Journal of Toxicology and Environmental Health Sciences Vol. 3(12), pp. 335-341, 5 November, 2011
Available online at http://www.academicjournals.org/JTEHS
ISSN 2006-9820 ©2011 Academic Journals

*Full Length Research Paper*

# Elucidate the origin of CYP flexible structural variation using molecular dynamics calculation

**Mohamed Ismael[1]*and Carlos A. Del Carpio[2]**

[1]Department of Chemistry, Faculty of Science, Sohag University, 82524, Sohag-Naser City, Egypt.
[2]Drosophila Genetic Resource Center, Kyoto Institute of Technology, Saga Ippongi-cho, Ukyo-ku, Kyoto 616-8354, Japan.

Accepted 20 September, 2011

**Human cytochrome P450 1A2 (CYP1A2) plays a critical role in the mechanism of drug metabolism the understanding of which constitutes a cornerstone in drug design processes. Its relevance has furthered in view of recent trends in tailor made medicine, since the enzyme's activity differs according to the mutations or single nucleotide polymorphisms (SNP's) in the respective genetic coding region. Here we investigate the structural variation induced by selected single mutations that have been reported on the CYP1A2 enzyme. We applied our graph theory approach and mainly focus on the differences in 3D structure between the "wild-type" and the mutant structures in terms of the structural flexibility changes that may arise as consequence of the mutations. Based on this information we have additionally inferred internal collective motions that may affect the function of the enzyme using molecular dynamics simulations. Our results show that most of the studied mutations are located in loops and regions of high flexibility and the simulation of their dynamic behavior sheds light on probable consequences of these mutations on the function of the enzyme.**

**Key words:** Cytochrome P450 1A2 (CYP1A2), single nucleotide polymorphism (SNP), molecular flexibility, molecular dynamics simulation, graph theory.

## INTRODUCTION

Human cytochorme P450 (CYP450) enzymes catalyze the metabolism of a wide variety of clinical, physiological, and toxicologically important compounds (Saito et al., 2005). CYP1A2 is a principal family 1 enzyme expressed in human liver, and it significantly contributes to the hepatic metabolism of drugs (Sansen et al., 2007). Liver drug-metabolizing CYP450-enzymes in general but CYP1A2 type ones in particular play a predominant role in the metabolic clearance of caffeine and melatonin as well as of marketed drugs such as flutamide, lidocaine, olanzapine, tacrine, theophylline, triamterene, and zolmitriptan (Agundez, 2004). However, several studies have reported individual variations in the activity of CYP1A2 amounting to up to 60 fold levels in drug clearance (Shimada et al., 1994; Saruwatari et al., 2002). Moreover, approximately 15 to 40 fold inter-individual variations in CYP1A2 mRNA and protein expression levels have been also observed in the human liver (Ikeya et al., 1999; Guengerich et al., 1999). These inter-individual differences are likely to influence the drug metabolism and to be associated with drug efficacy and safety as well as cancer susceptibility caused by procarcinogens. Environmental factors have been thought to influence the inter-individual differences. Cigarette smoking and intake of oral contraceptive steroids are well established modifiers of CYP1A2 activity (Rasmussen et al., 2002). However, it has been suggested that approximately 35 to 75% of the inter-individual variability in CYP1A2 activity is due to genetic factors (Kendler and Prescott, 1999). Thus several researches have focused their efforts on the identification of CYP1A2 genetic variants.

Large inter-individual differences in CYP1A2 stand for large differences in drug clearance. The basis for this variation is only partially understood. Structural characterization of human CYP1A2 has been reported recently. On the other hand, several investigators have reported the critical amino acids affecting CYP1A2 activity.

Murayama et al. (2004) reported six single subsequent mutations in human CYP1A2, T83M, e168Q, F186L,

*Corresponding author. E-mail: m.ismaelmohamed@gmail.com. Tel/Fax: +20-93-460-1159.

S212C, G299A, and T438I. Among these six CYP1A2 variants, F186L showed the most profound and statistically significant reduction in CYP1A2 oxidation reactivity. However all these reported mutations are located in regions far from the active site; and the majority is located on the surface.

The elucidation of the effects of these mutations on the structure of these enzymes and consequently their function gains in importance as recent trends in drug design underscore individualized medicine as the next challenging task in this field. Mapping the effects of single nucleotide polymorphisms (SNP's), especially those on coding regions of chromosomes, in terms of structural and functional changes in the expressed proteins constitute the basis to undertake this enterprise.

