Improved quantum mechanical model of P450-mediated aromatic oxidation

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Introduction

The Cytochrome P450 enzymes (P450s) are a large family of monooxygenases responsible for the phase I metabolism of 70-80% of drugs *via* oxidative reactions such as aliphatic and aromatic hydroxylation, epoxidation and heteroatom oxidation. It has become increasingly important, within drug discovery, to develop computer based methods to accurately predict P450-mediated metabolism of drugs and address issues such as:

- Rapid metabolism leading to poor bioavailability or rapid clearance
- Drug-drug interactions caused by inhibition or induction of P450 isoforms by a co-administered drug
- Variabilities in exposure due to genetic polymorphisms of P450 isoforms
- Formation of reactive or toxic metabolites

StarDrop™ P450 models

We have recently published a method that uses quantum mechanical simulations to predict the regioselectivity and lability of cytochrome P450 metabolism [1]. This method applies semi-empirical quantum mechanical calculations to estimate the activation energies for reactions leading to P450 metabolism, which are corrected for steric and orientation effects. The resulting models provide accurate predictions of the regioselectivity of metabolism; at least one site of metabolism is correctly identified in the top 2 predictions for 82-91% across 7 isoforms.

These models use semi-empirical, AM1 calculations and a Brønsted relationship to estimate the activation energy. For prediction of aromatic oxidation, a tetrahedral intermediate formed by addition of a hydrogen radical is optimised, as a substitute for the full oxy-haem responsible for oxidation in the P450 enzyme. This offers faster calculation time than first-principles Density Functional Theory (DFT) calculations, while retaining good accuracy. However, we have identified the potential for further improvements in the prediction of some aromatic oxidation reactions, as illustrated for diclofenac in Figure 1.

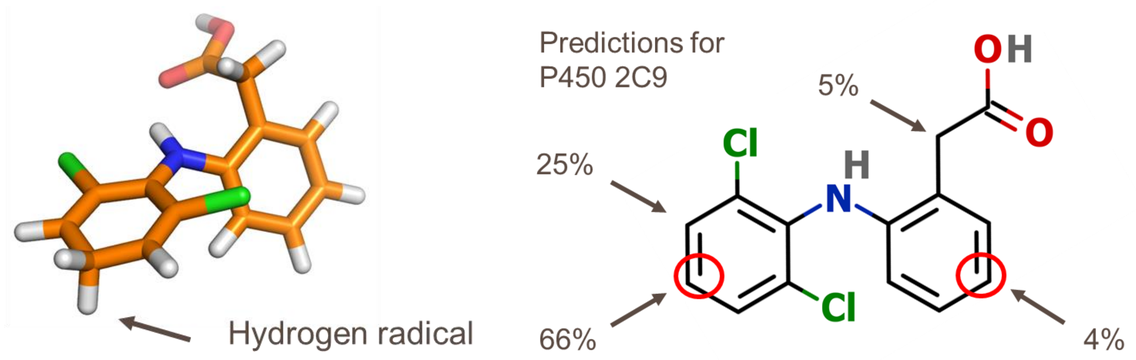

Figure 1. (left) The tetrahedral intermediate used for prediction of aromatic oxidation at the *para* position of diclofenac. (right) StarDrop site-of-metabolism prediction for diclofenac in percentages of lability. Experimentally-determined sites of metabolism are marked by red circles [2].

Modelling hydroxylation with DFT

In the present study, we have calculated activation energies from transition state geometries using DFT. The transition states are confirmed with frequency calculations. Calculations are performed with NWChem [3] at the B3LYP/6-31G* level of theory. By using DFT to evaluate activation energies for aromatic hydroxylation of diclofenac we can correctly predict both experimentally observed sites of metabolism.

SOM	Activation energy (kJ/mol)
C3'	42.9
C4'	38.8
C5'	43.0
C3	70.3
C4	44.0
C5	38.8
C6	49.5
Aliphatic	48.0

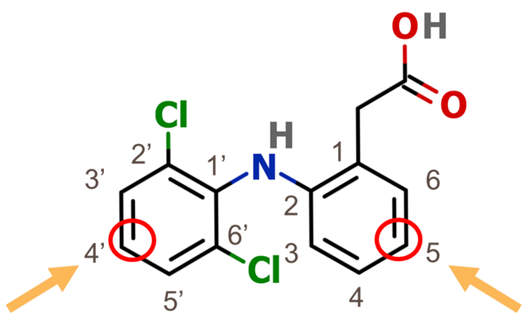

Figure 2. (left) Activation energies for hydroxylation of diclofenac calculated with DFT. (right) Diclofenac with experimentally-determined sites of metabolism marked by red circles and predicted sites of metabolism marked by arrows.

Predicting regioselectivity

DFT calculations can correctly predict aromatic regioselectivity. However, we must also understand the reactivity in the context of alternative, competing reactions performed by P450s. A common competing reaction is aliphatic hydroxylation. We have collected literature data on quantitative reaction rates for 6 cytochrome P450 3A4 substrates that undergo both aromatic and aliphatic hydroxylation. The differences in activation energies correlate well with the ratios of experimental reaction rates.

