Improved quantum mechanical model of P450-mediated aromatic oxidation

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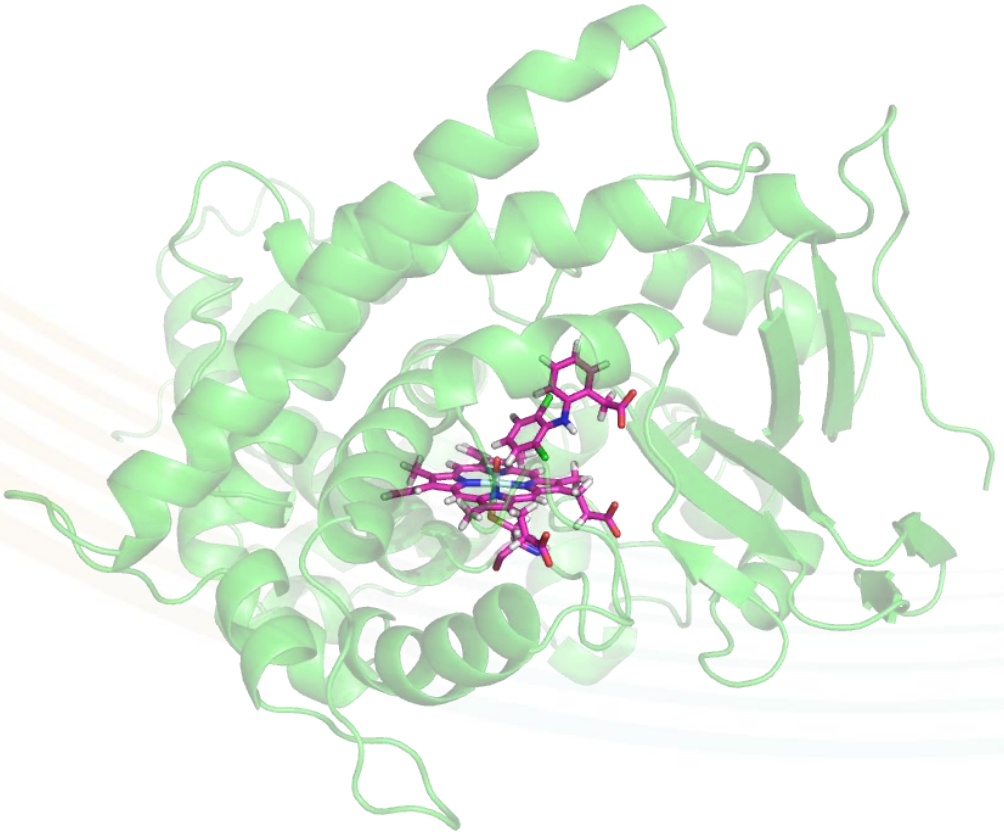
Overview

- Cytochrome P450
  - Why are these important in drug design?
- Modelling P450 metabolism with quantum mechanics
  - Austin Model 1 (AM1)
  - Density Functional Theory
- Improving the model for aromatic oxidation
  - Reducing model size
  - Optimizing protocol
- Conclusions

