

# The spin distribution in low-spin haem

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## Introduction

• In many low-spin ( $S=1/2$ ) iron porphyrins, electron spin resonance (ESR) spectra indicate that one of the  $d_{z^2}$ -orbitals of iron is as good as singly occupied; the unpaired electron is almost completely located at the metal.

• In contrast, nuclear magnetic resonance (NMR) and electron nuclear double resonance (ENDOR) spectroscopy convincingly show a significant share of the unpaired electron to be delocalised.

• Here we explain this apparent contradiction by density-functional-theory (DFT) calculations performed on a haem *a* model.  
• The calculations show that the integrated spin density at the iron atom is nearly one, in agreement with ESR measurements.  
• However, significant areas with opposite ( $\beta$ ) spin are found along the Fe–N bond axes, thus evoking a need for additional  $\alpha$ -spin density to be present in the porphyrin ring, ring substituents and the axial ligands in order to keep the net amount of unpaired spin exactly one.

• The gross spin density, *i.e.* the sum of unpaired  $\alpha$  and  $\beta$  spins, amounts to about 1.3 electrons.

## Computational methods

### Level

• The molecular structure and the spin density of the low-spin, bis-imidazole-ligated porphyrins have been calculated at the spin-unrestricted density functional theory (DFT) level.

• Both a gradient-corrected local-density approximation (BP) and a hybrid functional (B3LYP) have been employed.

• As the Becke-Perdew (BP) functional does not contain any explicit Hartree-Fock exchange interaction terms, the resolution of the identity (RI) approximation of the Coulomb interaction was used. RI-DFT calculations are much faster than ordinary DFT calculations without any significant loss of accuracy.

• Split-valence SV(P) and triple-zeta valence TZVP basis sets were used. The TZVP basis set has polarization functions on all atoms, while the SV(P) basis set lacks polarization functions on the hydrogens.

• No symmetry restrictions were used.

• All quantum chemical calculations were performed with the TURBOMOLE [1] program package.

### Spin density calculations

• The spin density is obtained as the difference between the  $\alpha$  and  $\beta$  spin contributions to the total electron density.

• The spin density was studied by evaluating it in an equidistant Cartesian grid.

• The radial distribution was explicitly obtained by performing numerical integration of the angular dependence of the spin density in the discrete representation.

• The accumulated net spin density inside a sphere of radius  $r$  can then be obtained as:

$$\rho_{\text{spin}}(r) = \frac{1}{N(r)} \frac{4}{3} \pi r^3 \sum_i \rho_{\text{spin},i}$$

•  $N$  is the number of integration points,  $\rho_{\text{spin},i}$  is the spin density at point  $i$ , and  $i$  runs over all three Cartesian coordinates.

• The core orbitals were left out of the integration;  $1s$  of C, N, and O, as well as  $1s$ ,  $2s$ , and  $2p$  of Fe were ignored.

• Though in principle an approximation, this actually improves the numerical accuracy of the integration. The integration will also be somewhat faster.

• Core orbitals are crucial for *e.g.* hyperfine coupling constants which depend on the point spin density at the nuclei.

