



In silico study of α -synuclein pathology in Parkinson's disease and the potential of H_2O_2 as complementary therapy

[Estudio *in silico* de la patología de la α -sinucleína en la enfermedad de Parkinson y el potencial del H_2O_2 como terapia complementaria]

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Abstract

Context: Parkinson's disease (PD) is caused by protein aggregates composed of α -synuclein (α Syn) and its pathologies, such as mitochondrial dysfunction, loss of dopamine neurons, dysregulation of the dopamine transporter (DAT), and absorption of α Syn. Treatment with L-DOPA could not overcome it. Therefore, it requires complementary therapy with H_2O_2 low dose.

Aims: To evaluate the potential of H_2O_2 as a complementary therapy with L-DOPA.

Methods: The three-dimensional (3D) ligand and protein target structures were retrieved from PubChem and Protein Data Bank (PDB). H_2O_2 , as ligand, was converted using the Open Babel, which integrated with PyRx v.0.9.8. The molecular docking program used AutoDock Vina as a plug-in in PyRx v.0.9.8. Docking visualization results were analyzed using PyMOL v.2.5.5 (Schrodinger, LLC) and Discovery Studio 2021 Client v21.1.0.20298. The protein-protein docking program used an HDock server (Lab of Biophysics and Molecular Modeling, Huazhong University). Molecular dynamics simulation used YASARA Structure v.23.9.29.

Results: H_2O_2 could prevent the formation of α Syn fibrils by reducing the binding affinity between α Syn proteins. H_2O_2 could avoid mitochondrial dysfunction and loss of dopamine neurons by preventing α Syn binding to VDAC. Dysregulation of the DAT could be avoided by the ability of H_2O_2 to prevent α Syn binding DAT and avoid α Syn uptake by preventing α Syn binding to LRP1.

Conclusions: The H_2O_2 low dose has the potential to be a complementary therapy with L-DOPA.

Keywords: α -synuclein; bioinformatics; hydrogen peroxide; neurodegenerative disease.

Resumen

Contexto: La enfermedad de Parkinson (EP) es causada por agregados proteicos compuestos por α -sinucleína (α Syn) y sus patologías, como disfunción mitocondrial, pérdida de neuronas dopaminérgicas, desregulación del transportador de dopamina (DAT) y absorción de α Syn, no han sido superadas por el tratamiento con L-DOPA, por lo que se requiere terapia complementaria con dosis bajas de H_2O_2 .

Objetivos: Evaluar el potencial del H_2O_2 como terapia complementaria con L-DOPA.

Métodos: Las estructuras tridimensionales (3D) del ligando y la proteína diana se recuperaron de PubChem y Protein Data Bank (PDB). El H_2O_2 , como ligando, se convirtió utilizando Open Babel, que se integró con PyRx v.0.9.8. El programa de acoplamiento molecular utilizó AutoDock Vina como complemento en PyRx v.0.9.8. Los resultados de la visualización del acoplamiento se analizaron utilizando PyMOL v.2.5.5 (Schrodinger, LLC) y Discovery Studio 2021 Client v21.1.0.20298. El programa de acoplamiento proteína-proteína utilizó un servidor HDock (Laboratorio de Biofísica y Modelado Molecular, Universidad de Huazhong). La simulación de dinámica molecular utilizó YASARA Structure v.23.9.29.

Resultados: El H_2O_2 podría prevenir la formación de fibrillas de α Syn al reducir la afinidad de unión entre las proteínas α Syn. El H_2O_2 podría evitar la disfunción mitocondrial y la pérdida de neuronas dopaminérgicas al prevenir la unión de α Syn a VDAC. La desregulación del DAT podría evitarse mediante la capacidad del H_2O_2 de prevenir la unión de α Syn al DAT y evitar la captación de α Syn al prevenir la unión de α Syn a LRP1.

Conclusiones: El H_2O_2 a dosis bajas tiene potencial de ser una terapia complementaria con L-DOPA.

Palabras Clave: α -sinucleína; bioinformática; enfermedades neurodegenerativas; peróxido de hidrógeno.

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Abbreviations: ADP/ATP: Adenosine diphosphate/Adenosine triphosphate; BBB: blood-brain barrier; DAT: dopamine transporter; H_2O_2 : Hydrogen peroxide; IMM: inner membrane mitochondria; L-DOPA: levo-3,4-dihydroxyphenylalanine; LRP1: low-density lipoprotein receptor-related protein 1; MD: Molecular dynamics; OMM: outer membrane mitochondria; PD: Parkinson's disease; PDB: Protein Data Bank; Rg: radius of gyration; TH: tyrosine hydroxylase; VDAC: Voltage-Dependent Anion Channel; α Syn: α -synuclein.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder (Agnello and Ciacio, 2022). PD causes memory and cognitive impairment, tremors, and painful muscle contractions. This disease has no cure and worsen over time, but therapies and medications can reduce symptoms (Gitler et al., 2017; WHO, 2023). The prevalence of PD has increased in the last 25 years. In 2019, more than 8.5 million people had PD (WHO, 2023). PD is caused by deficiency of dopamine and the presence of intraneuronal insoluble protein aggregates of α -synuclein (α Syn) (referred to as Lewy pathology) (Jones et al., 2014; Hijaz and Volpicelli-Daley, 2020; Simon et al., 2020), mitochondrial dysfunction (Park et al., 2020; Pathak et al., 2013), the loss of dopamine neurons in the substantia nigra pars compacta (Hijaz and Volpicelli-Daley, 2020), dopamine transporter (DAT) dysregulation (Butler et al., 2015; Swant et al., 2011; Vaughan and Foster, 2013), and α Syn uptake (Chen et al., 2022). α Syn is a protein expressed in the presynaptic terminals of the central nervous system, where it regulates neurotransmitter release (Burré et al., 2014).

PD treatment is carried out by therapy of L-DOPA (levo-3,4-dihydroxyphenylalanine) or levodopa, which is a precursor to dopamine, to overcome dopamine deficiency in PD. In recent years, there have been problems associated with long-term use of L-DOPA (Jones et al., 2014). L-DOPA requires energy to be converted into dopamine, but this can be disrupted due to insufficient available energy because of mitochondrial dysfunction. This is associated with α Syn pathology and its interaction with voltage-dependent anion channel (VDAC), specifically the ability of α Syn to bind VDAC. These proteins are found in the mitochondrial outer membrane, as the main gateway for ADP/ATP (Risiglione et al., 2021; Rosencrans et al., 2021; Rostovtseva et al., 2015). α Syn can block the VDAC pore and can translocation into the mitochondrial inner membrane. This leads to decreased ATP production, representing mitochondrial dysfunction (Rostovtseva et al., 2015). L-DOPA has been converted into dopamine, and a certain amount of dopamine is recycled by reuptake via the DAT (Jones et al., 2014). After exocytosis release, dopamine binds to DAT and transported into the cytosol (Chen et al., 2010). DAT controls dopamine neurotransmission and maintains dopamine homeostasis (Swant et al., 2011). In PD, the dopamine reuptake is not functioning properly, causing excess dopamine in the synaptic cleft. This causes a lot of dopamine to bind to presyn-

aptic D2 autoreceptors, and overstimulation occurs. Then, it inhibits tyrosine hydroxylase (TH) activation, limiting the rate of endogenous dopamine production (Blackstone, 2009). Aggregation and spreading of α Syn protein are major pathological features of PD (Chen et al., 2022). α Syn oligomers, fibrils, and monomers, can be transferred between cells and taken up by neighbor cells (Kang et al., 2022). Spreading mechanism can mediated by low-density lipoprotein receptor-related protein 1 (LRP1), which acts as the main regulator of α Syn absorption (Chen et al., 2022; Robert et al., 2020). Excessive amounts of α Syn enter neuronal cells, making PD worsen.

Treatment using only L-DOPA cannot overcome some of the problems above. Giving L-DOPA becomes ineffective and requires complementary therapy using H_2O_2 low dose, which has neuroprotective potential (Widiyarti et al., 2023). H_2O_2 is known to be a toxic compound responsible for free radical-dependent neuronal damage, but in recent years, the 'bad reputation' of H_2O_2 has changed (Nisticò et al., 2008). In research conducted by Nisticò et al. (2008), the rescuing action of H_2O_2 showed that neuroprotection against *in vitro* OGD (oxygen/glucose deprivation) on CA1 hippocampal neurons was observed in slices treated with H_2O_2 (3 mM). Research conducted by Wang et al. (2020) states that pretreatment with low concentrations of H_2O_2 (50 μ M) can improve the ability of anti-oxidative stress and reduce bone marrow mesenchymal stem cells (BMSC) apoptosis under oxidative stress. Studies from Blanc et al. (2003) showed that 100 μ M of H_2O_2 increased ERK 1/2 phosphorylation in vascular smooth muscle cells (VSMC). According to Jiang et al. (2017) low concentrations of H_2O_2 promote the proliferation, migration, and formation of human umbilical vein endothelial cells (HUVECs). From some of the research above, it can be seen that low concentrations of H_2O_2 are very important for several physiological processes. The aim of this research is to determine the potential of H_2O_2 low dose in preventing the formation of α Syn fibrils, preventing α Syn from binding to VDAC, DAT, and LRP1 using *in silico* methods. Therefore, it is known that H_2O_2 low dose is a candidate for complementary therapy with L-DOPA.

