DOI: 10.1002/ejic.200900231

Phosphate Ester Hydrolysis: Metal Complexes As Purple Acid Phosphatase and Phosphotriesterase Analogues

Lawrence R. Gahan,*[a] Sarah J. Smith,[a] Ademir Neves,[b] and Gerhard Schenk[a]

Keywords: Metalloenzymes / Biomimetics / Phosphoesterases / Iron / Zinc

This microreview describes the structures and properties of a number of bimetallic complexes designed as both structural and functional mimics of the active sites of some specific metallohydrolase enzymes. The metalloenzymes in question include the predominantly monoesterase-activity-displaying purple acid phosphatase (PAP) and di- and triesterase enzymes, which have significant roles in the bioremedial hydrolysis of organophosphate pesticides and nerve gases.

Introduction

Binuclear metallophosphatases are important in an array of biochemical processes involving the hydrolysis of phosphate ester bonds. These enzymes typically belong to one of three different classes, (i) mono-, (ii) di- and (iii) triesterases. Typical monoesterases include the purple acid phosphatases (PAPs) and are potential targets for drug design against a wide variety of human disorders, including osteoporosis, cancer, cystic fibrosis and depression. Contrastingly, the di- and triesterases (PTEs) are of significance in bioremediation, since they can be modified to degrade pesticides or organophosphorus nerve gases.

PAPs [Figure 1(a)[1]] (E.C. 3.1.3.2) are monoester-cleaving enzymes, members of the metallophosphoesterase superfamily [with a consensus sequence of **GD**(X)$_n$-G**D**XX**Y**XD(X)$_m$G**N**H(D/E)(X)G**H**X**H**, where metal-binding residues are shown in bold] and are among the only binuclear metallohydrolases in which the requirement for a heterovalent active site ($Fe^{III}M^{II}$, where M = Fe, Zn or Mn) for catalysis has been established. PAPs are glycosylated, resistant to inhibition by L-tartrate and catalyse the hydrolysis of phosphorylated substrates at acidic to neutral pH.[2–5] Their characteristic purple colour arises from a tyrosinate-to-Fe^{III} charge-transfer transition (λ_{max} = 510–560 nm; $\varepsilon \approx 3{,}000$–$4{,}000$ $M^{-1}\,cm^{-1}$) at the active site.[6,7] PAPs have been identified in mammals, plants, fungi and bacteria, although sequence conservation between kingdoms is low (<20% sequence homology) and limited predominantly to residues in the catalytic centre.[8] Animal PAPs have a redox-active $Fe^{III}Fe^{III/II}$ centre, where only the mixed-valent form is catalytically competent.[9] Mammalian PAPs can easily and reversibly be oxidised to the inactive diferric form because of the low redox potential (ca. 340 mV) of the divalent iron,[10,11] leading to the suggestion that enzyme ac-

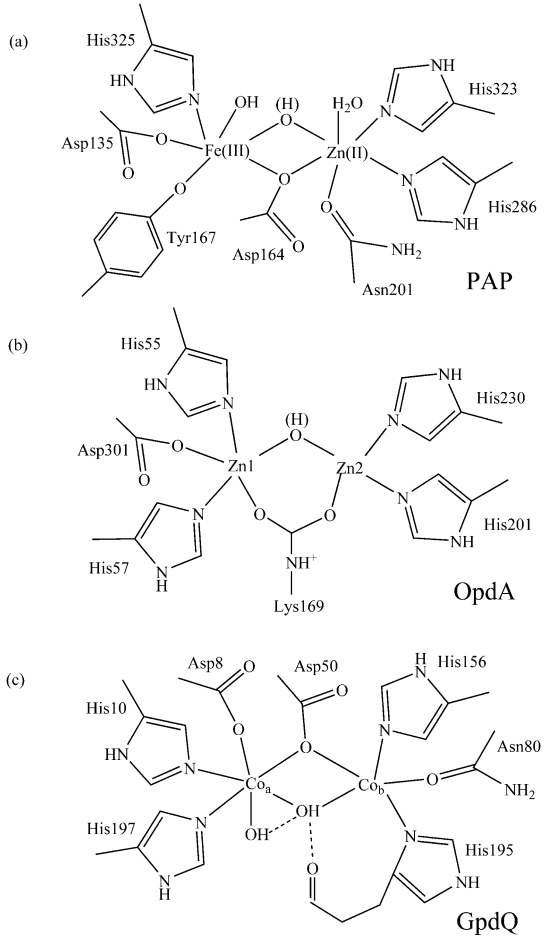


Figure 1. Active site structure of (a) red kidney bean PAP, (b) OpdA/OPH and (c) GpdQ [cobalt(II) analogue].

[a] School of Chemistry and Molecular BioSciences, The University of Queensland, 4072 Brisbane, Australia
[b] Laboratorio de Bioinorgânica e Cristalografica, Departamento de Química, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, SC, Brazil

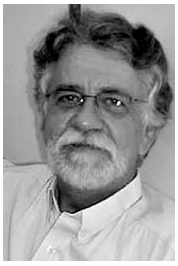
tivity may be regulated in vivo this way.[11] The metal ion composition in plant PAPs is the Fe^{III}-M^{II} type, where M is zinc or manganese, again exhibiting a chromophore centre similar to that of their animal counterparts ($\lambda_{max} \approx 550$ nm).[12–15] The activity of plant PAPs cannot be regulated by reversible oxidation/reduction. Recently, PAPs have been shown to exhibit some diesterase activity.[16]

Most organophosphate (OP) degrading enzymes are phosphotriesterases (PTEs), first characterised from *Flavobacterium sp*[17] and *Pseudomonas diminuta*.[18] These enzymes have identical gene sequences, and their isolation was initially of interest because of their ability to degrade the insecticide parathion.[17,18] PTEs are, however, promiscuous enzymes able to catalyse the hydrolysis of a wide range of OP substrates.[19] PTEs do not occur naturally; however, due to the widespread use of OP pesticides, enzymes have evolved that are capable of hydrolysing these compounds. The PTEs from *Pseudomonas diminuta* (OPH)[20–23] and *Agrobacterium radiobacter* [Organophosphate degrading enzyme from *Agrobacterium radiobacter* (OpdA)]; Figure 1(b)] [24–28] are examples of these, and they have gained increasing attention for their potential application in degrading phosphotriester nerve agents. A promiscuous glycerophosphodiesterase [GpdQ; Figure 1(c); E.C. 3.1.4.46] from *Enterobacter aerogenes* is of particular interest.[25,29–31] GpdQ was initially noted for its remarkable activity towards stable aliphatic diesters such as dimethyl phosphate (DMP) and ethyl methylphosphonate[30,31] and is at present the only known enzyme that is capable of hydrolysing all three types of phosphate esters. Its biological function and that of other members of the glycerophosphodiesterase (GPD) family is the hydrolysis of the 3′-5′ phosphodiester bond of glycerophosphodiesters such as glycerol-3-phosphoethanolamine.[32] Other known substrates for GpdQ include *p*-nitrophenyl phosphate (pNPP), bis(*p*-nitrophenyl) phosphate (BPNPP) and, notably, EA 2192, the toxic hydrolysis product of the nerve agent VX.[25,31,33]

This review will focus on our work on binuclear complexes, biomimetics of phospho-mono-, -di- and -triesterase metalloenzymes, especially PAPs and PTEs.

Lawrence Gahan is an Associate Professor of Chemistry in the School of Chemistry and Molecular Biosciences at the University of Queensland, Brisbane, Australia. He obtained his PhD in Inorganic Chemistry at La Trobe University, Melbourne, Australia, and, after postdoctoral positions in the United States and England, joined the group of Professor Alan Sargeson at the Research School of Chemistry at the Australian National University, Canberra, Australia. In 1984 he joined the Chemistry Department at the University of Queensland as a lecturer. His current research is related to the design of synthetic and functional models for the active site of metalloenzymes.

Sarah Smith received her Honours degree (First Class) in Chemistry at the University of Queensland (Australia) in 2004 and recently completed her PhD under the supervision of Lawrence Gahan and Gerhard Schenk at the University of Queensland. Her research is focused on the mechanism of purple acid phosphatases and biomimetics of binuclear metalloenzymes.



Ademir Neves is a Professor of Chemistry in the Department of Chemistry, Universidade Federal de Santa Catarina (UFSC), Brazil. He obtained his PhD in Inorganic Chemistry (summa cum laude) in 1983 at the University of Bochum, Germany, under the supervision of Professor Karl Wieghardt. From 1984 to 1986 he joined the faculty of chemistry at UFSC as an assistant professor of chemistry. After a one-year postdoctoral stay at the University of Bochum in 1987, again in the laboratory of Karl Wieghardt, he became a full professor of bioinorganic chemistry at UFSC. His current research is related to the design of unsymmetrical small molecules as synthetic and functional models for the active sites of metalloenzymes including catechol oxidases and purple acid phosphatases. The research also includes the application of these transition-metal complexes as tools to probe the recognition and reactions of double helical DNA and the design of novel chemotherapeutics.

Gerhard Schenk is an Associate Professor of Biophysical Chemistry in the School of Chemistry and Molecular Biosciences at the University of Queensland, Brisbane, Australia. He obtained a Diploma in Chemistry from the University of Berne, Switzerland, and was awarded a PhD in Biochemistry from the University of Queensland in 1997. Following a postdoctoral position in the same institution, Dr Schenk spent several months as a visiting research fellow in Prof. A. Geoffrey Sykes' laboratory at the University of Newcastle-upon-Tyne, England (2000). In 2001 he joined the group of Professor Edward I. Solomon in the Department of Chemistry at Stanford University. He has been a member of the Academic Staff at the University of Queensland since 2003. His current research is related to the investigation of the mechanism of binuclear metalloenzymes and the design of antiosteoporotic compounds.

Model Phosphatases

Low-molecular-weight mimics of phosphoesterases simplify the study of both the phosphorolysis and the environment of the metal ions and can provide insight into possible catalytic mechanisms. Typically, binucleating ligands are used to mimic the coordination environment of the metals in metallohydrolases. Two essential factors in the design of binucleating ligands have been outlined: (a) the ligand must accommodate two metals with a well-suited metal–metal separation allowing bridging by required ligands and (b) the coordination environment must dictate the steric and electronic features, in particular the redox properties of each metal centre.[34] Although the second and subsequent coordination spheres of protein systems are complex, and therefore many of the model complexes obtained cannot entirely reproduce the structure or function of an enzyme, they can be instructive in defining parameters that may guide enzyme reactivity.[35] The synergistic interplay between complementary enzyme and model complex studies increases the structural understanding of the enzyme system and can provide information for the design of better model complexes which may be catalytically more relevant, with a more easily accessible mechanism than that of the enzyme itself.[36]

Various complexes modelling the activity of the metallophosphoesterase enzymes have been reported.[37–76] In general, it has been noted that binuclear metal complexes are substantially more efficient phosphorolytic agents than their mononuclear counterparts.[77–82]

The spectroscopic properties of some of the most significant complexes employed to model mammalian and plant PAPs, which are discussed in this review and are most relevant to our work, are reported in Table 1. The kinetic properties of a number of mono-, di- and triphosphatase biomimetic complexes are reported in Table 2. The range of complexes reported is not exhaustive but does illustrate the kinetic properties of a range of hetero- and homobinuclear metal combinations employed as phosphatase models. The variety of different substrates, temperatures and solvent conditions used means that comparison between the activities of the systems is difficult. More interesting is consideration of the different mechanisms proposed, typically determined by the pH dependence of the hydrolysis. For example, the proposed nucleophile varies from a terminally bound hydroxide in Ni_2LH_{-2},[83] Zn_2LH_{-2},[83] $[Cu_2(L^2O)](CF_3SO_3)_3$[78] and Cu_2LH_{-3},[84] to an alkoxide associated with the ligand in $[Zn_2(\{HP\}_2B)(OAc)(H_2O)]PF_6$,[85] and a bridging hydroxide/oxide in $[Fe^{III}Cu^{II}(BPBPMP)(OAc)_2]ClO_4$, (Table 2),[86] $[Zn_2(\mu\text{-}OH)(\mu\text{-}PO_2Ph_2)(BPAN)](ClO_4)_2$ {BPAN = 2,7-bis[2-(pyridin-2-ylethyl)aminomethyl]-1,8-naphthyridine}[77] and dicobalt(III)–tacn (tacn = 1,4,7-triazacyclononane) complexes studied by Williams and others.[87–89] Interestingly, in $[Ni_2(L_2)(\mu\text{-}OAc)_2(CH_3CN)]BPh_4$ (Table 2)[54] both terminal and bridging nucleophiles are proposed to act in a sequential manner, the terminal hydroxide group hydrolysing the diester and the bridging hydroxide group hydrolysing the monoester.

