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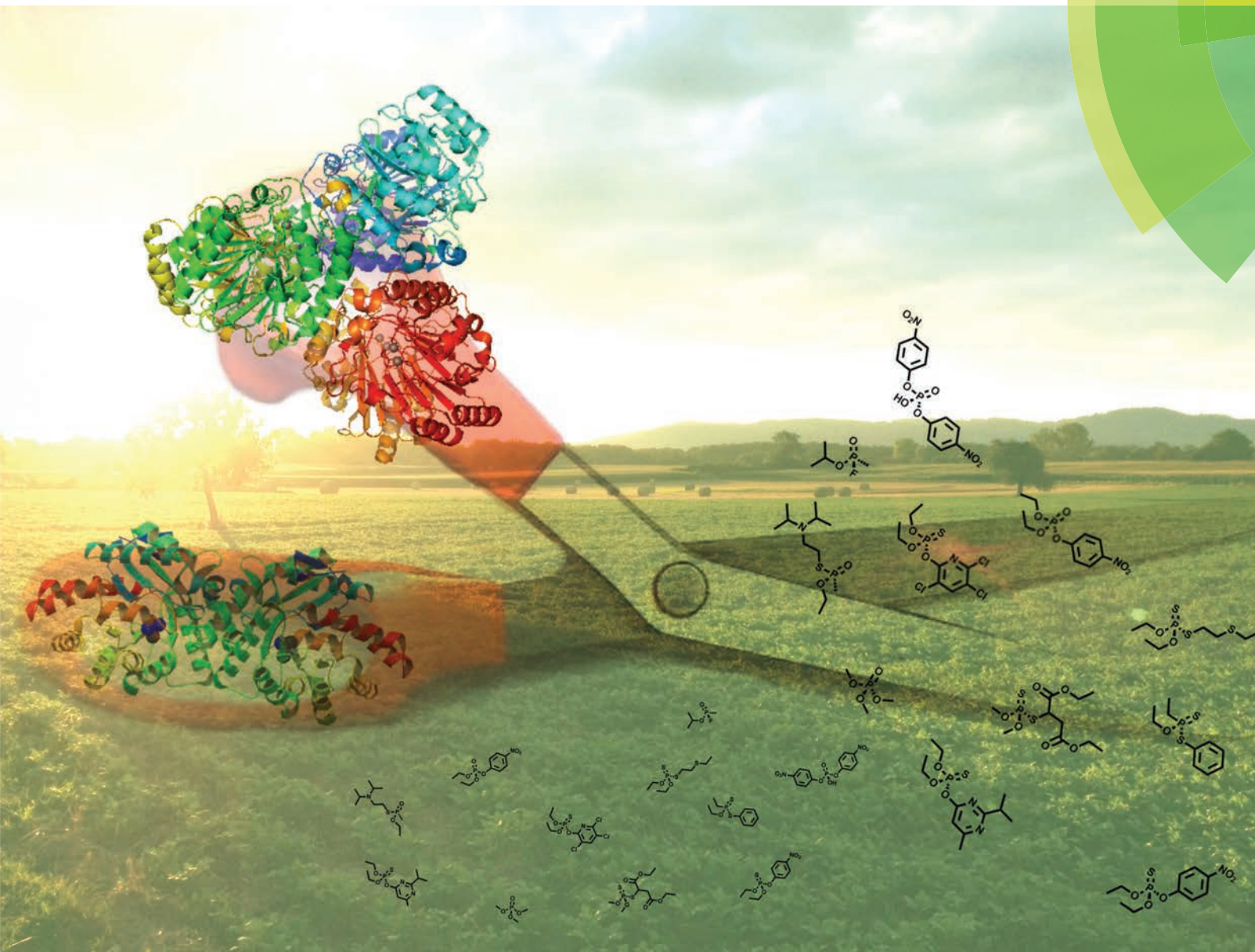
COVER ARTICLE
Gahan *et al.*
Spectroscopic and mechanistic studies of dinuclear metallohydrolases
and their biomimetic complexes

