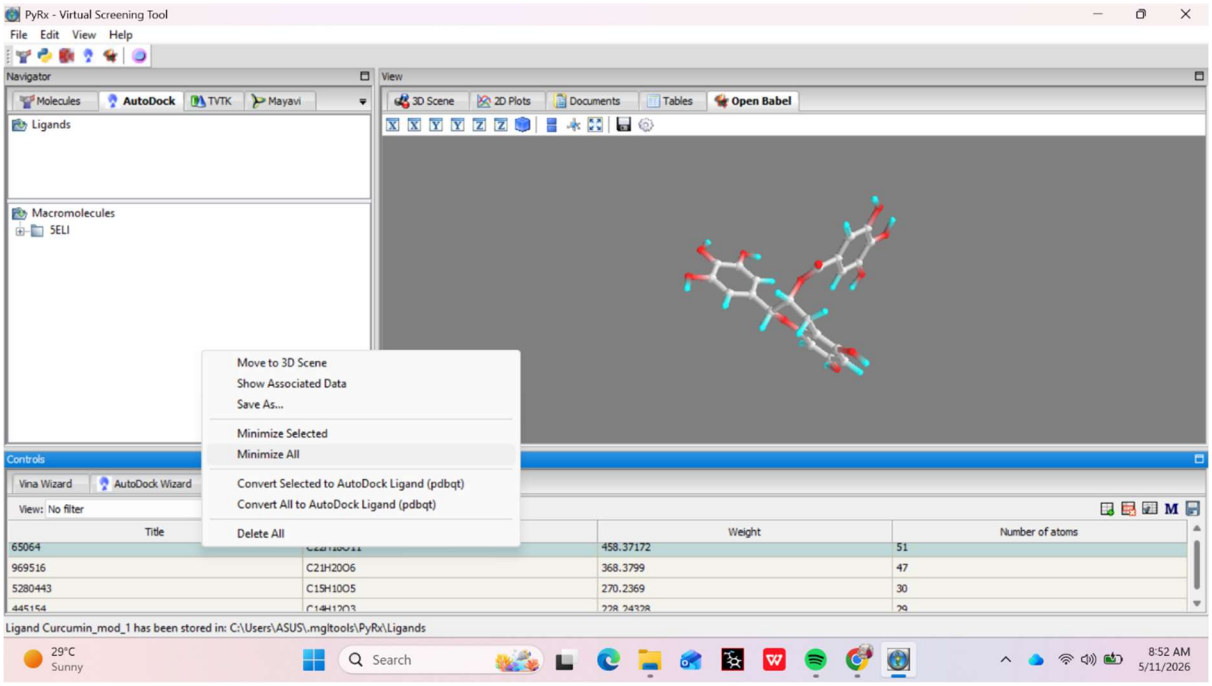


Fig 3.6: Screenshot showing minimization of energy of ligand

• Again, right click and select convert all to AutoDock ligand (pdbqt) to convert all ligands to pdbqt format.

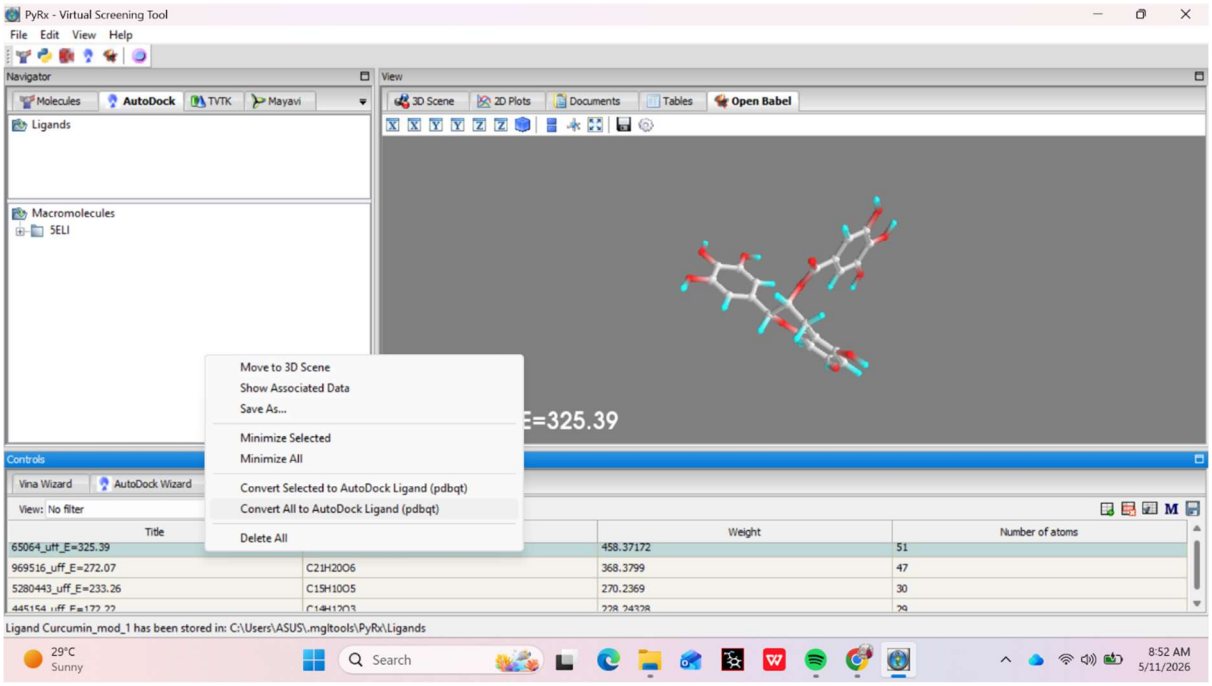


Fig 3.7: Screenshot showing conversion of all ligands to pdbqt

3 Defining ligands and proteins

The loaded protein and ligand are shown under the "Molecules" tab. It is now necessary to identify which is a ligand and which is a protein. • Right-click on the protein –> “Autodock” > “Make Macromolecule” to accomplish that. Perform a right-click on the ligand, select "Autodock," then "Make Ligand."After that, you'll see that it has automatically prepared their PDBQT files under the 'Autodock' page.