MATERIAL AND METHODS

Protein target preparation

Ligand drug-likeness properties were determined with Lipinski's rules of 5. These methods were done

to ensure that ligands meet the requirements of drug-likeness properties to be used as drug molecules (Banerjee et al., 2024; Lestari et al., 2023). These properties are analyzed using the Protox III database, which can be accessed via <https://tox.charite.de> by inputting the canonical SMILES of the ligand. It could also be used to see toxicity predictions. The compound used in this study is H_2O_2 at low concentrations because it has neuroprotective abilities (Widyarti et al., 2023). The protein target three-dimensional (3D) crystal structure of *Homo sapiens* was retrieved from Protein Data Bank (PDB) (<https://www.rcsb.org/>). The Protein target consists of α Syn (PDB ID: 8A9L), VDAC (PDB ID: 5XDO), and DAT (PDB ID: AF-Q01959-F1), which were retrieved in pdb format and prepared by removing water molecules using Discovery Studio 2021 Client v21.1.0.20298, operated under Microsoft Windows 11. The crystal structure of LRP1 had yet to be elucidated (Boder et al., 2023). To perform homology modeling to obtain a model of LRP1, we used SWISS-MODEL (<https://swissmodel.expasy.org/>). First, we used the webserver Uniprot to find the fully sequenced of LRP1 human sequence (Uniprot #Q07954) (Boder et al., 2023). The sequence was copied and inputted onto SWISS MODEL for homology modeling.

Molecular docking

Hydrogen peroxide (CID: 784) as a ligand was retrieved in SDF format from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). The ligand preparation process was also carried out using Open Babel (Price et al., 2014) integrated into PyRx 0.9.8 software to lower or minimize the free energy of the ligand (Lestari et al., 2023). Then, it was converted into pdbqt format. AutoDock Vina integrated in PyRx v.0.9.8. was used for docking H_2O_2 - α Syn proteins. We used a rigid docking method in this study, with 50 exhausted and nine modes parameters and visualization used PyMOL v.2.5.5 (Schrodinger, LLC) and Discovery Studio 2021 Client. In the body, α Syn, some are in monomer form, and some have formed polymer (aggregation of α Syn) (Moriarty et al., 2013). Thus, the potential of H_2O_2 was known to prevent α Syn monomers from α Syn aggregation and prevent the binding of monomers or polymers α Syn to proteins target (VDAC, DAT, and LRP1). HDock server (from Lab of Biophysics and Molecular Modeling, Huazhong University) (<http://hdock.phys.hust.edu.cn/>) was used for protein-protein docking to form complexes: VDAC- α Syn, DAT- α Syn, and LRP1- α Syn (all in monomeric or polymeric α -Syn form, with and without H_2O_2). Then, the binding energy and binding site of each complex were analyzed. Validation of the docking method: This could be seen from the binding energy, which

had a negative value, indicating that the receptor-ligand interaction took place spontaneously. Free energy analysis was obtained from docking results using HDock by looking at the docking score that showed the free energy value and then comparing protein complexes with and without H_2O_2 . Chemical interaction of ligand and target protein could be seen from the docking result complex opened with Discovery Studio, which saw the ligand interaction of non-bond and unfavorable non-bond sections (category and type, to know the type of interaction).

Molecular dynamic

Molecular dynamics (MD) was performed simulation using YASARA Structure v.23.9.29, with forcefield AMBER 14. The variables for simulation included the water solvent with a density of 0.997 g/mL; ion concentration of NaCl 0.9%; pH 7.4; temperature 310K (Lestari et al., 2023); save interval was set in 25000 fs and duration of simulation in 20 ns. To evaluate the stability of the simulated system, it was necessary to analyze: molecular mechanic Poisson Boltzmann surface area (MMPBSA), root mean square deviation (RMSD), backbone root mean square fluctuation (RMSF), radius of gyration (Rg), and superimpose (Biswas et al., 2023; Chaudhuri et al., 2019). From MD results in tab.file format was changed to Microsoft Excel. Then, the values of MMPBSA, RMSD, RMSF, Rg, and superimpose of each complex were compared. Then, the data presentation was displayed as a picture with an insert chart in Microsoft Excel.

RESULTS AND DISCUSSION

To advance the discovery and development of new drugs, evaluation of similar 'drug-like' properties of molecules at an early stage of the discovery-research process was undertaken. The simplest and most widely used approach was the Lipinski Rule or Rule of Five (ROF), developed by Lipinski et al. (2012). It was a rule of thumb for evaluating drug-likeness or determining whether a chemical compound with a particular pharmacological or biological activity had properties that would make it an active drug. In the discovery set, the 'rule of 5' predicted that poor absorption or permeation was more likely when there were more than 5 H-bond donors, 10 H-bond acceptors, a molecular weight (MWT) greater than 500, and a calculated Log P (CLogP), greater than 5 (or MlogP. 4.15) (Lipinski et al., 2012). Ligands met Lipinski's rule of 5 (Table 1) and are included as safe compounds because it has a toxicity class ≥ 3 , with topological polar surface area (TPSA) also being between 0 and 140 (Lestari et al., 2023).

Table 1. The result of Lipinski rule from Prottox III.

Name	Hydrogen peroxide
Molecular weight (g/mol)	34.01
Number of hydrogen bond acceptors	2
Number of hydrogen bond donors	2
Number of atoms	2
Number of bonds	1
Number of rotatable bonds	0
Molecular refractivity (m ³ /mol)	5.26
Topological polar surface area (Å ²)	40.46
Octanol/water partition	0.02

Source: <https://tox.charite.de>

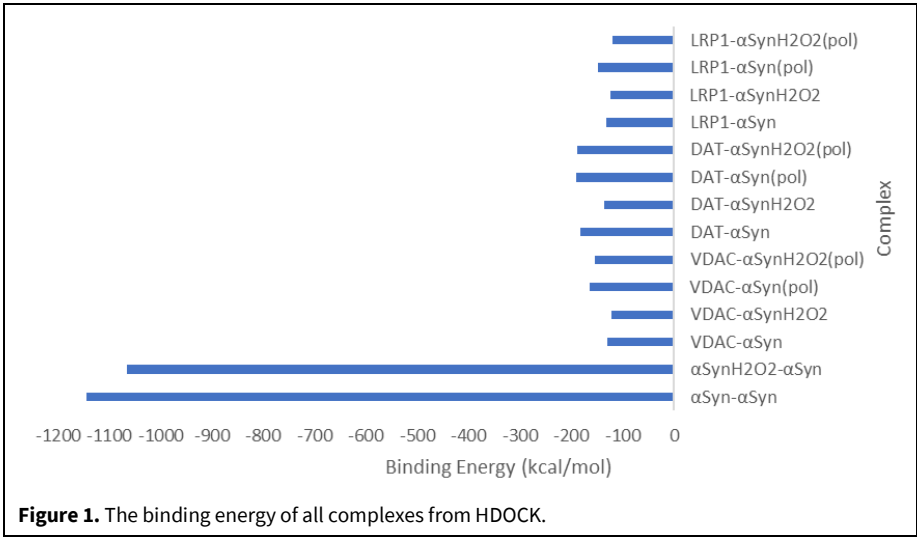


Figure 1. The binding energy of all complexes from HDock.

Potential of H₂O₂ molecule to reduce binding affinity

The potential of H₂O₂ to prevent the formation of αSyn fibrils and prevent αSyn from binding the protein target (VDAC, DAT, and LRP1) could be known by its ability to reduce the binding affinity value, change the interaction type and active site residues of the αSyn-protein target complex (all active site residues shown in supplementary Table S1). The docking result showed that the complexes without H₂O₂ molecules have a lower binding energy (Fig. 1), indicating that the complexes had a higher binding affinity than the complexes with H₂O₂. Because of the lower or more negative binding energy of the docking results, the binding affinity of ligand and protein target is higher (Li et al., 2021). There was a change in the interaction types and active sites on αSyn-protein target complexes (Table 2). This indicated that H₂O₂

can interfere with the active site residue on the target protein, making it not the active site residue that is bound.

Based on the results of the analysis showed the addition of unfavorable bonds, which were bonds that could affect stability. The formation of unfavorable bonds between protein-ligand complexes reduced the stability of the complex because this bond showed the repulsive force that occurs between two molecules and an atom (Dhorajiwala et al., 2019). Therefore, with the increase in unfavorable bonds, it was possible for αSyn to be released from its protein target. In the VDAC-αSynH₂O₂(pol) complex, the number of conventional hydrogen bonds and carbon-hydrogen bonds was reduced. This also reduced the stability of the complex because conventional hydrogen bonds were basically the fundamental stabilizing force in biomolecular structures (Horowitz and Trievel, 2012).

Table 2. Change in the active site and type of interaction of all complexes.

