ChemComm
Chemical Communications

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Mukherjee and A. Dey, *Chem. Commun.*, 2020, DOI: 10.1039/D0CC03468A.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/chemcomm

View Article Online
DOI: 10.1039/D0CC03468A

COMMUNICATION

A Heterogeneous Bio-Inspired Peroxide Shunt for Catalytic Oxidation of Organic Molecules

Manjistha Mukherjee, Abhishek Dey*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Heme enzymes are capable of catalytically oxidising organic substrates using peroxide via the formation of high valent intermediate. Iron porphyrins with three different axial ligands are created on self assembled monolayer modified gold electrodes which can oxidize C-H bonds and epoxidize alkenes efficiently. The kinetic isotope effects suggest that Hydrogen atom transfer reaction by a highly reactive oxidant is likely to be rate determining step. Effect of different axial ligands and different secondary structure of the iron porphyrin confirms that thiolate axial ligand with a hydrophobic distal pocket is most efficient for this oxidation chemistry.

Emulating the rich chemistry of cytochrome P-450 (Cyt P450) enzymes, which can catalyse a wide range of oxidation reactions, including the hydroxylation of un-activated primary C-H bonds of alkanes, in artificial systems has been a major goal for the catalysis community for decades. Cytochrome P450 is known to form a high valent Fe^{IV}=O bound to a porphyrin radical cation [$(Fe^{IV}=O)P^{+\cdot}$ or Compound I] species as the main oxidizing agent during the oxidation of the small organic molecules.[1, 2] This highly oxidizing intermediate compound I can be derived from the reaction of the resting ferric (Fe^{III}) state of the enzyme with H_2O_2, known as 'peroxide shunt', as well as via activation of dioxygen where the later pathway requires electron transfer from a reductase component (Figure 1). A group of Cyt P450 enzymes, known as peroxygenase, use peroxides as the surrogate of O_2. Though the use of peroxides can lead to inactivation of enzymes, there are several examples where peroxides have been used effectively in α-β hydroxylation, decarboxylation, oxidation of the cyano groups etc.[3-5]

Previous investigations using synthetic ferric porphyrin complexes for catalyzing alkane hydroxylation reactions have been conducted extensively with oxidants such as PhIO, $KHSO_5$, NaOCl, ROOH and ozone [6-11]. Since H_2O_2 is a biologically important and environmentally benign oxidant, selective oxidation of hydrocarbons with H_2O_2 using ferric porphyrin has always been a sought-after goal. However radical-free (enzyme mimetic) hydroxylation of alkanes and selective epoxidation with H_2O_2 is still difficult to achieve under physiological condition due to the undesired side reactions like catalyst decomposition and uncontrolled oxidation.[12-17] Several attempts have been made by changing the nature of the solvent and electronic structure of the porphyrin to improve both the reactivity and selectivity during the oxidation in homogeneous medium[18]. However, sluggish reactivity towards these demanding substrates rather allow the reactive intermediates to indulge in undesirable side reactions which includes catalyst degradation. Moreover, an axial thiolate ligand which is important for the reactivity of the native enzyme could not be investigated vividly due to the synthetic complexities and vulnerability of axial thiolate ligands[19, 20] towards oxidation in the presence of H_2O_2.

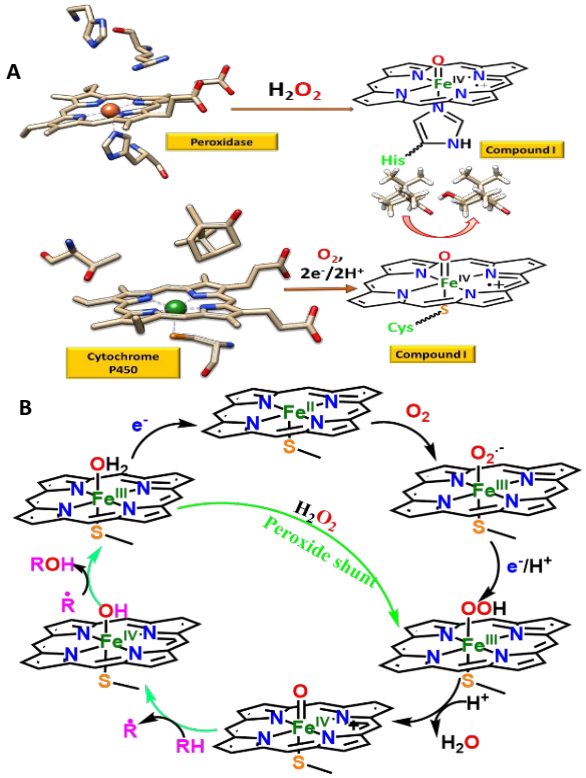


Figure 1: A) Primary reaction taking place in the active site of peroxidase and Cytochrome P450, evidence of similar intermediate B) Schematic representation of the peroxide shunt.

