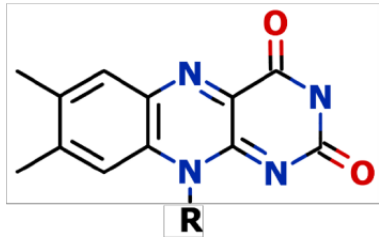
N- and *S*-Oxidation Model of the Flavin-containing MonoOxygenases

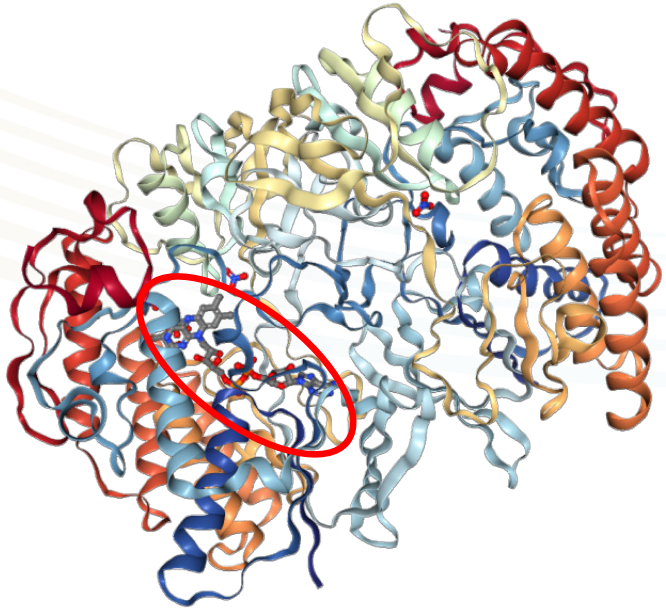
Peter J. Walton, Mario Öeren, Peter Hunt, Matthew Segall

Background

- **F**lavin-containing **M**ono**O**xygenase (FMO)
- 5 active isoforms (FMO1–5)
  - FMO3 major isoform found in adult liver
- Enzyme class found in multiple tissues
- Phase I enzyme involved in compound metabolism
- Metabolites commonly subject to Phase II metabolism



Flavin Moiety



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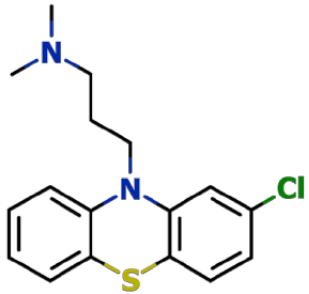
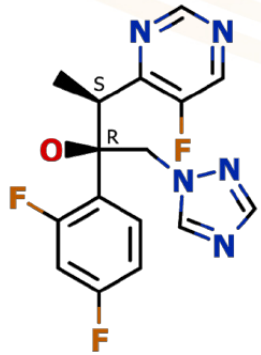
Role of Flavin-containing Monooxygenases

- Works in conjunction with Cytochrome P450 in Phase I metabolism
- FMO3 polymorphism responsible for trimethylaminuria
  - Decreased rate of metabolism leads to increased drug exposure
- Variety of reaction types possible:
  - ***N/S*-Oxidation ≈ 99% cases**
  - Demethylation, Bayer-Villiger Oxidation, Desulfuration

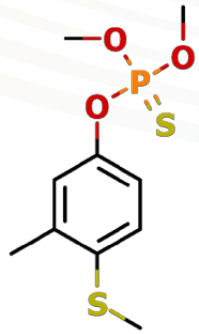
Trimethylamine



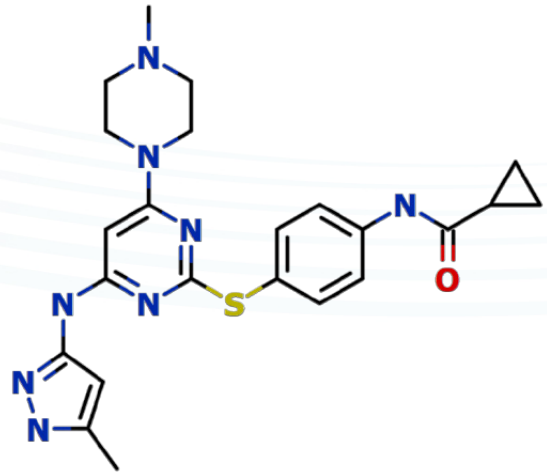
Chlorpromazine



Voriconazole



Fenthion



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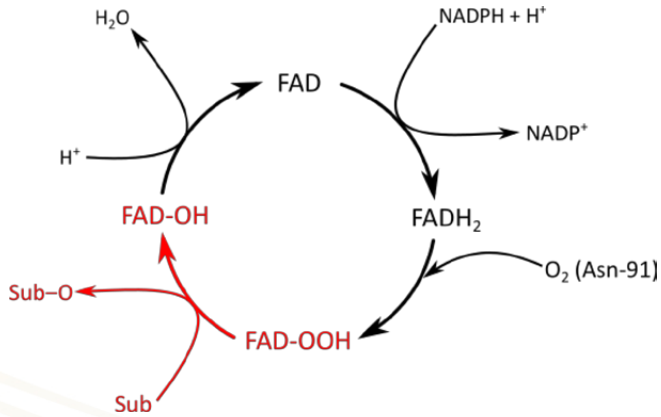
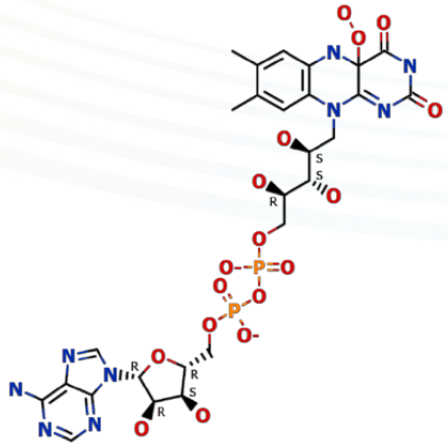
N/S–Oxidation

- *N/S*-Oxidation involves transfer of Oxygen from peroxide of FAD–OOH
- Debate in the literature regarding mechanism:
  - Radical
  - S_N2
- Our focus:
  - Better understanding of the mechanism substrate oxidation by FAD peroxide.



