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Comparison of docking procedures and its efficiency for Betasecretase, Aromatase and Pyruvate dehydrogenase kinase inhibitors

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Abstract

Proper docking protocols were presented for three known enzyme structures, human betasecretase (BACE1), Aromatase and pyruvate dehydogenase kinase (PDHK) using Autodock4.2 and Vina softwares. The validity of docking protocols was verified using a set of known active ligands and decoys for all three enzymes. Different energy minimization algorithms were performed prior to docking of each ligand in order to find out by which method a more reasonable correlation between binding energy and corresponding experimental activity of the compounds was obtained. The highest ROCAUC value was 0.916, 0.914 and 0. 833 when MM^+-PM3 methods were applied as minimization method, whereas without minimization it was 0.127, 0.187, and 0.51 for PDHK, BACE-1 and aromatase, respectively. So a combination of molecular mechanics (MM^+) and a semi-empirical method (PM3 or AM1) could promote the docking protocol in case of all targets. Protein ligand interaction studies using self-organizing map (SOM) were also conducted in order to reveal the validity of docking protocol and to evaluate its predictive ability in terms of distinguishing between ligands and decoys.

Keywords: Aromatase, Betasecretase, Energy minimization, Docking, Pyruvate Dehydrogenase Kinase, Virtual ligand screening.

1. Introduction

During the past decades, the tools available for either designing new bioactive molecules or improving the old structures have grown incredibly both in number and quality (1). Among them, computer-assisted drug design and quantitative structure activity relationship (QSAR) are the two main fields of modeling three-dimensional properties of molecules from which the whole procedure of drug design has emerged (1-3).

Docking programs are widely used to predict the binding mode and affinity of ligands in the binding site of receptors (4, 5). Due to biological andpharmaceuticalsignificance of molecular docking, considerable efforts have been directed to improve the methods used in docking process (4, 5).

Most docking programs are based on a stochastic algorithm to generate different conformations of ligands with different orientations and locations in the active site (6, 7). A global search method such as genetic algorithm is consequently

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used to find the best pose of ligand in the binding cavity (6, 7). One of the most important issues which is normally leading to different results in docking procedure is therefore the primary state of the ligands at the starting point. Since different minimization methods of molecular mechanics (MM^+) or quantum mechanics (QM) could be taken prior to docking run (2, 6), it is worth nothing by which method more realistic results are being obtained. In this study, different procedures of docking were performed for active ligands and decoys of three known targets using Autodock 4.2 and Vinasoftwares. The target enzymes were BACE1, aromatase and PDHK. The selected enzymes are among the most important topics in drug discovery (8-10). Some molecular docking and virtual ligand screening protocols on these targets have been described so far. However, the energy states of the ligands at the starting point has not been addressed in almost either of studies (11-15). Since the energy states of the ligands at starting point could result in different docking energy values, different protocols were investigated in this study. As it was shown in Figure 1, the docking protocols were different in terms of the minimization algorithms that were used prior to main docking run. The minimization algorithms included two semi-emprical methods (AM1 and PM3), the commonly used molecular mechanics (MM^+) and a combination of both methods. Two docking softwares,Autodock 4.2 and Vina were used in this study. The two known statistical metrics, receiver operating characteristic (ROC) and Enrichment Factor (EF) were used to evaluate the docking protocols in terms of their predictive ability to distinguish between active ligands and inactive decoys (16). A clustering based approach towards protein ligand interaction fingerprints using self-organizing map was also conducted to classify the ligands and decoys based on their contact maps (17). The obtained protocol of docking could be used to design inhibitors for the target enzymes in future studies.

