Article:
Horch, Marius (2019) Rational redox tuning of transition metal sites:Learning from superoxide reductase. Chemical Communications. pp. 9148-9151. ISSN 1364-548X

https://doi.org/10.1039/c9cc04004h

COMMUNICATION

Rational Redox Tuning of Transition Metal Sites: Learning from Superoxide Reductase

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

DOI: 10.1039/x0xx00000x

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Using superoxide reductase as a model system, a computational approach reveals how histidine tautomerism tunes the redox properties of metalloenzymes to enable their catalytic function. Inspired by these experimentally inaccessible insights, non-canonical histidine congeners are introduced as new versatile tools for the rational engineering of biological transition metal sites.

Histidine (His) is an *N*-heterocyclic aromatic amino acid with a substituted imidazole (Im) side chain that plays a major role as a ligand in metalloproteins.[1,2] In its neutral, single-protonated form, the Im side chain of His adopts two tautomeric forms with a proton bound either to N_δ or N_ε (Fig. 1).[‡] In aqueous solution, protonation of N_ε is favoured, most likely due to intramolecular interactions.[3–5] In proteins, however, these interactions cannot occur, and both tautomers are found, e.g., as conserved ligands of metal sites. So far, Nature's choice for either tautomer in individual proteins is not understood.

Superoxide reductase (SOR) is a non-heme iron enzyme that catalyses the reduction of $O_2^{-}\cdot$ to H_2O_2, thereby cycling between ferric and ferrous forms.[6–8] Apart from an axial cysteine (Cys) and four equatorial His ligands, the ferrous form has a vacant coordination site, which is typically occupied by the substrate, a solvent molecule, or a glutamate (Glu) ligand in the ferric state (Fig. 2a).[10–18] Containing a conserved pattern of one N_δ- and three N_ε-coordinated His ligands (Fig. 2a),[10–14] SOR provides a valuable model system to explore the role of His tautomerism in metalloenzymes.

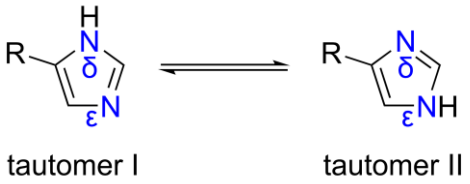

Figure 1: Tautomeric forms of His and related substituted imidazole compounds. Adopting a common nomenclature for His, N_δ and N_ε represent the two non-equivalent nitrogen atoms. In case of MeIm, tautomers I and II are typically designated as 5-MeIm and 4-MeIm, respectively.

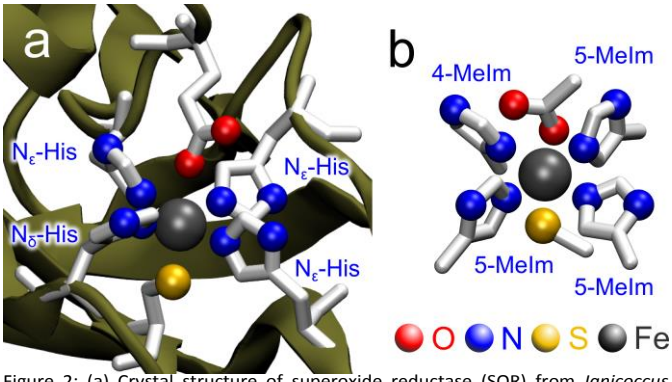

Figure 2: (a) Crystal structure of superoxide reductase (SOR) from *Ignicoccus hospitalis* exhibiting the conserved $(N_\delta)_1(N_\varepsilon)_3$ coordination pattern (PDB: 4BK8).[9] (b) Minimum model of the SOR active site featuring one 4-MeIm and three 5-MeIm ligands mimicking this pattern. Both structures correspond to the ferric resting state.

