Journal of Molecular Graphics and Modelling 52 (2014) 114–123

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Protein tyrosine phosphatases: Ligand interaction analysis and optimisation of virtual screening

Mohammad A. Ghattas[a], Noor Atatreh[a], Elena V. Bichenkova[b], Richard A. Bryce[b,*]

[a] College of Pharmacy, Al Ain University of Science and Technology, Al Ain 64141, United Arab Emirates
[b] Manchester Pharmacy School, University of Manchester, Oxford Road, Manchester M13 9PT, UK

ARTICLE INFO

Article history:
Accepted 26 June 2014
Available online 5 July 2014

Keywords:
Protein tyrosine phosphatase
PTP1B
Virtual screening
Docking
Pharmacophore
Constraint

ABSTRACT

Docking-based virtual screening is an established component of structure-based drug discovery. Nevertheless, scoring and ranking of computationally docked ligand libraries still suffer from many false positives. Identifying optimal docking parameters for a target protein prior to virtual screening can improve experimental hit rates. Here, we examine protocols for virtual screening against the important but challenging class of drug target, protein tyrosine phosphatases. In this study, common interaction features were identified from analysis of protein–ligand binding geometries of more than 50 complexed phosphatase crystal structures. It was found that two interactions were consistently formed across all phosphatase inhibitors: (1) a polar contact with the conserved arginine residue, and (2) at least one interaction with the P-loop backbone amide. In order to investigate the significance of these features on phosphatase-ligand binding, a series of seeded virtual screening experiments were conducted on three phosphatase enzymes, PTP1B, Cdc25b and IF2. It was observed that when the conserved arginine and P-loop amide interactions were used as pharmacophoric constraints during docking, enrichment of the virtual screen significantly increased in the three studied phosphatases, by up to a factor of two in some cases. Additionally, the use of such pharmacophoric constraints considerably improved the ability of docking to predict the inhibitor's bound pose, decreasing RMSD to the crystallographic geometry by 43% on average. Constrained docking improved enrichment of screens against both open and closed conformations of PTP1B. Incorporation of an ordered water molecule in PTP1B screening was also found to generally improve enrichment. The knowledge-based computational strategies explored here can potentially inform structure-based design of new phosphatase inhibitors using docking-based virtual screening.

© 2014 Elsevier Inc. All rights reserved.

Within the drug discovery process, computational design approaches are now integral [1,2]. An important computational medicinal chemistry tool is molecular docking, which seeks to predict the structure of a complex of two or more molecules. Protein–ligand docking is widely used in the drug discovery area, both in the hit identification and lead optimisation stages [3]. Generally, docking comprises two critical components: the search technique and the scoring function [4]. Although conformational search techniques are reasonably efficient, particularly when applied to small drug-like molecules, scoring functions still required further development [5]. This can pose a problem for virtual screening (VS) of a large library of compounds, where inaccurate scoring of the docked ligand library yields a large number of false positives. The subsequent investigation of these false positives experimentally wastes valuable resources.

Therefore, efforts have been made to optimise the docking approach prior to VS in the hope of limiting virtual false positives and improving experimental hit rates. Approaches to improve docking performance have included evaluating different scoring functions and their effect on ranking docked ligand libraries [6,7]. An alternative approach is to focus on identifying important structural features of ligand and protein, for example, key interactions, presence of water molecules, amino acid rotamers and protein flexibility [8]. As an example of the latter approach, Perola conducted a structural analysis of a large group of kinase enzymes [9] revealing key interactions formed between kinase inhibitors and the backbone amides of the hinge region. Following a series of

Abbreviations: VS, virtual screening; PTP, protein tyrosine phosphatase; PTP1B, protein tyrosine phosphatase-1B; IF2, isoform 2 of low molecular weight protein tyrosine phosphatase; PLIF, protein–ligand interaction fingerprint; EF, enrichment factor; RMSD, root mean square deviation.

* Corresponding author. Tel.: +44 0161 275 8345; fax: +44 0161 275 8360.
E-mail addresses: richard.bryce@manchester.ac.uk, R.A.Bryce@manchester.ac.uk (R.A. Bryce).

http://dx.doi.org/10.1016/j.jmgm.2014.06.011
1093-3263/© 2014 Elsevier Inc. All rights reserved.

docking experiments, it was found that constraining these interactions during docking led to retrieval of a significantly larger number of known kinase inhibitors in the top-ranked docked dataset in comparison to unconstrained docking.

In this study, we investigate the interactions of protein tyrosine phosphatases (PTPs) with their co-crystallized ligands in order to identify features that could enhance their virtual screening. PTPs are a group of enzyme that hydrolyse phosphate from a tyrosine residue [10]. All cysteine-based PTPs share the same signature motif, Cys(XXXXX)Arg, in their catalytic site. Cysteine-based PTPs are generally grouped into three classes according to the amino acid sequence of their catalytic domains [10]. Class I PTPs share a characteristic motif of 250 residues, including the conserved active site motif. PTP1B, a prototypical class I phosphatase, is considered a novel target for type II diabetes mellitus and diet-induced obesity [11]. Class II PTPs are known as low molecular weight PTPs due to their small size, of around 20 kDa. Human low molecular weight PTPs, for example isoform 2 (IF2), are also involved in the pathogenesis of type II diabetes mellitus [12]. Class III phosphatases include only Cdc25 phosphatases which are involved in cell-cycle progression. Cdc25 are cysteine-based PTPs which show catalytic activity against both phosphotyrosine- and phosphoserine/threonine-containing substrates [13]. PTPs are also considered as interesting targets for cancer since both PTP1B and Cdc25 are known to be overexpressed in breast tumour [14] and human low molecular PTPs such as IF2 are known to play a role in tumour onset and growth [15].

