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VALIDATION THROUGH RE-DOCKING, CROSS-DOCKING AND LIGAND ENRICHMENT IN VARIOUS WELL-RESOLUTED MAO-B RECEPTORS

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ABSTRACT: Molecular docking is one of the most utilized *in silico* techniques, which drastically reduces the cost and the time needed for the design of novel drugs. Recently, the tremendous growth of resolved crystallographic MAO-B structures, together with the rapidly rising computing power, has resulted in accelerated and moderately correct docking studies. However, initial validations of the docking protocols prior to virtual screening are required, considering the diverse set of scoring functions and the high number of crystallographic structures with different conformations. In this study, we conducted self- and cross-docking simulations of 21 highly resolute MAO-B receptors utilizing the docking software GOLD 5.3. The crystallographic structures with codes 1S3B, 3PO7, and 6FVZdemonstrated the most prominent ability to accommodate a chemically diverse set of ligands with good RMSD values. In some cases, higher enrichments were observed when rigid docking was carried out. The PDB code 1S3B demonstrated the highest modified enrichment value of 8.33. The latter structure could be further examined through ensemble and side-chain flexible dockings in order to obtain enhanced enrichments for a future virtual high-throughput screening.

INTRODUCTION: The application of MAO-B inhibitors in the treatment of Parkinson's disease has shown promising results in the early and stages of the condition, considering the conservation of high dopamine levels in the synaptic cleft [1]. Hence, the concentrations of both endogenous and exogenously administered neurotransmitters could be retained, and the mediator effects are clearly manifested [2].

Moreover, all monoamine oxidase inhibitors provide additional neuroprotective properties arising from the minimized amounts of neuronal toxic radicals and peroxides [3]. Monoamine

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oxidases (MAOs) are enzymes involved in the processes of deactivation of aliphatic and aromatic amines [4]. The enzymatic system encompasses two isoenzymes - MAO-A and MAO-B, which are crystallographically solved and described in detail. The similarities between both isoforms are over 70%; however, there are several significant differences in the amino residues present in the active sites of both enzymes [5]. MAO-B is characterized by three functional domains - entrance cavity, substrate cavity, and aromatic cage **Fig. 1.** The volume of the active gorge is estimated to be 700A3 [6]. In essence, the resolved crystallographic structures of MAO-B have provided insights into the active site of the latter receptor and have catalyzed the interest for further computational simulations [7, 8].

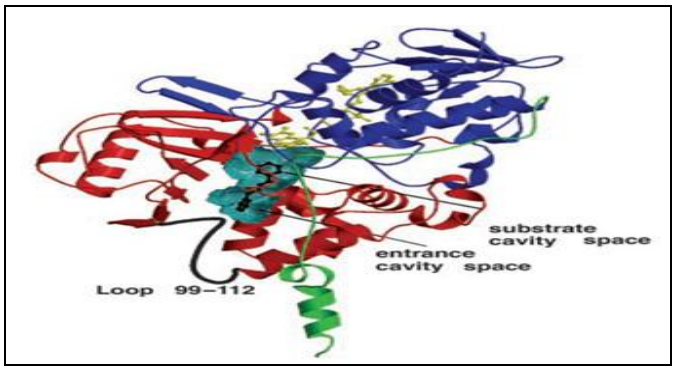

FIG. 1: 3D-STRUCTURE OF HMAO-B IN COMPLEX WITH 1, 4-DIPHENYL-1-BUTENE (PDB: 10J9) 6

Recently, molecular docking is emerging as a rapidly growing structural-based drug design (SBDD) technique, which could be utilized for hit identification and/or lead optimization [9]. In general, the core of each docking software are the search algorithm, which generates numerous ligand poses in the active site of the receptor, and the scoring function, which calculates the energies of the produced solutions and ranks them [10]. The readers are kindly forwarded to a paper published by Pagala *et al.* [11]. That provides detailed data about the different types of docking and the docking software.