For both electronic (*vide infra*) and steric reasons, metal coordination *via* N_ε is clearly favoured in non-proteinaceous complexes of alkyl-substituted imidazole.[19] Consistently, N_ε coordination dominates in metalloproteins as well, indicating a distinct role of N_δ-coordinated His in, e.g., SOR. Nonetheless, most previous studies on this enzyme have paid little attention to the four His ligands, despite their dominating contribution to a set of only six strictly conserved amino acids.[7,8] § Since tautomeric forms of amino acids cannot be interchanged by site directed mutagenesis, theoretical methods (see SI2 and SI3) are used in the present study to demonstrate how intrinsic differences between His tautomers affect the properties of (biological) metal sites, using SOR as an example. Isosteric His congeners are also studied and introduced as novel means for rational metalloenzyme design.

Bonding interactions of His with a transition metal ion are governed by the Lewis acid-base properties of the coordinating nitrogen site of the Im side chain. Thus, it is tempting to propose

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Electronic Supplementary Information (ESI) available: Supplementary figures and tables. Absolute energies and Cartesian coordinates of computational models. Supplementary References. See DOI: 10.1039/x0xx00000x

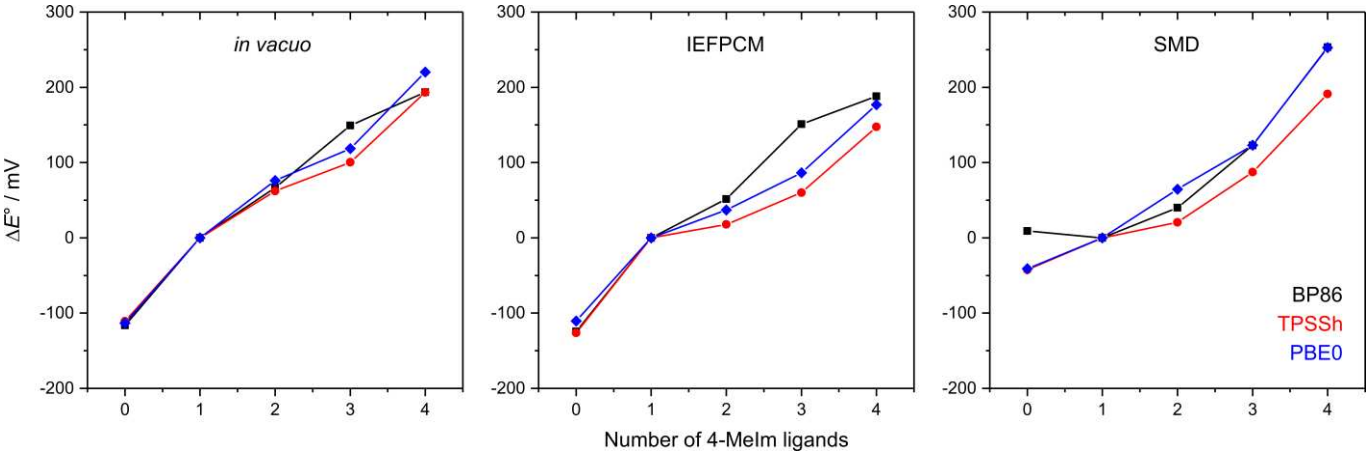

Figure 3: Calculated standard reduction potentials of SOR active site models with different numbers of 4-MeIm (and 5-MeIm) ligands. All values represent potential differences ΔE° between the species of interest and a model reflecting native SOR coordination, $(4\text{-MeIm})_1(5\text{-MeIm})_3$. ΔE° values were derived from gas-phase standard Gibbs free energies (left) as well as aqueous-solution standard Gibbs free energies obtained using IEFPCM (center) and SMD (right) solvation models. Calculations were performed using BP86 (black), TPSSh (red), and PBE0 (blue) density functionals.