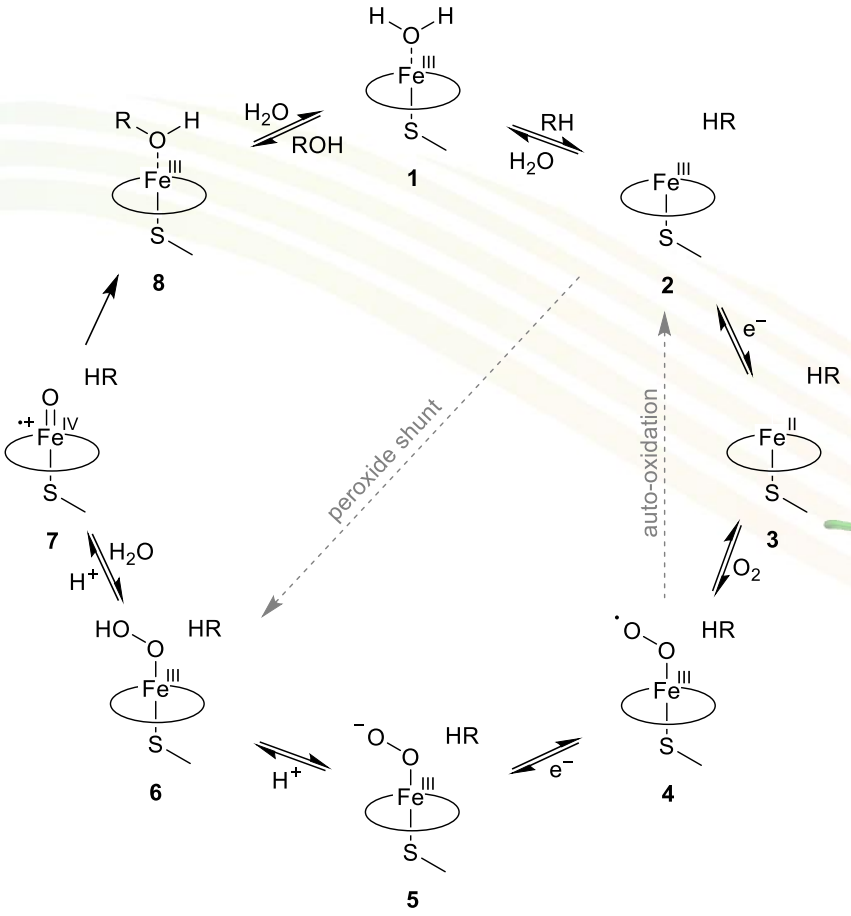
Cytochrome P450 enzymes

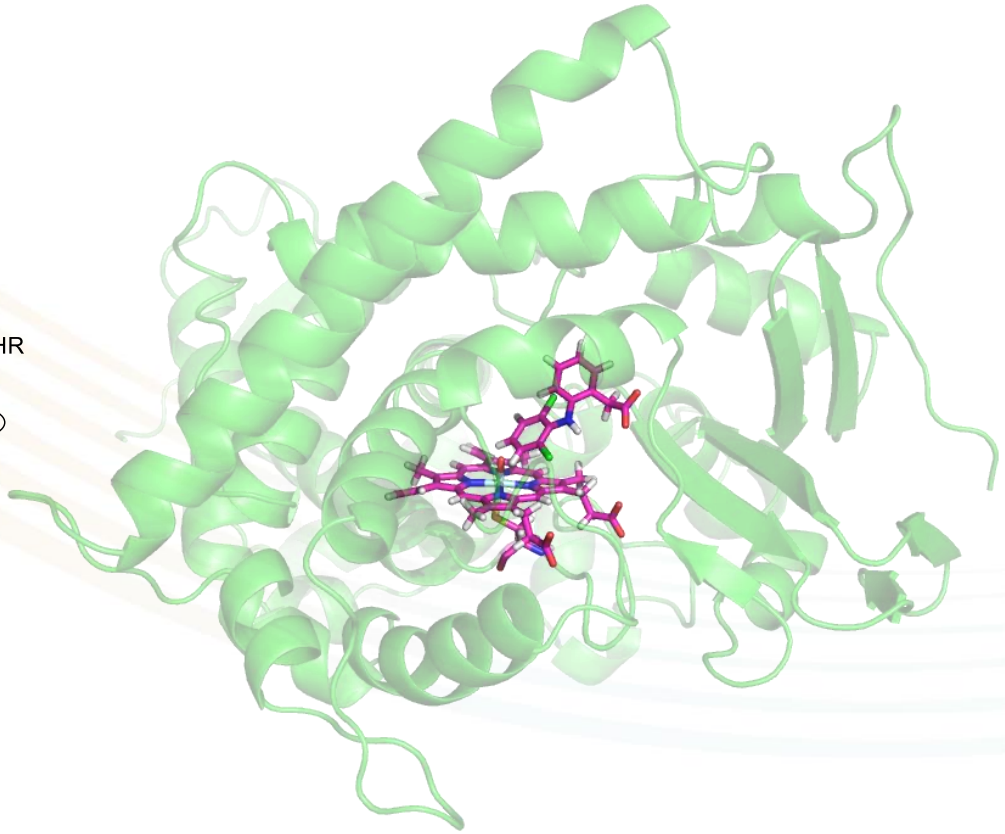
- Large family of enzymes responsible metabolism of drugs in humans
- Important in drug design
  - Drug-drug interactions
  - Rapid clearance
  - Genetic polymorphism
- Formation of reactive metabolites
  - Adverse effects
  - Failure in drug development



Compound I and substrate inside cytochrome P450 enzyme.

