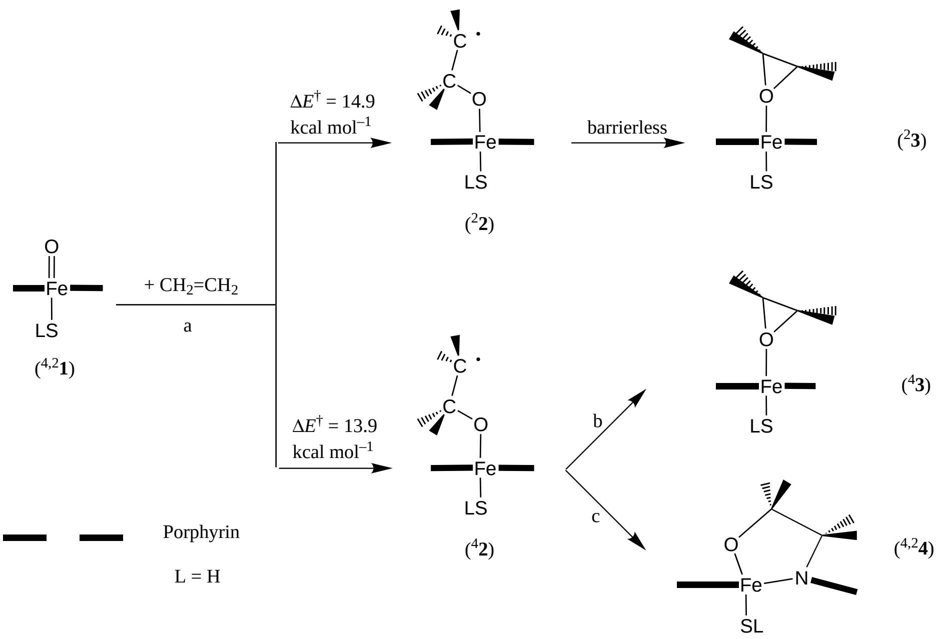
How Does Ethene Inactivate Cytochrome P450 En Route to Its Epoxidation? A Density Functional Study[**]

Sam P. de Visser, François Ogliaro, and Sason Shaik[*]

Cytochrome P450 is one of the principal enzymes in the "oxygen machinery" of biosystems. The enzyme functions as a mono-oxygenase in the biosynthesis of steroids, and in the transformation of hydrophobic molecules such as alkanes into alcohols, which are water soluble and thus secreted from the body.[1–3] A large turnover number of the enzyme is essential, and is limited inter alia by side reactions, which form dead-end compounds, so called suicidal complexes. Therefore, the enzyme itself is inactivated in the course of the vital processes. Identification of the mechanisms that "poison" the enzyme is an important task and constitutes the focus herein.



Scheme 1. Schematic description of the mechanisms of ethylene epoxidation (ref. [14]) and suicidal complex formation.

Olefin epoxidation is one of the processes that have been implicated to generate suicidal complexes as dead-end products.[1, 3] Indeed, heme-type enzymes were shown to produce suicidal products in reactions with pharmaceutical and environmental agents.[4] In particular, terminal olefins and acetylenes react by N-alkylation of the heme-group. The *N*-alkyl hemes have been shown to result in hepatic porphyrias accompanied by the formation of the so-called "green pigment". The formation of these suicidal complexes was observed in catalytic reactions of cytochrome P450[4–10] as well as chloroperoxidases (CPO).[11–13] Such a suicidal complex is schematically depicted as **4** in Scheme 1. The mechanism and energetics of its formation in relation to those for the epoxidation process itself are addressed in the present paper by means of density functional theoretical calculations (DFT).

The epoxidation mechanism was recently[14] investigated using the simplified iron–oxo species (**1**, L = H) and ethene (Scheme 1). Starting from either the quartet (high spin) ^{4}**1** or the doublet (low spin) ^{2}**1** species, the bond-activation step a, with barriers ($\Delta E^{\neq}$) of approximately 14–15 kcal mol^{-1}, leads to radical complex intermediates **2**, which upon ring closure lead to the epoxide complex **3**. The high-spin intermediate ^{4}**2** was shown[1, 14] to be responsible for stereochemical scrambling and formation of *cis*/*trans* mixtures of the epoxides. Depending on the lifetime of ^{4}**2**, the suicidal reaction c (Scheme 1) may be competitive with the ring-closure step b (Scheme 1). Other side reactions of ^{4}**2** are possible, but the present study focuses on the suicidal reaction.

