# Investigating the allosteric inhibition mechanism of I2 ligands in MAO-B using MD simulations with organic solvent/water mixtures

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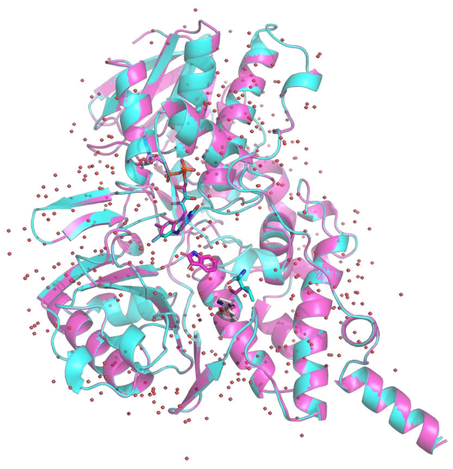
## Summary

Imidazoline receptors (I2-IRs) are linked to brain disorders like depression, Alzheimer's, Parkinson's, and tumors. Selective I2-IR ligands show neuroprotective potential but the molecular structure remains elusive. Evidence suggests neuroprotection might stem from binding I2-IR sites on various proteins, particularly MAO-B involved in dopamine. Our study used molecular dynamics simulations with water/organic solvent mixtures to explore I2-IR sites in potential receptors. Ethanol, iso-propanol, pyridine, and water revealed strong interaction areas. Density analysis of retained solvents highlighted binding spots. We aim to dissect MAO-B's entry/exit paths for Dopamine and Dopal. Comparing these paths to interactions facilitated by 2-BFI I2 ligand, we seek to reveal the intricate regulatory mechanisms by which I2 ligands modulate MAO-B. We investigate how 2-BFI I2 ligand likely disrupts normal enzyme turnover and potentially competes for binding sites along product pathways.

## Introduction

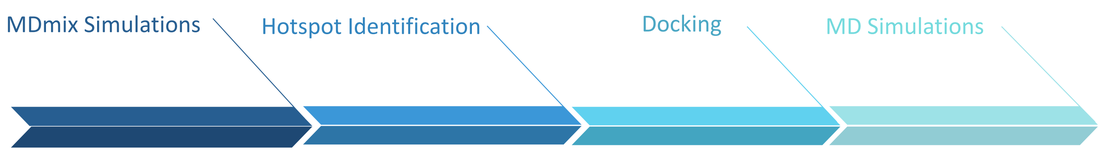


Monoamino Oxidase B

The increasing occurrence of Alzheimer's Disease (AD)[1] and Parkinson's Disease (PD)[2] drives innovative approaches against neurodegenerative disorders, supported by strong evidence linking Imidazoline receptors (IRs) to both conditions. These receptors are widespread in the brain, and I2 ligands have potential in countering neurodegeneration by alleviating cognitive and non-cognitive symptoms.[3] Yet, the structural nature of I2-IRs remains elusive, currently categorized as a diverse protein set (at least 4) interacting with I2 ligands like 2-BFI.[4,5]

Our study employs biomolecular simulations to reveal I2 ligand interactions with their targets. Cellular distribution studies highlight I2 receptors on the outer mitochondrial membrane, possibly as novel allosteric binding sites for monoamine oxidases MAO-B[6], linked to neurotransmitter oxidation and the potential antidepressant effects of I2 ligands. Using MDMix[7] and docking, we've pinpointed crucial binding sites for dopamine and I2 ligand-sized molecules on MAO-B's surface, near the enzyme's entrance. Our focus involves clarifying substrate entry, product exit, and the disruptive potential of I2 ligands.

## Methodology




**MD simulations in organic solvent/water mixtures**

- The MDmix methodology employs diverse solvent mixtures to identify pharmacophoric features within potential binding sites.

**Finding the Interaction spots and the binding pattern**

- Hotspots within the binding site of the 2BFI ligand have been utilized to create a probe pattern for the I2 binding site, revealing potential allosteric pockets identified through this binding pattern.

**Docking of I2 ligands using rDock programme**

- The 2BFI I2 ligand has been docked using aromatic and hydrophilic restraints within the identified hotspots.

**Analysis of the I2 ligand's stability**

- MD simulations have been conducted to visualize the stability of the I2 ligand in the docking poses.

## Hotspot Analysis

MDmix

Used to characterize the binding sites and druggability.

Identify high affinity interaction spots (hotspots) via retained solvent molecules along the MD simulations.