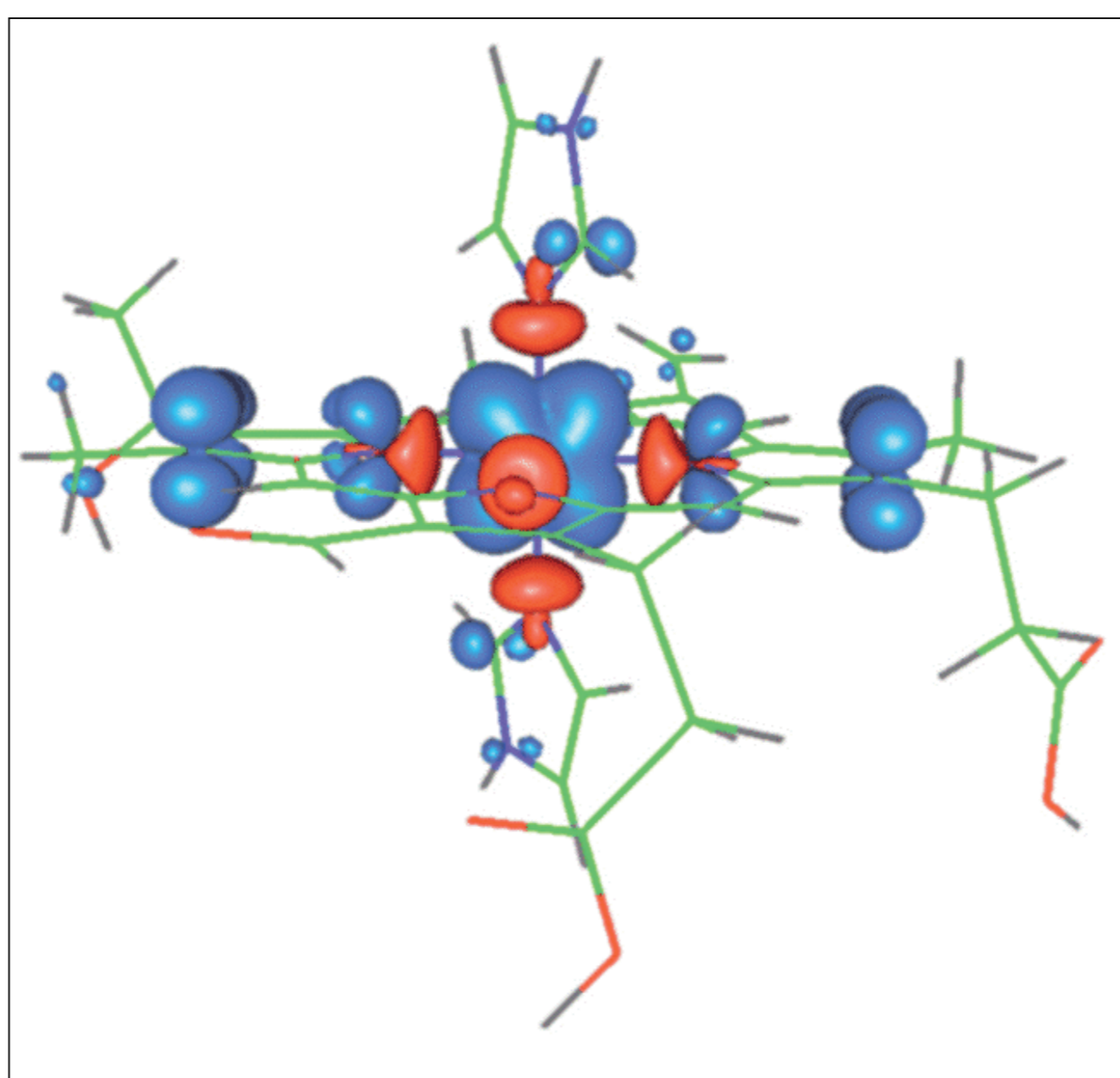
• The contribution from the very tight, concentrated core has however no bearing on the overall spin distribution in the system.

• Spin contamination was checked using the expectation value  $\langle S^2 \rangle$ , as it has been shown that it does, after all, have at least diagnostic value within DFT.

• The ideal value of  $\langle S^2 \rangle$ , assumed meaningful, would then be  $S(S+1)=0.75$ . It was never larger than 0.78, B3LYP consistently giving a somewhat higher value.