The following calculations were performed by using the Jaguar 4.0 program package[15] with the unrestricted hybrid density functional (UB3LYP) in combination with an LACVP basis set on iron and a 6-31G basis set on the rest of the atoms. Previous calculations with larger basis sets have shown that these types of basis sets are sufficient to give good qualitative results.[14, 16]

Since the previous study[14] revealed that the low-spin intermediates ^{2}**2** form the epoxide complex ^{2}**3** in a barrierless fashion (Scheme 1), we focus herein on the suicidal reaction nascent from ^{4}**2**. The intermediate ^{4}**2** has two electromeric forms, which differ in the oxidation state of the iron. The two forms are depicted in Figure 1 along with the orbital diagram of the key valence orbitals. These are the $\delta(d_{x^2-y^2})$ orbital and the $\pi^*(d_{xz}, d_{yz})$ orbitals on iron, the porphyrin a_{2u}-type orbital, the hybrid orbital of the radical of the intermediate ($\phi_{C^\cdot}$), and two vacant d orbitals on the iron. The ^{4}**2**-IV and ^{4}**2**-III intermediates differ in the occupation of the π^* and a_{2u} orbitals as indicated in Figure 1.

The electronic structure of the suicidal complex ^{4}**4** is quite different from that of the radical complexes ^{4}**2**-III and ^{4}**2**-IV. It involves $\delta(d_{x^2-y^2})^2$ $\pi^*(d_{xz})^1$ $\pi^*(d_{yz})^1$ $\sigma^*(d_{xy})^1$ $a_{2u}{}^2$ occupation (Figure 1). Thus, the transformation of **2** into **4** is attended by population of the high-lying $\sigma^*(d_{xy})$ orbital that involves antibonding interactions in the four Fe–N linkages in the iron–porphyrin plane. As shall be seen later it is this reorganization that is responsible for the formation of the suicidal complex and for gauging the corresponding suicidal barrier.

---

[*] Prof. S. Shaik, Dr. S. P. de Visser, Dr. F. Ogliaro
Department of Organic Chemistry and
The Lise Meitner-Minerva Center
for Computational Quantum Chemistry
The Hebrew University of Jerusalem
91904 Jerusalem (Israel)
Fax: (+972) 2-658-4680
E-mail: sason@yfaat.ch.huji.ac.il

[**] The research is supported in part by the ISF and in part by the Ministry of Science, Culture, and Sport. F.O. acknowledges the European Union for a Marie Curie Fellowship.

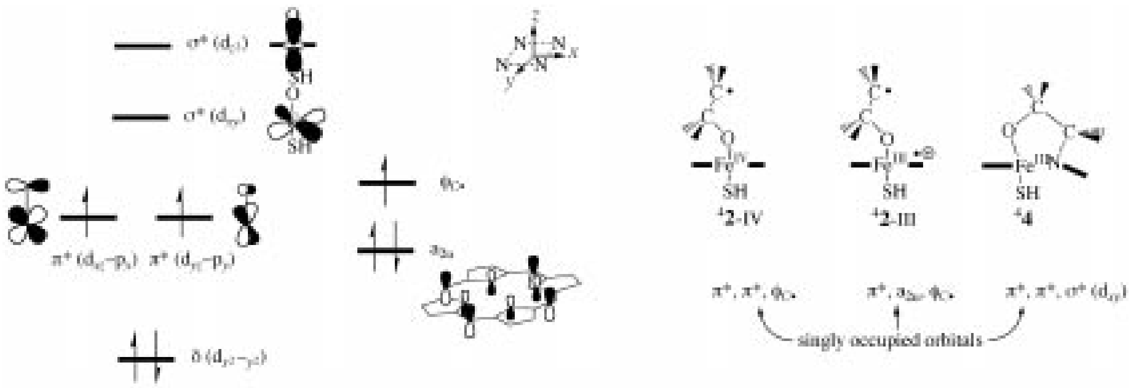


Figure 1. The highest occupied and lowest virtual orbitals with occupations for ^{4}**2**-IV (left). The coordinate axes are drawn vis-à-vis the nitrogen atoms of the porphyrin ring. The singly occupied orbitals for the various complexes (^{4}**2** and ^{4}**4**) are written on the right hand side.

