

# Change in Electron and Spin Density Upon Electron Transfer to Haem *a*

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# Introduction

Haem *a* is the low-spin, bis-imidazol-ligated iron-porphyrin cofactor in many of the respiratory haem-copper oxidases, which catalyse most of the biological consumption of O<sub>2</sub>. Its structure is related to many other haem groups, the typical redox cofactors of cytochromes. Haem *a*, like many other haems, functions in electron transfer, which involves continuous shuttling between its oxidised (“ferric”) and reduced (“ferrous”) states.

Here we show by quantum-chemical calculations that the unit charge by which these two states differ is evenly distributed throughout the haem's porphyrin ring, the charge difference at the haem iron being very small despite the formal unit difference in oxidation state. In contrast, the unpaired spin density in the oxidised haem is found to be exclusively located at the haem iron in agreement with spectroscopic observations. Thus, while addition of an electron to the oxidised haem pairs the spin on the haem iron, there is in addition substantial pairwise electron delocalisation towards the haem's periphery on reduction.

# Computational methods

## Level

The molecular properties for haem *a* were calculated using density functional theory (DFT) with a split valence polarisation quality basis set, SV(P), where polarisation functions are present on all atoms except hydrogens.

The structure of haem *a* in its “ferrous” state was optimized both using the B-P and B3-LYP functionals. The open-shell “ferric” state structure has so far been optimized only with the B-P functional.

The resolution of the identity (RI) approximation was employed at the B-P level[1]. With the RI-DFT method the DFT functional ( $r$ ) is approximated by an expansion in atom-centred auxiliary basis sets and the evaluation of four-centre two-electron integrals is avoided. The RI-DFT approach reduces the computational cost by a factor of three to ten.

The RI-method can not be employed in conjunction with B3-LYP as this functional calculates the exchange part using Hartree-Fock (HF) theory.

## Electron density calculations

By integrating the difference in electron density between the two states in a sphere around an atom the total change in electron density about the atom can be found.

The integration was carried out using an equidistant cartesian grid sampling the calculated total electron density. The total electron population inside a sphere of radius  $r$  is then defined by

$$e_{tot} = \frac{1}{N} \frac{4}{3} \pi r^3 \sum_i^N \rho_i$$

where  $N$  is the number of integration points and  $i$  runs over all three cartesian coordinates.

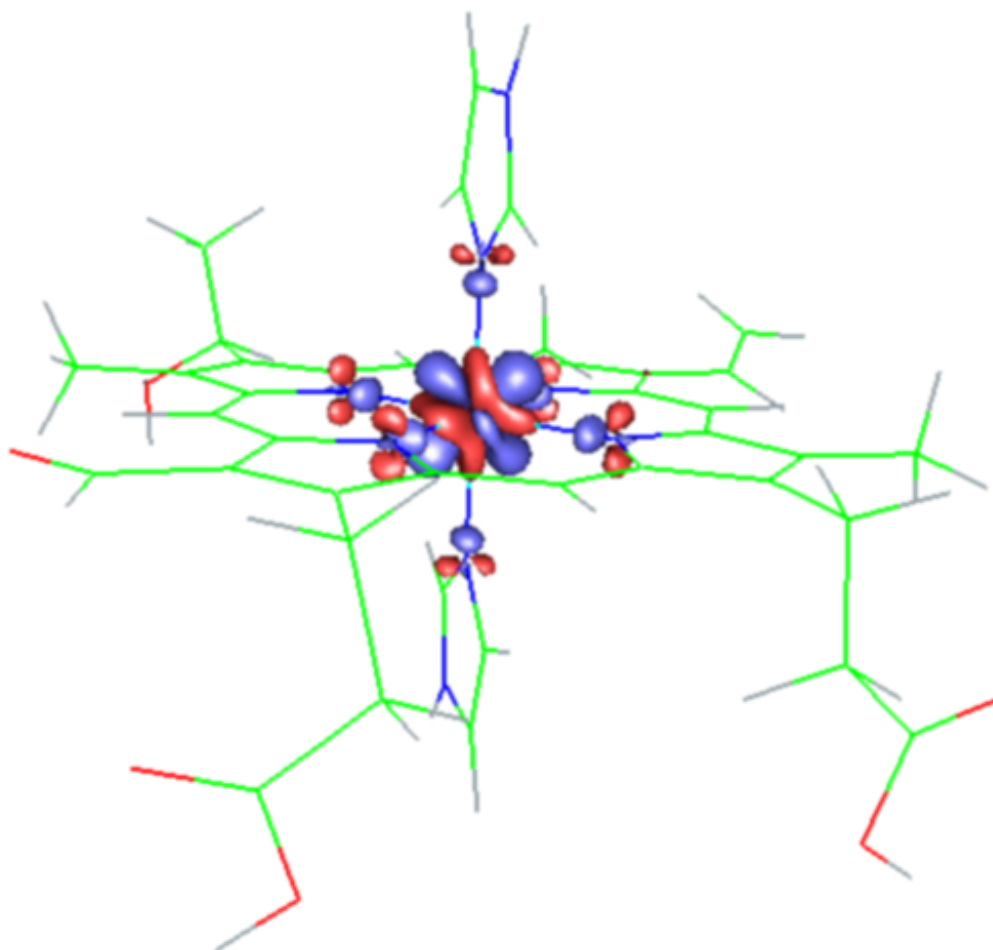
When calculating the electron densities some of the lowest molecular orbitals were left out. These were the orbitals that in effect represent the 1s orbitals of carbon, nitrogen and oxygen, as well as the 1s, 2s and 2p orbitals of iron. These orbitals are so tightly concentrated around their corresponding nuclei that leaving them out, though in principle an approximation, improves the quality of the numerical integration.

