



## Ab initio calculations on iron-porphyrin model systems for intermediates in the oxidative cycle of cytochrome P450s

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### Summary

Geometry optimizations for several spin states of the iron(III)-*S*-methyl-porphyrin complex, the iron(III)-oxo-*S*-methyl-porphyrin complex and the respective anions were performed in order to examine models for intermediates in the oxidative cycle of cytochrome P450. The aim of this study was to obtain insights into the ground states of the intermediates of this catalytic cycle and to use the ab initio calculated geometries and charge distributions to suggest better and more realistic parameters for forcefields which are generally used for modeling P450s. The results indicate that the ground states of both the iron(III)-*S*-methyl-porphyrin complex and the iron(III)-oxo-*S*-methyl-porphyrin complex are sextet spin states (high spin). The ground states of the anions of both complexes are probably quintet spin states. The fact that experimentally a shift from low spin to high spin is observed upon binding of the substrate suggests that the ab initio calculations for the iron(III)-*S*-methyl-porphyrin complex in vacuum give a correct representation of the (hydrophobic) substrate-bound state of the active site of P450. The ab initio geometries of the iron-porphyrin complexes are very similar to the experimentally observed geometries, except for the longer iron-sulfur bond in ab initio calculations, which is probably caused by the omission of polarization functions on the sulfur atom during the geometry optimization. The charge distribution in all ab initio calculated complexes can be described by a series of concentric rings of alternating charge, thus allowing a relatively large positive charge on the iron atom. The commonly used forcefields generally underestimate the charge differences between the iron atom and the different parts of the porphyrin moiety or ignore the charges completely. Although forcefield calculations can reproduce the experimental geometry of iron-porphyrin moieties, extension of the forcefields with charges obtained from ab initio calculations should give a better description of the heme moiety in protein modeling and docking experiments.

### Introduction

Cytochromes P450 (P450s) constitute a large superfamily of heme-containing enzymes [1], capable of oxidizing and reducing a wide variety of substrates, both of endogenous and exogenous origin. P450s

generally detoxify potentially dangerous compounds, but in a number of cases non-toxic compounds are bioactivated into toxic intermediates [2–4].

In the initial part of the catalytic monooxygenation cycle of P450 [5–11], the substrate first binds to the enzyme, thus inducing a shift in the heme moiety from low spin (six-coordinated iron) to high spin (five-coordinated iron) [5,10]. Consecutively, a one-electron reduction of the P450-substrate complex

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takes place, followed by binding of dioxygen. At a certain stage, a reactive oxygen intermediate is formed ( $\text{Fe}^{\text{V=O}}$  species [5,10]). Recombination of the substrate and oxygen radicals results in oxidation of the substrate, after which the oxygenated product is released from the active site and the enzyme returns to the ground state. Axial ligands play a major role in the chemistry of heme proteins [12]. In P450s the fifth (axial) ligand is a cysteine residue, as can be seen in various crystal structures [13–15], while in myoglobin this ligand is a histidine residue [16]. P450 activates dioxygen while myoglobin merely transports dioxygen. Due to the importance of metabolism by P450, a wide variety of homology models have been constructed for this class of enzymes, based on the available crystal structures [7].

A variety of theoretical molecular orbital (MO) studies have been performed on metalloporphyrins with bound atomic or molecular oxygen. Usually either the basis set, the level of electron correlation or the structure are treated below the desirable level of accuracy [18]. Often different spin states are completely ignored. Several studies have been reported on the geometry, electronic structure and spin states of metalloporphyrins with bound axial and proximal ligands, using methods ranging from semi-empirical to *ab initio* multiconfiguration self-consistent field (MCSCF). Two possible positions for the iron-bound oxygen atom in P450 (model systems) have been described: one perpendicular to the plane of the heme moiety (ferryl-oxygen intermediate) [19] and a second one with the oxygen atom bridged in between the iron atom and one of the four heme nitrogen atoms [7,20].

Relatively few *ab initio* calculations have been performed on metalloporphyrins due to the large computational resources that are required. Early *ab initio* calculations on a small and incomplete model system for a porphyrin radical cation of catalase compound I (with ammonia as axial ligand) indicated an oxygen atom axially above the iron atom to be favored over an oxygen atom bridged in between the iron atom and one of the pyrrole nitrogen atoms. These results contradicted a variety of experimental data [21], which could be expected as this system does not give an appropriate description of a porphyrin moiety [18]. Furthermore, as no geometry optimizations were performed, these theoretical results were highly dependent on the geometry selected for the models [21]. Restricted Hartree-Fock (RHF) and complete active space self-consistent field (CASSCF) studies on iron-porphyrin systems have been described with

either a bound ferryl-oxygen atom and pyridine as axial ligand [22,23], or with a bound oxygen molecule and ammonia as axial ligand [24]. In none of these RHF or CASSCF calculations was the geometry optimized and species with a bridged oxygen atom were not considered. The CASSCF results on the iron-oxo-porphyrin  $\pi$ -radical cation were consistent with experimentally obtained data, while the spin population on oxygen obtained with RHF methods was too small (0.03 for RHF compared to 0.43 for CASSCF [22]). Recently, RHF geometry optimizations of several metalloporphyrins were described [25,26]. RHF and single double configuration interaction (SDCI) calculations were performed on quartet and sextet singly charged cations and anions starting from the quintet ground state of the manganese(III)-chloro-porphyrin complex to test the RHF results [25,27]. The RHF sequence of energy levels equaled the SDCI sequence, indicating that RHF methods may be used to calculate the ground state [25,27] of these metalloporphyrins. Furthermore, an optimized geometry for a manganese(III)-oxo-chloro-porphyrin complex with an oxygen atom bridged between manganese and one of the pyrrole nitrogen atoms was obtained [25,28].