Figure 2 depicts the optimized geometries of the suicidal complex (^{2,4}**4**), the Fe^{III} (^{4}**2**-III) and Fe^{IV} (^{4}**2**-IV) radical complexes in the most stable *anti* conformations, and the corresponding *gauche* conformations, ^{4}**2**-III′ and ^{4}**2**-IV′, which are local minima on the rotational surface. Formation of the suicidal complex disrupts the planarity in the porphyrin ring. The nitrogen atom involved in the ring closure is lifted out of the plane of the remaining nitrogen atoms by 6.9°, leading to puckering of the corresponding pyrrole ring. This ring in turn loses its π-bonding as indicated by the C–N bond of 1.439 (1.474) Å compared with the other pyrrole rings in which these bond lengths are approximately 1.37 Å. Additionally, the Fe–O distance is augmented to 1.866 Å with respect to the radical complexes **2**-III and **2**-IV.

The most significant change in ^{4}**4** relative to its precursors is the Fe–N distance in the suicidal ring; 2.438 Å compared with 2.016 Å or 2.019 Å (on average) for ^{4}**2**-IV and ^{4}**2**-III, respectively. This long Fe–N bond is a result of the σ*(d_{xy}) occupancy and therefore the formation of ^{4}**4** is attended by C–N bond formation at the expense of Fe–N displacement and some rupture of the π-bonding in the pyrrole ring that involves the nitrogen site of attack. The data for the low-spin form of the suicidal complex ^{2}**4** are shown alongside the high-spin form in Figure 2. In ^{2}**4**, the σ*(d_{xy}) orbital is vacant, while the complex still is in the Fe^{III} oxidation state with a $\delta^2\,\pi^*(d_{yz})^2\,\pi^*(d_{xz})^1$ configuration. In accord, the Fe–N distance is now close to normal (2.195 Å), albeit somewhat elongated by the nitrogen pyramidality. The low-spin ^{2}**4** is