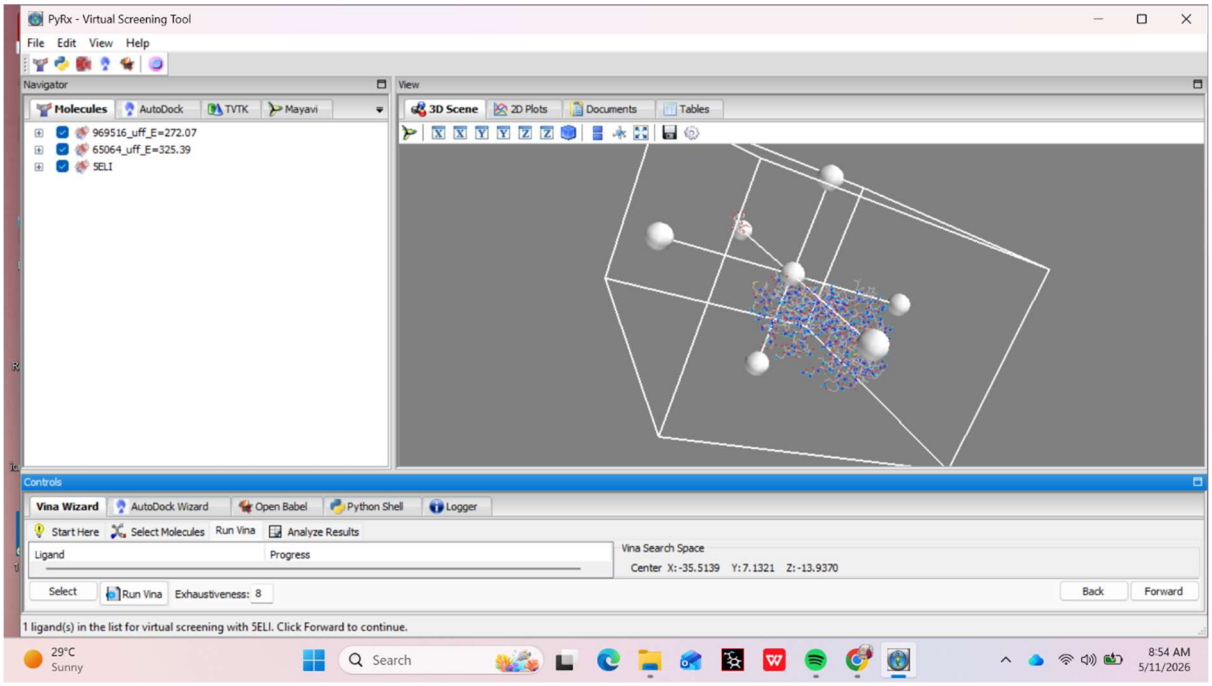


Fig 3.9: Screenshot showing grid box

4 Defining grid box

• Now click on Vina Wizard and select start option on bottom right corner. Start selecting protein and ligand one by one by pressing shift and control button.

• Click on forward. Grid box appears. Return to the 'Molecules' tab located on the right-hand side. Click the loaded protein's "+" symbol.

• All of the residues in the chain will be visible to you. To choose the binding residues, right-click on the residue and choose Atoms, Display, Label, and Atoms. The atoms will start to show up on the protein. Now make the appropriate adjustments to the grid box so that it contains all of the selected residues. The ligand does not need to be enclosed within the grid box.

5 Running vina for docking

• To adjust the exhaustiveness, simply enter the desired number in the box located in the left bottom corner. Once everything has been adjusted, press the "Forward" button.

• Docking will begin, and the processing will be shown. The bottom panel will display the poses and their binding affinities after the docking process is complete. It will show all poses along with RMSD values. Save your file in excel sheet.

• Now analyze the result and the one ligand which has the highest energy with negative sign is selected. Again, open pyrx tab and click on Auto Dock and select macromolecule and select the ligand with highest binding energy. Now right click and select display then all models of the ligands get displayed and select your desired model and save it in pdb format.

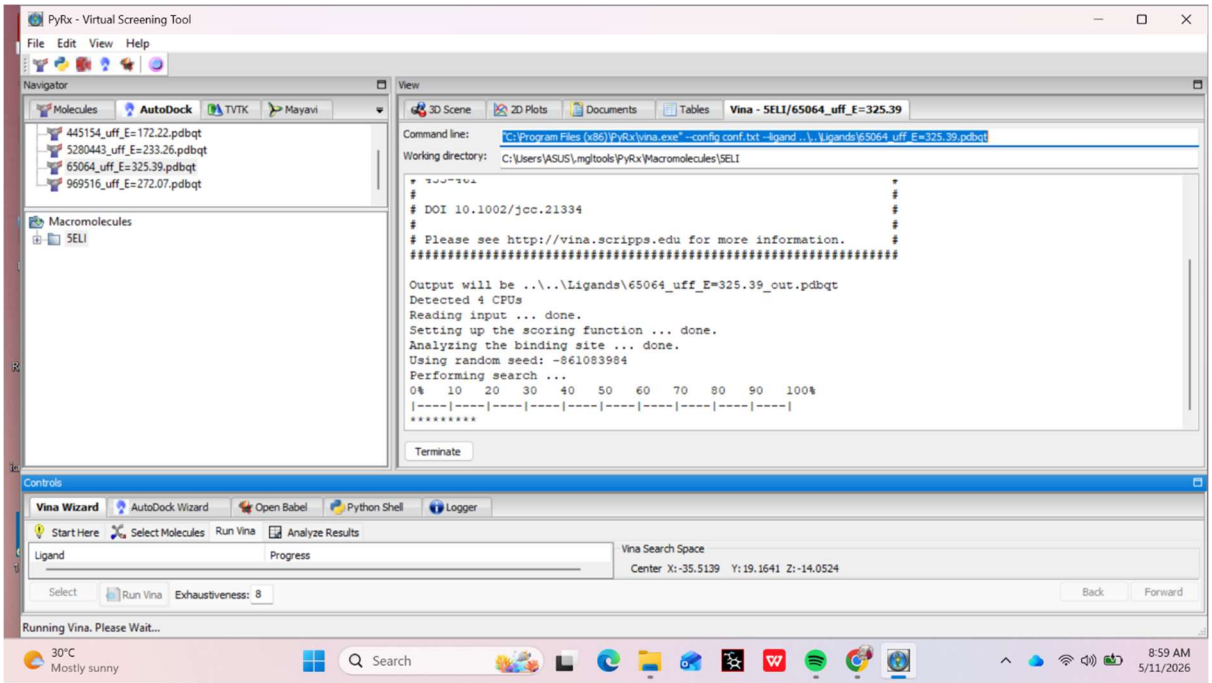


Fig 3.10: Screenshot showing running of vina wizard

6 Open Discovery Studio: -

Start a new project in Discovery Studio.

7 Protein Structure Import:

• Open Discovery Studio and import the structure of the target protein.

• Choose your protein structure file (such as *.pdb) by using File > Open.