Complex	Change in the active site and type of interaction
αSyn-αSyn compared to αSyn H ₂ O ₂ αSyn	• GLU57 from conventional hydrogen bond → carbon-hydrogen bond. • ALA91 from hydrophobic bonds → carbon-hydrogen bond. • The electrostatic bond possessed by the αSyn monomer changes from the active residue. GLU57 to LYS58. • ALA53 and VAL48, conventional hydrogen → unfavorable bond.
VDAC-αSyn compared to VDAC- αSynH ₂ O ₂	• Forming new bonds that did not exist before, SER44 and GLU189 form conventional hydrogen. bonds and LEU10 forms a new hydrogen carbon bond. • There was a change in electrostatic bond residues from LYS20 to LYS32.
VDAC-αSyn _(pol) compared to VDAC-αSynH ₂ O _{2(pol)}	• Conventional hydrogen bond and carbon-hydrogen bonds in the VDAC-AsynH₂O_{2(pol)} complex. are reduced when compared to the amino acid residues in the VDAC-αSyn_(pol) complex. • There is an addition of amino acid residues in the hydrophobic, electrostatic and unfavorable bonds in the VDAC-AsynH₂O_{2(pol)} complex. • THR107 from unfavorable bonds → conventional hydrogen bond. • ARG218 from hydrogen bonds → electrostatic bond. • THR80 conventional hydrogen bond→ unfavorable. • ASN185 carbon-hydrogen bond →unfavorable bond.
DAT-αSyn compared to DAT- αSynH ₂ O ₂	• GLU608 unfavorable bond → conventional hydrogen bond. • ARG606 hydrophobic bond → unfavorable bond. • VAL620 and ARG615 electrostatic bond → conventional hydrogen bond.
DAT-αSynH ₂ O _{2(pol)} compared to DAT-αSyn _(pol)	• DAT-αSynH₂O_{2(pol)} has unfavorable bonds, while the DAT-αSyn_(pol) complex does not have unfavorable bonds at ARG515.
LRP1-αSyn compared to LRP1- αSynH ₂ O ₂	• ASN202 conventional hydrogen bond → unfavorable bond. • In the LRP1-Asyn complex, there is a sulfur-X interaction at residue CYS208.
LRP1-αSyn _(pol) compared to LRP1-αSynH ₂ O _{2(pol)}	• The LRP1-αSynH₂O_{2(pol)} complex has fewer conventional hydrogen interactions. • GLN122 unfavorable bond → conventional hydrogen bond. • ALA123 carbon-hydrogen bond → hydrophobic bond.

The presence of H₂O₂ molecules had the potential to reduce the binding stability of the complex. In the LRP1-αSyn complex, there is a sulfur-X. These interactions belong to noncovalent interactions involving sulfur atoms and play an important role in protein structure and function by contributing significantly to protein stability, folding, and biological activity (Kojasoy and Tantillo, 2023). Thus, the LRP1-αSyn complex was more stable than the LRP1-αSynH₂O₂. In conclusion, changes in interactions indicated that the presence of H₂O₂ molecules affects the interaction of αSyn with the protein target, and H₂O₂ molecule had the potential to prevent αSyn from binding to the protein target.

The potential of H₂O₂ molecule to prevent αSyn formation of fibrils

Fibrillogenesis and aggregation on αSyn (oligomers and amyloid fibrils) are highly toxic (Lashuel et al., 2013; Michel et al., 2016). Therapeutic interventions directed at reducing toxicity and aggregation of αSyn are needed to develop future treatments for PD (Moriarty et al., 2013). The total RMSD was used to confirm the stable nature of the protein-ligand com-

plex under simulated conditions (Alom et al., 2023). Lower values of total RMSD indicated more stable molecules (Biswas et al., 2023). RMSD provided information related to protein conformational changes. RMSD >2.50Å indicated conformational changes in protein structure, and RMSD >8.0Å indicated unfolding (Sumaryada et al., 2016). The average of total RMSD complexes with H₂O₂ (αSynH₂O₂ and αSynH₂O₂-αSyn) was higher (Fig. 2A). The total RMSD value was also in accordance with the value of Cα RMSD (Fig. 2B). Cα RMSD could indicate a conformational change in the target protein. The presence of H₂O₂ molecules increased the total and Cα RMSD value and underwent unfolding (Sumaryada et al., 2016), which caused the protein to be non-functional (Alaei et al., 2021), and the potential to reduce αSyn pathology. In native condition, αSyn is in an unfolded state, so the presence of H₂O₂ molecules makes αSyn in its native state, which prevents folding. Because the presence of folded monomers αSyn facilitated the formation of fibrils and led to the formation of Lewy bodies and the development of PD (Uversky, 2008). The presence of H₂O₂ molecules can prevent the formation of αSyn fibrils.

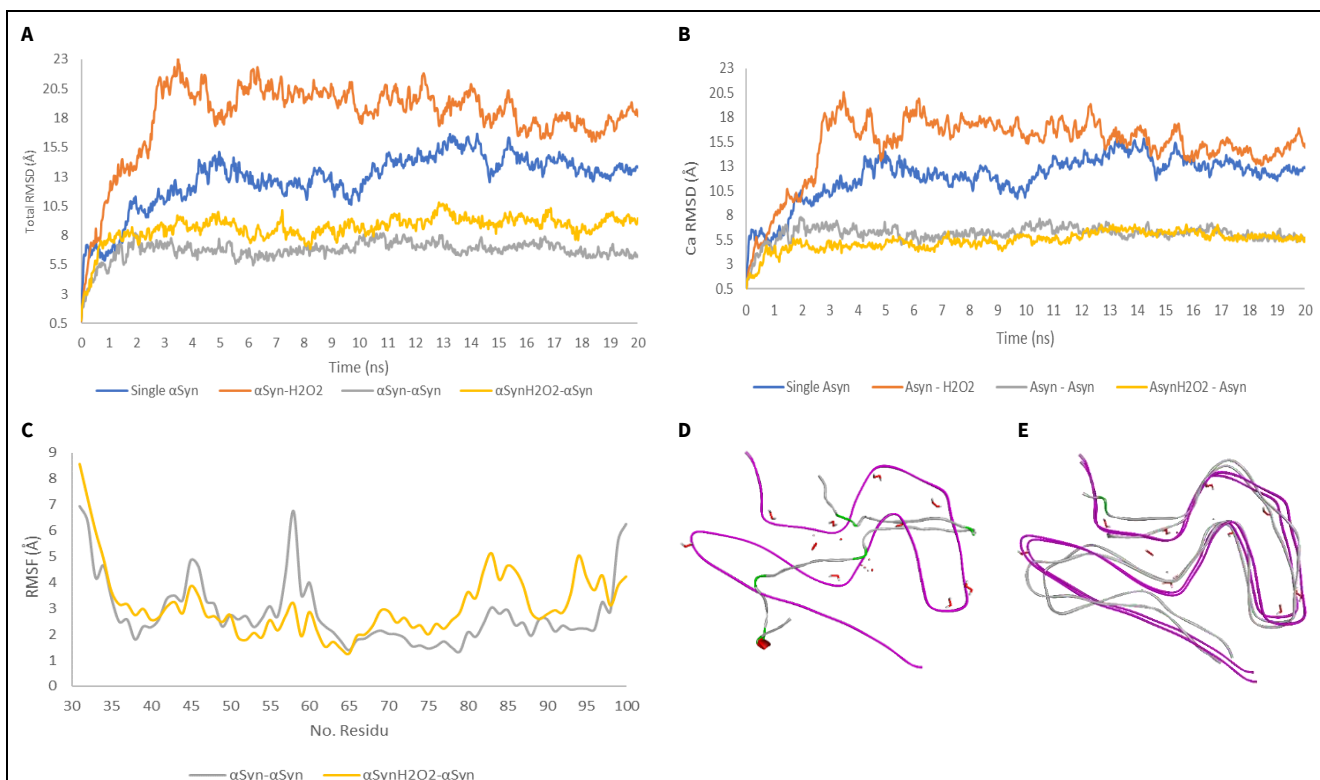


Figure 2. Molecular dynamics (MD) results of H_2O_2 potency to prevent the formation of α Syn fibrils.

(A) Total root mean square deviation (RMSD); (B) Ca RMSD; (C) root mean square fluctuation (RMSF) value of α Syn- α Syn and α Syn H_2O_2 - α Syn complex; (D) Superimpose of α Syn H_2O_2 complex; (E) Superimpose α Syn H_2O_2 - α Syn complex (purple: before MD simulation, grey: after MD simulation).

The RMSD result was supported by the RMSF analysis (Fig. 2C). H_2O_2 molecule increased the RMSF in the active site. RMSF was used to measure fluctuations in the interaction or position of ligands with amino acid residues (Farmer et al., 2017).