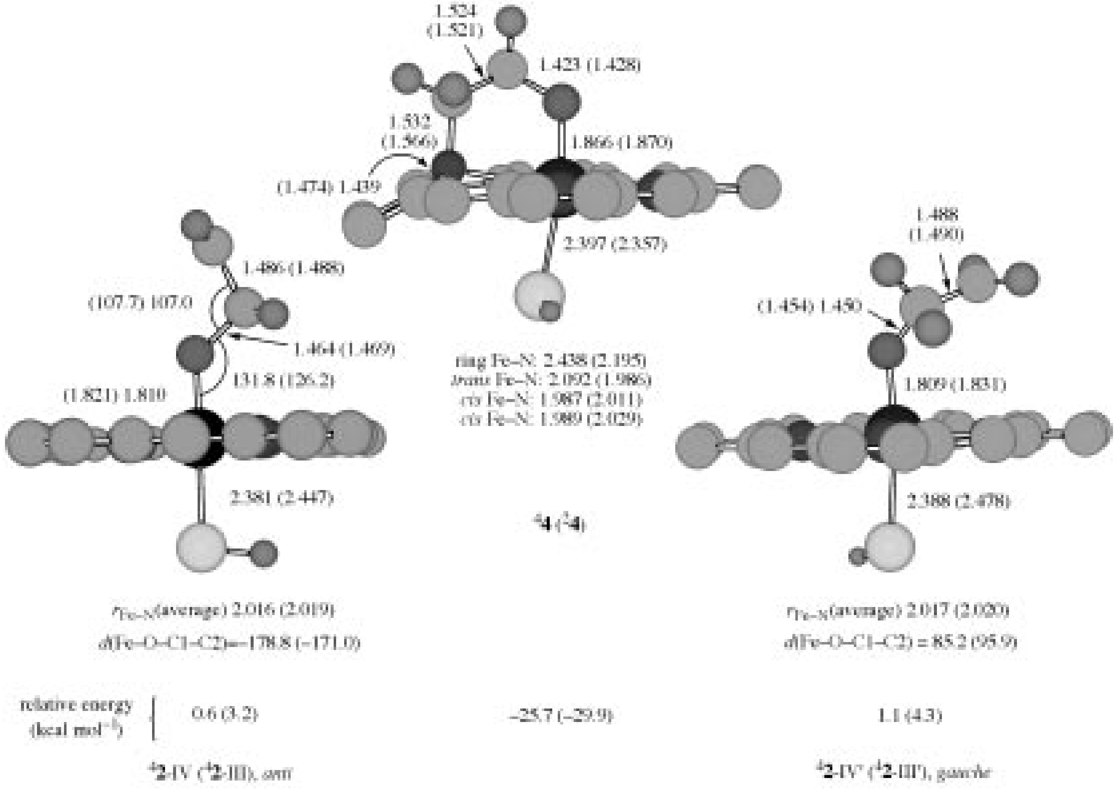


Figure 2. Optimized geometries of ^{4}**2**-IV (^{4}**2**-III), ^{2,4}**4** (middle) and ^{4}**2**-IV′ (^{4}**2**-III′) (right). Bond lengths are reported in angstroms and bond angles in degrees. The relative energies of the clusters are given with respect to the sum of the reactants (^{4}**1**+CH_2=CH_2) and are summarized at the bottom of the figure.

4.2 kcal mol^{-1} more stable than the high-spin suicidal complex, ^{4}**4**.

Figure 3 is an energy profile for a geometry scan from the radical complex toward the suicidal complex. Each point represents a full optimization with one fixed coordinate representing the C–N bond distance. The optimized radical complexes in the *gauche* orientation have a C–N interatomic distance of 3.306 Å (^{4}**2**-III′) and 3.245 Å (^{4}**2**-IV′), as shown in Figure 2. The *gauche* conformers are local minima and are 1.1 (Fe^{III}) and 0.5 kcal mol^{-1} (Fe^{IV}) higher in energy than their *anti* conformers. Adiabatic excitation from the lowest state (^{4}**2**-IV′) by 11.0 kcal mol^{-1} leads to the excited state that has a singly occupied $\sigma^*(d_{xy})$ orbital. This state has the correct electronic configuration and therefore falls in a barrierless fashion into the suicidal complex (^{4}**4**).

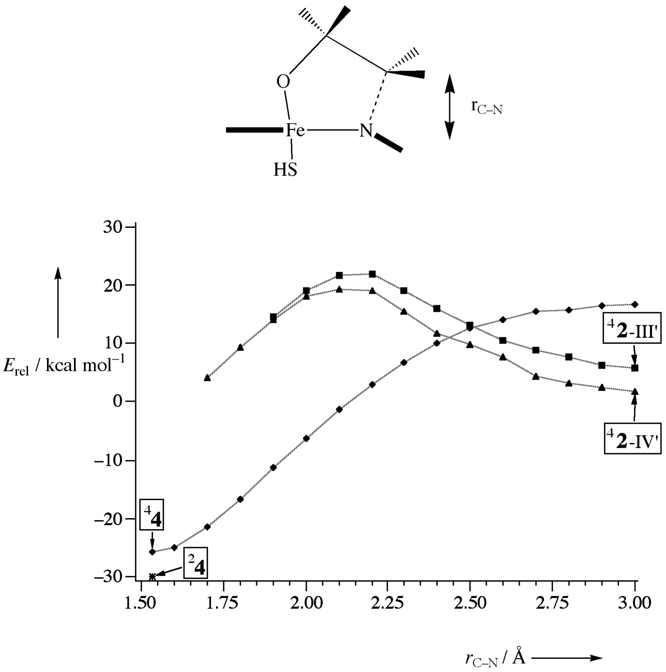


Figure 3. Energy profile for the reaction of ^{4}**4** leading to ^{4}**2**-IV′ and ^{4}**2**-III′. Each point represents a full geometry optimization with a fixed Fe–N bond distance. The energies are given relative to the sum of the reactants (kcal mol^{-1}). The relative energy of the ^{2}**4** complex has been marked with an asterisk.