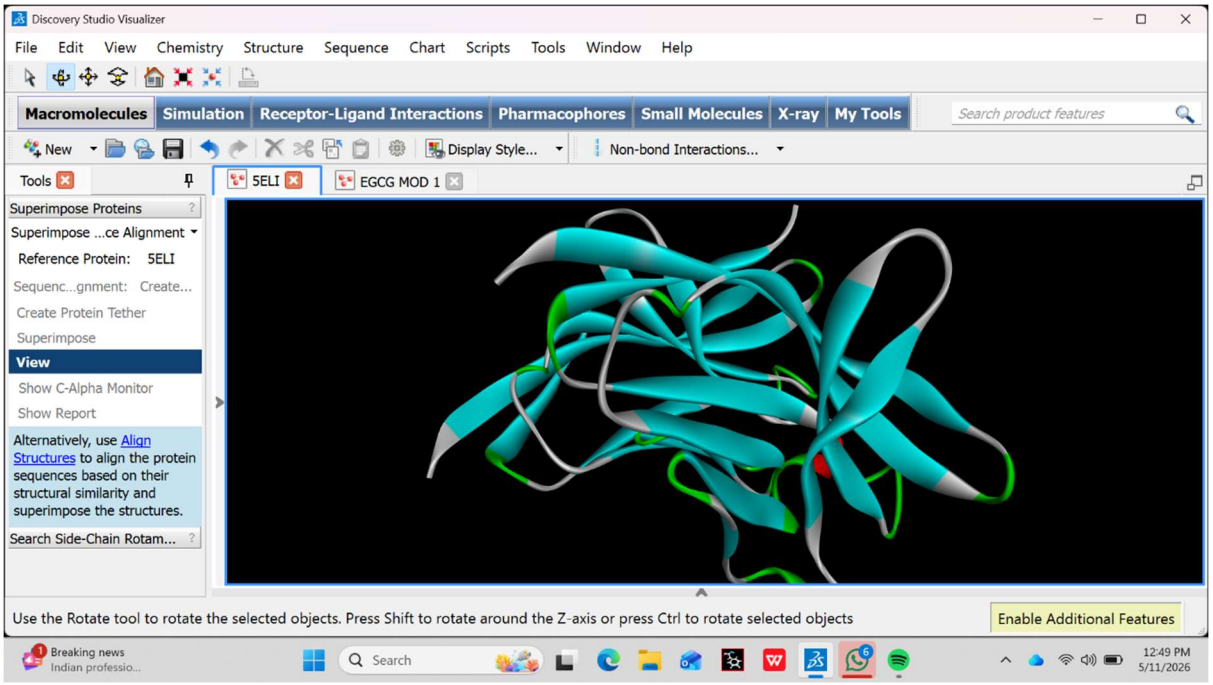


Fig 3.10: Screenshot showing visualization of protein structure in discovery studio

8 Import Docked Ligand Conformations:

• Import the docked ligand conformations.

• Use File > Open and select the converted ligand file (e.g., *.pdb or *.mol2).

9 Visualize Docked Poses:

• Display the protein and ligand together in the 3D workspace.

• Use the View > Sequence panel to ensure the correct structures are loaded.

• Adjust the display settings to show interactions clearly (e.g., stick or surface representations).

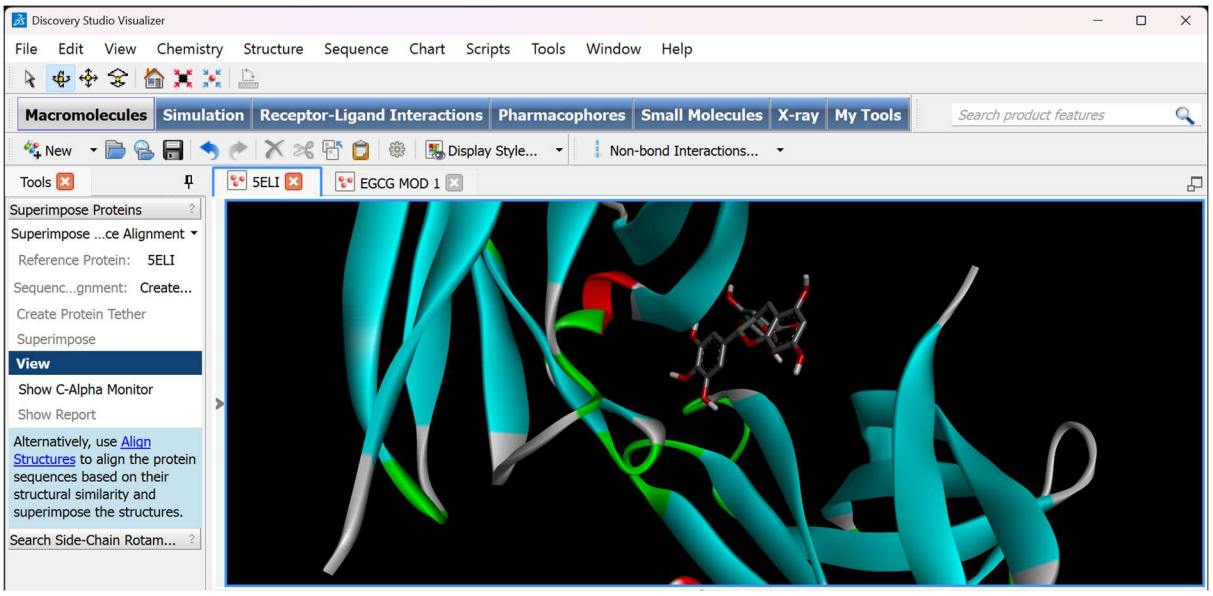


Fig 3.11: Screenshot showing EGCG binding with TREM 2

10 Analyse Binding Affinities:

• Check the binding affinities associated with each docked pose.

• This information can be found in the PyRx log files or the output summary from PyRx.

• Record the binding affinity scores (typically in kcal/mol) for reference.

11 Examine Binding Interactions:

• Use the Analyze > Receptor-Ligand Interactions tool to identify and visualize key interactions between the ligand and the protein.

20 • Highlight hydrogen bonds, salt bridges, and π-π stacking and hydrophobic interaction interactions. Check for consistency with known binding sites or important residues in the binding pocket.

12 Evaluate Docked Poses:

• Compare multiple docked poses to determine if there is a consensus binding mode.

• Use the View > Compare tool to overlay different conformations and evaluate their similarity.

