A theoretical study on the mechanism of a superficial mutation inhibiting the enzyme activity of CYP1A2

ARTICLE *in* INTERDISCIPLINARY SCIENCES COMPUTATIONAL LIFE SCIENCES · MARCH 2014

Impact Factor: 0.66 · DOI: 10.1007/s12539-014-0184-2 · Source: PubMed

Interdiscip Sci Comput Life Sci (2014) 6: 25–31
DOI: 10.1007/s12539-014-0184-2

A Theoretical Study on the Mechanism of a Superficial Mutation Inhibiting the Enzymatic Activity of CYP1A2

Li-Na MA[1,2], Zhen-Zhen DU[3], Peng LIAN[1*], Dong-Qing WEI[1*]
[1](School of Life Science and Biotechnology, Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai 200240, P. R. China)
[2](School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai 200240, P. R. China)
[3](Department of Radiation Oncology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, P. R. China)

Received 21 August 2013 / Revised 3 September 2013 / Accepted 10 September 2013

Abstract: CYP1A2, one of the major members of cytochrome P450 in human liver, participates in the metabolism of various drugs. While most harmful mutations are located near the catalytic core of CYP1A2, a recently found loss-of-function mutation, F186L, is on the surface. By far, function of this superficial residue remains unclear. In this paper, 7-ethoxyresorufin, a widely used agent in benchmarking the O-deethylation activities of CYP1A subfamily enzymes, was employed as a substrate to investigate the impact of the F186L mutation through ensemble docking and molecular dynamics simulations. It was found that the F186L mutation altered the binding inclination of the substrate through a series of changes on the catalytic pocket, which are, actually, long-range effects. The activities of access channels in the enzyme are also affected by the F186L mutation and the substrate binding. Based on these findings, a detailed mechanism of how F186 regulates the functions of CYP1A2 was proposed, and it may shed light on the diverse effects of SNPs and the personalized drug design.
Key words: F186L mutation, 7-ethoxyresorufin O-deethylation, long-range effects, access channels, ensemble docking, molecular dynamics simulations.

1 Introduction

The superfamily of cytochrome P450 (CYP) plays an important role in various organisms. Its functions range from detoxication of foreign compounds to biosynthesis of endogenous molecules (Estabrook, 1996). Subfamilies 1, 2 and 3 of P450 were reported to be responsible for the metabolism of more than 90% of clinical drugs (Hodgson, 2001). As one of the major members of CYPs in human liver (Eaton *et al.*, 1995), CYP1A2 is in charge of the metabolism of lots of marketed drugs, including caffeine, olanzapine, theophylline, *etc.* (Zhou *et al.*, 2009). On the other hand, P450 single nucleotide polymorphisms (SNPs), which are closely related to the individual variation of expressions and activities of P450 enzymes (Shimizu *et al.*, 2003a; Shimizu *et al.*, 2003b), are critical for clinical medication and personalized drug design (Ingelman-Sundberg *et al.*, 2007). Until now, more than 40 alleles of CYP1A2 have been discovered and defined (Sim, 2010). Recently, CYP1A2*11 allele, a mutation of cytosine to adenine at the site 558 of the CYP1A2 gene, was found to result in the replacement of the 186^{th} amino acid, phenylalanine, by leucine (F186L) (Murayama *et al.*, 2004). Without affecting the expression of the enzyme, this SNP shows an increase in substrate binding affinity of 7-ethoxyresorufin but a significant decrease in the velocity of O-deethylation (Murayama *et al.*, 2004). Interestingly, the F186 residue is conserved and located near the protein surface – approximately 26 Å away from the iron atom of the porphyrin in the active site. According to our previous study, the F186L mutation of CYP1A2 may increase the structural flexibility of the protein and regulate the catalytic activity through long-range effects (Zhang *et al.*, 2011). However, in order to understand the regulation mechanisms in detail, the substrate-induced conformational changes of the enzyme need to be taken into account (Baldwin *et al.*, 1979). Therefore, the enzyme-substrate complex of CYP1A2 and 7-ethoxyresorufin was built and studied via ensemble docking and molecular dynamics (MD) simulations. A more detailed mechanism on how the F186 residue is involved in the regulation of CYP1A2 was proposed, which may shed light on the diverse effects of SNPs and the personalized drug design. Similar approaches have been successfully applied to other

*Corresponding authors.
E-mail: penglian518@gmail.com (P. Lian)
dqwei@sjtu.edu.cn (D.-Q. Wei)

P450 enzymes and various bio-macromolecular systems (Li *et al.*, 2011; Lian *et al.*, 2010; Lian *et al.*, 2013a; Lian *et al.*, 2013b; Lian *et al.*, 2011).

