Quantum chemical analysis of reaction indices and reaction path for drug molecules

Takao Otsuka¹, Noriaki Okimoto¹, Hiroaki Saito¹ and Makoto Taiji¹

¹ RIKEN Center for Biosystems Dynamics Research (BDR), 6-2-4 Furuedai, Suita, Osaka 565-0874, Japan
totsuka@riken.jp

Abstract. We performed a quantum chemical study on evaluating chemical reactivity for drug molecules in Cytochrome P450 (CYP) metabolic reaction. In this study, we focused on two insights for analysing the chemical reactivity: one is the Fukui reaction indices for a molecule, and the other is the minimum energy paths of hydrogen transition reaction between drug molecule and FeO-Porphyrin ring part on the CYP metabolic reaction. The Fukui indices are obtained numerically by using the familiar population analysis method in quantum chemical calculations. We performed the assessments of some population analyses to clarify the numerical behaviour clearly and then evaluated the potential of the reactivity of drug molecule. On the other hand, in the analysis of the minimum energy path for the CYP metabolic reaction, we performed the nudged elastic band (NEB) calculations for the hydrogen transition reaction in the first step of the CYP metabolic reaction. We report the findings for the reactivity from the Fukui indices method and also show the NEB results for the reaction paths.

1. Introduction

In the field of drug discovery, molecular simulation technique become widely used as a means of understanding the mechanism of biological reaction and exploring novel drug molecules. For example, one of the challenging study is the one on Cytochrome P450 (CYP) metabolic reaction. The CYP reaction has two categorized metabolic reaction, Phase I (oxidation, reduction, hydrolytic reaction) and Phase II (conjugation). A lot of drug candidate molecules that cause the metabolic reaction on a specific atom have been synthesized from the detailed analysis for those metabolic reactions mechanism (typically, Phase I reaction) between CYP and a substrate molecule by using quantum mechanics and molecular mechanics (QM/MM) hybrid methods [1]. On the other hand, along with the rapid development of computer and/or computing technology, the knowledge-based studies on the prediction of drug metabolism have increased [2].

Along this line, we have been trying to establish the prediction scheme for the site of metabolism (SOM) of drug molecule by using molecular dynamics (MD) simulation and QM calculation technique [3]. Our strategy for predicting the site of metabolic reaction is to consider “accessibility” and “reactivity” on the CYP metabolic reaction. In accessibility analysis, we evaluate the “accessibility”, that is, the distribution probability against the distance between atoms in a drug molecule and FeO-Porphyrin ring part of CYP by the combination of molecular docking and MD calculations. In reactivity analysis, we evaluate the “reactivity” by using the empirical QM-based transition energies of atoms in a drug molecule, which has already been tabulated in previous study [4].
We found that these combination methodology works very well for predicting the SOM of a drug molecule [3].

As one of our successive studies, we performed a quantum chemical study on evaluating chemical reactivity for drug molecules in CYP metabolic reaction in order to explore further non-empirical parameters. In this study, we focused on two insights for analyzing the chemical reactivity for a drug molecule: one is the Fukui reaction indices for a molecule, and another is the minimum energy paths of hydrogen transition reaction between drug molecule and FeO-Porphyrin ring on the CYP metabolic reaction. Traditionally, the Fukui indices have been used as one of the potential index of chemical reaction for molecule only. The Fukui indices are obtained numerically by using the familiar population analysis method such as Mulliken, Löwdin, Hirschfeld, Iterative-Hirschfeld, and Natural population analysis in quantum chemical calculations. We performed the assessment of these population analyses to clarify the numerical behavior clearly and then evaluated the potential of the reactivity of drug molecule. On the other hand, in order to understand the first step mechanism of the metabolic reaction, we performed the nudged elastic band (NEB) calculations for the hydrogen transition reaction and analyzed the minimum energy path. Because the hydrogen transition reaction from a drug molecule to FeO-Porphyrin is the first step of the metabolic reaction and is also the rate-limiting step. In the NEB calculations, we used the cluster models for some structure poses of the drug molecule and FeO-Porphyrin ring part in CYP. We report some findings for the metabolic reactivity. Those were somewhat contrary results to the understanding from the Fukui indices method. We also show the NEB results for the reaction path for the hydrogen transition reaction.