CHAPTER 4 : RESULT

The binding affinities (kcal/mol) between TREM 2 and various natural plant polyphenols are summarized in the table below:

RESULTS

EXCIPIENT	BINDING ENERGY (kcal/mol)
Apigenin	-8.6
EGCG	-8.5
Curcumin	-7.1
Resveratrol	-6.3

Table: Binding Energies of TREM2 with selected excipients

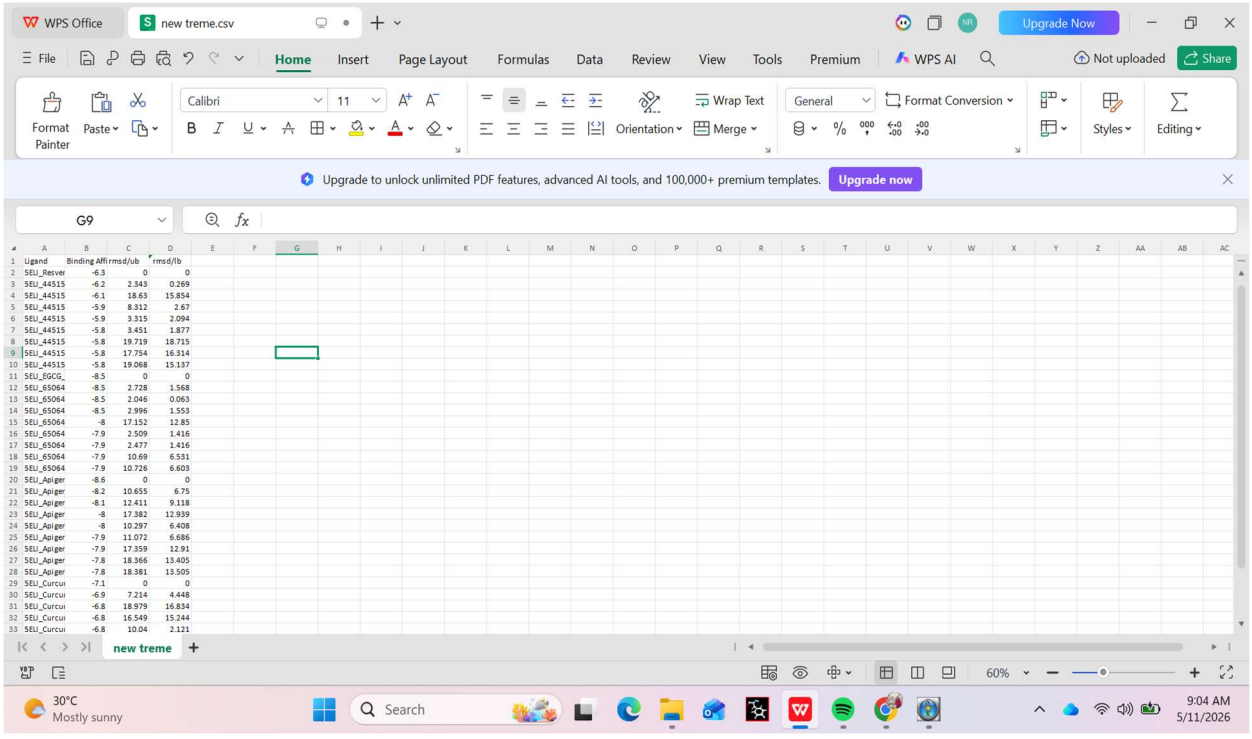


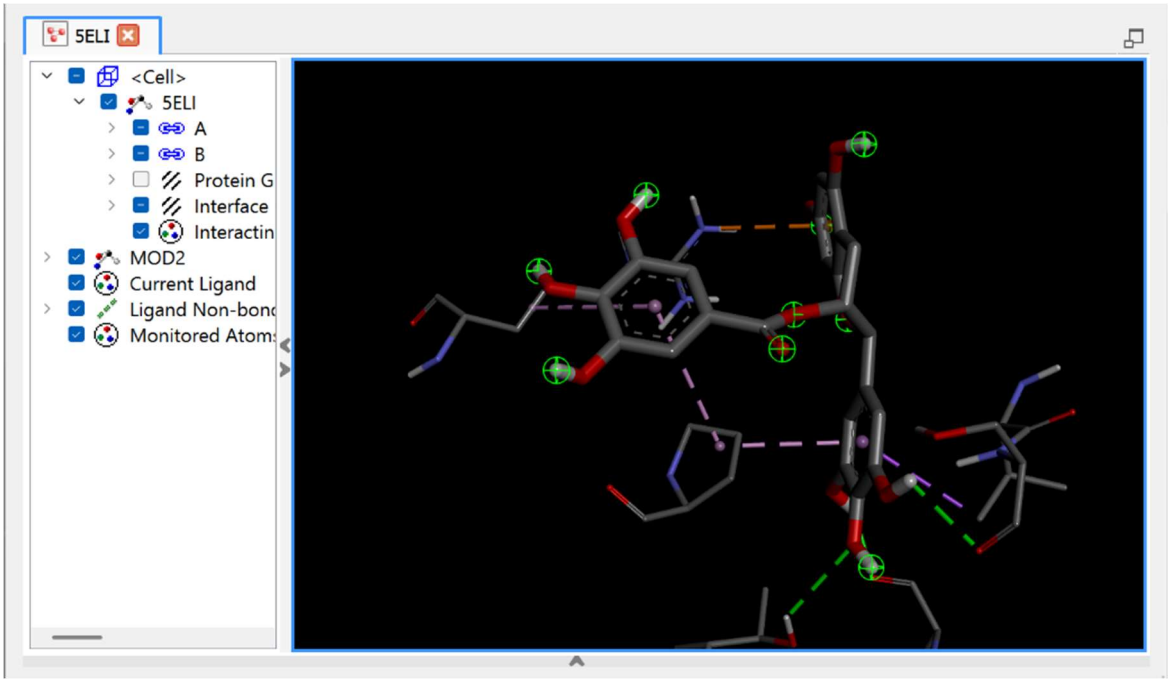
Fig 4.1: Screenshot showing Binding Affinities

These results indicate that Apigenin exhibited the strongest binding to TREM 2, closely followed by EGCG and Curcumin. In contrast, Resveratrol displayed weaker interactions, suggesting a reduced potential for stabilizing the protein.

Molecular docking showed that Apigenin(–8.6kcal/mol), EGCG (–8.5 kcal/mol), and had stronger binding affinities with TREM 2 compared to Curcumin (–7.1kcal/mol) and Resveratrol (–6.3 kcal/mol). Polyphenols formed multiple hydrogen bonds with key TREM 2 residues, suggesting better surface interaction and potential stabilizing effects.