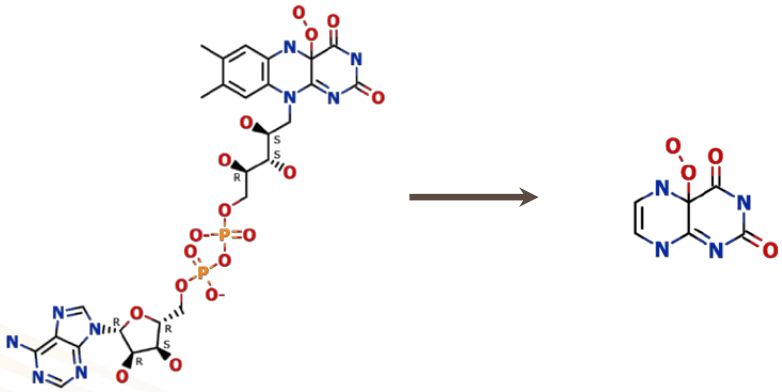


Model System

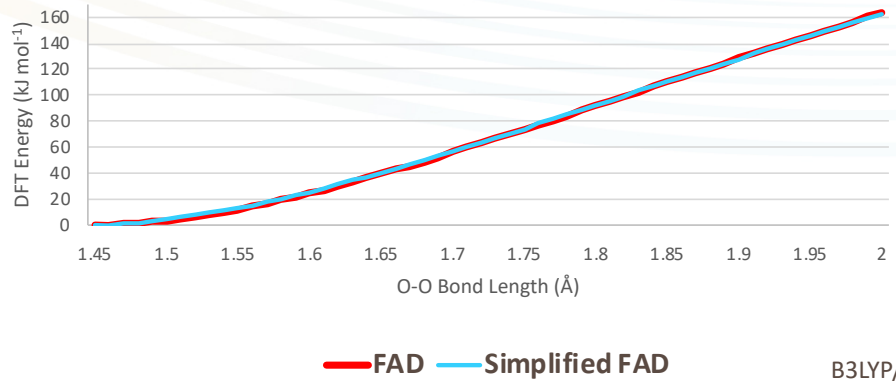
optibrium

Simplifying the System

- Full FAD too large for practical calculations
- “Tail” buried in groove and does not interact with substrate
- Compared bond stretching of the full FAD–OOH vs the simplified analogue
- Behaviour of simplified system at peroxide site analogous to larger molecule



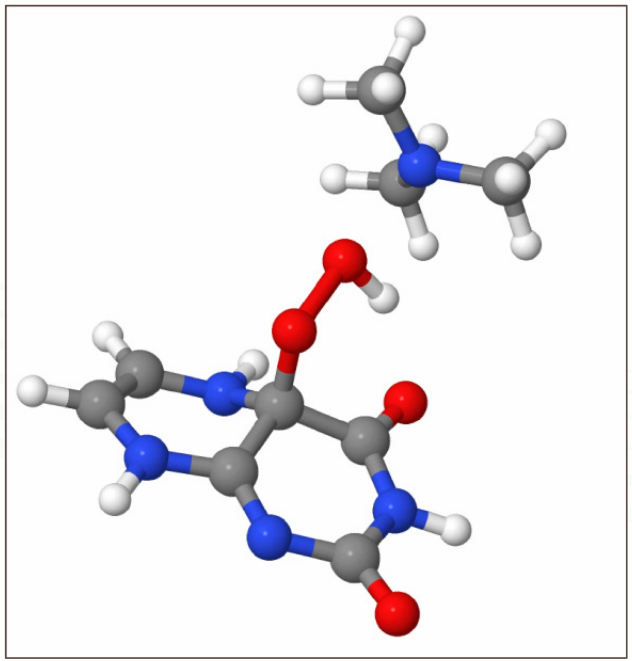
DFT Energy vs $r_{O\text{-}O}$ for FAD and Simplified FAD



B3LYP/def2-SVP

Finding the Transition State

- We searched for both S_N2 and radical transition state
- Despite multiple attempts we were unable to find a transition state for a radical mechanism
- Transition state found for closed-shell system, S_N2 mechanism