In this study, geometry optimizations for several spin states of the iron(III)-*S*-methyl-porphyrin complex, the iron(III)-oxo-*S*-methyl-porphyrin complex and the respective anions are performed. The aim of this study is to obtain insights into the ground states of the intermediates of the oxidative cycle of cytochrome P450 and to use the *ab initio* calculated geometries and charge distributions to allow the derivation of a better and more realistic parametrization of forcefields which can be used for modeling P450s.

## Computational methods

The quantum chemical program package GAMESS-UK (Generalised Atomic and Molecular Electronic Structure System UK-version [29–31]), implemented on IBM RS6000 workstations, a CRAY C98 supercomputer and a 76-node IBM SP-2, was used for the *ab initio* calculations.

The initial conformation of the iron(III)-*S*-methyl-porphyrin complex was based on the *ab initio* optimized conformation of an iron(III)-chloro-porphyrin complex [26]. The *S*-methyl group was positioned symmetrically below one of the pyrrole nitrogen atoms (labeled N\* in Figure 1). The porphyrin plane was kept

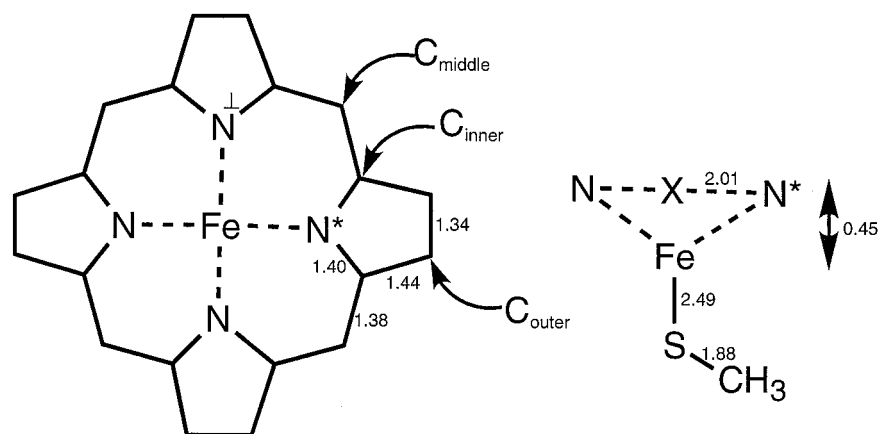


Figure 1. Bond lengths in the ab initio optimized sextet iron(III)-S-methyl-porphyrin complex. Due to (almost) fourfold symmetry of the porphyrin, only a few distances are shown (in Å). The fourfold symmetry is lowered to  $C_s$  only by the S-methyl moiety. Nomenclature of the different carbon atom types is indicated. X marks the geometrical center of the porphyrin moiety. The methyl group is located directly under the pyrrole nitrogen atom indicated with an asterisk.

planar and  $C_s$  symmetry was maintained during the geometry optimization.

The initial iron(III)-oxo-S-methyl-porphyrin complex was based on an ab initio optimized structure of a manganese(III)-oxo-chloro-porphyrin complex with an oxygen atom bridged between the manganese atom and one of the pyrrole nitrogen atoms [28]. Although during the geometry optimization the symmetry of the porphyrin moiety was maintained and the porphyrin plane was kept planar, no symmetry was present in the overall structure due to the bound S-methyl ligand and the bridged oxygen atom.

All complexes were optimized at the (open shell) RHF level with the STO-3G (Slater type orbitals comprising 3 Gaussians) basis set [32] for the carbon and hydrogen atoms and a split valence (SV) 3-21G basis set for the nitrogen, oxygen, sulfur and iron atoms [33–36]. These calculations consisted of 198 basis functions for the iron-S-methyl-porphyrin complex and 207 basis functions for the iron-oxo-S-methyl-porphyrin complex. The resulting optimized conformations were used to perform a single point energy and distributed multipole analysis (DMA) calculation [37] in an extended basis set: SV 3-21G for the carbon and hydrogen atoms [33], SV 6-31G with extra polarization functions for the nitrogen, oxygen and sulfur atoms [33–35], and double zeta (DZ) with an extra polarization function for the iron atom [38]. The recently added direct open-shell routines of GAMESS-UK [30,31] made these calculations possible, while the parallel version of GAMESS-UK [31] reduced the ‘real time’ required for the calculations.

For all spin states, the complexes with the lowest total energy are shown in the tables and figures. Several other doublet, quartet and sextet spin states have been calculated.

The ab initio calculated geometries are compared with the geometries obtained from several studies, i.e., a recent modeling study on P450 2D6 [39] using Quanta/CHARMm [40,41] employing the CHARMm forcefield [42,43], a molecular dynamics study using Discover [44] employing a set of specific parameters for describing the heme moiety of P450s [45,46] and a study using the MM2 forcefield [47] employing a recently derived set of iron-porphyrin parameters [48]. Charges obtained from the ab initio calculations are compared with charges used in the CHARMm forcefield [42,43], a set of specific parameters for the Discover forcefield [45,46], and a recently derived set of MM2 forcefield iron-porphyrin parameters [48].

## Results and discussion

### *Geometries and energies of iron-porphyrin complexes*

The geometry of the porphyrin moiety hardly changes with the spin state of the iron(III)-S-methyl-porphyrin complex. In Figure 1, the bond lengths in the sextet iron(III)-S-methyl-porphyrin complex are shown, as well as the labeling of the carbon atoms cited in the text. In the anion (quintet iron(II)-S-methyl-porphyrin complex) the bond lengths are slightly different ( $\pm 0.01$  Å) from those shown in Figure 1. Energies and some additional geometrical properties are listed in Table 1.