Under these circumstances, using a recent system called RIGIX developed in our laboratories we examine the effects of natural mutations on protein structure and function in terms of the gain or loss of flexibility of the 3D structure of the mutant as compared to that of the wild type (Del Carpio et al., 2005; Abdur Rauf et al., 2009). Based on this information we identify regions in the protein that may undergo collective motions important to the function of the protein, especially those affecting the active site. These calculations are applied to the six reported mutants of the CYP1A2 enzyme. Application of our graph theory approach to the 3D structures of the mutants and the WT reveals changes in the flexibility of the WT that differ in magnitude according to the type of mutation.

Finally, in order to evaluate the effects of these mutations on the dynamic behavior of the protein the models were subjected to molecular dynamics (MD) simulations. The simulation allowed the characterization of the time of evolution of the structure and averaging the values of structural properties. Structures to perform the present work were extracted from the RCSB protein data bank, and for those mutants whose structures can not be found in the data bank, we built a model by deletion and insertion of the mutated amino acid.

Our investigations on the CYP1A2 wild type and (T83M, e168Q, F186L, S212C, G299A and T438I) mutant structures reveal that mutant F186L shows the largest perturbation in terms of the flexibility adopted by the mutated structure. Analysis of the found structural differences arisen by the mutation of F186L and performing the MD simulation of these regions lead us to postulate that the internal collective motions originated as consequence of the mutated amino acid inhibit the expression of the enzymatic properties of the molecule.

## METHODS

### Modeling the mutant structures

The X-ray structure extracted from RCSB protein data bank (PDB:2HI4) of the human CYP1A2 was used as the wild-type (WT) model. The WT structure was used as the starting structure to generate mutant (MT) structures by replacing WT residues with the mutating residues. Figure 1 shows the WT structure with indication to the positions of mutations, referring to the names of residue prior mutation. Overall, six MT structures were generated corresponding to the six point mutations T83M, E168Q, F186L, S212C, G299A, and T438I (Murayama et al., 2004). The residue replacements were carried out using the MUTATE_MODEL command of Swiss-PdbViewer (Guex and Peitsch, 1997).

### Molecular dynamics simulation

The maestro program package adopting the OPLS-AA force field parameters was used for energy minimization (EM) and molecular dynamics (MD) simulations (Jorgensen et al., 1996; Zhang et al., 2001). The structures were energy minimized using the steepest descent method, terminating when maximum energy gradient is found to be smaller than 0.05 KJ/mol. The energy-minimized structures were subjected to MD simulation for 250 ps. All the simulations were performed in the NVT ensemble at constant temperature (300 K) and pressure (1 atm.) with a time step of 1.5 fs. The cell solvent was represented using cubic cell of water surrounding each model.

### Flexibility analysis of the WT and MT structures (RIGIX software)

It is a very well known fact that the dynamics of protein folds are dictated by the intra-molecular inter-atomic interactions energies. A protein fold is mainly stabilized by covalent bonding and intra-molecular electrostatic interactions, especially, hydrogen bonds play a critical role in the stabilization of the secondary structure of a protein, mainly helices and sheets conformations. A commonly used index to account for the stability of a molecule is the number of degrees of freedom (DOF) or the number of floppy modes in the network of interactions that hold the atoms at fix positions within the molecule.

Although enumerating the number of DOFs for a molecule involves computationally expensive procedures, a methodology based on network rigidity and constraint counting proposed by Jacobs et al. (2001) has proved a worst case performance of O(n2), for a network of n sites. We have developed an algorithm based on Jacobs algorithm to account for flexibility loss and rigidity gain not only in a protein but also in protein complexes formed by more than two interacting subunits (Del Carpio et al., 2005). The program expresses the flexibility of an amino acid calculating the index flex, the definition of which is expressed by the following equation:

$$flex = \frac{\text{Number of independent hinges in the amino acid}}{\text{Number of constraints for the amino acid}} \quad (1)$$