2 Materials and methods

2.1 Ensemble docking

Starting from the crystal structure of human CYP1A2 in the Protein Data Bank (ID: 2HI4, resolution 1.95 Å) (Sansen *et al.*, 2007), the inhibitor, α-naphthoflavone (ANF), was removed to generate the wild-type model (denoted as WT_apo). The F186L mutant structure (F186L_apo) was produced by mutating F186 to leucine manually. Both proteins were then solvated, respectively, in a rectangular box of TIP3P water molecules (Jorgensen *et al.*, 1983) with a minimum distance of 10 Å between the outermost protein atoms and the walls of the box. Six chloride ions were introduced to neutralize each system. Then, a short minimization followed by a 5 ns MD simulation was conducted on both systems to equilibrate the system. The root mean-square deviation (RMSD) of the Cα atoms of each protein is shown in Fig. S1 (Supplementary Material). During each simulation, 20 consecutive frames with equal time intervals of 250 ps were selected as targets to dock 7-ethoxyresorufin into the catalytic pocket. Autodock4.2 (Goodsell *et al.*, 1996; Huey *et al.*, 2007; Morris *et al.*, 1998) with the following docking procedure was employed. Firstly, a 40-point cubic grid spaced by 0.375 Å was defined and centered with respect to the iron atom of the heme group. Secondly, 100 independent runs using the genetic algorithm were performed for each docking. The top 100 configurations of the lowest binding energy were then clustered in terms of the RMS threshold of 2.0 Å. Up to this point, 54 clusters of wild-type and 71 of F186L were obtained. As shown in Fig. S2 (Supplementary Material), the distance between C13 atom of 7-ethoxyresorufin and the iron atom of heme (denoted as C13-Fe distance) is a key factor in the O-deethylation in CYP1A2. Previous docking studies indicated that 6 Å is the reactive distance between the site of metabolism and iron atom in CYP2D6 (Hritz *et al.*, 2008; Keizers *et al.*, 2005). However, as 7-ethoxyresorufin is larger and more rigid than the substrates investigated for CYP2D6 ...(Hritz *et al.*, 2008; Keizers *et al.*, 2005), a larger cut-off distance 7 Å was applied to the C13-Fe distance in this work. In the end, two types of configurations, type I for WT and type II for F186L were adopted for further investigation (Fig. 1).

2.2 Molecular dynamics simulations

Substrates with the lowest binding energy of configuration type I in wild-type and type II in F186L mutant were used as the initiate coordinates for the following MD simulations (Figs. S3a and S3b). Gromacs 4.5.4 (Pronk *et al.*, 2013; Van Der Spoel *et al.*, 2005), along with the force field of AMBER99SB (DePaul *et al.*, 2010; Hornak *et al.*, 2006; Sorin *et al.*, 2005) was employed for all molecular dynamics simulations. The parameters of 7-ethoxyresorufin were built using Antechamber (Wang *et al.*, 2006; Wang *et al.*, 2004) and amb2gmx.pl (Mobley *et al.*, 2006). Periodic boundary conditions were employed. A 10 Å cut-off distance was specified to deal with the van der Waals interactions. The particle mesh Ewald (PME) method was applied to calculate the long-range electrostatic interactions. All bonds involving hydrogen atoms were restrained by the Lincs algorithm (Hess *et al.*, 1997). A short energy minimization with a positional constraint on all protein atoms was carried out using steepest descent method (Fletcher *et al.*, 1963). After that, a 15 ns molecular dynamics simulation was performed under the condition of the NPT ensemble (298K, 1 atm). The time step for all the simulations was 2 fs.

3 Results and discussion

3.1 Binding modes of 7-ethoxyresorufin

7-Ethoxyresorufin is a planar, conjugated fluorometric molecule with two carbon aromatic rings and one heteroaromatic ring in the middle. It is a known suicide inhibitor for various cytochrome P450 enzymes except the CYP1A subfamily. On the contrary, the O-deethylation of 7-ethoxyresorufin has been widely used to determine the activities of CYP1A enzymes (Chang *et al.*, 1998).