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PERSPECTIVE

Spectroscopic and mechanistic studies of dinuclear metallohydrolases and their biomimetic complexes

Lena J. Daumann,[a] Gerhard Schenk,[a] David L. Ollis[b] and Lawrence R. Gahan*[a]

An enhanced understanding of the metal ion binding and active site structural features of phospho-esterases such as the glycerophosphodiesterase from *Enterobacter aerogenes* (GpdQ), and the organo-phosphate degrading agent from *Agrobacterium radiobacter* (OpdA) have important consequences for potential applications. Coupled with investigations of the metalloenzymes, programs of study to syn-thesise and characterise model complexes based on these metalloenzymes can add to our understanding of structure and function of the enzymes themselves. This review summarises some of our work and illus-trates the significance and contributions of model studies to knowledge in the area.

1. Introduction

The current knowledge about the structure, function, and mechanism of dinuclear metallohydrolases has been summar-ised by a number of authors.[1–7] Metallohydrolases are of con-siderable interest by virtue of their biological roles and their use in bioremediation. In some cases, their roles make them targets for drug design against a variety of human disorders while in other cases their catalytic activities make them ideal reagents for the bioremediation of organophosphate pesticides and nerve agents.[1–6,8–13] In addition, the capacity for mimicry of their properties through model complexes makes them attractive to the bioinorganic chemist.[14–34] In this perspective we firstly describe the general classes of metallohydrolases, we review the properties of two organophosphate-hydrolysing metalloenzymes of interest to our research and discuss the various model complexes of divalent metal ions synthesised in order to probe the structure, function and spectroscopic pro-perties of the metalloenzymes.

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Lena Daumann received her Diploma in Chemistry from the Ruprecht-Karls University of Heidelberg in 2009 under the supervision of Peter Comba. After an internship at the BASF SE in Ludwigshafen she moved to Australia where she recently completed her PhD under the supervision of Lawrence Gahan and Gerhard Schenk at The University of Queensland holding IPRS and UQCent scholarships. Her doctoral thesis focused on spectroscopic and mechanistic studies of dinuclear metallohydro-lases and related biomimetics. In 2013 she joined Kenneth Raymond at the University of California, Berkeley as a post-doctoral research fellow. Her current research focuses on lumines-cent lanthanides.

Gerhard Schenk

Assoc. Prof. Schenk's research interest is focused on the eluci-dation of the reaction mecha-nisms of metal ion-dependent enzymes, in particular dinuclear metallohydrolases, and their application as drug targets or agents for bioremediation. Fol-lowing doctoral studies at the University of Queensland and postdoctoral research at Stanford University Assoc. Prof. Schenk commenced an academic posi-tion at the University of Queens-land. Until recently he also held a joint professorial appointment at the National University of Ireland – Maynooth and now holds a Future Fellowship from the Australian Research Council.

2. Dinuclear metallohydrolases

The family of dinuclear metallohydrolases comprises a large number of enzymes with very different functionalities (Table 1). The dual action of two metals can have different advantages over catalysis by just one metal ion.[35] These advantages include that the thermodynamic driving force for redox reactions is lowered due to charge-delocalization; as well, the activation barrier is also lowered for solvent and enzyme re-organization.[35] In addition, substrates can be more effectively oriented and the electrostatic activation of substrates is more easily achieved.[35] Furthermore, two metal ions facilitate the formation of hydrolysis-initiating nucleophiles more readily, and lower lying energy transition states for hydrolysis reactions can be achieved.[35] The metal ion content and oxidation states of the dinuclear metallohydrolases are as diverse as their proposed mechanisms, nucleophiles and substrates utilized.