2. Materials and Methods

2.1. Preparation of the structures

The structures for all three target enzymes BACE1 (1W51, Resolution 2.55 Å), Aro-

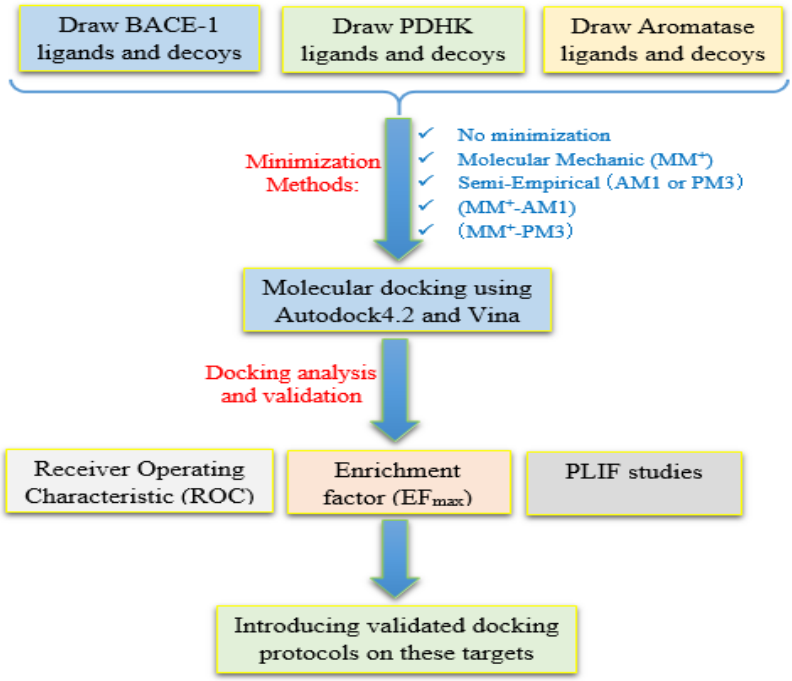

Figure 1. Flowchart for the entire work. The minimization algorithms included two semi-emprical methods (AM1 and PM3), the molecular mechanics (MM^+) and a combination of both methods. Two docking softwares, Autodock 4.2 and Vina were used in this study. The two known statistical metrics, ROC and EF_{max} were used to evaluate the docking protocols in terms of their predictive ability to distinguish between active ligands and inactive decoys. The obtained protocol of docking could be used to design inhibitors for the target enzymes in future studies.

matase (3EQM, Resolution 2.9 Å) and PDHK (2BU8 Resolution 2.5 Å) were retrieved from protein data bank (PDB) (18). The role of water molecules in all structures was studied using ligplot software (19). Water molecules and the co-crystal ligands were thereafter excluded from the structures and the PDBs were corrected in terms of missing atom types using modeller 9.12 (20). An *in house* application program interface (MODELFACE) was used for generation and running of python scripts within modeller software. The MODELFACE application can be retrieved from modeller website.21Subsequently, the enzymes were converted to PDBQT and gasteiger partial charges were added using MGLTOOLS 1.5.6. (6).

For each target, 20 active ligands and 70 inactive decoys were retrieved from ChEMBL database as SMILES format (22, 23, 24). Iterative runs of openbabel 2.3.2 through a shell script provided the primary 3D generation of the structures as mol2 format (25). Ionization states at PH=7 were also calculated for all structures. The shell script was provided by means of batch scripting in windows operating system.

2.2. Optimization of the active ligands and decoys

The active ligands and decoys were subjected to different minimization procedures by means of an *in house* TCL script using Hyperchem8 (Hypercube Inc). Polak-Ribiere algorithm with RMS gradient value of 0.1 was taken and the maximum number of cycles was set to 32767 in order to reach convergence with all structures. Each structure was separately saved after being minimized as any of the five different methods *viz*MM^+, AM1, PM3, MM^+-AM1 and MM^+-PM3. The output structures were thereafter converted to PDBQT using MGLtools 1.5.6. The structures were also saved at their primary unminimized energy states.

2.3. Docking procedure

A workstation with 8 processors running on Windows 7 was used during all experiments. The docking simulations were carried out by means of an in house batch script (DOCKFACE) for automatic running of AutoDock 4.2 and Vina in parallel mode using all system resources. DOCKFACE was designed to facilitate the virtual ligand screening in stepwise mode including ligand preparation, receptor preparation, grid maps generation, dpf files preparation and finalization of docking runs. Processing of docking with Vina was also implemented in DOCKFACE (26, 27). In all Autodock 4.2 experiments Genetic algorithm search method was used to find the best pose of each ligand in the active site of the target enzyme (28, 29). The Genetic Algorithm and grid box parameters are listed in Table 1 and 2, respectively. The exhaustiveness parameter in Vina was set to 100 (30). Random orientations of the conformations were generated after translating the center of the ligand to a specified position within the active site of the receptor and making a series of rotamers. This process was recursively repeated until the desired number of low-energy orientations was obtained. No attempt was made to minimize the ligand-receptor complex (rigid docking). All visualization of protein ligand complexes were done using VMD software (31).