Structural Models of Phosphomonoesterase Metalloenzymes (PAP Biomimetics)

A specific complication in the preparation of authentic PAP biomimetics is the requirement for the stabilisation of a heterovalent binuclear core. Although some heterovalent systems have been prepared by using symmetrical ligands,[40,60,61,90] reliable formation of a heterovalent core requires the use of an unsymmetrical ligand, ideally with different donor atoms of different hardness.[34,91] For example, Belle et al.[92,93] reported the ligand 2-{[bis(2-hydroxybenzyl)amino]methyl}-6-{[bis(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (**1**; numbered ligands referred to throughout this review are illustrated in Figures 2 and 3, where ligand abbreviations have been used as they are reported). This ligand has been used to regioselectively form

Table 1. Spectroscopic parameters of complexes employed to model mammalian (M) and plant (P) PAPs.

Complex	Model	λ_{max} [nm] (ε [$M^{-1}cm^{-1}$])	E_0[a] [V]	J [cm^{-1}]	δ [$mm\,s^{-1}$] (ΔE_Q [$mm\,s^{-1}$])
$[Fe^{III}Fe^{II}(BPBPMP)(\mu\text{-}OAc)_2](BF_4)$[37]	M	556 (4560)	–0.89	–7.4	(N_3O_3) 1.055 (1.751) (293 K) (N_2O_4) 0.423 (1.141)
$[Fe^{III}Cu^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[86]	P	546 (3400)		–0.5[b]	
$[Fe^{III}Mn^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[47]	P	544 (2680)	–0.87	–6.8	0.48 (1.04) (80 K)
$[Fe^{III}Ni^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[65]	P	538 (4813)	–0.94	–13.3	
$[Fe^{III}Zn^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[41]	P	540 (3700)	–0.91		
$[Fe(F)_2Fe(BPBP)(H_2O)_2](BF_4)_2{\cdot}4H_2O$[56,114]	M	356 (403)	–0.61[c]	–8	1.17 (3.26); 0.47 (0.20) (80 K)
$[Fe_2(BPBP)(\mu\text{-}OAc)_2](ClO_4)_2$[56,114]	M	555 (1040)	–0.334[d]	–4	0.98 (1.63); 0.44 (0.53) (280 K) 1.10 (2.03); 0.46 (0.47) (150 K) 1.13 (2.59); 0.48 (0.46) (80 K)
$[Fe(F)_2Cu(BPBP)(H_2O)](BF_4)_2{\cdot}4H_2O$[56,114]	P	494 (860)	–0.623[c]	+2	
$[FeCu(BPBP)(\mu\text{-}OAc)_2](ClO_4)_2$[56,114]	P	551 (776)	–0.308[d]	–20	
$[Fe(F)_2Co(BPBP)(H_2O)_2](BF_4)_2{\cdot}2.5H_2O$[56,114]	P	476 (650)	–0.569[c]	–10	0.47 (0.21) (80 K)
$[FeCo(BPBP)(\mu\text{-}OAc)_2](ClO_4)_2$[56,114]	P	588 (885)	–0.352[d]	–6	
$[FeNi(BPBP)(\mu\text{-}OAc)_2](ClO_4)_2$[56,114]	P	560 (806)		–11	

[a] $Fe^{III}M^{II}/Fe^{II}M^{II}$, CH_3CN, Fc/Fc^+. [b] EPR. [c] 20% (v/v) acetone/dichloromethane, Fc/Fc^+. [d] CH_3CN, Fc/Fc^+; $H_2BPBPMP$ = 2-{[bis(pyridin-2-ylmethyl)amino]methyl}-6-{[(2-hydroxybenzyl)(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (**12**); HBPBP = 2,6-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-*tert*-butylphenol (**11**).

Table 2. Kinetic properties of phosphatase biomimetic complexes.

Complex	k_{cat} [s^{-1}]	K_M [mM]	Substrate	pH optimum	T [K]	Kinetics solvent system	Kinetic pK_a	Potentiometric pK_a
$[Cu_2(LH_{-3})]^{+}$[a][84]	5.54×10^{-3}	26.2	DNPEP	8.60	298	aqueous		
$[Cu_2(L^2O)](CF_3SO_3)_3$[b][78]	11.2×10^{-6}	0.17	BNPP	6	313	aqueous	6.14	3.74; 6.14
$[Zn_2(LH_{-2})]$[c][83]	2.24×10^{-6}	12	BNPP	9.73	308	aqueous		8.15
$[Ni_2(LH_{-2})]$[c][83]	1.49×10^{-4}	14.8	BNPP	10.05	308	aqueous		9.51
$[Ni_2(L_2)\ (\mu\text{-}OAc)_2(CH_3CN)]^{+}$[d][54]	0.368	5.67	BDNPP	9	298	CH_3CN/water (1:1)	8.2	6.65; 8.2
$[Fe^{III}_2L_a]$[e][181]	5.2×10^{-5}	3.1	BNPP	7.36	323	DMSO/water (1:9)		
$[Fe^{III}Fe^{II}(BPBPMP)(\mu\text{-}OAc)_2](BF_4)$[f][37]	3×10^{-3}	13	BDNPP	6	298	CH_3CN/water (1:1)	4.74; 7.54	3.02; 4.13; 5.76; 7.53
$[Fe^{III}Cu^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[f][86]	1.77×10^{-3}	11	BDNPP	7	298	CH_3CN/water (1:1)	5.2; 8.5	5.25; 6.20; 7.82
$[Fe^{III}Mn^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[f][47]	4.51×10^{-4}	2.1	BDNPP	6.7	298	CH_3CN/water (1:1)	5.80; 7.76	
$[Fe^{III}Ni^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[f][65]	4.47×10^{-4}	3.85	BDNPP	6	298	CH_3CN/water (1:1)	4.91; 8.34	5.30; 6.80; 8.61
$[Fe^{III}Zn^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[f][41]	7.31×10^{-4}	8.1	BDNPP	6.1	298	CH_3CN/water (1:1)	4.80; 7.50	4.86; 6.00; 7.22
$[Ga^{III}Zn^{II}(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$[f][123]	1.41×10^{-3}	7.15	BDNPP	6.8	298	aqueous	5.35; 8.55	5.59; 6.19; 7.96
$[Fe^{III}(OH_2)(\mu\text{-}OH)Zn^{II}(BPBPMP)](ClO_4)_2$[f][131]	9.13×10^{-4}	4.2	BDNPP	6.5	298	CH_3CN/water (1:1)	5.3; 8.1	2.93; 4.81; 8.30
$[Zn_2(\{HP\}_2B)(\mu\text{-}OAc)(H_2O)](PF_6)$[g][85]	4.6×10^{-6}	61.5	BDNPP	7.2	323	DMSO/water (30:70)	7.13	7.20; 8.15
$[Zn_2(HL1)(\mu\text{-}OAc)](PF_6)$[h][171]	1.26×10^{-6}	1.96	BNPP	9.0	323	CH_3CN/water (1:1)	7.87	
$[Cd_2(\{HP\}_2B)(OAc)_2(OH_2)](PF_6)$[g][172]	3.8×10^{-3}	8.4	BDNPP	10.5	298	CH_3CN/water (1:1)	8.9	5.3; 8.3
$[Zn_2(L^1H_{-1})(OH)](ClO_4)_2$[i][153]	4.9×10^{-6}	51	BNPP	8.28	323	DMSO/water (1:1)	7.96	5.27; 7.96
$[Zn_2(L^2H_{-1})(MeOH)(OH)](ClO_4)_2$[j][153]	2.3×10^{-5}	56	BNPP	8.28	323	DMSO/water (1:1)	7.60	3.44; 4.36; 7.60
$[Zn_2(L^2H_{-1})(MeOH)(OH)](ClO_4)_2$[k][154]	1.9×10^{-6}	42	BNPP	8.28	323	DMSO/water (1:1)	7.57	7.57
$[Zn_2(L^4H_{-1})]$[l][154]	4.2×10^{-5}	55	BNPP	8.28	323	DMSO/water (1:1)	7.66	6.58; 6.53; 7.66
$[Zn_2(BPMP)(\mu\text{-}OH)]^{2+}$[m][155]	6.4×10^{-4}	13.5	HPNP	–[n]	298	DMSO/water (3:7)	7.4	7.60 (NMR)

[a] L = 1,3,5-trideoxy-1,3,5-tris(dimethylamino)-*cis*-inositol. [b] L^2OH = 1,3-bis{bis[2-(pyridin-2-yl)ethyl]amino}propan-2-ol. [c] LH = 1,1′-(1*H*-pyrazole-3,5-diyl)bis(methylene)bis[octahydro-1*H*-1,4,7-triazonine]. [d] L_2 = 2-{*N*-[2-(pyridin-2-yl)ethyl][1-methylimidazol-2-yl(aminomethyl)]}-4-methyl-6-{[*N*-(2-imidazol-4-yl)ethyl]aminomethyl}phenol. [e] L_a = 2,6-bis{[(2-hydroxybenzyl)(pyridin-2-ylmethyl)-amino]methyl}-4-methylphenol. [f] H_2BPBPMP = 2-{[bis(pyridin-2-ylmethyl)amino]methyl}-6-{[(2-hydroxybenzyl)(pyridin-2-ylmethyl)-amino]methyl}-4-methylphenol (**12**). [g] $\{HP\}_2B$ = 2,6-bis{[(2-hydroxyethyl)(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (**20**). [h] H_3L1 = *N*-(2-hydroxy-3-{[(2-hydroxyethyl)(pyridin-2-ylmethyl)amino]methyl}-5-methylbenzyl)-*N*-(pyridin-2-ylmethyl)aminoacetic acid (**25**). [i] L^1 = *N*,*N*′-(4*H*-pyrazole-3,5-diyl)bis(methylene)bis{2-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl]ethanamine} (**18**). [j] L^2 = *N*,*N*′-(4*H*-pyrazole-3,5-diyl)bis(methylene)bis{1-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl]methanamine} (**19**). [k] L^3 = N^1,$N^{1\prime}$-(4*H*-pyrazole-3,5-diyl)-bis(methylene)bis{N^1-[2-(diethylamino)ethyl]-N^2,N^2-diethylethane-1,2-diamine} (**15**). [l] L^4 = N^1,$N^{1\prime}$-(4*H*-pyrazole-3,5-diyl)bis(methyl-ene)bis(N^1,N^2,N^2-trimethylethane-1,2-diamine) (**17**). [m] HBPMP = 2,6-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (**7**). [n] pH not controlled.