It has been recently demonstrated that a terminal thiolate group at the end of a thiol functionalized gold electrode can bind iron picketfence (FePf) porphyrin (Figure2B) and can generate compound I like intermediate from molecular oxygen using the electrode as the reductase component and this can oxidize alkanes to alcohols and alkenes to epoxide selectively with high turnover numbers (TON)[21]. The selectivity is imparted by the sterically congested "picket fence" architecture which restricts access to the reactive ferryl centre and prefers the access of the secondary C-H bonds over tertiary C-H bonds. Thus, cyclohexane was only oxidized to cyclohexanol (no cyclohexanone) and the 1° C-H of ethyl benzene could be oxidized in the presence of the benzylic C-H bond. Compound I has been detected using in-situ surface enhanced Resonance Raman spectroscopy (SERRS) during the H_2O_2 disproportionation (HPD) reaction by phenolate ligated iron porphyrin attached to the gold electrodes where Fe-O vibration of the compound I species were detected at 796 cm^{-1} and 803 cm^{-1} for porphyrins containing hydrophilic (Fe-tetra ester porphyrin, $FeEs_4$) and hydrophobic (Fe-'picket fence' porphyrin, FePf) distal structures respectively (Figure 2A, Supporting Information Figure S.8).[22] The, compound I, so generated, can be used to oxidize organic substrates.

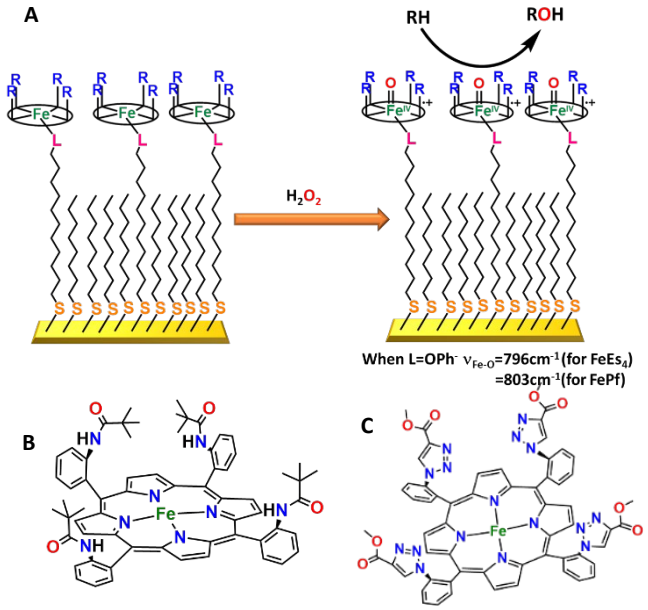
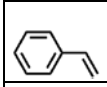
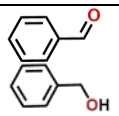
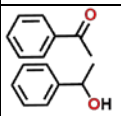
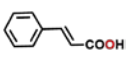


Figure 2: A) Schematic representation of generation of compound I on top of the SAM modified gold electrode using H_2O_2 as the oxidant. The structure of B) FePf and C) $FeEs_4$ are shown.

Generation of compound I with phenolate bound Fe-porphyrin, mentioned earlier, raises the possibility of the formation of compound I during the same HPD reaction by thiolate and imidazole ligated iron porphyrins in this heterogeneous construct. Such hypothesis gets support from the fact that cytP450 is known to generate compound I from H_2O_2 and Metmyoglobin (metMb) with Histidine as axial ligand assists in oxidative reactions of a variety of organic compounds such as lipids, styrenes and sulfides in presence of H_2O_2 (metMb) via the formation of compound I.[23-25] In this manuscript, the oxidation of a range of organic substrates by H_2O_2 in aqueous medium catalyzed by iron porphyrin immobilized on self-assembled monolayer on Au electrodes with varying axial ligands (thiolate, phenolate and imidazole) are reported. The role of different axial ligands and Kinetic Isotope effects (KIE) for those constructs are also evaluated to understand the reactivity properly.