B3LYP/def2-SVP

Analysis of the S_N2 Mechanism

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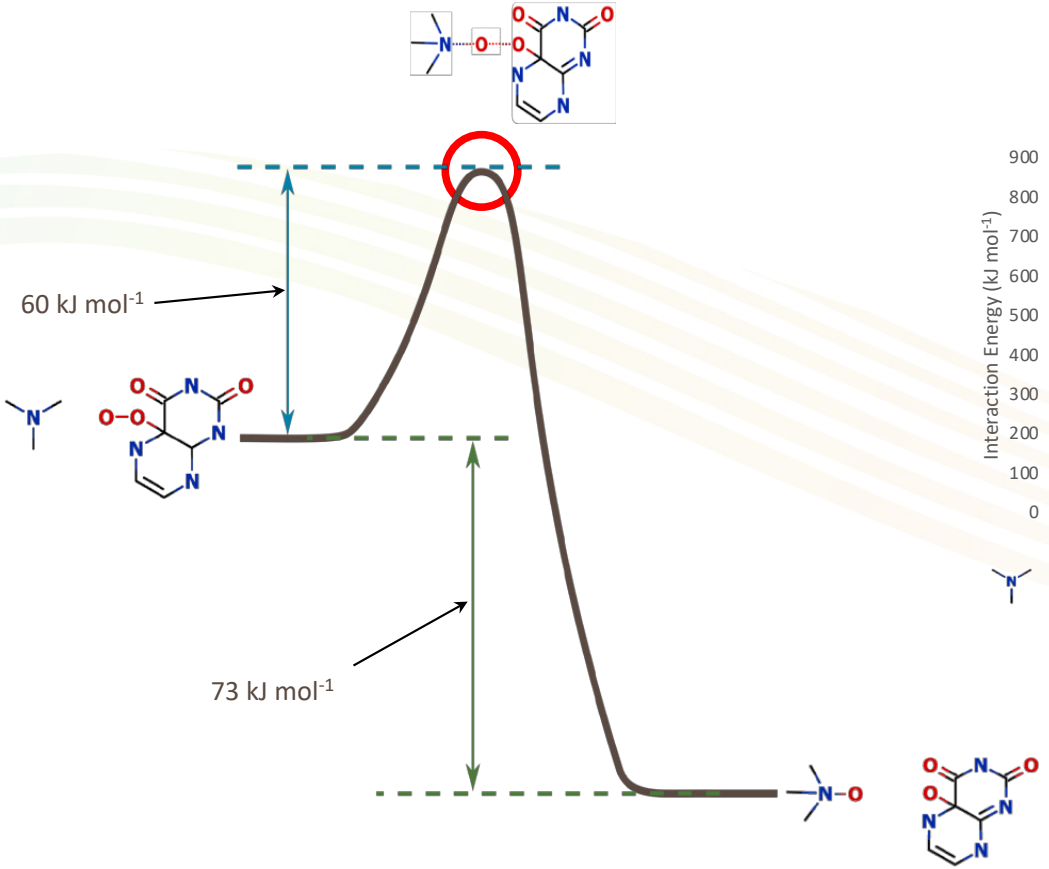
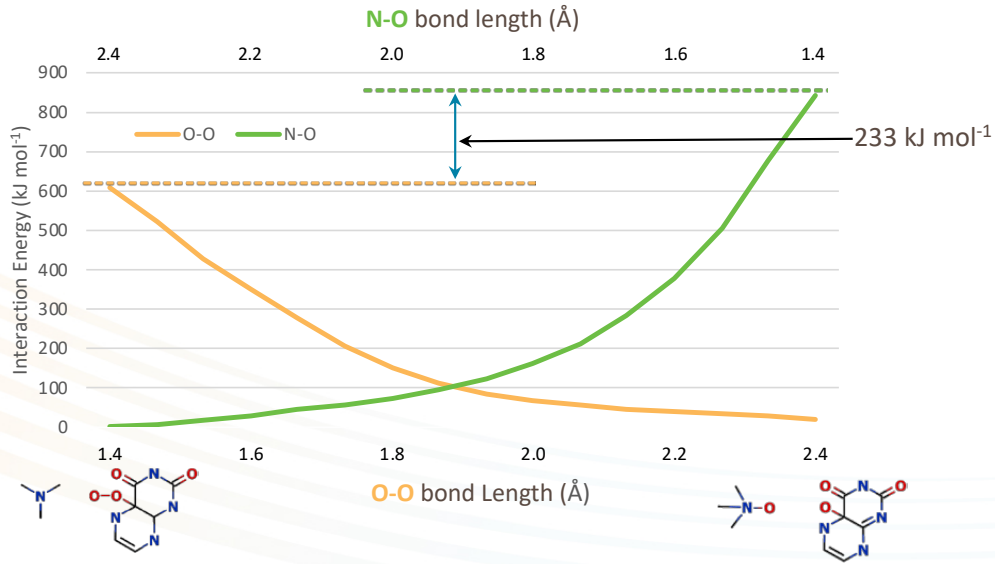
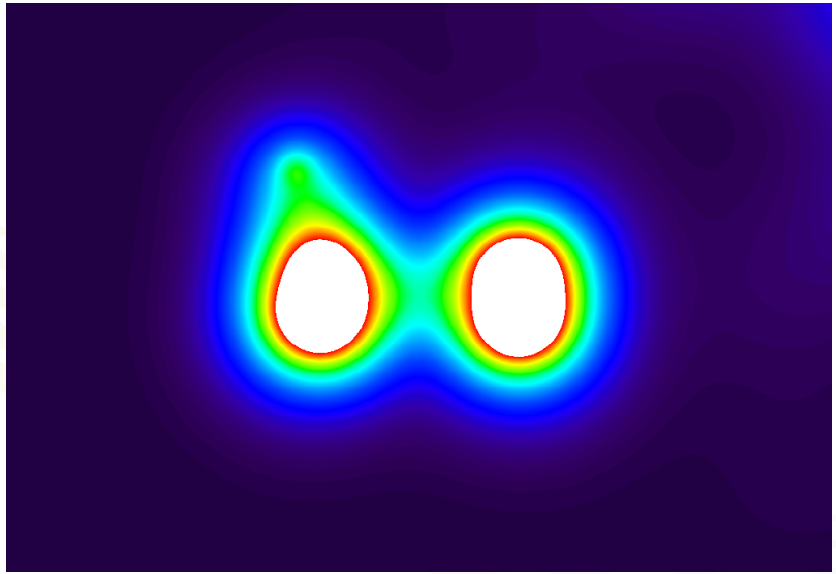
Thermodynamic Feasibility

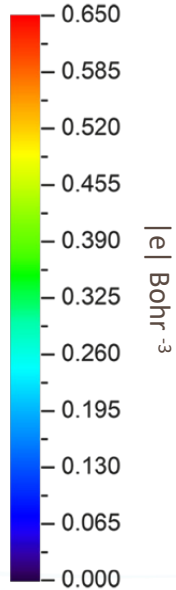


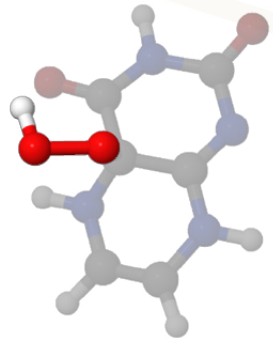


B3LYP/def2-SVP QTAIM

Electron Density – Reactant







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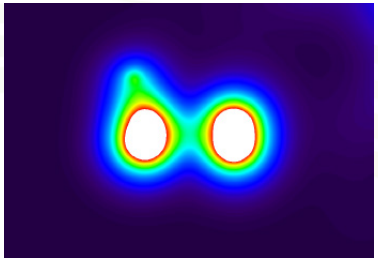
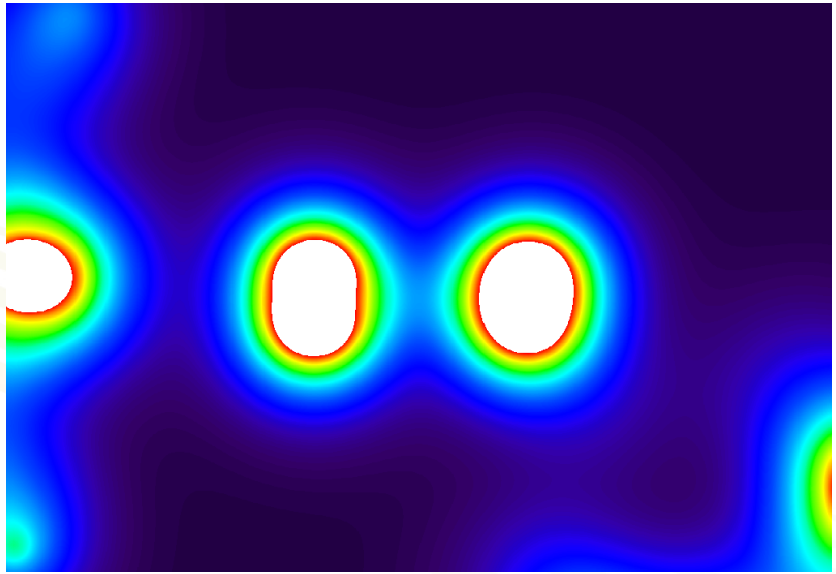
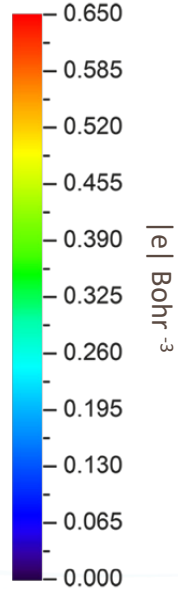
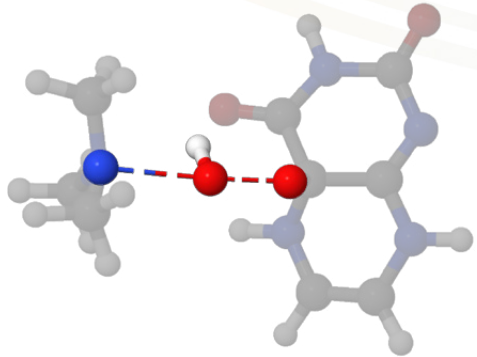
Electron Density – Pre-transition State