In contrast, the ^{4}**2**-IV′ and ^{4}**2**-III′ states rise up in energy along the same coordinate. An approximate real crossing point[17] between the suicidal energy profile and the profiles of the two radical states, was estimated to occur at $r_{C-N} \approx 2.7-2.8$ Å. Relative to the onset energies of the *anti* conformers ^{4}**2**-III and ^{4}**2**-IV, the height of the crossing point is approximately 10–12 kcal mol^{-1}. The suicidal barrier will be smaller as a result of avoided crossing.[17] While a multireference configuration interaction treatment will be required eventually to calculate the barrier for the suicidal process, this is still beyond reach. Therefore, the value determined here can be taken as an upper bound estimate of the barrier for the formation of the suicidal complex on the high-spin surface.[17]

In order to pinpoint the intermediate-radical state responsible for the suicidal process, we must consider the barriers of the various processes experienced by ^{4}**2**-III and ^{4}**2**-IV vis-à-vis the suicidal barrier as summarized in Table 1. It is clear that the ^{4}**2**-IV intermediate will participate mostly in epoxide formation. In contrast, for ^{4}**2**-III the suicidal barrier and the epoxidation barrier are approximately equal and therefore the ^{4}**2**-III intermediate will participate in two competing ring-closure processes; one leads to epoxide ^{4}**3** and the other to the suicidal complex ^{4}**4**. Furthermore, it is seen that the C–C rotational barrier of ^{4}**2**-III, responsible for stereochemical scrambling of the epoxide is lower than either ring-closure or suicidal-complex formation. Therefore, ^{4}**2**-III will participate in at least three competing reactions. Another event that may result in the low-spin suicidal complex ^{2}**4** must involve spin-state crossover from the quartet surfaces in Figure 3 to the doublet surface, and end in the ^{2}**4** complex. A spin crossover that leads to ^{2}**4** is likely to take place near the ^{4}**4**/^{4}**2**-III and ^{4}**4**/^{4}**2**-IV crossover points.[18]

Table 1. Barriers for individual processes starting at the radical complexes ^{4}**2**.[a]

Reactant	Product	$\Delta E^{\neq}$ [kcal mol^{-1}]	Reaction
^{4}**2**-III	^{4}**2**-III′	1.1	internal C–O rotation
^{4}**2**-III	^{4}**2**-III	1.4	internal CH$_2$ rotation
^{4}**2**-III	^{4}**3**	7.2	epoxidation
^{4}**2**-III	^{4}**4**	≤ 10[17]	suicidal complex
^{4}**2**-IV	^{4}**2**-IV′	0.5	internal C–O rotation
^{4}**2**-IV	^{4}**2**-IV	1.4	internal CH$_2$ rotation
^{4}**2**-IV	^{4}**3**	2.3	epoxidation
^{4}**2**-IV	^{4}**4**	≤ 10[17]	suicidal complex

[a] The barrier leading from ^{4}**1** to ^{4}**2**-IV is 13.9 kcal mol^{-1} (Scheme 1). Equilibration of ^{4}**2**-IV and ^{4}**2**-III is expected to be fast.[14]

In conclusion, theoretical studies on a model P450 system with ethene have shown that competing reactions can take place, leading to either epoxide or suicidal products.[19] Both these products are nascent from the same initial intermediate radical complex **2**. The ^{4}**2**-III complex has a substantial barrier for epoxide formation and a roughly equal barrier for crossing to the suicidal complex. This implies that competing epoxidation and suicidal-complex formation reactions can take place from the ^{4}**2**-III isomer. In the polar environment of the protein pocket and polar solvents such as water, this particular state is favored relative to the ^{4}**2**-IV isomer,[20] thus enhancing the suicidal-complex formation in polar solvents. Moreover, the same intermediate ^{4}**2**-III, which is responsible for *cis*/*trans* scrambling in epoxidation processes[1, 14, 21] is implicated in this study as a source of destruction of the enzyme cytochrome P450.[22] This specificity could hopefully be probed by experimental means.