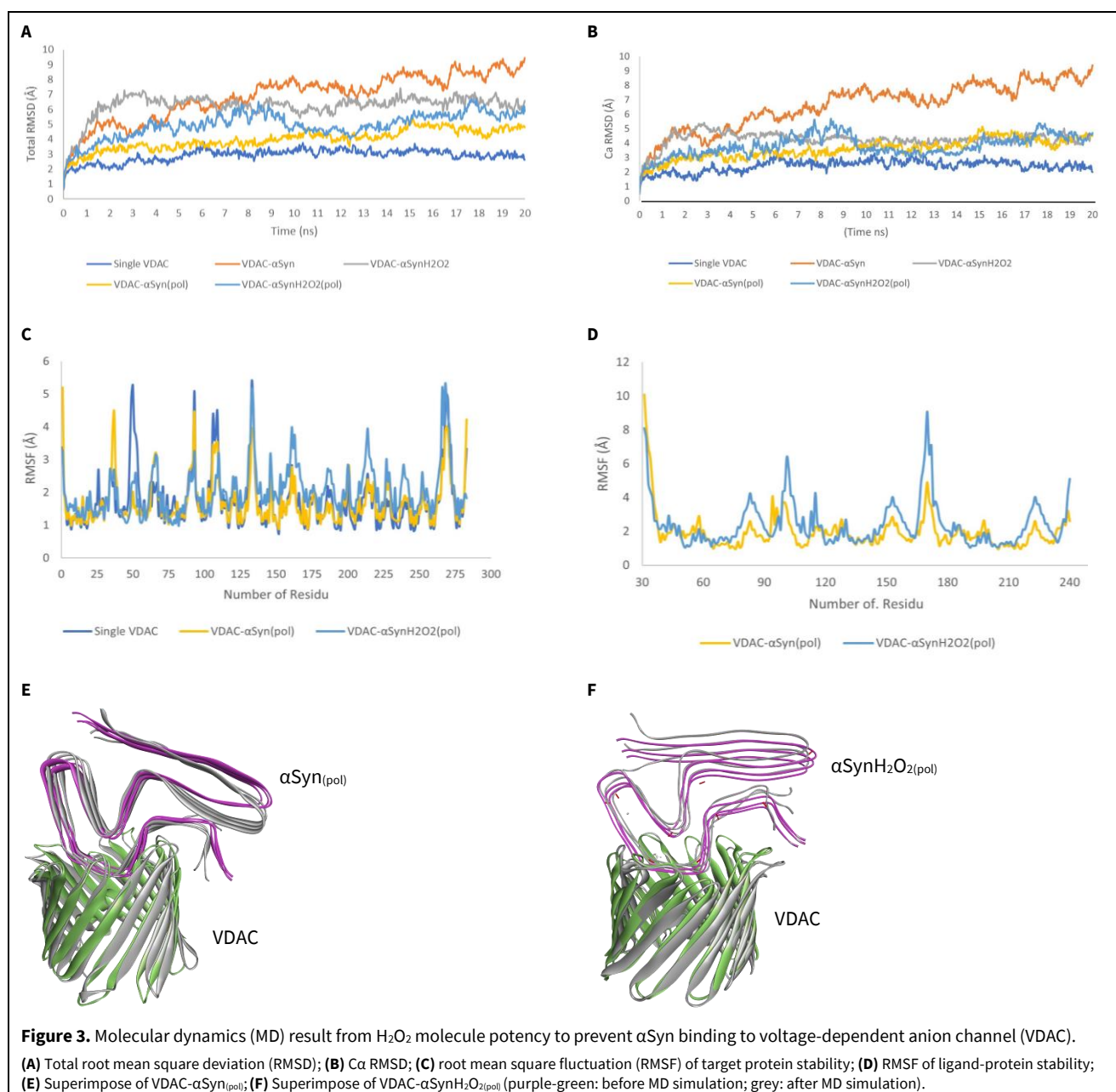
In the α Syn H_2O_2 - α Syn complex, the amino acid residues that fluctuated exceeded 3 Å, at numbers 45, 58, 80, 86, and 97, and the highest reached 5 Å residues at numbers 83 and 94, which were included in the active site residues, it was estimated that these residues have a major impact on ligand binding (Rampogu et al., 2022). A high RMSF value indicated greater flexibility with a maximum degree of fluctuation (Ajarapu et al., 2021). A lower RMSF value of the binding site residue indicated the stability of ligand binding to the protein (Soumia et al., 2022). When the active site residue fluctuated, it had the potential for α Syn protein to be released from the α Syn H_2O_2 - α Syn complex, so that the formation of α Syn fibrils could be prevented. Proteins that unfolded in the α Syn H_2O_2 complex (Fig. 2D) and α Syn H_2O_2 - α Syn (Fig. 2E) could be seen from the superimpose results. The superimpose showed the comparison of all protein samples before and after the simulation. This analysis provides information about the effect of ligand binding on the conformational stability of protein receptors (Bauer et al., 2008).

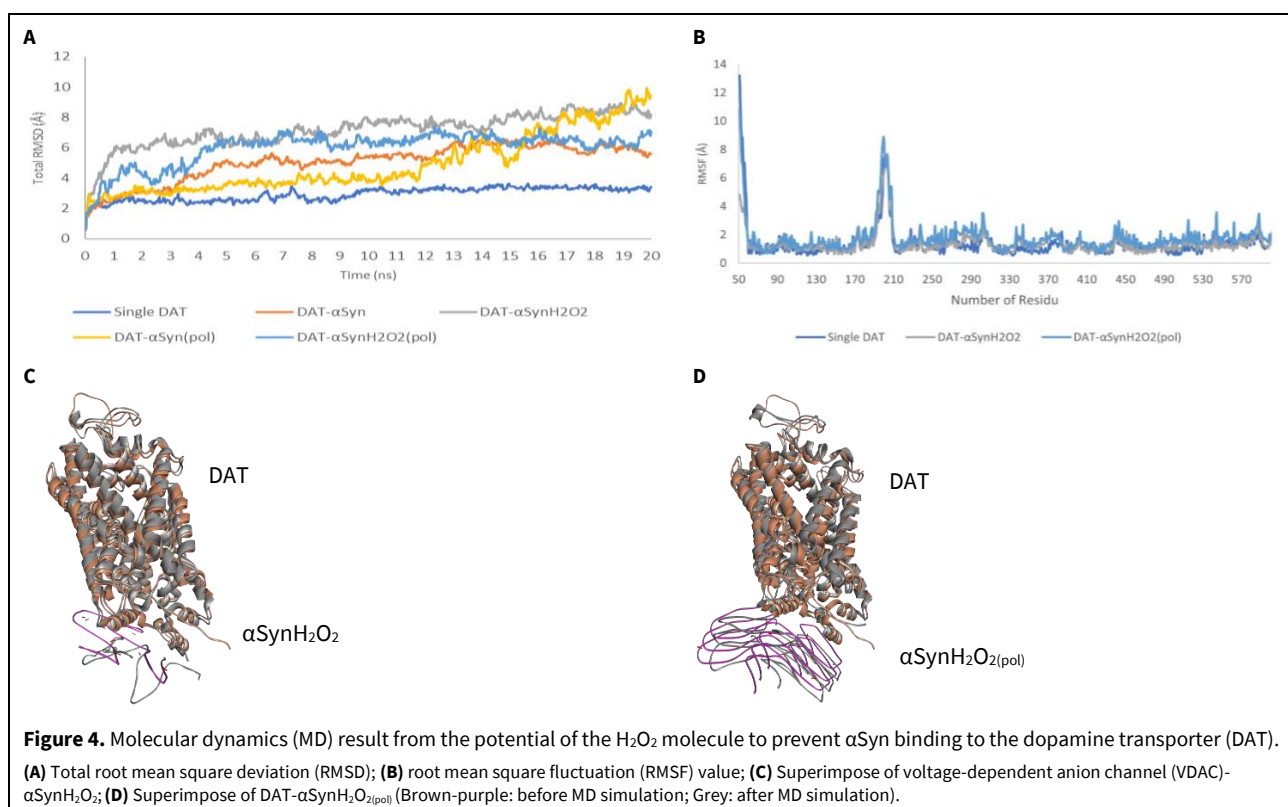
The potential of H_2O_2 molecule to prevent α Syn binding to VDAC

α Syn regulated mitochondrial function through the VDAC. When complexed with α Syn, the C-terminal domain was transiently captured within the VDAC pore and partially blocked, reducing ATP/ADP transport (Risiglion et al., 2021). Subsequently, α Syn could translocate to the mitochondria through the VDAC, interfere with mitochondrial respiration (interaction with the complex-I electron transport chain), decrease ATP production, and cause mitochondrial dysfunction (Rajendran et al., 2022). Therefore, it was possible to regulate the complexation of α Syn with VDAC with H_2O_2 low dose. Docking results showed the ability of H_2O_2 to reduce the binding affinity of α Syn protein with VDAC (Fig. 1). This result is supported by total RMSD (Fig. 3A). VDAC- α Syn complex had a high total RMSD value ($>8\text{Å}$) indicating the VDAC protein becomes unfunctional when it binds to α Syn (Sumaryada et al., 2016). VDAC- α Syn H_2O_2 complex has a lower total RMSD value of $<8\text{Å}$, potentially making the complex more functional. However, this result is not in accordance with the VDAC that binds to the α Syn polymer (VDAC- α Syn_(pol)), which has a lower total RMSD value than the VDAC- α Syn H_2O_2 (_{pol}) complex.

As a result of the $C\alpha$ RMSD (indicating a conformational change in the target protein) (Fig. 3B), the VDAC- α Syn H_2O_2 (pol) complex has a low $C\alpha$ RMSD value, although it does not show a significant decrease. However, when viewed from the results of RMSF analysis of protein stability (Fig. 3C). When compared to the VDAC- α syn(pol) complex, the VDAC- α Syn H_2O_2 (pol) complex has more active sites that experience high fluctuations exceeding 3 Å, at residues 212, 218, 186, 189, 168, and 185. These results indicated that the bond between VDAC and α Syn H_2O_2 (pol) becomes less stable (Ajjarapu et al., 2021; Rampogu et al., 2022), and the α Syn H_2O_2 (pol) ligand has the poten-

tial to detach from VDAC, this can be validated by the results of the RMSF analysis of ligand stability (Fig. 3D), which shows that the α Syn H_2O_2 (pol) ligand in the VDAC- α Syn H_2O_2 (pol) complex has a higher fluctuation value compared to the α Syn(pol) ligand in the VDAC- α Syn(pol) complex. Thus, the H_2O_2 molecule can increase the RMSF value at the binding active site and can reduce the stability of the complex, and has the potential to detach the α Syn protein binding with VDAC. Therefore, mitochondrial dysfunction can be prevented, and ATP production returns to normal. For more details can be seen from the superimpose of the complex (Fig. 3E-F).





