NOTE

PyPLIF-ASSISTED REDOCKING INDOMETHACIN-(*R*)-ALPHA-ETHYL-ETHANOLAMIDE INTO CYCLOOXYGENASE-1

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ABSTRACT

Identification of Protein-Ligand Interaction Fingerprints (PLIF) has been performed as the rescoring strategy to identify the best pose for the docked poses of indomethacin-(R)-α-ethyl-etanolamide (IMM) in the binding site of cyclooxygenase-1 (COX-1) from simulations using PLANTS molecular docking software version 1.2 (PLANTS1.2). Instead of using the scoring functions included in the docking software, the strategy presented in this article used external software called PyPLIF that could identify the interactions of the ligand to the amino acid residues in the binding pocket and presents them as binary bitstrings, which subsequently were compared to the interaction bitstrings of the co-crystal ligand pose. The results show that PyPLIF-assisted redocking strategy could select the correct pose much better compared to the pose selection without rescoring. Out of 1000 iterative attempts, PyPLIF-assisted redocking simulations could identify 971 correct poses (more than 95%), while the redocking simulations without PyPLIF could only identify 500 correct poses (50%).These works have also provided us with the initial step of the construction of a valid Structure-Based Virtual Screening (SBVS) protocol to identify COX-1 inhibitors.

Keywords: *PyPLIF; rescoring; Structure-Based Virtual Screening (SBVS); molecular docking*

ABSTRAK

Telah dilakukan identifikasi sidik jari interaksi antara ligan dan protein sebagai strategi penskoran ulang guna memilih posisi terbaik hasil penambatan molekuler ulang senyawa ko-kristal indomethacin-(R)-α-ethyl-etanolamide (IMM) ke dalam binding site siklooksigenase-2 (COX-1) luaran dari penggunaan aplikasi penambatan molekuler PLANTS versi 1.2 (PLANTS1.2). Sebagai pengganti penggunaan fungsi penskoran bawaan asli dari aplikasi penambatan molekuler PLANTS1.2, digunakanlah aplikasi eksternal bernama PyPLIF yang mampu mengidentifikasi sidik jari interaksi antara ligan dengan residu-residu asam amino di binding pocket dan menyajikannya dalam bentuk binary bitstrings, yang kemudian dibandingkan dengan binary bitstrings sidik jari interaksi pose asli ligan ko-kristal. Hasil penelitian menunjukkan bahwa simulasi penambatan ulang dengan berbantukan PyPLIF memiliki kemampuan jauh lebih baik dalam mereproduksi posisi asli ligan ko-kristal dibandingkan dengan simulasi penambatan ulang dengan menggunakan fungsi penskoran bawaan asli aplikasi penambatan molekul yang digunakan. Dari 1000 kali iterasi, simulasi penambatan ulang berbantukan PyPLIF mampu mereproduksi posisi asli ligan ko-kristal sebanyak 971 kali (lebih dari 95%) sementara simulasi penambatan ulang tanpa penskoran ulang dengan PyPLIF hanya mampu mereproduksi sebanyak 500 kali (50%). Penelitian ini memberikan fondasi awal untuk konstruksi protokol Penapisan Virtual Berbasis Struktur (PVBS) yang valid guna mengidentifikasi inhibitor COX-1.

Kata Kunci: *PyPLIF; penskoran ulang; Penapisan Virtual Berbasis Struktur (PVBS); penambatan molekuler*

INTRODUCTION

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs, *e.g.* aspirin, indomethacin, and diclofenac) play an important role by inhibiting cyclooxygenase (COX) enzyme from converting arachidonic acid (AA) to prostaglandin (PG) H [1-2]. This leads to the inhibition of the inflammation processes [1]. COX consists of two

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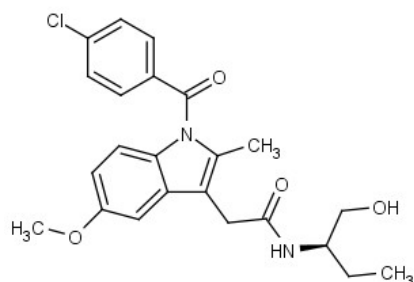Email address : enade.istyastono@molmod.org