Cytochrome P450 enzymes

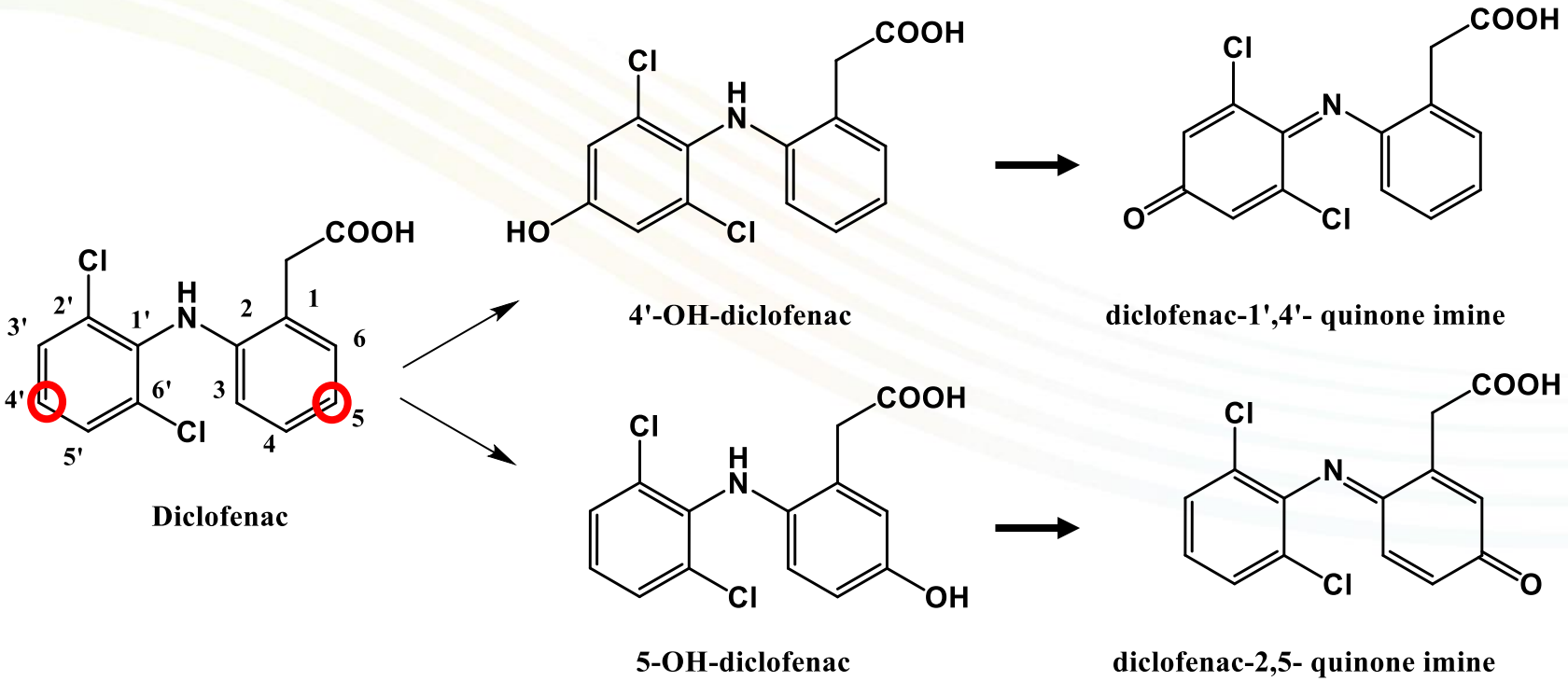




Compound I and substrate inside cytochrome P450 enzyme.

Cytochrome P450 metabolism of diclofenac

The P450 mediated metabolism of diclofenac can lead to the formation of reactive metabolites associated with hepatotoxicity



Mancy *et al.*, Biochemistry 1999, 38, 14264-14270

Modelling P450 metabolism

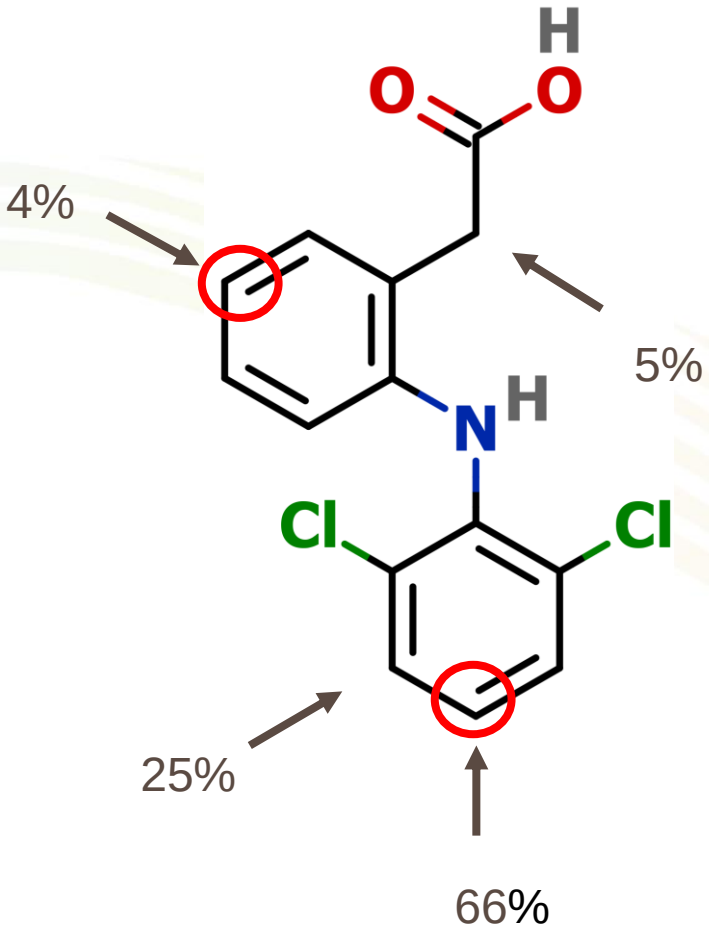
Two primary factors determine the site of metabolism:

- Chemical reactivity of heme and substrate
  - Many different reactions performed by P450
  - Aromatic- and aliphatic oxidation, epoxidation, dealkylation, etc.
  - Activation barrier of the given reaction
  - Independent of isoform
- Binding of substrate in active site
  - Dependent on isoform and substrate
  - Electrostatic and van der Waals forces between protein and substrate
  - Steric accessibility and orientation

Quantum mechanical modelling

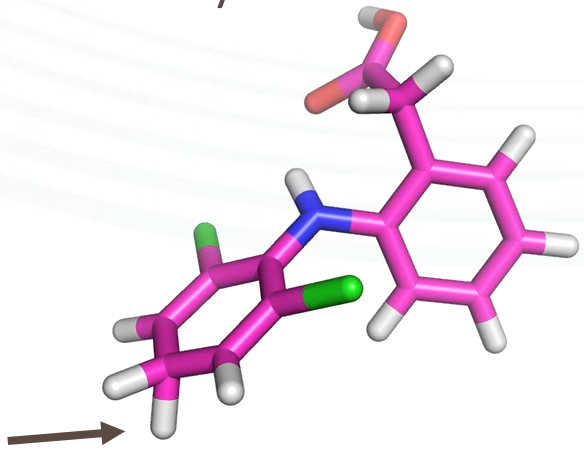
Austin Model 1 (AM1)	Density Functional Theory (DFT)
Neglect of Differential Diatomic Overlap	Energy is derived from the electron density
Uses fitted parameters	Usually includes an *ab initio* component
Low level of theory	High level of theory
Computationally fast	Computationally slow

