

**Supporting Information**  
for

**The mechanism for the reversible addition of oxygen to heme. A  
theoretical CASPT2 study.**

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**Table S1.** The most relevant geometrical parameters for the geometry of oxyheme in its  $^1A'$  state, computed at the BP86 level. Distances are given in angstrom and angles in degrees. The crystallographic data has been obtained from: Vojtechovsky, J.; Chu, K.; Berendzen, J.; Sweet, R. M.; Schlichting, I. *Biophysics J.* **1999**, *77*, 2153

	Fe-O <sub>1</sub>	O <sub>1</sub> -O <sub>2</sub>	Fe-N <sub>porphyrine</sub>	Fe-N <sub>imidazole</sub>	<FeOO
Optimized structure	1.82	1.27	2.015 <sup>a)</sup>	2.09	120.6
Crystallographic data	1.81	1.24	2.01 <sup>a)</sup>	2.06	122

<sup>a</sup> Average value of the four distances between the Fe ion and the porphyrin nitrogens.

**Table S2** Energy ordering of the lowest electronic states of oxyheme (relative to the  $^1A'$  state, taken as zero). Comparison between the multiconfigurational results of this work and some DFT results. All the energies are in kcal/mol.

Electronic state	BP86 adiabatic energies	B3LYP adiabatic energies <sup>a</sup>	CASSCF vertical energies	CASPT2 vertical energies
$^3A''$	23.4 <sup>b</sup>	4.7	12.1	3.2
$^5A''$	33.3 <sup>b</sup>	<sup>c</sup>	2.5	2.0
$^1A'$	0	0	0	0

<sup>a</sup> B3LYP calculations using optimum BP86 geometries (Jensen, K. P.; Ryde, U. *J. Biol. Chem.* **2004**, 279, 14561)

<sup>b</sup> This work (BP86 calculations on BP86 optimum geometries)

<sup>c</sup> A different state to that found in the CASPT2 is obtained.

**Table S3.** Energy ordering of the lowest electronic states of deoxyheme (relative to the  $^5A'$  state, taken as zero). All the energies are in kcal/mol. All values were computed in this work, using the same geometry employed in the CASPT2 calculations, and the Ahlrichs TZV basis set for Fe, and the 6-31g(d) set for C, H and N. Since the electronic states of the deoxyheme are essentially monoconfigurational, the MP2, MP4 and CCSD results agree with the CASPT2 results.

Electronic state	BP86	B3LYP <sup>a</sup>	CASSCF	CASPT2	MP2	MP4	CCSD
$^5A'$	0.	0.	<b>0.</b>	<b>0.</b>	<b>0.</b>	<b>0.</b>	<b>0.</b>
$^3A''$	-13.7	<b>-1.7</b>	22.8	13.6	18.5	17.5	14.6
$^1A'$	<b>-17.1</b>	3.9	41.1	21.9	25.4	25.2	21.1

<sup>a</sup> In some B3LYP calculations the ground state is the quintet and in others is the triplet, being all triplet-quintet energy differences placed within the (0.6-2.0) range.