Several papers have been published emphasizing the need for initial validation of the docking protocols and sufficient hardware information for the molecular docking studies [12, 14]. Common techniques for the evaluation of the accuracy of docking protocols are re-docking (self-docking), cross-docking, and ligand enrichment. Self-docking is an exceedingly utilized approach for the initial assessment of the accuracy of a docking program. As a validation method, it recreates the original pose of the co-crystalized ligand, while cross-docking evaluates the ability of the specific receptor to situate chemically diverse sets of ligands with acceptable RMSD values [15, 16]. During the database enrichment procedures, decoys are seeded in a set of active inhibitors, and the docking software is assessed for its ability to rank the active molecules. The decoy molecules resemble the active compounds by comprising the same physical properties; however, they should contain no-bind affinity towards the receptor. This study aimed to utilize re-docking, cross-docking, and ligand enrichments as docking validation procedures and select the most reliable MAO-B crystallographic structure or set of structures, which would accommodate a chemically diverse set of compounds applying GOLD 5.3 as a docking software.

MATERIAL AND METHODS:

Hardware: Operating system - Windows 10 Pro; CPU- AMD Ryzen 5 3600 6-core 3.60 GHz; GPU - GeForce GTX 1060 3 GB; Install memory (RAM) - 16 GB.

Software: GOLD applies a genetic search algorithm, and it examines the entire flexibility of the ligands [17]. The program allows side-chain flexibility and further freedom of the amine and hydroxyl-containing residues. Thus, intramolecular and intermolecular hydrogen bonds are permitted. The grid space is used to define all solvent-accessible atoms within the binding site are examined as active. The highest scored poses of the ligands are considered the best-bounded ones. Four scoring functions are defined-Chem PLP, Gold Score, Chem score, and ASP. GOLD is an automated ligand-docking program that uses a genetic algorithm to explore the full range of ligand conformational flexibility [17]. Moreover, it permits some protein conformational freedom in the sense that torsion angles of serine, threonine, and tyrosine hydroxyl groups, and lysine amine groups are optimized by the search algorithm during the posing. These groups are allowed to rotate freely to favor intra molecular (with other residues of the protein) and intermolecular (with the ligand trial solution) H-bond formation. GOLD requires a user-

defined binding site. It searches for a cavity within a defined zone and considers all the solvent-accessible atoms in that area as active-site atoms. On the basis of the GOLD score, for each molecule, a bound conformation with a high score is considered the best solution. The score function that was implemented in GOLD consisted of H-bonding, complex energy and ligand internal energy terms. A population of possible docked orientations of the ligand is set up at random. Each member of the population is encoded as a "chromosome", which contains information about the mapping of the ligand H-bond atoms onto protein H-bond atoms, mapping of hydrophobic points of the ligand into protein hydrophobic points, and the conformation around flexible ligand bonds and protein OH groups. A number of parameters control the precise operation of the genetic algorithm.

Pre-docking Methodology: 21 structures were downloaded from PDB [18] with the following codes: 1OJ9, 1OJA, 1OJC, 1S3B, 2BK3, 2BYB, 2C65, 2V5Z, 2VRL, 2VRM, 2VZ2, 2XFU, 3PO7, 4A7A, 4A79, 4CRT, 5MRL, 6FVZ, 6FW0, 6FWC and 6RLE. All protein and ligand refinements were carried out in the Hermes module (the 3D visualizer of GOLD 5.3 Suite). The proteins were further optimized by adding hydrogens and removing all water molecules applying the GOLD wizard setup.

The co-crystallized active ligands were extracted from the complex. The amino residues situated in the binding site were selected within 6Å from the original pose of the co-crystallized ligand. The pose of the latest was used as a template for the calculation of the RMSD values. All four scoring functions were evaluated with an early termination disallowed. The searching algorithm was set to default with a search efficiency of 100%. The top 10 solutions of each ligand were obtained, and all docking studies were repeated 10 times.