StarDrop P450 module



Predictions
for P450 2C9

- Estimate activation energies with AM1
  - Brønsted relationship
  - Energies corrected for steric and orientation effects
- A site of metabolism correctly predicted in top2 for 82-91% across 7 isoforms*
- Aromatic regioselectivity could be improved

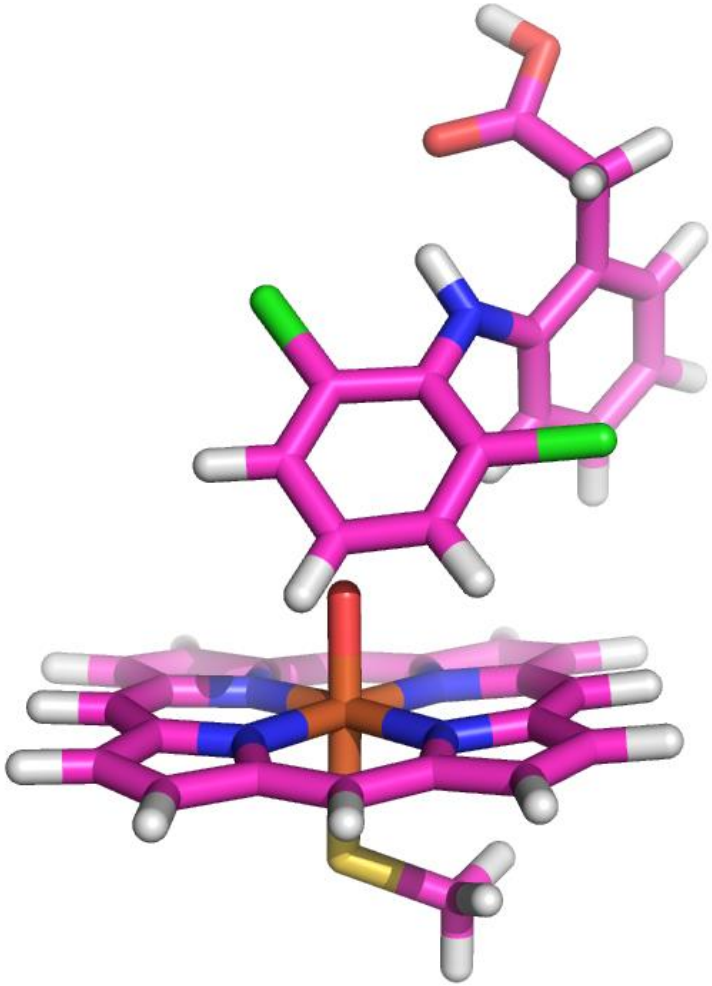


Hydrogen radical

*Tyzack *et al.*, Chem. Inf. Model 2016, 56, 2180-2193

Modelling hydroxylation with DFT

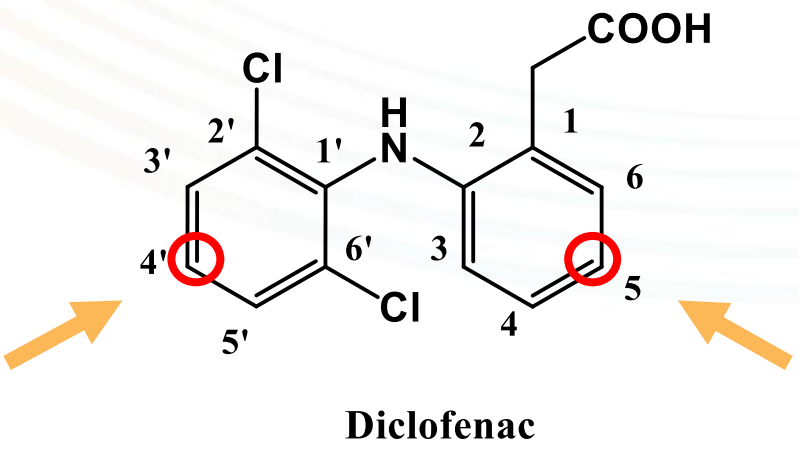
- Activation energies are determined from transition state geometries and confirmed with frequency calculations
- A truncated heme model is used to represent the enzyme
- Calculations performed with NWChem using B3LYP/6-31G*