All calculations were performed using the TURBOMOLE package[2].

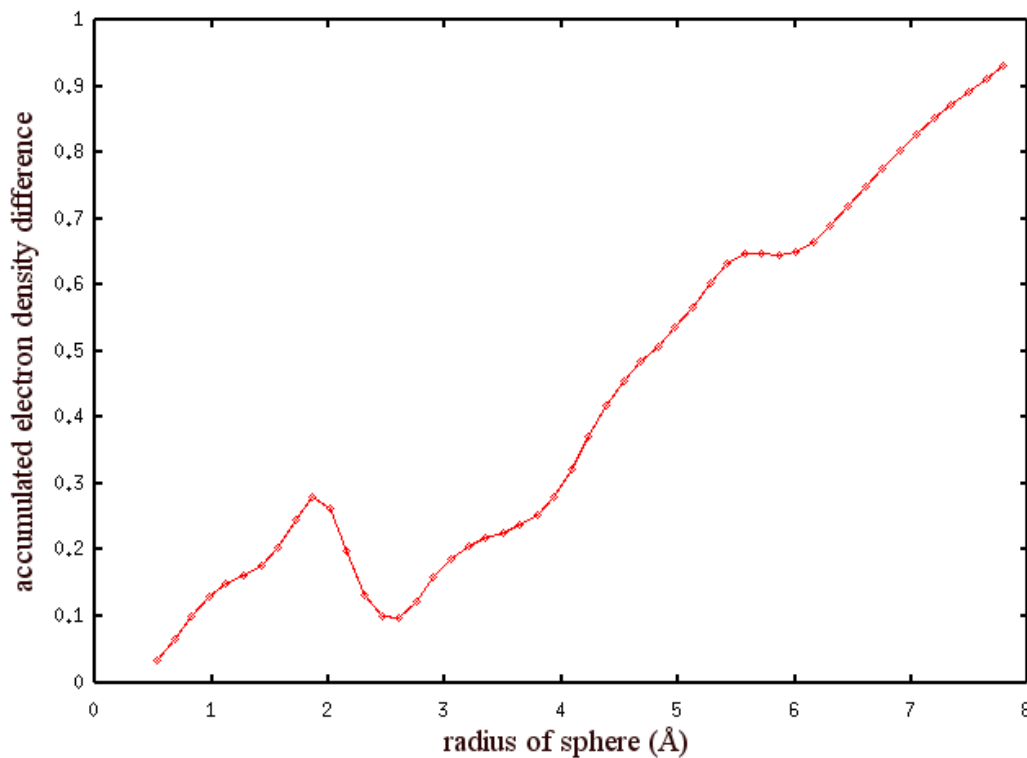
# Results

## Charge density

- First a fully optimized closed-shell “ferrous” structure was calculated at the B3-LYP level, followed by a single point calculation on the oxidised open-shell “ferric” state using the same geometry.
- When subtracting the electron density of the oxidised state from the density of the reduced state the extra electron of the latter can be located
- *Blue colour*: areas with more electrons in the reduced form (compared with the oxidised).
- *Red colour*: more electrons in the oxidised form.