2.4. Analysis of Docking Results

For each target, the resulted files were subjected to an in house application implemented in vigual.net and the minimum energies related to the most favourable pose of each ligand were extracted from Autodockdlg files and Vina out.txt files. Subsequently, the two metrics of virtual screening including the area under the curve (AUC) for receiver operating characteristic (ROC) plot and the maximum value of enrichment factor (EFmax) were calculated for active ligands and decoys of all

Table 1. Docking Parameters of Autodock 4.2 and Vina softwares.

Parameter Name	Value
Number of GA Runs (Autodock 4.2)	500
Population Size (Autodock 4.2)	200
Max. No. of evaluations (Autodock 4.2)	2500000
Exhaustivenes (Vina)	100

targets using our application (16, 32).

2.5. Protein ligand interaction fingerprint (PLIF)

In order to perform PLIF studies on docking results, the poses of docking were extracted from dlg files using an *in house* vb.net application (preAuposSOM) (33). The resulted pdbqts and the receptor were converted to mol2 by means of a batch script using OpenBabel 2.3.1. The resulted mol2 files were submitted to AuposSOM 2.1 web server (17, 34, 35). Two training phases with 1000 iterations were set in the self organizing map settings of AuposSOMconf files. Other parameters of the software were remained as default. The output files were subjected to Dendroscope 3.2.10 for visualization of the results (36).

3. Results and discussion

The x-ray resolution of all pdb structures used in this study were in the similar range of 2.5 to 2.9 Å. Based on the data obtained from x-ray crystallography, no interaction was seen with any water molecules in the active cavity of the studied target enzymes. As an instance the interaction of the co-crystal for BACE1 target is depicted in Figure 2 with regard to water molecules. Since the docked compounds were structurally relevant to co-crystal structures, removing water molecules could be more beneficial in terms of simulating the native interactions as seen for the co-crystal ligands.

The data for grid maps are displayed in Table 2. The grid box dimensions were defined based on two times the length of the largest ligand in the data set for each target to avoid any constrains and bias in docking procedure. The grid center was selected based on the centre of the co-crystal ligand in case of all targets.

The results of analysis are listed in figure 3 and Table 3 for all protocols in terms of the ROCAUC and EFmax metrics, respectively. The plots of ROC and EFmax are provided for BACE-1 enzyme in Figure 4. The application of ROC in computational medicinal chemistry was first reported by Triballeau *et. al*. (16). Since emergence of this method, it was widely used as a useful metric in order to evaluate the validity of docking scores in screening studies. In order to use this metric in a virtual screening study, the structures must be first categorized into two subsets of actives and decoys based on their experimental activities. The screening method should be therefore able to discriminate between active ligands and decoys. ROC value is the area under the curve

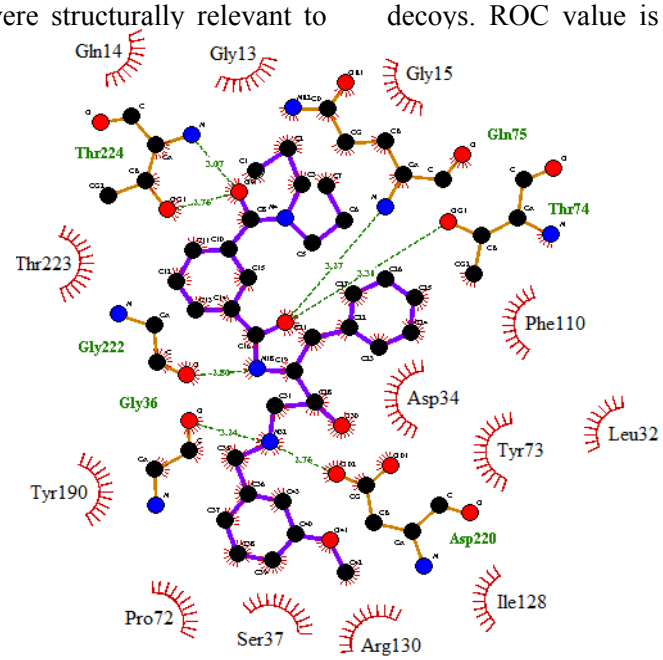

Figure 2. Two dimensional interaction map for the co-crystal ligand of BACE-1 target using ligplot software. No interaction with water molecules was observed in the active site. The key residues in the active site of BaCE-1 which play a great role in binding with ligands are Thr224, Gly222, Gly36, Asp220, Thr74 and Gln75.