As shown in Fig. 1, five binding modes (types I to V) were obtained after ensemble docking. Compared with the configuration of α-naphthoflavone (ANF) in the crystal structure of CYP1A2, these modes were divided into two classes, the Orthogonal and the Parallel. Conformations in the former group, including types I and II, are similar to that of ANF in crystal, where the poly-aromatic rings are placed almost orthogonal to the porphyrin. In addition, the ethyl fragment is close to the iron atom of the porphyrin and ready for O-deethylation. As for types III, IV and V, the substrate tends to be parallel to the heme plane. 7-Ethoxyresorufin is closer to the iron atom, but the ethyl group points against the active site. Moreover, the electron density map from the X-ray diffraction implies that there may be only one preferred orientation for the binding of large substrates to CYP1A2, *i.e.* ANF (Sansen *et al.*, 2007). Therefore, types I and II were considered exclusively in this study.

Types I and II both adopt similar alignment as ANF does in the crystal. The major difference is that in type I the 'oxygen side' of 7-ethoxyresorufin faces toward the heme group, while it is the 'nitrogen side' in type II. Types I and II were found in both WT and F186L. In

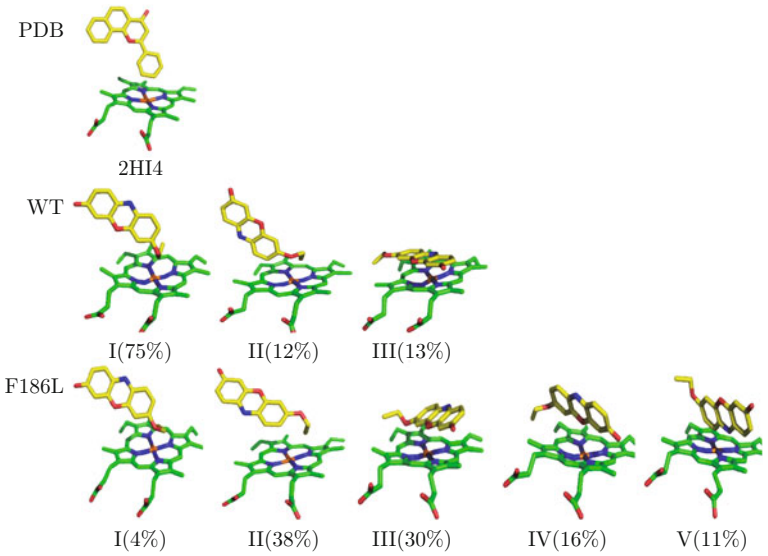

Fig. 1 Binding modes of 7-ethoxyresorufin in wild-type CYP1A2 and F186L mutant. The first row shows the binding mode of α-naphthoflavone (ANF) in the crystal structure of CYP1A2 (PDB 2HI4) (Sansen *et al.*, 2007). The second and third rows represent 7-ethoxyresorufin binding modes in WT and F186L, respectively, from ensemble docking. The population of each type is listed at the lower-right corner of each configuration. Figures in this article were prepared using PyMol (http://www.pymol.org).

WT, type I is dominant (up to 75%); however, type II has a higher population in F186L. The switch of the substrate binding inclination between these two different modes may be one of the consequences of the long-range effects of the superficial mutation of CYP1A2.

3.2 Binding sites and catalytic pocket

During the MD simulations, the RMSD of Cα atoms in both WT and F186L was calculated with respect to the crystal structure (Fig. S3c). Both reached a plateau of about 1.8 Å at 9 ns, which indicates that an equilibrated state has been achieved. Therefore, all the subsequent analysis is based on the last 6 ns (9-15 ns) of the simulation trajectories.

The C13-Fe distance was collected during the MD simulations (Fig. 2(a)). In both WT and F186L, it reached a plateau after the system became equilibrated, which indicates that the substrate was stabilized in both systems. In WT, the distance is approximately 4 Å. However, in F186L it is enlarged to around 8 Å, with which the possibility of the O-deethylation would be reduced significantly. Therefore, these observations may account for the substantial drop of the reaction velocity caused by F186L mutation (Murayama *et al.*, 2004).

The pocket of CYP1A2 is constructed by residues including I117, T118, T124, F125, F256, F260, N312, D313, G316, A317, D320, and L497. In order to investigate the open/close state and the size of the catalytic pocket, the total solvent accessible surface area (SASA) of these residues was measured. In Fig. 2(b), the relative SASA of the pocket was plotted with respect to that in the crystal structure. In both cases, a larger SASA was observed, which is known due to the substrate binding (Agnew *et al.*, 2012; Ekroos *et al.*, 2006). However, compared with the pocket in WT, SASA in F186L was relatively smaller, which further implies a lower possibility for water and substrates to access the active site. Therefore, it may lead to a small reaction velocity of CYP1A2 as measured in the experiments (Murayama *et al.*, 2004). The catalytic pocket in WT and F186L is further illustrated in Fig. 2(c) and 2(d), respectively. It is remarkable that the shapes and spatial positions of the pockets are different. The pocket in WT is flat and right above the heme group, while it shifts to the upper left of the heme group in F186L. These differences may be caused by long-range effects of the F186L mutation, which is known to increase the flexibility of D, E, F helices, and further affect the size and shape of the active pocket (Zhang *et al.*, 2011).