**ETA** : Ethanol 20% mixture

**ISO** : Isopropanol %20 mixture

Hydrophobic, and polar area (HB donor)

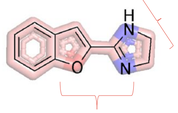
**PYR1** : Pyridine mixture %1

Hydrophobic, aromatic area, and HB acceptor

**WAT** : Water

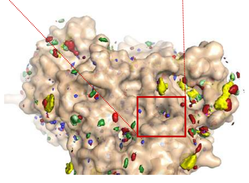
Polar area

Represented by alkyl groups of ethanol, isopropanol and pyridine solvents



Represented by Nitrogen atom in pyridine solvent

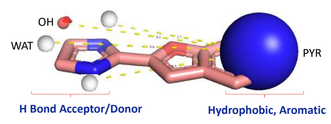
Represented by OH group of Ethanol and isopropanol solvents and water molecules

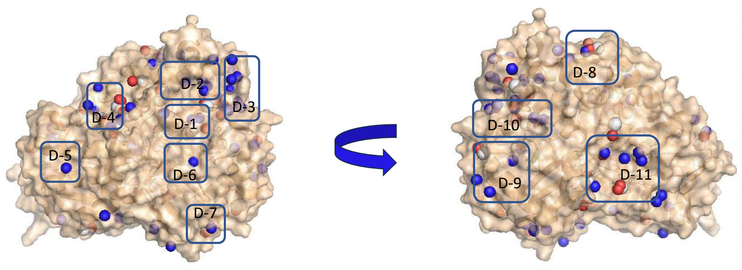


Through the utilization of Mdmix simulations, we successfully identified the pharmacophoric features of the entrance cavity, which notably accommodated the 2BFI ligand as observed in the crystal structure of the protein.

## Docking

Pharmacophoric restrains:

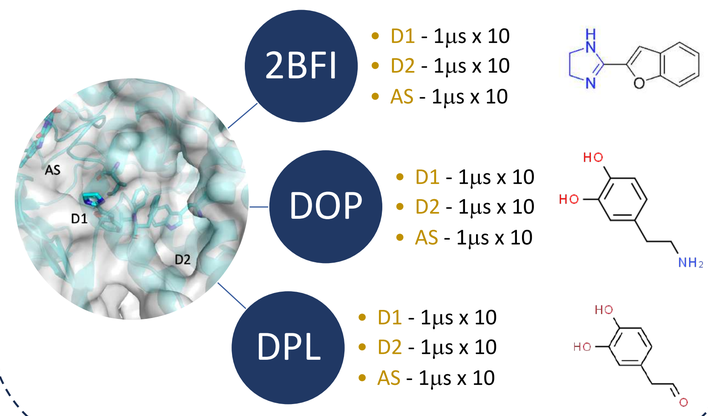


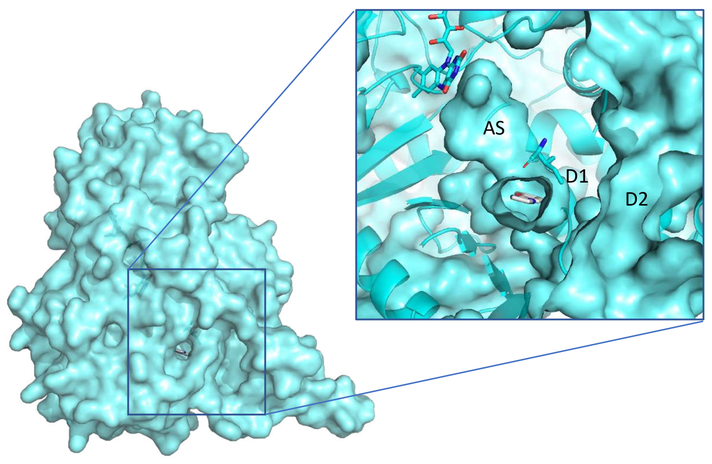

Utilizing the hotspots identified from the initial placement of 2BFI in the crystal structure, we established a probe pattern and explored its combination throughout the entire protein. This led to the selection of 11 docking positions. Subsequent short MD simulations were conducted with three replicas. Among these, only the D2 docking position exhibited stability for the 2BFI ligand.