The potential of H_2O_2 molecule to prevent α Syn binding to DAT

DAT regulates dopamine signaling and may interact with the neuronal protein α Syn, (Threlfell et al., 2021). The formation of DAT- α Syn complexes is an aberration and may contribute to the degeneration of dopamine neurons in PD (Longhena et al., 2018). α Syn may modulate DAT activity by regulating its presence on the cell surface. The interaction of DAT and α Syn can influence the membrane microenvironment near the transporter. DAT activity is inhibited when α Syn is overexpressed and can change dopamine neuron homeostasis (Butler et al., 2015; Swant et al., 2011). Therefore, we evaluated the potential of H_2O_2 to prevent α Syn binding to DAT. Docking results (Fig. 1) show that the presence of H_2O_2 molecules can decrease the binding affinity of α Syn protein with DAT. This is supported by the results of the total RMSD analysis (Fig. 4A).

The total RMSD of the DAT- α Syn(pol) complex increased significantly to $>8\text{\AA}$, indicating when DAT binds polymer α Syn, it becomes an unfolding protein and becomes non-functional (Alaei et al., 2021). DAT- α Syn H_2O_2 (pol) complex has lower total RMSD values $<8\text{\AA}$; this indicates the complex becomes more stable and allows DAT to become more functional. However, when looking at the total RMSD of the DAT- α Syn and DAT- α Syn H_2O_2 complexes, it shows the opposite result, where the DAT- α Syn H_2O_2 complex has a higher total RMSD value. The results of RMSF analysis

(Fig. 4B) show the DAT- α Syn H_2O_2 complex, all active binding site residues fluctuations (RMSF value $>3\text{\AA}$), at residue numbers 521, 606, 610, 615, 619, 620, 608 and 518. Fluctuations in the active site residue cause a decrease in the stability of α Syn H_2O_2 binding to the DAT (Ejaz et al., 2023) and allow α Syn H_2O_2 release from DAT. In the DAT- α Syn H_2O_2 (pol) complex, where all active sites also increase in fluctuations at 514, 606, 610, 615, 619, 608, 515, 518, 618, 619. This indicates that the H_2O_2 molecule can increase the RMSF value of the DAT- α Syn H_2O_2 and DAT- α Syn H_2O_2 (pol) complex, specifically at the active site residue, the complex becomes less stable and the bonds between amino acid residues become tenuous (Wargasetia et al., 2023). The presence of H_2O_2 molecules causes α Syn monomers and α Syn polymers to be released from DAT. More details can be seen in the superimpose results (Fig. 4C-D).

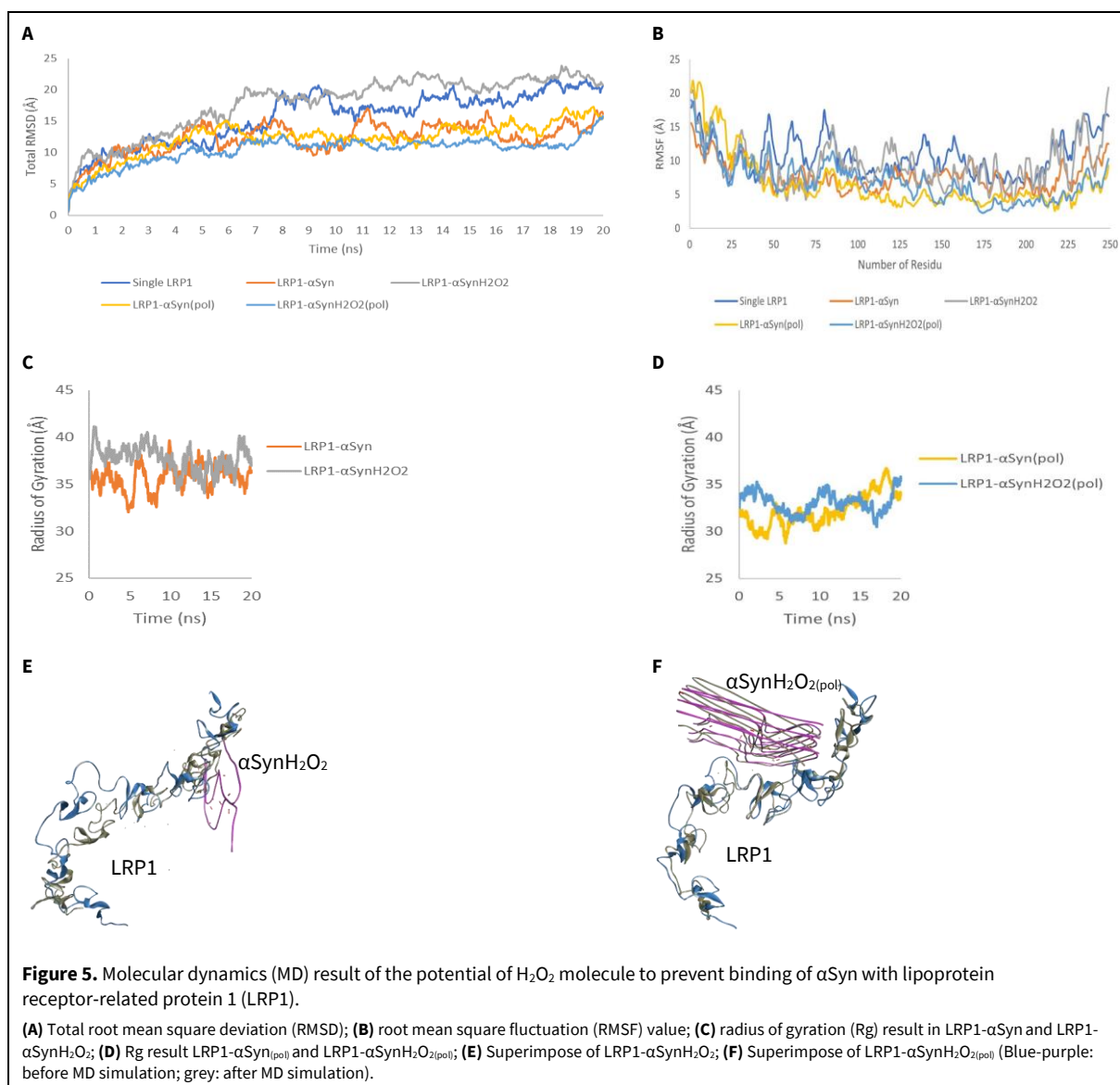
The potential of H_2O_2 molecule to prevent α Syn binding to LRP1

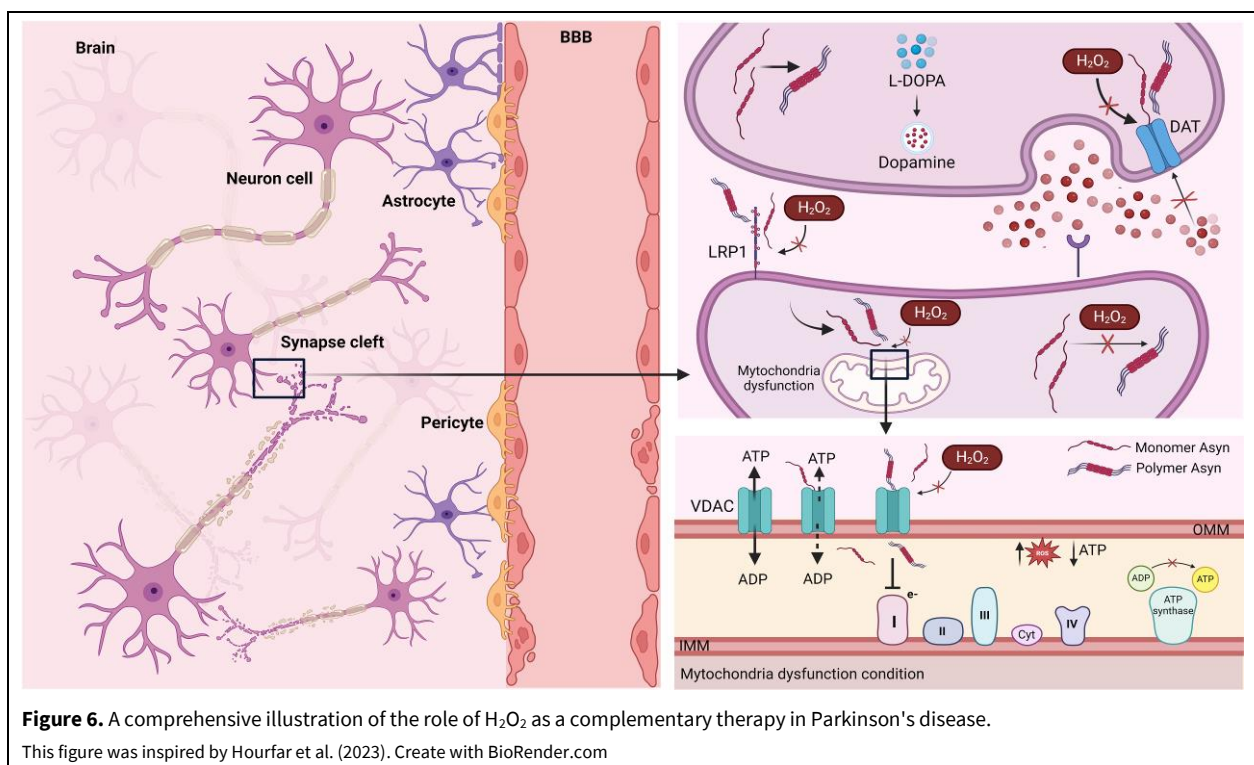
The clinical progression of PD is associated with the spread of α Syn protein aggregates. α Syn spreads between cells and causes neurotoxicity. LRP1 is a multifunctional endocytic receptor that is expressed in neurons. LRP1 regulates the uptake and spread of α Syn in the brain and may be a potential target for the treatment of α Syn spread-related diseases (Chen et al., 2022). H_2O_2 molecules can reduce the binding affinity of α Syn protein with LRP1 from the docking results with HDock (Fig. 1). From the total RMSD

analysis (Fig. 5A), which shows that the LRP1- α Syn H_2O_2 complex has a higher total RMSD value than LRP1- α Syn. H_2O_2 molecules can increase the total RMSD value; it makes the complex LRP1- α Syn H_2O_2 unfolding and unfunctional (Alaei et al., 2021) so that LRP1 cannot bind α Syn. This result is inversely proportional when LRP1 binds to α Syn polymers (LRP1- α Syn_(pol)) and LRP1- α Syn H_2O_2 _(pol)), because the LRP1- α Syn H_2O_2 _(pol) complex has a lower total RMSD value than LRP1- α Syn_(pol).