Ligand Enrichment: The enrichment factor (EF) represents the quantification of the reliability of the docking program. We have calculated both a modified enrichment factor (EF`) and the classical version - EF [19]. The latter enrichment value does not consider the rankings of the seeded actives, while EF` provides higher values when the active compounds are located in top positions. The formulas of EF and EF` (N) are defined as:

$$EF = (\text{Hits sampled}/\text{Hits total}) / (\text{N sampled}/\text{N total}),$$

Where Hits sampled are the actives located in the chosen dataset (N sampled). N total corresponds to all compounds included in the dataset, while Hits total is the number of the active molecules seeded in the decoys.

$$EF`(N) = (50\% / \text{APR sampled}) \times (\text{Hits sampeld} / \text{Hits total}),$$

Where N is the percent of the active compounds; ARP stands for ''average percentile rank'' of Hits total. In this study, we looked at 6% of the seeded active compounds; therefore, the value of Hits sampled corresponds to 10; Hits total equals 6931. In our case, if 10 of the ligands are situated in the top 10 docking solutions, the EF` (6) would be 37.6. The active structures and the decoys were taken from the Database of Useful Decoys: Enhanced website (DUD-E) [20]. The decoys possess physical properties similar to the active compounds; however, they are inactive. The docking protocol was set at 10% search efficiency considering the time-consuming simulation. The scoring algorithm was taken from the previous studies. Only the top 100 ranked solutions were retained and observed.

RESULTS AND DISCUSSION: After more than 2100 re-docking simulations, we obtained ten MAO-B receptors with optimal RMSD values. The docking protocols for each of the latter were further examined, and a suitable scoring function for each PDP structure was selected. Moreover, the flexibility of the active site residues, the presence of water molecules, and the size of the binding gorge were altered in order to obtain optimal docking parameters. Throughout the cross-docking procedure, we docked every co-crystallized ligand, from the first phase of the study, into the top ten receptors. The most prominent crystallographic structures were assessed to distinguish decoys from active compounds during the ligand enrichment simulation. A classical Enrichment factor and a modified version of it -EF`, were calculated after rigid and flexible docking simulations. All PDP codes of the examined crystallographic structures and information about the GOLD software are provided in the experimental part of the paper.

Analyzing and Pre-docking Refinements of the Selected MAO-B Crystallographic Structures: For the purpose of this study, 21 crystallographic structures of hMAO-B receptor in complex with co-crystallized ligands were taken from the Protein Data Bank (PDB) **Table 1.** The resolutions of the crystallographic structures were ranging from 1.6A to 2.4Å. Values under 2.5 Åunambiguously indicate that the structures are well resolved with accurate atomic coordinates 21. Each protein was initially prepared for the docking procedures by removing one of the two monomers together with the ligand and the co-factor lying in the binding cavity. We additionally extracted domains and other small molecules which were not present in the binding site.

TABLE 1: MOLECULAR DOCKING SIMULATIONS IN VARIOUS MAO-B RECEPTORS APPLYING DIFFERENT SCORING FUNTIONS

PDB	**ASP**			
	RMSD <1 Å	**RMSD 1-2 Å**	**RMSD 2-3 Å**	**RMSD >3 Å**
1OJ9	0	72	28	0
1OJA	0	0	8	92
1OJC	0	63	37	0
1S3B	99	1	0	0
2BK3	6	94	0	0
2BYB	0	94	6	0
2C65	**25**	24	5	46
2V5Z	40	60	0	0
2VRL	0	93	7	0
2VRM	0	0	80	20
2VZ2	0	79	19	2
2XFU	0	46	52	2
3PO7	1	35	10	54
4A7A	1	13	69	27
4A79	5	10	35	50
4CRT	3	32	62	3
5MRL	0	10	15	75
6FVZ	81	17	1	1
6FW0	72	28	0	0
6FWC	64	34	2	0
6RLE	2	98	0	0
PDB	**Chem Score**			
	RMSD <1 Å	**RMSD 1-2 Å**	**RMSD 2-3 Å**	**RMSD >3 Å**
1OJ9	8	92	0	0
1OJA	0	0	8	92
1OJC	0	0	0	100
1S3B	0	15	11	74
2BK3	0	5	74	21
2BYB	0	0	0	100
2C65	5	10	0	85
2V5Z	9	68	23	3
2VRL	0	5	31	64
2VRM	0	88	10	2
2VZ2	0	2	17	81
2XFU	0	0	54	46
3PO7	3	19	5	73
4A7A	2	87	7	4
4A79	2	87	7	4
4CRT	1	1	27	71
5MRL	0	0	9	91
6FVZ	56	4	2	38
6FW0	81	8	3	8
6FWC	8	40	46	6
6RLE	1	95	4	0
PDB	**Chem PLP**			