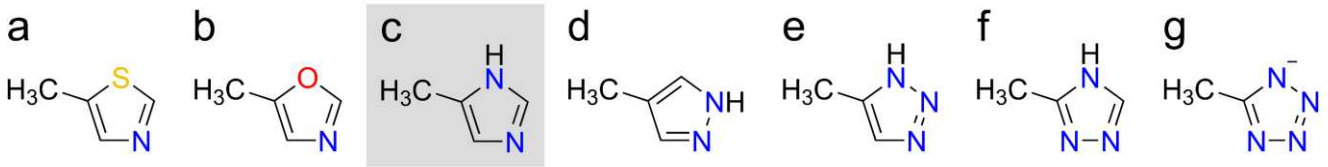

Figure 4: Structural comparison of 5-MeIm (highlighted in grey) with different methylazole compounds used as ligands in computational models of non-native SOR active site variants: (a) 5-methyl-1,3-thiazole, (b) 5-methyl-1,3-oxazole, (c) 5-methyl-1*H*-imidazole (5-MeIm), (d) 4-methyl-1*H*-pyrazole, (e) 5-methyl-1*H*-1,2,3-triazole, (f) 5-methyl-1*H*-1,2,4-triazole, and (g) 5-methyl-tetrazolate.

that characteristics of a (biological) metal centre, e.g. substrate specificity, binding, and conversion, are subtly tuned by the different donor-acceptor properties of the two His tautomers. To evaluate this proposal, the simpler case of His^- interacting with a proton (yielding neutral His) is considered first. Since intrinsic differences between the two nitrogen sites are masked by intramolecular interactions in non-proteinaceous His,[3–5] 5-methylimidazole (5-MeIm) and 4-methylimidazole (4-MeIm), respectively, are used as *in silico* models of N_δ- and N_ε-protonated His instead. 4-MeIm is found to exhibit a lower standard Gibbs free energy, a higher N–H stretch frequency, and a shorter N–H bond length (Table S1). Although subtle, the differences are self-consistent, largely insensitive towards computational details (Tables S1 and S2), and in line with experimental and other theoretical data.[5,20,21,22] Moreover, the same N–H bond-length trend is observed for the imidazolium cation, as also reported by others.[20,21] Since the N–H bond of Im derivatives is built from electron density of the Im ring, binding of a proton can be interpreted in terms of Lewis basicity. Thus, it can be concluded that N_ε is a slightly stronger and/or harder Lewis base than N_δ.

Since interactions of substituted imidazole derivatives with a transition metal ion might differ from those with a proton, *inter alia*, by involving π-interactions,[23] effects on metals sites might be more pronounced. Assuming a noticeable effect, different bonding properties of the two His tautomers are expected to alter the energies of metal *d*-orbitals, which could in principle affect substrate binding. For SOR, however, this scenario can be ruled out since the experimentally observed end-on binding of a dioxo species was well reproduced by computational models with unsubstituted Im ligands,[10,15,16,24]

Alternatively, altered energies of frontier orbitals could affect catalysis by tuning the standard reduction potential E° of the metal site. To evaluate this hypothesis, differences in E° between several variants of an SOR active site minimum model containing different numbers of 4-MeIm and 5-MeIm ligands (Fig. 1b; SI2 and SI3) were calculated. Specifically, models with an equatorial $(4\text{-MeIm})_x(5\text{-MeIm})_y$ coordination pattern ($0 \le x \le 4$; $y = 4 - x$) were compared with a $(4\text{-MeIm})_1(5\text{-MeIm})_3$ model reflecting the native SOR active site with one N_δ- and three N_ε-coordinated His ligands (Fig. 1). According to these calculations, E° increases systematically with the number of N_δ-coordinated 4-MeIm ligands (Fig. 3). This finding is independent from the level of theory, the inclusion and implementation of implicit aqueous solvation, and technical details of standard Gibbs free energy calculations (Fig. 3; Fig. S1), indicating that the observed trend is firmly defined by the intrinsic electronic properties of the two MeIm tautomers. Coordination by N_ε (N_δ) stabilizes the