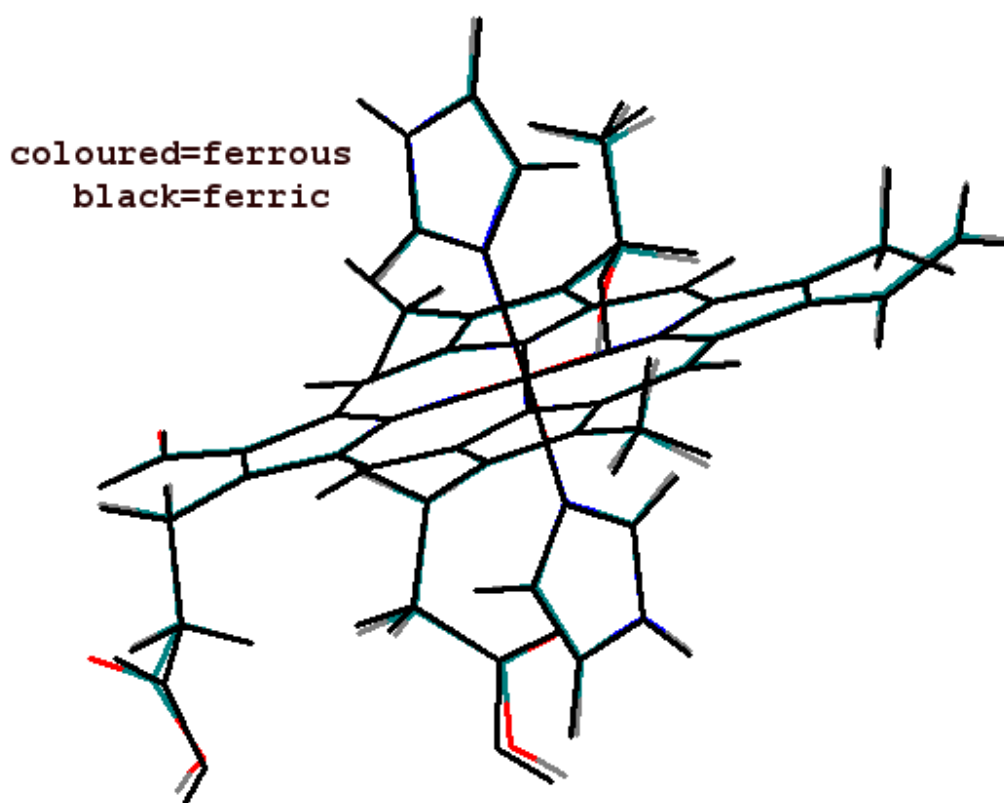
- A **massive reorganisation of the electron density** around the central iron atom is seen. It is however impossible to locate the extra electron from this picture.
- Formally the charge of the central iron changes from +3 to +2 upon reduction.
- However, integrating the **electron density difference** in a sphere around the iron gives the following curve:



- There is a **very small net density change** in the immediate vicinity of the iron atom.
- Inside the covalent radius of iron,  $\sim 1.2 \text{ \AA}$ , the reduced state of the system only has about 0.15 electrons more compared with the oxidised form.
- The bump at around  $2 \text{ \AA}$  is caused by the slight polarisation near the nitrogen atoms.
- Without this effect the curve would go almost linearly towards its maximum value, 1.
- In effect the added electron is very smoothly delocalised over the system, the whole electron “being found” only when the integrated sphere is so big that it includes the entire haem. The haem iron does not want to possess the electron.
- Compared with other atoms in the system the iron does however hog the biggest bite; the other atoms get an increase in electron density of about 5% of the increase around the iron.
- **The effect of the functional used** was also studied. The B-P functional reproduces the aforementioned behaviour of the system, the only difference being that the bump near the nitrogens is not quite as distinguished.