3.3 Stabilization of 7-ethoxyresorufin

Although the shape and position of the catalytic pocket of WT and F186L are different, 7-

Table 1 The average interaction energy (IE, kcal/mol) of 7-ethoxyresorufin with the heme group and the rest of the enzyme

	WT			F186L		
	VdW	Elec	IE	VdW	Elec	IE
HEME	−4±1	0	−5±1	−1±0	0	-1±0
Protein	−35±2	−2±2	−37±2	−35±2	−9±3	−44±3

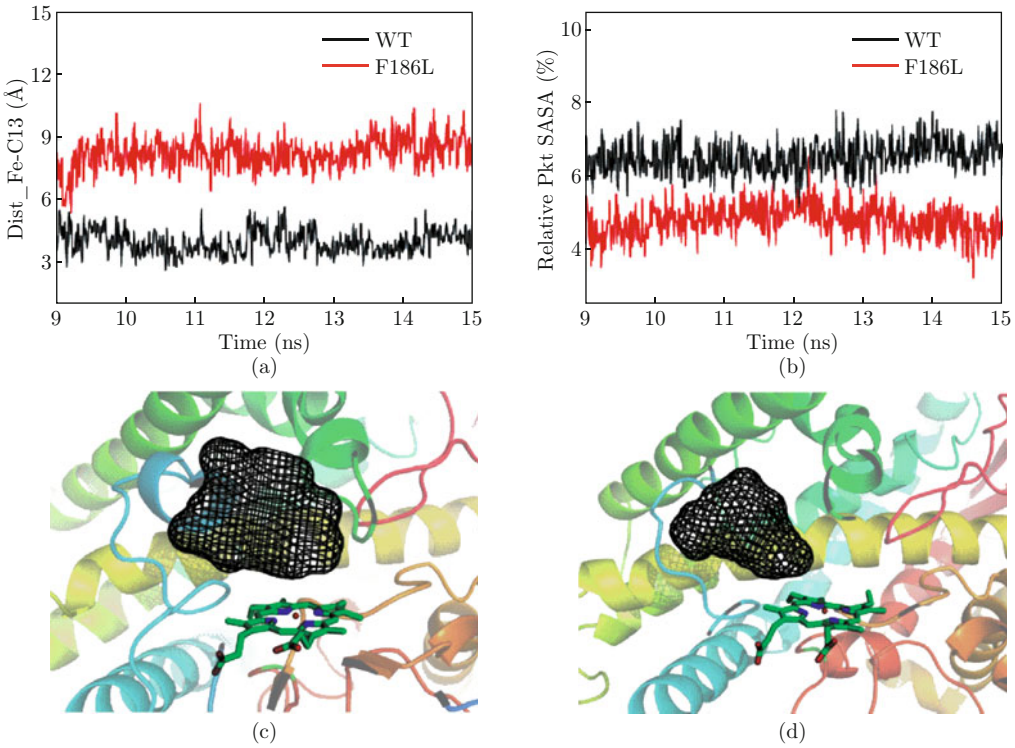

Fig. 2 Comparison of active sites between wild-type CYP1A2 and F186L mutant. The fluctuations of C13-Fe distance in the MD simulations are shown in panel a. The evolutions of the relative SASA of the catalytic pocket are plotted in panel b. Panel c and panel d illustrate the shape and spatial position of catalytic pocket in WT and F186L, respectively.

ethoxyresorufin is stabilized in both systems. Details of the interaction between the substrate and each enzyme were investigated. As shown in Table 1, the interaction energy (IE) between the substrate and the heme group is higher in WT than that in F186L. It may be resulted from the shorter distance between the substrate and the iron atoms in WT as discussed above (Fig. 2(a)). In F186L, a stronger interaction between 7-ethoxyresorufin and the rest of the enzyme was observed. It is consistent with the approximate four-fold smaller K_m value of 7-ethoxyresorufin in F186L (Murayama *et al.*, 2004).