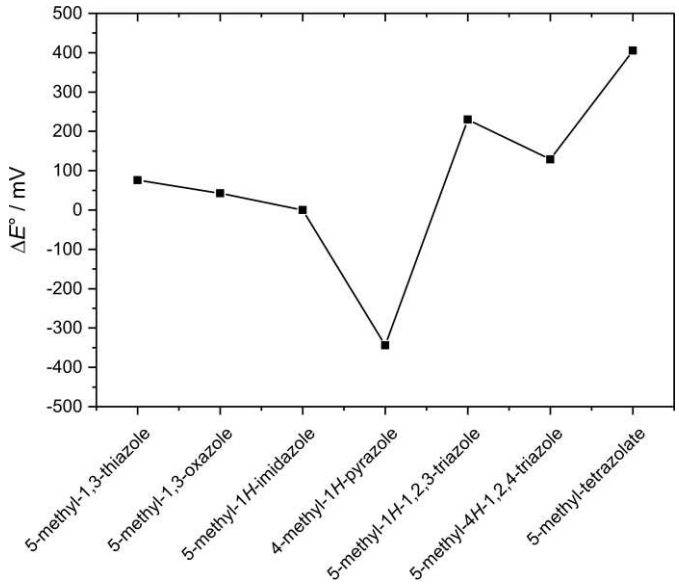

Figure 5: Calculated standard reduction potentials of SOR active site models with a non-native $(4\text{-MeIm})_1(5\text{-MeIm})_2(\text{MeAz})_1$ coordination pattern, where MeAz refers to a methylazole ligand, as indicated (see Fig. 4). All values represent potential differences $\Delta E°$ between the species of interest and a model reflecting native SOR coordination, $(4\text{-MeIm})_1(5\text{-MeIm})_3$. $\Delta E°$ values were derived from gas-phase standard Gibbs free energies obtained using the TPSSh density functional.

ferric (ferrous) forms of the models, which is in line with the finding that N_ε is a harder and/or stronger Lewis base than N_δ. Standard reduction potentials of the investigated variants cover a range of ca. 300 mV, i.e., on average, each included 4-MeIm ligand increases $E°$ by ca. 75 mV (Fig. 3; Fig. S1), demonstrating the (biological) relevance of the observed effect. In particular, inclusion of a single N_δ-coordinated 4-MeIm ligand, analogous to native SOR, increases $E°$ by up to 150 mV (Fig. 3; Fig. S1).

Despite the clear effects of His tautomerism, redox tuning in SOR might appear startling at first since reduction potentials of SORs from different organisms span a range of 170 mV.[18,25,26] However, the experimentally probed redox transition between the ferric resting form and the ferrous state of the enzyme involves Glu dissociation from the iron ion in most cases. Thus, experimental $E°$ values include $\Delta G°$ contributions from the actual redox transition as well as Glu dissociation and protein rearrangement. Since the latter term depends on the flexibility of the Glu-harbouring domain, experimentally derived $E°$ values are unsuited for evaluating the driving force for redox reactions with the substrate, antagonists, or electron donors.

From a thermodynamic point of view, tuning the SOR active site potential may be relevant to enable efficient and selective substrate conversion. On the one hand, the potential should be low enough to provide a driving force for $O_2^{-}\cdot$ reduction ($E°' = 910$ mV)[27]; on the other hand, it should be high enough to limit the formation of highly reactive HO· radicals from H_2O_2 ($E°' = 390$ mV)[27], the product of the SOR catalytic reaction. Notably, this situation implies that the SOR active site potential has to be finely tuned, and the inclusion of a single N_δ-coordinated His ligand appears to provide the best balance between the two requirements. Increasing the reduction potential relative to an all-N_ε coordination would also disfavour undesired high-valent iron species, as formed at the congeneric active site of cytochrome P450, where the four-histidine pattern of SOR is substituted by a porphyrin.[28] Thus, redox tuning of SOR may, together with other effects,[8,29,30] account for different reactivities of the two enzymes.

Tuning the SOR reduction potential could also be relevant from a kinetic point of view. Increasing the active site potential relative to an all-N_ε pattern would accelerate outer-sphere electron transfer between cellular reductants and the SOR active site without impeding the inner-sphere reduction of $O_2^{-}\cdot$.[29,31] This could be particularly relevant for 2Fe-SORs, where intramolecular long-range electron transfer from a second iron site likely proceeds *via* a redox-active tyrosine.[32]

Differences in the bonding properties of the two nitrogen sites also explain previous observations on SOR. N_δ-coordinated 4-ethylimidazole (4-EtIm) was found to dissociate from a computational active site model upon deprotonation of the EtIm ligand in *trans* position, but no such reaction was observed for the N_ε-coordinated 5-EtIm ligands.[26] Considering the *trans* influence arising from the more pronounced σ-donation of the imidazolate anion,[1,33] this effect can be explained by the lower Lewis basicity of the N_δ site. Remarkably, imidazolate formation is prevented in the enzyme by H-bonding to a conserved proline of previously unknown function,[26] thereby protecting the active site from dissociation of N_δ-coordinated histidine.