## Results

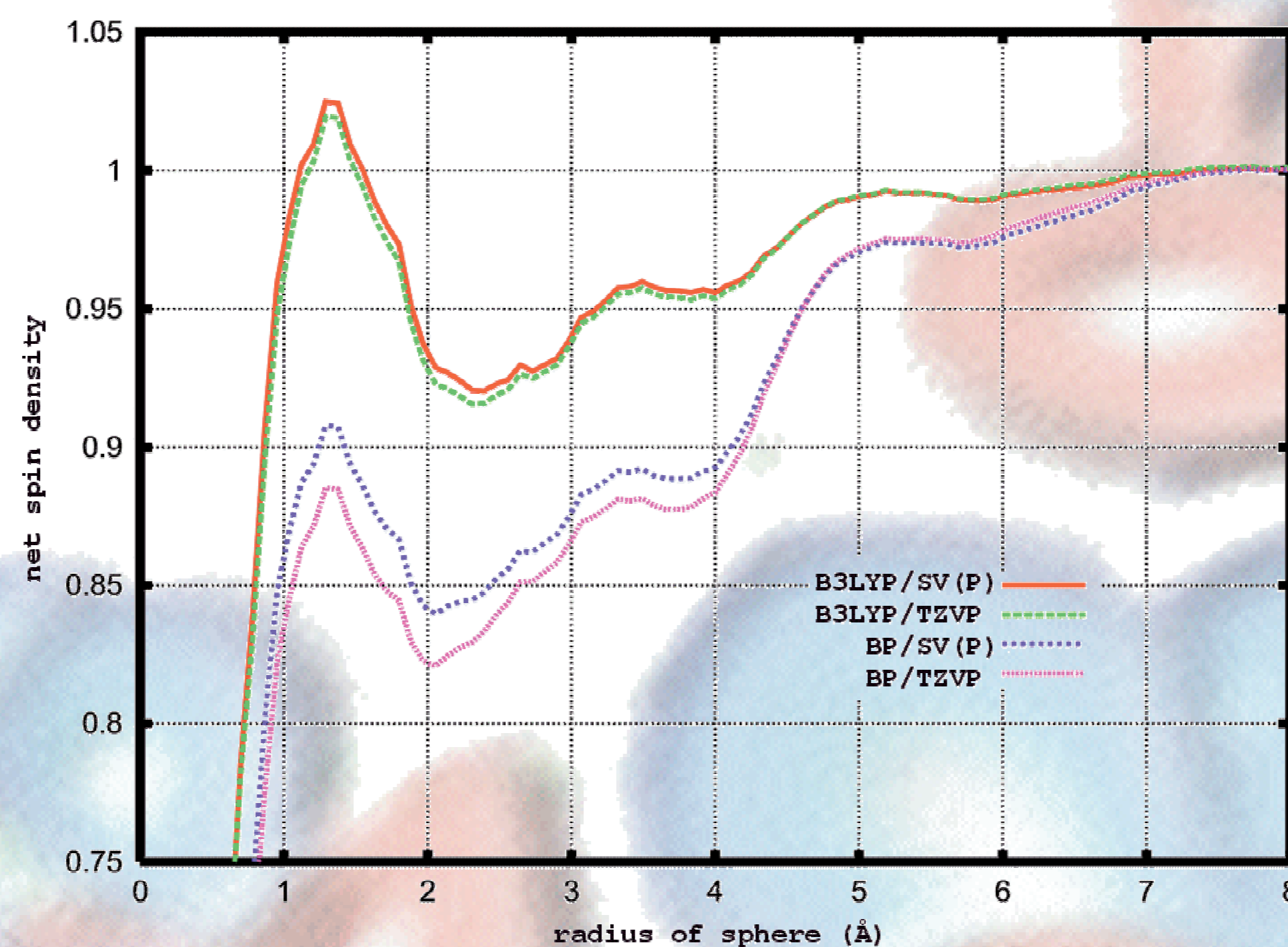
• The spatial spin density distribution is seen below. Blue colour represents excess  $\alpha$ -spin density, red colour excess  $\beta$ -spin.



• The densest region of  $\alpha$ -spin is found at Fe.  
• A striking presence of  $\beta$ -spin is seen along the Fe–N bonds  
• Also the pyrrole carbons have notable amounts of  $\alpha$ -spin.

