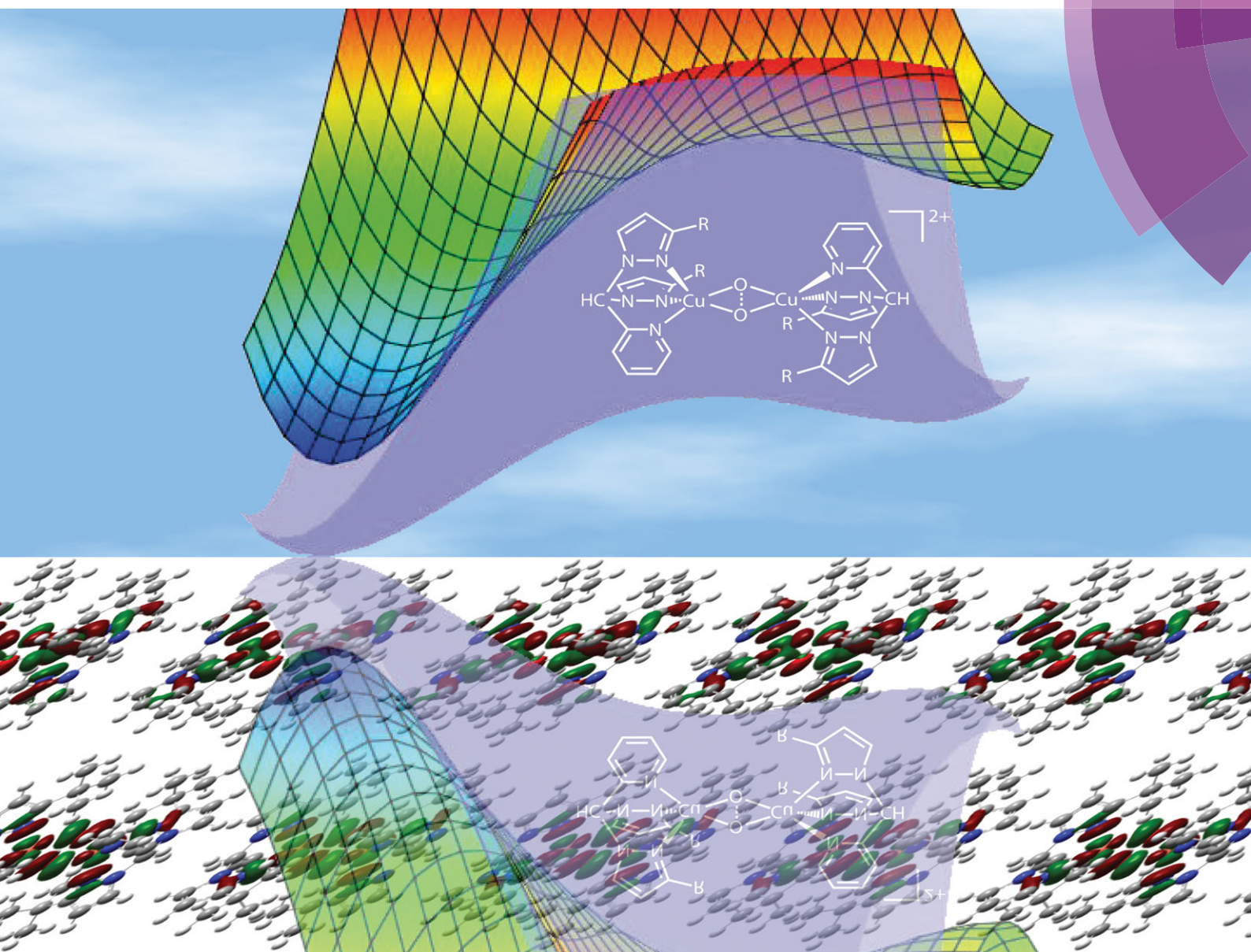


ChemComm

Chemical Communications

www.rsc.org/chemcomm



ISSN 1359-7345



COMMUNICATION

Alexander Hoffmann and Sonja Herres-Pawlis

Hiking on the potential energy surface of a functional tyrosinase model – implications of singlet, broken-symmetry and triplet description

Hiking on the potential energy surface of a functional tyrosinase model – implications of singlet, broken-symmetry and triplet description†

Cite this: *Chem. Commun.*, 2014, 50, 403Received 9th September 2013,
Accepted 24th October 2013

Alexander Hoffmann and Sonja Herres-Pawlis*

DOI: 10.1039/c3cc46893c

www.rsc.org/chemcomm

The singlet, open-shell singlet and triplet potential energy surfaces (PES) for the peroxy state of a catalytic functional tyrosinase model have been investigated by density functional theory calculations. The broken-symmetry solution exhibits considerable stabilisation over the whole PES but the importance of the triplet state is unravelled as well.