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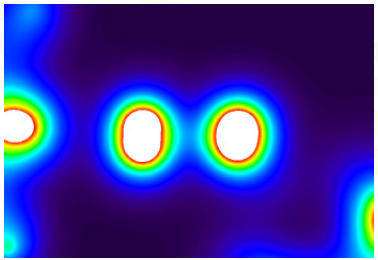
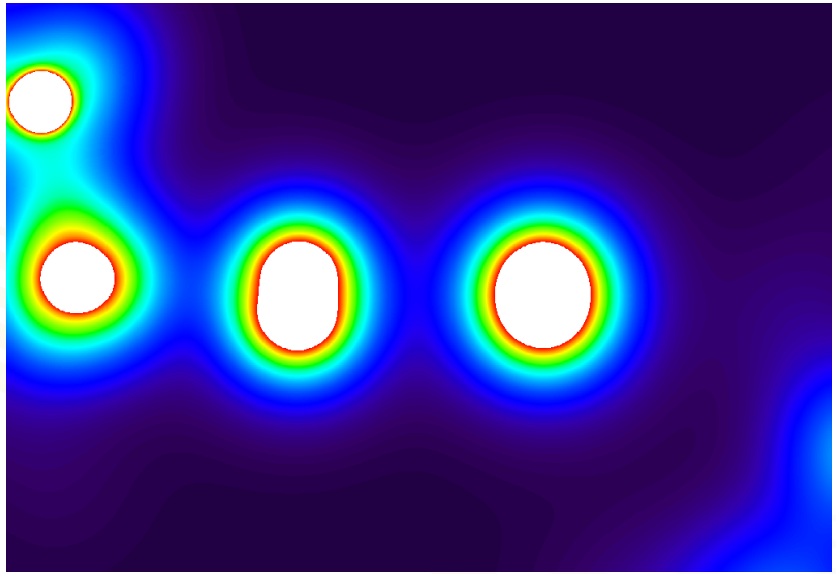
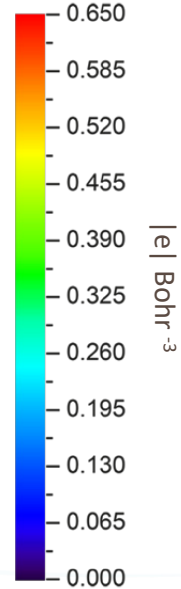
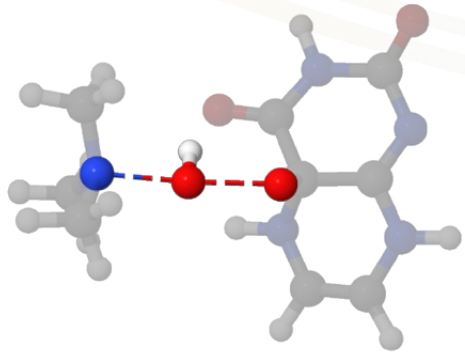
Electron Density – Transition State