Table 1. Energies and geometric properties of different spin states of *ab initio* calculated iron(III)-*S*-methyl porphyrin and the quintet state of its anion (iron(II)-*S*-methyl porphyrin) and comparison with an iron-*S*-cysteiny-porphyrin optimized using molecular mechanics

Program used Spin state	GAMESS-UK <sup>a</sup>				CHARMm <sup>b</sup> undefined
	Doublet	Quartet	Sextet	Quintet <sup>c</sup>	
E <sub>total</sub> (Hartree <sup>d</sup> )	-2676.7457	-2676.8506	-2676.9427	-2676.9923	not comparable
Fe-X (Å)	0.35	0.32	0.45	0.59	0.39
N-X (Å)	1.97	1.97	2.01	2.05	1.98
Fe-N(Å)	2.00	2.00	2.06	2.14	2.02
Fe-S (Å)	2.56	2.55	2.49	2.66	2.05
N-C <sub>inner</sub> (Å)	1.41	1.41	1.40	1.39	1.33
N*-Fe-S(*) <sup>e</sup>	109.7	103.2	106.0	110.8	ND <sup>f</sup>
Fe-S-C <sub>thiol</sub> (*)	112.0	112.5	112.0	107.3	ND <sup>f</sup>
F-N-C <sub>inner</sub> (*)	127.0	127.1	126.1	124.7	126.8

<sup>a</sup> C<sub>inner</sub>-C<sub>outer</sub> = 1.44 ± 0.01 Å; C<sub>inner</sub>-C<sub>middle</sub> = 1.38 ± 0.01 Å; C<sub>inner</sub>-C<sub>outer</sub> = 1.34 Å; S-C<sub>thiol</sub> = 1.88 Å; C-H = 1.08 Å; N-C<sub>inner</sub>-C<sub>outer</sub> = 109.3 ± 0.7°; C<sub>inner</sub>-C<sub>outer</sub>-C<sub>outer</sub> = 107.9 ± 0.1°; N-C<sub>inner</sub>-C<sub>middle</sub> = 125.5 ± 0.1°; N\*-Fe-S-C<sub>thiol</sub> = 0.0°.

<sup>b</sup> Taken from Ref. 39. C<sub>inner</sub>-C<sub>outer</sub> = 1.45 Å; C<sub>inner</sub>-C<sub>middle</sub> = 1.36 Å; C<sub>outer</sub>-C<sub>outer</sub> = 1.41 Å; C-H = 1.08 Å; N-C<sub>inner</sub>-C<sub>outer</sub> = 112.7°; C<sub>inner</sub>-C<sub>outer</sub>-C<sub>outer</sub> = 104.3°; N-C<sub>inner</sub>-C<sub>middle</sub> = 124.9°.

<sup>c</sup> Anion of iron(III)-*S*-methyl-porphyrin (iron(II)-*S*-methyl-porphyrin).

<sup>d</sup> 1 Hartree = 1 a.u. (atomic unit) of energy = 2625.5 kJ/mol.

<sup>e</sup> N\* indicates the nitrogen atom under which the *S*-methyl group is located.

<sup>f</sup> ND = not determined.

**Iron(III)-*S*-methyl-porphyrin complex** Table 1 shows that the iron atom is located under the plane of the porphyrin moiety (in the direction of the *S*-methyl axial ligand, Figure 1). At higher spin states of the iron(III)-*S*-methyl-porphyrin complex, the total energy of the complex decreases and the iron atom is located at a larger distance below the plane of the porphyrin moiety (Fe-X in Table 1). In our calculations (vacuum) a sextet ground state is found for the iron(III)-*S*-methyl-porphyrin complex (0.197 Hartree lower than the lowest doublet spin state). In the literature a sextet ground state has also been indicated for several iron(III)-porphyrin species [25–27,49]. Experimentally a shift from low spin to high spin is observed upon binding of the substrate, resulting in a high spin state for the (hydrophobic) substrate-bound state of the active site of P450 [5,10]. Combined, this indicates that the *ab initio* calculations on the iron(III)-*S*-methyl-porphyrin complex give a correct representation for the (hydrophobic) substrate-bound state of the active site of P450. Test calculations using a small basis set (STO-3G [32] for the carbon and hydrogen atoms and SV 3-21G for the nitrogen, oxygen, sulfur and iron atoms [33–36]) including a water molecule above the porphyrin moiety (in a position derived from a crystal structure for P450 101 [13]) indicated recently that upon incorporation of water a lower spin

state (doublet or quartet) will become the ground state of the system [50].

In the sextet ground state the iron is furthest from the porphyrin plane (Fe-X in Table 1) and the nitrogen atoms are 0.04 Å more distant from the geometric center of the porphyrin moiety (X) than in the quartet and doublet spin states (N-X in Table 1). This movement of the iron atom out of the plane towards the cysteine ligand when going from low spin (doublet) P450 to high spin (sextet) P450 has also been proposed based on experimentally observed spectroscopic changes occurring upon substrate binding [5,6]. The present *ab initio* results corroborate this proposal. When going from lower to higher spin states, the iron-sulfur bond is shortened.