Received: November 15, 2000 [Z16115]

---

[1] J. T. Groves, Y.-Z. Hang in *Cytochrome P450: Structure, Mechanisms and Biochemistry* (Ed.: P. R. Ortiz de Montellano), 2nd ed., Plenum Press, New York, **1995**, chap. 1, p. 3.

[2] I. Schlichting, J. Berendzen, K. Chu, R. M. Stock, S. A. Maves, D. E. Benson, R. M. Sweet, D. Ringe, G. A. Petsko, S. G. Sligar, *Science* **2000**, *287*, 1615.

[3] *The Porphyrin Handbook*, *Vol. 4* (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, San Diego, **2000**; in particular the following chapters: a) D. Mansuy, P. Battioni, chap. 26; b) J. T. Groves, K.

Shalyaev, J. Lee, chap. 27; c) K. S. Suslick, chap. 28; d) Y. Watanabe, chap. 30; e) B. Meunier, A. Robert, G. Pratviel, J. Bernadou, chap. 31.

[4] P. R. Ortiz de Montellano, H. S. Beilan, K. L. Kunze, B. A. Mico, *J. Biol. Chem.* **1981**, *256*, 4395.

[5] K. L. Kunze, B. L. K. Mangold, C. Wheeler, H. S. Beilan, P. R. Ortiz de Montellano, *J. Biol. Chem.* **1983**, *258*, 4202.

[6] P. R. Ortiz de Montellano, B. L. K. Mangold, C. Wheeler, K. L. Kunze, N. O. Reich, *J. Biol. Chem.* **1983**, *258*, 4208.

[7] T. Mashiko, D. Dolphin, T. Nakano, T. G. Traylor, *J. Am. Chem. Soc.* **1985**, *107*, 3735.

[8] J. P. Collman, P. D. Hampton, J. I. Brauman, *J. Am. Chem. Soc.* **1990**, *112*, 2977; J. P. Collman, P. D. Hampton, J. I. Brauman, *J. Am. Chem. Soc.* **1990**, *112*, 2987.

[9] B. T. Luke, J. R. Collins, G. H. Loew, A. D. McLean, *J. Am. Chem. Soc.* **1990**, *112*, 8686.

[10] Z.-Q. Tian, J. Richards, T. G. Traylor, *J. Am. Chem. Soc.* **1995**, *117*, 21.

[11] A. F. Dexter, L. P. Hager, *J. Am. Chem. Soc.* **1995**, *117*, 817.

[12] P. G. Debrunner, A. F. Dexter, C. E. Schulz, Y.-M. Xia, L. P. Hager, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12791.

[13] H.-I. Lee, A. F. Dexter, Y.-C. Fann, F. J. Lakner, L. P. Hager, B. M. Hoffman, *J. Am. Chem. Soc.* **1997**, *119*, 4059.

[14] S. P. de Visser, F. Ogliaro, N. Harris, S. Shaik, *J. Am. Chem. Soc.*, **2001**, *123*, 3037.

[15] Jaguar 4.0, Schrödinger Inc., Portland, OR, **1998**.

[16] F. Ogliaro, N. Harris, S. Cohen, M. Filatov, S. P. de Visser, S. Shaik, *J. Am. Chem. Soc.* **2000**, *122*, 8977.

[17] Since the two states have the same overall symmetry, they should have avoided the crossing and produced a normal saddle point. However, since the Kohn–Sham method uses a single determinant, the DFT calculation results in a surface crossing. Our attempts to evade the crossing by starting with a guess that had mixed orbitals (of the two species) were not successful. The "real" crossing point was estimated by a series of calculations of the ^{4}**2**-III and ^{4}**4** structures which have the same geometric parameters.