The RMSF results (Fig. 5B), the LRP1- α Syn H_2O_2 and LRP1- α Syn H_2O_2 _(pol) complexes show that the H_2O_2 molecule can increase the RMSF value at several active site residues (206, 216, 202 and 121). Fluctuations that occur in the active site residues affect the bonding of the LRP1 protein with the α Syn, allowing

the α Syn to be released. This result is supported by Rg analysis (Fig. 5C-D). Rg analysis represents the compactness of the protein structure (Dankwa et al., 2022; Wargasetia et al., 2023), where higher values Rg indicate unstable complex and lower values indicate the protein to be more compact or stable (Hasan et al., 2021; Lobanov et al., 2008; Mahmud et al., 2021). Where complexes with H_2O_2 molecules (LRP1- α Syn H_2O_2 and LRP1- α Syn H_2O_2 _(pol)) have higher Rg values than complexes without H_2O_2 (LRP1- α Syn and LRP1- α Syn_(pol)), this indicates that the presence of H_2O_2 molecules increases the Rg value, making the complex unstable and less compact, which indicates a loose bond between binding amino acid residues. More clearly can be seen from the superimpose result of the complexes (Fig. 5E-F).





From the results of molecular docking and MD analysis, it can be seen that the H_2O_2 molecule has the potential to prevent α Syn forming fibrils and prevent α Syn from binding to protein targets (VDAC, DAT, and LRP1) by reducing its binding affinity. H_2O_2 molecules can also affect interactions at the active site, which causes changes in interactions. Besides that, the presence of H_2O_2 molecules can also increase fluctuations in the active site of target proteins, making the binding of the α Syn protein with the target protein less stable. Potentially preventing the binding between α Syn and the target protein, as well as preventing mitochondrial dysfunction, dysregulation of dopamine reuptake, and the spread of α Syn from cell to cell. Thus, H_2O_2 can be used as a complementary therapy to L-DOPA in PD patients, will be easy to understand with comprehensive illustration (Fig. 6).

CONCLUSION

The docking result showed H_2O_2 potential to decrease the binding affinity and change the active site interaction of the α Syn-protein target complex. This indicated that the presence of H_2O_2 molecules affects interaction, and the H_2O_2 molecule has the potential to prevent α Syn from binding to the protein target. From the MD result, H_2O_2 increases the fluctuation in the active site, allowing α Syn release from the protein target. It can be seen that H_2O_2 low dose has the potential to be complementary therapy with L-DOPA because it can prevent α Syn aggregation, mitochondrial dysfunction, dysregulation of DAT, and spreading of α Syn.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

- Agnello L, Ciaccio M (2022) Neurodegenerative diseases: From molecular basis to therapy. *Int J Mol Sci* 23(21): 12854. <https://doi.org/10.3390/ijms232112854>
- Ajjarapu SM, Tiwari A, Taj G, Singh DB, Singh S, Kumar S (2021) Simulation studies, 3D QSAR and molecular docking on a point mutation of protein kinase B with flavonoids targeting ovarian Cancer. *BMC Pharmacol Toxicol* 22(1): 68. <https://doi.org/10.1186/s40360-021-00512-y>
- Alaei L, Ashengroph M, Moosavi-Movahedi AA (2021) The concept of protein folding/unfolding and its impacts on human health. *Adv Protein Chem Struct Biol* 126: 227-278. <https://doi.org/10.1016/bs.apcsb.2021.01.007>
- Alom MM, Bonna RP, Islam A, Alom MW, Rahman ME, Faruque MO, Khalekuzzaman M, Zaman R, Islam MA (2023) Unveiling neuroprotective potential of spice plant-derived compounds against Alzheimer's disease: Insights from computational studies. *Int J Alzheimers Dis* 2023: 8877757. <https://doi.org/10.1155/2023/8877757>
- Banerjee P, Kemmler E, Dunkel M, Preissner R (2024) ProTox 3.0: A webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res* 52(W1): W513-W520. <https://doi.org/10.1093/nar/gkac303>

- Bauer RA, Bourne PE, Formella A, Frömmel C, Gille C, Goede A, Guerler A, Hoppe A, Knapp EW, Pöschel T, Wittig B, Ziegler V, Preissner R (2008) Superimpose: A 3D structural superposition server. *Nucleic Acids Res* 36: 47–54. <https://doi.org/10.1093/nar/gkn285>
- Biswas P, Bibi S, Yousafi Q, Mehmood A, Saleem S, Ihsan A, Dey D, Hasan Zilani MN, Hasan MN, Saleem R, Awaji AA, Fahmy UA, Abdel-Daim MM (2023) Study of MDM2 as prognostic biomarker in Brain-LGG Cancer and Bioactive phytochemicals Inhibit the P53-MDM2 pathway: A Computational Drug Development approach. *Molecules* 28(7): 2977. <https://doi.org/10.3390/molecules28072977>
- Blackstone C (2009) Infantile parkinsonism-dystonia: A dopamine “transportopathy”. *J Clin Invest* 119: 1455–1458. <https://doi.org/10.1172/jci39632>
- Blanc A, Pandey N, Srivastava A (2003) Synchronous activation of ERK 1/2, p38mapk and PKB/Akt signaling by H_2O_2 in vascular smooth muscle cells: Potential involvement in vascular disease (Review). *Int J Mol Med* 11(2): 229–234. <https://doi.org/10.3892/ijmm.11.2.229>
- Boder EJ, Goncalves BG, Lebedenko CG, Banerjee IA (2023) Probing the Interactions of LRP1 ectodomain-derived peptides with fibrillar Tau protein and its impact on cellular internalization. *Appl Sci* 13(2): 853. <https://doi.org/10.3390/app13020853>
- Burré J, Sharma M, Südhof TC (2014) α -Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. *Proc Natl Acad Sci USA* 111(40): E4274–E4283. <https://doi.org/10.1073/pnas.1416598111>
- Butler B, Saha K, Rana T, Becker JP, Sambo D, Davari P, Goodwin JS, Khoshbouei H (2015) Dopamine transporter activity is modulated by A-Synuclein. *J Biol Chem* 290(49): 29542–29554. <https://doi.org/10.1074/jbc.m115.691592>
- Chaudhuri A, Biswas S, Chakraborty S (2019) Exploring protein-protein intermolecular recognition between meprin- α and endogenous protease regulator cystatinC coupled with pharmacophore elucidation. *J Biomol Struct Dyn* 37(2): 440–453. <https://doi.org/10.1080/07391102.2018.1429311>
- Chen K, Martens YA, Meneses A, Ryu DH, Lu W, Raulin AC, Li F, Zhao J, Chen Y, Jin Y, Linares C, Goodwin M, Li Y, Liu CC, Kanekiyo T, Holtzman DM, Golde TE, Bu G, Zhao N (2022) LRP1 is a neuronal receptor for α -synuclein uptake and spread. *Mol Neurodegener* 17(1): 57. <https://doi.org/10.1186/s13024-022-00560-w>
- Chen R, Furman CA, Gnegy ME (2010) Dopamine transporter trafficking: Rapid response on demand. *Future Neurol* 5(1): 123–134. <https://doi.org/10.2217/fnl.09.76>
- Dankwa B, Broni E, Ennifful KS, Kwofie SK, Wilson MD (2022) Consensus docking and MM-PBSA computations identify putative furin protease inhibitors for developing potential therapeutics against COVID-19. *Struct Chem* 33(6): 2221–2241. <https://doi.org/10.1007/s11224-022-02056-1>
- Dhorajiwala T, Halder S, Samant L (2019) Comparative *in silico* molecular docking analysis of L-threonine-3-dehydrogenase, a protein target against African trypanosomiasis using selected phytochemicals. *J Appl Biotechnol Rep* 6(3): 101–108. <https://doi.org/10.29252/jabr.06.03.04>
- Ejaz SA, Aziz M, Zafar Z, Akhtar N, Ogaly HA (2023) Revisiting the inhibitory potential of protein kinase inhibitors against NEK7 protein via comprehensive computational investigations. *Sci Rep* 13(1): 4304. <https://doi.org/10.1038/s41598-023-31499-7>
- Farmer J, Kanwal F, Nikulsin N, Tsimigras M, Jacobs D (2017) Statistical measures to quantify similarity between molecular dynamics simulation trajectories. *Entropy* 19(12): 646. <https://doi.org/10.3390/e19120646>
- Gitler AD, Dhillon P, Shorter J (2017) Neurodegenerative disease: models, mechanisms, and a new hope. *Dis Model Mech* 10(5): 499–502. <https://doi.org/10.1242/dmm.030205>
- Hasan R, Chowdhury SM, Aziz MdA, Shahriar A, Ahmed H, Khan A, Mahmud S, Emran TB (2021) *In silico* analysis of ciprofloxacin analogs as inhibitors of DNA gyrase of *Staphylococcus aureus*. *Inform Med Unlocked* 26: 100748. <https://doi.org/10.1016/j.imu.2021.100748>
- Hijaz BA, Volpicelli-Daley LA (2020) Initiation and propagation of α -synuclein aggregation in the nervous system. *Mol Neurodegener* 15(1): 19. <https://doi.org/10.1186/s13024-020-00368-6>
- Horowitz S, Trievel RC (2012) Carbon-oxygen hydrogen bonding in biological structure and function. *J Biol Chem* 287(50): 41576–41582. <https://doi.org/10.1074/jbc.r112.418574>
- Hourfar H, Aliakbari F, Aqdam SR, Nayeri Z, Bardania H, Otzen DE, Morshedi D (2023) The impact of α -synuclein aggregates on blood-brain barrier integrity in the presence of neurovascular unit cells. *Int J Biol Macromol* 229: 305–320. <https://doi.org/10.1016/j.ijbiomac.2022.12.134>
- Jiang S, Zhang D, Huang H, Lei Y, Han Y, Han W (2017) Extracellular signal-regulated kinase 5 is required for low-concentration H_2O_2 -induced angiogenesis of human umbilical vein endothelial cells. *BioMed Res Int* 2017: 6895730. <https://doi.org/10.1155/2017/6895730>
- Jones DR, Moussaoud S, McLean P (2014) Targeting heat shock proteins to modulate α -synuclein toxicity. *Ther Adv Neurol Disord* 7(1): 33–51. <https://doi.org/10.1177/1756285613493469>
- Kang SJ, Kim SJ, Noh HR, Kim BJ, Kim JB, Jin U, Park SA, Park SM (2022) Neuronal APOE regulates the cell-to-cell transmission of A-synuclein. *Int J Mol Sci* 23(15): 8311. <https://doi.org/10.3390/ijms23158311>
- Kojasoy V, Tantillo DJ (2023) Importance of noncovalent interactions involving sulfur atoms in thiopeptide antibiotics—glycothiohexide α and nocathiacin I. *J Phys Chem A* 127(9): 2081–2090. <https://doi.org/10.1021/acs.jpca.2c07600>
- Lashuel HA, Overk CR, Oueslati A, Masliah E (2013) The many faces of α -synuclein: from structure and toxicity to therapeutic target. *Nat Rev Neurosci* 14(1): 38–48. <https://doi.org/10.1038/nrn3406>
- Lestari EDP, Widyarti S, Santjojo DH, Widodo N, Sumitro SB (2023) Computational approach to determine the combination of polyherbs based on the interaction of their metal complexes on the mucoadhesive properties of type II mucin. *J Appl Pharm Sci* 13: 109–122. <https://doi.org/10.7324/japs.2023.35062>
- Li H, Hung A, Yang AWH (2021) Herb-target virtual screening and network pharmacology for prediction of molecular mechanism of Danggui Beimu Kushen Wan for prostate cancer. *Sci Rep* 11: 6656. <https://doi.org/10.1038/s41598-021-86141-1>
- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 64: 4–17. <https://doi.org/10.1016/j.addr.2012.09.019>
- Lobanov MY, Bogatyreva NS, Galzitskaya OV (2008) Radius of gyration as an indicator of protein structure compactness. *Mol Biol* 42(4): 623–628. <https://doi.org/10.1134/s0026893308040195>
- Longhena F, Faustini G, Missale C, Pizzi M, Bellucci A (2018) Dopamine transporter/A-synuclein complexes are altered in the post mortem caudate putamen of Parkinson’s disease: An *in situ* proximity ligation assay study. *Int J Mol Sci* 19(6): 1611. <https://doi.org/10.3390/ijms19061611>