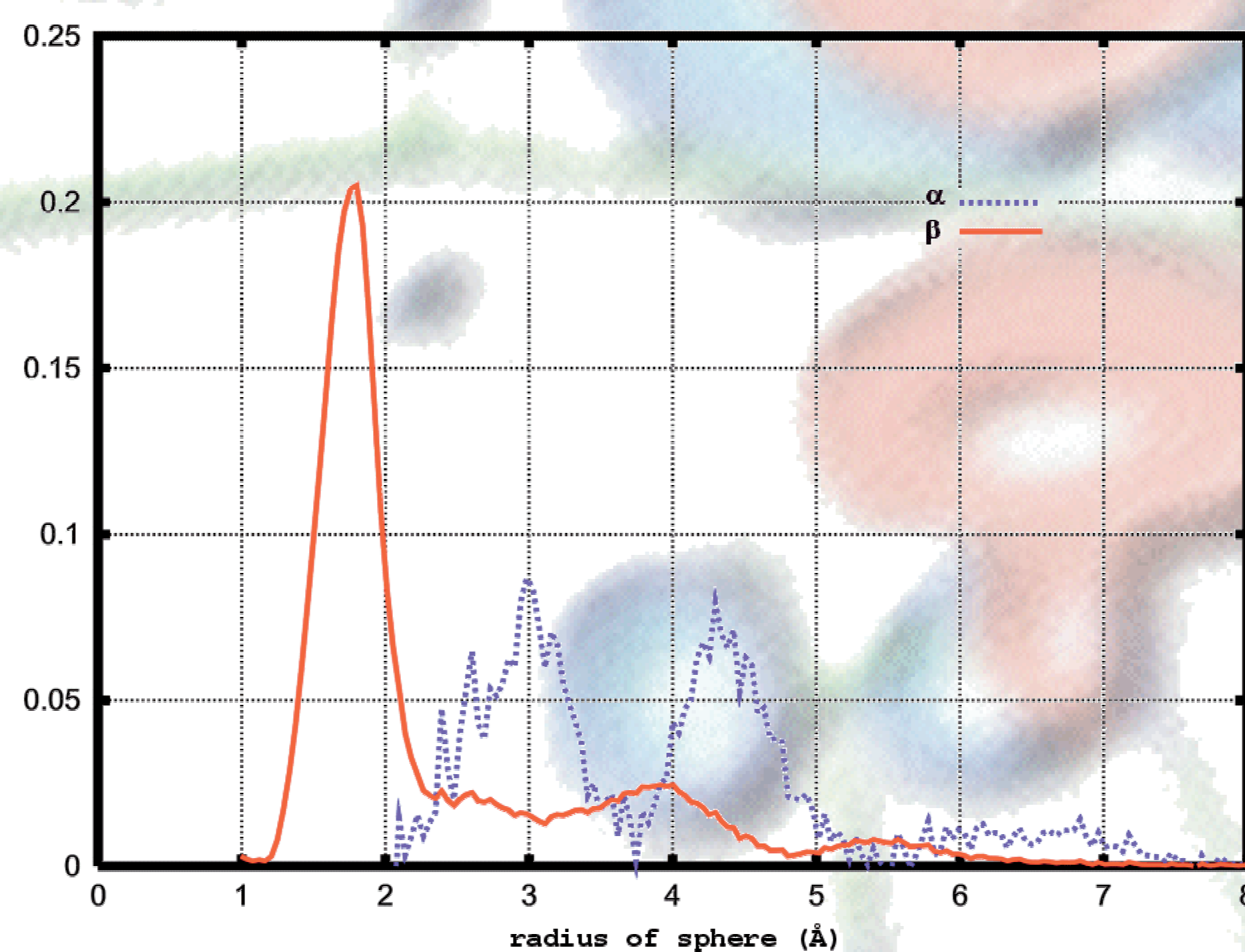
• The large excess of  $\alpha$ -spin at Fe leads to spin polarisation of the electron density outside the iron.  
• Large scale, molecular version of the spin polarisation observed for atoms.

• Integration of the accumulated spin density in a sphere around Fe yields the following curve:



• A maximum at 1.2 Å is seen, the covalent radius of Fe.  
• The region of excess  $\beta$ -spin decreases the net amount of  $\alpha$ -spin by about 0.10 electrons.  
• From 2.0–2.3 Å onwards the accumulated spin density steadily increases. A small decrease is seen around 3.5 Å and 5.7 Å.