DFT and Diclofenac

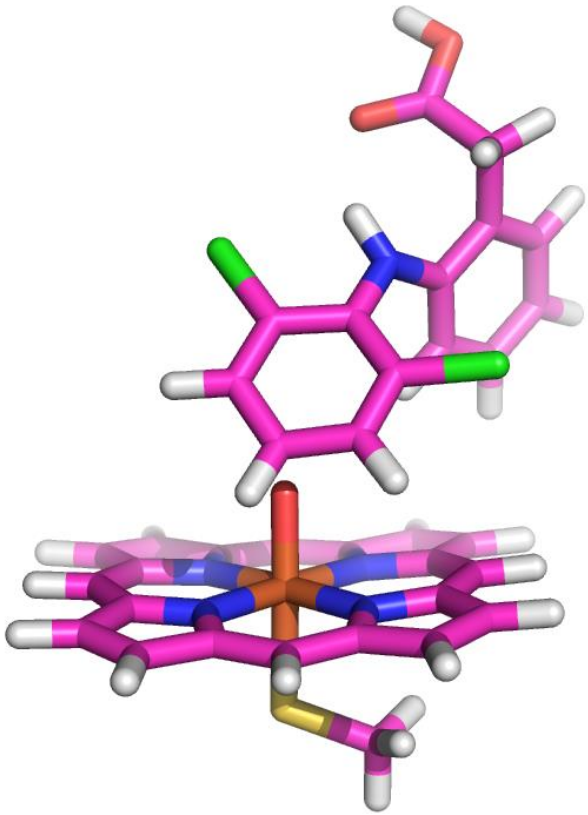
- DFT correctly predicts the aromatic regioselectivity
- The DFT model does not correct for steric and orientation effects

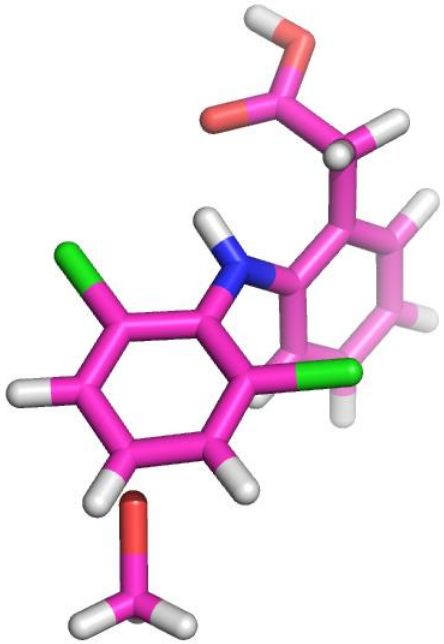
SOM	Activation energy (kJ/mol)
C3'	42.9
C4'	38.8
C5'	43.0
C3	70.3
C4	44.0
C5	38.8
C6	49.5
Aliphatic	48.0



Diclofenac

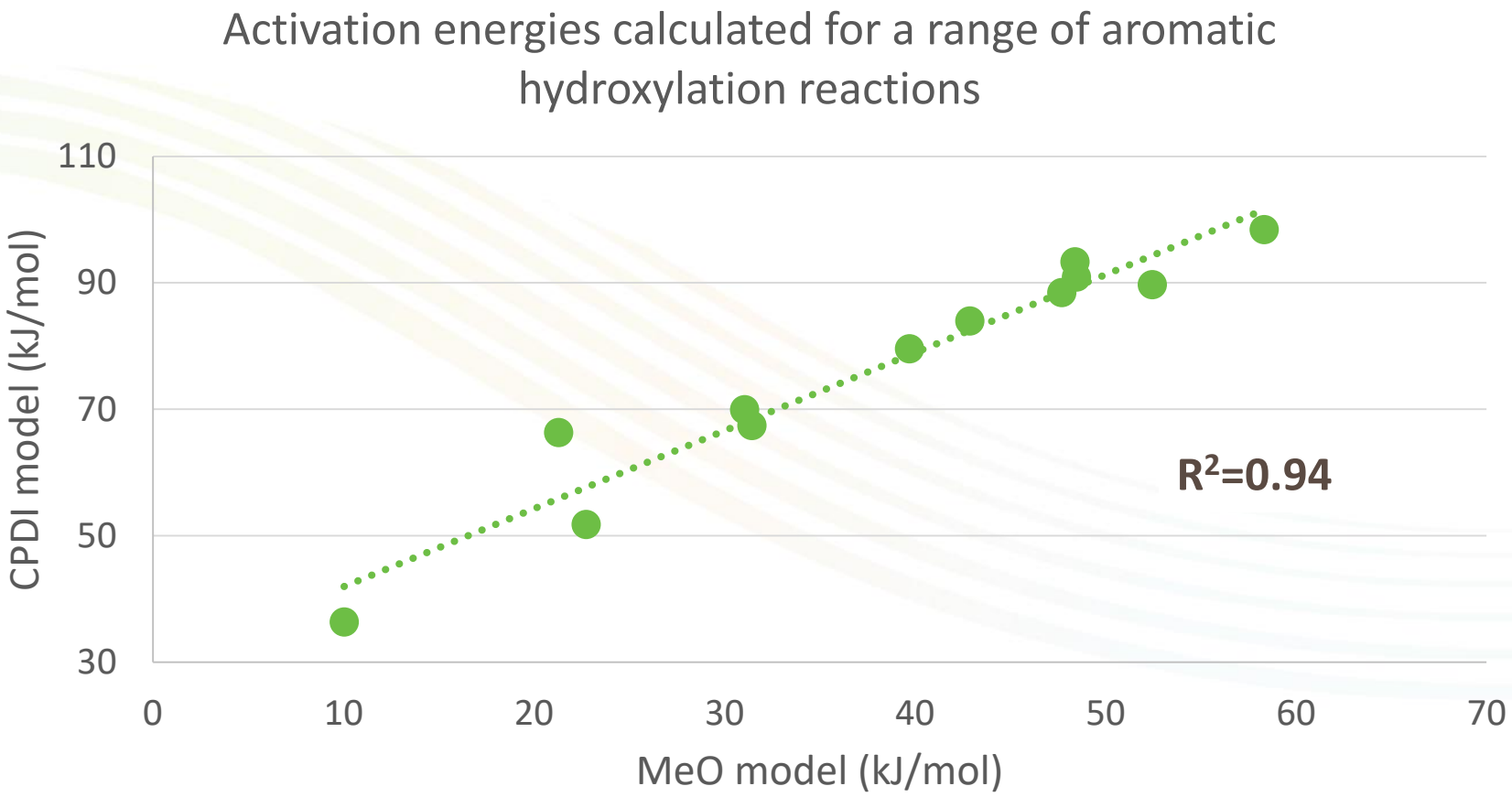
Reducing model size and computation time





