Electronic Supplementary Material (ESI) for RSC Advances.


# Mechanistic insights into the chemistry of compound I formation in heme peroxidases: Quantum Chemical investigations of cytochrome c peroxidase

**SUPPLEMENTARY INFORMATION:**

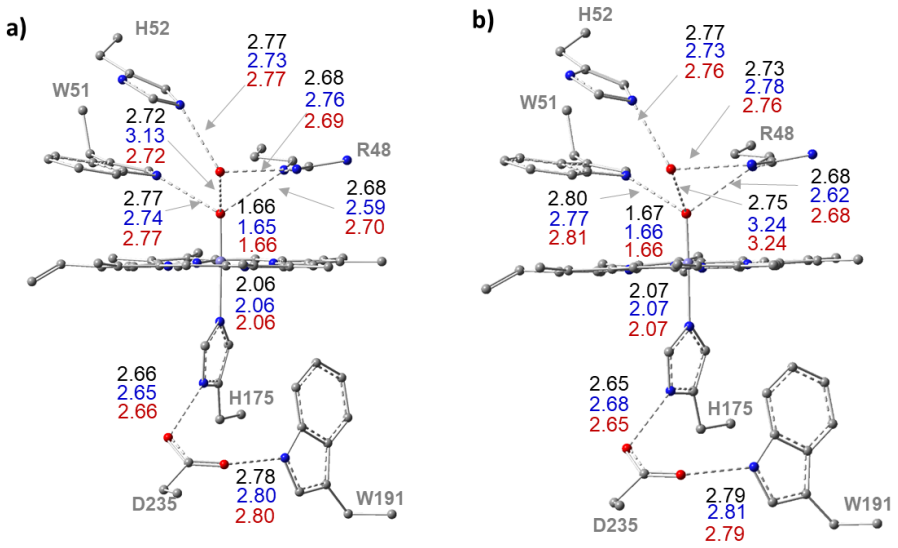


**Figure S1***:* Cluster structures of Cpd I complexes, with selected distances between heavy atoms (Å), for three different spin states: doublet (black), quartet (blue) and sextet (red); geometries optimized with a) UB3LYP-D3BJ/6-31G(d,p) and b) UB3LYP-D3BJ/6-31+G(d,p).