Van der Waals (VdW) interaction is the essential part of IE between 7-ethoxyresorufin and the enzyme. Residues including F125, F226 and V227 contribute most of the VdW interactions (Fig. 3(a)). Similar to interactions between CYP1A2 and ANF in the crystal, aromatic interaction between F125/F226 and 7-ethoxyresorufin was observed in both WT and F186L. The phenyl group of F125 exhibits an orthogonal contact with the aromatic rings of the substrate. The side-chain of F226 forms a relatively strong π-π interaction with the π-conjugated rings of 7-ethoxyresorufin, which is vital to the catalytic activity of CYP1A2 (Parikh *et al.*, 1999). However, V227 plays distinct roles in both systems. In WT, hydrophobic interaction between V227 and the third ring of 7-ethoxyresorufin is critical to maintain the proper binding orientation for O-deethylation. An approximate 65% decrease in activity is expected when V227 is mutated to glycine (Liu *et al.*, 2004). Contrarily, in F186L the side-chain of V227 tends to form a stable interaction with the ethyl group of 7-ethoxyresorufin, which keeps the ethyl moiety 8 Å away from the iron atom of the heme group (Fig. 2(a)), and thus lowers the O-deethylation velocity of the substrate.

In CYP1A2, T118 and T124 are conserved polar

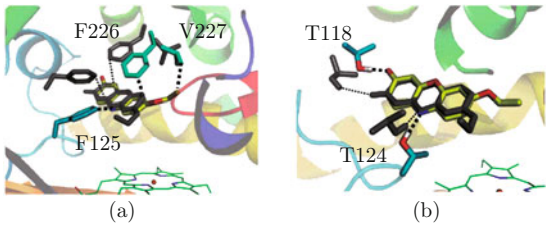

Fig. 3 Interactions between 7-ethoxyresorufin and the binding sites in WT and F186L. Active sites of both systems were superimposed. Residues and substrate in WT are represented in gray sticks; those from F186L are in color sticks. a: Hydrophobic interactions formed by 7-ethoxyresorufin with F125, F226 and V227. b: Hydrogen bonds between 7-ethoxyresorufin and T118/T124.

residues located at the distal bottom and the middle part of the hydrophobic pocket, respectively. As shown in Fig. 3(b), T118 forms a stable hydrogen bond with the carbonyl group of 7-ethoxyresorufin. T124 was observed to interact with the heterocyclic ring. Both residues play essential roles in substrate selectivity of the enzyme (Lewis *et al.*, 1996; Liu *et al.*, 2004; Sansen *et al.*, 2007). Compared to that in WT, 7-ethoxyresorufin formed stronger electrostatic interactions with T118 and T124 in F186L. Hydrogen bond lifetime of T118 and T124 in F186L is 4.6 ns and 0.5 ns, respectively, which is much longer than in WT (1 ns and 0.1 ns). These differences may lead to the smaller K_m value observed in the F186L mutation experiment (Murayama *et al.*, 2004). Furthermore, it is worth noting that T118, T124 and F125 are on the same loop, B'-C loop, which is gating some vital channels of P450 enzymes.

3.4 Effects on channels

Active sites of CYP450 enzymes are generally buried inside the enzyme. Channels that connect the active site to the solvent can provide access for substrates, oxygen, water molecules and products, *etc.* (Cojocaru *et al.*, 2007). In substrate-free enzymes, channels are proposed to be more likely open, while in enzyme-substrate complexes they tend to be relatively closed (Lee *et al.*, 2010). Herein, 30 consecutive conformations with equal time interval (200 ps) were extracted from each trajectory. Then, the open/close states of channels in these structures were analyzed using CAVER2.1.2 (Medek *et al.*, 2008) following the same protocol with our previous study (Zhang *et al.*, 2011). The results were summarized in Table S1, S2 and S3 (Supplementary Material).

In both systems, all the channels are closed during most of the simulation time, except channels 2a and 2c. Channel 2c is a predominant channel involved in the egress of soluble products in P450 enzymes (Li *et al.*, 2005; Schleinkofer *et al.*, 2005). In WT, the most active channel 2c stays open for 43% of the simulation time, which is similar to that in the apo enzymes in the previous study (38% opening in WT and 43% in F186L) (Zhang *et al.*, 2011). It seems that the binding of the substrate does not cause severe effects on channel 2c. However, in F186L, the ratio of opening time of channel 2c decreases to only 10%. That is probably due to the following reasons. The opening of channel 2c requires the separation of B'-C loop from G, I helices (Fig. 4). In F186L, because of the shifted binding site of 7-ethoxyresorufin discussed above, B'-C loop formed a stronger, direct interaction with the substrate through residues like T118, T124 and F125, *etc.* Therefore, B'-C loop is stabilized, which makes it difficult for channel 2c to open. Channel 2a is one of the most common channels among CYP450s and considered to be responsible for both substrate ingress and product egress (Wade *et al.*, 2004). A long last opening of this channel was found in the previous study (81% opening in WT and 71% in F186L) (Zhang *et al.*, 2011). However, in the current simulations, with the existence of 7-ethoxyresorufin in the catalytic pocket, channel 2a is closed in both systems except that only slight activity was observed in F186L. This is probably due to less opening of channel 2c in F186L and the different binding modes in both systems.