In crystal structures of high spin iron(III)-porphyrins, the following average bond lengths are found: Fe-X 0.51 Å, Fe-N 2.07 Å, N-C<sub>inner</sub> 1.38 Å, C<sub>inner</sub>-C<sub>middle</sub> 1.39 Å, C<sub>inner</sub>-C<sub>outer</sub> 1.44 Å and C<sub>outer</sub>-C<sub>outer</sub> 1.35 Å [48]. These values are in good agreement with those found in the geometry of the *ab initio* calculated sextet iron(III)-*S*-methyl-porphyrin complex presented in Table 1, except for the displacement of the iron atom from the plane of the porphyrin moiety (Fe-X) which is 0.06 Å larger in the crystal structures. In the crystal structures for low spin (doublet) and intermediate spin (quartet) iron(III)-porphyrins,

the Fe-X distances are smaller than those obtained in the calculations (Table 1) or observed in the crystal structures for high spin iron(III)-porphyrins, i.e., 0.26 Å and 0.22 Å [48], respectively. The Fe-N distances in the crystal structures for low spin (doublet) and intermediate spin (quartet) iron(III)-porphyrins were found to be 1.99 Å, while the other bond lengths are comparable to the bond lengths in crystal structures of high spin (sextet) iron(III)-porphyrins [48]. The *ab initio* data (Table 1) also show smaller values for the Fe-X and Fe-N distances in the low spin and intermediate spin iron(III)-porphyrins compared to the high spin iron(III)-porphyrins. Crystallographic and enhanced X-ray absorption fine structure (EXAFS) spectroscopy studies on low spin and high spin iron(III)-porphyrin moieties provided experimental information on the Fe-N and Fe-S bonds in these systems [51]. The observed Fe-N bond lengths, 2.00 Å in low spin porphyrins and 2.06 Å in high spin iron(III)-porphyrins [51], are in agreement with the theoretical results (doublet and sextet in Table 1). The calculated Fe-S bond lengths, however, are generally too long: 2.56 Å theoretically compared to 2.27 Å experimentally [51] for the low spin state, and 2.49 Å theoretically compared to 2.26 Å experimentally [51] for the high spin state. This deviation is probably a result of the omission of polarization functions (d-orbitals) on the sulfur atom during the geometry optimization. Inclusion of polarization functions on sulfur is expected to shorten the iron-sulfur bond considerably.

*Iron(II)-S-methyl-porphyrin complex* Within the iron(II)-S-methyl-porphyrin complex, the iron atom is even further displaced from the plane of the porphyrin. The pyrrole nitrogen atoms are 2.05 Å away from the center of the porphyrin (Fe-N in Table 1), enlarging the cavity with 0.08 Å compared to the sextet iron(III)-S-methyl-porphyrin complex. The geometry of the quintet state of the iron(II)-S-methyl-porphyrin complex (Table 1) resembles the structure of the quintet singly charged anion of an iron(III)-chloro-porphyrin complex (Fe-X 0.55 Å, Fe-N 2.60 Å and Fe-Cl 2.12 Å [25,26]). Upon adding an electron to these two systems, the bond to the axial S-methyl ligand is lengthened (Table 1), whereas the bond to the axial chlorine ligand is shortened [25,26]. In crystal structures of the high spin iron(II)-porphyrin complex, the following average bond lengths are found: Fe-X 0.51 Å, Fe-N 2.09 Å, N-C<sub>inner</sub> 1.38 Å, C<sub>inner</sub>-C<sub>middle</sub> 1.39 Å, C<sub>inner</sub>-C<sub>outer</sub> 1.44 Å and C<sub>outer</sub>-C<sub>outer</sub> 1.35 Å [48]. These data are again in good agreement with the *ab initio*

results shown in Table 1, except for the Fe-X distance (0.08 Å difference). Experimental information on the Fe-N and Fe-S bonds in high spin iron(II)-porphyrin moieties was again obtained using crystallography and EXAFS spectroscopy [51] revealing that the calculated Fe-N bond length (quintet state in Table 1) is in agreement with the experimental value of 2.08 Å [51], while omission of polarization functions (d-orbitals) on the sulfur atom in the *ab initio* calculations again incorrectly lengthens the Fe-S bond: 2.66 Å theoretically (Table 1) compared to 2.34 Å experimentally [51].

*Forcefield methods* Comparison of the bond lengths in Table 1 suggests that the heme moiety parametrized in the CHARMM forcefield probably represents an average between the low spin (doublet) state and the high spin (sextet) state of the heme. Still, the CHARMM geometry reveals some marked differences from both the low spin and the high spin states presented in Table 1, i.e., both the distances between the pyrrole nitrogen atoms and the inner carbon atoms (N-C<sub>inner</sub>), and the iron-sulfur bond (Fe-S) are considerably shorter in the CHARMM geometry (Table 1). Recently, preliminary iron-porphyrin parameters for the MM2 forcefield [47] for several spin states were proposed [48]. The adjusted forcefield was capable of reproducing the experimental geometry for a variety of spin states (see above) closely (bonds within 0.015 Å, bond angles within 1.5°, and torsion angles within 4°). However, all electronic properties were ignored (e.g., all electrostatic parameters were set to zero [48]). As will be discussed below, ignoring the electronic properties of a porphyrin moiety is hardly warranted.

#### *Charge distribution in iron-porphyrin complexes*

The differences in the charge distribution of the various spin states of an iron-porphyrin complex are small. Differences between different complexes and different computational methods will be discussed below.