2. Methods
In this study, in order to analyze the chemical reactivity of drug molecules on the CYP metabolic reaction, we focused on two quantum chemical methods. Here, we briefly describe some key points of our computational approaches, that is, the Fukui reaction indices (or Fukui function) method for the potential index for chemical reaction, and the NEB method for the evaluation of hydrogen transition. Although these methods are independent each other, we assessed and evaluated the possibility for applying the understanding the metabolic reaction of drug molecules.

2.1. Fukui reaction indices
The Fukui reaction indices (or Fukui function) is well known as one of the analysis method for a molecular reactivity [5] and can evaluate the site of high or low reactivity in a molecule. Theoretically, the Fukui function is derived from the framework of density functional theory (DFT) exactly,

\[ f(r) = \left( \frac{\delta \mu}{\delta \rho(r)} \right)_N = \left( \frac{\delta \rho(r)}{\delta N} \right)_\nu, \]

where the Fukui function \( f \) is evaluated by the electron density \( \rho \) and the number of electrons \( N \) in a molecule. If some appropriate approximations are applied, the Fukui function can evaluate the nucleophilic or the electrophilic reactions, respectively. In practical quantum chemical calculations, the Fukui function can be obtained from the electron density of the Fukui’s frontier orbitals [6,7] by using \( \phi_{HOMO} \) and \( \phi_{LUMO} \), or the population analysis on \( i \)-th atoms in a molecule. The former is expressed as the electron density map. The latter is expressed by the population analysis numerically,

\[ f^+(r) \equiv q_i \ (N+1) - q_i \ (N), \text{(Nucleophilic attack)} \]
\[ f^-(r) \equiv q_i \ (N) - q_i \ (N-1), \text{(Electrophilic attack)} \]
where the Fukui function $f^+$ (or $f^-$) represents the case of the nucleophilic attack (or the case of the electrophilic attack), and the charge $q_i$ of $i$-th atom is evaluated by the quantum chemical calculations of a molecule for $N, N+1$ and $N-1$ electrons systems [8].

From the definition of the Fukui function, the numerical value by the Fukui function has positive value conventionally, and therefore, it has been considered traditionally that the negative value by the Fukui function is meaningless. However, some recent studies discuss the negative values of Fukui function [9].

### 2.2. Nudged elastic band calculation

The NEB calculation is one of the methods for finding a saddle point (near a saddle point) and the minimum energy path between reactant and product in chemical reaction [10,11]. In the NEB calculation, the saddle point search is performed by optimizing a number of intermediate images, $R_i$ along the reaction path. Here, the intermediate images, $R_i$ are generated as the interpolating structures between the reactant structure and the product one, and connected as an elastic band. Practically, the elastic band optimization by the NEB calculation is performed by summing the spring forces along the band between the intermediate images, and the projection component of the force due to the potential perpendicular of the band. Therefore, the total force acting on an intermediate image is expressed by using the energy of the system,

$$F_i = F_i^s |\gamma| - \nabla E(R_i)|_\perp.$$

Then the true force is given by,

$$\nabla E(R_i)|_\perp = \nabla E(R_i) - \nabla E(R_i) \cdot \tau_i,$$

where $\tau_i$ is the normalized local tangent at $i$-th image. The spring force is

$$F_i^s |\gamma| = k(|R_{i+1} - R_i| - |R_i - R_{i-1}|)\tau_i,$$

where $k$ is the spring constant. The movements of the intermediate images according the forces in above equations are performed by using an optimization algorithm such as a projected velocity Verlet algorithm [10,11]. In recent study, a lot of optimization algorithm have been tested [12].

### 2.3. Computational details

Three drug molecules were selected as the test molecules in this study, as shown in Figure 1. These selected drug molecules are mainly metabolized by CYP 1A2 as the oxidation in Phase I reaction, and are well known experimentally that the metabolic site is majorly one in each molecule. These molecules has also been used well for evaluating the site of metabolic reaction of a molecule theoretically. We also used these molecules in our previous study [3].