Phosphoesterases such as the **g**lycero**p**hospho**d**iesterase from *Enterobacter aerogenes* (GpdQ), the **o**rgano**p**hosphate-**d**egrading **a**gent (OpdA) from *Agrobacterium radiobacter* and the **p**hospho**t**riesterase (PTE) from *Pseudomonas diminuta* have attracted attention due to their ability to degrade toxic pesti-cides, whereas for the purple acid phosphatases (PAPs), metallo-β-lactamases (MβLs) and ureases the interest is mainly due to their role in human disorders and therapy.[7,36,37] The active site structures of dinuclear metallohydrolases typically have the metal ions coordinated to histidine, glutamic acid, aspartate, asparagine and (carboxylated) lysine amino acid residues, in addition to terminal or bridging water/hydroxide ligands. The two metal binding sites are often defined as the α- and β-site, differentiated by their coordination environments.[38]

In this review we focus on the dinuclear metalloenzymes OpdA and, in particular, GpdQ. OpdA is an extremely efficient

Table 1 Overview of hydrolytic metalloenzymes

Enzyme	Metal ion composition	Biological function	Research incentive
Glycerophospho-diesterase (GpdQ)[8,11,38,41,42,77–80]	Fe(II)–Zn(II), Co(II)–Co(II), Mn(II)–Mn(II), Cd(II)–Cd(II)	Hydrolysis of 3′-5′ phosphodiester bond of glycerophosphodiesters	Potential bioremediator
Purple acid phosphatases (PAP)[191–199]	Fe(III)–Fe(II), Fe(III)–Mn(II), Fe(III)–Zn(II)	Bone metabolism (animals) and phosphate uptake (plants)	Target for anti-osteoporotic drugs
Metallo-β-lactamases[117]	Zn(II)–Zn(II)	Hydrolysis of β-lactam substrates (antibiotics)	Study potential inhibitors to fight antibiotic resistance
Rv0805[39,40]	Fe(III)–Mn(II)[a]	Cleavage of cAMP/cGMP phosphodiester bonds	Potential bioremediator
Phosphotriesterases (OpdA, OPH)[49,64,68,71]	Co(II)–Co(II), Mn(II)–Mn(II), Cd(II)–Cd(II), Fe(II)–Zn(II)	Organophosphate hydrolysis	Potential bioremediator
Ureases[200]	Ni(II)–Ni(II)	Hydrolysis of urea	Treatment of bacterial infections, regulation of nitrogen uptake in plants
Ser/Thr phosphatase[2,191]	Fe(II)–Fe(II), Fe(II)–Zn(II), Mn(II)–Mn(II)	Gene expression	Target in chemotherapy
Leucine aminopeptidases[201]	Zn(II)–Zn(II)	Amino acid synthesis	Target for drugs against leukemia
Arginase[35,202]	Mn(II)Mn(II)	Hydrolysis of Arginine	Involved in vascular diseases

[a] Proposed *in vivo*.

David Ollis graduated from the University of NSW before under-taking doctoral studies at Sydney University under the direction of Professor Hans Freeman and Dr Cyril Appleby of the CSIRO. He worked on the first DNA poly-merase structure during his post-doctoral studies at Yale under Professor Tom Steitz. He then established a laboratory in the Biochemistry and Molecular Biology Department at North-western University in Chicago

David L. Ollis

before taking his present position in the Research School of Chemistry at the ANU. During the course of his career the empha-sis of his research has shifted from structural biology to evolving proteins using directed evolution.

Lawrence Gahan obtained a BSc in chemistry and a PhD from La Trobe University, Melbourne, Australia. After postdoctoral positions in the United States and England he was a research fellow in the Research School of Chemistry at the Australian National University, Canberra, Australia, with Professor Allan Sargeson. Following a position at Monash University, Mel-bourne, he moved to the Univer-sity of Queensland in Brisbane,

Lawrence R. Gahan

Australia, in 1984 where he is currently a Professor of Chemistry. His current research focuses on bioinorganic and transition metal chemistry.

phosphotriesterase with low levels of activity towards phosphodiesters. GpdQ rapidly degrades a variety of phosphodiesters and has weak activity towards mono- and triphosphate ester substrates. Attempts to duplicate the catalytic, spectroscopic and mechanistic properties of phosphoesterases have been one of our goals, and the goals of many other workers in the area.[14–29] The metal ions of special interest in our own work (zinc(II), cobalt(II) and cadmium(II)) will be discussed. We acknowledge (i) the contributions of all previous authors upon which our contributions are built on, and (ii) the efforts of contemporary researchers in our area, whose work parallels ours. The review is selective in its coverage and we extend our apologies to everyone whose contributions may not have been covered by this review.