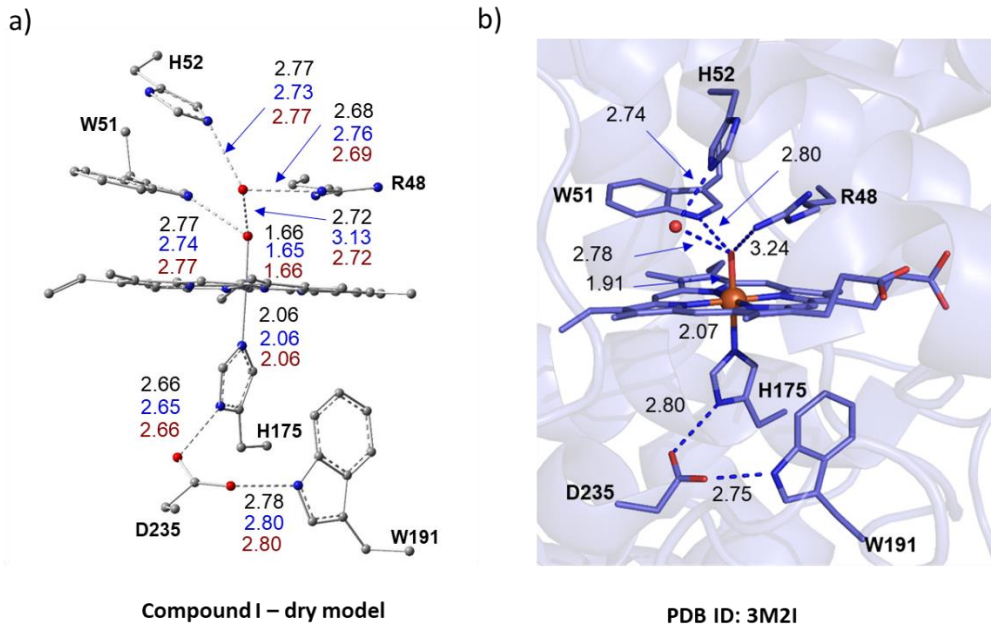


**Figure S2**: Cluster structures of Cpd I complexes, with selected distances between heavy atoms (Å), for a) three different spin states: doublet (black), quartet (blue) and sextet (red); b) the heme pocket of crystalized ferryl-intermediate Ccp1.

**Table S1**: Gibbs Free-energy (kcal/mol) for both the wet and dry mechanisms of Cpd I formation for three different spin states: Free energies are estimated with B3LYP-GD3BJ/6-311+G(2d,2p)+ΔG with ε=4.

| | Wet-mechanism | | | Dry-Mechanism | | |
|---|---|---|---|---|---|---|
| | **Doublet** | **Quartet** | **Sextet** | **Doublet** | **Quartet** | **Sextet** |
| **RC** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **TS1** | 1.0 | 16.6 | 2.1 | 3.0 | 0.7 | 0.0 |
| **IC1** | 8.5 | 18.3 | 6.8 | -5.8 | 0.7 | 1.0 |
| **IC2** | 1.4 | 18.6 | 9.0 | -2.8 | 14.6 | 2.8 |
| **TS2** | 11.8 | 25.0 | 10.5 | 7.6 | 31.1 | 25.6 |
| **PC** | -18.5 | -17.8 | -9.7 | -28.4 | -21.9 | -14.8 |

**Table S2**: Relative-energy (kcal/mol) for both the wet and dry mechanisms of Cpd I formation for three different spin states: Free energies are estimated with UB3LYP-GD3BJ/6-311+G(2d,2p) with ε=4.

| | Wet-mechanism | | | Dry-Mechanism | | |
|---|---|---|---|---|---|---|
| | **Doublet** | **Quartet** | **Sextet** | **Doublet** | **Quartet** | **Sextet** |
| **RC** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **TS1** | 1.8 | 19.5 | 4.1 | 3.9 | 1.1 | 1.0 |
| **IC1** | 5.4 | 13.4 | -2.0 | -1.7 | 1.0 | 0.2 |
| **IC2** | -4.4 | 11.9 | -2.5 | -8.7 | 11.6 | 0.7 |
| **TS2** | 8.4 | 20.4 | 42.7 | 7.7 | 30.6 | 27.4 |
| **PC** | -16.9 | -21.5 | -13.4 | -26.6 | -20.5 | -14.6 |

**Table S3:** Relative-energy (kcal/mol) for the dry mechanisms of Cpd I formation in doublet spin states: Electronic and Free energies are estimated with BS1 (UB3LYP-GD3BJ/6-311+G(2d,2p) with ε=4) and BS2 (UB3LYP-GD3BJ/6-311+G(2df,2p).

| | BS1 | | BS2 | |
|---|---|---|---|---|
| | **Electronic** | **Gibbs** | **Electronic** | **Gibbs** |
| **RC** | 0.0 | 0.0 | 0.0 | 0.0 |
| **TS1** | 3.9 | 3.0 | 3.9 | 3.0 |
| **IC** | -1.7 | -0.3 | -1.7 | -0.4 |
| **IC2** | -8.7 | -2.8 | -8.7 | -2.7 |
| **TS2** | 7.7 | 7.7 | 8.0 | 8.0 |
| **PC** | -26.6 | -28.4 | -26.5 | -28.3 |

**Table S4**: Relative Free-energy (kcal/mol) for both cpd0 and Cpd I calculated in three different spin states estimated with UB3LYP-GD3BJ/6-311+G(2d,2p) in four different dielectric constants.