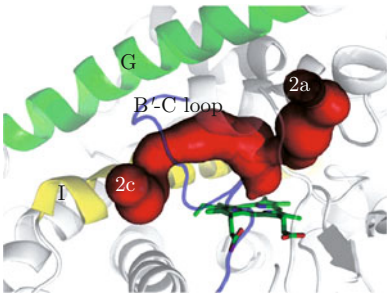

Fig. 4 The active channels in the MD simulations.

According to this study, channels are more active in the wild-type complex than in F186L mutant. Therefore, in WT, reactants have easier access to the active site so that products could be released more smoothly. On the contrary, the relatively "closed" mode of F186L inhibits the reaction cycle to a certain extent, which may explain the 72% drop of the O-deethylation rate in the F186L mutation experiment (Murayama *et al.*, 2004).

4 Conclusion

In summary, different binding modes of 7-ethoxyresorufin in CYP1A2 and its F186L mutant were found via ensemble docking. Further dynamics simulations indicate that a series of catalytic pocket changes, long-range effects of F186L mutation, are responsible for the binding mode switch of 7-ethoxyresorufin. In addition, these changes lead to different interactions between the substrate and the enzyme. In F186L, newly found hydrophobic interaction between V227 and the ethyl group of the substrate plays an essential role in keeping a doubled Fe-C13 distance. These findings may account for the experimental observations that the F186L mutation enhances the binding affinity but lowers the O-deethylation velocity of 7-ethoxyresorufin, that in V227G mutant the enzyme activity decreases to only about 35%. Furthermore, activities of CYP450 channels are affected by the F186L mutation and the binding of the substrate. The main active channel in WT, channel 2c, seems to be closed because of the stabilization of the B'-C loop in F186L. As a result, the relatively closed state of the mutant enzyme probably makes it more difficult to go through the normal O-

deethylation cycle. This research explored the details of a superficial mutation that influences the enzyme activity via theoretical methods. It may provide useful information for personalized drug design and protein engineering as well.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s12539-014-0184-2 and is accessible for authorized users.

Acknowledgements

This work was supported by grants from the National High-Tech R&D Program (863 Program Contract No. 2012AA020307), the National Basic Research Program of China (973 Program) (Contract No. 2012CB721000), the Key Project of Shanghai Science and Technology Commission (Contract No. 11JC1406400), and the Ph.D. Research Funds, Ministry of Education (20120073110057) which were awarded to Prof. D.-Q. Wei.

References

[1] Agnew, C.R., Warrilow, A.G., Burton, N.M., Lamb, D.C., Kelly, S.L.,Brady, R.L. 2012. An enlarged, adaptable active site in CYP164 family P450 enzymes, the sole P450 in Mycobacterium leprae. Antimicrob Agents Chemother 56, 391-402.

[2] Baldwin, J., Chothia, C. 1979. Haemoglobin: The structural changes related to ligand binding and its allosteric mechanism. J Mol Biol 129, 175-220.

[3] Chang, T.K., Waxman, D.J. 1998. Enzymatic analysis of cDNA-expressed human CYP1A1, CYP1A2, and CYP1B1 with 7-ethoxyresorufin as substrate. In: Enzymatic Analysis of cDNA-Expressed Human CYP1A1, CYP1A2, and CYP1B1 with 7-Ethoxyresorufin as Substrate, Springer, 103-110.

[4] Cojocaru, V., Winn, P.J., Wade, R.C. 2007. The ins and outs of cytochrome P450s. Biochim Biophys Acta 1770, 390-401.

[5] DePaul, A.J., Thompson, E.J., Patel, S.S., Haldeman, K., Sorin, E.J. 2010. Equilibrium conformational dynamics in an RNA tetraloop from massively parallel molecular dynamics. Nucl Acid Res 38, 4856-4867.

[6] Eaton, D.L., Gallagher, E.P., Bammler, T.K., Kunze, K.L. 1995. Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. Pharmacogenetics 5, 259-274.

[7] Ekroos, M., Sjogren, T. 2006. Structural basis for ligand promiscuity in cytochrome P450 3A4. Proc Nat Acad Sci USA 103, 13682-13687.