The iron porphyrin complex, Fe-"picket-fence" (FePf) is attached to undecane-1,11-dithiol ($SHC_{11}SH$) modified gold electrode thinly dispersed (surface coverage=$5.23x10^{-13}$moles/cm^2) between octanethiol (C_8SH) self-assembled monolayer (SAM) to mimic the active site of Cytochrome P450 (Cyt P450). In the presence of dissolved organic substrates this Cytochrome P450 analogue could oxidize a number of organic substrates using H_2O_2. The products were then extracted in organic solvent (Chloroform) and analysed using GC-MS. Both hydroxylation of C-H bonds, having BDE as high as 100 kcal/mol, and epoxidation can be achieved with H_2O_2 with TON of ~10^3 (Table 1). The TON is ~10^4 for the oxidation of weaker C-H bonds of toluene (89.8 kcal/mol) and Cinnamaldehyde to yield benzaldehyde and Cinnamic acid, respectively.

Table1: Turnover numbers (TON) for the oxidation of substrates by thiolate ligated FePf complex.

Bond Dissociation Energy (kcal/mole)	Product	Turnover Number for three different axial ligands		
		RS^-	OPh^-	Imd
(100)		$3.4x10^3$ $2.1x10^3$	$1.6x10^3$ $7.4x10^2$	$5.8x10^2$ $9.2x10^2$
		$1.9x10^3$	$3.3x10^2$	$1.3x10^2$
(89.8)		$5.3x10^4$ $5.2x10^2$	$4.6x10^4$ $1.7x10^2$	$2.2x10^4$ 74.5
(2° C-H = 85)		$1.5x10^4$ $1.2x10^4$	ND	ND
(N.A)		$8.2x10^4$	$4.1x10^4$	$7.9x10^3$

These high TONs with respect to the amount of catalyst on the surface are an less than those reported in a recent work where substrate oxidation was achieved using this same construct by electrochemical reductive activation of oxygen, i.e. when molecular oxygen is used as the oxidant instead of H_2O_2 which replicates exactly what happens during the peroxygenation by the enzymatic system.[21] The lower reactivity with H_2O_2 is due to competing HPD reaction (Fig. 4A) by the compound I formed during the reaction of ferric porphyrin with H_2O_2. Notably, the oxidation of cyclohexane with H_2O_2 offer both Cyclohexanol and cyclohexanone with a ratio of 17:10 whereas

[a] School of Chemical Science, Indian Association for the Cultivation of Science, Kolkata, india, E-mail: icad@iacs.res.in

†Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

when previously, using molecular O_2, only cyclohexanol was obtained as the exclusive product. The primary oxidant formed when oxygen was used as an oxidant was compound I and the selectivity was imparted by the steric hindrance of the four tertiary butyl group limiting access of the substrate to the high-valent oxo species at the centre. Accordingly, when the steric hindrance was minimized by decreasing the number of bulky pivolyl group in the distal structure of the porphyrin geometry (iron half-picket, FehPf) (Supporting information, Figure S.7) the selectivity decreased and cyclohexanol and cyclohexanone were found with 26:10 ratio under the same conditions the same catalytic cycle (Figure 3).[21] With H_2O_2 as an oxidant, the ratio of cyclohexanol:cyclohexanone increased to 3:7 for the same porphyrin i.e. FehPf. The over oxidation increases as the concentration of H_2O_2 is increased (Figure 4B) in the reaction medium and in control experiments H_2O_2 in buffered solution could oxidize cyclohexanol to cyclohexanone without any catalyst (Supporting information, Figure S.5) indicating that the selectivity is lost due to the excess H_2O_2 present in the medium. Note that H_2O_2 cannot oxidize C-H bond of cyclohexane without a catalyst (Supporting information Figure S.6).