The difference in flexibility among the wild type and mutants can be expressed by the sum of the differences in flexibility of the amino acids at corresponding positions in the mutant and the wild type (Equation 2) as expressed in Equation 3:

$$\Delta flex_i = flex_i^{mutant} - flex_i^{WT} \quad (2)$$

$$\Delta flex = \sum_{i=1}^{N} \Delta flex_i \quad (3)$$

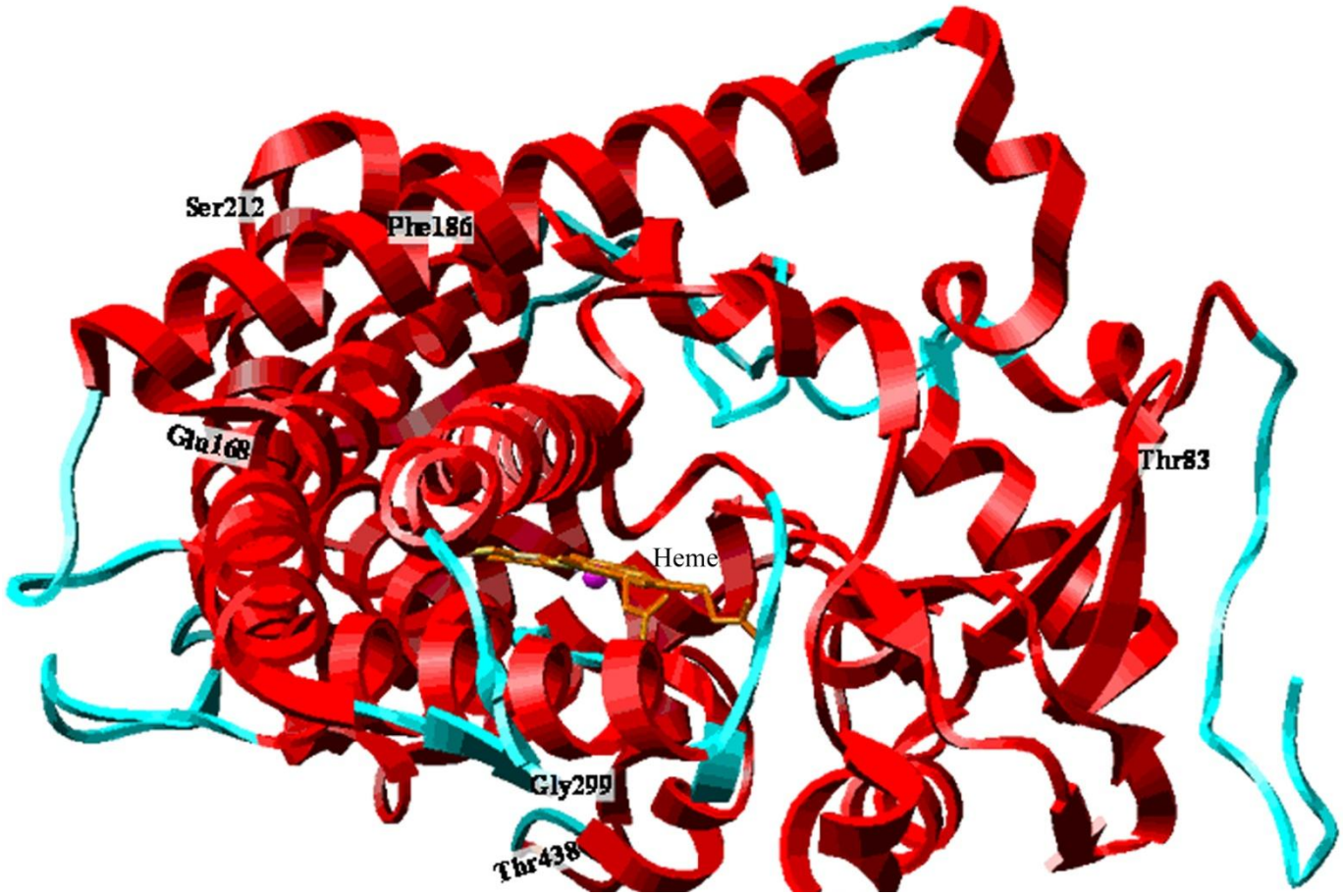


**Figure 1.** Flexible and rigid regions in CYP1A2 are shown with cyan and red colors respectively.

Where $\Delta flex_i$ is the difference in flexibility at the amino acid level and $\Delta Flex$ is the total difference in flexibility for the mutant; N being the total number of amino acids in the WT and mutant structures. While the former index.

#### Visualization and analysis

Molecular graphics images were created with UCSF Chimera code (Pettersen et al., 2004), and interactions between protein and ligand were analyzed by schematic representation using the LIGPLOT program (Wallace et al., 1995).

## RESULTS AND DISCUSSION

### Flexibility of the CYP1A2 WT structure

Figure 1 shows the mapping of the flexible regions on a ribbon model of the 3D structure of the WT regions of high flexibility are shown in red. It can be observed that only G299 belongs to a flexible region while all other mutation sites are rigid. The rigidity of the substrate binding pocket comes from the fact that this region is conserved and it explains why substrate interacting with the heme group in the protein remains in a perpendicular plane through the metabolic reaction.