DOCKING OF DIFFERENT EXCIPIENTS WITH TREM 2

Docking of TREM 2 with EGCG



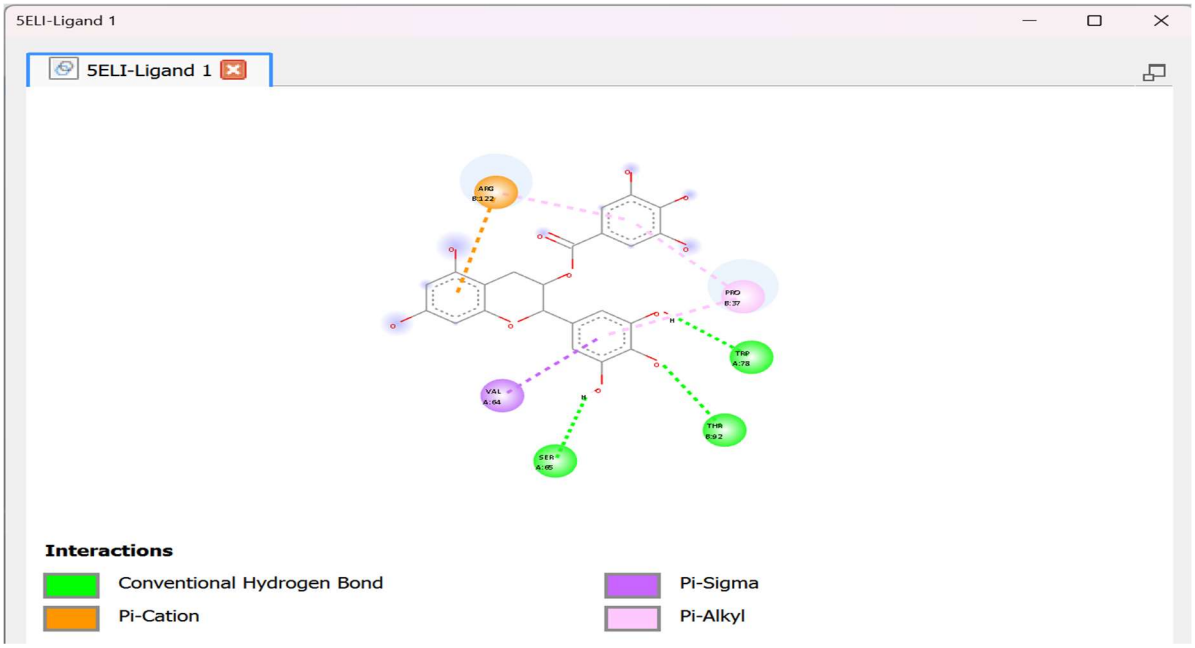


Figure 1: 2D and 3D structures of the TREM2–EGCG complex. EGCG binds with TREM2 through a Pi–Cation bond (ARG122) and a Pi–Sigma interaction (VAL). Several hydrophobic interactions, including Pi–Alkyl interactions (PRO), also contribute to the stabilization of the complex. In addition, conventional hydrogen bonds with THR residues further support the stability of the complex, suggesting a good fit of EGCG within the binding pocket of TREM2.

Docking of TREM 2 with Apigenin

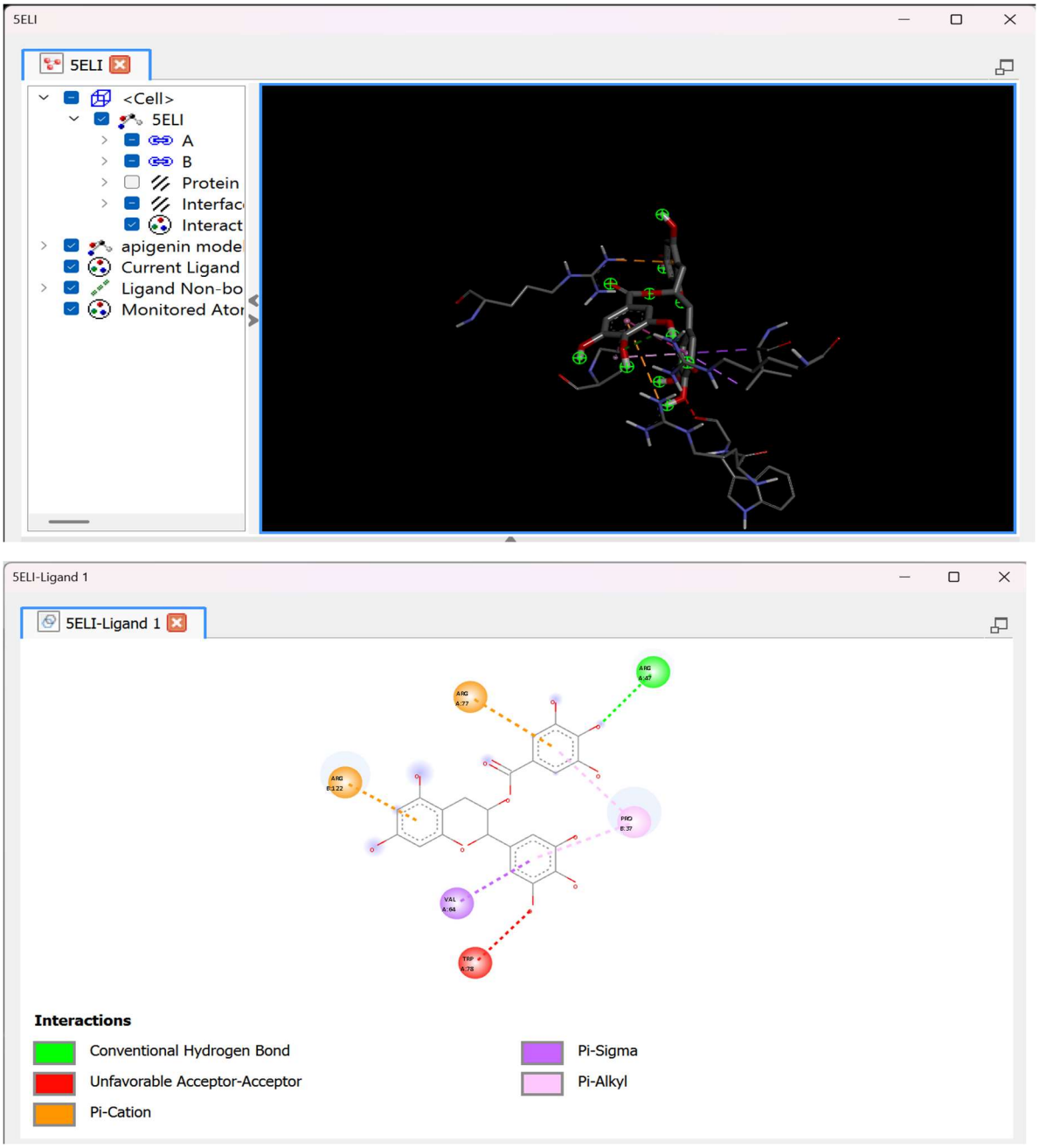


Figure 2: 2D and 3D structures of the TREM2–Apigenin complex. Apigenin interacts with TREM2 through Pi–Cation interactions (ARG47, ARG122) and a Pi–Sigma interaction (VAL64). Several hydrophobic interactions, including Pi–Alkyl interactions (PRO59), also contribute to the stabilization of the complex. A conventional hydrogen bond with ASN residues further supports ligand binding, while an unfavorable acceptor acceptor interaction with TRP residues is also observed. These interactions suggest that Apigenin fits well within the binding pocket of TREM2, contributing to the overall stability of the protein–ligand complex.