- Mahmud S, Biswas S, Kumar Paul G, Mita MA, Afrose S, Robiul Hasan M, Sharmin Sultana Shimu M, Uddin MAR, Salah Uddin M, Zaman S, Kaderi Kibria KM, Arif Khan M, Bin Emran T, Abu Saleh M (2021) Antiviral peptides against the main protease of SARS-CoV-2: A molecular docking and dynamics study. *Arab J Chem* 14(9): 103315. <https://doi.org/10.1016/j.arabjc.2021.103315>
- Moriarty GM, Janowska MK, Kang L, Baum J (2013) Exploring the accessible conformations of N-terminal acetylated α -synuclein. *FEBS Lett* 587(8): 1128–1138. <https://doi.org/10.1016/j.febslet.2013.02.049>
- Michel PP, Hirsch EC, Hunot S (2016) Understanding dopaminergic cell death pathways in Parkinson disease. *Neuron* 90(4): 675–691. <https://doi.org/10.1016/j.neuron.2016.03.038>
- Nisticò R, Piccirilli S, Cucchiaroni ML, Armogida M, Guatteo E, Giampà C, Fusco FR, Bernardi G, Nisticò G, Mercuri NB (2008) Neuroprotective effect of hydrogen peroxide on an *in vitro* model of brain ischaemia. *Br J Pharmacol* 153(5): 1022–1029. <https://doi.org/10.1038/sj.bjp.0707587>
- Park JH, Burgess JD, Faruqi AH, DeMeo NN, Fiesel FC, Springer W, Delenclos M, McLean PJ (2020) Alpha-synuclein-induced mitochondrial dysfunction is mediated via a sirtuin 3-dependent pathway. *Mol Neurodegener* 15: 5. <https://doi.org/10.1186/s13024-019-0349-x>
- Pathak D, Berthet A, Nakamura K (2013) Energy failure: Does it contribute to neurodegeneration? *Ann Neurol* 74(4): 506–516. <https://doi.org/10.1002/ana.24014>
- Price GW, Gould PS, Marsh A (2014) Use of freely available and open source tools for *in silico* screening in Chemical Biology. *J Chem Educ* 91(4): 602–604. <https://doi.org/10.1021/ed400302u>
- Rajendran M, Queralt-Martín M, Gurnev PA, Rosencrans WM, Rovini A, Jacobs D, Abrantes K, Hoogerheide DP, Bezrukov SM, Rostovtseva TK (2022) Restricting α -synuclein transport into mitochondria by inhibition of α -synuclein-VDAC complexation as a potential therapeutic target for Parkinson's disease treatment. *Cell Mol Life Sci* 79(7): 368. <https://doi.org/10.1007/s00018-022-04389-w>
- Rampogu S, Lee G, Park JS, Lee KW, Kim MO (2022) Molecular docking and molecular dynamics simulations discover curcumin analogue as a plausible dual inhibitor for SARS-COV-2. *Int J Mol Sci* 23(3): 1771. <https://doi.org/10.3390/ijms23031771>
- Risiglione P, Zinghirino F, Di Rosa MC, Magri A, Messina A (2021) α -Synuclein and mitochondrial dysfunction in Parkinson's disease: The emerging role of VDAC. *Biomolecules* 11(5): 718. <https://doi.org/10.3390/biom11050718>
- Robert J, Button EB, Martin EM, McAlary L, Gidden Z, Gilmour M, Boyce G, Caffrey TM, Agbay A, Clark A, Silverman JM, Cashman NR, Wellington CL (2020) Cerebrovascular amyloid angiopathy in bioengineered vessels is reduced by high-density lipoprotein particles enriched in apolipoprotein E. *Mol Neurodegener* 15(1): 23. <https://doi.org/10.1186/s13024-020-00366-8>
- Rosencrans WM, Aguilera VM, Rostovtseva TK, Bezrukov SM (2021) α -Synuclein emerges as a potent regulator of VDAC-facilitated calcium transport. *Cell Calcium* 95: 102355. <https://doi.org/10.1016/j.ceca.2021.102355>
- Rostovtseva TK, Gurnev PA, Protchenko O, Hoogerheide DP, Yap TL, Philpott CC, Lee JC, Bezrukov SM (2015) α -Synuclein shows high affinity interaction with voltage-dependent anion channel, suggesting mechanisms of mitochondrial regulation and toxicity in Parkinson disease. *J Biol Chem* 290: 18467–18477. <https://doi.org/10.1074/jbc.m115.641746>
- Simon DK, Tanner CM, Brundin P (2020) Parkinson disease epidemiology, pathology, genetics, and pathophysiology. *Clin Geriatr Med* 36(1): 1–12. <https://doi.org/10.1016/j.cger.2019.08.002>
- Soumia M, Hajji H, El Mzibri M, Younes FZ, Mohammed B, Mohamed B, Benaissa M (2022) In-silico molecular modeling studies to identify novel potential inhibitors of HPV E6 protein. *Vaccines* 10(9): 1452. <https://doi.org/10.3390/vaccines10091452>
- Sumaryada T, Hati J, Wahyudi ST, Malau ND, Sawitri KN (2016) Elucidation of GB1 protein unfolding mechanism via a long-timescale molecular dynamics simulation. *IOP Conf Ser Earth Environ Sci* 31: 012008. <https://doi.org/10.1088/1755-1315/31/1/012008>
- Swant J, Goodwin JS, North A, Ali AA, Gamble-George J, Chirwa S, Khoshbouei H (2011) α -Synuclein stimulates a dopamine transporter-dependent chloride current and modulates the activity of the transporter. *J Biol Chem* 286(51): 43933–43943. <https://doi.org/10.1074/jbc.m111.241232>
- Threlfell S, Mohammadi AS, Ryan BJ, Connor-Robson N, Platt NJ, Anand R, Serres F, Sharp T, Bengoa-Vergniory N, Wade-Martins R, Ewing A, Cragg SJ, Brimblecombe KR (2021) Striatal dopamine transporter function is facilitated by converging biology of α -Synuclein and cholesterol. *Front Cell Neurosci* 15: 658244. <https://doi.org/10.3389/fncel.2021.658244>
- Uversky V (2008) α -Synuclein misfolding and neurodegenerative diseases. *Curr Protein Pept Sci* 9(5): 507–540. <https://doi.org/10.2174/138920308785915218>
- Vaughan RA, Foster JD (2013) Mechanisms of dopamine transporter regulation in normal and disease states. *Trends Pharmacol Sci* 34(9): 489–496. <https://doi.org/10.1016/j.tips.2013.07.005>
- Wang L, Zhang F, Peng W, Zhang J, Dong W, Yuan D, Wang Z, Zheng Y (2020) Preincubation with a low-dose hydrogen peroxide enhances anti-oxidative stress ability of BMSCs. *J Orthop Surg Res* 15: 392. <https://doi.org/10.1186/s13018-020-01916-y>
- Wargasetia TL, Ratnawati H, Widodo N, Widyandana MH (2023) Antioxidant and anti-inflammatory activity of sea cucumber (*Holothuria scabra*) active compounds against KEAP1 and iNOS protein. *Bioinform Biol Insights* 17: 11779322221149613. <https://doi.org/10.1177/11779322221149613>
- WHO (2023) Parkinson disease. World Health Organization. Available at: <https://www.who.int/news-room/fact-sheets/detail/parkinson-disease> (Accessed: 17 April 2024).
- Widyarti S, Wibowo S, Sabarudin A, Abhira I, Sumitro SB (2023) Dysfunctional energy and future perspective of low dose H₂O₂ as protective agent in neurodegenerative disease. *Heliyon* 9(7): e18123. <https://doi.org/10.1016/j.heliyon.2023.e18123>