3. Phosphoesterases in general

Phosphoesterases are ubiquitous in nature. Amongst other activities they are involved in signal transduction in bacteria (Rv0805),[39,40] phospholipid degradation (GpdQ),[8,41,42] as well as regulation of phosphate levels in plants, fungi and mammals (PAPs).[2,36,43–47] Some phosphoesterases such as the organophosphate-degrading agent OpdA have apparently recently evolved due to the widespread use of organophosphate pesticides.[48,49] Another well studied enzyme of this type is the PTE from *P. diminuta* and *Flavobacterium* species, which is also known under the name OPH (organophosphorus hydrolase).[50,51] It is proposed that these enzymes provide bacteria living in soil with essential nutrients like phosphate by hydrolysing pesticides. These enzymes have attracted attention as they are also able to hydrolyse highly toxic organophosphate nerve agents.[8–11]

3.1 Organophosphates

In developing countries especially, the benefits of pesticides have to be compared with the risks involved with handling and applying them. The question is how to eliminate the residues of these compounds from the environment to avoid hazards for aquatic organisms and human health?[52–55] As well as use as pesticides, OPs have also been used as nerve agents, for example, sarin or VX.[54] It is proposed that the mode of action of these compounds involves the organophosphate

molecule undergoing nucleophilic attack by a serine-OH moiety in the active site of the enzyme acetylcholine esterase (AChE), subsequently blocking the site for the neurotransmitter acetylcholine.[54,55] With the enzyme inhibited, acetylcholine builds up in the synapses and nerve impulses are continually transmitted. The resulting dysfunction of the parasympathetic nervous system may lead to the death of the organism. Fig. 1 shows some examples of organophosphates used as pesticides and nerve agents.[54–57] Most pesticides bear a P = S moiety, whereas nerve agents contain the P = O motif. Thiophosphates are less potent AChE inhibitors than the corresponding oxophosphates.[54,55] This is due to the former being more stable and thus less likely to react with AChE. However, desulfurization *in vivo* leads to a high acute toxicity of the thiophosphate pesticides.[58] A typical example is the conversion of parathion to paraoxon. Mesomeric and inductive effects of substituents affect the stability and electrophilicity further; electron withdrawing groups/atoms such as fluorine enhance the reactivity of the phosphorus and thus the toxicity and AChE inhibitory effects.[58] The phosphate ester bond is prone to cleavage, mostly by hydrolysis, but photolytic degradation by sunlight is also possible;[59] however, biological pathways for decontamination have also attracted attention.[11,53,60–62]

3.2 The phosphotriesterase OpdA

OpdA is one of the most efficient enzymes known to degrade phosphate triesters, working at near diffusion limited rates towards favored substrates.[49] It has been commercially introduced to Australia by CSIRO and Orica Watercare under the name Landguard™ OP-A, as bioremediator in agriculture (treatment of sheep dip, water run-off and soil contamination).[63,64] Landguard™ OP-A is capable of degrading common pesticides like chlorpyriphos or diazinon and is to date the only enzyme-based product for pesticide bioremediation in Australia. Therapeutic use of OpdA against OP poisoning has also been recently demonstrated.[65–67] For example, rats poisoned with a lethal dose of the pesticide dichlorvos showed 100% survival after OpdA was included in the treatment.[65] The pharmacokinetics of this enzyme has also been studied in the African green monkey to develop new therapeutic approaches for OP poisoning.[66] OpdA has a similar active site to OPH with a more buried 5-coordinate α-site in the resting state, and a
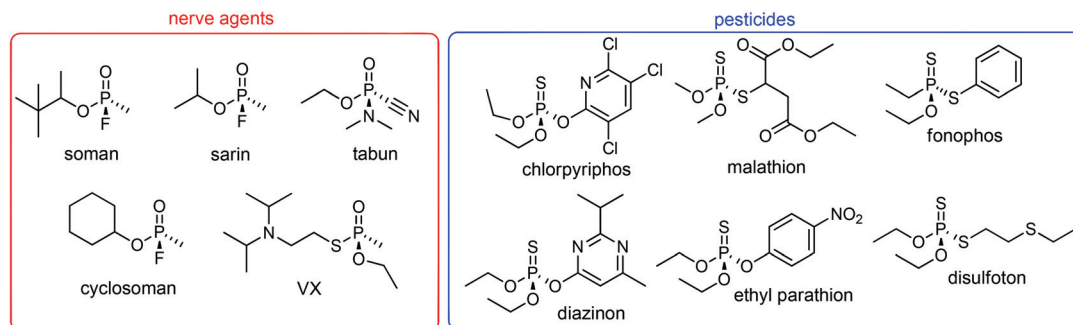