23

Docking of TREM 2 with Curcumin

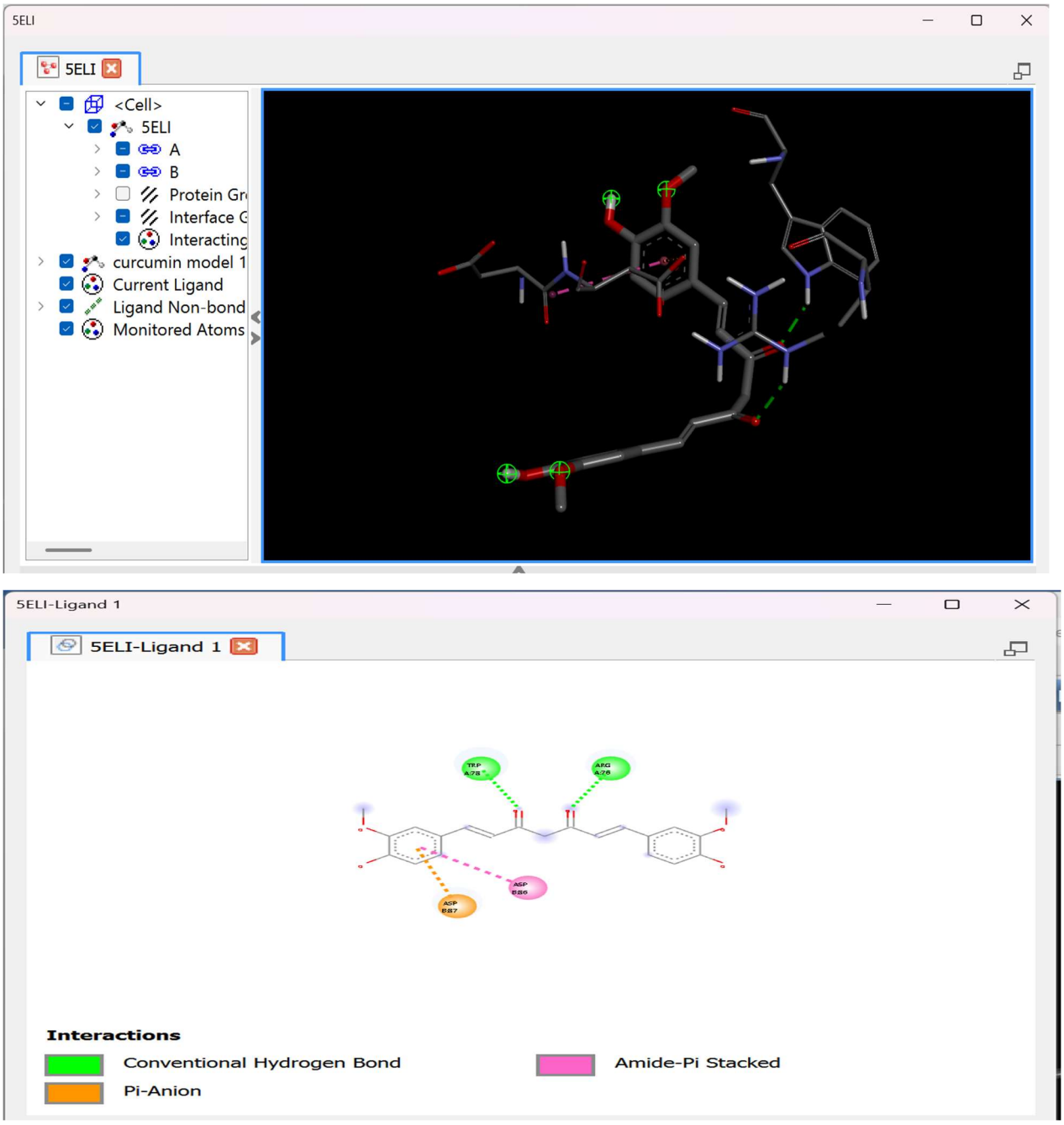


Figure 3: 2D and 3D structures of the TREM2–Curcumin complex. Curcumin interacts with TREM2 through conventional hydrogen bonds (ASN residues), which contribute to stabilizing the ligand with binding pocket. In addition, an Amide–Pi stacked interaction (PRO) are observed, further supporting the stability of the complex. These interactions suggest that Curcumin fits well within the binding pocket of TREM2, contributing to the formation of a stable protein–ligand.

