Supporting information

Simulation protocol

Docking. The chromophore Ru(II)(bpy)$_2$(im) was first docked to His33 of Fe(II)cyt c (pdb code 2GIW) as there was no experimental structure available with Ru(II)(bpy)$_2$(im) bound to cyt c. The Ru-chromophore was manually placed in the simulation box so that the angle between ϵN of His33, Ru and the axial nitrogen atom N$_{\mathrm{ax}}$ of the bpy ligand was 180 degrees. Eighteen initial docking structures were then created by rotating the chromophore about the ϵN-Ru-N$_{\mathrm{ax}}$ axis in increments of 20 degrees. The system was neutralized with 9 Cl$^-$ ions and each of the structures were energy minimized using the AMBER99 force field for the protein and a special parametrization of the heme c cofactor and Ru(II)(bpy)$_2$(im)His33. The minimization was carried out with the sander program of the AMBER9 simulation package. The protonation states of the ionizable groups were taken from the experimental (NMR) structure and no water was included in the energy minimization. The structure with the lowest energy was selected as an initial configuration for the MD simulation.

MD simulation with nonpolarizable force field. The minimum energy structure obtained from docking was solvated with 5148 TIP3P water molecules and neutralized by adding 11 Cl$^-$ and 10 Na$^+$ ions were added. The salt concentration in the simulation cell (0.1 M) is comparable to the one in experiment.[98] However, the concentration of protein is three orders of magnitude higher than in experiment. The atomic positions of the protein and cofactors in state Fe(II)Ru(III) were fixed and the ionic solution equilibrated for 100 ps using a MD time step of 1 fs, a barostat with target pressure 1.01325 bar and a temperature rescaling to 300 K. Then the atomic positions of protein and cofactors were restrained to the position of the structure 2GIW and the system simulated for 400 ps with successively decreasing force constants of 99, 50, 25 and 10 kcal/mol. Finally all position restraints on protein and cofactors were released and the protein equilibrated for a further 6.5 ns in the NPT ensemble using a time step of 2 fs. The next 50 ns of dynamics was taken for calculation of configurational averages. The classical MD simulation of the charge transferred complex Fe(III)Ru(II) was carried out similarly using as initial geometry a snapshot of the simulation of Fe(II)Ru(III). After equilibration for 4 ns in the NPT ensemble, the next 50 ns of dynamics were taken for calculation of configurational averages. The MD simulation were carried out with the NAMD simulation program.

MD simulation with polarizable force field. A snapshot of Fe(II)Ru(III) from the nonpolarizable simulation was taken as an initial configuration for the simulation with the polarizable force field Amber02. The TIP3P model was replaced by POL3 water. Only the protein and the water were treated polarizable, the polarizability of all ionizable atoms of the two cofactors was set equal to zero. The system was equilibrated in the NPT ensemble using a Car-Parrinello scheme for the dynamics of the induced dipoles with the default value for the fictitious mass and a time step of 1 fs. The following 11 ns of dynamics was used for calculation of configurational averages. The simulation of Fe(III)Ru(II) was carried out similarly. The Car-Parrinello dynamics was stable during the entire simulations. A test simulation in the NVE ensemble showed that the energy conservation of the Car-Parrinello algorithm was comparable with the one of an (exact) iterative scheme used in combination with a tight convergence criterion of the induced dipoles.