Figure 2 shows the results of applying RIGIX program to the structure of the CYP1A2 WT which is used as reference for comparison with MT structures. Analysis of the residue's flexibility indicates that most residues are rigid (flexibility index less than 0.15). Heme moiety is surrounded by highly rigid residues (118, 122-125, 220-223, 226, 256-260, 313, 317- 321) which is essential for the substrate binding site pocket.

### Flexibility analysis of the CYP1A2 MT structures

Figure 3 summarize schematically the differences in flexibility ($\Delta Flex$) for the six mutants studied in the present work calculated relative to the WT. In these plots, T438I shows minor difference because it is located in a high flexible loop and near the C terminal. Global flexibility changes for the mutants T83M, E168Q, F186L, S212C and G299A show increase in the flexibility of the residues 102, 400 and 513, with almost the same value. For the flexibility change on the MT site itself, F186L and G299A

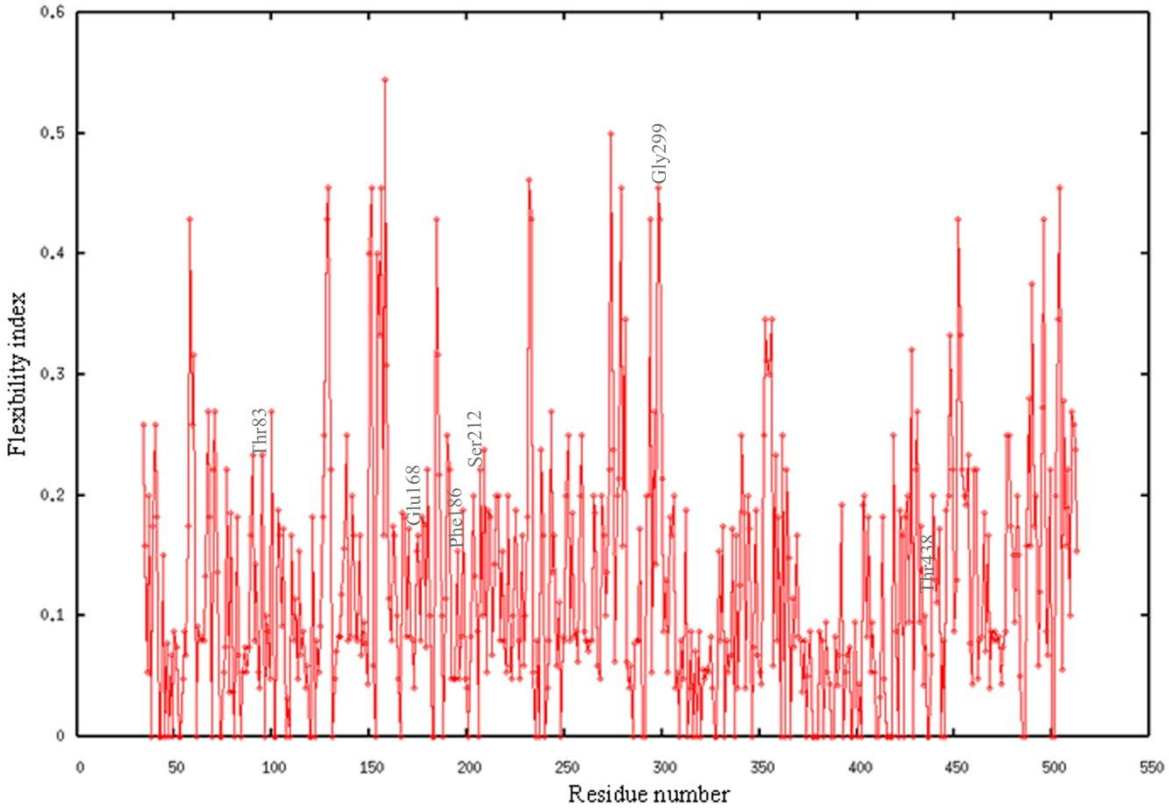


**Figure 2.** Graph of the flexibility index versus the amino acid numbers in the WT of CYP1A2.

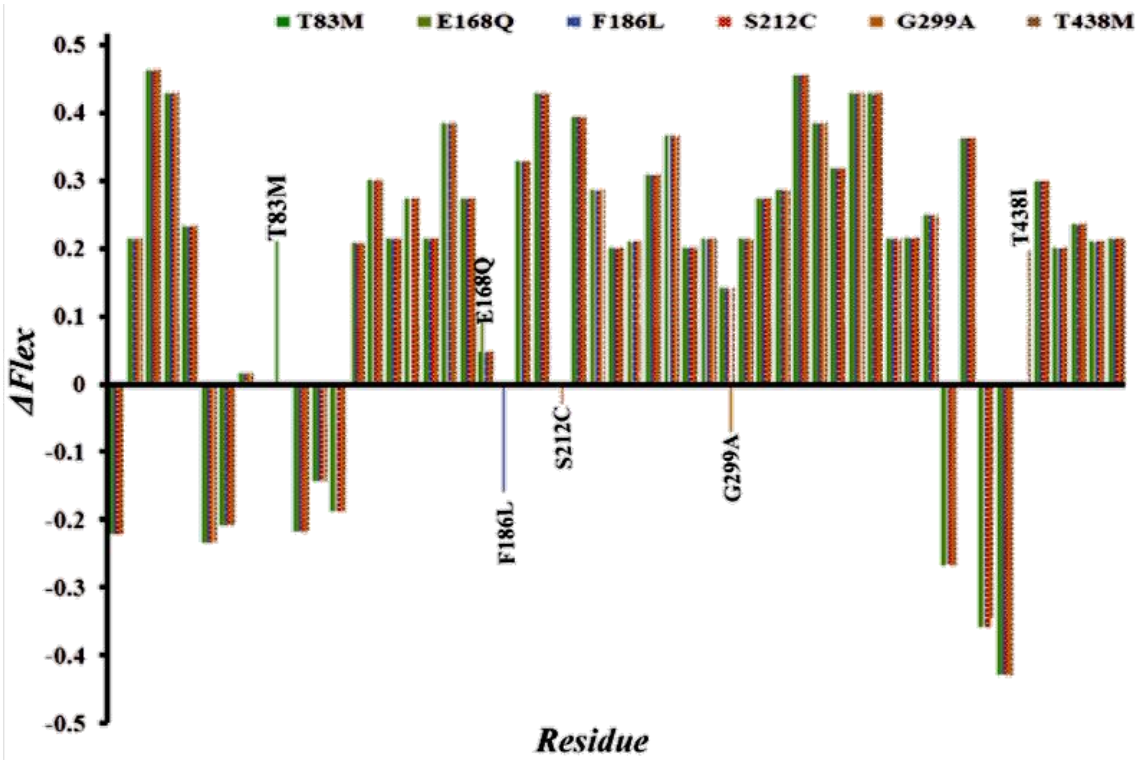


**Figure 3.** Comparing the rigidity difference between the MT structures.

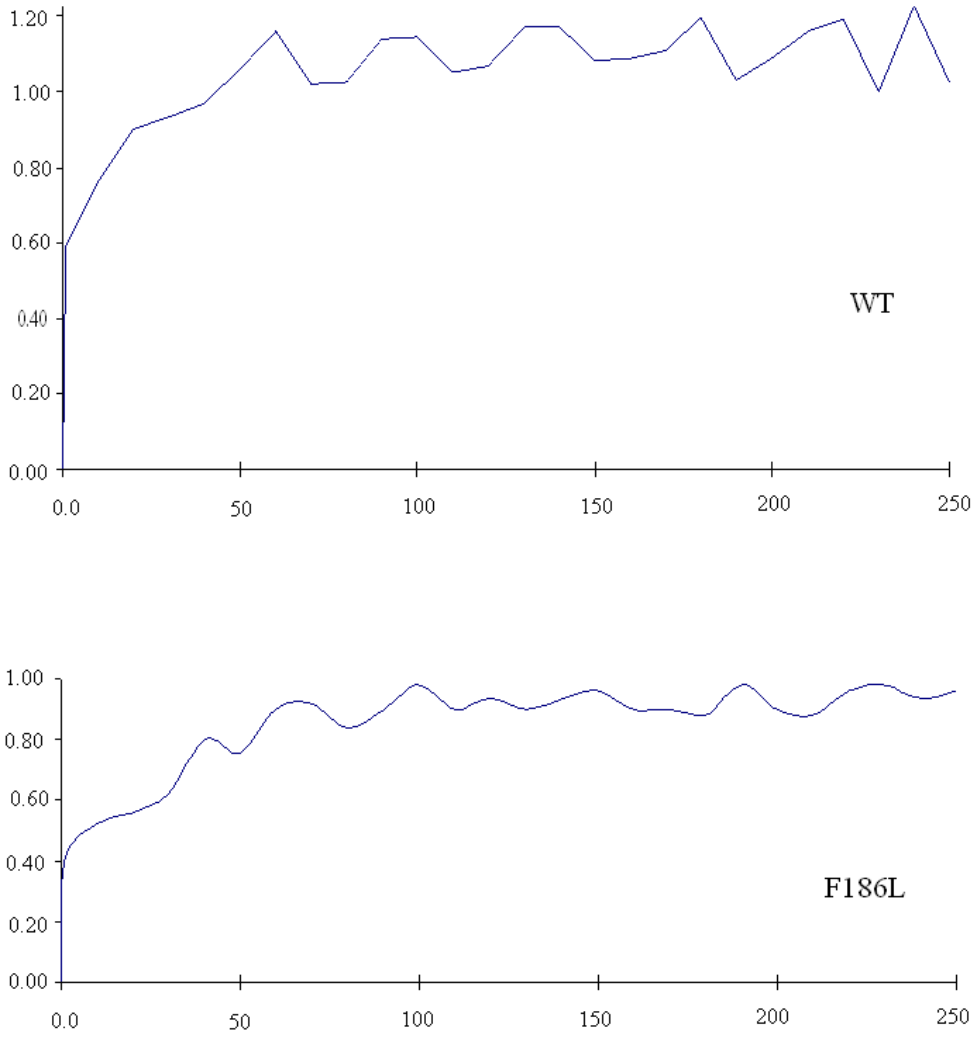


**Figure 4.** The RMSD of the Cα of the WT and F186L mutant versus the simulation time.

show increasing in the flexibility (*ΔFlex are* -0.06 and -0.03 respectively). The other MT sites became rigid (*ΔFlex* for T83M, E168Q, F186L, and G299A are 0.15, 0.05, 0.10 respectively. S212C had no changes. Increase in rigidity will preserve the main skeleton of the protein structure and thus preserve its activity. While increasing the flexibility will lead to a distortion in the structure and inhibiting the activity.

## MD simulation studies

The results of applying the structural analysis to the mutants and wild type molecules are in fact a static assessment of the rigidity/flexibility of the structures, and also point out those flappy amino acids present in a determined structure that may lead to particular motions within the protein structure. How these motions proceed and how these movements propagate through the protein structure leading to a disruption in function however can only be estimated performing the dynamic simulation of these motions. Here we have applied molecular dynamic simulations (MD) to the structure of the MTs as well as the WT. Hence, the WT and the MT structures were subjected to MD simulations for 250 ps. The instantaneous structures saved at every successive 2.5 ps from the beginning of the simulation.

## Overall structural changes

The overall structural deviation between the WT and the F186L from their initial structures during the entire simulations was observed by calculating the RMSD of the $C_\alpha$. Figure 4 shows the RMSD versus the simulation time. It should be noted that the starting structures were