Table 2. Gridbox parameters in Autodock 4.2 and Vina softwares.

Parameter Name	PDHK[1]	BACE1[2]	Aromatase
No. of points in x	50	50	60
No. of points in y	50	50	60
No. of points in z	50	50	60
Grid spacing	0.375	0.375	0.375
Box X center	55.685	69	85
Box Y center	46.509	48	54
Box Z center	80.992	8	46

[1]Pyruvate dehydrogenase kinase, [2]human betasecretase.

(AUC) for the plot of selectivity versus specificity in a screening method. The two metrics selectivity (Se) and specificity (Sp) for each docking score are calculated according to the Equations 1 & 2.

Se=No. selected actives/No. total actives

Se=TP/TP+FN (Eq. 1)

Sp=No. Discarded actives/No. total inactives

Sp=TN/TN +FP (Eq. 2)

In the above equations TP denotes true positive ligands for any of the docking scores while FP and FN denote false positive and false negative structures, respectively.

ROC curves were obtained by plotting (Se) versus (1-Sp) for all docking scores. The area under the curve for ROC is calculated by trapezoidal integration method as implemented in our *in house* application.

The more values of ROCAUCmeans that the docking protocol is able to discriminate active ligands from decoys. Enrichment Factor is another tool to evaluate the efficiency of docking protocol in virtual screening studies. Compared to ROC curves, EF_{max} factor is highly dependent to the number of actives in a data set (16). It means that early enrichment can be easily obtained if the number of active ligands is increasing in a dataset. Enrichment factor values for all experiments were calculated according to Equation 3:

EF=(No.SA/No.SC)/(Total No.Actives/No. SC) (Eq. 3)

Where SA denotes for screened actives and SC for screened compounds. As displayed in Table1, the docking parameters were converged in the current study so that obtained ROC values were merely dependent to the parameters of optimization rather than stochastic implementations of Autodock software. Higher values of ROCAUC or EFmax are therefore representative of better minimization methods leading to more reasonable starting points before the docking procedure.

The probability to obtain a randomly high ROC value was also measured by a post test of chance correlation. During the test, the ROC values were recalculated 100 times with randomly arranged docking scores. If any of the generated scores of post test was more than the primary values, the docking scores for that protocol were considered as statistically meaningful. Based on the obtained values in Figure 3, it was seen that the procedures with minimization step such as AM1 or PM3 preceded by mm+led to higher values of ROC. The highest ROCAUC value for this study was observed in PDHK target (Autodock 4.2;MM^{+}-PM3=0.916, Vina MM^{+}-AM1=0.886). Using a direct AM1 or PM3 semi-empirical minimization method, resulted in lower ROCAUC values in comparison with the hybrid method of MM^{+}-AM1 or MM^{+}-PM3 in case of most targets. This finding was extensively similar in case of both softwares used in this study. Regarding the data displayed in Figure 3, the area under the curve values for Autodock 4.2 software were more sensitive to energy states than Vina software. In case of Vina the minimum ROC value was pertained to aromatase target in the experiment which no minimization was taken. However with Autodock 4.2 software the minimum ROC value was seen in PDHK target(0.127). According to the data in Table 3, it