QM/MM calculation. In states R$_{\mathrm{qm}}$O$_{\mathrm{mm}}$ and O$_{\mathrm{qm}}$O$_{\mathrm{mm}}$ (Eq. 22) the QM region is comprised of the heme c cofactor and the residues His18 and Met80 ligating the Fe atom in axial directions. In the smallest QM model (58 atoms) all substituents of the heme c ring are replaced by a QM dummy hydrogen atom and the substituents treated at the classical force field level. Similarly, for His18 and Met80 the βC and γC atoms, respectively, were saturated with a QM dummy hydrogen atom. The bond length of all C-H$_{\mathrm{dummy}}$ bonds was set to 1.09 Å. The charge of both the QM and total system is equal to 0 in state R$_{\mathrm{qm}}$O$_{\mathrm{mm}}$ and equal to 1 in state O$_{\mathrm{qm}}$O$_{\mathrm{mm}}$. The chromophore Ru(III)(im)(bpy)$_2$His33 ("O$_{\mathrm{mm}}$") is part of the MM subsystem which is modeled with the AMBER99 force field (nonpolarizable). In states O$_{\mathrm{mm}}$O$_{\mathrm{qm}}$ and O$_{\mathrm{mm}}$R$_{\mathrm{qm}}$ (Eq. 21) the QM region is comprised of the Ru(bpy)$_2$(im) chromophore and His33, where again the βC atom of His33 was saturated with a dummy hydrogen atom. The Fe(III)heme c cofactor and ligands ("O$_{\mathrm{mm}}$") are treated as part of the MM system. The charge of the QM and total system are 1 in state O$_{\mathrm{mm}}$O$_{\mathrm{qm}}$ and 0 in state O$_{\mathrm{mm}}$R$_{\mathrm{qm}}$. The QM/MM calculations were carried out with the CPMD code. 27 equidistantly spaced snapshots were selected from the 50 ns classical MD trajectories of Fe(II)Ru(III) (see above) and the QM/MM ground state energy calculated for all four states R$_{\mathrm{qm}}$O$_{\mathrm{mm}}$, O$_{\mathrm{qm}}$O$_{\mathrm{mm}}$, O$_{\mathrm{mm}}$O$_{\mathrm{qm}}$ and R$_{\mathrm{qm}}$O$_{\mathrm{mm}}$ using wavefunction optimization. The electron transfer energy ΔE was then calculated according to Eqs. 24-26 and $\langle \Delta E \rangle_{\mathrm{A}}$ obtained by averaging over all 27 snapshots. The ET energy average for the charge transferred state, $\langle \Delta E \rangle_{\mathrm{B}}$, was calculated similarly by selecting 27 equidistantly spaced snapshots from the 50 ns classical MD trajectory of Fe(III)Ru(II). The QM calculation was carried out with the PBE functional for the experimental low spin ground states of Fe-heme c and the Ru-chromophore using the same pseudopotentials and reciprocal space cutoffs as in the gas phase calculations of the model compounds (see below). The interaction between the QM system and the MM system is computed using a Hamiltonian electrostatic coupling scheme.[95] The electrostatic interaction energy between the electron+nuclei density of the QM subsystem and the point charges of all MM atoms within $r_{\mathrm{NN}} = 10\,\mathrm{a.u.}$ of any QM atom (=MM1 atoms) is calculated on a real space grid. All other MM atoms (=MM2 atoms) interact with RESP charges assigned to QM atoms. The latter are calculated from the electron+nuclei density.[96]

Gas phase calculations. Gas phase calculations were carried out to test the accuracy of density functionals and pseudopotentials used for CPMD QM/MM calculations. The heme c cofactor was modeled as Fe-porphin(mim)(dms) (mim=methylimidazole, dms=dimethylsulfide) and $Ru(bpy)_2$(im)His33 as $Ru(bpy)_2$(im)(mim). The geometries of the Fe(II) and Ru(II) complexes were optimized for the singlet spin state and the geometries of the Fe(III) and Ru(III) complexes in the doublet spin state without symmetry constraints using the CPMD and the Gaussian program packages. For CPMD optimizations the orbitals were expanded in plane waves with a reciprocal space cutoff of 90 Ry for the Fe-complexes and 70 Ry for the Ru-complexes. The PBE and BP[124,125] exchange correlation functionals were used and Troullier-Martins type[127] valence pseudopotentials for second row atoms (see e.g. Ref. 130 for specification) and semi-core pseudopotentials for Ru (see Ref. 131 for specification) and Fe. The latter was generated from all electron calculations for the Fe^{2+} reference configuration $[Ar]d^6$ using pseudization radii of 1.15, 1.15 and 1.2 a.u. for 3s, 3p, and 3d channels, respectively. The geometry optimizations were carried out until the gradient of all atomic nuclei was smaller than 5×10^{-4} Hartree/bohr. Vertical ionization potentials of the reduced states (IP) and vertical electron affinities of the oxidized states (EA) were calculated at the respective energy minimum structure. All electron geometry optimizations for the Fe-complexes were carried out with the Gaussian program for BP, PBE and B3LYP exchange-correlation functionals using the 6-31G(d) basis set. For Ru-complexes the LANL2DZ basis set was used. The optimizations were carried until the default convergence criteria were reached. Vertical ionization potentials and electron affinities of the Fe-complexes were calculated on the optimized geometries using the 6-31++G(d,p) basis set; for Ru-complexes the LANL2DZ basis was used.