Inspired by these findings, the impact of alkyl-substituted azoles that can serve as isosteric substitutes for His (Fig. 4) were considered as well. Using advanced genetic engineering,[34] non-canonical amino acids with such side chains could be utilized, e.g., to selectively tune the (redox) properties of SOR and other His-containing metalloproteins without interfering with their overall structure. To illustrate this strategy, standard reduction potentials were calculated for a series of SOR active site models in which one of the 5-MeIm ligands was replaced by another methylazole (Fig. 4 and Fig. 5; Fig. S2). Overall, changes of the standard reduction potential observed for these models are more pronounced than those obtained upon single-tautomer exchange (*vide supra*), confirming that steric contributions to the calculations are low. Subtle effects on $E°$ are observed for methylated thiazole and oxazole congeners, while two methyltriazole tautomers are found to increase the standard reduction potential of the native SOR model by up to 200 mV. The largest effects can be observed for methylated pyrazole and tetrazolate variants, whose standard reduction potentials differ from that of the native SOR model by ca. −350 and +400 mV, respectively. Within the protein, potential changes will also depend on factors not included in these computational models, but the calculations clearly demonstrate the possibility to considerably tune biological transition metal sites by sterically conservative exchange of a single coordinating amino acid.

In the present study, microscopic Lewis basicities were assigned to the nitrogen sites of His, demonstrating that N_ε is a stronger and/or harder Lewis base than N_δ. This effect was shown to systematically tune the standard reduction potential of a computational SOR model, and, thus, it is proposed that His tautomerism is relevant for the catalytic function of this enzyme and the redox tuning of biological metal sites in general. Building on this idea, standard reduction potentials of SOR models containing non-native azole ligands were evaluated. These calculations show that a drastic change of redox

properties can be evoked by substituting a single His ligand with an isosteric non-canonical amino acid. This approach can be expanded towards polysubstituted *N*-heterocycles, applied to other amino acids, and used to design tailored metalloenzymes with higher catalytic activity or altered spectra of substrates and products. The author thinks that this so-far unexplored strategy provides an interesting perspective for bioinorganic research as well as synthetic biology and chemistry.

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ N_δ and N_ε have also been termed N_π and N_τ, respectively. N(1)–H and N(3)–H abbreviations have also been used to designate the two His tautomers, but atom numbering is ambiguous: In biochemical literature, N(1) and N(3) refer to N_δ and N_ε, respectively, while the opposite assignment is used in most other fields.

§ A recent amino acid alignment indicates that only three of the four histidines are strictly conserved.[6] However, it is not known whether sequences lacking the fourth His represent functional SORs and how these may differ from their canonical counterparts.