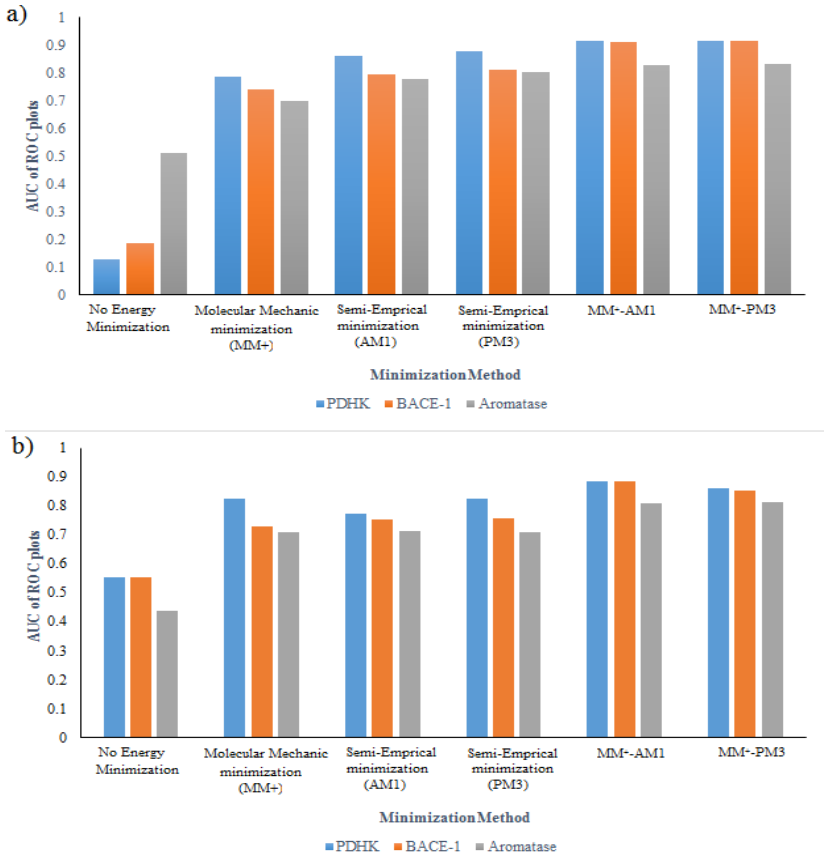

Figure 3. a) The area under the curve of ROC plots for different docking protocols using Autodock 4.2 software. b) The area under the curve of ROC plots for different docking protocols using vina software. Based on the obtained values, it was seen that the procedures with minimization step such as AM1 or PM3 preceded by MM^+ led to higher values of ROC. The AUC values for Autodock 4.2 software were more sensitive to energy states than Vina software.

was observed that regardless of the minimization algorithm, using a molecular mechanics or semi-empirical method could increase the EFmax value compared to protocols wherein no minimization methods were taken. Since ROC values do not depend to the number of active ligands and decoys, they are more reliable in making decision about the validity of the methods than EFmax analysis. In order to perform a more convincing validation test for the presented protocol and to check out the selectivity of docking for each target, cross docking studies were conducted. During this procedure the ligands and decoys of each target were cross docked on the other targets. Finding insignificant ROC values or more positive energy scores in cross docking studies is indicative of validity and selectivity for the presented docking protocols. Cross docking of BACE1 ligands in the active site of PDHK has resulted in positive energy values. In other cases negative energy values were obtained but the ROC values were low and significant. For instance the cross docking result of aromatase li-

Table 3. EF_{max} values for different docking protocols using Autodock 4.2 software

Targets	No Energy Minimization	Molecular Mechanic minimization	Semi-Emprical minimization		Molecular Mechanic+Semi-Empirical	Molecular Mechanic+Semi-Empirical
		MM^+	AM1	PM3	MM^+-AM1	MM^+-PM3
PDHK	0.94	3.53	3.38	3.44	3.07	3.37
BACE1	1.45	4.62	4.62	4.29	4.75	4.68
Aromatase	1.02	2.17	2.26	3.48	3.48	3.43