| | Cpd 0 | | | Cpd I | | |
|---|---|---|---|---|---|---|
| | **Doublet** | **Quartet** | **Sextet** | **Doublet** | **Quartet** | **Sextet** |
| **ε =4** | 1.3 | 0.0 | 5.1 | 0.0 | 5.3 | 13.8 |
| **ε =8** | 2.7 | 0.0 | 7.3 | 0.0 | 9.9 | 14.5 |
| **ε =16** | 0.0 | 15.6 | 3.7 | 0.0 | 9.0 | 13.8 |
| **ε =78.4** | 0.0 | 16.2 | 3.0 | 0.0 | 2.5 | 13.5 |

**Table S5**: Selected distances between heavy atoms (Å) obtained for Cpd 0, for three different spin states: doublet (black), quartet (blue) and sextet (red). Geometries are optimized with UB3LYP-D3BJ/6-31G(d,p).

| | **Fe-O1** | **1O···1N$_{R48}$** | **2O···2N$_{R48}$** | **1O···N$_{W51}$** | **1O···O2** | **2O···N$_{H52}$** | **Fe–N2$_{H175}$** | **$_{H175}$N2···O$_{D235}$** | **$_{W191}$N···O$_{D235}$** |
|---|---|---|---|---|---|---|---|---|---|
| | 1.97 | 2.65 | 2.87 | 2.76 | 1.83 | 2.74 | 1.88 | 2.68 | 2.86 |
| **ε =1** | 1.97 | 2.67 | 2.88 | 2.77 | 1.76 | 2.76 | 1.89 | 2.67 | 2.86 |
| | 2.31 | 2.59 | 2.90 | 2.73 | 1.74 | 2.72 | 1.98 | 2.66 | 2.87 |
| | 1.93 | 2.72 | 2.87 | 2.77 | 1.48 | 2.78 | 1.91 | 2.62 | 2.85 |
| **ε =4** | 2.31 | 2.62 | 2.93 | 2.72 | 1.48 | 2.78 | 2.08 | 2.60 | 2.85 |
| | 2.22 | 2.66 | 2.88 | 2.72 | 1.47 | 2.75 | 2.03 | 2.60 | 2.86 |
| | 1.92 | 2.73 | 2.87 | 2.77 | 1.48 | 2.87 | 1.92 | 2.61 | 2.86 |
| **ε =8** | 1.92 | 2.73 | 2.87 | 2.77 | 1.48 | 2.79 | 1.92 | 2.61 | 2.86 |
| | 2.21 | 2.72 | 2.88 | 2.66 | 1.47 | 2.76 | 2.03 | 2.60 | 2.86 |
| | 1.92 | 2.75 | 2.87 | 2.77 | 1.48 | 2.81 | 1.92 | 2.61 | 2.86 |
| **ε =16** | 1.93 | 2.75 | 2.88 | 2.78 | 1.47 | 2.82 | 1.93 | 2.61 | 2.86 |
| | 2.21 | 2.67 | 2.88 | 2.72 | 1.47 | 2.76 | 2.04 | 2.60 | 2.85 |
| | 1.91 | 2.76 | 2.86 | 2.77 | 1.47 | 2.81 | 1.93 | 2.60 | 2.86 |
| **ε =78.4** | 1.92 | 2.76 | 2.88 | 2.78 | 1.47 | 2.82 | 1.94 | 2.61 | 2.86 |
| | 2.19 | 2.68 | 2.89 | 2.73 | 1.47 | 2.76 | 2.05 | 2.58 | 2.84 |

**Table S6**: Selected distances between heavy atoms (Å) obtained for Cpd I, for three different spin states: doublet (black), quartet (blue) and sextet (red). Geometries are optimized with UB3LYP-D3BJ/6-31G(d,p).