Tyrosinase is a ubiquitous copper enzyme that catalyses the hydroxylation of phenols to catechols and the subsequent oxidation of catechols to quinones by activating dioxygen in the form of a side-on-bonded peroxide dicopper(II) species (**P** core).¹ With regard to the unique and impressive catalytic oxidation chemistry of tyrosinases, decades of effort have been directed to reproduce their reversible dioxygen binding and oxidative reactivity in small synthetic complexes.^{2,3} Besides the biologically occurring side-on-bonded peroxide, the isomeric bis(μ -oxo) dicopper(III) core (**O** core) has often been found in model complexes.^{3,4} The most accepted mechanism, based on both theory and experimental data, proposes that a **P** core species performs the hydroxylation reaction through an electrophilic aromatic substitution reaction and that the cleavage of the O–O bond occurs either concerted with or after the C–O bond formation.⁵ However, radical mechanisms have been proposed⁶ and several examples of highly active pure **O** core complexes kept the discussion about the truly hydroxylating species in tyrosinase alive.⁷ To complicate matters, the isomeric equilibrium (Fig. 1) between **O** and **P** cores can be shifted by the choice of the ligands, temperature and counteranions.³ Moreover this equilibrium is theoretically regarded as a torture track for computational chemistry,^{8,9} since the amount of exact exchange heavily influences the predicted energies for the **O/P** equilibrium.⁹ Several studies with multi-reference (MR) calculations based on a wave functional theory (WFT) method such as CASPT2,¹⁰ MRCI + Q,¹¹ DMRG,¹² and RASPT2¹³ have been carried out with ammonia ligands as a substitution for histidine residues. However, the small model does not reproduce experimental Cu–Cu and O–O distances of “real” peroxy complexes. Further DFT studies

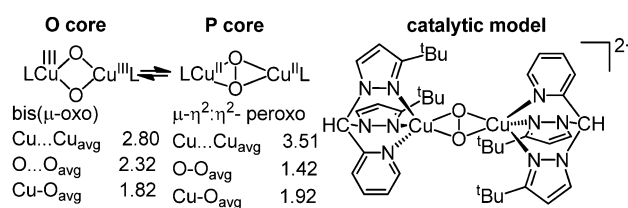


Fig. 1 Equilibrium between **O** and **P** core species, catalytic model²⁰ $[\text{Cu}_2\text{O}_2(\text{HC}(3\text{-tBuPz})_2(\text{Py}))_2]^{2+}$.

focused on real-life systems,^{7,14} but still “cutting” of the ligands or substrates yielded deviating results. In a very recent study utilising DFT and the full complex system, Liu and Blomberg gave an explanation for the early cleavage of the O–O bond with subsequent phenolate coordination.¹⁵ These results fit excellently with the experimental data provided by Stack *et al.*¹⁶ and emphasize the importance of the **O** core for substrate activation. Moreover, they point out the importance to simulate “full” systems without simplifications.

In spite of decades of effort, only four complexes achieved significant catalytic phenol hydroxylation using dioxygen: a dinucleating, polydentate imine complex reported by Réglier,¹⁷ another dinucleating benzimidazole system with 1.2 turnovers reported by Casella¹⁸ and with the first mononucleating analogues, Tuzcek *et al.* found systems capable of 30 turnovers at maximum in the presence of triethylamine.¹⁹ They propose the **P** core species as active species. Recently, we presented a new catalytic tyrosinase model based on bis(pyrazolyl)pyridylmethane ligands.²⁰ The model complex $[\text{Cu}_2\text{O}_2(\text{HC}(3\text{-tBuPz})_2(\text{Py}))_2]^{2+}$ could be unambiguously identified as the **P** core by spectroscopic methods such as UV/Vis, Raman, X-ray absorption spectroscopy (XAS) and mass spectrometry. For the theoretical description, geometrical data from XAS and optical data from UV/Vis spectroscopy have been used for benchmarking purposes. The hybrid functional TPSSH²¹ has been found to treat this 108-atom system reasonably in combination with the 6-31g(d) basis set (see ESI†). To shed light on the PES, a 64-point grid was used by equidistant variation of the Cu–Cu and O–O distances, setting the chosen value as constant and full relaxation of the rest of the molecule.