[8] Estabrook, R.W. 1996. The remarkable P450s: A historical overview of these versatile hemeprotein catalysts. FASEB J 10, 202-204.

[9] Fletcher, R., Powell, M.J. 1963. A rapidly convergent descent method for minimization. Comput J 6, 163-168.

[10] Goodsell, D.S., Morris, G.M., Olson, A.J. 1996. Automated docking of flexible ligands: Applications of AutoDock. J Mol Recognit 9, 1-5.

[11] Hess, B., Bekker, H., Berendsen, H.J., Fraaije, J.G. 1997. LINCS: A linear constraint solver for molecular simulations. J Comput Chem 18, 1463-1472.

[12] Hodgson, J. 2001. ADMET-turning chemicals into drugs. Nat Biotechnol 19, 722-726.

[13] Hornak, V., Abel, R., Okur, A., Strockbine, B., Roitberg, A., Simmerling, C. 2006. Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins 65, 712-725.

[14] Hritz, J., de Ruiter, A., Oostenbrink, C. 2008. Impact of plasticity and flexibility on docking results for cytochrome P450 2D6: A combined approach of molecular dynamics and ligand docking. J Med Chem 51, 7469-7477.

[15] Huey, R., Morris, G.M., Olson, A.J., Goodsell, D.S. 2007. A semiempirical free energy force field with charge-based desolvation. J Comput Chem 28, 1145-1152.

[16] Ingelman-Sundberg, M., Sim, S.C., Gomez, A., Rodriguez-Antona, C. 2007. Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 116, 496-526.

[17] Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W., Klein, M.L. 1983. Comparison of simple potential functions for simulating liquid water. J Chem Phys 79, 926.

[18] Keizers, P.H., de Graaf, C., de Kanter, F.J., Oostenbrink, C., Feenstra, K.A., Commandeur, J.N., Vermeulen, N.P. 2005. Metabolic regio- and stereoselectivity of cytochrome P450 2D6 towards 3,4-methylenedioxy-N-alkylamphetamines: *In silico* predictions and experimental validation. J Med Chem 48, 6117-6127.

[19] Lee, Y.T., Wilson, R.F., Rupniewski, I., Goodin, D.B. 2010. P450cam visits an open conformation in the absence of substrate. Biochemistry 49, 3412-3419.

[20] Lewis, D.F., Lake, B.G. 1996. Molecular modelling of CYP1A subfamily members based on an alignment with CYP102: Rationalization of CYP1A substrate specificity in terms of active site amino acid residues. Xenobiotica 26, 723-753.

[21] Li, W., Liu, H., Scott, E.E., Grater, F., Halpert, J.R., Luo, X., Shen, J., Jiang, H. 2005. Possible pathway(s) of testosterone egress from the active site of cytochrome P450 2B1: A steered molecular dynamics simulation. Drug Metab Dispos 33, 910-919.

[22] Li, J., Wei, D.-Q., Wang, J.-F., Li, Y.-X. 2011. A negative cooperativity mechanism of human CYP2E1 inferred from molecular dynamics simulations and free energy calculations. J Chem Inf Model 51, 3217-3225.

[23] Lian, P., Liu, A.L., Shi, Y., Bu, Y., Wei, D. 2010. Tethered-hopping model for protein-DNA binding and unbinding based on Sox2-Oct1-*Hoxb*1 ternary complex simulations. Biophys J 98, 1285-1293.

[24] Lian, P., Guo, H.-B., Smith, J.C., Wei, D.-Q., Guo, H. 2013a. Catalytic mechanism and origin of high activity of cellulase *Tm*Cel12A at high temperature: A quantum mechanical/molecular mechanical study. Cellulose, 1-13.

[25] Lian, P., Li, J., Wang, D.Q., Wei, D.Q. 2013b. Car-Parrinello molecular dynamics/molecular mechanics (CPMD/MM) simulation study of coupling and uncoupling mechanisms of cytochrome P450cam. J Phys Chem B 117, 7849-7856.

[26] Lian, P., Wei, D.Q., Wang, J.F., Chou, K.C. 2011. An allosteric mechanism inferred from molecular dynamics simulations on phospholamban pentamer in lipid membranes. PLoS ONE 6, e18587.

[27] Liu, J., Ericksen, S.S., Sivaneri, M., Besspiata, D., Fisher, C.W., Szklarz, G.D. 2004. The effect of reciprocal active site mutations in human cytochromes P450 1A1 and 1A2 on alkoxyresorufin metabolism. Arch Biochem Biophys 424, 33-43.