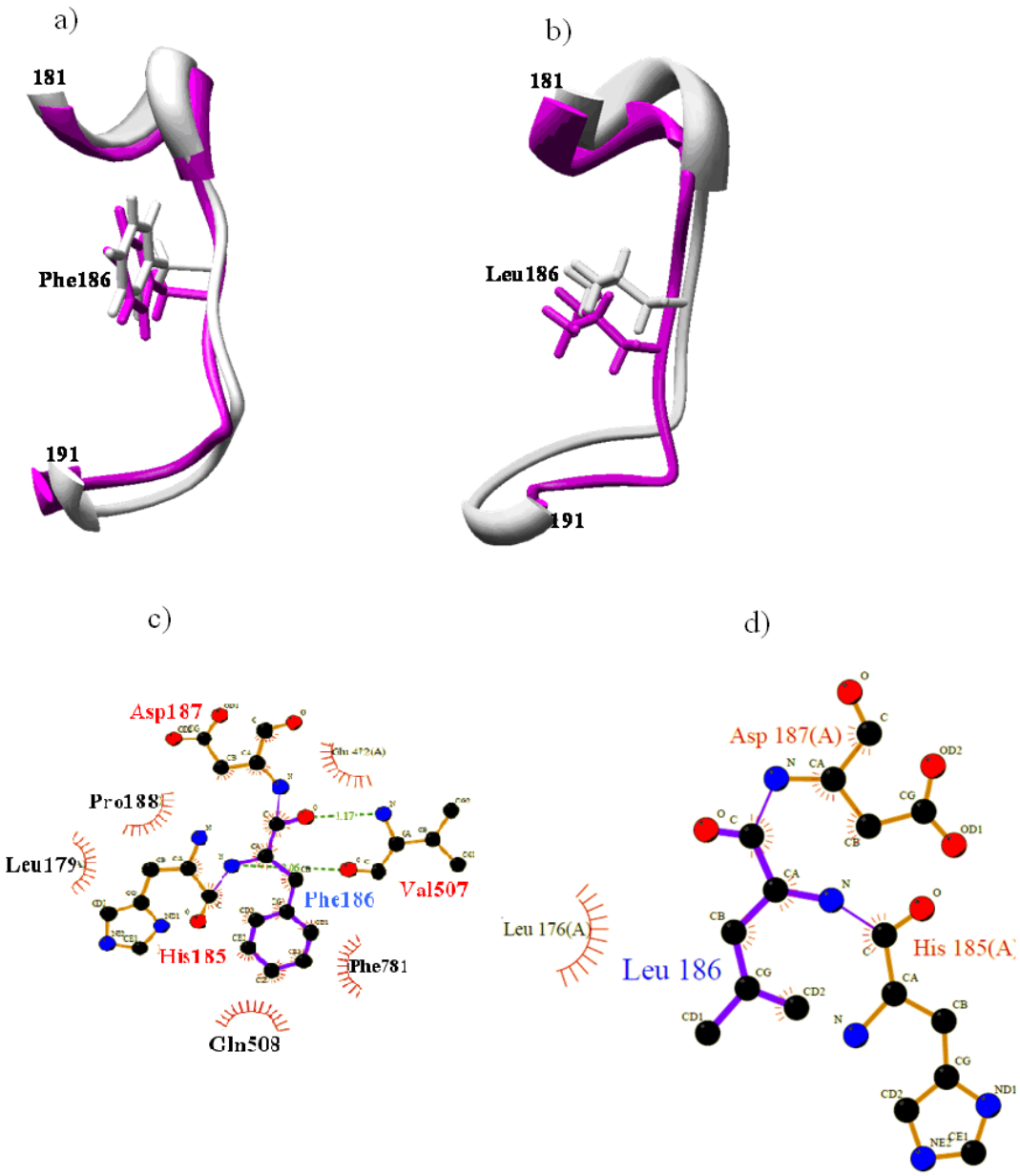


**Figure 5.** Superimposition of the final "pink" and initial "gray" structures of the (a) WT and (b) F186L mutant. 2D schematic diagram of the Hydrogen bonds in the final structure of the (c) WT and (d) F186L mutant.

identical except for the side chains of the residues at the mutation site. Both structures initially evolve rapidly during the first 50 ps and seem to stabilize at different times. All analyses were carried out using the data collected after RMSD stabilization. The average value of the overall RMSD after stabilization is of 1.06 Å in WT, whereas in the mutant F186L mutant it is 0.45 Å.

## Structural changes at the mutation sites

Substitutions of residues at the mutation sites are expected to bring out certain structural changes at the mutation sites. To know changes brought out by the F186L mutation, we calculated the RMSD in the mutation flexible region recognized by the first graph theoretical procedure as mentioned above. Furthermore, since these changes are brought up mainly by disruption of the network of hydrogen bonds we have compared the hydrogen bond (Hb) interaction networks in both the WT and the mutant.

Figure 5 shows the superposition between the final and initial structures of the WT and F186L mutant and the 2D scheme of the network of Hb of two final structures. From the Figure it can be seen that the F186L mutation is

characterized by a major conformational change at the mutation site as compared to WT. The mutation site is marked by a large RMSD of $C_\alpha$ (res. 181 ~ res. 191) 1.21 Å compared with that of the WT which is 0.57 Å. Similarly, the Hb interactions present in Phe186 of the WT were not formed by Leu186 in the mutant model (Figure 5a and d). The increased flexibility in the structure of the mutant can thus be attributed to the loss of this interaction and decreases reactivity of the cytochrome (Murayama et al., 2004; Rosaert, 2008).

## Conclusions

Through this study we have been able to map the effects of single mutations on the three dimensional structure of proteins, and by consequence in its function. The key step in our study is the graph theoretical analysis of the rigidity of the 3D structures of the wild type and mutant which leads to the determination of the most flexible regions on the proteins. We have seen here for example, that while five out of six mutations have no relevant effect on the structural stability of the protein, the F186L does, and the underlying cause for the change in rigidity of this mutant is the difference in the internal network of hydrogen bonds in the WT and the mutant. Molecular dynamics simulation of the structures corroborates these results, and shows that the loop containing the mutated amino acid changes of conformation even at small simulation times.