$Fe^{III}Zn^{II}$[93] and $Fe^{III}Cu^{II}$[92] complexes with μ-diphenylphosphato and μ-ethoxy bridges, respectively. Particularly elegant in these studies is the illustration of regioselectivity, by crystallisation of the monocopper species to which iron is added in the case of the iron–copper system and by following the selective binding of the zinc atom by NMR in the iron–zinc system.[92,93] Unfortunately, functional studies relating these complexes to the active site of PAPs were not undertaken. However, a μ-methoxido diferric complex with the same ligand has been reported,[94] and the mixed-valence form of this complex is stabilised. Bernard et al.[42,63] reported the use of the ligand 2-[(3-{[bis(pyridin-2-ylmethyl)amino]methyl}-2-hydroxy-5-methylbenzyl)(pyridin-2-ylmethyl)amino]phenol (**2**) to generate a heterovalent binuclear iron complex. Electrochemical studies of this system indicated that the ligand stabilised the heterovalent and diferric forms, while destabilising the diferrous form, and Mössbauer parameters similar to those of the enzyme were obtained.[63]

The metal complexes of symmetric and unsymmetrical examples of mammalian PAP biomimetics display electrochemical properties that typically depend on the ligand employed[42] and on the nature of the bridging (either carboxylate or phosphate) ligand.[95] The $Fe^{III}Fe^{III}$ complexes of the symmetrical ligands 2,6-bis{[(2-hydroxybenzyl)(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (H_3BBPMP; **3**),[96] 1,3-bis[(2-hydroxybenzyl)(pyridin-2-ylmethyl)amino]-2-propanol (H_3BHPP; **4**),[43] *N*,*N*,*N*′,*N*′-tetrakis(2-benzimidazolylmethyl)-2-hydroxy-1,3-diaminopropane (HTBPO; **5**),[58] 2,6-bis({(2-hydroxybenzyl)[(1-methylimidazol-2-yl)-methyl]amino}methyl)-4-methylphenol (H_3BIOMP; **6**)[97] display electron-transfer processes attributed to Fe^{III}_2/$Fe^{II}Fe^{III}$ and $Fe^{II}Fe^{III}$/Fe^{II}_2 couples, which display a significant cathodic shift when compared to the corresponding redox processes observed in PAPs. $Fe^{II}Fe^{III}$ complexes of symmetrical ligands have also been studied. $Fe^{II}Fe^{III}$ complexes of 2,6-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (HBPMP; **7**),[61,98,99] 4-methyl-2,6-bis({[(6-methylpyridin-2-yl)methyl](pyridin-2-ylmethyl)amino}-methyl)phenol (HBPLMP; **8**),[90] 2,6-bis({[(6-methylpyridin-2-yl)methyl](pyridin-2-ylmethyl)amino}methyl)-4-nitrophenol (HBPLNP; **9**)[40] and 2,6-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-methoxyphenol (HBPMOP; **10**)[100] have been reported. The syntheses are often, but not always, reported as being undertaken under an atmosphere of nitrogen. These mixed-valence complexes typically display one-electron redox processes assigned to Fe^{II}_2/$Fe^{II}Fe^{III}$ and $Fe^{II}Fe^{III}$/Fe^{III}_2 couples with comproportionation constants (K_{comp}) of the order of 10^{10}–10^{12}, indicating substantial stability of the mixed-valence $Fe^{II}Fe^{III}$ complex

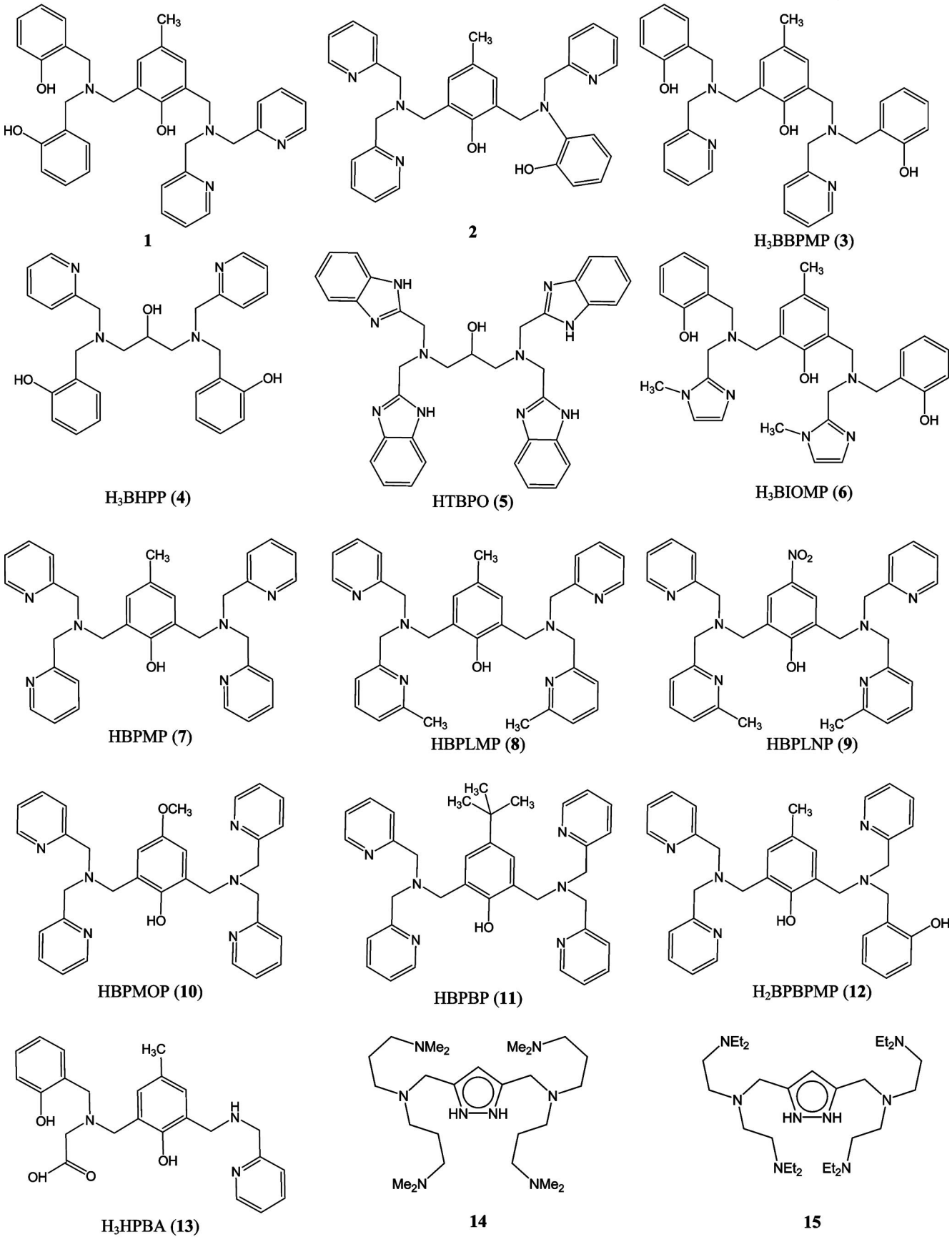


Figure 2. Ligands **1**–**15** referred to in the text and their abbreviations.

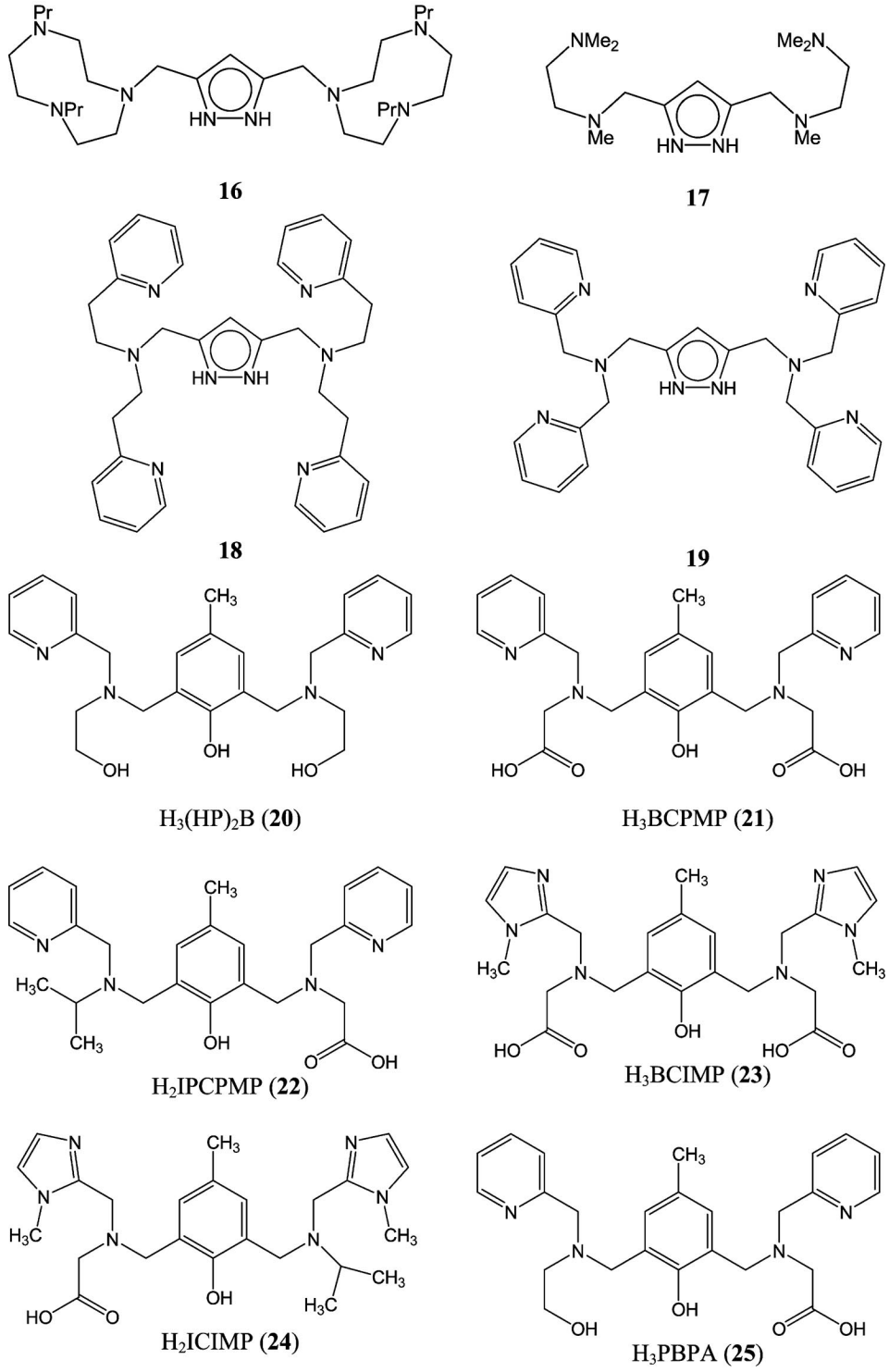


Figure 3. Ligands **16**–**25** referred to in the text and their abbreviations.

over the corresponding Fe^{II}_2 or Fe^{III}_2 complexes.[40,42,56,61,90,98,100,101] Given the facile formation of diferric oxoanion-bridged forms of PAP, which have been characterised through various spectroscopic techniques, including crystallography[102–105] and extended X-ray absorption fine structure (EXAFS),[106,107] and the inhibitory properties of these anions, substantial attention has previously been focussed on the preparation of model complexes of these systems in order to probe the likely inhibitory binding mode. Diferric model complexes with arsenato,[58] phosphato,[62,108,109] chromato,[110] molybdato[111] and sulfato[112] bridges have been reported. Fluoride is an interesting inhibitor of PAPs, displaying a pH-dependent mode of inhibition,[113] which suggests that there are mul-

tiple binding modes for the fluoride ion, and some attention has been focussed on preparation of complexes with a fluoride group[56,114] by employing the ligand 2,6-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-*tert*-butylphenol (HBPBP; **11**). These complexes displayed a terminal binding mode for the fluoride group,[114] while a bridging mode is proposed in the enzymes.[113,115–117] In addition, heterovalent complexes of the form $[FeM(BPBP)(\mu\text{-}OAc)_2](ClO_4)_2$ (M = Co, Ni, Cu, Zn, Fe) and $[(BPBP)Fe(F)_2Cu(H_2O)][BF_4]_2$ were reported,[114] in which the two terminal fluoride ions are bound to the Fe atom and one is strongly hydrogen-bonded to a water molecule on the adjacent Cu ion. The Mössbauer data for the $[Fe_2(BPBP)(\mu\text{-}OAc)_2](BF_4)_2$ complex are consistent with distinguishable iron(III) and iron(II) sites.[114]

Perhaps the best and most widely employed ligand for mimicking the mixed-valence coordination environment of the metals in the active site of PAP is the unsymmetrical ligand 2-{[bis(pyridin-2-ylmethyl)amino]methyl}-6-{[(2-hydroxybenzyl)(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (H_2BPBPMP; **12**).[37,42,63] When complexed with two metal ions in the presence of a suitable bridging ligand (e.g. acetate), the ligand furnishes a (μ-phenoxido)-bis(μ-carboxylato) core with a soft site (N_3O_3) for the divalent metal and a harder site (N_2O_4) for the trivalent metal. Consideration of the reported structures of the complexes of $BPBPMP^{2-}$ reveals some consistent structural features in addition to the soft (N_3O_3) and hard (N_2O_4) site mimics. The metal–metal distance for the $Fe^{III}Fe^{II}$ acetate-bridged complex [3.483(2) Å][118] is comparable to the 3.26 Å separation reported for red kidney bean and sweet potato PAP[103,119] and the 3.31 Å reported in uteroferrin (Uf), the PAP extracted from pig uterine fluid.[102] The key visible spectroscopic feature of the complexes is a terminal phenolate-to-iron(III) charge transfer, which gives rise to an intense deep purple colour (ca. 550 nm), mimicking the tyrosinate-to-iron(III) charge transfer in the protein. The charge transfer is also strongly dependent on pH,[41] and variation of the bridging ligand effects spectroscopic shifts.[42] Susceptibility measurements show that both Fe^{III} and Fe^{II} sites are high-spin and weakly antiferromagnetically coupled, yielding an S = ½ ground state, for which *J* is reported as –7.4 cm^{-1} (μ-acetato)[37] and –4.5 cm^{-1} (μ-diphenylphosphato).[42] Mössbauer parameters of the μ-acetato and μ-diphenylphosphato $Fe^{III}Fe^{II}$ complexes[37,42] clearly indicate the presence of high-spin iron(III) and iron(II) and confirm that iron valences are localised in the solid state. The Mössbauer data are comparable with those of the reduced form of uteroferrin.[120] Importantly, the Fe^{III}/Fe^{II} complex of H_2BPBPMP displays a reversible redox $Fe^{III}Fe^{II}/Fe^{II}Fe^{II}$ couple which is comparable to that observed in uteroferrin.[37,47,65]

The process of carboxylate/diphenylphosphate exchange at the Fe^{III}-Fe^{II} centre has been studied, and it was found that carboxylate ligands readily displace diphenylphosphate (dpp^-) in both the heterovalent and diferric states of the complex, although ligand exchange in the diferric complex is concomitant with reduction to the mixed-valent state.[95] Diphenylphosphate is able to displace acetate ligands when the complex is in the diferric state, and ligand exchange is also accompanied by reduction to the mixed-valent state. It is proposed that liberated carboxylate is the reducing agent in this instance.[95] This has potential ramifications for the investigation of kinetic activity of the complexes, as it is clearly important that at least one of the bridging carboxylates is displaced by the solvent system used for kinetic studies, leaving sites available for phosphate binding and subsequent phosphorolysis.