Perfomance of density functionals and pseudopotentials

Structure. Metal-ligand bond lengths of Fe-porphin(mim)(dms) and $Ru(bpy)_2$(im)(mim) are summarized in table 2 in the main text. The distances obtained from CPMD optimizations with pseudopotential/plane wave basis sets differ by not more than 0.02 Å from the values obtained with all-electron/Gaussian basis set optimization showing that the semi-core pseudo potentials used for Fe and Ru give reliable geometries if a sufficiently large plane wave cutoff is used (90 Ry for Fe and 70 for Ru). Optimizations with the BP and PBE density functionals give very similar bond lengths while the distances obtained with the B3LYP functional are somewhat larger. The Fe(II)-S and Fe(II)-N_{eq} bond lengths obtained with PBE and plane wave basis differ by not more than 0.02 Å from the bond lengths of the crystal structure while the Fe(II)-N_{ax} distance is underestimated by 0.04 Å. No major changes of the geometry are observed when Fe-porphin(mim)(dms) is oxidized from the ferrous to the ferric state. Most significantly, the Fe-S distance is increased by 0.03 Å. This is in agreement with previous calculations on heme c cofactor models[53,63] but somewhat smaller than the 0.08 Å increase obtained from crystal structures. The distances computed for the Ru(II)$(bpy)_2$(im)(mim) cofactor model are also in reasonably good agreement with crystal structure. Though the Ru(II)-N_{ax} and Ru(II)-N_{im} distances are consistently overestimated by about 0.05-0.08 Å for all functionals and basis sets. At the absence of any other reason one might attribute this deviation to steric strain or electrostatic effects of the protein environment that are not taken into account in the gas phase calculations.

Ionization energies. Ionization potentials and electron affinities of the cofactor models are summarized in table 1. Comparing the all-electron calculations for Fe-porphin(mim)(dms) we find that the ionization energies are similar for PBE and B3LYP functionals but about 0.1 eV higher for the BP functional. Interestingly, the same tendency among functionals is obtained for the ionization energies of $Ru(bpy)_2$(im)(mim) leading to almost identical reaction energies (= inner sphere driving force) of −6.45 eV for the full reaction Fe(II)Ru(III)→ Fe(III)Ru(II) of the infinitely separated gas phase model compounds. The pseudopotential calculations for Fe-porphin(mim)(dms) give ionization energies that are consistently smaller than the values obtained from all-electron calculations. The deviation is significant, −0.16 and −0.19 eV for PBE and BP functionals. For $Ru(bpy)_2$(im)(mim) the agreement with all electron calculations is much better with errors less than 0.03 eV. Therefore the pseudopotential error is not cancelled for the reaction energy of the full reaction but can be estimated to be −0.18 and −0.19 eV for PBE and BP density functionals, respectively.

Reorganization energies. Very consistent values for reorganization energy are obtained for all basis sets and density functionals (except B3LYP), 23-26 meV for Fe-porphin(mim)(dms) and 108-120 meV for Ru(bpy)$_2$(im)(mim) giving a total reorganization energy of 132-144 meV for the full inner sphere reaction. The negative reorganization energy obtained with the B3LYP functional for the oxidized state of Fe-porphin(mim)(dms) is probably due to the fact that the geometry was optimized with a 6-31Gd basis set while the final potential energies are calculated with a 6-31++G(d,p) basis set. However, the problem persisted when the geometry was optimized with a larger basis set (6-31+G(d)).

Potential energy in QM/MM

In the CPMD/Gromos QM/MM interface all atoms (QM+MM) are at first treated as MM atoms and the total classical potential energy $E_{\rm cl}$ calculated in periodic boundary conditions using Ewald summation. The classical potential is the sum of all bonded terms (bonds,bends,dihedrals), Lennard-Jones and electrostatic interactions. All classical interactions between QM-QM atom pairs, $E_{\rm cl}^{\rm QM\text{-}QM}$, and all electrostatic interactions between QM-MM atom pairs, $E_{\rm cl,ele}^{\rm QM\text{-}MM}$, are then subtracted in the central cell to give the total potential energy of the MM subsystem.

$$E_{\rm mm}(\mathbf{R}_i,\mathbf{R}_j) = E_{\rm cl} - E_{\rm cl}^{\rm QM\text{-}QM} - E_{\rm cl,ele}^{\rm QM\text{-}MM} \tag{45}$$

where the positions of the nuclei of QM and MM atoms are denoted $\mathbf{R}_i$ and $\mathbf{R}_j$, respectively. The energies $E_{\rm cl}^{\rm QM\text{-}QM}$ and $E_{\rm cl,ele}^{\rm QM\text{-}MM}$ are then replaced by the energy terms obtained from the QM calculation, $E_{\rm qm}$ and $E_{\rm qm/mm}$, respectively. The MM energy $E_{\rm mm}$ includes all interactions between MM-MM atom pairs and all bonded and Lennard-Jones interactions between QM-MM atom pairs in the central cell. It also includes interactions of all atoms (QM+MM) with the periodic images at the classical level. The QM energy is calculated by expanding the orbitals of the QM atoms in plane-waves and minimizing the DFT energy functional $E_V[n(\mathbf{r}),\mathbf{R}_i]$ at the presence of the static electrostatic potential $V(\mathbf{r},\mathbf{R}_j)$ created by the point charges of a subset of the MM atoms (= MM1 atoms).

