

# Peculiarities of the Electronic Structure of Cytochrome P450 Active Site Compound I: DFT and CASPT2 Modelling

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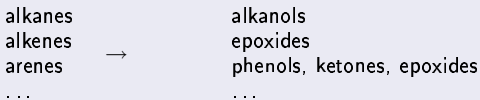
alkanes  
alkenes  
arenes  
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alkanols  
epoxides  
phenols, ketones, epoxides  
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- Active form, **Compound I (Cpd I) is very strong oxidant** (e.g. [O]-insertion into inert C-H bond)

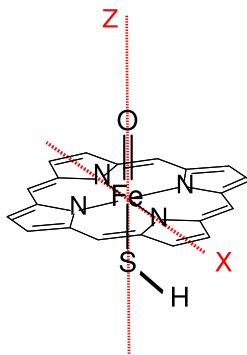
# Why Cytochrome P450?

Why to study P450 and its reactivity?

- Biological and medicinal importance (many biological processes, including drug metabolism)
- Elusive active form: difficulties for experiments  $\Rightarrow$  theory may be useful
- Difficult (interesting) electronic structure – challenge for theoretical models

# QM Model of the Active Site

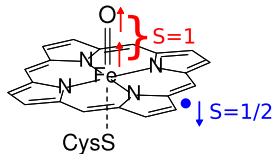
Well separable active site (all heme enzymes)



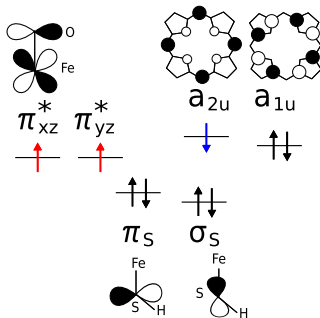
# Electronic Structure of Cpd I: Model

Low-lying states of Cpd I – 3 unpaired electrons model:

- 2 unpaired electrons in



- 1 electron located in  $a_{2u}$ ,  $a_{1u}$ ,  $\pi_S$  or  $\sigma_S$  (as *free radical*)



Ferro- or antiferromagnetic coupling  $\Rightarrow$  quartet or doublet

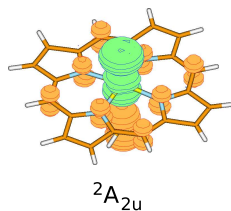
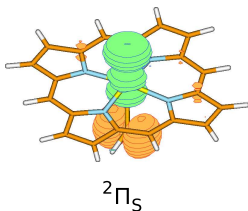


# Electronic Structure of Cpd I: Model

- States of **various radical character** are candidates for the ground state:
  - porphyrine-like:  $A_{2u}$ ,  $A_{1u}$
  - sulphur-like:  $\Pi_S$ ,  $\Sigma_S$
- For each type (e.g.  $A_{2u}$ ) one has both doublet and quartet state (e.g.  $^2A_{2u}$ ,  $^4A_{2u}$ ).

# Electronic Structure of Cpd I: Illustration

Spin densities for 2 lowest states in DFT (B3LYP):



# Electronic Structure of Cpd I: Experiments

Experimental results:

- R. Rutter et al., *Biochemistry* **1984**, 23, 6809–6916
- C.M. Hosten et al., *J. Biol. Chem.* **1994**, 269, 13966–13978
- D. Kellner et al., *J. Biol. Chem.* **2002**, 277, 9641–9644.

⇒ ground state is porphyrine radical like, presumably  $^2A_{2u}$

# DFT Modelling

Theoretical works: in most calculations **hybrid** DFT (B3LYP, B3PW91) was used

- F. Ogliaro et al., *Angew. Chem. Int. Ed.* **2000**, 39, 3851–3855.
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## Purpose

Compare results from widely used hybrid DFT with the ones from non-hybrid functional (BLYP)

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*(detailed methodology and results can be found in our poster)*

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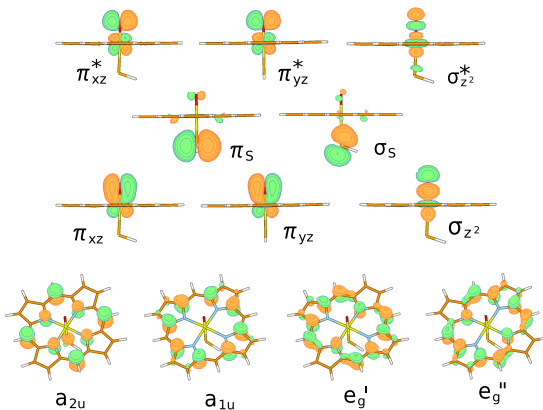
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  - doublets from unrestricted B3LYP have indeed 3 unpaired electrons (analysis by *natural orbitals* and *spin orbitals*) – support for 3 electron model
- However, non-hybrid DFT (BLYP) gives qualitatively different picture
- Doubts from DFT + multiconfigurational nature of doublets  
⇒ attempt to use multiconfigurational ab initio method