## Structural difference between states

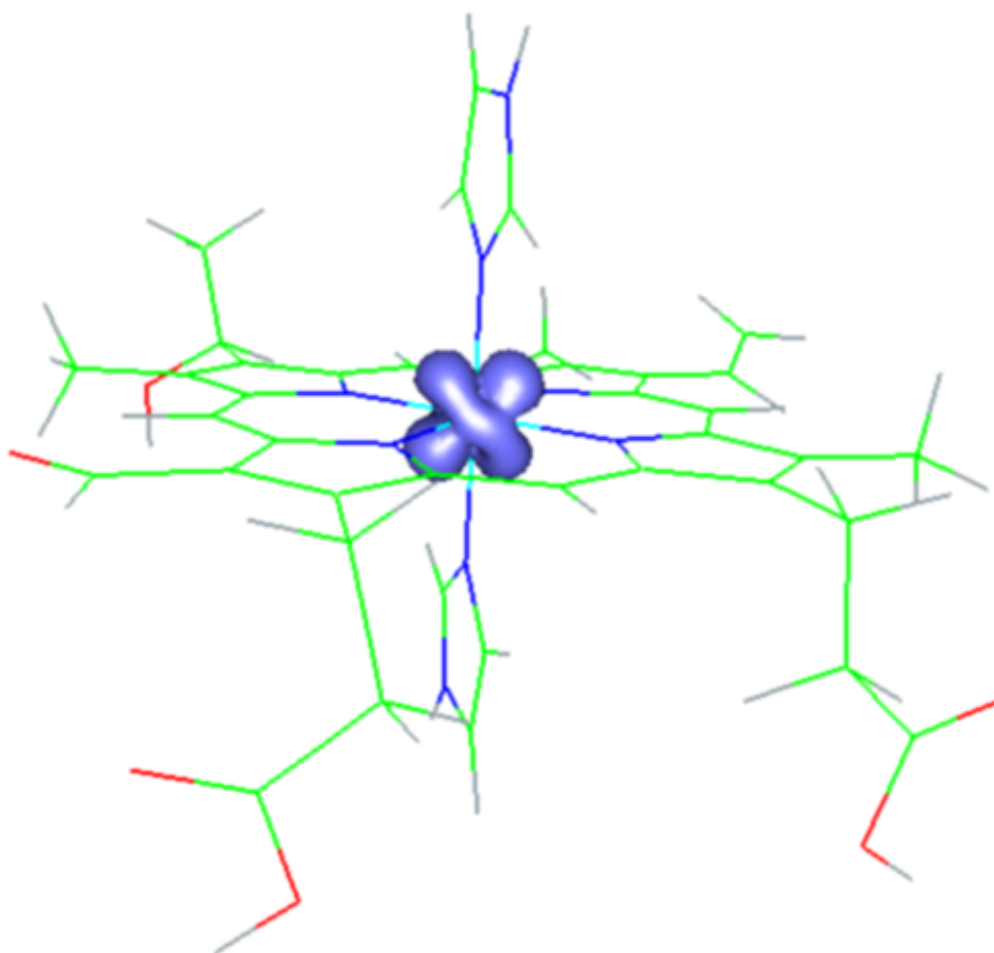
On B-P level the system was also studied with the “ferric” structure being optimized while the “ferrous” electron density was calculated with “ferric” structure, the reverse of the previous calculations. The electron density difference between the two states was in effect identical regardless of the structure chosen. This is no surprise as the structures are very similar:



About the only difference is found in the outermost ligands, the porphyrin ring staying intact.