| | Fe-O1 | 1O···1N$_{R48}$ | 2O···2N$_{R48}$ | 1O···N$_{W51}$ | 1O···O2 | 2O···N$_{H52}$ | Fe–N2$_{H175}$ | $_{H175}$N2···O$_{D235}$ | $_{W191}$N···O$_{D235}$ |
|---|---|---|---|---|---|---|---|---|---|
| | 1.97 | 2.65 | 2.87 | 2.76 | 1.83 | 2.74 | 1.88 | 2.68 | 2.86 |
| **ε =1** | 1.97 | 2.67 | 2.88 | 2.77 | 1.76 | 2.76 | 1.89 | 2.67 | 2.86 |
| | 2.31 | 2.59 | 2.90 | 2.73 | 1.74 | 2.72 | 1.98 | 2.66 | 2.87 |
| | 1.93 | 2.72 | 2.87 | 2.77 | 1.48 | 2.78 | 1.91 | 2.62 | 2.85 |
| **ε =4** | 2.31 | 2.62 | 2.93 | 2.72 | 1.48 | 2.78 | 2.08 | 2.60 | 2.85 |
| | 2.22 | 2.66 | 2.88 | 2.72 | 1.47 | 2.75 | 2.03 | 2.60 | 2.86 |
| | 1.92 | 2.73 | 2.87 | 2.77 | 1.48 | 2.87 | 1.92 | 2.61 | 2.86 |
| **ε =8** | 1.92 | 2.73 | 2.87 | 2.77 | 1.48 | 2.79 | 1.92 | 2.61 | 2.86 |
| | 2.21 | 2.72 | 2.88 | 2.66 | 1.47 | 2.76 | 2.03 | 2.60 | 2.86 |
| | 1.92 | 2.75 | 2.87 | 2.77 | 1.48 | 2.81 | 1.92 | 2.61 | 2.86 |
| **ε =16** | 1.93 | 2.75 | 2.88 | 2.78 | 1.47 | 2.82 | 1.93 | 2.61 | 2.86 |
| | 2.21 | 2.67 | 2.88 | 2.72 | 1.47 | 2.76 | 2.04 | 2.60 | 2.85 |
| | 1.91 | 2.76 | 2.86 | 2.77 | 1.47 | 2.81 | 1.93 | 2.60 | 2.86 |
| **ε =78.4** | 1.92 | 2.76 | 2.88 | 2.78 | 1.47 | 2.82 | 1.94 | 2.61 | 2.86 |
| | 2.19 | 2.68 | 2.89 | 2.73 | 1.47 | 2.76 | 2.05 | 2.58 | 2.84 |

**Table S7:** Selected distances between heavy atoms (Å) obtained for the structures of cpd0 (IC2), for WT Ccp1 and the explored in-silico generated variants. Geometries are optimized with UB3LYP-D3BJ/6-31G(d,p).

| | Fe-O1 | 1O···1N$_{R48}$ | 2O···2N$_{R48}$ | 1O···N$_{W51}$ | 1O···O2 | 2O···N$_{H52}$ | Fe–N2$_{H175}$ | $_{H175}$N2···O$_{D235}$ | $_{W191}$N···O$_{D235}$ |
|---|---|---|---|---|---|---|---|---|---|
| **WT** | 1.99 | 2.76 | 2.81 | -- | 1.48 | 2.69 | 1.90 | 2.70 | 2.85 |
| **W51A** | 1.97 | 2.74 | 2.85 | | 1.48 | 2.55 | 1.91 | 2.72 | 2.86 |
| **R48A** | 1.93 | -- | -- | 2.81 | 1.47 | 2.70 | 2.09 | 2.57 | 2.84 |
| **W51F/ W191F** | 2.00 | 2.71 | 2.85 | -- | 1.48 | 2.71 | 1.90 | 2.69 | -- |
| **W191Y** | 2.01 | 2.76 | 2.82 | -- | 1.48 | 2.91 | 1.89 | 2.63 | 2.75 |
| **W191A** | 1.97 | 2.74 | 2.85 | -- | 1.48 | 2.87 | 1.88 | 2.70 | -- |
| **D235H** | 1.92 | 2.79 | 2.82 | -- | 1.48 | 2.84 | 1.95 | 2.82 | -- |
| **D235N** | 1.95 | 2.76 | 2.84 | -- | 1.48 | 3.01 | 1.93 | 2.74 | -- |
| **D235E** | 1.97 | 2.77 | 2.83 | -- | 1.48 | 2.81 | 1.92 | 2.62 (O1) | 2.72 (O1) |
| **D235A** | 1.90 | 2.81 | 2.85 | -- | 1.47 | 2.69 | 1.95 | -- | -- |