Ludwig-Maximilians-Universität München, Department Chemie, Butenandtstr. 5-13, 81377 München, Germany. E-mail: sonja.herres-pawlis@cup.uni-muenchen.de

† Electronic supplementary information (ESI) available: Computational details, additional tables and structures. See DOI: 10.1039/c3cc46893c

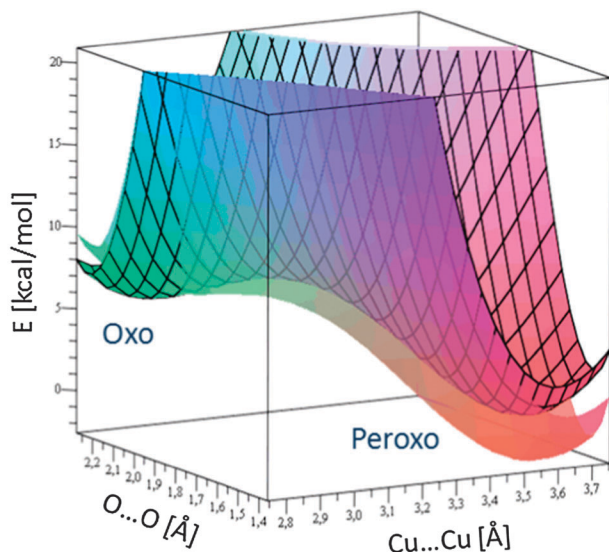


Fig. 2 Potential energy surfaces of the catalytic model system; with grid: singlet PES; transparent: open-shell singlet PES (singlet **P** set as zero).

The distances have been varied between 2.89 and 3.79 Å for the Cu...Cu vector and 1.36 and 2.15 Å for the O...O vector. Since the **P** core is not adequately described by a pure closed-shell singlet,^{8,9,15,22} we have calculated the full broken-symmetry (BS) PES. Fig. 2 depicts the closed-shell and open-shell singlet PES. The effect of spin contamination was found to be negligible (0.5 kcal mol⁻¹). Both PES represent a remarkably narrow valley with steep sides. The **O** core lies in a shallow high valley whereas the **P** core lies in a deep immersion. The pass between both species seems not to be unsurmountable with 8.0 kcal mol⁻¹ from the **P** side. Applying the broken-symmetry approach changes this picture: the **P** core gains 3.3 kcal mol⁻¹ stabilisation yielding a difference in electronic energies of 9.3 kcal mol⁻¹ and a barrier height of 11.2 kcal mol⁻¹. With regard to the two-determinant description of the **P** species, the triplet state has been studied by single-point calculations up to now and was found to be close in energy in some cases.^{8,9,22} We provide with a fully relaxed triplet PES (Fig. 3) and find that this PES is not so steep as the singlet PES (as was expected for an excited state) and lies approximately 10–20 kcal mol⁻¹ above the BS PES.

It is highly noteworthy that the triplet **P** minimum is only 3.7 kcal mol⁻¹ higher than the singlet **P** species and 7.0 kcal mol⁻¹ higher than the BS **P** species. The triplet **P** minimum is shifted towards a slightly shorter Cu...Cu distance (3.51 Å in comparison to 3.56 Å for the closed-shell singlet).

After inspection of the three PES, we focused on the minimum path between **O** and **P** cores by varying the O...O distance and keeping it at fixed values between 1.36 and 2.15 Å. In order to prove the influence of the functional and basis set, we relaxed the complex molecules with fixed O...O distances (Fig. 4A). Remarkably, the minimum profile does not change significantly when using hybrid or pure functionals or applying a PCM solvent model. When using Ahlrichs' triple-zeta basis set, the **O** state cannot be found as a local minimum at all. For the singlet minimum path, we were able to obtain the **O** → **P** transition state **TS** with a transition normal mode