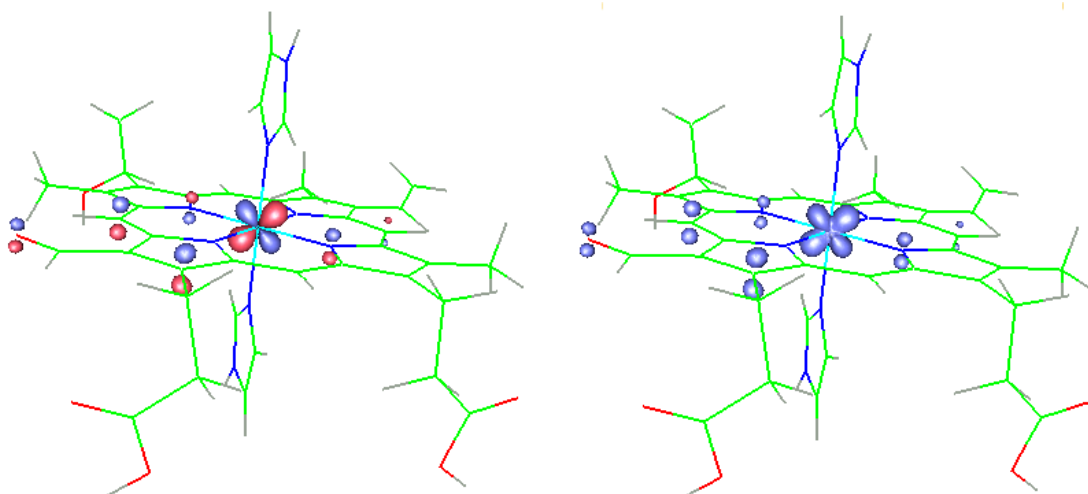
## Spin density

- It has been spectroscopically shown that the spin density of the open-shell, “ferric”, state of haem *a* is exclusively located at the central iron atom.
- So even if oxidation of the system removes the electron evenly from the whole haem it should result in a very localised concentration of spin difference.
- When subtracting the *beta*-spin density from the *alpha*-spin density (where the system is defined as having one more *alpha*- than *beta*-electrons) the following is left over:



- All of the unpaired *alpha*-electron is now found within the covalent radius of the iron atom, in excellent agreement with experiment.
- **The spin density is not delocalised** in contrast with the total electron density

Looking at the situation the other way around, starting from the oxidised form which is about to be reduced, the **LUMO-orbital** should be a first alternative for the incoming electron:



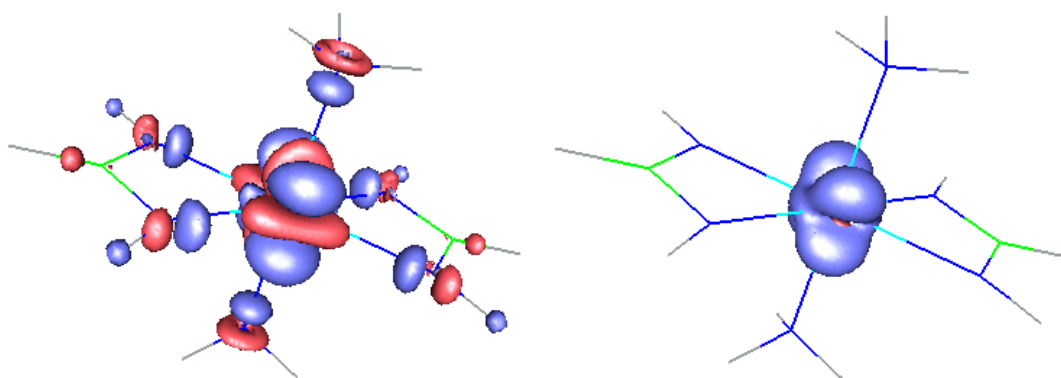
- *On the left* the somewhat distorted *d*-orbital,
- *On the right* its square.
- Ignoring the small *p*-contribution from some atoms in the ring, the LUMO is quite similar to the observed existing spin-density.
- Filling the LUMO pairs the spin.
- At the same time an immediate pairwise electron delocalisation takes place and the iron atom experiences a very small change in charge.