*Iron(III)-S-methyl-porphyrin complex* The calculated charge distribution of the (sextet) ground state of the iron(III)-S-methyl-porphyrin complex is shown in Figure 2. The axial ligand distorts the fourfold symmetry of the charge distribution only slightly. The singly occupied MOs of the various *ab initio* calculated spin states of the iron(III)-S-methyl-porphyrin complex are shown in Figure 3. In the doublet and quartet spin state, the charges are slightly different (0.01–0.11 electrons) from the charges in the sextet spin state

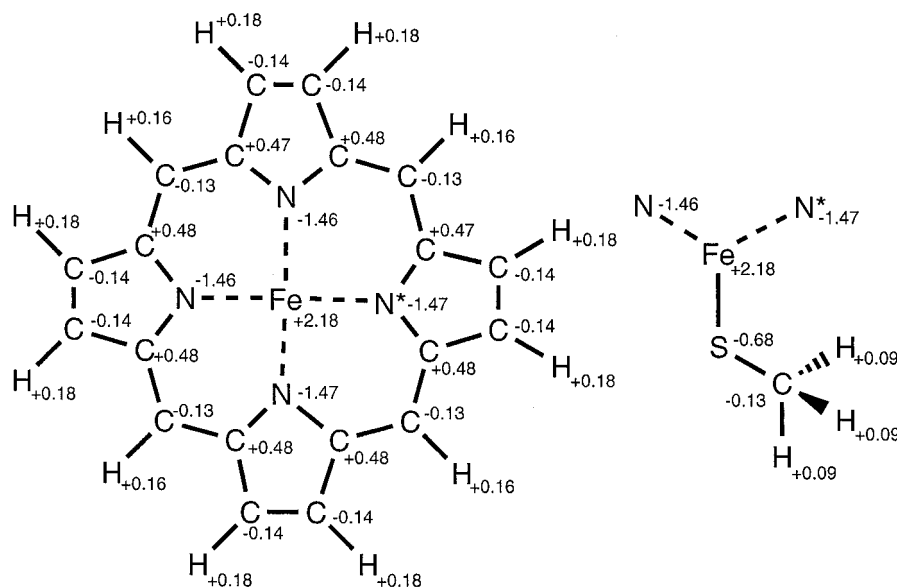


Figure 2. Charge distribution of the (sextet) ground state of the iron(III)-S-methyl-porphyrin complex (in electrons).

(Figure 2). The largest differences are present in the charges of the iron atom, the sulfur atom, the pyrrole nitrogen atoms and the carbon atoms labeled C<sub>middle</sub> (see Figure 1 for labeling).

In analogy to the recently described manganese(III)-chloro-porphyrin complex [25,26], the charge distribution of the iron-S-methyl-porphyrins is ordered in a circular pattern, as depicted in Table 2.

A higher spin state results in a larger distance between the pyrrole nitrogen atoms (larger N-X, Table 1) and a shorter iron-sulfur bond length (Table 1). In parallel, the charge differences between the various atoms become more pronounced. It is expected that enlarging the basis set even more would make these differences still larger, as can be seen when comparing the present results to those obtained using a smaller basis set on an iron(III)-chloro-porphyrin complex [25,26].

**Iron(II)-S-methyl-porphyrin complex** In the iron(II)-S-methyl-porphyrin complex an additional electron is present compared to the iron(III) complex. This extra electron is delocalized over the complete molecule, thereby merely reducing the total positive charge on the iron atom from +2.18 to +1.72 (Table 2). The charge distribution, however, remains ordered in a circular pattern. For the quintet state of the iron(II)-S-methyl-porphyrin complex, the MOs containing an unpaired electron are  $d_{yz}$  (−0.4759 Hartree),  $d_{xz}$  (−0.4686 Hartree),  $d_{x^2}$  (−0.4400 Hartree) and  $d_{z^2-y^2}$  (−0.4323 Hartree).

**Forcefield methods** Table 2 summarizes the differences in charge distribution between the sextet iron(III)-S-methyl-porphyrin and quintet iron(II)-S-methyl-porphyrin complexes obtained with ab initio methods, together with the charges as used in the CHARMm forcefield and a new set of charges used to augment the Discover forcefield.

In general, the charge distributions used in the molecular mechanical calculations are very different from those obtained from ab initio calculations. Although in the P450 protein model [39] the charge on the central iron atom (CHARMm forcefield) is comparable to the charge obtained for the iron atom in the ab initio calculated iron(III)-S-methyl-porphyrin complex, the charges on the pyrrole nitrogen atoms and the carbon atoms differ significantly, resulting in the absence of a pattern with alternating charges. Although the new set of parameters for the Discover forcefield [45] does yield a charge distribution with concentric rings of alternating charge, our results, which we believe to be lower bounds to the charges, suggest that this forcefield severely underestimates the charges on the different atoms. The recently proposed iron-porphyrin parameters [48] for the MM2 forcefield [47] ignore all electronic properties of the system. Although this latter set of parameters reproduces the experimental geometries for a variety of spin states of low spin iron-porphyrins closely, the omission of electrostatic parameters will considerably influence the results of heme-protein modeling studies such as

LUMO	↑↓	-0.0014	LUMO	↑↓	-0.0018	LUMO	↑↓	-0.0079
HOMO	↑↓	-0.2384	HOMO	↑↓	-0.2394	HOMO	↑↓	-0.2386
$d_{z^2-y^2}$	↑	-0.7628	$d_{x^2}$	↑	-0.6861	$d_{x^2}$	↑	-0.6688
$d_{x^2}$	↑	-0.7931	$d_{xz}$	↑	-0.7597			
$d_{xy}$	↑	-0.8480	$d_{xy}$	↑	-0.7654			
$d_{xz}$	↑	-0.8487						
$d_{yz}$	↑	-0.8762						
sextet ( $S=5/2$ )			quartet ( $S=3/2$ )			doublet ( $S=1/2$ )		

Figure 3. Electronic configuration (with orbital energies) of the sextet, quartet and doublet spin states of the iron(III)- *S*-methyl-porphyrin complex. Only the singly occupied MOs and the HOMO and LUMO are shown (energies in Hartree; HOMO = highest occupied MO, LUMO = lowest unoccupied MO).

docking experiments. As charges (and dipole moments generated by charges) are very important for reactivity in proteins, it is not unlikely that reparametrization of the forcefields making the charge differences between atoms in different rings (cf. Table 2) more extreme would give a better description of the heme moiety in protein modeling and would have a large influence on docking experiments in models of the P450 active site.