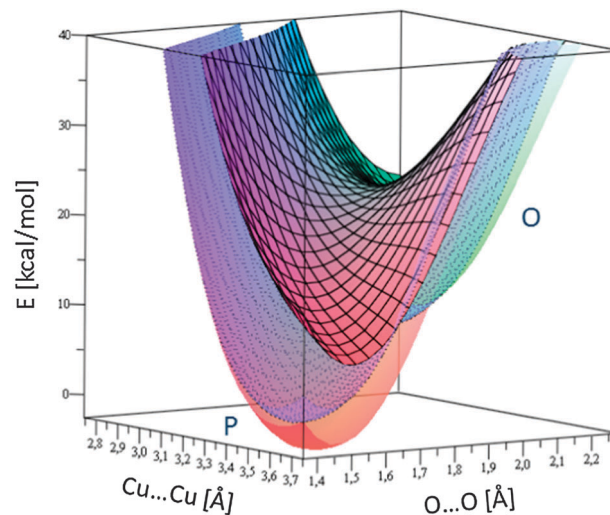


Fig. 3 Potential energy surfaces of the catalytic model system; with light grid: singlet PES; transparent: open-shell singlet PES; with dark grid: triplet PES (singlet **P** set as zero).

of 185 cm⁻¹ in the form of a Cu₂O₂ **P** → **O** core deformation vibration. Inspection of the minimum path towards the energies of the closed-shell singlet, open-shell singlet (broken-symmetry) and triplet (Fig. 4B) states gives a similar surprising picture as the PES: the BS **P** species is stabilised by 3.3 kcal mol⁻¹, the triplet **P** just lying 3.7 kcal mol⁻¹ over the singlet **P**.

Hence, the great relevance of triplet states for the activity of tyrosinase and its models has to be underscored since such an excited state can be easily attained by optical excitation in the π_v* → d_{xy} transition. On the **O** side, the triplet is 11.0 kcal mol⁻¹ higher in energy. This can be explained by the d⁸ configuration of the Cu atoms which try to avoid the triplet state.

In order to understand the electronic situation of the **P** core, we performed NBO analysis²³ (Fig. 4C and Table S7, ESI[†]) and charge decomposition analysis (CDA, Fig. 5).²⁴ The HOMO of the **P** core is the bonding interaction between peroxide σ* and Cu d_{xy} orbitals whereas the LUMO is the antibonding combination of π_{σ*} and Cu d_{xy}.^{3,25} When the **P** core is transformed to the **O** core, these electrons are shifted more into the σ* orbital of the peroxide moiety yielding the oxido bridges but the resulting HOMO is still a bonding combination of oxygen σ* and Cu d_{xy} orbitals with more oxygen contribution.²⁵ The NBO charges in Fig. 4C illustrate that the charge on the copper atoms changes only by 0.12 *e*-units whereas the oxygen charge changes by 0.24 *e*-units during this transition. The overall charge is then compensated by the pyrazolyl and pyridine donors. The CDA allows a detailed insight into (back)bonding of the **P**, **TS** and **O** species. The backbonding of the Cu to the unoccupied σ* orbital is thus very small in the **P** species with 1.7% and grows to a contribution of 40.4% in the **O** core, since here, this orbital is now formally filled and represents the HOMO (mainly consisting of the filled σ*). The increasing charge on the oxygen atoms is also reflected in the increase in back-donation of peroxide π* to Cu in the **P** → **O** pathway. The marginal σ* population in the **P** species is a hallmark for an enhanced electrophilic hydroxylation activity which is found experimentally.²⁰

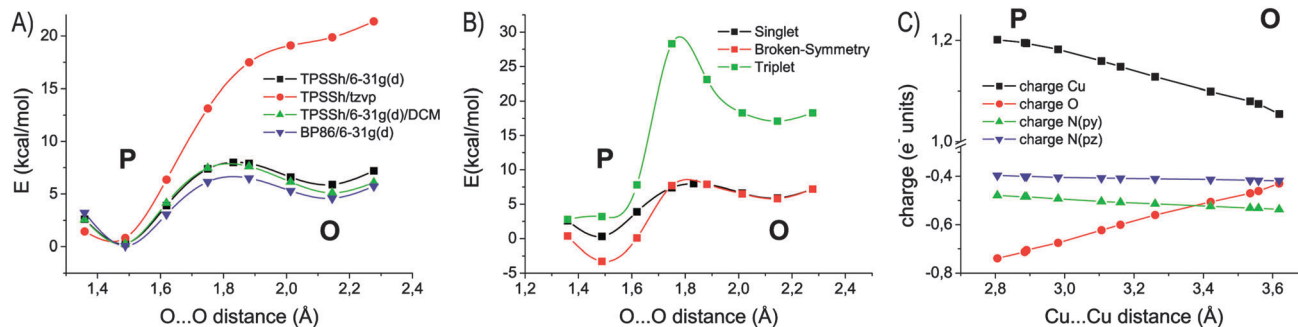


Fig. 4 (A) Singlet minimum path for functional/basis set combinations (**P** with TPSSH/6-31g(d) set as zero); (B) minimum path (TPSSH/6-31g(d)) for the three states (singlet **P** set as zero); (C) NBO charges for the **O/P** transition (TPSSH/6-31g(d)).