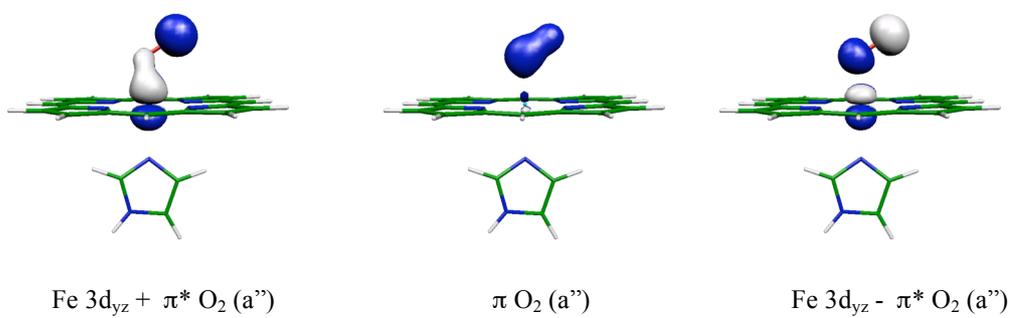
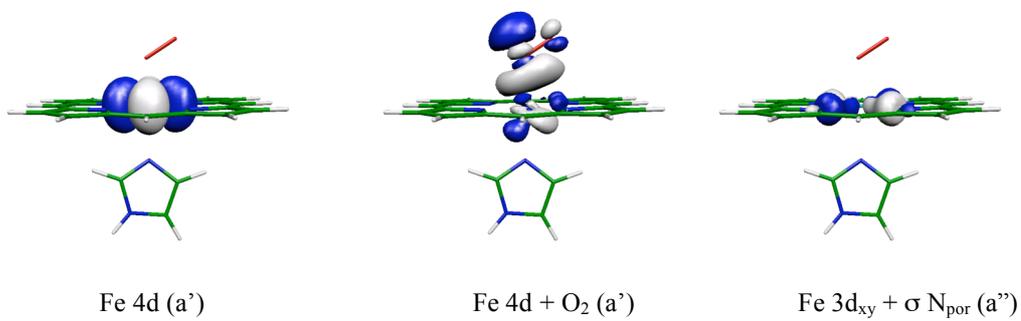
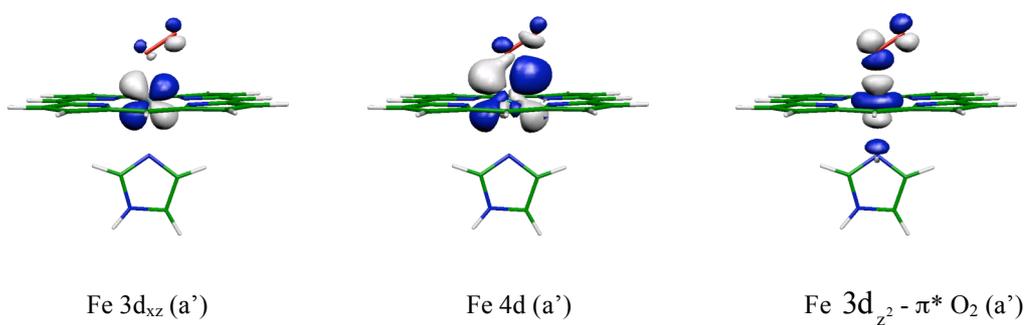
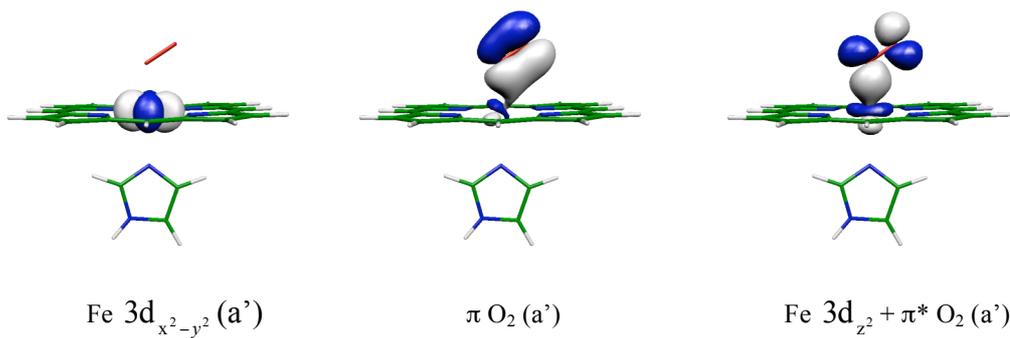
**Table S4.** Occupation numbers for the natural orbitals that differ (in absolute value) by more than 0.05 electrons from 2 or 0. The values in this table refer to the three lowest states of the oxyheme.