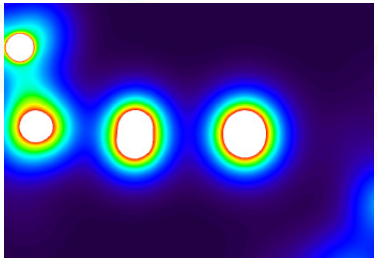


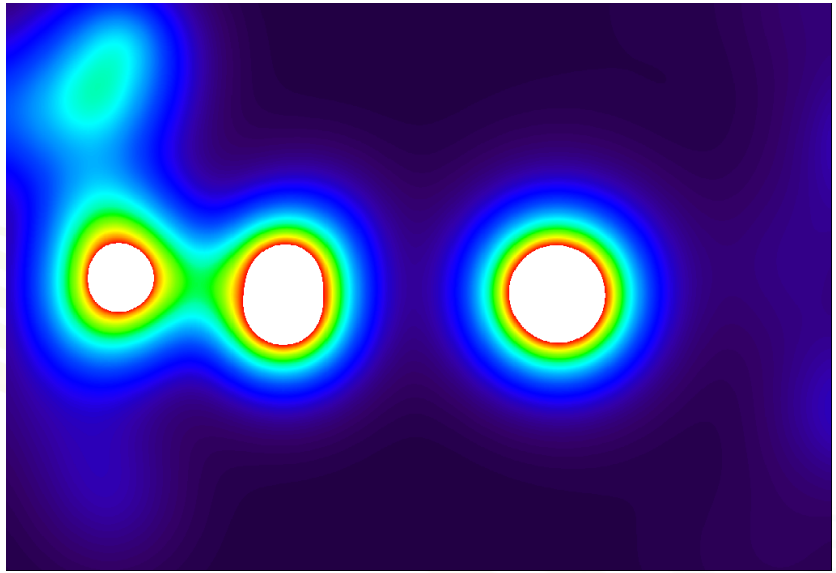


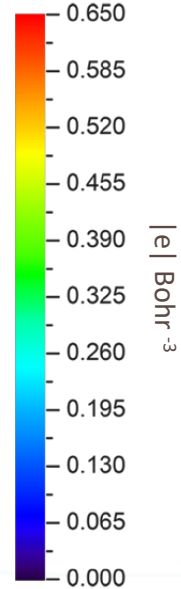


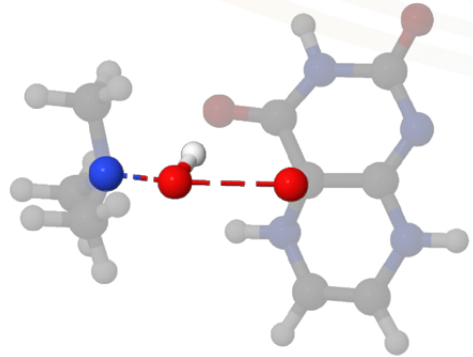
B3LYP/def2-SVP

Electron Density – Post-transition State





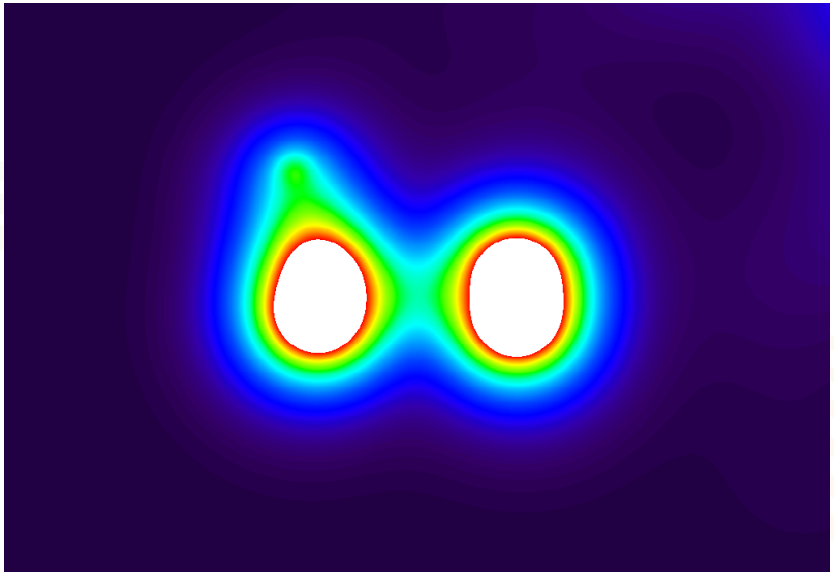


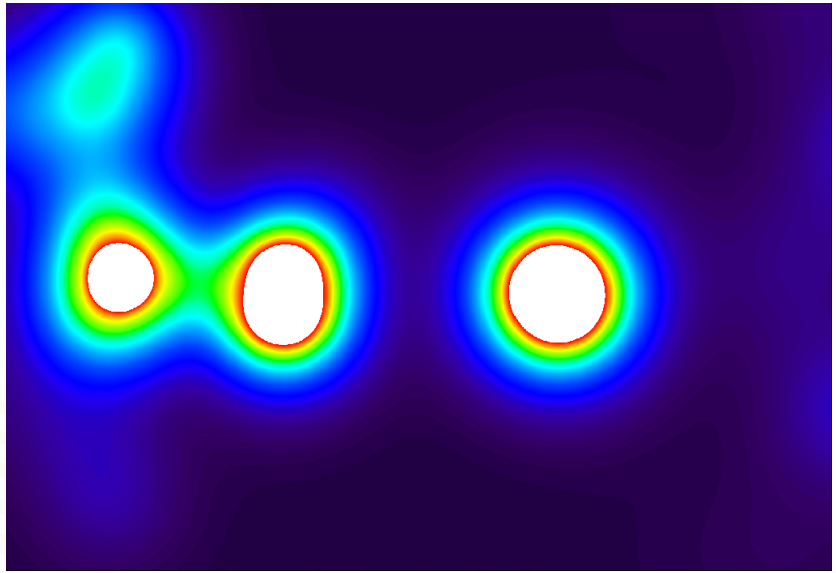


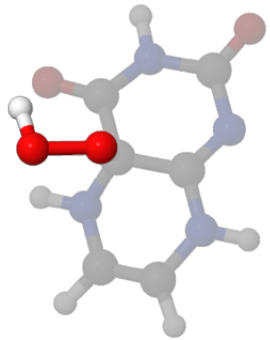
B3LYP/def2-SVP

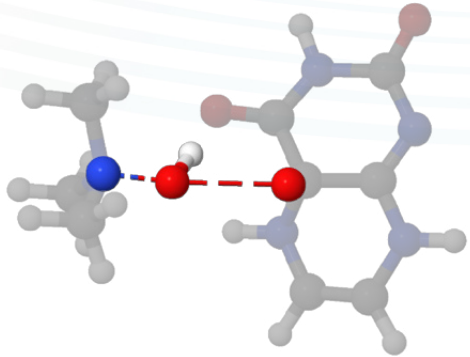
Electron Density

Comparison of reactant and post-transition state structures







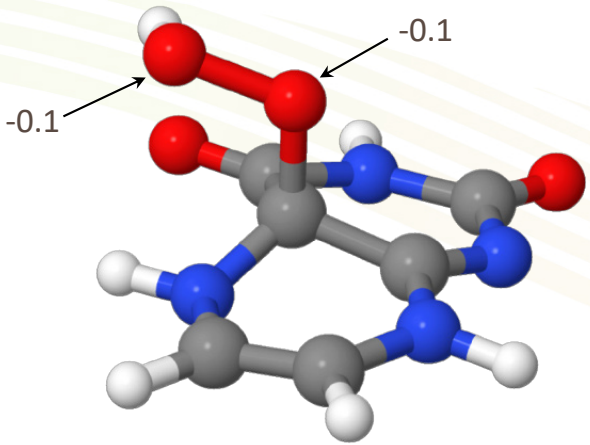


B3LYP/def2-SVP

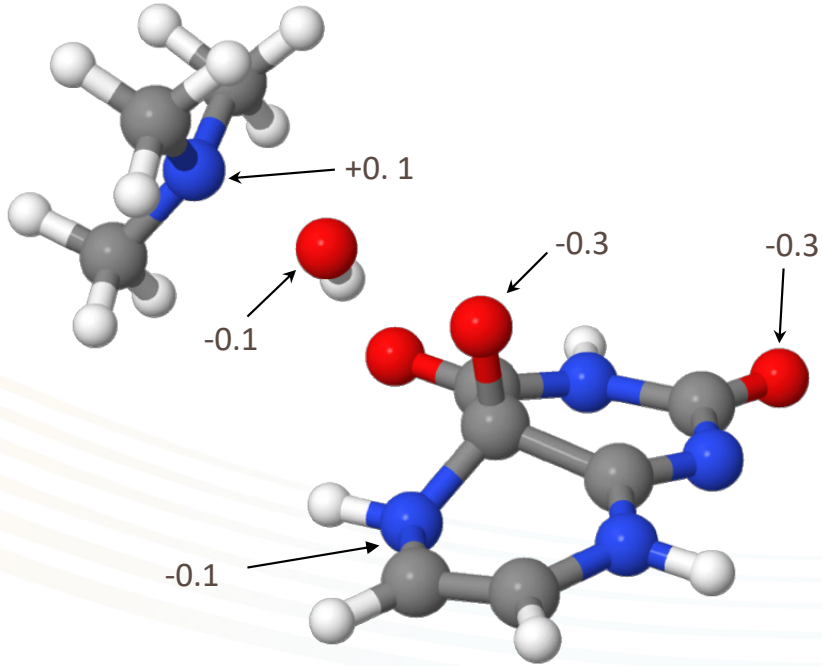
Properties Derived from Electron Density