The $Fe^{III}Fe^{II}$ complex of $BPBPMP^{2-}$ has been used as an electrochemical biomimetic sensor for the determination of phenolic compounds.[121] For a mixture of the complex, Nujol and graphite powder, the analytical curve for dopamine was linear in the region 0.05 to 6.5 mM, the sensor being accurate for six months (≥ 800 determinations).

Heterovalent complexes of $BPBPMP^{2-}$ with iron(III) or manganese(III) with a range of divalent metal ions have been reported.[37,41,42,47,65,86,122] $Fe^{III}Zn^{II}$[41,66,100] and Fe^{III}-Mn^{II}[47] complexes mimicking the red kidney bean and sweet potato PAPs, respectively, have been prepared, in addition to $Fe^{III}Ni^{II}$[65] and $Fe^{III}Cu^{II}$[86] complexes. The heterodinuclear (Fe^{III}/M^{II}, M = Zn, Mn, Ni) complexes of $BPBPMP^{2-}$ display redox couples typical of $Fe^{III}M^{II}/Fe^{II}M^{II}$.[37,47,65] The spectroscopic data for complexes of $BPBPMP^{2-}$ are consistent with those reported for complexes of $BPBP^-$.[56,114]

The analogous symmetric ligands 2,6-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (**7**)[61,98,99] and A2,6-bis{[(2-hydroxybenzyl)(pyridin-2-ylmethyl)amino]-methyl}-4-methylphenol (**3**)[96] permitted an assessment of the site-specificity of the putative trivalent and divalent sites. The mixed $Ga^{III}Zn^{II}$ complex of $BPBPMP^{2-}$ and the digallium complexes of ligands **3** and **7** have been prepared and characterised.[123] Comparison of the ^{71}Ga NMR spectra of these complexes suggests that the Ga^{III} ion is located exclusively in the N_2O_4 hard site of the asymmetric ligand $BPBPMP^{2-}$; ES-MS confirms that no $Ga^{III}Ga^{III}$ or Zn^{II}-Zn^{II} complexes are formed. The study suggests that the zinc(II) ion is exclusively coordinated in the softer N_3O_3 site, and the crystal structure of $[GaZn(BPBPMP)(\mu\text{-}OAc)_2](ClO_4)$ also supports a mixed metal centre. By analogy, the structurally related $Ga^{III}Zn^{II}$ complex in Uf is also anticipated to display the same metal ion selectivity.[123]

Potentiometric titrations of the $[Fe^{III}M^{II}(BPBPMP)(\mu\text{-}OAc)_2]^+$ complexes in water/ethanol (30:70) have revealed three protonation equilibria in the pH range 4–10.[37,41,47,65,86] It has been proposed[65] that, upon dissolving $[Fe^{III}Ni^{II}(BPBPMP)(\mu\text{-}OAc)_2]^+$, dissociation of the first carboxylate ion leads to a species described as $[(OH)Fe^{III}(\mu\text{-}OAc)Ni^{II}(OH_2)]$, where the pK_{a1} (5.30) value is consistent with the dissociation of the Fe^{III}-bound terminal water molecule.[65] Dissociation of the second carboxylate bridge results in the formation of a species in which a second water molecule is bound to the Fe^{III} centre, and upon its deprotonation (pK_{a2} = 6.80) an $[(HO)Fe^{III}(BPBPMP)(\mu\text{-}OH)Ni^{II}(OH_2)]^+$ species is formed. This second pK_a presumably does not affect the rate of catalysis by the complex. Finally,

deprotonation of the Ni^{II}-bound terminal water molecule (pK_{a3} = 8.61) leads to the [(OH)Fe^{III}(μ-OH)Ni^{II}(OH)] species. Assignment of this pK_a is supported by the observation that it is the most substantially affected by changes of the divalent metal ion.[41,47,65,86] It is also relevant to note that the pK_{a1} values related to the deprotonation of the Fe^{III}–OH_2 bond are comparable to pK_a values determined from the acidic limbs of the pH dependence parameters for PAPs.[124–127]

Functional Models of Phosphomonoesterase Metalloenzymes (PAP Biomimetics)

A mechanistic model for PAPs that invokes a direct transfer of the phosphoryl group of the substrate to a metal-coordinated, nucleophilic solvent molecule has emerged.[1] A useful method for probing the structure and function of metalloenzymes is metal ion replacement, essentially removing the native metal ions from the enzyme, to generate the apo-enzyme, and replacing them with other metals of interest. There are two key reasons for altering the metal ion composition of an enzyme. The first is to investigate the geometric and electronic properties of the active site by replacing the native metal ion(s) with a different metal ion which provides a better spectroscopic probe. The second is to assess the impact of altering the metal ion composition on reactivity. Specifically, if the metal ion plays a key role in reactivity, for example, by furnishing the nucleophile, alteration of the metal ion would be expected to affect the reactivity and kinetic parameters of the enzyme. In the case of the biomimetics, exactly the same philosophy can be applied.

The substrates used for model complex assays are illustrated in Figure 4. pNPP is a phosphomonoester substrate also used in enzyme assays, the phosphodiester counterpart of which is BNPP. BDNPP is a highly activated phosphodiester, which has been used exclusively for model complex studies.[128–130] HPNP is a phosphodiester mimic of RNA that has been used in model studies for RNAase models (this kind of activity is very similar to phosphatase activity, and so these complexes will be included in this review).

The phosphatase activities of the mixed-valence complexes $Fe^{III}M^{II}$ (M = Zn, Cu, Mn and Ni) of $BPBPMP^{2-}$ have been measured with the activated substrate BDNPP (Table 2).[86,131,132] These reactions are strongly dependent on pH, giving rise to bell-shaped pH vs. rate profiles and pH optima in the range 6.0 to 7.0[41,47,65] and show modest activity ($k_{cat} \approx 10^{-3}$–10^{-4} s^{-1}). The two kinetically relevant pK_a values typically correspond to the highest and lowest pK_a values measured in the potentiometric titrations (pK_{a1} and pK_{a3}). In each case, the catalytically active species is proposed to be of the type [(OH)Fe^{III}(μ-OH)M^{II}(OH_2)].[1] Although not as active as the PAPs themselves, these biomimetics are approximately three orders of magnitude more efficient in hydrolysis relative to the uncatalysed hydrolysis of the substrate.[133]

In an extensive study of the complex [Fe^{III}(BPBPMP)(μ-$OAc)_2Zn^{II}$]$^+$, Neves et al.[131] shed significant light on the mechanism of these biomimetics. These authors were able to isolate and crystallographically characterise the dinuclear complex [(H_2O)Fe^{III}(BPBPMP)(μ-OH)Zn^{II}]$^{2+}$ with a terminal, Fe-bound water molecule at a position equivalent to that of the proposed nucleophile in red kidney bean PAP.[119] Potentiometric studies revealed pK_as consistent with deprotonation of a bridging water molecule (2.93), a terminal Fe–OH_2 (4.81) and Zn–OH_2 (8.30) group. The phosphatase activity of this complex was studied with BDNPP. The resulting bell-shaped pH rate profile exhibited an optimum at pH 6.5, with pK_a values of 5.3 and 8.1, demonstrating that the catalytically active species was [(OH)Fe^{III}(μ-OH)Zn^{II}(OH_2)].[131] An EPR study indicated that the substrate did not interact with the Fe^{III} site, and the measured kinetic isotope effect of k_H/k_D = 1.34 suggested that no proton transfer was involved in the rate-limiting step, supporting an intramolecular nucleophilic attack by the Fe^{III}-bound hydroxide. The authors were also able to conclude that the μ-OH was a significantly poorer nucleophile for the hydrolysis of the diester substrate than the terminal, Fe^{III}-bound hydroxide.

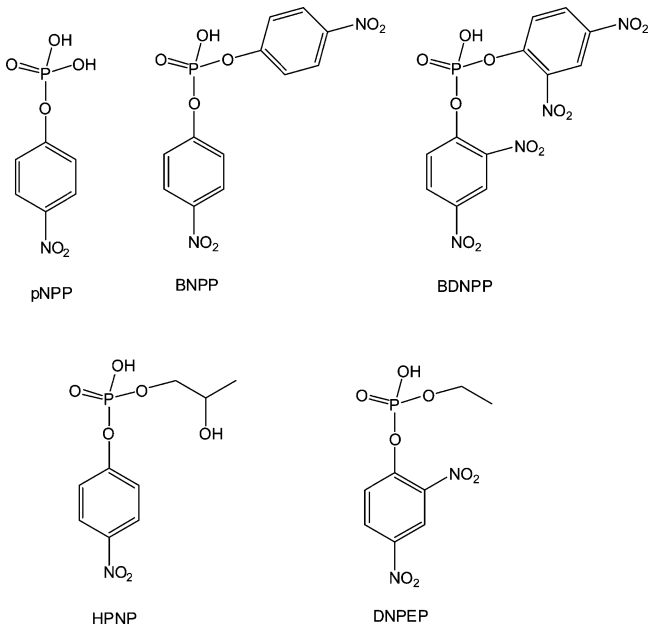


Figure 4. Substrates used in phosphatase assays.

While it is believed that PAPs are only active in the heterovalent state,[134] phosphatase activity is reported for various Fe^{III}_2 model complexes.[51,129,135–139] In some of these systems, asymmetry has been introduced by substitution of a 2,2′-bipyridyl ligand with a substituted 1,10-phenthroline, for example [$Fe_2O(bipy)_3$(4,4′-Me_2Phen)-(OH)(OH_2)](NO_3)$_3$;[137] in other cases symmetric complexes such as [$Fe_2O(phen)_4(OH_2)_2$](NO_3)$_4$[129] were employed. In each case the catalytically active species was determined to be a nucleophilic hydroxide bound to one Fe^{III} site and an exchangeable (H_2O)Fe^{III} site. The ligand *N*-(2-hydroxybenzyl)-*N*-(2-hydroxy-5-methyl-3-{[(pyridin-2-ylmethyl)amino]-methyl}benzyl)aminoacetic acid (H_3HPBA; **13**) mimics that of the active site of PAP. With iron(III) or iron(II), the tet-

ranuclear complex $[Fe_4(HPBA)_2(\mu\text{-}OAc)_2(\mu\text{-}O)(\mu\text{-}OH)(OH_2)_2]ClO_4{\cdot}5H_2O$ forms.[140] The phosphoesterase-like activity of this complex in 50:50 acetonitrile/water, under which conditions the complex exists as an $Fe^{III}Fe^{III}$ dimer, was investigated by using the substrate BDNPP. Preliminary analysis of the data reveals a K_M of 8.7 ± 0.6 mM and k_{cat} of 3.5 $(\pm 0.2) \times 10^{-3}$ s^{-1}, with kinetically relevant pK_as of 5.0 and 10.5.[118] The magnitude of k_{cat} is comparable to that reported for any of the other heterovalent PAP biomimetics. The results of this and other studies with homotrivalent Fe^{III} models suggest that, in the model systems the slow exchange of product is not a significant mechanistic impediment to the hydrolysis reaction, whereas in the PAP enzyme systems a homotrivalent active site would be.