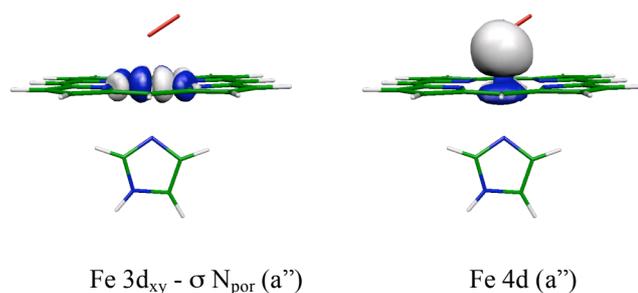
STATE	Orbital	Orbital symmetry	Occupation
<sup>1</sup> A'	Fe 3d <sub>z2</sub> + π* O <sub>2</sub>	a'	1.84
	Fe 3d <sub>z2</sub> - π* O <sub>2</sub>	a'	0.18
<sup>3</sup> A''	Fe 3d <sub>yz</sub> + π* O <sub>2</sub>	a''	1.61
	Fe 3d <sub>yz</sub> - π* O <sub>2</sub>	a''	0.44
	Fe 3d <sub>z2</sub> + π* O <sub>2</sub>	a'	1.55
	Fe 3d <sub>x2-y2</sub>	a'	1.00
<sup>5</sup> A''	Fe 3d <sub>z2</sub> + π* O <sub>2</sub>	a'	0.47
	Fe 3d <sub>yz</sub> + π* O <sub>2</sub>	a''	1.31
	Fe 3d <sub>xy</sub> - σ N <sub>porf</sub>	a''	1.00
	Fe 3d <sub>yz</sub> + π* O <sub>2</sub>	a''	0.72
	Fe 3d <sub>xz</sub>	a'	1.00
	Fe 3d <sub>x2-y2</sub>	a'	1.00
<sup>5</sup> A''	Fe 3d <sub>z2</sub>	a'	1.00
	Fe 3d <sub>yz</sub> + π* O <sub>2</sub>	a''	1.17
	Fe 3d <sub>yz</sub> - π* O <sub>2</sub>	a''	0.82
	Fe 3d <sub>xy</sub>	a''	1.00

**Table S5.** Occupation numbers for the natural orbitals that differ (in absolute value) by more than 0.05 electrons from 2 or 0. The values in this table refer to the three lowest states of the deoxyheme.

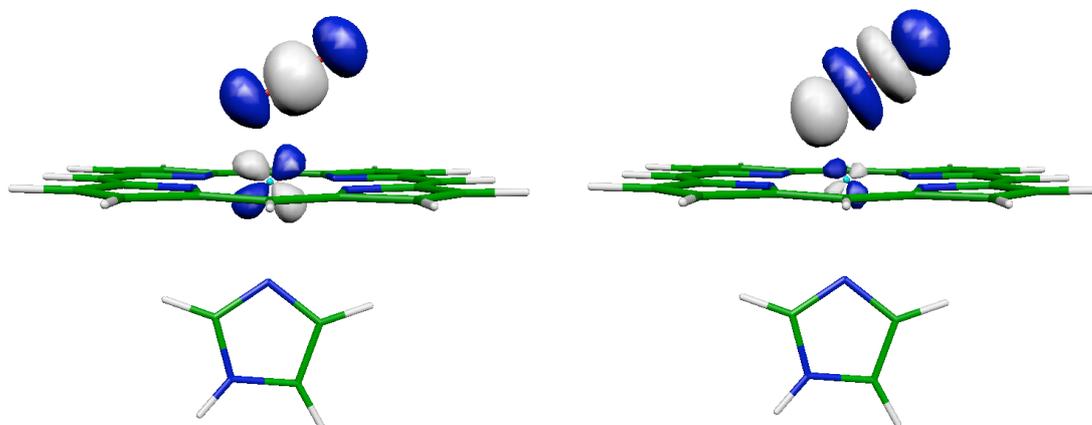
STATE	Orbital	Orbital symmetry	Occupation
$^5A'$	Fe3d <sub>x<sup>2</sup>-y<sup>2</sup></sub>	a'	1.00
	Fe 3d <sub>z<sup>2</sup></sub>	a'	1.00
	Fe 3d <sub>yz</sub>	a''	1.00
	Fe 3d <sub>xy</sub> - $\sigma$ N <sub>porf</sub>	a''	1.00
$^3A''$	Fe 3d <sub>z<sup>2</sup></sub>	a'	0.98
	Fe 3d <sub>yz</sub>	a''	1.00
$^1A'$	*	*	*

\* There are no orbitals whose occupation differs by more than 0.05 electrons from 2 or 0, thus indicating a pure closed-shell singlet state.