Heme Compound I (CPDI) model → Methoxide (MeO) model

Correlating data from the CPDI and MeO model



Experimental data

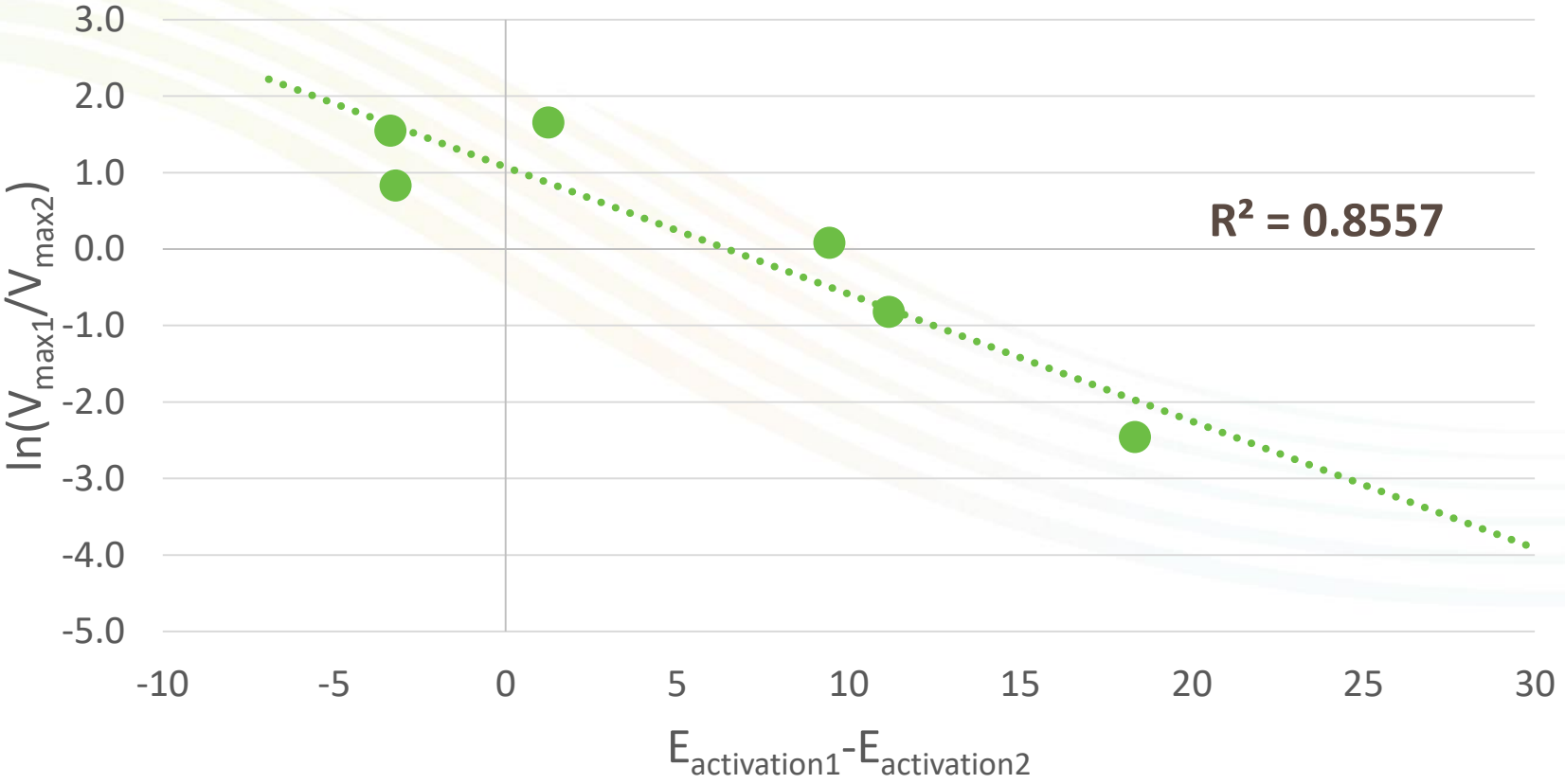
The DFT model correctly predicts the aromatic regioselectivity

Can the DFT model predict the regioselectivity between aliphatic and aromatic hydroxylation for 3A4 substrates where steric and orientation effects are minimized?