Typical Mimics of Phosphodi- and Triesterase Metalloenzymes (PTE Biomimetics)

The study of synthetic analogues of the di- and triesterases has generally focussed on two themes. The first and most common is the study of the hydrolysis of phosphodiester bonds of nucleic acids. This area has been recently reviewed,[73,75,141] and only brief mention will be made here. Studies in this area include the use of dinickel(II),[142] dicopper(II),[57,142–147] dizinc(II)[148–151] and monocopper(II)[152] systems. Meyer and co-workers prepared and characterised a series of pyrazolate-based ligands (**14**–**19**) and their corresponding dizinc(II) complexes as functional models for the hydrolytic cleavage of nucleic acids.[153,154] By controlling the topology of the compartmental ligand scaffold, they were able to modulate the Zn···Zn distance, the bridging or nonbridging position of the zinc-bound hydroxide nucleophile as well as the coordination numbers of the individual metal ions.[154] Amongst other outcomes, these authors were able to demonstrate that the involvement in strong hydrogen bonding, as exemplified by an O_2H_3 bridge, can promote a dramatic decrease in the pK_a of the zinc-bound water molecule when compared to that observed for a μ-hydroxido ligand, suggesting that the O_2H_3 moiety may play a significant role in oligozinc hydrolases.[76,154] In a subsequent report, the same authors investigated the functional role of the Zn–(H)O···HO(H)–Zn motif.[153] In both cases BNPP was employed as the substrate. Two ligands, *N*,*N*′-(4*H*-pyrazole-3,5-diyl)bis(methylene)bis{2-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl]ethanamine} (**18**) and *N*,*N*′-(4*H*-pyrazole-3,5-diyl)bis(methylene)-bis{1-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl]methanamine} (**19**), differing in the length of the pyridyl side arms, were employed. The shorter side arms in the latter ligand restrain the two zinc(II) ions, imposing a longer intermetallic separation.[153] The authors concluded that enforcing a large Zn···Zn separation, thus prohibiting the formation of a tightly bridged Zn–O(H)–Zn arrangement, promoted the formation of the Zn–(H)O···HO(H)–Zn unit, which is considered to be the crucial motif in oligozinc enzyme chemistry.[153] These authors also investigated the transesterification of phosphate diesters by using these metal complexes.[153] The addition of 2-amino substituents to the pyridine donors of a binucleating ligand has been shown to enhance substrate binding and catalytic activity for phosphodiester transesterification.[148–150] These systems combine hydrogen-bonding interactions and double Lewis activation. Chen et al.[85] employed the symmetric ligand 2,6-bis{[(2-hydroxyethyl)(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol ($H_3(HP)_2B$; **20**] as a model nuclease. The X-ray crystal structure has shown that the dizinc(II) complex contains a μ-acetato-μ-cresolatodizinc(II) core composed of one quasi-trigonal-bipyramidal zinc site and a distorted six-coordinate zinc(II) site.[85] The phosphodiesterase activity of the complex was investigated, again by employing BNPP as the model nucleic acid substrate; the conclusion reached was that the nucleophile was not a metal-bound hydroxide but rather a metal-bound alkoxide.[85] Belle and co-workers[155] in very elegant work combined structural, kinetic and theoretical studies of these zinc biomimetics to investigate both the hydrolysis of the substrate HPNP and the transesterification reaction. The ligand employed was 2,6-bis{[bis(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol (**7**), and the authors were able to show that the catalytically active species was the Zn–O(H)–Zn rather than the nonbridged $(H_2O)Zn(H_2O)Zn$ form. Nordlander[48] reported the synthesis of a symmetric (2,6-bis-{[(carboxymethyl)(pyridin-2-ylmethyl)amino]methyl}-4-methylphenolate, trisodium salt; Na_3BCPMP; **21**) and an asymmetric [2-{[*N*-isopropyl-*N*-(pyridin-2-ylmethyl)-amino]methyl}-6-{[*N*-(carboxymethyl)-*N*-(pyridin-2-ylmethyl)amino]methyl}-4-methylphenol, hexafluorophosphate salt; $H_4IPCPMP(PF_6)_2$; **22**] ligand and their respective dizinc(II) complexes and studied the transesterification reaction of HPNP. In line with previous results, the unsymmetrical complex was more active than the symmetrical.

The second, and less well-explored, theme in the study of synthetic multinucleases is that the design of biomimetics to model the metalloenzymes which hydrolyse organophosphates, typically pesticides and warfare agents. As mentioned in the Introduction, phosphate triesters do not occur naturally, but enzymes capable of hydrolysing these compounds have evolved. The phosphotriesterases from *P. diminuta* (OPH), *A. radiobacter* (OpdA) and the promiscuous glycerophosphodiesterase (GpdQ) from *E. aerogenes* are examples of these. The active sites of these PTEs contain a binuclear metal centre and were thought to natively contain a single Zn^{II} in the active site,[156] but recent analysis by atomic absorption spectroscopy and anomalous scattering suggests that OpdA, at least, is natively a Zn^{II}/Fe^{II} enzyme.[157] PTEs are also catalytically active as Co^{II}-, Cd^{II}-, Mn^{II}- or Ni^{II}-substituted forms.[158] Structures of several metal-substituted OPHs (Zn^{II}/Zn^{II}, Zn^{II}/Cd^{II}, Cd^{II}/Cd^{II}, Mn^{II}/Mn^{II}, Co^{II}/Fe^{II}) have been obtained.[28,157,159–161] For Cd^{II}/Cd^{II}, Zn^{II}/Cd^{II} and Mn^{II}/Mn^{II} OPHs, the more buried metal site (α-site) is five-coordinate, the four amino acids His55, His57, Asp301 and Lys169 and a hydroxide molecule forming a trigonal bipyramidal geometry (Figure 1). Both Lys169 and the hydroxide molecule act as bridges to the second more solvent-exposed metal

site (β-site), which has additionally His201, His230 and two terminal water molecules as ligands, forming a distorted octahedral geometry.[159,162] Co^{II}/Co^{II} OpdA, crystallised in the presence of polyethylene glycol (PEG), has a similar structure, except that the α-metal adopts an octahedral geometry upon monodentate coordination of an ethylene glycol (EGL) molecule, possibly from degradation of PEG.[163] In comparison, the β-metal ion of Zn^{II}-substituted OPH adopts a five-coordinate, trigonal bipyramidal geometry in which one of the terminal water ligands are moved away from Zn^{II} into a noncoordinating position.[159] The protonation state of the bridging water molecule was probed in the Mn^{II}-substituted form of OPH with EPR spectroscopy. At pH 8.3, the spectroscopic data are consistent with the presence of a μ-hydroxide (as evidenced from the relatively weak exchange coupling constant $|J| = 2.7 \pm 0.02\ cm^{-1}$); decreasing the pH to 7.0 leads to its protonation, forming uncoupled Mn^{II} species.[164] Molecular simulation studies, including ab initio and density functional theory calculations, with Cd^{II}- and Zn^{II}-substituted OPH have also demonstrated that a μ-hydroxide group is present in the catalytically active enzyme.[165,166]

The crystal structures of Zn^{II}- and Co^{II}-substituted GpdQ have initially been solved to 2.9 Å and 3.0 Å, respectively,[29] and an improved structure (1.9 Å) of the diZn^{II} form illustrates the presence of an extensive hydrogen-bonding network in the active site.[29] The oligomeric structure of the protein is hexameric, forming a trimer of dimers.[29] Each subunit contains a binuclear metal centre (Figure 1), and each metal ion is coordinated by four amino acid side chains, two aspartate groups and two histidines for the metal in the M1 or α site, and two histidines, one aspartate and one asparagine for the metal in the M2 or β site. One of the aspartates acts as a bridging ligand (Asp50). Structural and spectroscopic studies suggest that the α site has a higher affinity for metal ions than the β site.[167] The active site structure of GpdQ is remarkably similar to that of a number of binuclear phosphomonoesterases, including PAPs,[102,105,117,119,168] 5′-nucleotidase[169] and Mre11 nuclease.[170]

Using the symmetrical ligand 2,6-bis({[*N*-(carboxymethyl)-*N*-(1-methyl-1*H*-imidazol-2-yl)methyl]amino}-methyl)-4-methylphenol (H_3BCIMP; **23**) and the asymmetric ligand 2-({[*N*-isopropyl-*N*-(1-methyl-1*H*-imidazol-2-yl)-methyl]amino}methyl)-6-({[*N*-(carboxymethyl)-*N*-(1-methyl-1*H*-imidazol-2-yl)methyl]amino}methyl)-4-methylphenol (H_2ICIMP; **24**), Nordlander et al.[151] prepared five new zinc(II) complexes as structural and functional models of the active site of a phosphotriesterase. One complex, $[Zn_4(ICIMP)_2(Ph_2Ac)_2](ClO_4)_2$, was structurally characterised as a dimer of dimers, although in solution the complex dissociates to form a structural model of the enzyme active site. Functional studies involving the hydrolysis and transesterification of HPNP have shown that the complex formed in solution from $[Zn_4(ICIMP)_2(Ph_2Ac)_2](ClO_4)_2$ displays a significantly higher rate of catalysis than the complex formed from the symmetrical ligand $[Zn_2(BCIMP)(Ph_2Ac)]$. The difference is attributed to the vacant and/or labile coordination site available in the former complex. The authors concluded that the open coordination site afforded by the ICIMP ligand was significant in terms of the ability of the metal complexes to efficiently catalyse the hydrolysis of the substrate, in this case HPNP. The hydrolysis reaction was suggested to occur through a terminally bound hydroxy group although the authors conceded that the evidence was not conclusive. In terms of the metal-catalysed transesterification, the authors suggested a mechanism involving either deprotonation of the HPNP alcohol moiety by a metal-bound hydroxide or coordination and deprotonation of the HPNP alcohol moiety to the metal and subsequent intramolecular nucleophilic attack.[151]

A structural model for the glycerophosphodiester-degrading enzyme GpdQ, employing the ligand *N*-(2-hydroxy-3-{[(2-hydroxyethyl)(pyridin-2-ylmethyl)amino]methyl}-5-methylbenzyl)-*N*-(pyridin-2-ylmethyl)aminoacetic acid (H_3PBPA; **25**), has been reported.[171] Two di-Zn^{II} complexes were prepared: $[Zn_2(PBPA)(CH_3COO)](PF_6).H_2O$ and $Li[Zn_2(PBPA)]_4(PO_4)_2(PF_6)_3.(CH_3OH)$. The X-ray crystal structure of $Li[Zn_2(PBPA)]_4(PO_4)_2(PF_6)_3(CH_3OH)$ revealed a tetramer of dinuclear complexes, bridged by two phosphate molecules and bifurcating acetic acid arms. Potentiometric titrations of Zn^{II} and H_3L1 with base in the pH range 2.0–8.5 in an acetonitrile/water solution (1:4, v/v) yielded $\log K_1 = 5.98$ and $\log K_2 = 3.35$ as the simplest model. The relative magnitudes of the binding constants suggest that one Zn^{II} is bound relatively tightly to $HL1^{2-}$ and the second more loosely. Functional studies of the zinc complex with BNPP determined the complex with $PBPA^{3-}$ to be a competent catalyst with $k_{cat} = 1.26 \pm 0.06 \times 10^{-6}\ s^{-1}$.[171] For the analogue $[Zn_2(\{HP\}_2B)(\mu\text{-}OAc)(H_2O)](PF_6)$[85] under similar reaction conditions, $K_M = 6.15 \times 10^{-2}$ M and $k_{cat} = 4.60 \times 10^{-6}\ s^{-1}$ have been reported. In the case of both HPBPA and $(HP)_2B$,[85] the zinc complexes mimic aspects of the features of the metallobiosites of OPH, OpdA and GpdQ, HPBPA in particular appearing to present a strong and loose binding site, analogous to the situation observed in the enzymes.