Docking of TREM 2 with Resveratrol

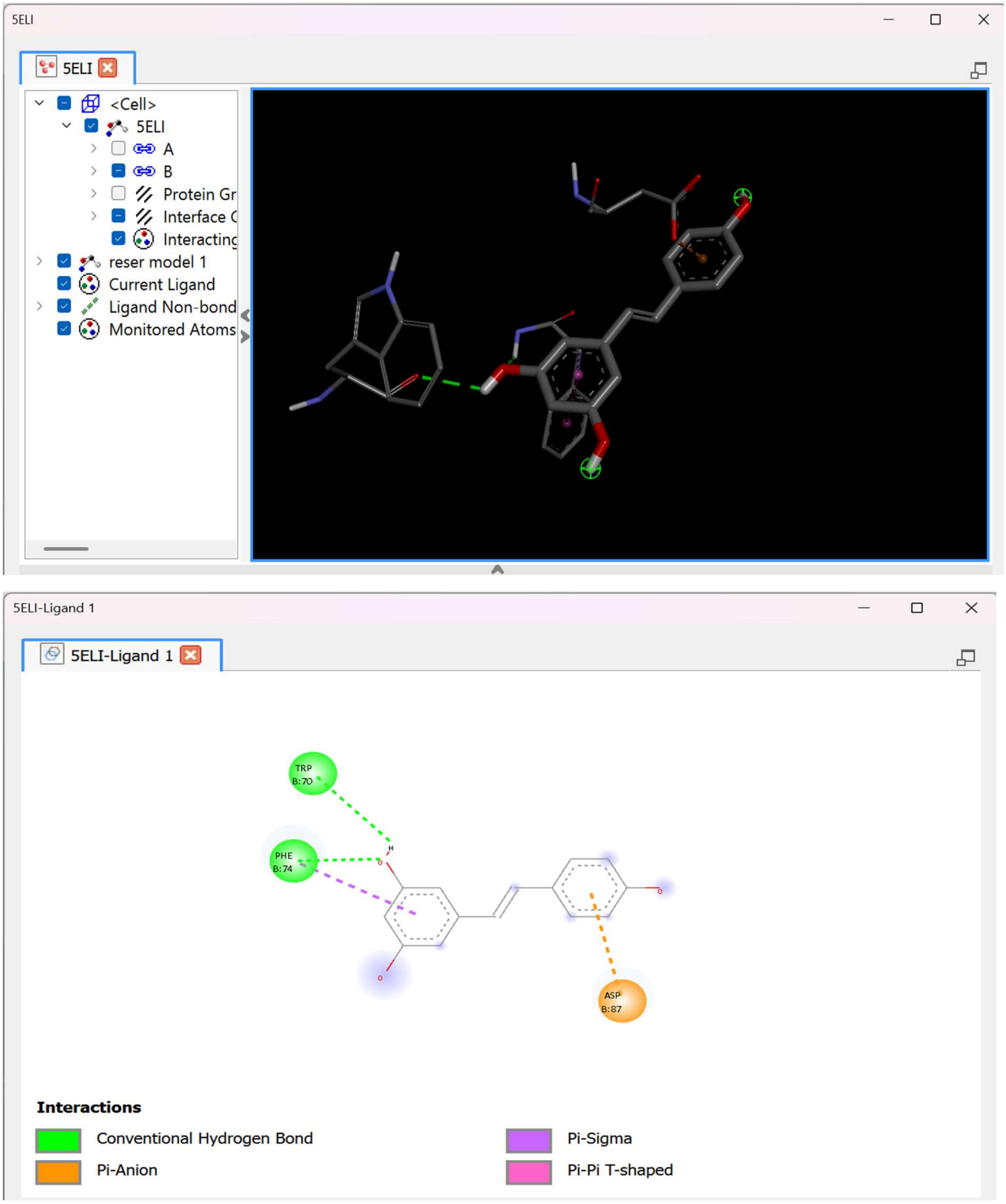


Figure 4: 2D and 3D structures of the TREM2–Resveratrol complex. Resveratrol interacts with TREM2 through conventional hydrogen bonds (TRP and PHE residues), which help stabilize the ligand binding pocket. In addition, a Pi–Sigma interaction (PHE) and a Pi–Pi T-shaped interaction further contributes to the stabilization of the complex. A Pi–Anion interaction with ASP is also observed, suggesting that Resveratrol fits well within the binding pocket of TREM2 and forms a stable protein–ligand complex.

DISCUSSION

TREM2 is a key player in the current knowledge of the mechanisms underlying Disease (AD) pathogenesis. TREM2 is a transmembrane glycoprotein that is mainly expressed on microglia cells and is a key sensor of the microenvironment of the brain that orchestrates microglial' state. Overall, the results of this study support the hypothesis that TREM2 plays an important role in microglial regulation of survival, phagocytosis of amyloid-β (Aβ) and neuroinflammation. In literature, impaired TREM2 signaling has been described as a disruption of microglial activation and lower clearance of Aβ plaques causing chronic inflammation and increased neurodegeneration. Therefore, the activation of this receptor to promote microglial metabolic fitness and shift to protective phenotypes is an important therapeutic approach.

Four natural phytochemicals namely epigallocatechin gallate (EGCG), apigenin, curcumin, and resveratrol were tested in the present molecular docking study for their interactions with the TREM2 receptor (PDB ID: 5ELI). The results from the computational analysis showed that Apigenin and EGCG had the binding affinities, of −8.6 kcal/mol and −8.5 kcal/mol,. These values indicate much stronger and more stable interactions in the receptor binding pocket for these compared to the Curcumin and Resveratrol (−7.1 and −6.3, respectively), both of which are naturally-derived antioxidants. These binding energies indicate that both Apigenin and EGCG are thermodynamically stable enough to interact with the V-set immunoglobulin domain of TREM2 in the correct way to be able to bind to the ligand and trigger immune signaling.

These particular ligands were chosen for their well known cytoprotective, and neurorestorative properties. Phenolics have recently been shown to regulate neuroinflammatory mechanisms and to reduce the toxic effects of amyloid in diverse models of AD. These general neuroprotective effects are complemented by our docking results, which implicate direct interaction with TREM2-associated pathways as a means by which Apigenin and EGCG may exert its effects. This may have a beneficial effect on microglial functions that are important to AD pathology, such as enhancing clearance of Aβ and the control of chronic neuroinflammation.

While these results are obtained by in silico computational analysis, it provides a sound basis for proposing a mechanistic justification for the prioritization of Apigenin and EGCG for experimental validation. These compounds in this study have a high binding affinity and stability, pointing to their potential as therapeutic modulators of TREM2 activity. Overall, the present experiment shows that natural phytochemicals can be effective modulators of microglial receptors and molecular docking is an effective preliminary screening technique to investigate candidate compounds that could modify the course of Alzheimer's Disease.

CHAPTER 5: CONCLUSION

5.1 SUMMARY OF FINDINGS

The present dissertation has made a systematic computational study of the binding interactions between four well characterized natural polyphenols (EGCG), apigenin, curcumin and resveratrol) and the extracellular immunoglobulin like domain of TREM2, PDB ID: 5ELI), an important microglial receptor that has recently been implicated in disease (AD) as a high-value target. The study created a detailed computational map of polyphenol–TREM2 interactions using molecular docking conducted via the virtual screening platform PyRx, with AutoDock Vina, followed by detailed interaction profiling in BIOVIA Discovery Studio Visualizer, with the aim to finding possible lead phytochemical candidates for TREM2-targeted neuroprotective therapy.

Through the docking process, a sequence of binding affinity was found for the four polyphenols. Apigenin was the most tightly bound compound with an affinity of −8.6 kcal/mol due to the presence of a dense network of such non-covalent interactions as Pi–Cation bonds with ARG47 and ARG122, hydrogen bonds with ASN residues and THR residues, and favorable hydrophobic Pi–Alkyl contacts with PRO59. The binding affinities of curcumin and resveratrol were moderate (−7.1 kcal/mol and −6.3 kcal/mol, respectively), with relatively less extensive binding networks, suggesting that the thermodynamic fit of both compounds in the TREM2 binding site was not as strong as that of the others under the conditions studied.

These computational results align with and expand upon the already established preclinical evidence, which shows that both apigenin and EGCG can be effective in the anti-neuroinflammatory and neuroprotective categories. The highly predicted binding of apigenin and EGCG to the TREM2 ligand-recognition interface provides a new level of molecular evidence for apigenin's and EGCG's reported effects on microglial activation, neuroinflammatory cytokine release, and ameliorating effects on amyloid-beta (Aβ) toxicity, which are tightly linked to the activities of TREM2 downstream in the context of AD pathogenesis. Combined these results are adequate for the first research goal of this thesis, and provide a useful computational basis for the nomination of apigenin and EGCG as high-priority candidates for TREM2 targeted drug discovery.

5.2 INTERPRETATION IN THE CONTEXT OF TREM2 BIOLOGY AND AD PATHOLOGY

The biological relevance of the docking results could best be understood within the context of TREM2's complex function in microglial physiology during AD progression. The TREM2-DAP12-PI3K-Akt, TREM2-DAP12-MAPK/ERK, and TREM2-DAP12-mTOR pathways drive microglial activation from the homeostatic state to a disease-associated microglial (DAM) phenotype, characterized by increased phagocytic capacity, metabolic reprogramming, and local containment of amyloid plaques. The high binding affinities at key residues such as ARG47, ARG122, which are located at or near the positively charged surface that binds to the TREM2 ligands, indicate that apigenin and EGCG may structurally mimic the endogenous TREM2 ligands from lipids or phospholipids, which would likely permit or enhance receptor activation in a disease context where lipids are dysregulated and Aβ is deposited.