Fig 1. Structure of 2-(1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl)-N-[(1*R*)-1-(hydroxyme-thyl)propyl]acetamide (IMM) [9,15]

isoforms, which are cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [3-4]. It is believed that COX-1 plays an important role in the anti-inflammatory processes since inhibiting this enzyme can lead to gastrointestinal (GI) toxicities while COX-2 in the pro-inflammatory processes [2,4]. However, selective inhibition COX-2 can lead to cardiovascular side effects [2]. To avoid both the GI toxicities from selective COX-1 inhibition and the cardiovascular side effects from selective COX-2 inhibition of the anti-inflammatory agents, a new strategy involving discovery and development dual COX-1/COX-2 inhibitors has been proposed [5-6].

Discovery of dual COX-1/COX-2 inhibitors can be more effective and efficient by employing valid Structure-Based Virtual Screening (SBVS) protocols. Interestingly, recent publications on the enhanced version (DUD-e) of directory of useful decoys (DUD) as well as the original version of DUD have retrospectively validated SBVS protocols either to identify COX-1 or COX-2 inhibitors [7-8]. The quality of SBVS protocols to discover COX-2 inhibitors are acceptable since the enrichment factor at 1% false positives ($EF_{1\%}$) value can reach more than 20 [7-8]. However, both [7-8] showed that the construction of SBVS protocols to discover COX-1 inhibitors remains challenging since the protocols resulted in the EF1% values of less than 5 [7-8]. Recent attempts by Istyastono [9] to initially construct and validate an SBVS protocol to discover COX-1 inhibitors by redocking the co-crystal ligand showed that the co-crystal ligand can be docked in two possible poses with a 50:50 chance. This information can be useful in the *de novo* design but to have more confidence to employ the constructed SBVS protocol further [10], the protocol should be able to redock the co-crystal ligand correctly most of the time [11]. Previous attempts to construct a valid SBVS protocols to discover COX-1 inhibitors did not involve rescoring processes by using protein-ligand interaction fingerprints (PLIF) identification [7–9], which on the other hand were reported could enhance the quality of SBVS protocols [12-14]. Therefore, more attempts assisted by

PLIF identification for rescoring are needed to have a valid SBVS protocol in order to identify COX-1 inhibitors since some high resolution COX-1 crystal structures are recently publicly available [15-17].

The construction of SBVS protocols to identify COX-1 inhibitors using PLANTS docking software version 1.2 (PLANTS1.2) [18-19] was reported by Istyastono [9]. These attempts incorporated iterative procedures 1000 times to optimize the protocols [9]. Although the iterative optimization procedures were able to obtain a docking protocol with an RMSD value of 0.633 Å, the iterative procedures have suggested two possibilities of the docked poses with a chance of 50-50 [9-10]. These indecisive protocols can mislead the selection of the best pose in a virtual screening campaign [10]. Further attempts by employing PyPLIF [14] to identify PLIF as the rescoring functions to have a better first step in the development of a good quality SBVS protocol to identify COX-1 inhibitors are presented in this article. These PyPLIF-assisted redocking simulations could identify 971 correct poses (more than 95%) out of 1000 redocking attempts.

METHODS

Materials

The docked poses from 1000 iterative redocking simulations of of indomethacin-(R)-α-ethyl-etanolamide (IMM; Fig. 1) in the COX-1 binding site performed by Istyastono [9], the output of the *bind* module of PLANTS1.2 [18-19] performed by Istyastono [9] stored as *PLANTSactiveSiteResidues.mol2* used as the binding site and the output of the *splitpdb* module of SPORES [20] stored as *ligand_IM8700_0.mol2* used as the reference co-crystal pose were obtained from previous works [9].