	RMSD <1 Å	RMSD 1-2 Å	RMSD 2-3 Å	RMSD >3 Å
1OJ9	7	84	9	0
1OJA	3	87	10	0
1OJC	0	69	27	4
1S3B	96	1	0	3
2BK3	0	82	4	14
2BYB	0	88	6	6
2C65	**0**	11	0	89
2V5Z	4	61	0	35
2VRL	0	100	0	0
2VRM	0	82	17	1
2VZ2	0	55	21	24
2XFU	0	66	28	6
3PO7	10	79	0	11
4A7A	30	69	1	0
4A79	57	43	0	0
4CRT	10	75	9	6
5MRL	30	32	25	13
6FVZ	96	4	0	0
6FW0	88	12	0	0
6FWC	65	34	1	0
6RLE	0	100	0	0
PDB		**Gold Score**		
	RMSD <1 Å	**RMSD 1-2 Å**	**RMSD 2-3 Å**	**RMSD >3 Å**
1OJ9	0	14	30	53
1OJA	0	0	1	99
1OJC	0	4	82	14
1S3B	0	0	25	75
2BK3	5	52	22	21
2BYB	0	73	22	5
2C65	52	13	0	35
2V5Z	0	12	0	88
2VRL	0	0	92	8
2VRM	0	0	0	100
2VZ2	0	78	9	13
2XFU	0	0	0	100
3PO7	0	1	0	99
4A7A	0	5	14	80
4A79	13	4	64	19
4CRT	0	0	96	4
5MRL	0	10	13	77
6FVZ	5	8	7	80
6FW0	80	6	3	11
6FWC	69	11	10	10
6RLE	0	95	4	0

Validating the Molecular Docking Study: We initiated the validation process applying self-docking procedures to assess the ability of the docking program to recreate the original co-crystallized poses of the ligands employing all GOLD scoring functions (Chem PLP, Chem Score, Gold Score, ASP). In the re-docking protocol, the co-crystallized ligands of the receptors were removed and without any minimization procedures, re docked back into the original receptors. Detailed parameters of the docking protocols are discussed later in the paper. The poses of the top-scored compounds and the initial pose of the co-crystallized ligand were compared to each other and final RMSD values were obtained. We defined four different RMSD thresholds. Values under 1 Å were determined as excellent, from 1-2 Å as good, 2-3Å as moderate, and above 3Å as wrong/incorrect. To obtain optimal RMSD values, we altered some changeable variables such as waters in the active site (toggle on/off), side-chain flexibility, and size of the binding cavity. **Table 1** provides information about the success rate of each simulation utilizing different scoring functions.

After analyzing the results, we selected the best scoring algorithms for each receptor.

TABLE 2: SUMARIZED TABLE OF THE RE-DOCKING SIMULATIONS

PDP codes	Scoring function	Binding site size	Number of solutions with RMSD <2 Å
1OJ9	Chemscore	6 Å	100/100
1OJA	ChemPLP	6Å	90/100
1OJC	ChemPLP	6 Å	69/100
1S3B	ChemPLP	8 Å	97/100
2BK3	ASP	8 Å	100/100
2BYB	ChemPLP	6 Å	88/100
2C65	GoldScore	6 Å	65/100
2V5Z	ASP	8 Å	100/100
2VRL	ChemPLP	8 Å	100/100
2VRM	Chemscore	6 Å	88/100
2VZ2	ASP	6 Å	79/100
2XFU	ChemPLP	8 Å	66/100
3PO7	ChemPLP	6 Å	89/100
4A7A	ChemPLP	6 Å	99/100
4A79	ChemPLP	8 Å	100/100
4CRT	ChemPLP	6 Å	85/100
5MRL	ChemPLP	8 Å	62/100
6FVZ	ChemPLP	6 Å	100/100
6FW0	ChemPLP	6 Å	100/100
6FWC	ChemPLP	6 Å	99/100
6RLE	ChemPLP	8 Å	100/100