The cadmium analogue of the above complex, $[Cd_2(\{HP\}_2B)(\mu\text{-}OAc)_2(OH_2)](PF_6)$, has also been reported.[172] Whilst cadmium is not common in metalloproteins, a carbonic anhydrase from the marine diatom *Thalassiosira weissflogii* has recently been found to have cadmium bound in the active site, and there is growing evidence that cadmium has some biological relevance.[173–176] $[Cd_2(\{HP\}_2B)(OAc)_2(OH_2)]^+$ models the N,O donor ions of the active site of the enzyme GpdQ and also shows a difference in the coordination numbers of the metal observed in the Zn^{II} analogue.[85] The X-ray structure shows that one cadmium ion is seven-coordinate with a distorted pentagonal bipyramidal geometry, with two nitrogen donors and an oxygen donor from one binding site of the ligand, an oxygen donor from the bridging oxygen of the ligand, two oxygen donors from bidentate acetate and an oxygen donor from a bound water molecule. This is an important feature, since a terminal water molecule is the likely nucleophile in GpdQ. The second cadmium ion displays a distorted six-

coordinate geometry with two nitrogen donors and an oxygen donor from one binding site of the ligand, an oxygen donor from the bridging oxygen of the ligand and two oxygen donors from a bidentate acetate ion. Potentiometric studies showed that the two cadmium(II) ions exhibit significantly different affinities, with $\log K_1$ = 13.6 and $\log K_2$ = 3.2, again mimicking the different affinities of the native enzyme.[29,172] Significantly, the complex can efficiently catalyse the cleavage of BDNPP. The phosphoesterase-like activity of $[Cd_2(\{HP\}_2B)(OAc)_2(OH_2)](PF_6)$ was studied by using the substrate BDNPP, yielding a kinetically relevant pK_a of 8.9, with k_{cat} = 0.004 s^{-1}. In the same work, the cadmium derivative of GpdQ was also prepared by reconstituting the apoenzyme.[172] Catalytic measurements with $(Cd^{II})_2$-GpdQ and the phosphodiester substrate BNPP yielded k_{cat} = 15 s^{-1}, again with a catalytically relevant pK_a of 9.4. For both the biomimetic and the enzyme, a hydroxide ligand is implicated as the catalytic nucleophile. Interestingly, Chen et al.[85] reported the analogous zinc(II) complex, $[Zn_2(\{HP\}_2B)(\mu\text{-}OAc)(OH_2)](PF_6)\cdot 2H_2O$, and with the same substrate k_{cat} was approximately three orders of magnitude smaller, with $k_{cat} = 4.6 \times 10^{-6}$ s^{-1}. Whilst the biological relevance of Cd^{II} is relatively unexplored, its potential as an analogue for Zn^{II} in metalloenzyme systems and biomimetics is attractive. Clearly, the cadmium-containing GpdQ metalloenzyme exhibits enhanced activity over its di-Zn^{II} and di-Co^{II} derivatives.[29]

Conclusions

Effective biomimetics are expected to display both the structural and functional characteristics of the metallobiosite.[47,48,85,140,151,177] In some reported systems, the complexes mimic aspects of the features of the metallobiosites of PAPs, OPHs, OpdA and GpdQ,[1,85,171,172] $HPBPA^{2-}$ in particular appearing to present a strong and loose binding site, analogous to the situation observed in the enzymes. Although the complexes formed with H_2BPBPMP address some of the limitations of other PAP biomimetics, such as stabilisation of a heterovalent active site by an asymmetric coordination environment, there are still limitations in the use of these model complexes as mimics of the protein active site. For example, the catalytic activity of the complexes is many orders of magnitude lower than that of the enzyme, despite the use of a more activated substrate. Additionally, the complexes hydrolyse a highly activated phosphodiester substrate, whereas the enzyme is primarily a phosphomonoesterase, and the extent to which carboxylates are removed under kinetic conditions is unclear. Finally, although the $BPBPMP^{2-}$ ligand does furnish two distinct sites, they are not exact mimics of the donor atoms in PAPs. Modelling the activity and the exact nucleophilic agent is more problematic. The catalytic activities of the Zn^{II} complexes of $HPBPA^{2-}$ and $(HP)_2B$ appear typical of those exhibited by similar complexes, although it is difficult to make definitive comparisons because of the different reaction conditions employed.[85,178] Whilst the k_{cat} of approximately 1.5×10^{-6} s^{-1} represents considerable enhancement over the uncatalysed rate (1.1×10^{-11} s^{-1}),[179] the mimetic does not approach the catalytic rate of the metalloenzymes (the catalytic rates for OPH and OpdA are greater than 1000 s^{-1}).[29,158,159] There are strategies, however, employing hydrogen-bonding substituents in concert with the double Lewis activation of a bimetallic metal binding system that lead to enhanced activity for biomimetic systems,[148–150] mimicking the hydrogen-bonding interactions in the second coordination sphere of the enzyme metal coordination sites.[117] Considering the potential applications of OPH, OpdA and GpdQ for bioremediation,[27,180] it is an attractive endeavour to develop more robust biomimetic systems employing these second coordination sphere interactions and exhibiting sufficient structural and functional stability to be used practically.

Acknowledgments

The authors acknowledge funds awarded by the Australian Research Council through grants DP0986292, DP0986613, DP0664039 and DP0558652, which supported this work. A. Neves acknowledges Instituto Nacional de Ciência e Tecnologia (INCT)-Catálise and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil for financial support.

[1] N. Mitić, S. J. Smith, A. Neves, L. W. Guddat, L. R. Gahan, G. Schenk, *Chem. Rev.* **2006**, *106*, 3338–3363.
[2] T. Klabunde, B. Krebs, *Struct. Bonding (Berlin)* **1997**, *89*, 177–198.
[3] G. W. Oddie, G. Schenk, N. Z. Angel, N. Walsh, L. W. Guddat, J. de Jersey, A. I. Cassady, S. E. Hamilton, D. A. Hume, *Bone* **2000**, *27*, 575–584.
[4] B. Antanaitis, T. Strekas, P. Aisen, *J. Biol. Chem.* **1982**, *257*, 3766–3770.
[5] K. Doi, C. Bradley, P. Aisen, *Struct. Bonding (Berlin)* **1988**, *70*, 1–26.
[6] B. C. Antanaitis, P. Aisen, H. R. Lilienthal, *J. Biol. Chem.* **1983**, *258*, 3166–3172.
[7] B. A. Averill, J. C. Davis, S. Burman, T. Zirino, J. Sanders-Loehr, T. M. Loehr, J. T. Sage, P. G. Debrunner, *J. Am. Chem. Soc.* **1987**, *109*, 3760–3767.
[8] G. Schenk, M. L. J. Korsinczky, D. A. Hume, S. Hamilton, J. de Jersey, *Gene* **2000**, *255*, 419–424.
[9] M. Merkx, B. A. Averill, *Biochemistry* **1998**, *37*, 8490–8497.
[10] P. V. Bernhardt, G. Schenk, G. J. Wilson, *Biochemistry* **2004**, *43*, 10387–10392.
[11] D. L. Wang, R. C. Holz, S. S. David, L. Que Jr, M. T. Stankovich, *Biochemistry* **1991**, *30*, 8187–8194.
[12] E. W. W. Leung, M. Teixeira, L. W. Guddat, N. Mitić, G. Schenk, *Curr. Top. Plant Biol.* **2007**, *8*, 21–31.
[13] A. Durmus, C. Eicken, B. H. Sift, A. Kratel, R. Kappi, J. Hötterman, B. Krebs, *Eur. J. Biochem.* **1999**, *260*, 709.
[14] G. Schenk, Y. Ge, L. E. Carrington, C. J. Wynne, I. R. Searle, B. J. Carroll, S. Hamilton, J. de Jersey, *Arch. Biochem. Biophys.* **1999**, *370*, 183–189.
[15] G. Schenk, C. L. Boutchard, L. E. Carrington, C. J. Noble, B. Moubaraki, K. S. Murray, J. de Jersey, G. R. Hanson, S. Hamilton, *J. Biol. Chem.* **2001**, *276*, 19084–19088.
[16] R. S. Cox, G. Schenk, N. Mitić, L. R. Gahan, A. C. Hengge, *J. Am. Chem. Soc.* **2007**, *129*, 9550–9551.
[17] N. Sethunathan, T. Yoshida, *Can. J. Microbiol.* **1973**, *19*, 873.
[18] D. M. Munnecke, *Appl. Environ. Microbiol.* **1976**, *32*, 7.
[19] F. M. Raushel, *Curr. Opin. Microbiol.* **2002**, *5*, 288–295.