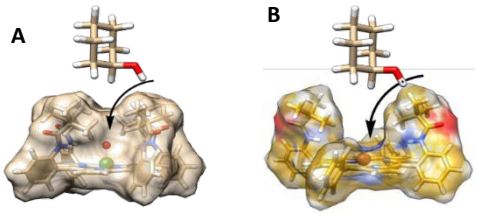


Figure 3: Access of cyclohexanol for over oxidation in case of A) FePf and B) FehPf

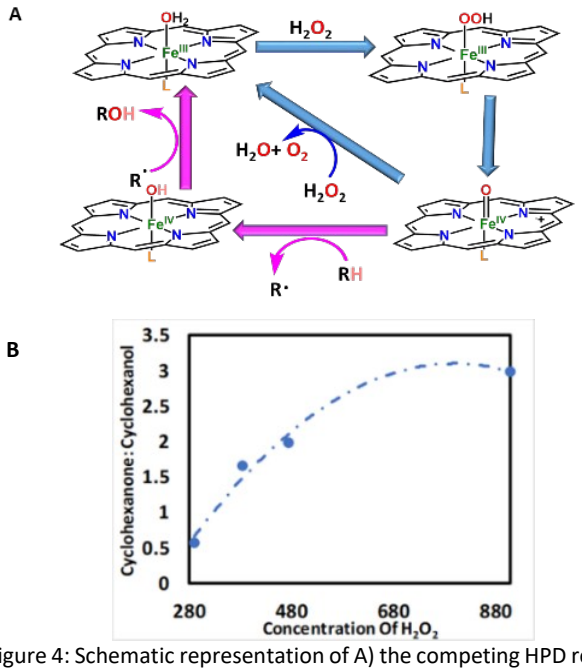


Figure 4: Schematic representation of A) the competing HPD reaction along with substrate oxidation by compound I and B) increase in the overoxidation with increase in the H_2O_2 concentration

The effect of axial ligands on the substrate oxidation activity is evaluated with Imidazole (Imd) and Phenolate (OPh^-) ligated FePf (Figure 5) along with the thiolate (RS^-) ligated analogue (already discussed). The results show that both Imd and OPh bound FePf can catalyze the oxidation of very strong C-H bonds of substrates like Cyclohexane (with BDE˜100kcal/mole) and epoxidize alkenes with high TONs using H_2O_2 as the oxidant. This directly implicates compound I as the key oxidizing species as most theoretical and experimental data suggest that other oxidants like compound 0 and compound II are incapable to achieve both these oxidations.[26-28]The decrease in the TON for both the hydroxylation and epoxidation (Table 1 and figure 5) with axial imidazole and phenolate ligands suggest that the thiolate axial ligand works best for the monooxygenation reactions. This is consistent with site directed mutants of cytochrome P450cam where replacement of the axial cysteine with histidine reduced the oxidation activity by an order of magnitude.[29]

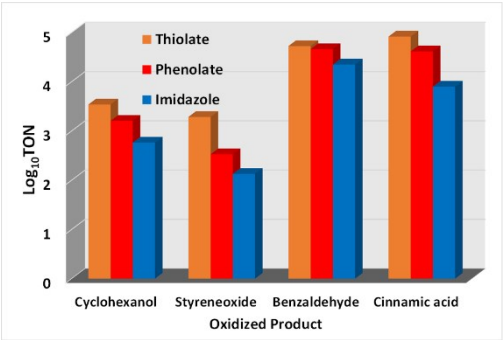


Figure 5: Comparison between the Log(TON) of cyclohexane, styrene, toluene and cinnamaldehyde to form cyclohexanol, Styrene oxide, benzaldehyde and cinnamic acid by thiolate (orange), phenolate (red) and imidazole (blue) ligated FePf.