• Differentiation of  $\alpha$ - and  $\beta$ -spin contributions (B3LYP/TZVP):

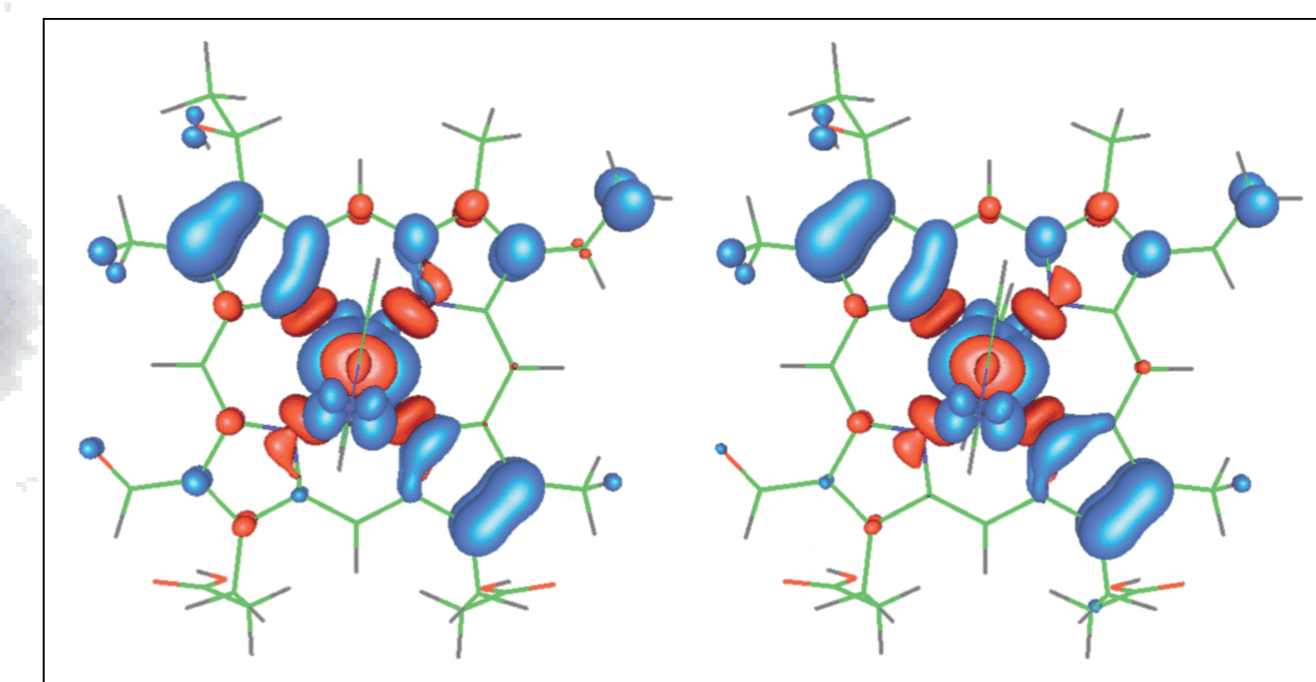


• The main region of excess  $\beta$ -spin is clearly seen, but also small contributions peaking at 3.9 Å and 5.4 Å are seen.  
• Excluding the  $\alpha$ -spin at iron (not in the plot), the largest peaks are found at 3.0 Å and 4.3 Å.  
• This can be related to the molecular frame:  
• pyrrole  $C_{\alpha}$ : 3.0 – 3.1 Å  $C_{\text{meso}}$ : 3.4 Å  
• pyrrole  $C_{\beta}$ : 4.3 Å

• Integration of all unpaired spin, *i.e.* the sum of  $\alpha$ - and  $\beta$ -spin, amounts to 1.28–1.34 unpaired electrons, at BP and B3LYP levels, respectively.