optibrium

Charge Analysis



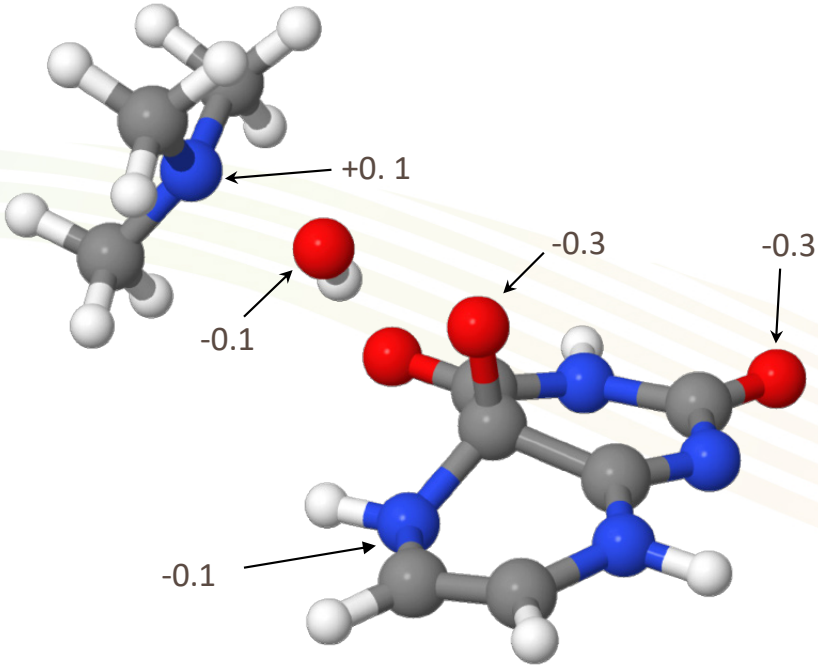
Isolated FAD−OOH



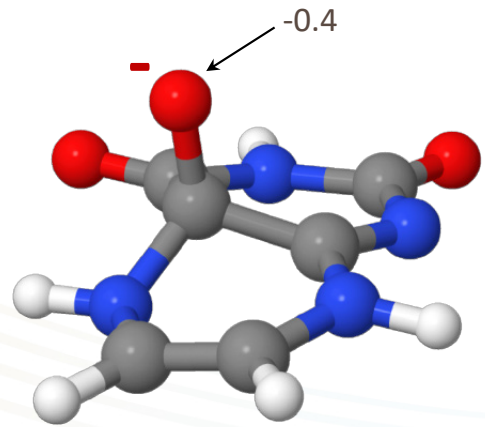
Transition State

Hirshfeld Charge

Charge Analysis



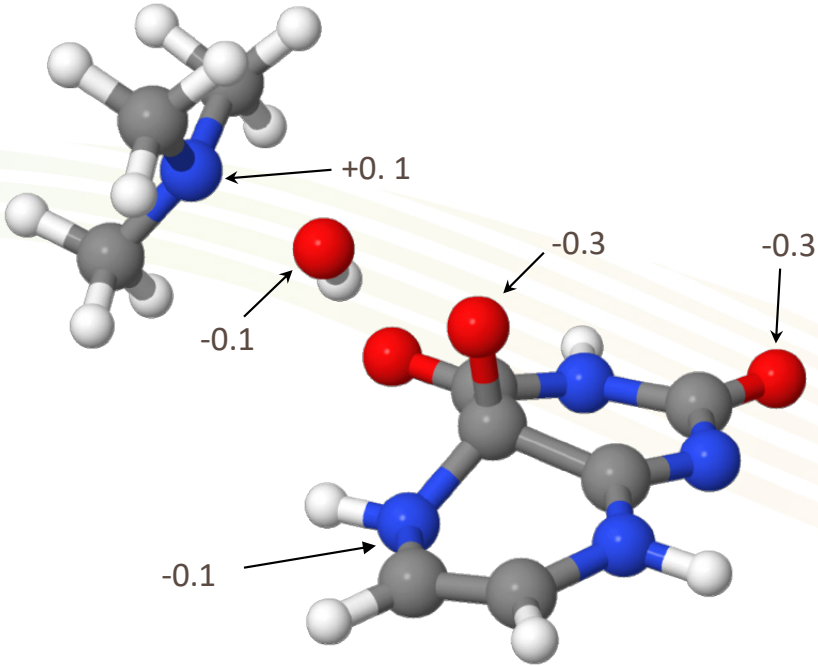
Transition State



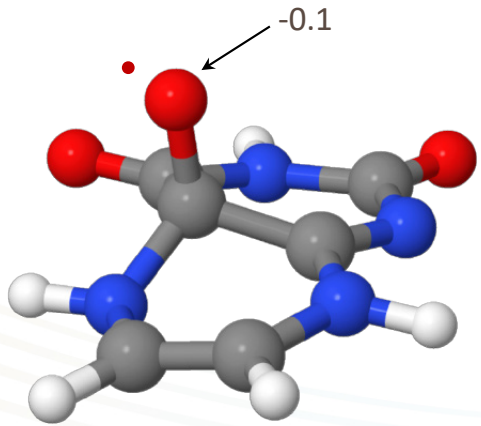
FAD–O⁻ Anion

Hirshfeld Charge

Charge Analysis



Transition State



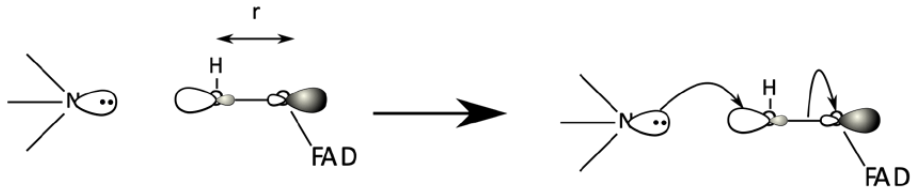
FAD–O• Radical

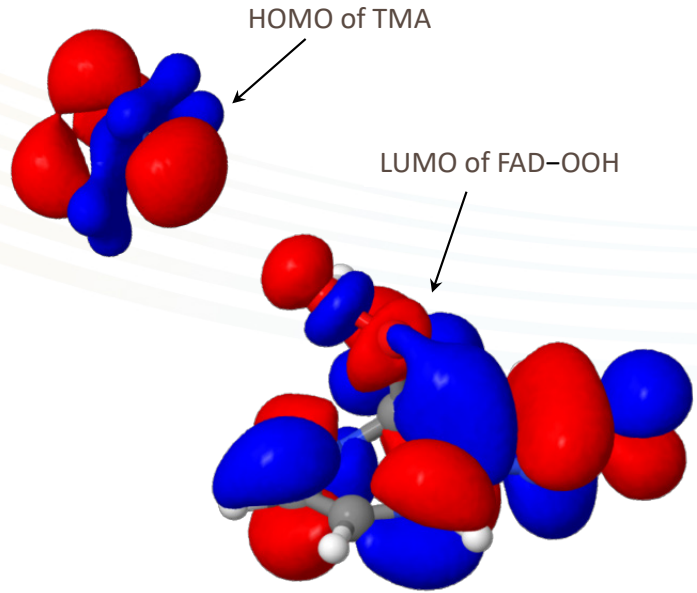
Charges seen in transition state more consistent with S_N2 mechanism

Hirshfeld Charge

Orbital Investigation

- Orbital shape and orientation conducive to successful interaction
  - Lone pair of trimethylamine (TMA) large component of HOMO
  - O–O σ* component of the LUMO of FAD–OOH accessible