[20] S. D. Aubert, Y. Li, F. M. Raushel, *Biochemistry* **2004**, *43*, 5707–5715.
[21] H. Shim, F. M. Raushel, *Biochemistry* **2000**, *39*, 7357–7364.
[22] D. P. Dumas, H. Durst, W. G. Landis, F. M. Raushel, J. R. Wild, *Arch. Biochem. Biophys.* **1990**, *277*, 155–159.
[23] H. Shim, S.-B. Hong, F. M. Raushel, *J. Biol. Chem.* **1998**, *273*, 17445–17450.
[24] C. J. Jackson, J.-W. Liu, M. L. Coote, D. L. Ollis, *Org. Biomol. Chem.* **2005**, *3*, 4343–4350.
[25] S. Y. McLoughlin, C. J. Jackson, J.-W. Liu, D. L. Ollis, *Appl. Environ. Microbiol.* **2004**, *70*, 404–412.
[26] S. Y. McLoughlin, C. J. Jackson, J.-W. Liu, D. L. Ollis, *Protein Expr. Purif.* **2005**, *41*, 433–440.
[27] F. Ely, J. L. Foo, C. J. Jackson, L. R. Gahan, D. L. Ollis, G. Schenk, *Curr. Top. Biochem. Res.* **2007**, *9*, 63–78.
[28] C. Jackson, H. K. Kim, P. D. Carr, J. W. Liu, D. L. Ollis, *Biochim. Biophys. Acta* **2005**, *752*, 56–64.
[29] C. J. Jackson, P. D. Carr, J.-W. Liu, S. J. Watt, J. L. Beck, D. L. Ollis, *J. Mol. Biol.* **2007**, *367*, 1047–1062.
[30] J. A. Gerlt, F. H. Westheimer, *J. Am. Chem. Soc.* **1973**, *95*, 8166–8168.
[31] J. A. Gerlt, G. J. R. Whitman, *J. Biol. Chem.* **1975**, *250*, 5053–5058.
[32] T. J. Larson, M. Ehrmann, W. Boos, *J. Biol. Chem.* **1983**, *258*, 5428–5432.
[33] E. Ghanem, Y. Li, C. Xu, F. M. Raushel, *Biochemistry* **2007**, *46*, 9032–9040.
[34] C. Belle, J.-L. Pierre, *Eur. J. Inorg. Chem.* **2003**, 4137–4146.
[35] A. L. Gavrilova, B. Bosnich, *Chem. Rev.* **2004**, *104*, 349–384.
[36] D. E. Fenton, *Chem. Soc. Rev.* **1999**, *28*, 159–168.
[37] A. Neves, M. A. de Brito, V. Drago, K. Griesar, W. Haase, *Inorg. Chim. Acta* **1995**, *237*, 131–135.
[38] M. S. Mashuta, J. Webb, J. K. McCusker, E. A. Schmitt, K. J. Oberhausen, J. F. Richardson, R. M. Buchanan, D. N. Hendrickson, *J. Am. Chem. Soc.* **1992**, *114*, 3815–3827.
[39] M. Leivers, R. Breslow, *Bioorg. Chem.* **2001**, *29*, 345–356.
[40] J. Lee, D. J. Jung, H. J. Lee, K.-B. Lee, N. H. Hur, H. G. Jang, *Bull. Korean Chem. Soc.* **2000**, *21*, 1025–1029.
[41] M. Lanznaster, A. Neves, A. J. Bortoluzzi, B. Szpoganicz, E. Schwingel, *Inorg. Chem.* **2002**, *41*, 5641–5643.
[42] E. Lambert, B. Chabut, S. Chardon-Noblat, A. Deronzier, G. Chottard, A. Bousseksou, J.-P. Tuchagues, J. Laugier, M. Bardet, J.-M. Latour, *J. Am. Chem. Soc.* **1997**, *119*, 9424–9437.
[43] B. Krebs, K. Schepers, B. Bremer, G. Henkel, E. Althaus, W. Muller-Warmuth, K. Griesar, W. Haase, *Inorg. Chem.* **1994**, *33*, 1907–1914.
[44] R. Kramer, *Coord. Chem. Rev.* **1999**, *182*, 243–261.
[45] R. Kramer, T. Gajda, *Persp. Bioinorg. Chem.* **1999**, *4*, 209–240.
[46] E. Kimura, *Curr. Opin. Chem. Biol.* **2000**, *4*, 207–213.
[47] P. Karsten, A. Neves, A. J. Bortoluzzi, M. Lanznaster, V. Drago, *Inorg. Chem.* **2002**, *41*, 4624–4626.
[48] M. Jarenmark, S. Kappen, M. Haukka, E. Nordlander, *Dalton Trans.* **2008**, 993–996.
[49] M. Jarenmark, H. Carlsson, E. Nordlander, *C. R. Chimie* **2007**, *10*, 433–462.
[50] J. A. Ibers, R. H. Holm, *Science* **1980**, *209*, 223–235.
[51] A. Horn, I. Vencato, A. J. Bortoluzzi, R. Horner, R. A. N. Silva, B. Szpoganicz, V. Drago, H. Terenzi, M. C. B. de Oliveira, *Inorg. Chim. Acta* **2005**, *358*, 339–351.
[52] C. He, V. Gomez, B. Spingler, S. J. Lippard, *Inorg. Chem.* **2000**, *39*, 4188–4189.
[53] J. R. Hartman, R. L. Rardin, P. Chaudhuri, K. Pohl, K. Wieghardt, B. Nuber, J. Weiss, G. C. Papaefthymiou, R. B. Frankel, S. J. Lippard, *J. Am. Chem. Soc.* **1987**, *109*, 7387–7396.
[54] A. Greatti, M. Scarpellini, R. A. Peralta, A. Casellato, A. J. Bortoluzzi, F. R. Xavier, R. Jovito, M. A. de Brito, B. Szpoganicz, Z. Tomkowicz, M. Rams, W. Haase, A. Neves, *Inorg. Chem.* **2008**, *47*, 1107–1119.
[55] N. S. Goncalves, A. Horn Jr, M. Lanznaster, M. Lanznaster, L. K. Noda, A. Neves, *J. Braz. Chem. Soc.* **2006**, *17*, 1658–1663.
[56] M. Ghiladi, C. J. McKenzie, A. Meier, A. K. Powell, J. Ulstrup, S. Wocadlo, *J. Chem. Soc., Dalton Trans.* **1997**, 4011–4018.
[57] F. H. Fry, A. J. Fischmann, M. J. Belousoff, L. Spiccia, J. Brugger, *Inorg. Chem.* **2005**, *44*, 941–950.
[58] B. Eulering, F. Ahlers, F. Zippel, M. Schmidt, H.-F. Nolting, B. Krebs, *J. Chem. Soc., Chem. Commun.* **1995**, 1305–1307.
[59] M. A. de Brito, *Sth. Braz. J. Chem.* **1996**, *4*, 19–26.
[60] A. S. Borovik, B. P. Murch, L. Que Jr, *J. Am. Chem. Soc.* **1987**, *109*, 7190–7191.
[61] A. S. Borovik, V. Papaefthymiou, L. F. Taylor, O. P. Anderson, L. Que Jr, *J. Am. Chem. Soc.* **1989**, *111*, 6183–6195.
[62] B. Bremer, K. Schepers, P. Fleischhauer, W. Haase, G. Henkel, B. Krebs, *J. Chem. Soc., Chem. Commun.* **1991**, 510–512.
[63] E. Bernard, W. Moneta, J. Laugier, S. Chardon-Noblat, A. Deronzier, J.-P. Tuchagues, J.-M. Latour, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 887–889.
[64] J. K. Bashkin, *Curr. Opin. Chem. Biol.* **1999**, *3*, 752–758.
[65] S. C. Batista, A. Neves, A. J. Bortoluzzi, I. Vencato, R. A. Peralta, B. Szpoganicz, V. V. E. Aires, H. Terenzi, P. C. Severino, *Inorg. Chem. Commun.* **2003**, *6*, 1161–1165.
[66] S. Albedyhl, D. Schnieders, A. Jancso, T. Gajda, B. Krebs, *Eur. J. Inorg. Chem.* **2002**, 1400–1409.
[67] B. N. Trawick, A. T. Daniher, J. K. Bashkin, *Chem. Rev.* **1998**, *98*, 939–960.
[68] R. Than, A. A. Feldmann, B. Krebs, *Coord. Chem. Rev.* **1999**, *182*, 211–241.
[69] A. Sreedhara, J. A. Cowan, *J. Biol. Inorg. Chem.* **2001**, *6*, 337–347.
[70] S. Striegler, *Curr. Org. Chem.* **2007**, *11*, 1543–1565.
[71] N. H. Williams, B. Takasaki, M. Wall, J. Chin, *Acc. Chem. Res.* **1999**, *32*, 485–493.
[72] A. Blasko, T. C. Bruice, *Acc. Chem. Res.* **1999**, *32*, 475–484.
[73] F. Mancin, P. Scrimin, P. Tecilla, J. Tonellato, *Chem. Commun.* **2005**, 2540–2548.
[74] J. Weston, *Chem. Rev.* **2005**, *105*, 2151–2174.
[75] F. Mancin, P. Tecilla, *New J. Chem.* **2007**, *31*, 800–817.
[76] F. Meyer, *Eur. J. Inorg. Chem.* **2006**, 3789–3800.
[77] C. He, S. J. Lippard, *J. Am. Chem. Soc.* **2000**, *122*, 184–185.
[78] K. Selmeczi, M. Giorgi, G. Speier, E. Farkas, M. Reglier, *Eur. J. Inorg. Chem.* **2006**, 1022–1031.
[79] S. Parimala, M. Kandaswamy, *Inorg. Chem. Commun.* **2003**, *6*, 1252–1254.
[80] J.-Z. Li, H.-B. Li, F.-M. Feng, J.-Q. Xie, S.-X. Li, B. Zhou, S.-Y. Qin, *Chin. J. Chem.* **2005**, *23*, 678–684.
[81] O. Iranzo, A. Y. Kovalevsky, J. R. Morrow, J. P. Richard, *J. Am. Chem. Soc.* **2003**, *125*, 1988–1993.
[82] C. Bazzicalupi, A. Bencini, C. Bonaccini, C. Giorgi, P. Gratteri, S. Moro, M. Palumbo, A. Simionato, J. Sgrignani, C. Sissi, B. Valtancoli, *Inorg. Chem.* **2008**, *47*, 5473–5484.
[83] C. Vichard, T. A. Kaden, *Inorg. Chim. Acta* **2002**, *337*, 173–180.
[84] T. Gajda, Y. Dupre, I. Torok, J. Harmer, A. Schweiger, J. Sander, D. Kuppert, K. Hegetschweiler, *Inorg. Chem.* **2001**, *40*, 4918–4927.
[85] J. Chen, X. Wang, Y. Zhu, J. Lin, X. Yang, Y. Li, Y. Lu, Z. Guo, *Inorg. Chem.* **2005**, *44*, 3422–3430.
[86] M. Lanznaster, A. Neves, A. J. Bortoluzzi, V. V. E. Aires, B. Szpoganicz, H. Terenzi, P. C. Severino, J. M. Fuller, S. C. Drew, L. R. Gahan, G. R. Hanson, M. J. Riley, G. Schenk, *J. Biol. Inorg. Chem.* **2005**, *10*, 319–332.
[87] D. Wahnon, A.-M. Lebuis, J. Chin, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2412–2414.
[88] N. H. Williams, W. Cheung, J. Chin, *J. Am. Chem. Soc.* **1998**, *120*, 8079–8087.
[89] N. H. Williams, *Biochim. Biophys. Acta* **2004**, 279–287.
[90] S. H. Yim, H. J. Lee, K.-B. Lee, S. J. Kang, N. H. Hur, H. G. Jang, *Bull. Korean Chem. Soc.* **1998**, *19*, 654–660.