- Collected literature data for 6 Cytochrome P450 3A4 substrates
- Compounds with both aromatic and aliphatic metabolites
- Kinetic data for reaction rates also available

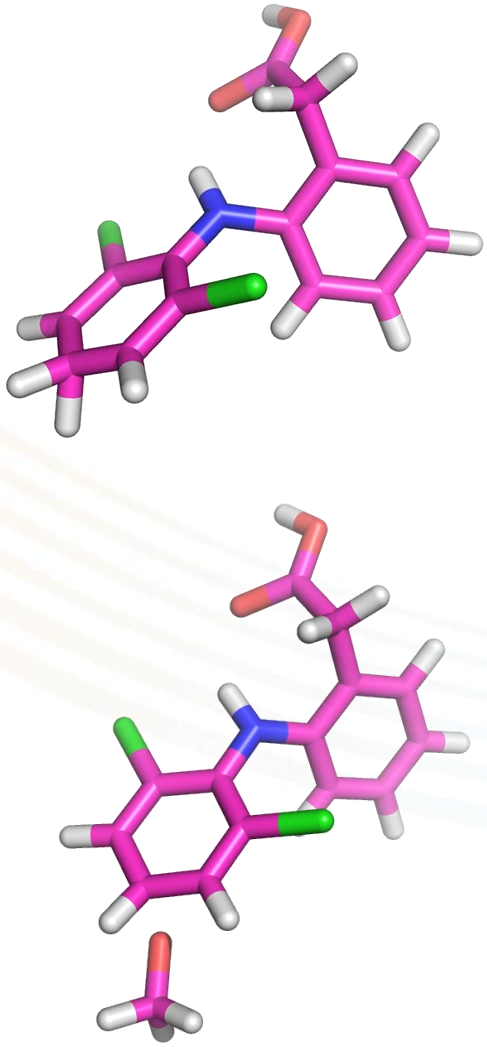
Results

Ratios in experimental reaction rates correlated with the differences in activation energies for aliphatic and aromatic hydroxylation, respectively



AM1 and regioselectivity

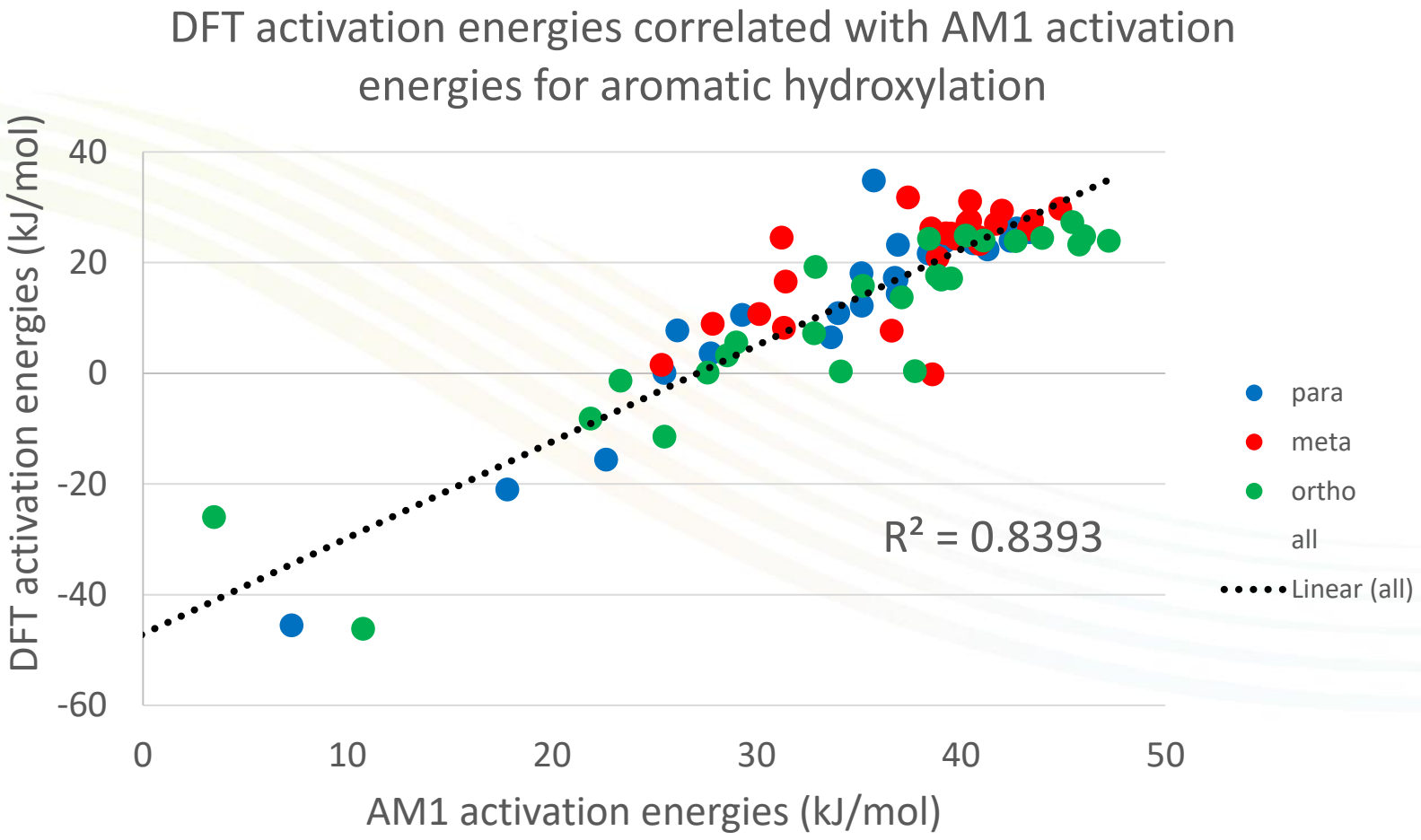
- DFT methoxide model is still too slow
- Can we improve the current AM1 model to provide fast results comparable to DFT?
- We have developed an improved AM1 protocol using transition state search and a methoxide model