This mechanistic hypothesis is further corroborated by the pharmacological profile of both the lead compounds. Apigenin has been shown to modulate TREM2-regulated neuroinflammatory signaling, and to enhance hippocampal neurogenesis, which are all processes downstream of or overlapping with microglial activation in the TREM2 pathway. The major bioactive tea catechin, EGCG, has been demonstrated to have abilities to inhibit Aβ aggregation, disaggregate preformed fibrils, chelate redox-active metal ions, and block multiple pro-inflammatory signaling axes in microglial and neuronal cell models. In the present study, the

additional mechanistic dimension is added that direct engagement of the TREM2 receptor may be one of the contributing mechanisms underpinning these broad neuroprotective effects; an entirely new and testable hypothesis.

Given that the binding affinities of curcumin and resveratrol are relatively weak, these compounds do not appear to be the primary targets of TREM2 in the brain; the other molecular mechanisms they are known to engage are additional targets and mechanisms that likely account for their experimentally demonstrated beneficial effects. This subtle interpretation highlights the importance of computational prioritisation in prioritising experimental resources to the most mechanistically plausible candidates.

5.3 LIMITATIONS OF THE STUDY

It is evident that there are some important limitations in the present investigation. Second, the molecular docking approach is a static, simplified view of the protein–ligand interaction process, which might not reflect the true dynamics of the process in a physiological context, and may not include conformational changes induced in the TREM2 receptor by the bound ligand. The stability of the predicted and under conditions that better reflect the membrane-associated, glycosylated microglial environment in which TREM2 normally functions would be assessed using molecular dynamics (MD) simulations, which were not undertaken in the present study.

Second, the docking simulation was done for the isolated extracellular immunoglobulin-like domain of TREM2 (PDB ID: 5ELI) without the transmembrane part, intracellular tail, and the DAP12 adaptor complex. This crystal structure is well understood and suitable for extracellular domain docking studies but does not fully capture the quaternary structural context in which TREM2 is found at the microglial plasma membrane in the context of the functional TREM2–DAP12 signaling complex. Finally, the tested polyphenols possess features that pose major translation challenges, especially their low aqueous solubility, poor oral bioavailability, and first-pass metabolism by hepatic enzymes in the gut, which are not taken into account during computational docking. To ensure sufficient in vivo brain exposure, in silico compound binding to TREM2 does not necessarily yield compounds with sufficient in vivo properties and may need in vivo pharmacokinetic optimization or nanoformulation strategies.

Last but not least, this present analysis was limited to four representative polyphenols. A more comprehensive virtual screening campaign that expands the phytochemical library to include other polyphenol semi-synthetic derivatives with better pharmacokinetic properties could result in even more candidates that have higher predicted TREM2 affinity and selectivity.

5.4 FUTURE DIRECTIONS

Results of this dissertation provide several scientifically promising and tractable avenues for further research. To assess the conformational stability, binding free energy and dynamic interaction profiles of the top-ranked complex of apigenin–TREM2 and EGCG–TREM2 over physiologically relevant timescales, molecular dynamics simulations of these predicted complexes should be performed in the immediate future. The binding free energy calculated will be useful in addition to the static docking scores and will give a more rigorous thermodynamic characterization of these interactions.

Experimental validation via in vitro biochemical assays should be used to directly measure the binding affinities of apigenin and EGCG for the recombinant TREM2 protein and to validate the computational predictions. Next, cell-based functional studies in primary microglia (michaels) and human iPSC-derived microglia (iMCHs) and TREM2-overexpressing

microglial cell lines should be performed to see if apigenin and EGCG treatment are able to recapitulate the functional outcomes of TREM2 (such as enhanced Aβ phagocytosis; reduced pro-inflammatory cytokine secretion; improved microglia survival under metabolic stress; and facilitated DAM transition in the presence of amyloid stimuli).

The evaluation in well-established transgenic models of AD (e.g., 5xFAD, APP/PS1 or 3xTg-AD), with outcomes measured as amyloid plaque burden, tau pathology, microglial activation state, and cognition in behavioral paradigms, would be a crucial translational step at the preclinical level. In addition, formulation approaches like prodrugs, lipid-based drug delivery systems, and encapsulation of polyphenols in nanoparticles should be investigated in parallel to enhance their BBB penetration and CNS bioavailability to overcome the pharmacokinetic limitations.

The present study can be further expanded in terms of computation by using virtual screening of larger natural product library in the binding site of TREM2, and then selecting potential modulators. Scaffold optimization by structure–activity relationship (SAR) analysis of apigenin and EGCG can further inform the rational design of improved apigenin and EGCG with better predicted TREM2 binding and metabolic stability, as well as better drug-likeness.

5.5 CONCLUDING REMARKS

Alzheimer's disease is one of the biggest unmet medical needs of the 21st century and the continued failure of amyloid-centric monotherapy approaches has highlighted the need for urgently identifying and validating new targets for therapeutic intervention that can overcome the full mechanistic complexity of disease. One of the emerging targets is TREM2, which receives input from microglial sensing, phagocytosis, metabolic adaptation and neuroprotection, which are all important processes needed to control and possibly reverse the progression of AD pathology. This study is a relevant scientific contribution to this new therapeutic frontier, demonstrating the strong and structurally specific predicted interactions of the naturally occurring, broadly safe anti-neuroinflammatory and blood–brain barrier permeable phytochemicals apigenin and EGCG with the TREM2 ligand-binding domain.

Although the in silico approach is not necessarily translatable to the clinic until further experimental studies are performed, the scientific and strategic rationale outlined in this study is relevant as a prioritization approach in the initial stages of drug discovery. In this way, natural polyphenols, which are pleiotropic neuroprotective agents, could have an unknown, but newly identified mechanism of action by direct modulation of TREM2, a receptor that is located at the intersection between neuroinflammation, microglial homeostasis and amyloid clearance in disease.

In conclusion, this dissertation shows that apigenin and EGCG are both computationally appealing phytochemicals able to interact with TREM2, and provides a computational basis for understanding the structural basis of predicted TREM2 interactions, as well as a solid computational platform for their subsequent incorporation into their respective experimental validation pipelines for development of effective plant-derived therapeutic strategies to treat disease. In doing so, this study adds to the accumulating evidence for the therapeutic modulation of microglial TREM2 signaling as a viable and scientifically compelling course of action in the battle against this crippling neurodegenerative disease.