#### Geometries and energies of iron-oxo-porphyrin complexes

Calculations on one sextet and one quartet spin state of the iron(III)-oxo-*S*-methyl-porphyrin complex have been completed. The geometries of the porphyrin moiety of these two spin states differ only slightly and are very similar ( $\pm 0.01$  Å) to the geometry of the sextet spin state of the iron(III)-*S*-methyl-porphyrin complex (Figure 1 and Table 1). The geometry optimization for the quintet spin state of the iron(II)-oxo-*S*-methyl-porphyrin complex converged very poorly. Table 3 gives the energies and some geometric properties for the geometry optimized quartet and sextet spin states of the iron(III)-oxo-*S*-methyl-porphyrin complex and the partially geometry optimized quintet spin state of the iron(II)-oxo-*S*-methyl-porphyrin complex.

**Iron(III)-oxo-*S*-methyl-porphyrin complex** Table 3 shows that the iron atom is located under the plane of the porphyrin moiety in the direction of the *S*-methyl axial ligand (Fe-X). The sextet spin state of the iron(III)-oxo-*S*-methyl-porphyrin complex is more stable than the quartet spin state. In the sextet ground state the iron atom is further from the porphyrin plane

(Fe-X) while the iron-sulfur bond (Fe-S) is shortened with respect to the quartet state. In the sextet state the nitrogen atoms are 0.05 Å more distant (N-X in Table 3) from the geometric center of the porphyrin (X) than in the quartet spin state. These differences are comparable to those observed between the various spin states of the iron(III)-*S*-methyl-porphyrin complex (Table 1). Due to the presence of a bridged oxygen atom, the iron atom is drawn (approximately 0.16 Å) into the plane of the porphyrin moiety relative to the iron(III)-*S*-methyl-porphyrin complex (compare Fe-X in Tables 1 and 3). In the sextet spin state the oxygen atom, is closer to both the nitrogen and the iron atom than in the quartet spin state (cf. N-O and Fe-O in Table 3). The atomic spin populations on the bridged oxygen atom were 0.040 and 0.046 for the quartet and sextet states of the iron(III)-oxo-*S*-methyl-porphyrin complex, respectively, which is close to the spin population on the oxygen atom observed by Yamamoto and Kashiwaga [22] (0.03) for RHF calculations on the iron-oxo-porphyrin  $\pi$ -radical cation [22].

**Iron(II)-oxo-*S*-methyl-porphyrin complex** The quintet spin state of the iron(II)-oxo-*S*-methyl-porphyrin complex differs in several aspects from the sextet spin state of the iron(III)-oxo-*S*-methyl-porphyrin complex: the iron atom is further away from the porphyrin ring (Fe-X), the cavity in the porphyrin ring is larger (N-X), and the iron-sulfur bond (Fe-S) is longer (see Table 3). These results are in strong analogy with the data obtained for the oxygen-free complexes (Table 1). Reducing the sextet iron(III)-oxo-*S*-methyl-porphyrin complex with one electron to obtain the

Table 2. Average atom charges in iron(III)-*S*-methyl-porphyrin, iron(II)-*S*-methyl porphyrin and iron(III)-*S*-cysteiny-porphyrin complexes<sup>a</sup>

Program used Spin state	GAMESS-UK		CHARMm undefined <sup>d,e</sup>	Discover undefined <sup>d,f</sup>
	Sextet <sup>b</sup>	Quintet <sup>c</sup>		
Iron atom	+2.18	+1.72	+2.00	+1.28
Pyrrole nitrogen atoms	−1.47	−1.43	−0.40	−0.50
Inner ring carbon atoms (C <sub>inner</sub> )	+0.47	+0.52	0.00	+0.10
Outer ring carbon atoms (C <sub>middle</sub> )	−0.13	−0.17	−0.13	−0.10
Outer ring carbon atoms (C <sub>outer</sub> )	−0.14	−0.17	0.00	−0.05
Ring of hydrogen atoms	+0.17	+0.14	+0.13	Na <sup>g</sup>
Sulfur atom	−0.68	−0.90	NA <sup>g</sup>	−0.10

<sup>a</sup> Atom types refer to Figure 1 and ‘rings’ refer to Refs. 25 and 26.

<sup>b</sup> Iron(III)-*S*-methyl-porphyrin complex, see also Figure 2 for the detailed charge distribution.

<sup>c</sup> Iron(II)-*S*-methyl-porphyrin complex, anion of the iron(III)-*S*-methyl-porphyrin complex.

<sup>d</sup> Probably iron(III)-*S*-cysteiny-porphyrin complex.

<sup>e</sup> Taken from Ref. 39.

<sup>f</sup> Taken from Ref. 45.

<sup>g</sup> NA = not available.

quintet iron(II)-oxo-*S*-methyl-porphyrin complex displaces the iron atom 0.24 Å further out of the plane of the porphyrin moiety. This displacement might well be the reason for the poor convergence observed for the quintet state, since additional strain is introduced on the system as the bridged oxygen atom is pulled towards the porphyrin ring by the iron atom. The influence on the Fe-O bond strength remains unclear. Furthermore, the iron-nitrogen bond in the iron(II)-oxo-*S*-methyl-porphyrin complex (Fe-N = 2.15 Å) is the longest observed so far in our calculations, possibly caused by the interfering position of the bridged oxygen atom.

