Journal of Atomic and Molecular Sciences

ISSN 2075-1303

Global Science Press

Configurational Prediction Of CYP2A6 Substrate Would Guide The Screening Of Potential Substrate

Received Nov. 15, 2018,
Accepted Dec. 28, 2018,

DOI: 10.4208/jams.111518.122818a

http://www.global-sci.org/jams/

Huizi Man[a], Yan Jia[a,*]

Abstract. Cytochrome P450 2A6 (CYP2A6) substrate database constitute a potential class of disease related molecules as well as therapeutic molecules, primarily expressed in liver and lungs. The prediction of CYP2A6-related metabolism is of great interest. In this study, a docking protocol was presented which made use of poses of known substrate to help guide the configurational search and to rank predicted poses of test substrates. As a result, a 68% success rate was obtained. Predicting ideal configurations of compound would make significant impact on screening potential substrate, as the predicted bound conformations of 3 Tanshinone IIa(CYP2A6 substrate) analogues revealed differences among them and the inappropriate characteristic to be selected as substrates which was confirmed experimently.

1. Introduction

As high attrition rate has become a conundrum in drug development1, the prediction of metabolism properties is of considerable interest2. Cytochrome P450 (CYP) constitutes a large family of heme-containing proteins that play an important role in the oxidative metabolism of a wide variety of endogenous and exogenous compounds. Each isozyme exhibits its own, usually overlapping, substrate specificity3. The prediction of CYP-related metabolism properties finds its significance in the screening of new drugs, drug application and therapeutics with respect to drug-drug interactions, as well as patients with CYP polymorphisms4. Human CYP2A6 contributes extensively to nicotine detoxication but also activates tobacco-specific procarcinogens to mutagenic products 5, as well as therapeutic drugs.

Previous works have revealed that CYP2A6 crystal structure shows a clearly well-adapted enzyme for the oxidation of small, planar substrates that can be accommodated within the compact, small, and hydrophobic active site (with a volume of only 260 Å^3). Inside the active site, N297 serves as one hydrogen bond donor and thus orients ligands such as coumarin for regio-selective oxidation. Yano et al. suggest that a CH-π interaction is evident between Phe107 and courmarin or methoxsalen6. With the aids of this crystal structure and a pharmacophore model, Leong et al7 propose that Phe480 might also involve in π-π interactions of the protein with its substrates. And other active site residues such as F118 and T305 also play an important role in ligand orientation. Yet, few studies have specified the common bioactive configuration among different CYP2A6 substrates.

Except several substrate configurations available in reported CYP2A6 co-crystal structures, most biocomformations of CYP2A6 substrates in the pocket remain unknown and would only be speculated by experimentally-proved reaction site. Prediction of the bound configuration of small-mol. ligands that differ substantially from the cognate ligand of a protein co-crystal structure is of great challenging than redocking of the cognate ligand. Here, we present a docking protocol that made use of poses of known substrate to help guide the configurational search and to rank predicted poses of test substrates. A set of 47 substratess for testing the docking protocol was tested. Overall, the top-five scoring pose family was correct over 55% of the time, with the top-ten pose families approaching a 68% success rate.

Predicting ideal configurations of compound in the pocket would make a significant impact on screening potential substrate. Lately, we have reported on the ability of CYP2A6 to accept Tanshinone IIa as high-affinity substrate8. In this assay, docking study revealed its bioactive configuration in the CYP2A6 pocket. Considered on the structural similarities to Tanshinone IIa, three pharmacological component Tanshinone I, Cryptotanshinone, Dihydrotanshinone were docked into the CYP2A6 pocket. It turned out that the incoming configuration in the active site differ substantially from tanshinone IIa though sharing the same skeleton. In vitro experiment confirmed that the three mol. are not selected as substrate of CYP2A6.

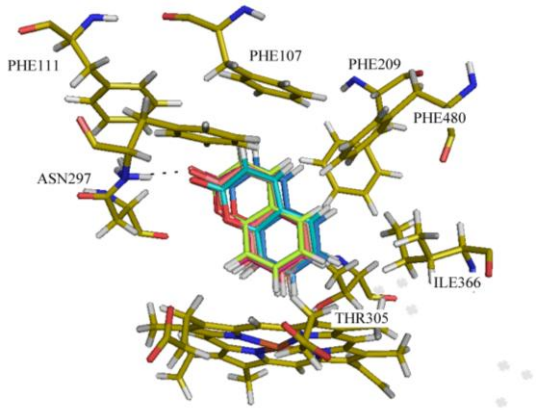

Figure 1. Binding mode of Coumarin(Top ten poses) predicted in CYP2A6 pocket versus crystal structure of Coumarin-CYP2A6 co-structure.

[a] *State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS).457 Zhongshan Road, Dalian 116023, P. R. China*
Corresponding author: Email: jiayan@dicp.ac.cn

2. Results and Discussion

2.1 Validation of docking simulations.

Reproducing the crystallographically observed conformation of the ligand (Coumarin) is a requirement to determine whether the docking setup is applicable to a given system. Initially, coumarin was prepared as described in the ligand preparation section(Supporting Information) and docked using the standard mode into the active site. Subsequently we compared the conformation and position with the bound ligand conformation in crystal structure measured in terms of the root-mean-square-deviation (RMSD). The top-ranked 10 poses reproduced the crystal bound conformation with a RMSD below 1 Å and the first two ranked poses had a RMSD of 0.73-0.75Å (**Figure 1**). By analyzing the binding mode of first-ranked docking pose, Coumarin shows very similar interaction with residues as observed in the reported crystal structures of 1Z10, for instances, oxygen atoms of Coumarin forms hydrogen bonding with Asn297 with a distance of 2.02Å, which in fact is smaller than the observed distance in the crystal structure (2.21 Å). As shown in the bound conformation, a favorable interaction is also evident between the pi-electron system of Phe107 and the aromatic hydrogens of coumarin10. In addition, Hydrogen bonding interaction orients coumarin for regioselective oxidation result in a distance of 3.41 Å from the hydroxylated location to the heme iron, smaller than the observed distance in the crystal structure (3.61 Å). In a word, the protocol was realiable and we would suggest it be used in predicting configurations of CYP2A6 substrates, as we have applied in the following prediction case.