In the assessment and the evaluation of the Fukui reaction indices by quantum chemical calculation, we used the DFT calculations with B3LYP exchange-correlation function and 3-21G basis set (denoted as B3LYP/3-21G). The initial structures were relaxed using the GAFF of the classical force field by AMBER software [13]. The Fukui reaction indices were estimated by using five population analysis methods: Mulliken, Löwdin, Hirshfeld, Iterative-Hirshfeld, and Natural population analysis. In this study, we only evaluated the Fukui reaction indices $f^+$ (the nucleophilic attack) because the present test molecules show the oxidation reaction, that is, the nucleophilic reaction. These calculations were performed by Gaussian09 software [14]. We also checked the case of the QM optimized structures by B3LYP/6-31G(d,p).
In the evaluation of the transition energy from the hydrogen transition path with the orientation of drug molecule (7-Ethoxycoumarin) by the NEB method, the initial structures were prepared as follows. The CYP structure from X-ray structure (PDB ID: 2HI4) was used. Firstly, the Fe-Porphyrin part in CYP structure was replaced with the oxygen added FeO-Porphyrin that reproduces a reactant in metabolic reaction, as shown in our previous study [3]. Then the binding structures of the drug molecule on the CYP structure were obtained by the molecular docking calculations using the GOLD software [15] with the scoring function of ChemScore. At this time, thirty docking poses per a drug molecule were considered. After the molecular docking calculations, the structure relaxation of only drug molecule was performed by using the GAFF classical force field in AMBER software [13]. We selected three initial orientations of a drug molecule by considering the nearest distances between the SOM of drug molecule and the FeO-Porphyrin part in CYP. Then we prepared for the drug molecule and the FeO-Porphyrin part with hydrogen termination as the cluster model, which corresponds to the reactant structure (the reaction coordinate, rc = 0) in the NEB calculation. For the product structure (the reaction coordinate, rc = 1) in the NEB calculation, we just moved the hydrogen atom of the SOM atom in drug molecule to the oxygen atom of FeO-Porphyrin part. The distance between the hydrogen and the oxygen atom was set to 0.95 Å. The NEB calculations were performed by using the nine intermediated images of the system including the reactant and the product, and the dumped Verlet optimization algorism [10-12] with the B3LYP/3-21G level of theory. In this study, we set the maximum value of force of 0.09 hartree/bohr or less as the optimization convergence condition. We only analyzed the case of 7-Ethoxycoumarin molecule in this study. The NEB calculations were performed by NWChem software [16].

**Figure 1.** Structures of test molecules (a) 7-Ethoxycoumarin, (b) 7-Methoxyresorufin, (c) Tacrine. The circle with pink color shows the SOM of the molecules. The lowers shows the atom ID for each molecules.

![Figure 1](image1.png)

**Figure 2.** Structure of FeO-Porphyrin part of CYP (PDB ID: 2HI4). The spheres show carbon (gray), nitrogen (blue), oxygen (red), sulfur (yellow), iron (purple) and hydrogen (white).

![Figure 2](image2.png)
In this study, although we limited our computational conditions in order to know the behavior the computational reaction indices and the hydrogen transition energies for the orientation of drug molecule, we will improve the computational accuracy using the higher quality of theory. Now we are going on further studies.

3. Reactivity analysis by Fukui reaction indices
First, we verified the numerical values of the Fukui reaction indices by five population analysis methods. Figure 3(a) shows the calculated Fukui reaction indices $f^+$ of the test molecule, 7-Ethoxycoumarin. Each value by five population analyses showed almost same tendency except for some parts. In order to specify the numerical features of these five population analysis methods, we performed the cluster analysis with a familiar Ward method. Figure 3(b) shows the dendrogram by the cluster analysis. As seen in Figure 3(b), Löwdin and Natural population analysis can be categorized as similar behavior, while Mulliken and Iterative-Hirshfelt population analysis seem to be close behavior numerically. From the cluster analysis of three test molecules, the similar tendency was confirmed. For the Fukui indices by five population analysis methods by the B3LYP/6-31G(d,p), the cluster analysis was almost similar result. We could mention that the Fukui reaction indices has less dependency of the basis set.

Next we evaluated the prediction of SOM by the Fukui reaction indices. As stated in the previous section, the values by the Fukui reaction indices mean the intensity of reactivity of a molecule. In the case of 7-Ethoxycoumarin illustrated in Figure 3, the SOM of 7-Ethoxycoumarin is the carbon atom of atom ID, 11 (see in Figure 2(a)). From Figure 3(a), the reactivity of the atom ID, 11 by the Fukui reaction indices showed the very lower and negative value in some population analysis. We can also see the other lower values of the atom ID, 4 and 14 with the same tendency. From the values of the Fukui reaction indices, the atoms with the low Fukui values are very stable for the nucleophilic reaction. We also checked the Fukui reaction indices $f^-$ of this molecule, which mean the reactivity for the electrophilic attack. The result is shown in Figure 4. As seen in Figure 4, it is found that the atoms with the low Fukui values are the atom ID, 11 and 14. From these two Fukui reaction indices, we can see that the atoms of the atom ID, 11 and 14 are very stable for the nucleophilic and electrophilic reactions. We also obtained the similar results from other two test molecules (not shown in this study). It is considered that the Fukui reaction indices could be applicable for finding the potential of the SOM in drug molecule, although it could not specify the SOM. From the analysis of the Fukui reaction indices using more molecules, we would also derive newly findings that in the metabolic reaction the FeO-Porphyrin attacks the relatively stable atom in drug molecule and the reaction is progressing.