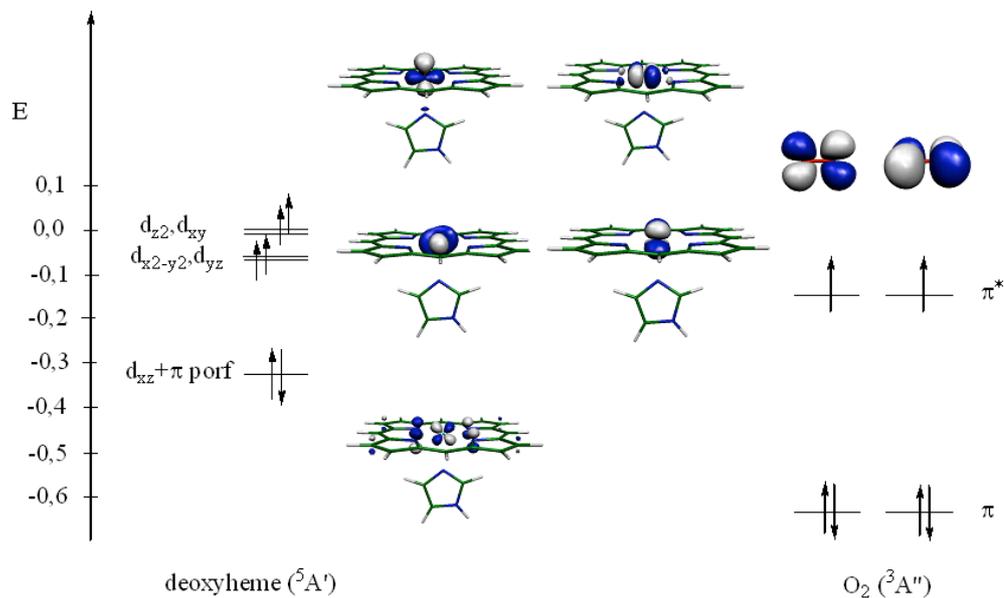




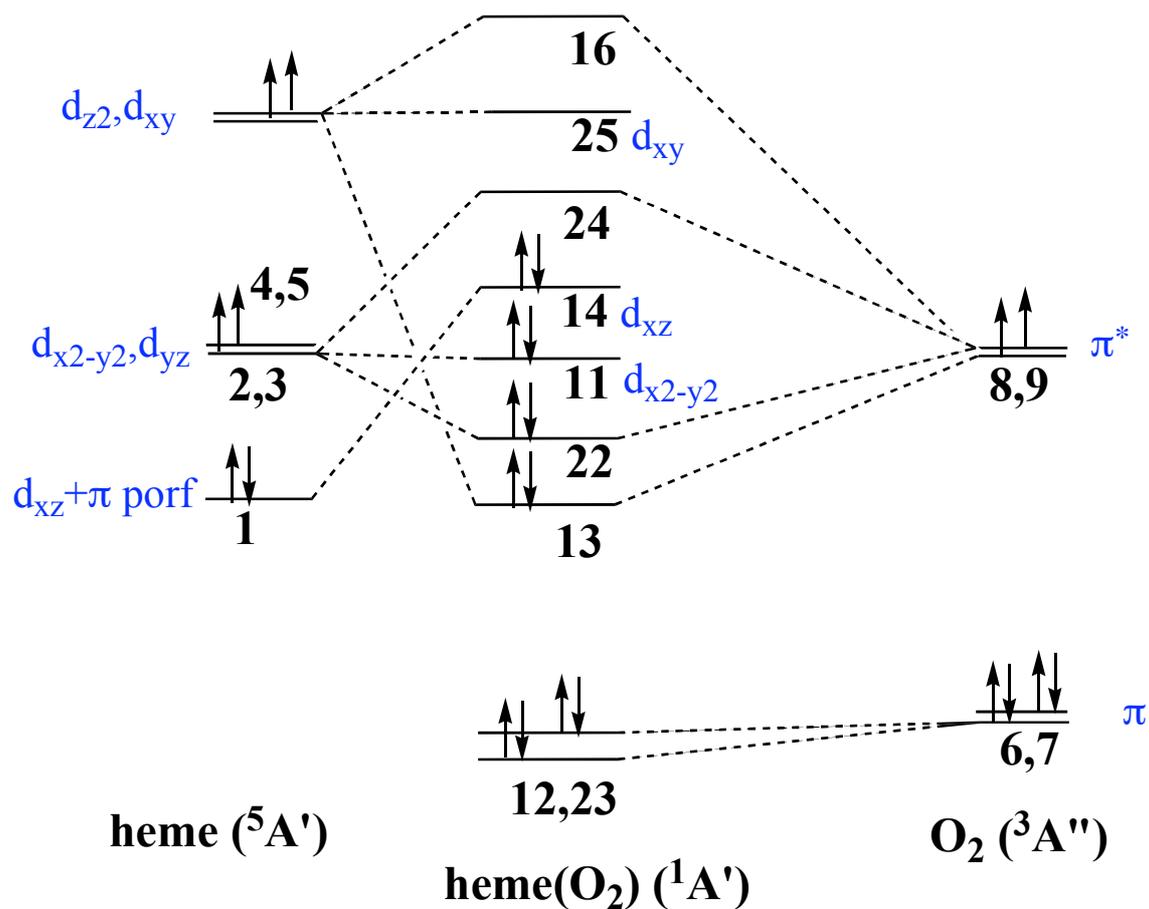
**Figure S1.** Orbitals included in the (14,14) active space. The orbitals shown here are taken from the  $^1A'$  state calculations. The calculations of the electronic states of the oxyheme complex were initially performed with a (14,14) active space. For the calculation of the potential energy curves we have had to use the (16,14) active space because the (14,14) space was not stable (against rotations between orbitals) along all the points of the potential curves. The (16,14) and (14,14) results for the electronic states at the optimum geometry of oxyheme are the same.