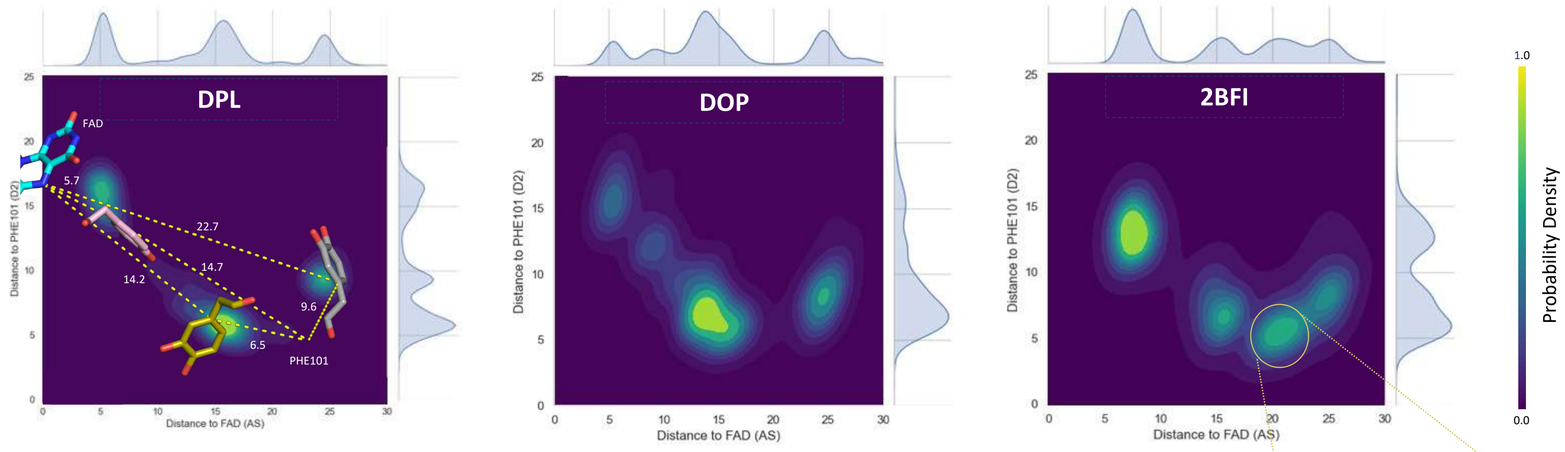
## MD Simulations

Two crystal structures of MAO-B have been employed in this project, distinguished by the position of an ILE residue that closes the gap between the entrance cavity and the active site cavity. Within these structures, dopamine, its metabolite Dopal, and the 2BFI I2 ligand were situated within the D1, D2, and active site (AS) cavities. Each simulation, utilizing 10 replicas, lasted for 1 μs. In total, each simulation contributed to a cumulative duration of 180 μs.




## Results

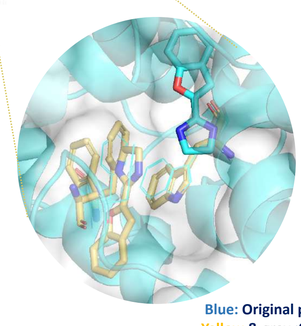



In addition to the active site and the entrance cavity D1, where the 2BFI ligand was located in the crystal structure, our investigation utilizing the MDmix and rDock programs unveiled a novel potential allosteric binding site, designated as D2. Over the course of 180 μs of molecular dynamics (MD) simulations, we conducted an analysis of probability density. This analysis was centered around the distances of molecules to both the FAD and PHE101 residues. These residues are in closer proximity to the active site (AS) and the allosteric position D2, respectively.

Through this analysis, we were able to observe the inclination of molecules to gravitate towards the AS, D1, or the newly discovered D2. In the visualization lighter shades indicate regions where molecules predominantly navigate along the exit-entrance path of the substrate. Our obsrvations unvailed a dynamic interchange of positions among these cavities by the molecules, occurring at varying rates. Notably, dopal and dopamine exhibited a propensity to predominantly occupy D1, whereas 2BFI displayed a higher probability density in the active site.

Furthermore, along the entrance/exit path, we noticed that the movement of 2BFI was impeded by the aromatic cavity formed by PHE101, TRP117, and PHE166 residues. This aromatic barrier could conceivably engage in competition with the dopamine substrate by obstructing its entrance. This phenomenon might provide insight into its influence on enhancing cognitive and non-cognitive behaviors.



Blue: Original position
Yellow & grey :Simulated

## Future work:

To observe the dual effect, we will conduct simulations with the coexistence of dopamin, dopal, and 2BFI in the same system.

We will employ QM/MM methodology to elucidate the reaction mechanism, focusing on the transition state, and explore the impact of 2BFI on the energy barrier of dopamine's reaction with FAD.

## References:

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## Acknowledgement:

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