Computation details

PyPLIF [14] which was developed based on protein-ligand interaction conventions [11] were used to identify the PLIF of each docked pose and to calculate the similarity to the PLIF of the co-crystal ligand by using Tanimoto coefficient (Tc-PLIF) [14]. Instead of based on the ChemPLP score [9,18], the best pose of each redocking simulation was selected from the highest Tc-PLIF value [14]. The RMSD values calculations and pictures generations were then performed using PyMOL (http://www.pymol.org/) [9,21]. All calculations and computational simulations were performed on a Linux (Ubuntu 10.04 LTS Lucid Lynx) machine with Intel® Xeon® Duo 5150 (@ 2.66 GHz) as the processors and 1.00 GB of RAM.
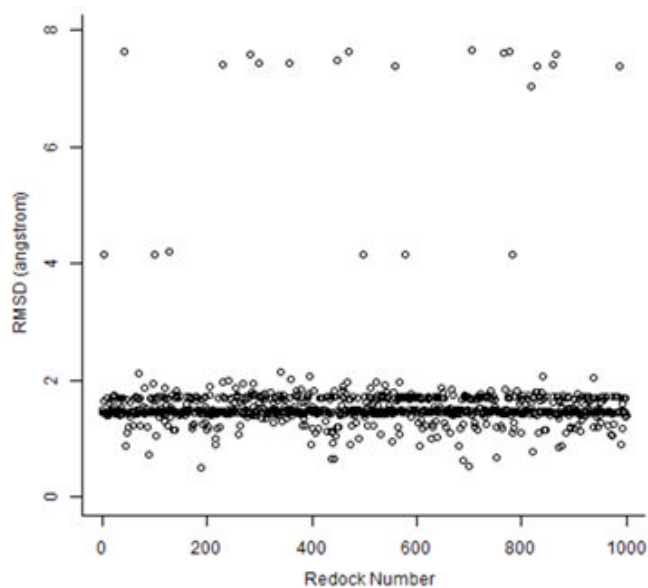
Fig 2. The RMSD values over 1000 iterations of the PyPLIF-assisted re-docking simulations
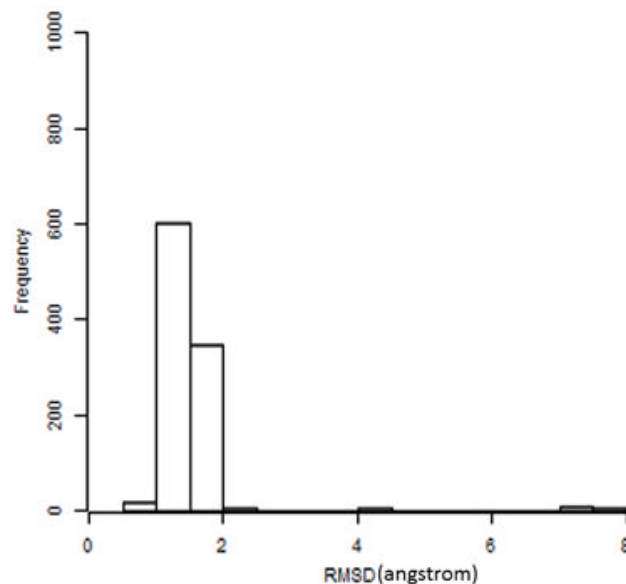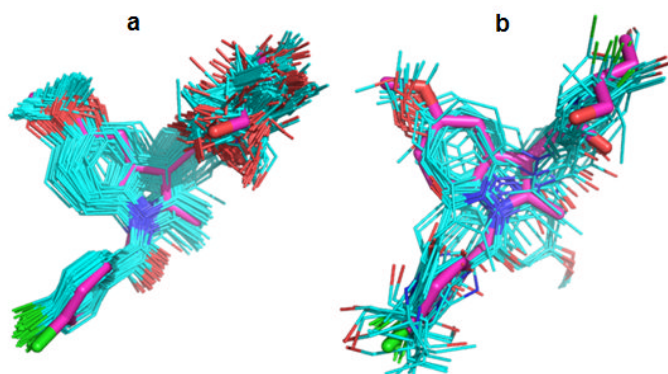