**Table S8:** Selected distances between heavy atoms (Å) obtained for the structures of cpd0 (IC2), for WT Ccp1 and the explored variants Geometries are optimized with UB3LYP-D3BJ/6-31G(d,p).

| | Fe-O1 | 1O···1N$_{R48}$ | 2O···2N$_{R48}$ | 1O···N$_{W51}$ | 1O···O2 | 2O···N$_{H52}$ | Fe···N2$_{H175}$ | $_{H175}$N2···O$_{D235}$ | $_{W191}$N···O$_{D235}$ |
|---|---|---|---|---|---|---|---|---|---|
| **WT** | 1.97 | 2.65 | 2.87 | 2.76 | 1.48 | 2.74 | 1.88 | 2.68 | 2.86 |
| **W51A** | 1.95 | 2.69 | 2.88 | -- | 1.49 | 2.61 | 1.91 | 2.70 | 2.85 |
| **R48A** | 1.90 | -- | -- | 2.76 | 1.48 | 2.65 | 1.93 | 2.62 | 2.83 |
| **W51F/ W191F** | 1.95 | 2.60 | 2.91 | 2.71 | 1.48 | 2.71 | 1.89 | 2.66 | -- |
| **W191Y** | 1.97 | 2.65 | 2.90 | 2.76 | 1.48 | 2.76 | 1.88 | 2.61 | 2.74 |
| **W191A** | 1.96 | 2.63 | 2.87 | 2.77 | 1.50 | 2.73 | 1.88 | 2.71 | -- |
| **D235H** | 1.91 | 2.70 | 2.94 | 2.79 | 1.48 | 2.87 | 1.94 | 2.82 | -- |
| **D235N** | 1.91 | 2.68 | 2.91 | 2.79 | 1.48 | 2.80 | 1.93 | 2.74 | -- |
| **D235E** | 1.94 | 2.66 | 2.90 | 2.76 | 1.48 | 2.75 | 1.91 | 2.64 | -- |
| **D235A** | 1.91 | 2.70 | 2.94 | 2.81 | 1.48 | 2.88 | 1.95 | -- | -- |

**TableS 9:** Selected distances between heavy atoms (Å) obtained for the structures of TS2, for WT Ccp1 and the explored variants Geometries are optimized with UB3LYP-D3BJ/6-31G(d,p).

| | Fe-O1 | 1O···1N$_{R48}$ | 2O···2N$_{R48}$ | 1O···N$_{W51}$ | 1O···O2 | 2O···N$_{H52}$ | Fe–N2$_{H175}$ | $_{H175}$N2···O$_{D235}$ | $_{W191}$N···O$_{D235}$ |
|---|---|---|---|---|---|---|---|---|---|
| **WT** | 2.77 | 2.67 | 2.77 | 2.77 | 1.83 | 2.53 | 1.92 | 2.70 | 2.85 |
| **W51A** | 1.74 | 2.72 | 2.78 | -- | 1.83 | 2.52 | 1.94 | 2.71 | 2.85 |
| **R48A** | 1.76 | -- | -- | 2.82 | 1.74 | 2.55 | 1.93 | 2.59 | 2.90 |
| **W51F/ W191F** | 1.74 | 2.62 | 2.79 | 2.59 | 1.84 | 2.59 | 1.92 | 2.68 | -- |
| **W191Y** | 1.77 | 2.66 | 2.78 | 2.77 | 1.81 | 2.77 | 1.91 | 2.63 | 2.71 |
| **W191A** | 1.77 | 2.69 | 2.75 | 2.85 | 1.82 | 2.59 | 1.91 | 2.72 | -- |
| **D235H** | 1.71 | 2.71 | 2.82 | 2.80 | 1.86 | 2.80 | 1.99 | 2.83 | -- |
| **D235N** | 1.71 | 2.71 | 2.83 | 2.79 | 1.86 | 2.70 | 1.99 | 2.74 | -- |
| **D235E** | 1.74 | 2.79 | 2.67 | 2.77 | 1.84 | 2.63 | 1.95 | 2.62 | 2.74(N1) |
| **D235A** | 1.71 | 2.72 | 2.82 | 2.82 | 1.86 | 2.71 | 2.00 | -- | -- |