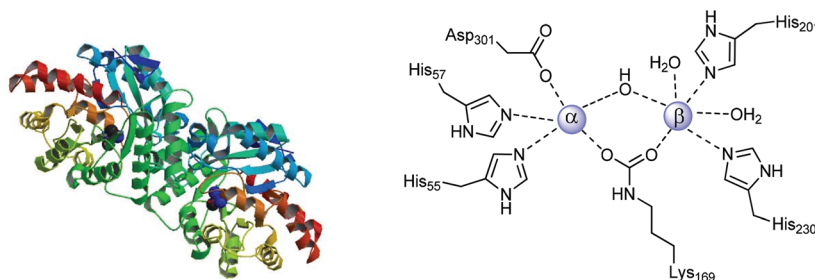**Fig. 1** Phosphoesters that have been used as nerve agents or pesticides.

Fig. 2 Overall structure of OpdA (left) and active site (right). PDB number 2D2G.[186,187]

more solvent exposed 6-coordinate β-site.[49,68–71] The metals are bridged by a carboxylated lysine residue in addition to a μ-hydroxide. The active site of OpdA is shown in Fig. 2 along with the overall structure of the enzyme. The native metal ion content *in vivo* is currently unknown for this enzyme and depending on the metal ion composition; OpdA displays a high mechanistic flexibility for OP degradation.[71] This mechanistic flexibility might be important for a rapid evolution of OpdA towards environmental changes. For the Zn(II) and Cd(II) derivatives one kinetically relevant protonation equilibrium was observed in ethyl paraoxon hydrolysis at a low pK_a range of around 4–5, as expected for a μ-hydroxide.[71] For the Co(II) derivative however, a terminal hydroxide is the proposed nucleophile.[71] Although OpdA is an extremely efficient enzyme it has drawbacks. The pH optimum is at a very limited pH range (so the pH has to be adjusted in many cases before water treatment to use the full potential of the enzyme), and it is only capable of degrading triester substrates. It also lacks stability which is essential for a bioremediator.

3.3 Discovery of GpdQ and its overall structure

The ability of GpdQ to degrade a variety of stable phosphate diesters first came to attention in the 1970s when Gerlt and co-workers purified GpdQ and investigated its function.[72] The natural substrate is glycerol-phosphoethanolamine[72] but GpdQ is reported to be also able to degrade other phosphodiester substrates along with phosphomonoester, phosphotriester and phosphorothiolate substrates (Fig. 3).[8,42,73,74] Its potential as bioremediator for nerve agents was noted in 2007 when Raushel reported the GpdQ-catalysed degradation of EA 2192, a degradation product of the nerve agent VX.[8] The crystal structure of GpdQ was first solved by Ollis and co-workers.[41,42]

A structure with higher resolution (1.9 Å) is, however, now available.[38,75] The structure shows that GpdQ is a hexamer which consists of a trimer of dimers, having overall D_3 symmetry.[38] Fig. 4 displays the hexamer (a) and the structure of the dimeric subunit (b). Each dimer is comprised of a cap domain (yellow), a dimerisation domain shown in red and two catalytic domains (blue), each of them hosting a dinuclear active site. The active site in the resting state is mononuclear with only the six-coordinate α-site being occupied by a metal ion.[38]