AUTHOR CONTRIBUTION:

Contribution	Layla A	Widyarti S	Sumitro BS
Concepts or ideas			x
Design	x		x
Definition of intellectual content	x	x	x
Literature search	x	x	x
Experimental studies	x		
Data acquisition	x		
Data analysis	x		
Statistical analysis	x		
Manuscript preparation	x	x	x
Manuscript editing	x	x	x
Manuscript review	x	x	x

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Supplementary data

Table 1S. Active site of all complexes from HDock.

Complex	Binding energy	Residue active site
α Syn- α Syn	-1144.50	Conventional hydrogen: LYS32, LYS34, VAL37, TYR39, GLY41 LYS43, LYS45, VAL48, GLY51, ALA53, VAL55, GLU57, THR59, GLU61, VAL63, ASN65, GLY68, VAL70, THR72, GLY73, THR75, VAL77, GLN79, THR81, GLY84, GLY86, ILE88, ALA90, THR92, PHE94, LYS96, ASP98, GLY36, LYS60, GLN62, GLY67, GLU83, LEU100
		Carbon-hydrogen: VAL37, GLY41, GLU46, THR72, ALA90, GLU35, SER42, GLU83
		Hydrophobic: TYR39, PHE94, LYS32, LYS34, VAL37, LEU38, VAL40, VAL71, LYS43, LYS45, VAL48, VAL49, VAL66, VAL52, VAL55, LYS58, LYS60, VAL63, VAL66, VAL70, VAL74, VAL77, LYS80, VAL82, ILE88, ALA91, VAL95, LYS96, LYS97, LEU100, ALA76, ALA78
		Electrostatic: GLU61, LYS80, GLU57, LYS60
		Unfavorable: GLY31, LYS32, LYS34, LYS43, LYS45, LYS80, LYS96, LYS97, GLU35, GLU61, GLU8, ASP98
α Syn- α Syn H_2O_2	-1066.09	Conventional hydrogen: LYS34, VAL37, TYR39, GLY41, LYS43, LYS45, GLY51, VAL55, LYS58, GLU61, VAL63, ASN65, GLY68, VAL70, THR72, GLY73, THR75, VAL77, GLN79, THR81, GLY84, GLY86, ILE88, ALA90, THR92, PHE94, LYS96, GLY36, THR44, VAL48, HIS50, ALA53, GLU57, LYS60, GLN62, GLY67, GLU83, ASP98, LEU100
		Carbon-hydrogen: VAL37, TYR39, GLY41, LYS43, LYS45, GLU46, VAL48, ALA53, VAL55, GLU57, GLY68, THR72, VAL77, ALA90, LYS96, GLU35, SER42, THR44, GLN62, THR64, VAL66, YS80, VAL82, ALA91, VAL95, LYS97
		Hydrophobic: TYR39, PHE94, LYS32, LYS34, VAL37, LEU38, VAL40, VAL71, LYS43, LYS45, VAL48, VAL49, VAL66, VAL52, VAL55, LYS58, LYS60, VAL63, VAL66, VAL74, VAL77, LYS80, VAL82, ILE88, VAL95, LYS96, LYS97, LEU100, ALA76, ALA78
		Electrostatic: GLU61, LYS80, LYS58, LYS60
		Unfavorable: GLY51, ALA53, GLY31, LYS32, LYS34, LYS43, LYS45, LYS80, LYS96, LYS97, GLU35, GLU61, GLU83, ASP98, VAL48
VDAC- α Syn	-130.59	Conventional hydrogen: THR55, GLN166
		Carbon-hydrogen: TYR7, THR77,
		Hydrophobic: LEU10
		Electrostatic: LYS20, ASP30
		Unfavorable: VAL54, THR55, THR77, ASN106, ARG15, GLY56, SER57, GLN166
VDAC- α Syn H_2O_2	-122.70	Conventional hydrogen: GLN166, SER44, THR55, GLU189
		Carbon-hydrogen: TYR7, LEU10, THR77, ALA56
		Hydrophobic: VAL54, LEU10
		Electrostatic: LYS32, ASP30
		Unfavorable: VAL54, THR77, ARG15, SER57
VDAC- α Syn _(pol)	-164.42	Conventional hydrogen: LYS109, ASN185, ARG218, THR52, LYS53, THR55, ASN216, ASP78, THR80
		Carbon-hydrogen: ARG218, ASN185
		Hydrophobic: LYS53, ALA209
		Electrostatic: GLU189
		Unfavorable: THR107, ASN76, ARG218, ASP186
VDAC- α Syn H_2O_2 _(pol)	-154.13	Conventional hydrogen SER104, ASN106, THR107
		Carbon-hydrogen: SER57
		Hydrophobic: LEU26, LYS53, ALA212, PRO105

		Electrostatic: ARG218, ASP186, GLU189
		Unfavorable: LEU26, THR51, THR80, THR107, ALA212, ASN48, ASN168, ASN185
DAT- α Syn	-182.74	Conventional hydrogen: GLN514, LEU518, ARG606, LYS619, LEU618
		Hydrophobic: ARG606, ARG615, LEU518
		Electrostatic: ARG615, VAL620
		Unfavorable: GLN510, ARG615, ARG515, GLU608
DAT- α Syn H_2O_2	-135.36	Conventional hydrogen: ARG521, ARG606, ARG610, ARG615, LYS619, VAL620, GLU608
		Hydrophobic: ARG615, LYS619, EU518
		Unfavorable: ARG515, ARG615, ARG606
DAT- α Syn _(pol)	-190.86	Conventional hydrogen: GLN514, ARG610, ARG615, LYS619, GLU608
		Carbon-hydrogen: ARG515
		Hydrophobic: ARG606, LEU518, LEU618, LYS619
		Electrostatic: ARG615
DAT- α Syn H_2O_2 _(pol)	-189.02	Conventional hydrogen: GLN514, ARG606, ARG610, ARG615, LYS619, GLU608
		Hydrogen carbon: ARG515
		Hydrophobic: ARG606, LEU518, LEU618, LYS619
		Electrostatic: ARG615
		Unfavorable: ARG515
LRP1- α Syn	-131.42	Conventional hydrogen: ASN202, ARG214, GLU206, GLU205, CYS227, SER217, ASP194
		Carbon-hydrogen: GLY195
		Hydrophobic: PRO216, PRO229
		Electrostatic: ARG214, ASP194
		Unfavorable: ASP194, ASP196, GLU205
		Other: CYS208
LRP1- α Syn H_2O_2	-123.32	Conventional hydrogen: SER217, GLU206, CYS227
		Carbon-hydrogen: PRO216
		Hydrophobic: VAL226, PRO229, PRO216
		Electrostatic: GLU209, ASP239
		Unfavorable: GLU205, GLU209, ASN202
LRP1- α Syn _(pol)	-149.0	Conventional hydrogen: ASN121, THR132, VAL171, THR132, SER210
		Carbon-hydrogen: ALA123
		Hydrophobic: VAL168, PHE178
		Electrostatic: ASP173
		Unfavorable: GLN122, VAL171, ARG147, GLN12, SER175
LRP1- α Syn H_2O_2 _(pol)	-120.39	Conventional hydrogen ASN121, GLN122
		Carbon-hydrogen: ASN223
		Hydrophobic: ALA123
		Electrostatic: LYS189

(pol): polymer