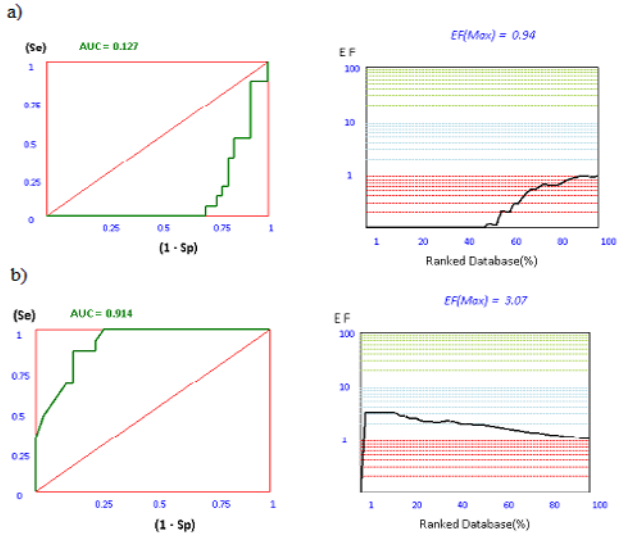

Figure 4. ROC and EF diagrams for PDHK target. a) Docking protocol without minimization. b) Protocol with minimization using MM^{+}- AM1. Higher values of ROCAUC or EFmax are representative of better minimization methods leading to more reasonable starting points before the docking procedure.

gands in the active site of BACE1 is depicted in Figure 5. As seen in the figure, the insignificant ROC value is indicating that the described protocol of BACE1 is sensitive to the structures of its own ligands.

As explained, the ROC curves and to some extent enrichment factor could explain the more efficiency of docking protocols with semi-empirically minimized structures as the starting point. The most important issue with these analysis methods was this fact that the best poses of ligands and decoys were merely used during analysis and other generated poses were ignored. In addition to validation methods such as ROC curves and enrichment factor, protein ligand interaction fingerprint (PLIF) studies could be used as a more reliable analysis technique.17 PLIF study is therefore a validation technique to evaluate the efficiency of the presented protocol. Finding rational classification of ligands and decoys is indicative of a docking protocol which is able to distinguish between active ligands and decoys. This method also makes it possible to study the effect of different starting states of the structures on generated poses as well as their corresponding vector of contacts towards receptor during docking procedure. For this purpose, two docking protocols with MM^{+} and MM^{+}-AM1 minimization

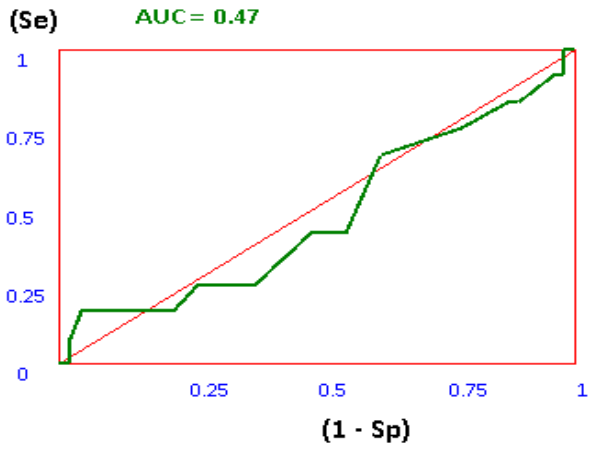

Figure 5. Cross docking of Aromatase ligands and decoys in the active site of BACE1 target. The insignificant ROC value is indicating that the described protocol of BACE1 is sensitive to the structures of its own ligands.

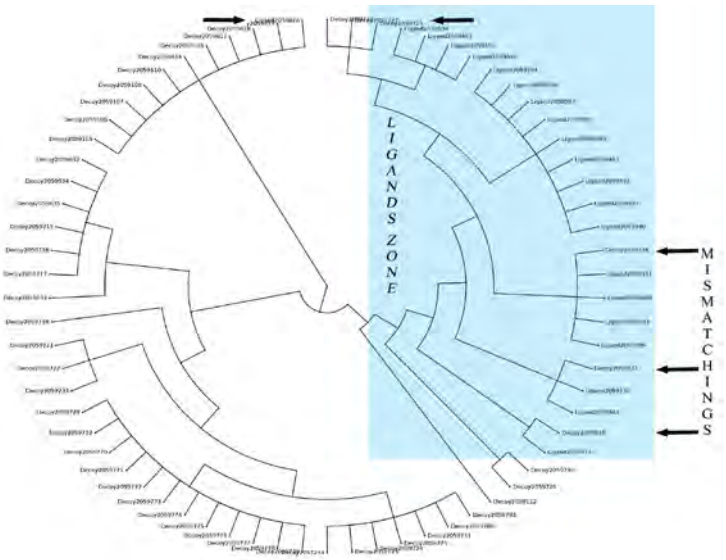

Figure 6. AuposSOM results for poses of docking with MM+ minimization method; classification with less resolution and more mismatchings.