[91] C. Belle, G. Gellon, C. Scheer, J.-L. Pierre, *Tetrahedron Lett.* **1994**, *35*, 7019–7022.
[92] C. Belle, I. Gautier-Luneau, G. Gellon, J.-L. Pierre, I. Morgenstern-Badarau, E. Saint-Aman, *J. Chem. Soc., Dalton Trans.* **1997**, 3543–3546.
[93] C. Belle, I. Gautier-Luneau, L. Karmazin, J.-L. Pierre, S. Albedyhl, B. Krebs, M. Bonin, *Eur. J. Inorg. Chem.* **2002**, 3087–3090.
[94] C. Belle, I. Gautier-Luneau, J.-L. Pierre, C. Scheer, E. Saint-Aman, *Inorg. Chem.* **1996**, *35*, 3706–3708.
[95] E. Bernard, S. Chardon-Noblat, A. Deronzier, J.-M. Latour, *Inorg. Chem.* **1999**, *38*, 190–193.
[96] A. Neves, M. A. de Brito, I. Vencato, V. Drago, K. Griesar, W. Haase, Y. P. Mascarenhas, *Inorg. Chim. Acta* **1993**, *214*, 5–8.
[97] H. Nie, S. M. J. Aubin, M. S. Mashuta, C.-C. Wu, J. F. Richardson, D. N. Hendrickson, R. M. Buchanan, *Inorg. Chem.* **1995**, *34*, 2382–2388.
[98] Y. Maeda, A. Ishida, M. Ohba, S. Sugihara, S. Hayami, *Bull. Chem. Soc. Jpn.* **2002**, *75*, 2441–2448.
[99] M. Suzuki, A. Uehara, H. Oshio, K. Endo, M. Yanaga, S. Kida, K. Saito, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3547–3555.
[100] S. Albedyhl, M. T. Averbuch-Pouchot, C. Belle, B. Krebs, J.-L. Pierre, E. Saint-Aman, S. Torelli, *Eur. J. Inorg. Chem.* **2001**, 1457–1464.
[101] A. Neves, M. A. de Brito, I. Vencato, V. Drago, K. Griesar, W. Haase, *Inorg. Chem.* **1996**, *35*, 2360–2368.
[102] L. W. Guddat, A. S. McAlpine, D. Hume, S. Hamilton, J. de Jersey, J. L. Martin, *Structure* **1999**, *7*, 757–767.
[103] G. Schenk, L. R. Gahan, L. E. Carrington, N. Mitic, M. Valizadeh, S. E. Hamilton, J. de Jersey, L. W. Guddat, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 273–278.
[104] J. Uppenberg, F. Lindqvist, C. Svensson, B. Ek-Rylander, G. Andersson, *J. Mol. Biol.* **1999**, *290*, 201–211.
[105] N. Sträter, B. Jasper, M. Scholte, B. Krebs, A. P. Duff, D. B. Langley, R. Han, B. A. Averill, H. C. Freeman, J. M. Guss, *J. Mol. Biol.* **2005**, *351*, 233–246.
[106] X. Wang, L. Que Jr, *Biochemistry* **1998**, *37*, 7813–7821.
[107] A. E. True, R. C. Scarrow, C. R. Randall, R. C. Holz, L. Que Jr, *J. Am. Chem. Soc.* **1993**, *115*, 4246–4255.
[108] L. Yin, P. Cheng, X. Yao, H. Wang, *J. Chem. Soc., Dalton Trans.* **1997**, 2109–2112.
[109] P. N. Turowski, W. H. Armstrong, S. Liu, S. N. Brown, S. J. Lippard, *Inorg. Chem.* **1994**, *33*, 636–645.
[110] S. Drueke, K. Wieghardt, B. Nuber, J. Weiss, H.-P. Fleischhauer, S. Gehring, W. Haase, *J. Am. Chem. Soc.* **1989**, *111*, 8622–8631.
[111] R. C. Holz, T. E. Elgren, L. L. Pearce, J. H. Zhang, C. J. O'Connor, L. Que Jr, *Inorg. Chem.* **1993**, *32*, 5844–5850.
[112] A. Horn, I. Vencato, A. J. Bortoluzzi, V. Drago, M. A. Novak, A. Neves, *J. Braz. Chem. Soc.* **2006**, *17*, 1584–1593.
[113] T. W. Elliot, N. Mitić, L. R. Gahan, L. W. Guddat, G. Schenk, *J. Braz. Chem. Soc.* **2006**, *17*, 1558–1565.
[114] M. Ghiladi, K. B. Jensen, J. Jiang, C. J. McKenzie, S. Morup, I. Sotofte, J. Ulstrup, *J. Chem. Soc., Dalton Trans.* **1999**, 2675–2681.
[115] X. Wang, R. Y. N. Ho, A. K. Whiting, L. Que Jr, *J. Am. Chem. Soc.* **1999**, *121*, 9235–9236.
[116] A. Dikiy, E. G. Funhoff, B. A. Averill, S. Ciuril, *J. Am. Chem. Soc.* **2002**, *124*, 13974–13975.
[117] G. Schenk, T. W. Elliott, E. Leung, L. E. Carrington, N. Mitić, L. R. Gahan, L. W. Guddat, *BMC Structural Biology* **2008**, DOI:10.1186/1472–6807–1188–1186.
[118] S. J. Smith, G. Schenk, L. R. Gahan, unpublished results, **2009**.
[119] T. Klabunde, N. Sträter, R. Fröhlich, H. Witzel, B. Krebs, *J. Mol. Biol.* **1996**, *259*, 737–748.
[120] P. G. Debrunner, M. P. Hendrich, J. de Jersey, D. T. Keough, J. T. Sage, B. Zerner, *Biochim. Biophys. Acta* **1983**, *745*, 103–106.
[121] I. R. W. Z. de Oliveira, A. Neves, I. C. Vieira, *Sensors Actuators, B* **2008**, *129*, 424–430.
[122] P. Karsten, A. Neves, A. J. Bortoluzzi, J. Strahle, C. Maichle-Mossmer, *Inorg. Chem. Commun.* **2002**, *5*, 434–438.
[123] S. J. Smith, A. Casellato, K. S. Hadler, N. Mitić, M. J. Riley, A. J. Bortoluzzi, B. Szpoganicz, G. Schenk, A. Neves, L. R. Gahan, *J. Biol. Inorg. Chem.* **2007**, *12*, 1207–1220.
[124] M. A. S. Aquino, J.-S. Lim, A. G. Sykes, *J. Chem. Soc., Dalton Trans.* **1994**, 429–436.
[125] M. B. Twitchett, G. Schenk, M. A. S. Aquino, D. T. Y. Yiu, T.-C. Lau, A. G. Sykes, *Inorg. Chem.* **2002**, *41*, 5787–5794.
[126] M. Valizadeh, G. Schenk, K. Nash, G. W. Oddie, L. W. Guddat, D. A. Hume, J. de Jersey, J. Burke, R. Terrence, S. Hamilton, *Arch. Biochem. Biophys.* **2004**, *424*, 154–162.
[127] N. Mitić, M. Valizadeh, E. W. W. Leung, J. de Jersey, S. Hamilton, D. A. Hume, A. I. Cassady, G. Schenk, *Arch. Biochem. Biophys.* **2005**, *439*, 154–164.
[128] M. J. Young, D. Wahnon, R. C. Hynes, J. Chin, *J. Am. Chem. Soc.* **1995**, *117*, 9441–9447.
[129] C. Duboc-Toia, S. Menage, J.-M. Vincent, M. T. Averbuch-Pouchot, M. Fontecave, *Inorg. Chem.* **1997**, *36*, 6148–6149.
[130] M. Scarpellini, A. Neves, R. Horner, A. J. Bortoluzzi, B. Szpoganicz, C. Zucco, R. A. N. Silva, V. Drago, A. Mangrich, W. A. Ortiz, W. A. C. Passos, M. C. B. de Oliveira, H. Terenzi, *Inorg. Chem.* **2003**, *42*, 8353–8365.
[131] A. Neves, M. Lanznaster, A. J. Bortoluzzi, R. A. Peralta, A. Casellato, E. E. Castellano, P. Herrald, M. J. Riley, G. Schenk, *J. Am. Chem. Soc.* **2007**, *129*, 7486–7487.
[132] G. Schenk, R. A. Peralta, S. C. Batista, A. J. Bortoluzzi, B. Szpoganicz, A. K. Dick, P. Herrald, G. R. Hanson, R. K. Szilagyi, M. J. Riley, L. R. Gahan, A. Neves, *J. Biol. Inorg. Chem.* **2008**, *13*, 139–155.
[133] A. J. Kirby, W. P. Jencks, *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216.
[134] M. Merkx, B. A. Averill, *Biochemistry* **1998**, *37*, 11223–11231.
[135] E. Longhinotti, J. B. Domingos, B. Szpoganicz, A. Neves, F. Nome, *Inorg. Chim. Acta* **2005**, *358*, 2089–2092.
[136] G. L. Parrilha, C. Fernandes, A. J. Bortoluzzi, B. Szpoganicz, M. d. S. Silva, C. T. Pich, H. Terenzi, A. Horn, *Inorg. Chem. Commun.* **2008**, *11*, 643–647.
[137] F. Verge, C. Lebrun, M. Fontecave, S. Menage, *Inorg. Chem.* **2003**, *42*, 499–507.
[138] C. Liu, S. Yu, D. Li, Z. Liao, X. Sun, H. Xu, *Inorg. Chem.* **2002**, *41*, 913–922.
[139] C. Fernandes, A. Neves, A. J. Bortoluzzi, A. S. Mangrich, E. Rentschler, B. Szpoganicz, E. Schwingel, *Inorg. Chim. Acta* **2001**, *320*, 12–21.
[140] A. K. Boudalis, R. E. Aston, S. J. Smith, R. E. Mirams, M. J. Riley, G. Schenk, A. G. Blackman, L. R. Hanton, L. R. Gahan, *Dalton Trans.* **2007**, 5132–5139.
[141] C. Liu, L. Wang, *Dalton Trans.* **2009**, 227–239.
[142] K. Yamaguchi, F. Akagi, S. Fujinami, M. Suzuki, M. Shionoya, S. Suzuki, *Chem. Commun.* **2001**, 375–376.
[143] M. J. Belousoff, M. B. Duriska, B. Graham, S. R. Batten, B. Moubaraki, K. S. Murray, L. Spiccia, *Inorg. Chem.* **2006**, *45*, 3746–3755.
[144] L. M. Rossi, A. Neves, R. Horner, H. Terenzi, B. Szpoganicz, J. Sugai, *Inorg. Chim. Acta* **2002**, *337*, 366–370.
[145] M. J. Belousoff, B. Graham, L. Spiccia, *Eur. J. Inorg. Chem.* **2008**, 4133–4139.
[146] M. J. Belousoff, A. R. Battle, B. Graham, L. Spiccia, *Polyhedron* **2007**, *26*, 344–355.
[147] M. J. Belousoff, L. Tjioe, B. Graham, L. Spiccia, *Inorg. Chem.* **2008**, *47*, 8641–8651.
[148] G. Feng, J. C. Mareque-Rivas, N. H. Williams, *Chem. Commun.* **2006**, 1845–1847.
[149] G. Feng, J. C. Mareque-Rivas, R. Torres Martin de Rosales, N. H. Williams, *J. Am. Chem. Soc.* **2005**, *127*, 13470–13471.
[150] G. Feng, D. Natale, R. Prabaharan, J. C. Mareque-Rivas, N. H. Williams, *Angew. Chem. Int. Ed.* **2006**, *45*, 7056–7059.

[151] H. Carlsson, M. Haukka, E. Nordlander, *Inorg. Chem.* **2004**, *43*, 5681–5687.
[152] J. N. Burstyn, K. A. Deal, *Inorg. Chem.* **1993**, *32*, 3585–3586.
[153] B. Bauer-Siebenlist, F. Meyer, E. Farkas, D. Vidovic, S. Dechert, *Chem. Eur. J.* **2005**, *11*, 4349–4360.
[154] B. Bauer-Siebenlist, F. Meyer, E. Farkas, D. Vidovic, J. A. Cuesta-Seijo, R. Herbst-Irmer, H. Pritzkow, *Inorg. Chem.* **2004**, *43*, 4189–4202.
[155] K. Selmeczi, C. Michel, A. Milet, I. Gautier-Luneau, C. Philouze, J.-L. Pierre, D. Schnieders, A. Rompel, C. Belle, *Chem. Eur. J.* **2007**, *13*, 9093–9106.
[156] D. Dumas, S. Caldwell, J. Wild, F. Raushal, *J. Biol. Chem.* **1989**, *264*, 19659.
[157] C. Jackson, P. D. Carr, H. K. Kim, J.-W. Liu, P. Herrald, N. Mitić, G. Schenk, C. A. Smith, D. L. Ollis, *Biochem. J.* **2006**, *397*, 501–508.
[158] G. Omburo, K. J. L. Mullins, F. Raushel, *J. Biol. Chem.* **1992**, *267*, 13278–13283.
[159] M. M. Benning, H. Shim, F. M. Raushel, H. M. Holden, *Biochemistry* **2001**, *40*, 2712–2722.
[160] L. Holm, C. Sander, *Proteins Struct., Funct., Genet.* **1997**, *28*, 72.
[161] C. M. Seibert, F. M. Raushal, *Biochemistry* **2005**, *44*, 6383.
[162] M. M. Benning, J. M. Kuo, F. M. Raushel, H. M. Holden, *Biochemistry* **1995**, *34*, 7973.
[163] H. Yang, P. D. Carr, S. Y. McLoughlin, J. W. Liu, I. Horne, X. Qiu, C. M. J. Jeffries, R. J. Russell, J. G. Oakeshott, D. L. Ollis, *Protein Eng.* **2003**, *16*, 135–145.
[164] C. R. Samples, T. Howard, F. M. Raushel, V. J. DeRose, *Biochemistry* **2005**, *44*, 11005.
[165] F. Zheng, C.-G. Zhan, R. L. Ornstein, *J. Phys. Chem. B* **2002**, *106*, 717–722.
[166] M. Krauss, *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 8–17.
[167] K. S. Hadler, E. A. Tanifum, S. H.-C. Yip, N. Mitić, L. W. Guddat, C. J. Jackson, L. R. Gahan, K. Nguyen, P. D. Carr, D. L. Ollis, A. C. Hengge, J. A. Larrabee, G. Schenk, *J. Am. Chem. Soc.* **2008**, *130*, 14129–14138.
[168] Y. Lindqvist, E. Johansson, H. Kaija, P. Vihko, G. Schneider, *J. Mol. Biol.* **1999**, *291*, 135–147.
[169] T. Knöfel, N. Sträter, *Nat. Struct. Mol. Biol.* **1999**, *6*, 448–453.
[170] K. P. Höpfner, A. Kärcher, L. Craig, T. T. Woo, J. P. Carney, J. A. Tainer, *Cell* **2001**, *105*, 473–485.
[171] R. R. Buchholz, M. E. Etienne, A. Dorgelo, R. E. Mirams, S. J. Smith, S. Y. Chow, L. R. Hanton, G. B. Jameson, G. Schenk, L. R. Gahan, *Dalton Trans.* **2008**, 6045–6054.
[172] R. Mirams, S. J. Smith, K. S. Hadler, D. L. Ollis, G. Schenk, L. R. Gahan, *J. Biol. Inorg. Chem.* **2008**, *13*, 1065–1072.
[173] T. W. Lane, M. M. Morel, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 4627–4631.
[174] Y. Xu, L. Feng, P. D. Jeffrey, Y. Shi, M. M. Morel, *Nature* **2008**, *452*, 56–62.
[175] T. Marino, N. Russo, M. Toscano, *J. Am. Chem. Soc.* **2005**, *127*, 4242–4253.
[176] T. W. Lane, M. A. Saito, G. N. George, I. J. Pickering, R. C. Prince, M. M. Morel, *Nature* **2005**, *435*, 42.
[177] R. Kramer, T. Gajda, *Persp. Bioinorg. Chem.* **1999**, *4*, 209–240.
[178] M. Arca, E. Bencini, C. Berni, F. A. Caltagrione, F. Devillanova, A. Isaia, C. Garau, V. Giorgi, A. Lippolis, L. T. Perra, B. Valtancoli, *Inorg. Chem.* **2003**, *42*, 6929–6939.
[179] B. K. Takasaki, J. Chin, *J. Am. Chem. Soc.* **1995**, *117*, 8582–8585.
[180] R. M. Dawson, S. Pantelidis, H. R. Rose, S. E. Kotsonis, *J. Hazard. Mater.* **2008**, *157*, 308–314.
[181] X.-Q. Chen, X.-J. Peng, J.-Y. Wang, Y. Wang, S. Wu, L.-Z. Zhang, T. Wu, Y.-K. Wu, *Eur. J. Inorg. Chem.* **2007**, 5400–5407.

Received: March 12, 2009
Published Online: June 8, 2009