1 R. J. Sundberg and R. B. Martin, *Chem. Rev.*, 1974, **74**, 471.
2 A. Messerschmidt, ed., *Handbook of Metalloproteins*, John Wiley & Sons, Inc, Hoboken, New Jersey, 2004.
3 S. Li and M. Hong, *J. Am. Chem. Soc.*, 2011, **133**, 1534.
4 W. F. Reynolds, I. R. Peat, M. H. Freedman and J. R. Lyerla, *J. Am. Chem. Soc.*, 1973, **95**, 328.
5 I. Ashikawa and K. Itoh, *Biopolymers*, 1979, **18**, 1859.
6 M. C. Martins, C. V. Romão, F. Folgosa, P. T. Borges, C. Frazão and M. Teixeira, *Free Rad. Biol. Med.* 2019.
7 A. F. Pinto, J. V. Rodrigues and M. Teixeira, *Biochim. Biophys. Acta*, 2010, **1804**, 285.
8 Y. Sheng, I. A. Abreu, D. E. Cabelli, M. J. Maroney, A. F. Miller, M. Teixeira and J. S. Valentine, *Chem. Rev.*, 2014, **114**, 3854.
9 a) M. Horch, A. F. Pinto, T. Utesch, M. A. Mroginski, C. V. Romao, M. Teixeira, P. Hildebrandt and I. Zebger, *Phys. Chem. Chem. Phys.*, 2014, **16**, 14220; b) C. V. Romão, P. M. Matias, C. M. Sousa, F. G. Pinho, A. F. Pinto, M. Teixeira and T. M. Bandeiras, *Biochemistry*, 2018, **57**, 5271;
10 G. Katona, P. Carpentier, V. Niviere, P. Amara, V. Adam, J. Ohana, N. Tsanov and D. Bourgeois, *Science*, 2007, **316**, 449.
11 V. Adam, A. Royant, V. Niviere, F. P. Molina-Heredia and D. Bourgeois, *Structure.*, 2004, **12**, 1729.
12 A. P. Yeh, Y. Hu, Jenney,F. E. Jr., M. W. Adams and D. C. Rees, *Biochemistry*, 2000, **39**, 2499.
13 T. Santos-Silva, J. Trincao, A. L. Carvalho, C. Bonifacio, F. Auchere, P. Raleiras, I. Moura, J. J. Moura and M. J. Romao, *J. Biol. Inorg. Chem.*, 2006, **11**, 548.
14 A. V. Coelho, P. Matias, V. Fülöp, A. Thompson, A. Gonzalez and M. A. Carrondo, *J. Biol. Inorg. Chem.*, 1997, **2**, 680.
15 C. Mathe, T. A. Mattioli, O. Horner, M. Lombard, J. M. Latour, M. Fontecave and V. Niviere, *J. Am. Chem. Soc.*, 2002, **124**, 4966.
16 C. Mathe, V. Niviere, C. Houee-Levin and T. A. Mattioli, *Biophys. Chem.*, 2006, **119**, 38.
17 C. Berthomieu, F. Dupeyrat, M. Fontecave, A. Vermeglio and V. Niviere, *Biochemistry*, 2002, **41**, 10360.
18 J. V. Rodrigues, B. L. Victor, H. Huber, L. M. Saraiva, C. M. Soares, D. E. Cabelli and M. Teixeira, *J. Biol. Inorg. Chem.*, 2008, **13**, 219.
19 a) C.-C. Su, H. C. Jan, H. Kuo-Yih, L. Shyh-Jiun, W. Shion-Wen, W. Sue-Lein and L. Sheng-Nan, *Inorg. Chim. Acta*, 1992, **196**, 231; b) M. Andersson, J. Hedin, P. Johansson, J. Nordström and M. Nydén, *J. Phys. Chem. A*, 2010, **114**, 13146;
20 K. Hasegawa, T. a. Ono and T. Noguchi, *J. Phys. Chem. B*, 2000, **104**, 4253.
21 G.-S. Li, M. F. Ruiz-López and B. Maigret, *J. Phys. Chem. A*, 1997, **101**, 7885.
22 a) G. A. Worth, P. M. King and W. G. Richards, *Biochim. Biophys. Acta Gen. Subj.*, 1989, **993**, 134; b) R. E. Wasylishen and G. Tomlinson, *Can. J. Biochem.*, 1977, **55**, 579;
23 a) C. R. Johnson, W. W. Henderson and R. E. Shepherd, *Inorg. Chem.*, 1984, **23**, 2754; b) W. W. Henderson, R. E. Shepherd and J. Abola, *Inorg. Chem.*, 1986, **25**, 3157;