methods were compared in case of BACE1 target using Autodock 4.2 software. As described in the methods section, all generated poses of ligands and decoys were subjected to AuposSOM 2.1 to calculate their contact vectors within the receptor binding cavity. The efficiency of AuposSOM for Surflex-Dock 2.0 from the Sybyl 1.2 package was previously reported.17In this method, the contacts between the structures and the protein include hydrophobic, hydrogen bonding and coulombic interactions. The resulted vectors of contacts are subsequently analyzed using self-organizing map as implemented in AuposSOM software. The output of self-organizing map is a classification pattern for ligands and decoys. If all ligands were classified in subgroups different from decoys, the docking protocol could have been considered as a perfect model in terms of its ability to discriminate between ligands and decoys. As displayed in Figure 6 and 7, using MM^{+} led to a PLIF pattern wherein ligands and decoys were clustered in different subgroups and low branching resolution. Mismatching of ligands and decoys was however seen in case of some structures (Figure 6). On the other hand by using a molecular mechanics/semi-empirical hybrid method such as MM^{+}-AM1, the

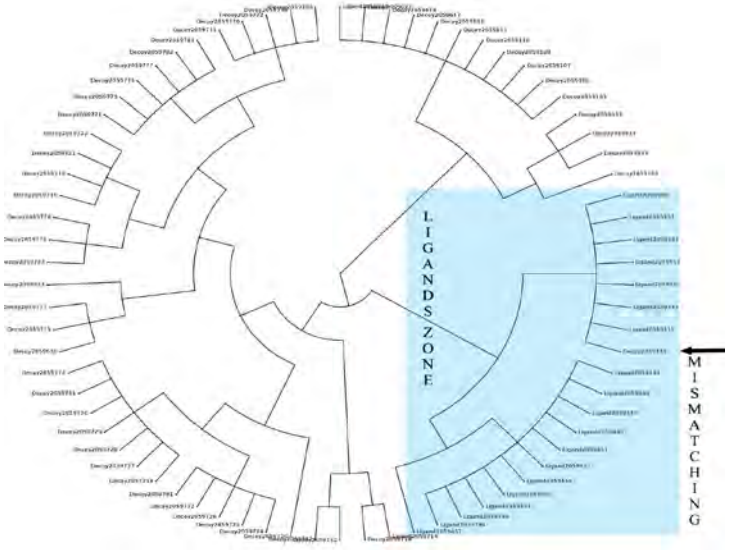

Figure 7. AuposSOM results for poses of docking with AM1 minimization method; classification with more resolution and less mismatchings.

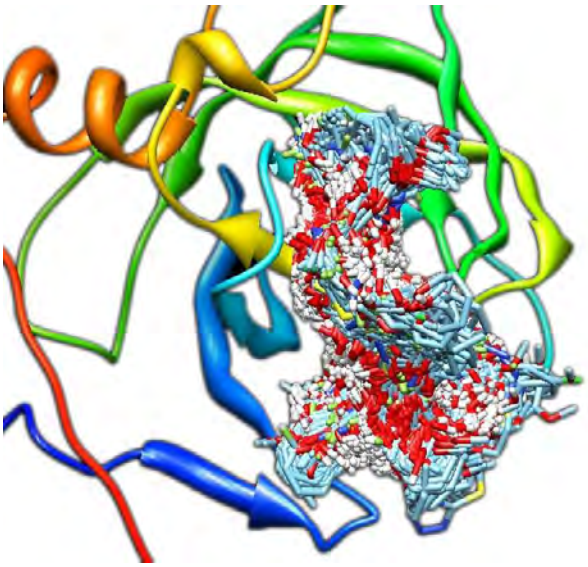

Figure 8. The best docking poses of some structures together with the co-crystal ligand of BACE1 target. Docking poses of ligands for BACE1 are in accord with its co-crystal structure in the active site of the enzyme.