Fig 4. All PyPLIF-selected docking poses of the reference ligand MIM aligned to the crystal structure pose: The docking poses resulted in the protocol that have RMSD value of < 2.0 Å (**a**) and the docking poses resulted in the protocol that RMSD value of ≥ 2.0 Å (**b**). The docked poses are presented in the lines mode, while the crystal structure pose is presented in the sticks mode. Hydrogens are not shown, carbon atoms are presented in magenta and in cyan for the crystal structure pose and the docking poses respectively, chlorine atoms are presented in light green, nitrogen atoms are presented in blue, and oxygen atoms are presented in red

Procedure

PyPLIF [14] was subjected to each docked pose obtained from previous works [9] by using *PLANTSactiveSiteResidues.mol2* as the binding pocket. PyPLIF converted the PLIF into binary bitstrings and calculated the similarity of the bitstrings to those

Fig 3. The frequency distribution of the RMSD values presented in a histogram

obtained from the PLIF of the co-crystal ligand resulted in Tc-PLIF values. For each redocking simulation, the docked pose with the highest Tc-PLIF was selected as the best docked pose. The root mean square distances (RMSD) value between the selected docked pose and the pose of the co-crystal ligand was calculated using *rms_cur* module in PyMOL [9]. The protocols were considered as acceptable in reproducing the co-crystal ligand pose if resulted in the RMSD value of less than 2.0 Å [11].

RESULT AND DISCUSSION

This research aimed to examine whether PyPLIF as a rescoring strategy can increase the quality of the validation initial step of the SBVS protocols to discover COX-1 inhibitors which were built using PLANTS1.2 as the docking software. In the previous attempts by [9] showed that to reproduce the co-crystal IMM into the binding pocket of COX-1 (prepared from the crystal structure with pdb code of 2OYE [9,15] remains challenging since Istyastono [9] could sample two possible binding poses of the ligand with a chance of 50-50 instead of correctly reproduce the co-crystal pose only. Although Istyastono [9] could offer the opportunities to determine the important interactions that lead to the optimization of COX-1 inhibitors in a *de novo* design, the results of the previously developed protocols reflected the indecisive nature of using the default scoring function to identify the correct binding pose of inhibitors to COX-1 binding pocket. The results of using PyPLIF as the rescoring strategy are presented in Fig. 2 and 3.

By employing PyPLIF as the rescoring strategy, the protocols increase the ability to reproduce the pose of the co-crystal ligands from 50% out of 1000 iterative runs to 97.1% (Fig. 2 and 3). Interestingly, different with the previously published attempts [9], which showed exactly similar binding poses for the rest 50%, by using PyPLIF the rest of 2.9% showed binding poses diversity (Fig. 4). The rescoring strategy used in this research has remarkably increased the quality of the initial validation of the SBVS protocols. Similar success stories in employing PLIF identification to enhance the SBVS protocols quality have been reported by [9-10,12-14,22]. Remarkably, PyPLIF used in this research was published by [14] as an open source software under GNU General Public License version 3 (GNU GPL v3) that can be adapted for further or other purposes in the drug design and discovery area [14,22].

The retrospective validation of these PyPLIF-assisted SBVS protocols to discover COX-1 inhibitors are being performed by using DUD-e datasets [8] and are going to be evaluated prospectively by employing ZINC database [23-24]. The quest to construct valid SBVS protocols to identify COX-1 inhibitors is challenging since some previous attempts resulted in poor $EF_{1\%}$ values [7-8]. Moreover, the availability of the valid protocols will be beneficial not only to discover COX-1 inhibitors, but also to discover selective COX-2 inhibitors or even dual COX-1/COX2 inhibitors [9,25].

CONCLUSION

The application of PLIF identification by employing PyPLIF as the rescoring strategy increases the quality of the initial step in the construction of valid SBVS protocols employing PLANTS1.2 as the docking software to identify COX-1 inhibitors.

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