[18] A spin crossover near the minima of ^{4}**2** and ^{2}**2** will simply lead to low-spin epoxidation through the low-energy path available to ^{2}**2** (see Scheme 1).

[19] It is likely that the fate of ^{4}**2**-III is more complex in view of the fact that propylene undergoes an unusual hydrogen/deuterium exchange during its epoxidation. See: J. T. Groves, G. E. Avaria-Neisser, K. M. Fish, M. Imachi, R. L. Kuczkowski, *J. Am. Chem. Soc.* **1986**, *108*, 3837.

[20] a) J. T. Groves, Z. Gross, M. K. Stern, *Inorg. Chem.* **1994**, *33*, 5065; b) M. Filatov, N. Harris, S. Shaik, *Angew. Chem.* **1999**, *111*, 3730; *Angew. Chem. Int. Ed.* **1999**, *38*, 3512.

[21] Z. Gross, S. Nimri, C. M. Barzilay, L. Simkhovich, *J. Biol. Inorg. Chem.* **1997**, *2*, 492.

[22] The ^{2}**2** intermediates have very small barriers for ring closure to epoxide formation (^{2}**3**). If these barriers grant them finite lifetimes, we cannot rule out some bifurcation of ^{2}**2** to the corresponding suicidal complex ^{2}**4**. However, most of the suicidal process is likely to occur from the high-spin intermediate.

Chameleon States: High-Valent Metal–Oxo Species of Cytochrome P450 and Its Ruthenium Analogue[**]

François Ogliaro, Samüel P. de Visser, John T. Groves,* and Sason Shaik*

There is a continuing search for efficient and robust catalysts that can perform monooxygenation of organic compounds.[1] High-valent metal–oxo porphyrin species **1** (Figure 1) constitute such an important family.[2] The members of this family are analogous to the principal oxidant of enzymes such as cytochrome P450 (CP450) or chloroperoxidase (CPO), and this analogy endows the (L)PorM^{z}O species with an added allure. While iron and manganese are the most ubiquitous metal centers in biological systems, ruthenium is present in artificially designed systems which are attractive potent catalysts.[2c] A fundamental question concerns the nature of the ground state of these catalysts, and the relationship between their electronic structure and monooxygenation capability. This is the focus of the present paper which uses density functional (DFT) calculations to compare the electromeric states for [(L)PorM^{z}O] (M = Fe, Ru; z = III–V; L = SH and SCH$_3$). As shall be seen, the Fe and Ru

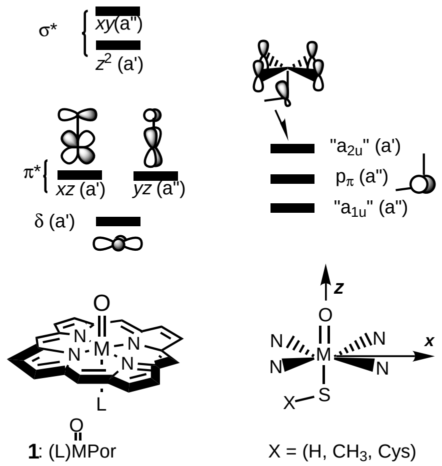


Figure 1. Key orbitals for **1** with thiolate ligands.

---

[*] Prof. S. Shaik, Dr. F. Ogliaro, Dr. S. P. de Visser
Department of Organic Chemistry and
The Lise Meitner-Minerva Center for
Computational Quantum Chemistry
The Hebrew University of Jerusalem
91904 Jerusalem (Israel)
Fax: (+972) 2-658-4680
E-mail: sason@yfaat.ch.huji.ac.il

Prof. J. T. Groves
Department of Chemistry, Princeton University
Princeton, NJ 08544-1009 (USA)
Fax: (+1) 60-9-258-0348
E-mail: jtgroves@pop.princeton.edu

[**] The research in HU was sponsored by the Binational German Israeli Foundation (GIF) and by the Israeli Ministry of Science, Culture and Sport. Partial support by the US National Science Foundation (CHE-9814301) to J.T.G. is acknowledged. F.O. thanks the EU for a Marie Curie Fellowship.

1433-7851/01/4015-2874 $ 17.50+.50/0