[28] Medek, P., Benes, P., Sochor, J. 2008. Computation of tunnels in protein molecules based on Delaunay triangulation. J WSCG 15, 107-114.

[29] Mobley, D.L., Chodera, J.D., Dill, K.A. 2006. On the use of orientational restraints and symmetry corrections in alchemical free energy calculations. J Chem Phys 125, 084902.

[30] Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A.J. 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 19, 1639-1662.

[31] Murayama, N., Soyama, A., Saito, Y., Nakajima, Y., Komamura, K., Ueno, K., Kamakura, S., Kitakaze, M., Kimura, H., Goto, Y., Saitoh, O., Katoh, M., Ohnuma, T., Kawai, M., Sugai, K., Ohtsuki, T., Suzuki, C., Minami, N., Ozawa, S., Sawada, J. 2004. Six novel nonsynonymous CYP1A2 gene polymorphisms: Catalytic activities of the naturally occurring variant enzymes. J Pharmacol Exp Ther 308, 300-306.

[32] Parikh, A., Josephy, P.D., Guengerich, F.P. 1999. Selection and characterization of human cytochrome P450 1A2 mutants with altered catalytic properties. Biochemistry 38, 5283-5289.

[33] Pronk, S., Pall, S., Schulz, R., Larsson, P., Bjelkmar, P., Apostolov, R., Shirts, M.R., Smith, J.C., Kasson, P.M., van der Spoel, D., Hess, B., Lindahl, E. 2013. GROMACS 4.5: A high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics 29, 845-854.

[34] Sansen, S., Yano, J.K., Reynald, R.L., Schoch, G.A., Griffin, K.J., Stout, C.D., Johnson, E.F. 2007. Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2. J Biol Chem 282, 14348-14355.

[35] Schleinkofer, K., Sudarko, P.J.W., Lüdemann, S.K., Wade, R.C. 2005. Do mammalian cytochrome P450s show multiple ligand access pathways and ligand channelling? EMBO Rep 6, 584-589.

[36] Shimizu, T., Ochiai, H., Asell, F., Shimizu, H., Saitoh, R., Hama, Y., Katada, J., Hashimoto, M., Matsui, H., Taki, K., Kaminuma, T., Yamamoto, M., Aida, Y., Ohashi, A., Ozawa, N. 2003a. Bioinformatics research on inter-racial difference in drug metabolism I. Analysis on frequencies of mutant alleles and poor metabolizers on CYP2D6 and CYP2C19. Drug Metab Pharmacokinet 18, 48-70.

[37] Shimizu, T., Ochiai, H., Asell, F., Yokono, Y., Kikuchi, Y., Nitta, M., Hama, Y., Yamaguchi, S., Hashimoto, M., Taki, K., Nakata, K., Aida, Y., Ohashi, A., Ozawa, N. 2003b. Bioinformatics research on inter-racial difference in drug metabolism II. Analysis on relationship between enzyme activities of CYP2D6 and CYP2C19 and their relevant genotypes. Drug Metab Pharmacokinet 18, 71-78.

[38] Sim, S.C. 2010. The Human Cytochrome P450 (CYP) Allele Nomenclature Database. http://www.cypalleles.ki.se/cyp1a2.htm.

[39] Sorin, E.J., Pande, V.S. 2005. Exploring the helix-coil transition via all-atom equilibrium ensemble simulations. Biophys J 88, 2472-2493.

[40] Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A.E., Berendsen, H.J. 2005. GROMACS: Fast, flexible, and free. J Comput Chem 26, 1701-1718.

[41] Wade, R.C., Winn, P.J., Schlichting, I., Sudarko. 2004. A survey of active site access channels in cytochromes P450. J Inorg Biochem 98, 1175-1182.

[42] Wang, J., Wang, W., Case, D.A. 2006. Automatic atom type and bond type perception in molecular mechanical calculations. J Mol Graphics Modell 25, 247-260.

[43] Wang, J., Wolf, R.M., Caldwell, J.W., Kollman, P.A., Case, D.A. 2004. Development and testing of a general AMBER force field. J Comput Chem 25, 1157-1174.

[44] Zhang, T., Liu, L.A., Lewis, D.F., Wei, D.Q. 2011. Long-range effects of a peripheral mutation on the enzymatic activity of cytochrome P450 1A2. J Chem Inf Model 51, 1336-1346.

[45] Zhou, S.F., Yang, L.P., Zhou, Z.W., Liu, Y.H., Chan, E. 2009. Insights into the substrate specificity, inhibitors, regulation, and polymorphisms and the clinical impact of human cytochrome P450 1A2. AAPS J 11, 481-494.