24 R. Silaghi-Dumitrescu, I. Silaghi-Dumitrescu, E. D. Coulter and Kurtz,D. M. Jr., *Inorg. Chem.*, 2003, **42**, 446.
25 a) L. Chen, P. Sharma, G. J. Le, A. M. Mariano, M. Teixeira and A. V. Xavier, *Eur. J. Biochem.*, 1994, **226**, 613; b) P. Tavares, N. Ravi, J. J. Moura, J. LeGall, Y. H. Huang, B. R. Crouse, M. K. Johnson, B. H. Huynh and I. Moura, *J. Biol. Chem.*, 1994, **269**, 10504; c) I. A. Abreu, L. M. Saraiva, C. M. Soares, M. Teixeira and D. E. Cabelli, *J. Biol. Chem.*, 2001, **276**, 38995; d) V. Niviere, M. Asso, C. O. Weill, M. Lombard, B. Guigliarelli, V. Favaudon and C. Houee-Levin, *Biochemistry*, 2004, **43**, 808; e) T. Jovanovic, C. Ascenso, K. R. Hazlett, R. Sikkink, C. Krebs, R. Litwiller, L. M. Benson, I. Moura, J. J. Moura, J. D. Radolf, B. H. Huynh, S. Naylor and F. Rusnak, *J. Biol. Chem.*, 2000, **275**, 28439; f) A. F. Pinto, C. V. Romão, L. C. Pinto, H. Huber, L. M. Saraiva, S. Todorovic, D. Cabelli and M. Teixeira, *J. Biol. Inorg. Chem.*, 2015, **20**, 155;
26 M. Horch, A. F. Pinto, M. A. Mroginski, M. Teixeira, P. Hildebrandt and I. Zebger, *RSC Adv.*, 2014, **4**, 54091.
27 W. H. Koppenol, D. M. Stanbury and P. L. Bounds, *Free Rad. Biol. Med.*, 2010, **49**, 317.
28 I. G. Denisov, T. M. Makris, S. G. Sligar and I. Schlichting, *Chem. Rev.*, 2005, **105**, 2253.
29 L. M. Brines and J. A. Kovacs, *Eur. J. Inorg. Chem.*, 2007, **2007**, 29.
30 a) J. A. Kovacs and L. M. Brines, *Acc. Chem. Res.*, 2007, **40**, 501; b) N. Lehnert, R. Y. Ho, Que,L. Jr. and E. I. Solomon, *J. Am. Chem. Soc.*, 2001, **123**, 12802; c) O. Horner, J. M. Mouesca, J. L. Oddou, C. Jeandey, V. Niviere, T. A. Mattioli, C. Mathe, M. Fontecave, P. Maldivi, P. Bonville, J. A. Halfen and J. M. Latour, *Biochemistry*, 2004, **43**, 8815; d) D. L. Harris and G. H. Loew, *J. Am. Chem. Soc.*, 1998, **120**, 8941; e) E. I. Solomon, A. Decker and N. Lehnert, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3589; f) A. Decker and E. I. Solomon, *Curr. Opin. Chem. Biol.*, 2005, **9**, 152; g) R. David, H. Jamet, V. Nivière, Y. Moreau and A. Milet, *J. Chem. Theory Comput.*, 2017, **13**, 2987;
31 R. A. Marcus and N. Sutin, *Biochim. Biophys. Acta Rev. Bioenerg.*, 1985, **811**, 265.
32 a) M. D. Clay, Jenney,F. E. Jr., H. J. Noh, P. L. Hagedoorn, M. W. Adams and M. K. Johnson, *Biochemistry*, 2002, **41**, 9833; b) F. Bonnot, S. Duval, M. Lombard, J. Valton, C. Houee-Levin and V. Niviere, *J.Biol.Inorg.Chem.*, 2011, **16**, 889; c) M. Horch, T. Utesch, P. Hildebrandt, M. A. Mroginski and I. Zebger, *Phys.Chem.Chem.Phys.*, 2016, **18**, 23053;
33 A. Blackman, *Advances in Heterocyclic Chemistry*, Academic Press, San Diego, 1993, Volume 58.
34 a) Y. Yu, C. Cui, J. Wang and Y. Lu, *Sci. China Chem.*, 2017, **60**, 188; b) C. C. Liu and P. G. Schultz, *Annu. Rev. Biochem., Vol 80*, 2010, **79**, 413; c) Y. Lu, N. Yeung, N. Sieracki and N. M. Marshall, *Nature*, 2009, **460**, 855;