**Table S10*:*** Selected distances between heavy atoms (Å) obtained for Cpd I, for WT Ccp1 and the explored variants Geometries are optimized with UB3LYP-D3BJ/6-31G(d,p).

| | Fe-O1 | $1O^{\cdots}1N_{R48}$ | $2O^{\cdots}2N_{R48}$ | $1O^{\cdots}N_{W51}$ | $1O^{\cdots}O2$ | $2O^{\cdots}N_{H52}$ | $Fe{-}N2_{H175}$ | $_{H175}N2^{\cdots}O_{D235}$ | $_{W191}N^{\cdots}O_{D235}$ |
|---|---|---|---|---|---|---|---|---|---|
| **WT** | 1.66 | 2.68 | 2.68 | 2.77 | 2.72 | 2.77 | 2.06 | 2.66 | 2.78 |
| **W51A** | 1.65 | 2.70 | 2.66 | -- | 2.74 | 2.76 | 2.09 | 2.67 | 2.85 |
| **R48A** | 1.70 | -- | -- | 2.81 | 2.29 | 2.78 | 1.97 | 2.60 | 2.92 |
| **W51F/ W191F** | 1.64 | 2.67 | 2.69 | | 2.68 | 2.82 | 2.09 | 2.80 | -- |
| **W191Y** | 1.66 | 2.66 | 2.72 | 2.80 | 2.70 | 2.81 | 2.06 | 2.71 | 2.71 |
| **W191A** | 1.67 | 2.71 | 2.66 | 2.79 | 2.72 | 2.74 | 2.06 | 2.80 | -- |
| **D235H** | 1.67 | 2.74 | 2.70 | 2.66 | 2.73 | 2.75 | 2.05 | 2.86 | -- |
| **D235N** | 1.67 | 2.74 | 2.70 | 2.66 | 2.73 | 2.75 | 2.05 | 2.77 | -- |
| **D235E** | 1.67 | 2.66 | 2.71 | 2.72 | 2.70 | 2.82 | 2.03 | 2.65 | 2.59 (N1) |
| **D235A** | 1.67 | 2.74 | 2.69 | 2.67 | 2.73 | 2.75 | 2.06 | -- | -- |

**Table S11**: Spin density sum Values for the major spin contaminated components of Cpd I obtained for WT Ccp1 and its studied variants calculated with UB3LYP-D3BJ/6-31G(d,p).

| | WT | R48A | W51A | W191Y | W191A | D235H | D235N | D235E | D235A | W51F/ W191F |
|---|---|---|---|---|---|---|---|---|---|---|
| $\pi_{FeO}$ | 2.11 | 2.07 | 2.07 | 2.07 | 2.07 | 2.08 | 2.08 | 2.08 | 2.08 | 0.96 |
| $\pi_{His}$ | -0.49 | 0.51 | -0.60 | -0.66 | -0.62 | -- | -- | -- | -- | -0.88 |
| $\pi_{Trp}$ | -0.45 | -0.21 | -0.41 | -0.24 | -- | -- | -0.14 | -0.55 | -- | -- |
| $\pi_{por}$ | **--** | -0.29 | -- | -- | -0.36 | -0.35 | -0.34 | -0.30 | -0.38 | -0.17 |
| $\pi_{W51}$ | -- | 0.12 | -- | -- | -0.12 | -0.65 | -0.61 | -0.25 | -0.66 | -- |