In an attempt to evolve GpdQ towards the non-physiological substrate bis(4-nitrophenyl)phosphate (BPNPP) a range of mutants were obtained by directed evolution.[76] These mutants exhibited increased activity towards BPNPP and the previously found hexameric structure was broken down in some mutants.[76] The mutation of a cysteine residue (C269) in the dimerization domain led to the formation of monomer and dimer forms of GpdQ. The smaller oligomeric (dimer) forms of GpdQ were shown to have enhanced activity towards bis(2,4-dinitrophenyl)phosphate (BDNPP) compared to the wild type form. For example, the 8–3 mutant dimer (8th generation) showed an over 500-fold higher catalytic efficiency compared to the wild type.[76] This was attributed to the opening of the active site towards solvent and substrate molecules.[76] The more recent crystal structure at higher resolution illustrates an extensive hydrogen bonding network in the metal binding active site connecting it with the second coordination sphere.[38,75] The α-site features four amino acid side chains, two aspartates (Asp50, Asp8) and two histidines (His10, His197), whereas the metal in the β-site, vacant in the resting state, is coordinated by two histidines (His156, His195), one aspartate (Asp50) and one asparagine (Asn80).[75,77,78] The crystal structure also suggests the presence of a terminal H_2O
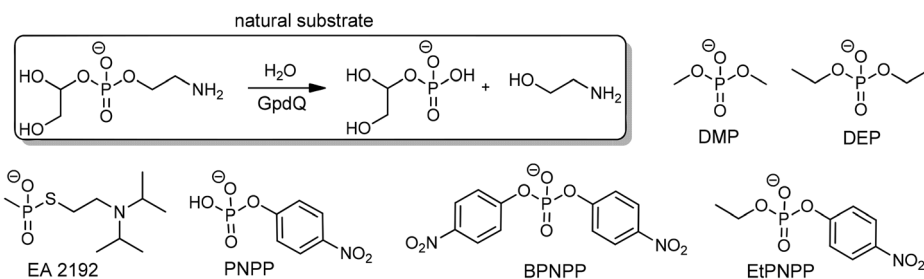

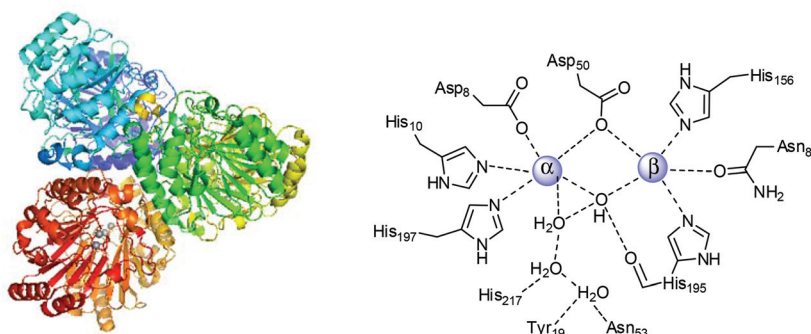**Fig. 3** Physiological substrate of GpdQ and other phosphoesters used in kinetic assays.[8,38,72–74]

Fig. 4 Hexamer of GpdQ (left) and active site structure (right). PDB number 3D03.[38,186]

bound to the α-metal and a bridging water/hydroxide molecule. The *in vivo* metal ion content in GpdQ has, to date, not been determined, although studies using anomalous scattering experiments suggest Fe(II) bound in the α-site.[75] Catalytic activity can be reconstituted with a variety of divalent metal ions including Co(II), Zn(II), Fe(II), Mn(II) and Cd(II),[75,79,80] and heterodinuclear derivatives of GpdQ have been reported in an attempt to explore the *in vivo* metal ion content.[75,81] As Co(II) serves as an excellent spectroscopic probe, the Co(II)Co(II) derivative is the most well studied metal derivative.[77,78] Given the potential use of GpdQ in bioremediation much work has been undertaken to understand (i) the nature of its reaction mechanism, and (ii) factors which influence substrate-and metal ion binding.