#### Charge distribution in iron(III)-oxo-porphyrin complexes

The charge distribution of the (sextet) ground state of the iron(III)-oxo-*S*-methyl-porphyrin complex is shown in Figure 4. The axial ligand and the bridged oxygen atom distort the twofold symmetry of the charge distribution only slightly. In the sextet ground state the MOs containing a single electron are  $d_{xy}$  (−0.8676 Hartree),  $d_{yz}$  (−0.8435 Hartree),  $d_{xz}$  (−0.7968 Hartree),  $d_{x^2-z^2}$  (−0.7869 Hartree) and  $d_{y^2}$  (−0.7820 Hartree), and in the quartet state the singly occupied MOs are  $d_{xz}$  (−0.7540 Hartree),  $d_{yz}$  (−0.7248 Hartree) and  $d_{z^2}$  (−0.7120 Hartree). In the quartet state of the iron(III)-oxo-*S*-methyl-porphyrin complex, the charges are slightly different (0.01–0.21 electrons) from the charges in the sextet spin state (Figure 4); the largest differences concern the charges

of the iron atom, the sulfur atom, the pyrrole nitrogen atoms and the carbon atoms labeled C<sub>middle</sub> (Figure 1). This is in analogy with the results obtained for the different spin states of the iron(III)-*S*-methyl-porphyrin complex.

The axial cysteine residue has been suggested to release electron density through the iron into the O<sub>2</sub> bound at the opposite side of the porphyrin ring, thereby making O<sub>2</sub> more electron rich [51], while reducing the formal oxidation state of iron, and possibly creating a radical cation on the axial sulfur ligand [52]. As is evident from the ground state charge distributions of the iron(III)-*S*-methyl-porphyrin and iron(III)-oxo-*S*-methyl-porphyrin complexes, the oxygen atom has a charge of −0.55, which is in agreement with the above-mentioned suggestion. However, the axial sulfur atom is also negatively charged (Figures 2 and 4) and the sulfur ligand is therefore *not* a radical cation. The oxidation state of the iron is very similar in all complexes calculated (even after adding an additional electron, see Table 2).

The charge distribution of the various spin states of the iron(III)-oxo-*S*-methyl-porphyrin is ordered in a distorted circular pattern, similar to the one depicted in Table 2 for the sextet ground state of the iron(III)-*S*-methyl-porphyrin complex. The average charges for the sextet spin state are +2.12 for the iron atom, −1.31 for the pyrrole nitrogen atoms, +0.46 for the carbon atoms in the inner ring (C<sub>inner</sub>), −0.11 and −0.13 for the different carbon atoms in the outer ring (C<sub>middle</sub>



Table 3. Geometric properties of quartet and sextet spin states of iron(III)-oxo-*S*-methyl-porphyrin and the quintet spin state of its anion (iron(II)-oxo-*S*-methyl-porphyrin)<sup>a,b</sup>

Spin state	Quartet	Sextet	Quintet <sup>c,d</sup>	Spin state	Quartet	Sextet	Quintet <sup>c,d</sup>
Fe-X (Å)	0.21	0.29	0.53	Fe-O-N (*) <sup>e</sup>	65.9	69.3	69.1
N-X (Å)	2.00	2.05	2.08	N*-Fe-S (*) <sup>e</sup>	99.6	101.5	109.4
Fe-N (Å)	2.01	2.07	2.15	Fe-S-C <sub>thiol</sub> (*)	114.2	113.8	114.0
Fe-S (Å)	2.59	2.54	2.63	Fe-NC <sub>inner</sub> (*)	127.0	126.1	124.5
Fe-O (Å)	2.04	2.02	2.16	N*-O-Fe-N <sup>⊥</sup> (*) <sup>e</sup>	266.4	265.1	261.7
N-O (Å)	1.59	1.56	1.52				

<sup>a</sup> N-C<sub>inner</sub> = 1.40 Å; C<sub>inner</sub>-C<sub>outer</sub> = 1.44 ± 0.01 Å; C<sub>inner</sub>-C<sub>middle</sub> = 1.39 ± 0.01 Å; C<sub>outer</sub>-C<sub>outer</sub> = 1.35 Å; S-C<sub>thiol</sub> = 1.88 Å; C-H = 1.08 Å; N-C<sub>inner</sub>-C<sub>outer</sub> = 108.3 ± 0.8°; C<sub>inner</sub>-C<sub>outer</sub>-C<sub>outer</sub> = 108 ± 0.2°; N-C<sub>inner</sub>-C<sub>middle</sub> = 125.2 ± 0.1°; N\*-Fe-S-C<sub>thiol</sub> = 0.0°.

<sup>b</sup> E<sub>total</sub>(quartet) = -2751.5689 Hartree (GAMESS-UK); E<sub>total</sub>(sextet) = -2751.6690 Hartree (GAMESS-UK).

<sup>c</sup> Anion of iron(III)-oxo-*S*-methyl-porphyrin (iron(II)-oxo-*S*-methyl-porphyrin).

<sup>d</sup> The quintet spin state of the iron(II)-oxo-*S*-methyl-porphyrin complex shows very poor convergence. The geometry shown is not completely geometry optimized (see text).

<sup>e</sup> N\* indicates the nitrogen atom to which the oxygen is bridged. The *S*-methyl group is also located directly under this nitrogen atom. N<sup>⊥</sup> is indicated in Figure 1.

and C<sub>outer</sub>, respectively), and +0.17 for the hydrogen atoms (cf. Table 2).