$$\begin{aligned} E_{\rm qm}(\mathbf{R}_i,\mathbf{R}_j) &= \min[E_V[n(\mathbf{r}),\mathbf{R}_i]] && (46)\\ V(\mathbf{r},\mathbf{R}_j) &= \sum_{j\in MM1} q_j \frac{r_c^4 - |\mathbf{R}_j-\mathbf{r}|^4}{r_c^5 - |\mathbf{R}_j-\mathbf{r}|^5} && (47) \end{aligned}$$

The QM calculation is carried out for the isolated system (no periodic boundary conditions, the interactions with the QM images are included in $E_{\rm mm}$ at the classical level). The potential V is a Coulomb potential modified at short range to avoid electron spill out. The QM/MM energy is then given by

$$E_{\rm qm/mm}(\mathbf{R}_i,\mathbf{R}_j) = \int_{QMbox} d\mathbf{r} V(\mathbf{r},\mathbf{R}_j)[n(\mathbf{r}) + \sum_{i\in QM} Z_i\delta(\mathbf{r}-\mathbf{R}_i)] + \sum_{i\in QM, j\in MM2} \frac{q_i^{resp} q_j}{r_{ij}} \tag{48}$$

where the first term on the RHS is the electrostatic energy between MM1 atoms and the nuclei+ground state electron density of the QM subsystem. The second term on the RHS is the Coulomb interaction between the RESP charges of the QM atoms that are derived from the ground state electron density and the point charges of the MM atoms that are not included in the group of MM1 atoms (= MM2, MM=MM1+MM2). The total potential energy in QM/MM is then the sum of $E_{\rm qm}$, $E_{\rm qm/mm}$, and $E_{\rm mm}$, Eq. 27.

Table S6: Potential energy of a single Ru ion as obtained from CPMD QM/MM calculation in periodic boundary conditions. The Ru ion is specified as quantum atom. In CPMD QM/MM the QM energy E_{qm} is then the energy of the isolated Ru ion and E_{mm} the energy of the periodic crystal of classical point charges. $E_{\mathrm{qm/mm}} = 0$ because there are no MM atoms in the central unit cell. E_{wc} is the Wigner-crystal energy, $E_{\mathrm{wc}} = -\alpha q^2/2L$, where q is the charge of the Ru ion, L the length of the unit cell and $\alpha = 2.837297$ the Madelung constant. Note that E_{mm} correctly approaches E_{wc} for large enough grid sizes. grid: number of grid points per dimension for the total unit cell. L: length of total unit cell.

	grid	L(Å)	E_{qm} (a.u.)	E_{mm} (a.u.)	E_{wc} (a.u.)
Ru^{2+}	64	100	-93.62997954	-0.02980275	-0.03002864
Ru^{2+}	256	100	-93.62997954	-0.03002340	-0.03002864
Ru^{2+}	64	50	-93.62997954	-0.06001724	-0.06005729
Ru^{3+}	64	100	-92.57679805	-0.06705617	-0.06756445

Table S7: Convergence of the ionization energy IE for the heme c cofactor of Ru(bpy)$_2$(im)His33 cyt c as a function of the cutoff radius R_{MM1} determining the number of MM1 atoms explicitly coupled to the QM region (first term on the RHS of Eq. 30 in the main text). $R_{\mathrm{MM1}} = 0$: no MM1 atoms, all MM atoms are MM2 atoms coupled via the second term on the RHS of Eq. 30. $R_{\mathrm{MM1}} = 100$: all MM atoms are MM1 atoms. The ionization energies are calculated according to Eqs. 28-31 in the main text. All energies are given in eV.

R_{MM1} (a.u.)	IE	IE_{qm}	$IE_{\mathrm{qm/mm}}$	IE_{mm}
0	4.58	5.27	-0.98	0.28
2.5	5.29	5.34	-0.33	0.28
5	5.30	5.33	-0.31	0.28
10	5.33	5.33	-0.28	0.28
25	5.34	5.33	-0.28	0.28
50	5.33	5.33	-0.28	0.28
100	5.33	5.33	-0.28	0.28