From the obtained results, it could be concluded that the receptors, which recreated the pose of the co-crystalized ligands with the best possible RMSD values, are 1OJ9, 1S3B, 2BK3, 2V5Z, 3PO7, 4A7A, 4A79, 6FVZ, 6FW0, and 6FWC. Chemscore was suitable for 1OJ9 and 2VRM, while the ASPscoring function recovered the co-crystallized ligands in 2BK3, 2V5Z and 2VZ2 with good results. Goldscore performed best when applied on 2C65, while for the rest of crystallographic structures, Chem PLP was the most fitting choice. The latter observations were expected, considering the results from recent performance tests done by Liebeschuetz *et al.* [22]. We also noted that the Chem PLP was the fastest scoring function concerning the time scale of the simulations, while Gold Score was the slowest. No improvements were achieved when rotatable side chains and water molecules were introduced in the active sites of the receptors. The data from the self-docking simulations is summarized in **Table 2.** Only the top 10 receptors were included.

Cross-docking: For further validation of the docking protocols, cross-docking procedures were carried out. In the cross-docking study, each co-crystallized ligand was docked in the receptors above. The purpose of the cross-docking simulation was to determine how many of the receptors could accommodate a chemically diverse set of co-crystallized ligands with low RMSD. The optimal parameters for every single receptor were taken from the previous re-docking simulation. The top 10 solutions of the docking runs were retained, each docking test was repeated 10 times and the average RMSD of the best-scored solutions were taken. The results from the cross-docking study are summarized in **Table 3.**

MAO-B receptor with PDB code 3PO7 demonstrated the best results as it accommodated 9 of the 21 chosen co-crystallized inhibitors with good RMSD values. Furthermore, two of the inhibitors (chorophenyl chromone and fluorophen chrom one) showed excellent RMDS values when docked into the 3PO7 receptor. Interestingly, the latter receptor was the only one, out of the 10 used in this study, to recreate the original pose of phenylethlhydrazine with “good” RMSD value of 1,75. The cross-docking simulation in the 3PO7 also displayed that the receptor is the best choice for docking molecules with hydrazine moiety, considering the prominent values of benzylhydrazine and phenylethlhydrazine. The worst results were represented by the receptor with PDB code 1OJ9. Considering the above-mentioned data, we selected the top three receptors for further enrichment simulations.

Ligand Enrichments: In this section of the study, we took the top-ranked receptors from the cross-docking study and applied them for the simulation of 6,900 decoys and 169 seeded active compounds (detailed description in the experimental section). The aim was to see how good the receptors were in distinguishing the inactive from the seeded active molecules by defining enrichment values.

The results for 3PO7 showed that EF` (6) equals 8.6. Seven of the top-ranked solutions (fitness scores 100-96) were obtained by decoys, which determines the weak capacity of the receptor in differentiating false positives from true positives. We repeated the docking procedure with the same set of parameters which led to similar results. The ordinary enrichment factor, which does not

consider the rankings of the ligands, equals to 4.18. During the modification of the docking protocol, we obtained interesting results when we fixed the ligand's rotatable bonds. In the latest case, five of the top 10 ranked solutions were occupied by the active ligands and more interestingly, the first 3 solutions (fitness score 82-81) were taken by them. Overall, the EF` (6) value of 3PO7, when the ligands were set as rigid, was 6.9, which is significantly higher compared to the flexible docking. We continued the enrichment simulations applying 6FVZ as a docking receptor. The ligands were freely rotatable, however, the results were unsatisfactory. As shown in **Table 4.** the EF` (6) value dropped to 3.96. When we fixed the rotatable bonds, five additional ligands were located in the top 100 scores, and hence the value of the enrichment factor increased. Lastly, we examined the enrichment of 1S3B, which demonstrated the highest EF` (6) value out of the 3 MAO-B receptors. Here the best score was obtained with flexible ligands. The rigid docking did not show any improvements.