In an early INDO study on an iron-oxo-porphyrin complex (with the oxygen atom bound above the iron atom instead of bridged between the iron atom and one of the pyrrole nitrogen atoms), Loew et al. [53] found a charge of only +1.58 on the iron atom. In a more recent molecular dynamics investigation of a similar iron-oxo-porphyrin complex (using the Discover forcefield [44]), the same forcefield parameters were used for the iron-porphyrin complex with and without an oxygen atom bound [46]. As is evident from both the geometric properties (Tables 1 and 3) and the charges (Figures 2 and 4), this is not warranted.

## Conclusions

The ground states for both the iron(III)-*S*-methyl-porphyrin complex and the iron(III)-oxo-*S*-methyl-porphyrin complex appear to be sextet spin states. The sextet ground state found for the iron(III)-*S*-methyl-porphyrin complex in vacuum (0.197 Hartree lower in energy than the most stable doublet spin state) indicates that in a hydrophobic environment (vacuum) a high spin (sextet) state is preferred. Experimentally a shift from low spin to high spin is observed upon binding of the substrate, resulting in a high spin state for the (hydrophobic) substrate-bound state of the active site of P450 [5,10]. Combined, this indicates that the ab initio calculations on the iron(III)-*S*-methyl-

porphyrin complex in vacuum give a correct representation for the (hydrophobic) substrate-bound state of the active site of P450. Test calculations including a water molecule above the porphyrin moiety indicated recently that upon incorporation of water a lower spin state (doublet or quartet) will become the ground state of the system [50].

The ground states of the respective anions of both complexes (iron(II)-*S*-methyl-porphyrin and iron(II)-oxo-*S*-methyl-porphyrin, respectively) are probably quintet spin states, although more calculations on different spin states are necessary to be sure that the quintet spin states are the ground states for these complexes. These theoretical investigations indicate the necessity of calculating various spin states since the spin state of the ground state is not directly apparent. In previous calculations often the higher spin states were neglected or were predicted not to be the ground state (e.g., INDO predicts a quartet ground state [53]).

Comparison of the geometries of the different spin states obtained with ab initio methods on the one hand and the geometries obtained from forcefield calculations on the other hand indicates that for the parametrization of the heme moiety in the CHARMM forcefield probably an average between the low spin doublet state and the high spin sextet state was used. Still, there are some remarkable differences between the ab initio and CHARMM geometries. Recently obtained iron-porphyrin parameters for the MM2 forcefield reproduce the experimental geometry closely, although all electronic properties are ignored [48]. The ab initio

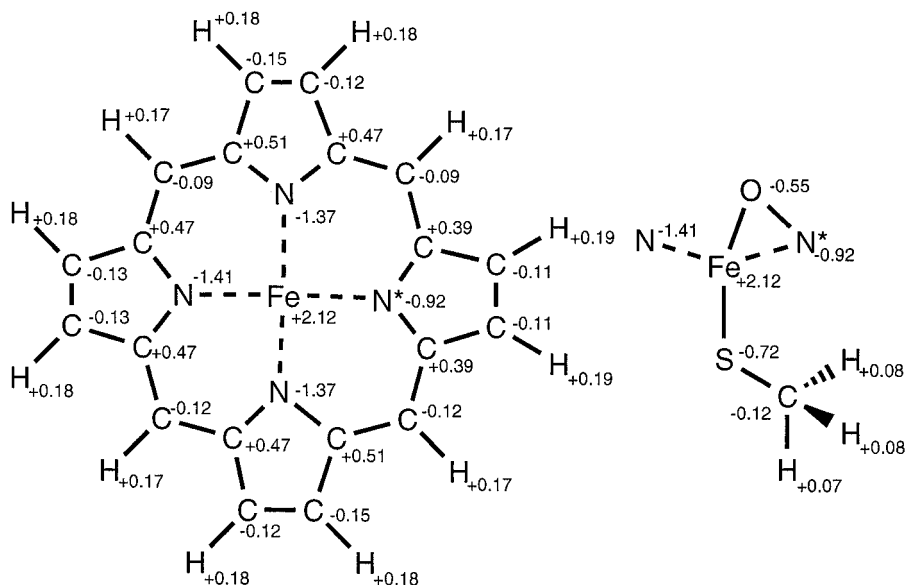


Figure 4. Charge distribution of the (sextet) ground state of the iron(III)-oxo-S-methyl-porphyrin complex (in electrons).

geometries are in very good agreement with experimentally obtained geometries, except for the length of the iron-sulfur bond. This is probably the result of the omission of polarization functions (d-orbitals) on the sulfur atom during the geometry optimization. Since forcefields are developed using experimental data, it is not surprising that the iron-sulfur bond length is better reproduced with forcefield methods.

The *ab initio* charge distribution in all obtained complexes can be described by a series of concentric rings of alternating charge, thus allowing a relatively large positive charge on the iron atom. However, *ab initio* calculations on low spin porphyrins using a small basis set will underestimate the differences present in the charge distribution of the porphyrin moiety considerably. Forcefields often underestimate the charge differences between the iron atom and the porphyrin moiety (e.g. the CHARMM forcefield [39]) or ignore these charges completely (e.g. modified MM2 forcefield [48]). Only the new parameter set for the Discover forcefield [45] approximates the *ab initio* charge distribution pattern of concentric circles with alternating charge, although the charges on the atoms are severely underestimated. The omission or underestimation of charges on the porphyrin moiety will have considerable effects when used in homology modeling and docking experiments. As charges (and dipole moments generated by charges) are very important for reactivity in proteins, it is not unlikely that reparametrization of the forcefields making the

charge distribution of the heme moiety more extreme would give a better description of the heme moiety in protein modeling and would have a large influence on docking experiments in models of the P450 active site. The influence of the charge distribution described in this work for the heme moiety on the orientations of the substrates docked in a protein model, like a recent protein model for P450 2D6 [39], still has to be examined.

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