Brønsted relationship and hydrogen radical

Transition state calculations and methoxide radical

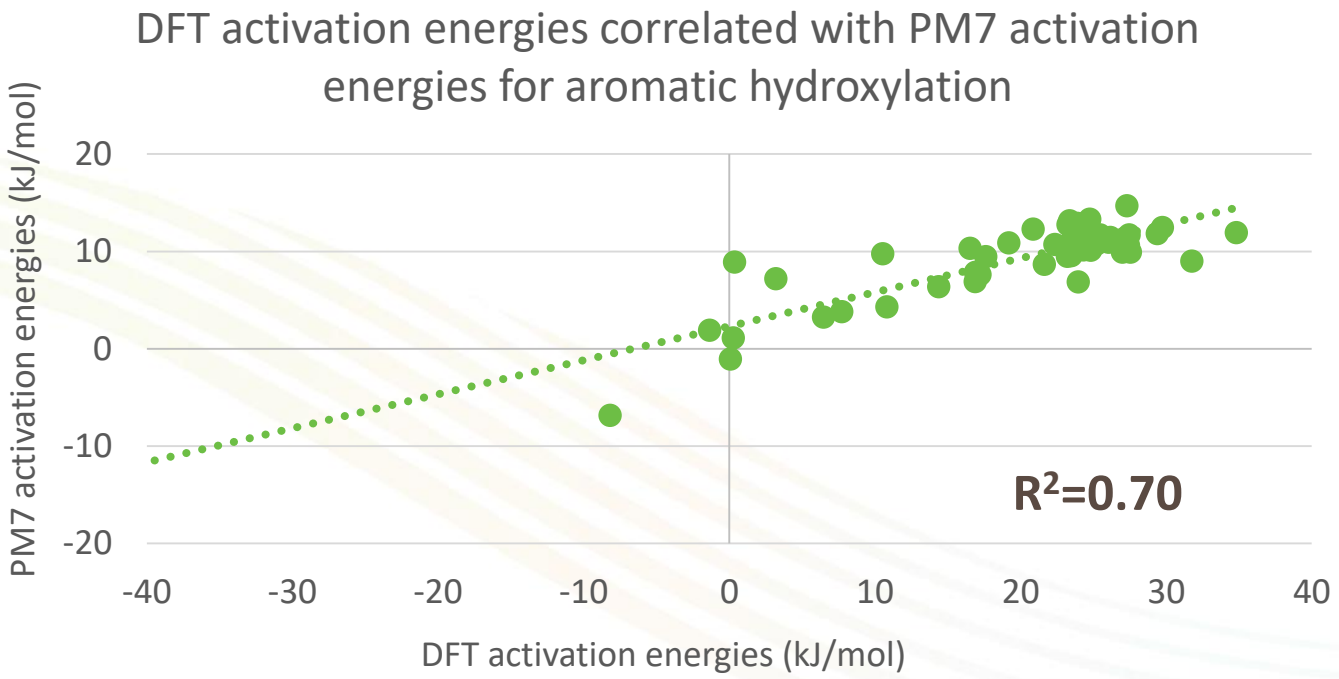
Correlating AM1 and DFT activation energies



Transition state calculations and AM1

- AM1 regioselectivity results comparable to DFT
- Inconsistencies in zero-point vibration energies
- Large variations in reactant complex geometries
  - Use separated species
- Alternative semi-empirical methods are available (RM1, PM3, PM6, PM7, …) are there better alternatives?

Alternative semi-empirical methods



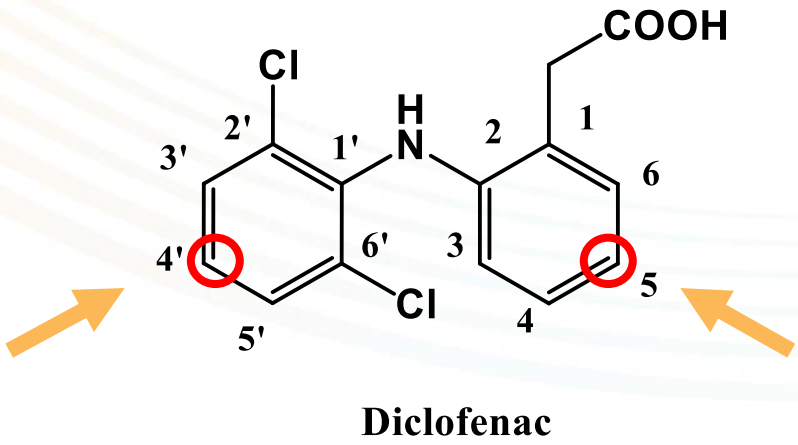
- Newer methods do not correlate better with DFT for aromatic hydroxylation
- Less robust when handling charged substitutions

Diclofenac and the AM1 MeO model

The top sites of metabolism are predicted correctly

The aromatic regioselectivity is correct

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



Diclofenac

Conclusion and Perspectives

- We have developed a 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 correlates well with DFT data
- Larger scale tests are underway to expand the applicability domain

Acknowledgement

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HeCaToS

Hepatic and Cardio Toxicity Systems modelling

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