Kinetic Isotope effect (KIE) on the oxidation is determined using d_8-Toluene. The H/D isotope effect is determined to be 3.6 for a thiolate bound FePf and this value falls in the classical range for Hydrogen atom transfer (HAT) reaction and indicates that HAT of the C-H bond is the rate determining step (rds). Moreover, the imidazole bound FePf shows a KIE of 13.02 for Toluene-d_8 oxidation and the Phenolate bound FePf shows KIE of 22.8 (Supporting information, Figure S.5). The large KIE values suggest that HAT from the benzylic C-H bond of Toluene by compound I is the rate-limiting step for both the catalytic cycles and there is substantial tunnelling in the Transition state (TS). These large isotope effects are also suggestive of tunnelling through a TS having very different polarity relative to the thiolate axial ligand.[30] The higher KIE for phenolate bound FePf than the Imidazole bound analogue supports the hypothesis of Shaik et. Al.[30] The barrier of hydrogen atom tunnelling depends on the electrostatic stabilization of the $H^{\delta+}$as it moves between the $C^{\delta-}$ and $O^{\delta-}$ (Figure 6B) moieties in the Transition state (TS)[30]. The neutral Imidazole axial ligand results in a lesser electrostatic stabilisation in the TS than the anionic phenolate axial ligand in the rate-limiting step. Thus, the lesser electrostatically stabilised transition state for imidazole bound compound I go through a wider tunnelling barrier and thus results in lower KIE values than more electrostatically stabilised T.S of phenolate bound compound I. The strong covalent interaction

between the thiolate and the iron is likely to results in lesser ionic character in the TS and less KIE than phenolate as observed experimentally.

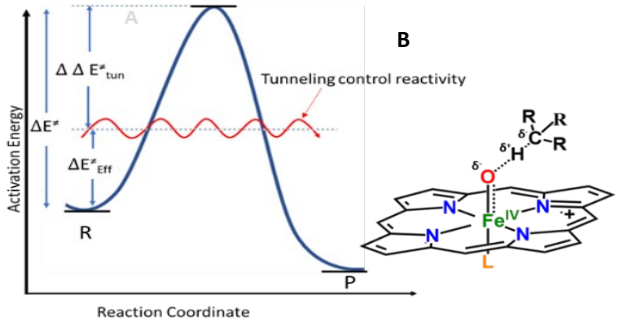


Figure 6: A. Tunnelling lowers the observed barrier relative to the semi classical TS. B. schematic representation of the TS where L represents the different axial ligations.

In summary, the catalytic oxidation of strong C-H bonds and epoxidation of alkene was demonstrated using H_2O_2 with high TON at room temperatures and in aqueous medium by thiolate bound FePf. The in situ generated compound I via a peroxide shunt abstracts hydrogen atom from C-H bond as evident from the isotope effects. Unlike with O_2, the excess H_2O_2 in the medium led to overoxidation of alcohols. Moreover, variation of the axial ligand from thiolate to phenolate to imidazole does not only result in the decrease in the yield but also results in significantly high isotope effect suggesting an involvement of a transition state of very different polarity.[30] The effect of hydrophobic distal pocket was also carried out which shows a show a significant decrease in both the TON and TOF of the oxidation hydrophobic substrates like cyclohexanol and styrene (Supporting information Figure S.9).These results open up new reaction engineering approaches for harnessing a green oxidant like H_2O_2 for useful chemical oxidations and also offer direct insight into the role of axial ligands in tuning the reactivity of very reactive intermediate compound I.

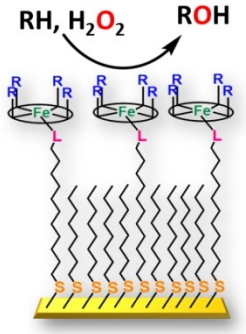
Conflicts of interest

There are no conflicts to declare.