# CASSCF/CASPT2

- Complete Active Space (CAS) approach:
  - CASSCF Self Consistent Field  $\Rightarrow$  static correlation
  - CASPT2 Perturbation Theory  $\Rightarrow$  dynamic correlation
- Reliable *ab initio* method for small and intermediate size molecules
- Simple model of Cpd I has 41 atoms and over 100 valence electrons  $\Rightarrow$  big challenge for CASSCF/CASPT2

# Active Space

15 electrons in 12 orbitals:



# CASSCF/CASPT2 Details

- **State-Average CASSCF (SA-CASSCF)** to cover all states of interest (i.e.  $A_{2u}$ ,  $A_{1u}$ ,  $\Pi_S$ ,  $\Sigma_S$ )

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- **Multi-State CASPT2 (MS-CASPT2)**<sup>1</sup> with all states included in SA-CASSCF
- Molcas 5.4 package
- basis set – small version of *Atomic Natural Orbitals* (ANO-S)<sup>2</sup>:  
ANO-S...6s4p3d2f (Fe), ANO-S...3s2p1d (C,N,O), ANO-S...4s3p2d (S),  
ANO-S...2s1p (H)

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<sup>1</sup>J. Finley et al., *Chem. Phys. Lett.* **1998**, 280, 299–306

<sup>2</sup>K. Pierloot et al., *Teor. Chim. Acta* **1995**, 90, 87–114 

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Due to adding dynamic correlation states from CASSCF not only change energies (**shift**) but also may change their character (**mix**)

# Results at CASSCF level

- ground state is  $^2\Pi_g$  (with quartet 3 kcal/mol above)
- states of expected  $A_{2u}$  character are extremely *high-lying* (about 60-70 kcal/mol)
- the same concerns  $A_{1u}$  (about 50 kcal/mol)
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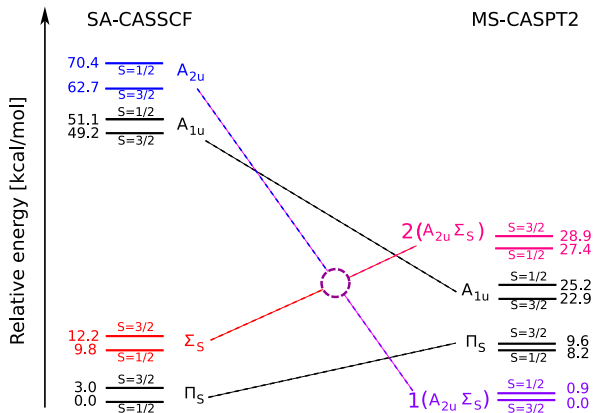
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Without dynamic correlation results are **qualitatively inconsistent with experimental data**.

# CASPT2: Influence of dynamic correlation

## MS-CASPT2:

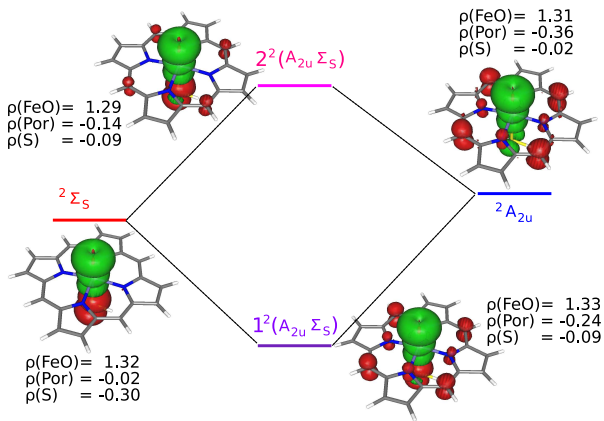
- state **reordering**  
( $\Pi_S$  is no longer ground state)
- mixing**  
between reference states  $A_{2u}$  and  $\Sigma_S$





# Spin densities analysis

Spin densities and Mulliken spin populations: good support for state identification



# Final results

Ground state is  $1(A_{2u}, \Sigma_S)$ , having porphyrine radical character  
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- $A_{2u}$  state, though high-lying in CASSCF, is of crucial importance for the final ground state.

# Conclusions

- In spite of *technical difficulties* due to problem size and the need to compute high-lying  $A_{2u}$  states ( $^2A_{2u}$  is 13th root in CASSCF!) **reasonable active space was found**.
  - Computed ground state has *expected porphyrine radical character* in agreement with experimental results.
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  - We note that no successful studies on Cpd I at CASSCF/CASPT2 level have been reported in literature so far.
- To obtain proper states character and energies in CASSCF/CASPT2:
  - **high-lying  $A_{2u}$  should be included in SA-CASSCF**
  - **multi-state treatment in CASPT2 is mandatory**

# Conclusions

- B3LYP ground state is the same as in CASSCF/MS-CASPT2 (including mixing between sulphur and porphyrine radical)
- Big role of dynamic correlation and qualitative agreement between CASPT2 and B3LYP results  $\Rightarrow$  **a good forecast to use B3LYP for similar problems**
- However, one should be aware that non-hybrid functional (BLYP) yields wavefunctions and energies that are inconsistent with B3LYP and CASPT2, and seem spurious.

Thank you for your attention!