TABLE 3: CROSS-DOCKING STUDIES OF THE MOST PROMINENT CRYSTALLOGRAPHIC MAO-B RECEPTORS. THE RE-DOCKING SCORES ARE BOLDED AND UNDERLINED

	1OJ9	**1S3B**	**2BK3**	**2V5Z**	**3PO7**	**4A7A**	**4A79**	**6FVZ**	**6FW0**	**6FWC**
1OJ9	2.1	2.6	3.35	3.67	2.72	2.5	2.24	2.63	2.8	2.7
1OJA	10.2	3.17	3.8	3.45	2.99	3.61	3.87	2.7	2.76	2.84
1OJC	6.42	2.27	3.15	2.6	1.65	4.13	4.53	2.27	2.12	1.8
1S3B	7.6	1.4	2.1	1.68	1.65	3.85	11	3.88	4.28	3.54
2BK3	3.4	1.96	1.74	1.81	1.94	2.28	9.55	3.56	4.45	4
2BYB	5.4	3.07	3.6	3.4	3.26	2.32	8.6	4.1	3.82	3.85
2C65	5.1	3.5	4	5.2	3.1	4.5	7	2.1	2.7	2.5
2V5Z	2.4	1.7	5	1.6	2.1	4	3	1.8	3	2.9
2VRL	12.19	2.13	2.8	3.01	1.87	2.2	11.55	3.84	4.29	4.15
2VRM	11.18	3.04	-	3.65	1.75	3.62	13.68	3.9	4.04	4.43
2VZ2	8.48	1.9	3.17	5.48	5.25	5.37	10.37	5.6	6.25	5.86
2XFU	11.95	3.51	4.36	4.57	4.1	6.93	14.2	6.04	5.65	5.9
3PO7	4.39	3.35	3.92	5.06	1.48	3.14	3.45	1.9	2.1	2.15
4A7A	1.6	2.84	4.9	5	1.41	1.17	0.88	1.4	1.8	1.85
4A79	9	3.48	9.1	7.7	7.88	8.79	0.69	7.5	7.03	6.05
4CRT	6.7	3.62	2.05	3.2	3.72	4.63	8.59	5.07	7.11	7.15
5MRL	8.3	4.69	5.15	4.96	1.96	2.28	9.55	3.56	4.45	4
6FVZ	4.3	0.78	1.72	2.34	1.19	1.33	0.52	0.69	1.73	1.8
6FW0	5.9	0.6	1.36	1.35	0.61	0.7	1.45	0.46	0.75	0.88
6FWC	6	0.93	1.89	1.83	0.53	0.79	2.14	0.69	0.6	0.64
6RLE	2.5	1.53	1.72	1.24	1.66	1.28	7.09	2.48	1.99	2.16

* The RMSDs from the re-docking simulations were underlined. ** All the unsuccessfully docked ligands are denoted with "-".

TABLE 4: ENRICHMENTS OF MAO-B RECEPTORS WITH FLEXIBLE AND RIGID DOCKING

	EF`(6)		**EF**
	Flexible docking	**Rigid docking**	
3PO7	4.1	6.9	4.18
6FVZ	3.96	8.84	4.58
1S3B	8.33	7.44	5.41

Considering the recent work of Sheng-You Huang [23], it is evident that in some receptors, flexible docking does not provide higher enrichments compared to rigid simulations. In the first two cases, the results demonstrated better MAO-B enrichments when rigid docking was carried out. Furthermore, the computational efficacy was tremendously lowered compared to flexible docking. However, the best value was acquired after flexible docking simulations towards the crystallographic MAO-B receptor with the code - 1S3B.

CONCLUSION: In this work, we presented the validation of a molecular docking protocol utilizing [21] highly resolute crystallographic structures of MAO-B. Re-docking, cross-docking and ligand enrichments towards freely available, highly resolute crystallographic structures taken from the Protein Data Bank were conducted. After the self-

docking simulations, we filtered most of the receptors, acquiring 10 structures with RMSD values of the re-docked ligands under 2 Å. Furthermore, within this part of the study, we applied all four scoring algorithms of GOLD 5.3 and selected the most prominent one considering the obtained RMSD scores and the computational expenses.

After the cross-docking procedures, we demonstrated that the receptors with PDB codes 1S3B, 3PO7 and 6FVZ have the most promising capacity of accommodating diverse set of chemically distinct ligands with "good" RMSD values and were further used for database enrichments.

A modified enrichment factor (EF`) was calculated, which considers the rankings of the actives. 3PO7 and 6FVZ demonstrated drastically higher EF` (6) values when the rotatable bonds were fixed. In the case of 1S3B, a higher enrichment value was obtained after flexible docking simulations. Further molecular dynamics and rigid-docking simulations should be carried out, considering the results discussed in this paper.

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