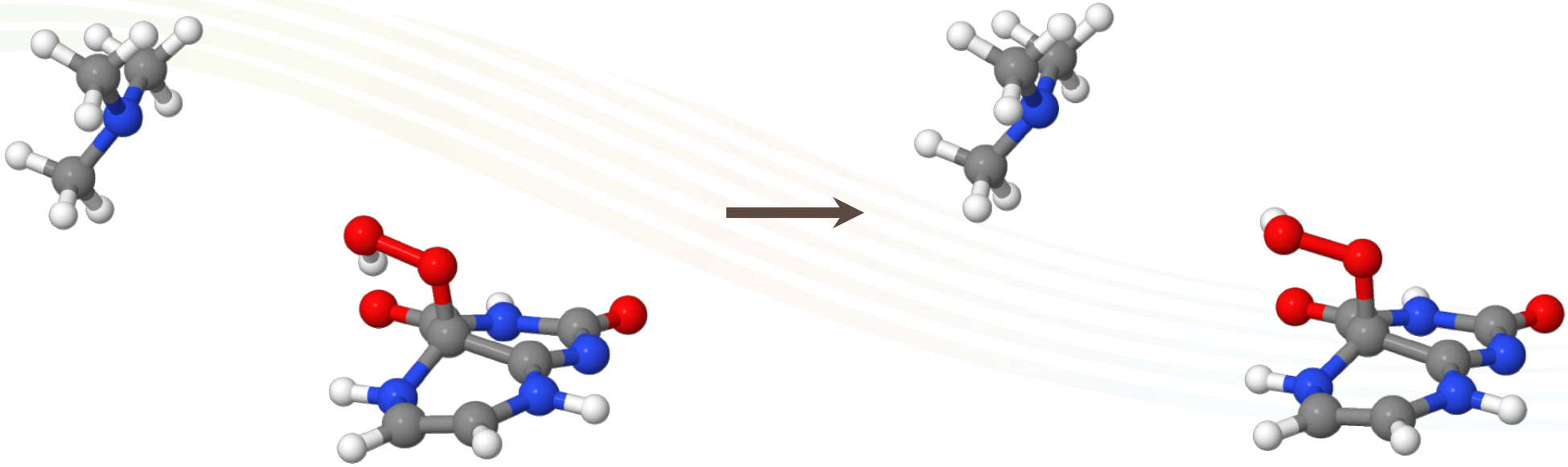
B3LYP/def2-SVP

Full S_N2 Mechanism

optibrium

Constrained Optimisation

Fixed N–O bond length = 5.00 Å



Pre-optimised Structure

Post-optimised Structure

B3LYP/def2-SVP

Constrained Optimisation

Fixed N–O bond length = 1.85 Å

Pre-optimised Structure

Post-optimised Structure

B3LYP/def2-SVP

Constrained Optimisation

Fixed N–O bond length = 1.70 Å

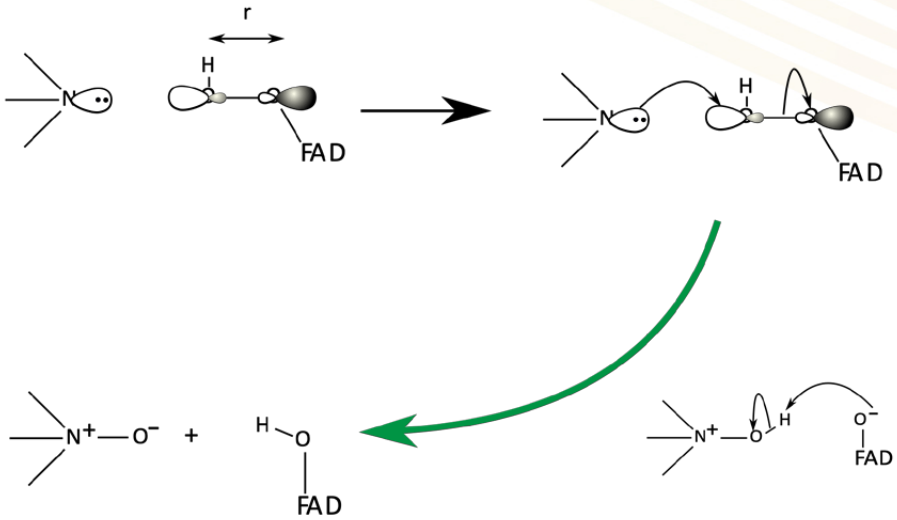
Pre-optimised Structure

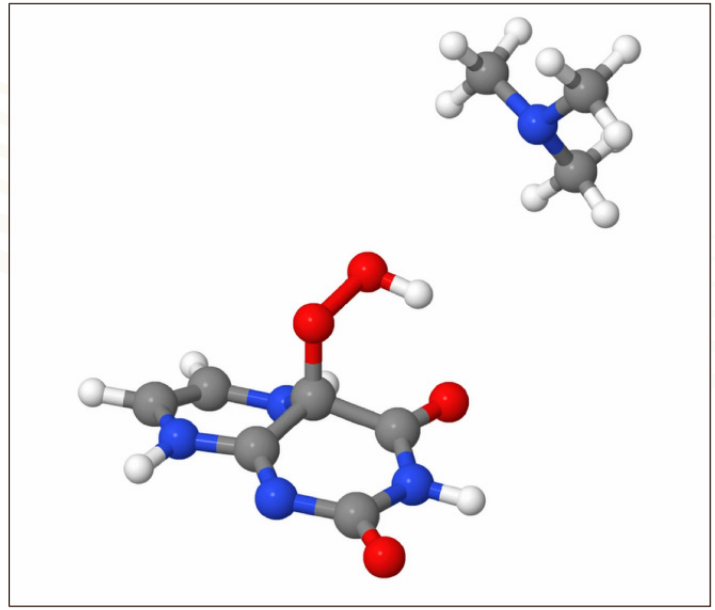
Post-optimised Structure

B3LYP/def2-SVP

Full Concerted Mechanism

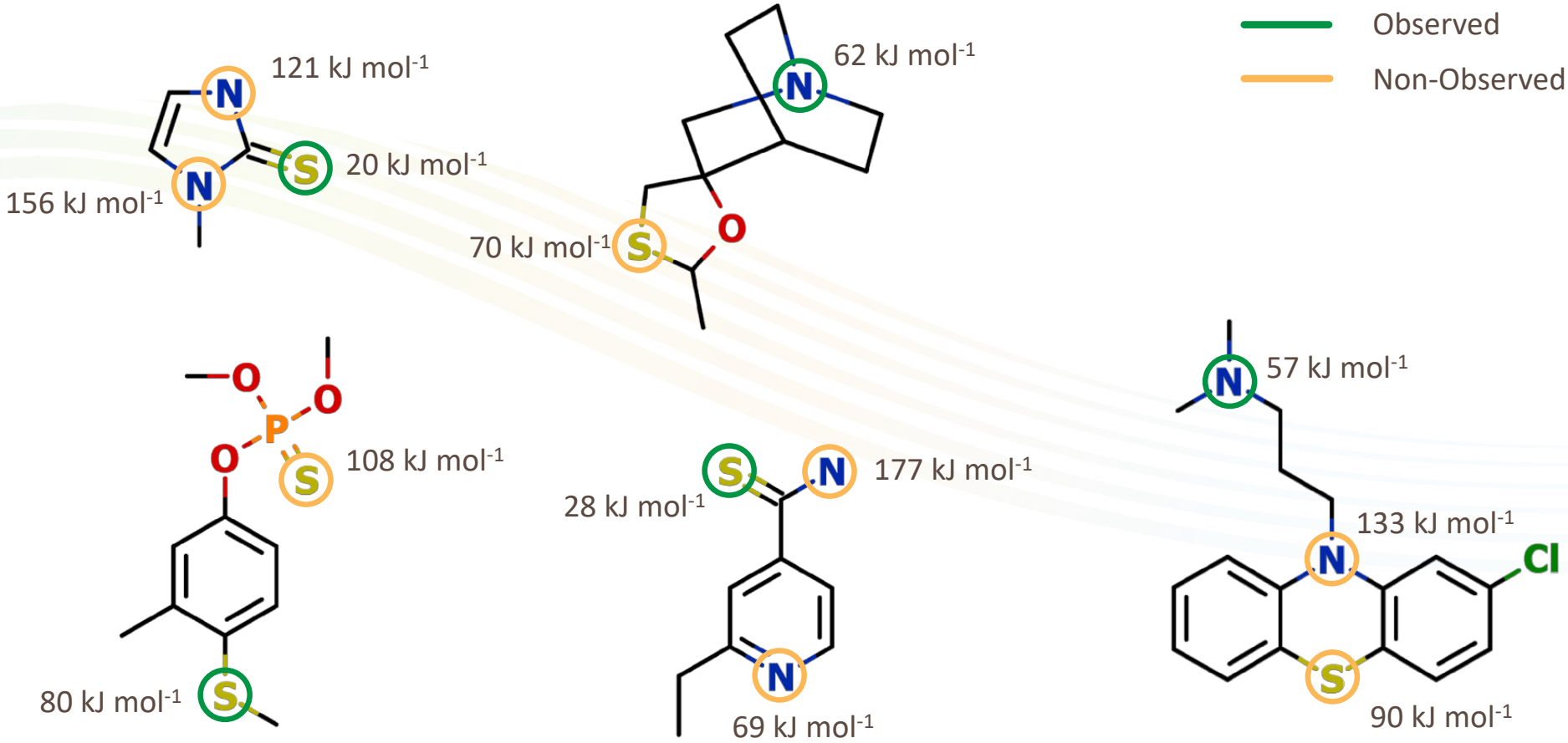