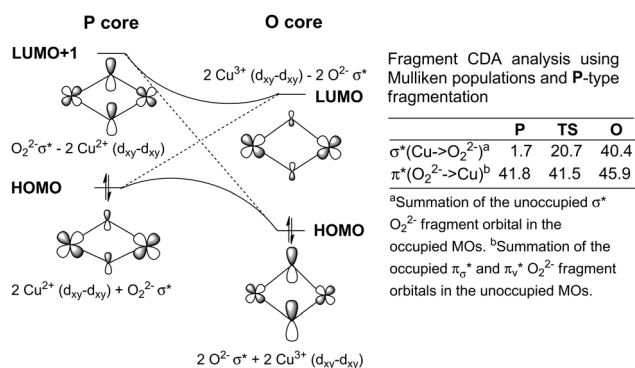


Fig. 5 **P** → **O** correlation diagram²⁵ and CDA results.

In summary, we herein present the first full PES analysis of a **P/O** core equilibrium for the three important states. Moreover, this complete analysis has been accomplished for a real-life catalytic tyrosinase model. We could show that the **P** core lies in a deep global minimum with additional broken-symmetry stabilisation. With regard to the simple optical excitation by visible light, the low-lying triplet **P** state appears as a viable alternative for induction of biological hydroxylation activity.

This work has been supported by the Deutsche Forschungsgemeinschaft (FOR1405 and SFB749, subproject B10). Calculation time at the PC² Paderborn and help with the grid construction by M. Rohrmüller are gratefully acknowledged.