Figure 3. Reactivity analysis of 7-Ethoxycoumarin; (a) the calculated Fukui reaction indices $f^+$ using five population analysis methods, (b) the classification of five population analysis methods by the cluster analysis with Ward’s method.
4. Reaction path analysis by NEB calculation

In order to evaluate the hydrogen transition energy of 7-Ethoxycoumarin molecule, three orientations of the molecule by molecular docking calculations were selected from the order of the nearest distance between the SOM of the molecule and the oxygen in FeO-Porphyrin (g1 < g2 < g3), as shown in Figure 5. As stated in the previous section, the reactant structure and the product structure were prepared as the cluster models, which corresponds to the first image and the last image in the NEB calculations (see Figure 5(a)).

Figure 5. Three initial structures of 7-Ethoxycoumarin in the NEB calculation (denoted as g1, g2 and g3); (a) the distance between carbon atom of the molecule and the oxygen atom of FeO-Porphyrin, C-O: 2.87 Å. Hydrogen atom moves form the reactant structure (left) to the product structure (right), (b) C-O: 3.03 Å, (c) C-O: 4.12 Å. The circle (orange) show the moving hydrogen atom.
Figure 6 shows the reaction paths of the hydrogen transition reaction for three orientation of 7-Ethoxycoumarin molecule. The reaction coordinates, \( rc = 0 \) and \( rc = 1 \) correspond to the reactant and the product structures, respectively. The seven points between \( rc = 0 \) and \( rc = 1 \) show the structures of the intermediated images. In Figure 6, the energy values are plotted with the energy of g1 orientation as a reference value. Table 1 also show the hydrogen transition energies of each orientation as reference value at \( rc = 0 \) of each orientation.

For the initial structures at the reaction coordinate, \( rc = 0 \) in Figure 6, the nearest orientation to FeO-Porphyrin, g1 (0.0 kcal/mol) is slightly unstable, compared with the g2 (-4.8 kcal/mol) and g3 (-4.2 kcal/mol) orientations energetically. However, along the reaction coordinate to the product structure, the transition energy from the g1 orientation showed the lowest one, 37.5 kcal/mol at around \( rc = 0.75 \). The transition energy from the g2 orientation was 41.0 kcal/mol at around \( rc = 0.75 \). These transition energies by the NEB calculation were estimated less than 62.2 kcal/mol obtained by the empirical QM-based energy [3,4]. This implies that it is important to consider the good orientation of drug molecule in CYP metabolic reaction. On the other hand, the transition energy by g3 orientation shows the largest one, 114.3 kcal/mol at around \( rc = 0.625 \). The reason for this is that not only the distance between the hydrogen atom and the oxygen of FeO-Porphyrin is so long (C-O: 4.12 Å) but also the steric hindrance of other atoms in the molecule (the carbon of atom ID, 14 and the attached hydrogen atom, see Figure 5(c)) occurred on the transition path of the hydrogen atom. In our previous study, we have evaluated the distribution probability against the distance between atoms in a drug molecule and FeO-Porphyrin ring part of CYP by the combination of molecular docking and MD calculations. In this process, we have neglected the orientation of hydrogen atom of a drug molecules. By considering the orientation of hydrogen atom and/or the steric hindrance of other atoms in a drug molecule, we would be able to further improve our proposed SOM predicting scheme.