References

1. M. Sono, M. P. Roach, E. D. Coulter and J. H. Dawson, *Chem. Rev.*, 1996, **96**, 2841-2888.
2. J. T. Groves, in *Cytochrome P450: Structure, Mechanism, and Biochemistry*, ed. P. R. Ortiz de Montellano, Springer US, Boston, MA, 2005, DOI: 10.1007/0-387-27447-2_1, pp. 1-43.
3. T. Fujishiro, O. Shoji, S. Nagano, H. Sugimoto, Y. Shiro and Y. Watanabe, *J. Biol. Chem.*, 2011, **286**, 29941-29950.
4. A. W. Munro, K. J. McLean, J. L. Grant and T. M. Makris, *Biochemical Society Transactions*, 2018, **46**, 183-196.
5. O. Shoji and Y. Watanabe, *JBIC Journal of Biological Inorganic Chemistry*, 2014, **19**, 529-539.
6. B. Meunier, in *Metalloporphyrins Catalyzed Oxidations*, eds. F. Montanari and L. Casella, Springer Netherlands, Dordrecht, 1994, DOI: 10.1007/978-94-017-2247-6_1, pp. 1-47.
7. F. Montanari, S. Banfi, G. Pozzi and S. Quici, in *Metalloporphyrins Catalyzed Oxidations*, eds. F. Montanari and L. Casella, Springer Netherlands, Dordrecht, 1994, DOI: 10.1007/978-94-017-2247-6_5, pp. 149-173.
8. J. T. Groves, R. C. Haushalter, M. Nakamura, T. E. Nemo and B. J. Evans, *J. Am. Chem. Soc.*, 1981, **103**, 2884-2886.
9. J. T. Groves and T. E. Nemo, *J. Am. Chem. Soc.*, 1983, **105**, 6243-6248.
10. T. J. McMurry and J. T. Groves, in *Cytochrome P-450: Structure, Mechanism, and Biochemistry*, ed. P. R. O. de Montellano, Springer US, Boston, MA, 1986, DOI: 10.1007/978-1-4757-9939-2_1, pp. 1-28.
11. Z. Gross and L. Simkhovich, *Journal of Molecular Catalysis A: Chemical*, 1997, **117**, 243-248.
12. B. S. Lane and K. Burgess, *Chem. Rev.*, 2003, **103**, 2457-2474.
13. T. G. Traylor, C. Kim, W.-P. Fann and C. L. Perrin, *Tetrahedron*, 1998, **54**, 7977-7986.
14. T. G. Traylor, C. Kim, J. L. Richards, F. Xu and C. L. Perrin, *J. Am. Chem. Soc.*, 1995, **117**, 3468-3474.
15. T. G. Traylor, S. Tsuchiya, Y. S. Byun and C. Kim, *J. Am. Chem. Soc.*, 1993, **115**, 2775-2781.
16. T. G. Traylor, W. P. Fann and D. Bandyopadhyay, *J. Am. Chem. Soc.*, 1989, **111**, 8009-8010.
17. W. Nam, H. J. Han, S.-Y. Oh, Y. J. Lee, M.-H. Choi, S.-Y. Han, C. Kim, S. K. Woo and W. Shin, *J. Am. Chem. Soc.*, 2000, **122**, 8677-8684.
18. W. Nam, S.-Y. Oh, Y. J. Sun, J. Kim, W.-K. Kim, S. K. Woo and W. Shin, *J. Org. Chem.*, 2003, **68**, 7903-7906.
19. J. P. Collman, T. N. Sorrell and B. M. Hoffman, *J. Am. Chem. Soc.*, 1975, **97**, 913-914.
20. F. Tani, M. Matsu-ura, S. Nakayama, M. Ichimura, N. Nakamura and Y. Naruta, *J. Am. Chem. Soc.*, 2001, **123**, 1133-1142.
21. M. Mukherjee and A. Dey, *ACS Cent. Sci.*, 2019, DOI: 10.1021/acscentsci.9b00046.
22. K. Sengupta, S. Chatterjee and A. Dey, *ACS Catal.*, 2016, **6**, 1382-1388.
23. T. Egawa, H. Shimada and Y. Ishimura, *J. Biol. Chem.*, 2000, **275**, 34858-34866.
24. S.-i. Ozaki, T. Matsui and Y. Watanabe, *J. Am. Chem. Soc.*, 1997, **119**, 6666-6667.
25. T. Matsui, S.-i. Ozaki and Y. Watanabe, *J. Biol. Chem.*, 1997, **272**, 32735-32738.
26. E. Derat, D. Kumar, H. Hirao and S. Shaik, *J. Am. Chem. Soc.*, 2006, **128**, 473-484.
27. R. Raag, S. A. Martinis, S. G. Sligar and T. L. Poulos, *Biochemistry*, 1991, **30**, 11420-11429.
28. I. G. Denisov, T. M. Makris, S. G. Sligar and I. Schlichting, *Chem. Rev.*, 2005, **105**, 2253-2278.
29. K. Auclair, P. Moënne-Loccoz and P. R. Ortiz de Montellano, *J. Am. Chem. Soc.*, 2001, **123**, 4877-4885.
30. J. E. M. N. Klein, D. Mandal, W.-M. Ching, D. Mallick, L. Que and S. Shaik, *J. Am. Chem. Soc.*, 2017, **139**, 18705-18713.

View Article Online
DOI: 10.1039/D0CC03468A



338x190mm (96 x 96 DPI)