### Effect of axial ligand orientation

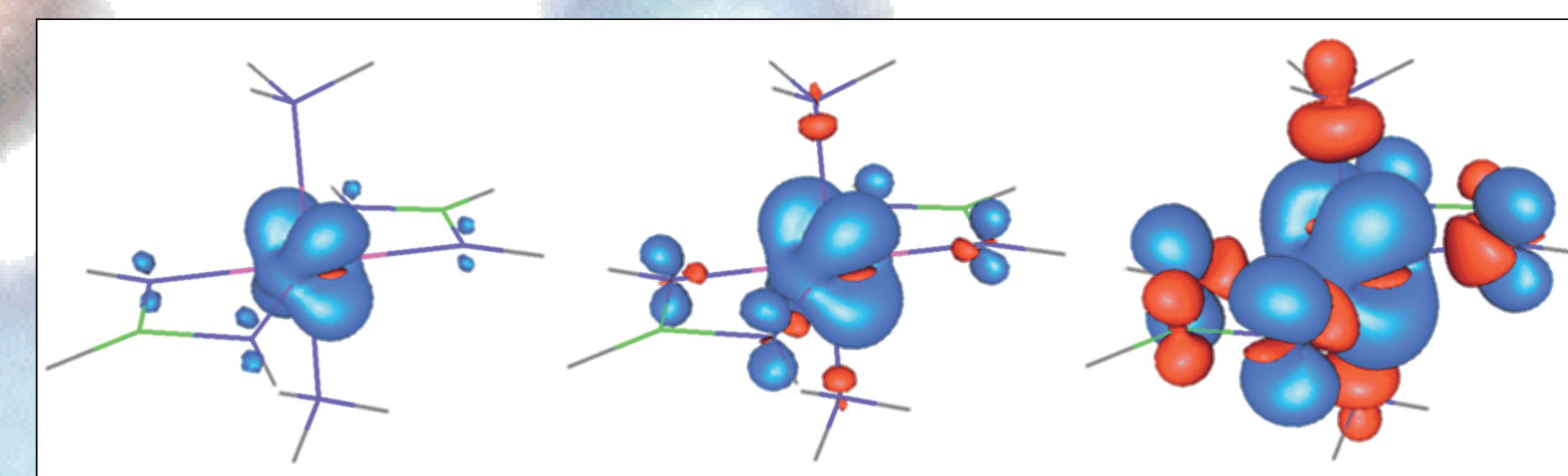
• In a model system, the axial imidazoles relax to almost parallel mutual orientation  
• In the enzyme, the orientation is effectively fixed.  
• The orientation however affects the spin distribution remarkably.  
• The spin distribution for a fully relaxed structure (left) and with experimental imidazole torsion angles (right), BP/TZVP-level:



• even larger differences are seen when the optimised and experimental structures differ more, like in haem *b*.  
• The largest effects of the protein backbone can be accounted for by re-turning the axial ligands.  
• The energy required for rotation is very low, so no computational caveats are expected to arise.  
• If differences in the Fe– $L_{\text{axial}}$  bond lengths are seen experimentally, an adjustment of the optimised structure to reflect this might be in place.

### Effect of the model size

• To check the generality of the spin polarisation and distribution, a very small model system was studied:



• All the major features of the larger model are reproduced.

## Final remarks

• Axial ligand orientation is the most important structural parameter affecting the spin distribution. They dictate the orientation of the  $d$ -hole, which in turn dictates the spin occupation in the macrocycle.  
• Ring substituents perturb the distribution significantly.  
• Solvent effects were studied with both the continuum model COSMO and with explicit water around haem *a*. Very similar distributions compared with gas phase are obtained.  
• SV(P) and TZVP give almost quantitatively the same results.  
• Of the computational parameters, the choice of functional affects the results the most. B3LYP polarises slightly more.

• The spin distribution is very different from the change in charge distribution upon reduction of haem [2]. A similar polarisation effect at the nitrogens can however be noted.

## Acknowledgements

• Dr. H. Kanschun contributed with enlightening comments.  
• The plots were made with gOpenMol [3].  
• The graphs with gnuplot.  
• CSC – Scientific Computing Ltd. provided ample computer time.

## References

- [1] <http://www.turbomole.com/>
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