The repercussion of this change in the conformation of the binding site can thus be expressed in terms of the rigidity of this loop, which apparently far away from the receptor region, affects the amino acids surrounding the heme group. Since several studies, including one of ours, have found that conformation at the receptor site has to be rigid so that ligands must approach the heme group at a perpendicular plane, the imbalance brought up by the internal motion originated by this mutation can influence the rigidity of the pocket region in CYP1A2.

### REFERENCES


Abdur Rauf SMd, Ismael M, Sahu KK, Suzuki A, Sahnoun R, Koyama M, Tsuboi H, Hatakeyama N, Endou A, Takaba H, Del Carpio CA, Kubo M, Miyamoto A (2009). A graph theoretical approach to the effect of mutation on the flexibility of the DNA binding domain of p53 protein, Chem. Papers, 63(6): 654-661.

Agundez JA (2004). Cytochrome P450 Gene Polymorphism and Cancer, Curr. Drug Metab., 5: 211-224.

Del Carpio CA, Shaikh AR, Ichiishi E, Koyama M, Kubo M, Nishijima K, Miyamoto A (2005). A graph theoretical approach for analysis of protein flexibility change at protein complex formation, Genome Informatics, 16: 148-160.

Guengerich FP, Parikh A, Turesky RJ, Josephy PD (1999). Inter-individual differences in the metabolism of environmental toxicants: cytochrome P450 1A2 as a prototype, Mutat. Res., 428: 115-124.

Guex N, Peitsch MC (1997). SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling, Electrophoresis, 18: 2714-2723.

Ikeya K, Jaiswal AK, Owens RA, Jones JE, Nebert DW, Kimura S (1989). Human CYP1A2: Sequence, gene structure, comparison with the mouse and rat orthologous gene, and differences in liver 1A2 mRNA expression, Mol. Endocrinol., 3: 1399-1408.

Jacobs DJ, Rader A, Kuhn LA, Thorpe MF (2001). Graph Theory Predictions of Protein Flexibility, Protein: Struct. Funct. Genet., 44: 150-155.

Jorgensen WL, Maxwell DS, Tirado-Rives J (1996). Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids, J. Am. Chem. Soc., 118: 11225-11236.

Kendler KS, Prescott CA (1999). Caffeine intake, tolerance, and withdrawal in women: A population-based twin study, Am. J. Psychiatry, 156: 223-228.

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. Pharm. Exp. Thera., 308: 300-306.

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004). UCSF Chimera - a visualization system for exploratory research and analysis, J. Comp. Chem., 25: 1605-1612.

Rasmussen BB, Brix TH, Kyvik KO, Brøsen K (2002). The interindividual differences in the 3-demthylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors, Pharmacogenetics, 12: 473-478.

Rosaert FAJ, Covian R, Trumpower BL (2008). Mutations in cytochrome b that affect kinetics of the electron transfer reactions at center N in the yeast cytochrome bc1 complex, Biochim. Biophys. Acta., 1777: 239-29.

Saito Y, Hanioka N, Maekawa K, Isobe T, Tsuneto Y, Nakamura R, Soyama A, Ozawa S, Kagawa TT, Jinno H, Narimatsu S, Sawada J (2005). Functional analysis of three cyp1a2 variants found in a Japanese population, Drug Metab. Dispos., 33: 1905-1910.

Sansen S, Yano JK, Reynald RL, Schoch GA, Griffin KJ, Stout CD, Johnson EF (2007). Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2, J. Biol. Chem., 282: 14348-14355.

Saruwatari J, Nakagawa K, Shindo J, Tajiri T, Fujieda M, Yamazaki H, Kamataki T, Ishizaki T (2002). A population phenotyping study of three drug-metabolizing enzymes in Kyushu, Japan, with use of the caffeine test, Clin. Pharmacol. Ther., 72: 200-208.

Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP (1994). Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians, J. Pharmacol. Exp. Ther., 270: 414-423.

Wallace AC, Laskowski RA, Thornton JM (1995). A program to generate hematic diagrams of protein-ligand interactions, Prot. Eng., 8: 127-134.

Zhang L, Gallicchio E, Friesnerp R, Levy RM (2001). Solvent models for protein-ligand binding: comparison of implicit solvent poisson and surface generalized born models with explicit solvent simulations, J. Comp. Chem., 22: 591-607.