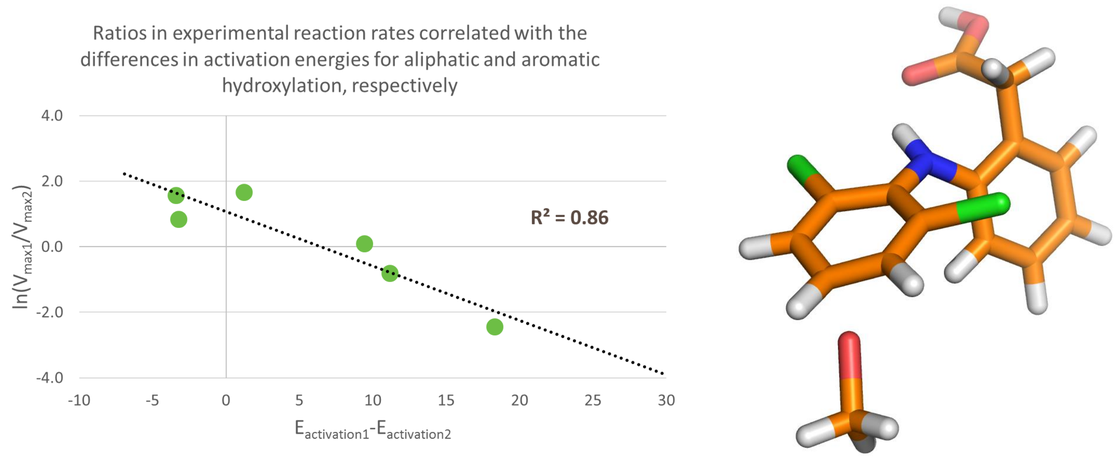

Figure 3. (left) Ratios in experimental reaction rates correlated with the differences in activation energies for aliphatic and aromatic hydroxylation, respectively. (right) The model system applied in our DFT calculations uses a methoxide radical as the reactive species.

Improving the current model

DFT calculations can take several days and are too slow to guide immediate decisions in drug design. We have therefore developed an improved semi-empirical AM1 protocol using transition state search and a methoxide model. The model provides results within minutes and produces activation energies that correlate well with DFT data.

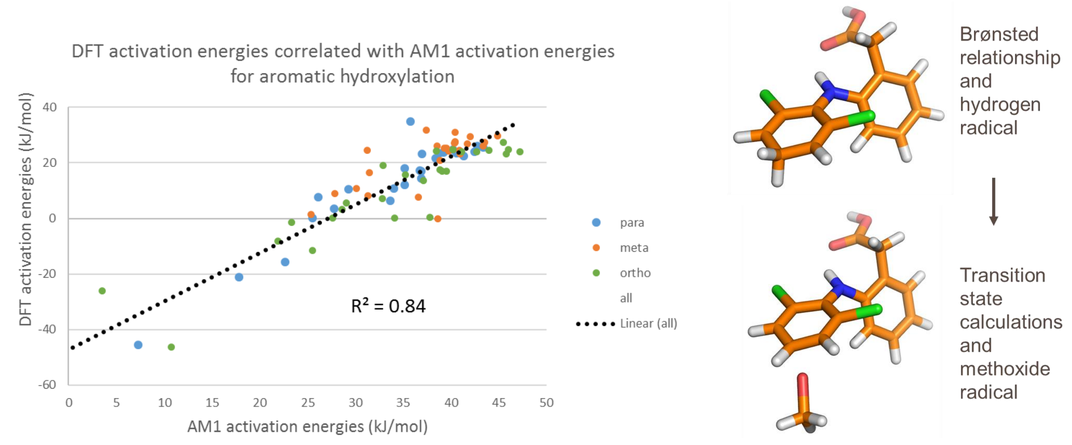

Figure 4. (left) Correlation between activation energies calculated with DFT and AM1. (right) The current and new model applied to aromatic hydroxylation.

Conclusions

We have developed an improved model for P450 mediated aromatic oxidation using AM1 and a methoxide radical. The model uses a single saddle point search to calculate the transition state energy. The model is very fast and produces energies for aromatic hydroxylation that correlate well with accurate DFT results and experimental observations.

SOM	Activation energy (kJ/mol)
C3'	39.0
C4'	36.0
C5'	39.5
C3	37.1
C4	40.7
C5	36.3
C6	45.8
Aliphatic	70.5

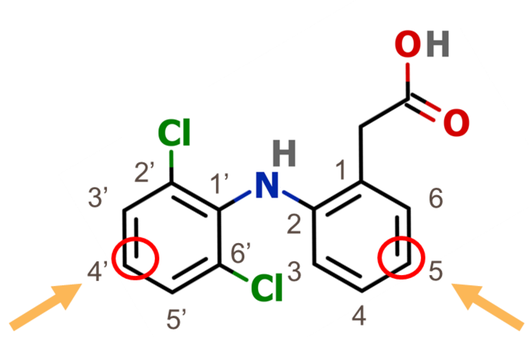

Figure 6. (left) Activation energies for hydroxylation of diclofenac calculated with AM1 using the MeO model. (right) Diclofenac with experimentally-determined sites of metabolism marked by red circles and predicted sites of metabolism marked by arrows.

References

[1] Tyzack *et al.*, Chem. Inf. Model. 2016, 56, 2180-2193
[2] Mancy *et al.*, Biochemistry 1999, 38, 14264-14270
[3] Valiev *et al.*, Comput. Phys. Commun. 2010, 181, 1477-1489

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