results were much improved in terms of branching and the number of mismatching was decreased (Figure 7). This finding is also another convincing reason for greater efficiency of minimization methods using quantum based approaches such as AM1 before docking procedures. The results obtained by AuposSOM scoring function was also in accord with ROC curve studies. As optimization of small ligands using semi-empirical methods is not a very time consuming task due to the progress in hardware development, it is highly suggested to perform both molecular mechanics and semi-empirical minimization prior to docking run.In order to investigate whether the suggested protocols of virtual screening is able to find the best pose of each ligand in its correct binding mode; some visual inspections were done for all used targets in case of both softwares. The results for each target were compared with its corresponding co-crystal ligand. As depicted in Figure 8 for Vina software, docking poses of ligands for BACE1 are in accord with its co-crystal structure in the active site of the enzyme. The docking protocol is therefore a reasonable procedure for prediction of orientation

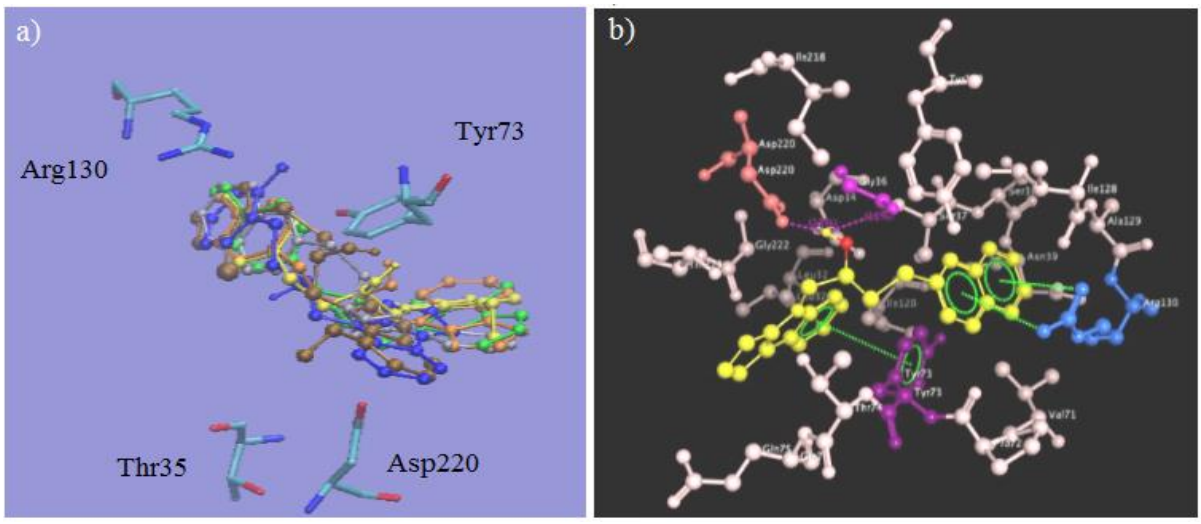

Figure 9. The key residues such as Arg130 and Tyr73 involved in the binding site of BACE-1. a) Superposed best poses of some ligands, b) the best pose for a representative structure in the active site visualizations were performed with VMD.

and location of the correct conformations for the ligands in the active site.

The most important residues for BACE1 target using Autodock4.2 are displayed in Figure 9a. Among them, Tyr73 seems to be important in π-π interaction with structures bearing aromatic moieties. The basic side chain of Arg130 can also participate in cation-π interaction with those structures tethering anionic fragments. (Figure 9b).

4. Concluding

It is worth knowing by which docking protocol proper scores are being obtained during docking procedure for betasecretase, aromatase and pyruvate dehydrogenase kinase targets. For this purpose, some ligand screening studies were performed on all three targets. The docking protocols were different from each other in terms of the minimization methods used prior to docking procedures. Based on the obtained data, having minimized by MM+-PM3 methods, the ROC value was 0.916, 0.914 and 0. 833 whereas it was 0.127, 0.187, and 0.51without minimization for PDHK, BACE-1 and aromatase, respectively. It was suggested that using a hybrid method of molecular mechanics/semi-empirical method could result in more realistic values during docking simulation studies. The result of this study showed applicability of the presented docking protocol using more converged starting points and can be used to design inhibitors of these targets in future studies.

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Conflict of Interest

None declared.

5. References

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