**Figure 6.** NEB reaction paths of hydrogen transition of 7-Ethoxycoumarin. The transition energies of the g2 and g3 orientation were converted based on the transition energy of the g1 orientation. The reaction coordinates, \( rc =0 \) and \( rc = 1 \) correspond to the reactant and product structures, respectively.
Table 1. Hydrogen transition energies of each orientation (referenced at rc = 0 of each orientation).

| Orientation | Reaction coordinate (rc) | Transition Energy (kcal/mol) |
|-------------|--------------------------|-----------------------------|
| g1          | 0.75                     | 37.5                        |
| g2          | 0.75                     | 41.0                        |
| g3          | 0.625                    | 114.3                       |

5. Conclusion
In this study, we performed a quantum chemical analysis for the chemical reactivity of drug molecules in CYP metabolic reaction motivated on two insights. In the reactivity analysis by the Fukui reaction indices (Fukui function), we first assessed the numerical values obtained by five population analysis methods, such as Mulliken, Löwdin, Hirschfeld, Iterative-Hirschfeld, and Natural population analysis. From the cluster analysis, we could categorize the groups of Mulliken and Iterative-Hirschfeld, and Löwdin and Natural population analysis. We also confirmed the less basis set dependency of the Fukui reaction indices, different from the conventional population analysis. Based on the findings, we evaluated the reaction potentials for the site of metabolic reaction using three test molecules. Although the Fukui reaction indices could not predict the SOMs completely, we could read the tendency that in the metabolic reaction the FeO-Porphyrin attacks the relatively stable atom of a drug molecule. Furthermore, in the analysis of the minimum energy path for the CYP metabolic reaction, we performed the NEB calculations for the hydrogen transition reaction in the first step of the CYP metabolic reaction. Although we evaluated only one test molecule case, we found that the minimum energy path of hydrogen transition would depend on the orientations of a drug molecule, that is, the orientation of hydrogen atom and the steric hindrance of other atoms. We expect that these findings would be useful for improving our prediction scheme for the site of metabolic reaction.

Acknowledgements
This work was partly supported by the JSPS KAKENHI projects: Grant Numbers, 26860084, 16KT0168 to T. O. and 16K05648 H. S.. The calculations were performed in part on the RIKEN HOKUSAI supercomputer. The authors also acknowledge Prof. Y. Okamoto (Nagoya Univ.) and Prof. H. Akai (Univ. of Tokyo) for critical comments and fruitful discussion.

References
[1] For example, S. Shaik, S. Cohen, Y. Wang, H. Chen, D. Kumar, W. Thiel, Chem. Rev. 110, 949-1027, (2010).
[2] For example, J. Kirchmair, A. H. Göller, D. Lang, J. Kunze, B. Testa, I. D. Wilson, R. C. Glen, G. Schneider, Nature Reviews Drug Discovery, 14, 387-404 (2015).
[3] H. Saito, T. Otsuka, N. Okimoto, M. Taiji et al., to be appread.
[4] P. Rydberg, D. E. Gloriam, J. Zaretzki, C. Breneman, L. Olsen, ACS Med. Chem. Lett., 1, 96-100 (2010).
[5] R. G. Parr, W. Yang, Density-functional theory of atoms and molecules, Oxford University Press, 1989.
[6] W. Yang, W. J. Mortier, J. Am. Chem. Soc., 108, 5708-5711 (1986).
[7] K. Fukui, T. Yonezawa, H. Shingu, J. Chem. Phys., 20, 772 (1952).
[8] K. Fukui, T. Yonezawa, C. Nagata, H. Shingu, J. Chem. Phys., 22, 1433 (1954).
[9] For example, P. Bultink, R. Carbó-Dorca, W. Langenaeker, J. Phys. Chem., 118, 4349-4356 (2003).
[10] G. Henkelman, B. P. Uberuaga, H. Jónsson, J. Chem. Phys., 113, 9901 (2000).
[11] G. Henkelman, H. Jónsson, J. Chem. Phys., 113, 9978 (2000).
[12] D. Sheppard, R. Terrell, G. Henkelman, J. Chem. Phys., 128, 134106 (2008).
[13] D. A. Case et al., AMBER 12; University of California: San Francisco, 2012.
[14] M. J. Frisch et al., Gaussian 09, revision A.08; Gaussian, Inc.: Wallingford, CT, 2009.
[15] G. Jones, P. Willett, R. C. Glen, A. R. Leach and R. Taylor, J. Mol. Biol., 267, 727-748, (1997).
[16] M. Valiev, E.J. Bylaska, N. Govind, K. Kowalski, T.P. Straatsma, H.J.J. van Dam, D. Wang, J. Nieplocha, E. Apra, T.L. Windus, W.A. de Jong, Comput. Phys. Commun. 181, 1477 (2010).