2.2 Docking-based Pose prediction.

47 substrate(All molecular structures would be found in Figure S1) were obtained from literature and each molecule was prepared as described in the ligand preparation part. For each compound, 50 poses were generated and top 10 poses were used to compare with the observed configuration in reported crystal structures. If the crystal structure is not available, speculated poses with the reaction site oriented near the heme iron (guided by reported experiment result) was used. When 4 similar poses out of top 10 poses (or 3 poses out of top 5 poses) consisted with the ideal configuration, we regarded it as a success prediction. 68%(55%) success rate was obtained as seen in Table S1(Supporting Information). As is seen in the ideal bound configurations, hydrogen bonding interaction were observed between Asn297 and H-bond acceptor, eg. carbonyl oxygen atom(Cpd.1/3/5/9/10/17/31/32/33/41/43/45), ether oxygen atom(Cpd.5), furan oxygen atom(Cpd.30), hydroxyl oxygen atom(Cpd.19), pyridine nitrogen atom(Cpd.8/11/13) or imidazole nitrogen atom(Cpd.10). H-bond interaction stabilized the orientation of substrate for regioselective metabolism, which is proved by the fact that the catalytic site always lies 6-8 atoms distance from the H-bond site. These results provide further demonstration of the reliability of the docking protocol we've used, and detailed docking cases of several substrates(Nicotin, NNK, Methoxsalen, Phenacetin, Pilocarpine) which have been discussed a lot were used in comparison with observed crystal structures as below.

2.3 Binding mode of Nicotin predicted in CYP2A6 versus crystal structure of Nicotin-CYP2A6 co-structure.

CYP2A6 oxidize nicotine at several locations on the methylpyrrolidine ring. The dominant oxidation reaction for CYP2A6 is 5'-hydroxylation to form 5'-hydroxynicotine, which is further oxidized to cotinine. CYP2A6 can also oxidize nicotine on the methyl to ultimately generate nornicotine11. Consistent with this, nicotine binds with the methyl-pyrrolidine ring oriented toward the heme was observed both in the crystal structure(PDB:4EJJ. 2.30 Å) and predicted structures (**Figure 2**). The predicted conformation reproduced the observed comformation with an RMSD of 0.47Å for equivalent Cα positions throughout the structures, with an RMSD of 0.31Å within the active site. In the 4EJJ complex, the pyrrolidine 5' and methyl carbons are at similar distances from the heme iron (3.4 and 5.8Å, respectively), but the 4'-carbon is even closer at only 2.5Å，and the pyridine nitrogen is too far (3.8 Å) from Asn-297 for even a weak hydrogen bonding interaction. In the predicted docking poses, the methyl pyrrolidine ring is oriented more vertical to the heme plane, and pyrrolidine 5'

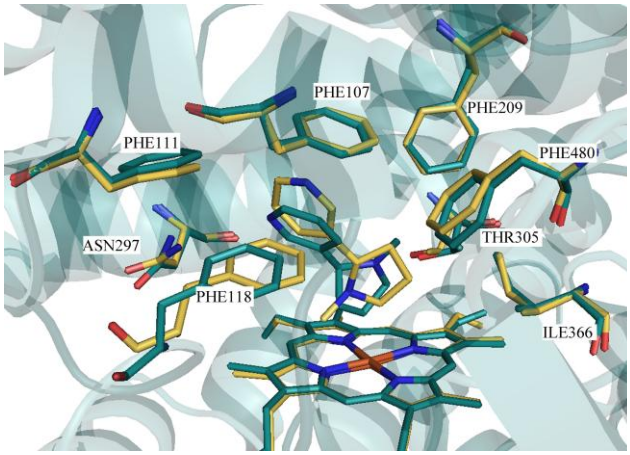

Figure 2. Binding mode of Nicotin predicted in CYP2A6(yellow) versus crystal structure(green) of Nicotin-CYP2A6 co-structure.

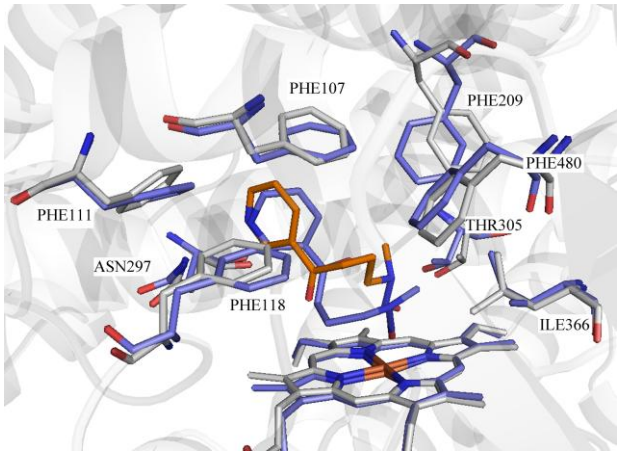

Figure 3. Binding mode of Nicotin predicted in CYP2A6(purple) versus crystal structure(white) of Nicotin-CYP2A6 co-structure.