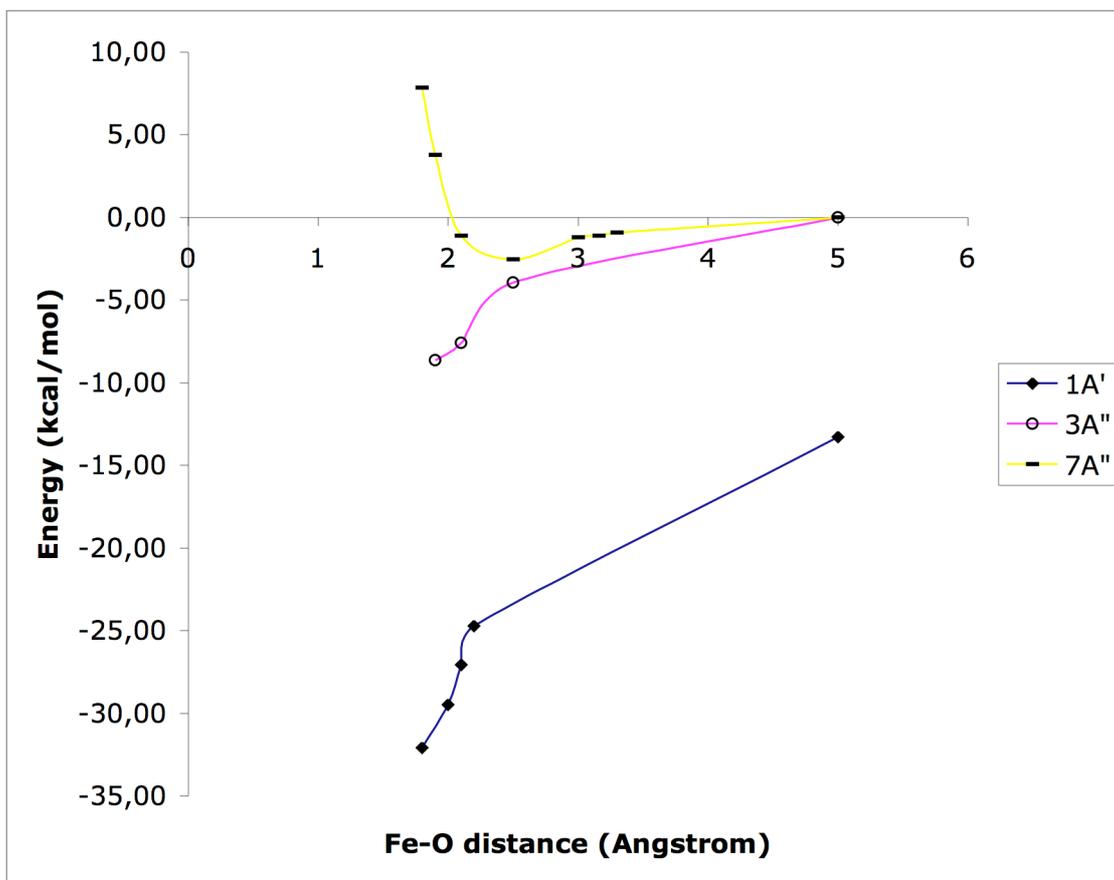
**Figure S2.** New orbitals included in the (16,14) active space that are not included in the (14,14) active space. The orbitals shown here are taken from the  $^1A'$  state calculations. The active orbitals included in the (16,14) active space are the following ones: the five Fe 3d atomic orbitals, the four  $O_2$   $\pi$  orbitals (a bonding orbital of symmetry  $a'$ , the corresponding antibonding of symmetry  $a'$ , a bonding orbital of symmetry  $a''$ , and the corresponding antibonding orbital of symmetry  $a''$ ), the highest occupied linear combination of the porphyrin nitrogen  $\sigma$  orbitals (this orbital has the appropriate symmetry and energy to interact with the Fe  $d_{xy}$  orbital), the highest occupied  $\sigma$  orbital of  $O_2$  and its corresponding  $\sigma^*$  and 2 Fe 4d virtual orbitals (symmetry  $a'$ ) included to account for the important double-shell effect (the dynamical radial correlation in the d shell). We have not been able to include more Fe 4d virtual because the corresponding active spaces are not stable against rotation of orbitals. The two Fe 4d orbitals that have been included are those which the two doubly occupied Fe 3d orbitals in the  $^1A'$  state can correlate with. The (14,14) active space is very similar to the (16,14) one, but does not include the highest occupied  $\sigma$  orbital of  $O_2$  and its corresponding  $\sigma^*$ , and includes two extra Fe 4d virtual orbitals. The (8,11) active space for the calculations of the isolated deoxyheme, in its turn, is the same as the (16,14) active space, but without the oxygen-based orbitals, and with the five Fe 4d virtual orbitals. For a reference on the double shell effect see: K. Pierloot in *Nondynamic correlation effects in transition metal coordination compounds*. *Computational organometallic chemistry*. 2001 ed. Thomas R. Cundari (Marcel Dekker, Inc.)