- Hydrogen transfer during optimisation shows no secondary local minima





B3LYP/def2-SVP

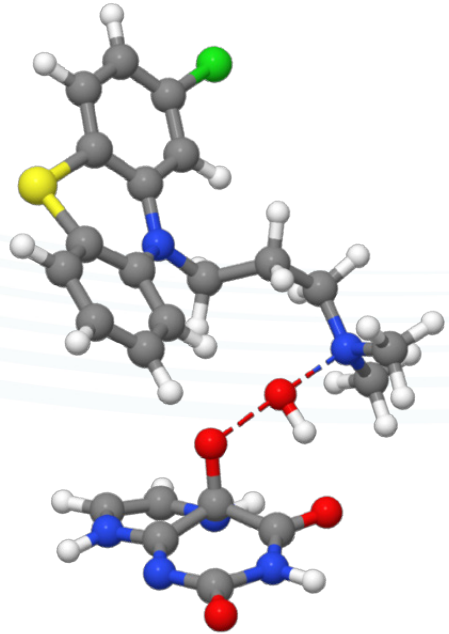
Application to Other Molecules



B3LYP/def2-SVP

Summary

- Identified transition state for closed-shell (S_N2) mechanism
- Extensive tests further support S_N2 mechanism
- Modelling of reaction coordinate provides a greater understanding of the *N/S*-Oxidation process
- Further tests indicate that this mechanism is applicable to larger substrates
- This enables further efforts to predict FMO metabolism



Thank You!

- Mario Öeren
- Peter Hunt
- Matthew Segall
- Jonathan Hirst