Notes and references

- 1 Y. Matoba, T. Kumagai, A. Yamamoto, H. Yoshitsu and M. Sugiyama, *J. Biol. Chem.*, 2006, **281**, 8981.
- 2 E. I. Solomon, U. M. Sundaram and T. E. Machonkin, *Chem. Rev.*, 1996, **96**, 2563; M. Rolf, J. Schottenheim, H. Decker and F. Tuczek, *Chem. Soc. Rev.*, 2011, **40**, 4077; S. Itoh and S. Fukuzumi, *Acc. Chem. Res.*, 2007, **40**, 592; *Copper-Oxygen Chemistry*, ed. K. D. Karlin, S. Itoh and S. Rokita, John Wiley & Sons, Inc., Hoboken, New Jersey, 2011; T. D. P. Stack, *Dalton Trans.*, 2003, 1881; W. B. Tolman, *Acc. Chem. Res.*, 1997, **30**, 227; P. L. Holland and W. B. Tolman, *Coord. Chem. Rev.*, 1999, **192**, 855; S. Schindler, *Eur. J. Inorg. Chem.*, 2000, 2311; L. Q. Hatcher and K. D. Karlin, *Adv. Inorg. Chem.*, 2006, **58**, 131.
- 3 L. M. Mirica, X. Ottenwaelder and T. D. P. Stack, *Chem. Rev.*, 2004, **104**, 1013.
- 4 J. A. Halfen, S. Mahapatra, E. C. Wilkinson, S. Kaderli, V. G. Young, L. Que, Jr., A. D. Zuberbühler and W. B. Tolman, *Science*, 1996, **271**, 1397.
- 5 L. Que, Jr. and W. B. Tolman, *Nature*, 2008, **455**, 333; V. Mahadevan, R. J. M. Gebbink and T. D. P. Stack, *Curr. Opin. Chem. Biol.*, 2000, **4**, 228.
- 6 T. Inoue, Y. Shiota and K. Yoshizawa, *J. Am. Chem. Soc.*, 2008, **130**, 16890.
- 7 A. Company, S. Palavicini, I. Garcia-Bosch, R. Mas-Balleste, L. Que, E. V. Rybak-Akimova, L. Casella, X. Ribas and M. Costas, *Chem.-Eur. J.*, 2008, **14**, 3535; S. Herres-Pawlis, P. Verma, R. Haase, P. Kang, C. T. Lyons, E. C. Wasinger, U. Flörke, G. Henkel and T. D. P. Stack, *J. Am. Chem. Soc.*, 2009, **131**, 1154.
- 8 C. J. Cramer, M. Wloch, P. Pieruch, C. Puzzarini and L. Gagliardi, *J. Phys. Chem. A*, 2006, **110**, 1991; P. E. M. Siegbahn, *JBIC, J. Biol. Inorg. Chem.*, 2006, **11**, 695; J. L. Lewin, D. E. Heppner and C. J. Cramer, *JBIC, J. Biol. Inorg. Chem.*, 2007, **12**, 1221; A. Poater and L. Cavallo, *Inorg. Chem.*, 2009, **48**, 4062; T. Saito, Y. Kataoka, Y. Nakanishi, T. Matsui, Y. Kitagawa, T. Kawakami, M. Okumura and K. Yamaguchi, *Int. J. Quantum Chem.*, 2009, **109**, 3649.
- 9 B. F. Gherman and C. J. Cramer, *Coord. Chem. Rev.*, 2009, **253**, 723.
- 10 M. Flock and K. Pierloot, *J. Phys. Chem. A*, 1999, **103**, 95.
- 11 M. F. Rode and H.-J. Werner, *Theor. Chem. Acc.*, 2005, **114**, 309.
- 12 K. H. Marti, I. M. Ondik, G. Moritz and M. Reiher, *J. Chem. Phys.*, 2008, **128**, 014104.
- 13 P. Å. Malmqvist, K. Pierloot, A. R. M. Shahi, C. J. Cramer and L. Gagliardi, *J. Chem. Phys.*, 2008, **128**, 204109.
- 14 B. T. Op't Holt, M. A. Vance, L. M. Mirica, D. E. Heppner, T. D. P. Stack and E. I. Solomon, *J. Am. Chem. Soc.*, 2009, **131**, 6421.
- 15 Y. F. Liu, J. G. Yu, P. E. M. Siegbahn and M. R. A. Blomberg, *Chem.-Eur. J.*, 2013, **19**, 1942.
- 16 L. M. Mirica, M. Vance, D. J. Rudd, B. Hedman, K. O. Hodgson, E. I. Solomon and T. D. P. Stack, *Science*, 2005, **308**, 1890.
- 17 M. Réglie, C. Jorand and B. Waegell, *J. Chem. Soc., Chem. Commun.*, 1990, 1752.
- 18 S. Palavicini, A. Granata, E. Monzani and L. Casella, *J. Am. Chem. Soc.*, 2005, **127**, 18031; L. Casella, M. Gullotti, R. Radaelli and P. Di Gennaro, *J. Chem. Soc., Chem. Commun.*, 1991, 1611.
- 19 M. Rolf, J. Schottenheim, G. Peters and F. Tuczek, *Angew. Chem.*, 2010, **122**, 6583 (*Angew. Chem., Int. Ed.*, 2010, **49**, 6438); J. Schottenheim, N. Fateeva, W. Thimm, J. Krahmer and F. Tuczek, *Z. Anorg. Allg. Chem.*, 2013, **639**, 1491.
- 20 A. Hoffmann, C. Citek, S. Binder, A. Goos, M. Rübhausen, O. Troeppner, I. Ivanović-Burmazović, E. C. Wasinger, T. D. P. Stack and S. Herres-Pawlis, *Angew. Chem.*, 2013, **125**, 5508 (*Angew. Chem., Int. Ed.*, 2013, **52**, 5398).
- 21 V. N. Staroverov, G. E. Scuseria, J. Tao and J. P. Perdew, *J. Chem. Phys.*, 2003, **119**, 12129.
- 22 A. Poater and L. Cavallo, *Theor. Chem. Acc.*, 2013, **132**, 1336.
- 23 F. Weinhold and C. Landis, *Valency and Bonding – A Natural Bond Orbital Donor-Acceptor Perspective*, Cambridge University Press, New York, 2005.
- 24 S. Dapprich and G. Frenking, *J. Phys. Chem.*, 1995, **99**, 9352.
- 25 G. Y. Park, M. F. Qayyum, J. Woertink, K. O. Hodgson, B. Hedman, A. A. N. Sarjeant and E. I. Solomon, *J. Am. Chem. Soc.*, 2012, **134**, 8513.