**Figure S3.** Molecular orbital diagram of the dissociation products of oxyheme (left: deoxyheme, right: oxygen). Only the molecular orbitals relevant in the formation of oxyheme from its constituent fragments are plotted. Their relative energetic ordering (obtained from ROHF calculations, in atomic units) is also given for orientational purposes.



**Figure S4.** Molecular orbital diagram (obtained from ROHF calculations of the oxyheme, deoxyheme and oxygen molecules) describing the main changes in the electronic structure of deoxyheme when an oxygen molecule is added.



**Figure S5.** BP86 potential energy curves for the  $^1A'$ ,  $^3A''$  and  $^7A''$  electronic states of oxyheme. The energy of the system ( $E$ ) is referred to the energy of the dissociation products of oxyheme ( $^5Fe^{2+} + ^3O_2$ ).  $E$  is given in kcal/mol, and  $r$  (the Fe-O distance) is given in Angstroms. The curves are similar to the B3LYP curves published in the literature.<sup>7,8</sup> The differences are due to the fact that while at the B3LYP level the  $^1A'$ ,  $^3A''$  and  $^7A''$  states have similar energy at dissociation, this is not the case at the BP86 level. Neither the B3LYP nor the BP86 curves reproduce the CASPT2 curves of Figure 2. The minimum found in the  $^7A''$  curve at 2.5 Å disappears when the BSSE is corrected, as already reported in the literature for other van der Waals complexes computed using DFT methods.