## Effect of system size on delocalisation

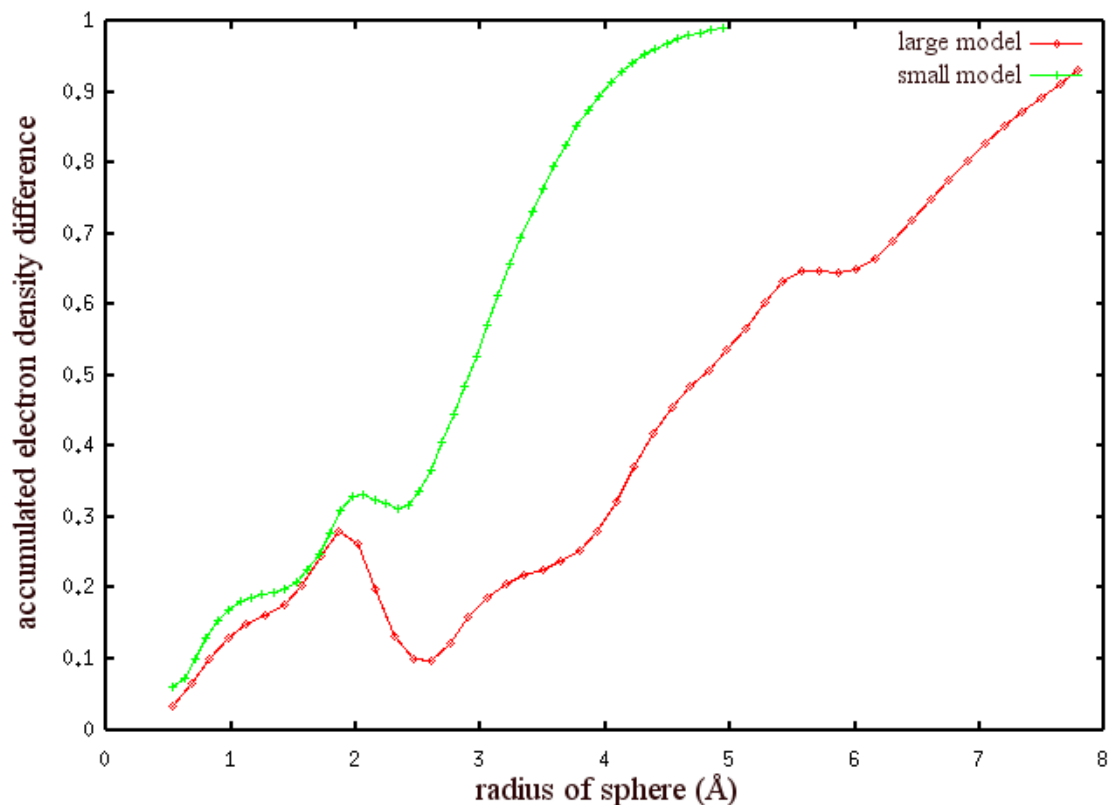
It is of interest to see how the system size affects the delocalisation, in part also because detailed DFT-calculations on systems as large as the studied haem are quite cumbersome.

A subsystem where the haem was modelled with the iron centre surrounded by two diformamidate ( $\text{NHCHNH}^-$ ) ligands and two ammonia units ( $\text{NH}_3$ ) was studied. This approximation has recently been used in a functional study of haem  $a_3$ , the sibling of haem  $a[3]$ .

- The resulting electron and spin density differences reproduce all general features of the larger model:



- The accumulated electron density difference between the oxidised and reduced forms superimposed upon the curve of the larger model:



- The same delocalisation away from the iron is seen.
- Only difference is that the whole electron is located inside a smaller sphere, the smaller system size preventing further delocalisation.
- **The electron is as diffuse as possible.**
- The spin density is again completely located inside the covalent radius of iron.

## Acknowledgements

The nice molecular plots were made using the **gOpenMol** package[4], while the curves were obtained with **gnuplot**.

## References

- [1] K. Eichkorn, O. Treutler, H. Öhm, M. Häser and R. Ahlrichs, *Chem. Phys. Letters* **1995**, 240, 283
- [2] R. Ahlrichs, M. Bär, M. Häser, H. Horn and C. Kölmel, *Chem. Phys. Letters* **1989**, 162, 165
- [3] M. Blomberg, P. Siegbahn, G. Babcock and M. Wikström, *J. Am. Chem. Soc.* **2000**, 122, 12848
- [4] L. Laaksonen, gOpenMol web page <http://www.csc.fi/~laaksonen/gopenmol>