In recent years, virtual screening has been applied to the discovery of new PTP inhibitors [16–19]. These computational studies have adopted a conventional preparation of the protein structures for docking, such that all solvent molecules were deleted and no geometric constraints were applied, although there was investigation of scoring function selection. Here, we seek to enhance these approaches by investigating whether there are any amino acid residues common to the PTP family that consistently interact with PTP inhibitors; and then assessing how important these interactions are for improving ligand docking and library ranking. To achieve this, we select a representative of each PTP class, i.e. PTP1B, IF2 and Cdc25b, for structural investigation and subsequent docking experiments.

1. Methods

1.1. Analysis of PTP–ligand complexes

We obtained 51 PTP1B-ligand complexes (listed in *Supporting Information*) and another four complexes, of Cdc25b (PDB: 1QB0, 1CWT and 1CWS [20]) and IF2 (PDB: 1XWW [21]) from the Protein Data Bank [22]. All 55 crystal structures were graphically analysed via the PyMOL program [23] in order to assess ligand–protein interactions. Automated protein–ligand analysis was carried out for the PTP1B–ligand complexes. This was achieved by categorising these crystal structures into two clusters. The first cluster, named 'open', included 7 PTP1B structures whose WPD loop is in the open conformation. The second cluster, named 'closed', included 44 structures whose WPD loop is in the closed conformation. The WPD loop takes the open conformation when PTP1B is in the apo form and, upon substrate binding, it closes downwards to take its part in the catalytic mechanism [24].

For each complex, a protein–ligand interaction fingerprint (PLIF) was generated using the MOE software package [25]. For the PLIF, protein–ligand hydrogen bonds were scored based on heavy atom type, interatomic distance and orientation, derived using a statistical approach trained using a set of experimental protein structures [26]. This score is then expressed as a percentage probability of being a good hydrogen bond. Ionic interactions were scored by calculating the inverse square of the distance between atoms with opposite formal charge (e.g. a carboxylate oxygen atom and a protonated amine), and expressing this as a percentage (such that 100% corresponds to a separation of 1 Å) [25]. Minimum score thresholds for hydrogen bonding and ionic interactions were taken as the default settings of 1% and 5% respectively. Interactions between the active site residues and the co-crystallised ligand atoms that were not able to achieve a higher value than the minimum scores were not considered in the PLIF. The PLIF graph generated then displays the interaction occupancy of all residues in the PTP1B active site. This occupancy is defined as the percentage of ligands interacting with the side chain or main chain of a given amino acid.

1.2. Preparation of test sets

Three test sets were prepared for use in seeded virtual screening against the three PTP enzymes, PTP1B, IF2 and Cdc25b. Each test set consists of two main components: firstly, known PTP inhibitors (PTP1B: 67 ligands; Cdc25b: 38 ligands; IF2: 25 ligands) which were obtained from literature and had a molecular weight of not more than 500 Da (listed in *Supporting Information*). The known inhibitors were considered as active ligands only if they had IC_{50} or K_i values of 50 μM or less. These compounds were either extracted from their original crystal structures or, alternatively, created using MarvinSketch [27]. Secondly, decoy ligands were selected from a commercial database (TimTec [28]) in order to represent inactive ligands. The process of choosing these compounds began by randomly selecting 50,000 ligands with molecular weights of less than 500 Da, each of which was assigned a fingerprint using MACCS structural keys [29]. This algorithm gives a fingerprint based on how many predefined substructures (one to ten non-hydrogen atoms) exist in each ligand. Ligands were then clustered using the Tanimoto coefficient in MOE (thresholds of similarity and overlap of 50%) [25]. Five thousand ligands were taken from 5000 different clusters and then processed via the *wash* module in MOE [25] in order to assign their ionisation state at pH 7. All ligands were assigned partial charges using the MMFF94x force field and energy minimised [30–36].

1.3. Preparation of protein structures for seeded VS experiments

The protein structures, all of resolution <2 Å, were obtained from the PDB: for PTP1B-closed (PDB: 1C88 [37]), for PTP1B-open (PDB: 1G7F [38]), IF2 (PDB: 1XWW [21]) and Cdc25b (PDB: 1QB0 [20]). Using the MOE *3D protonate* module [25], hydrogen atoms were added to each enzyme and partial charges were assigned on each atom based on the MMFF94 force field [31–35]. All water molecules were removed from the PTP1B open, IF2 and Cdc25b structures prior protein preparation. For the PTP1B closed conformation, two structures were prepared: the first was constructed without water; and the second with all water removed, apart from the ordered water molecule that was observed to be buried behind the WPD loop.

1.4. Docking protocols

Prior to docking, an Arg/P-loop pharmacophore was designed using the MOE Pharmacophore Elucidation module for use in the constrained docking protocol [25]. Two pharmacophore points were set up based on the coordinates of the two terminal oxygen atoms bound to the conserved Arg side chain and/or the P-loop backbone amide. Both pharmacophore points accept an anionic group (An) and/or a hydrogen bond acceptor (HBA). Consequently, co-crystallised ligands of the three PTP enzymes used in the seeding experiments were employed to define the protein catalytic site.

Download English Version:

https://daneshyari.com/en/article/443336

Download Persian Version:

https://daneshyari.com/article/443336

Daneshyari.com