4.   Catalytic mechanism of GpdQ

We, and others, have made extensive use of magnetic circular dichroism (MCD) to probe the mechanism as well as the geometric and electronic structure of biological metal systems and the metal binding sites.[68,78,82–86] MCD measures the differential absorption of left and right circular polarised light, similar to circular dichroism (CD), but in the presence of a magnetic field.[84] Application of a magnetic field parallel to the direction of light causes all matter to exhibit MCD activity so it is not restricted to paramagnetic materials.[84] Zero-field splitting, *g*-tensors and spin states can also be obtained, in addition to magnetic coupling interaction information for dimetallic sites, by analysing variable temperature, variable field (VTVH) data.[84,87,88] MCD studies of Co(II)₂-GpdQ, have revealed the two distinct binding sites, a 6-coordinate site with high affinity and a 5-coordinate site with low affinity.[38,78] These studies showed that GpdQ exists as a mononuclear resting state enzyme with only the α-site occupied by metal, as evidenced by the single band in the MCD spectrum at 495 nm (typical for 6-coordinate Co(II)).[38,75] Upon binding of substrate it is proposed that the active site ($E_M'\cdot S$) (Fig. 5) undergoes rapid structural rearrangement facilitated by the hydrogen bond network that connects the first and second coordination sphere.[79] The studies demonstrated that the dinuclear Co(II)Co(II) metal centre in GpdQ is only formed in the presence of a substrate or in this case phosphate, a substrate analogue ($E_B\cdot S$).[29,79] The fact that bands from 5- and 6-coordinate Co(II) were observed in the MCD led to the proposal that the bond between Co(II) and Asn80 in the β-site is broken upon substrate coordination ($E_B^*\cdot S$).[38,75,79] A terminal hydroxide then acts as nucleophile, product and the β-metal are released and the active site returns to its mononuclear resting state.[38,79] The formation of a dinuclear centre in the presence of substrate was substantiated by electron paramagnetic resonance (EPR) spectroscopic studies of the Mn(II)Mn(II) derivative of GpdQ.[79] EPR data
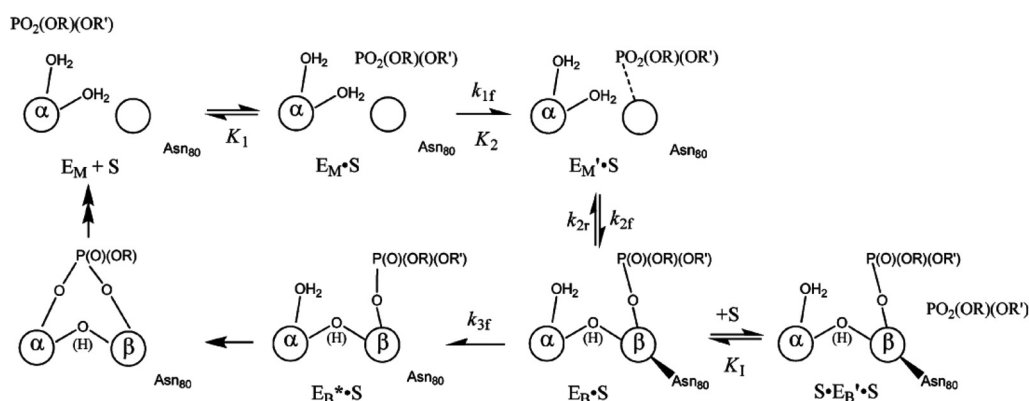

Fig. 5 Proposed mechanism of hydrolysis of phosphate esters by GpdQ (determined from stopped-flow fluorescence studies, picture taken from ref. 79).

revealed K_d values for the metal ions in the α- and β-sites to be 29 and 344 μM, respectively, in the absence of a substrate analog.[79] In the presence of phosphate the affinity of the β-site increased significantly (K_d = 56 μM).[79] The metal ion affinity of the β-site can be increased by changing the Asn80 residue to an aspartate, resulting in a fully occupied dinuclear centre after the addition of two equivalents of Co(II) to the apo-enzyme, even in the absence of substrate.[75] However, the enzyme activity was impaired as shown by kinetic measurements, leading to the proposal that the coordination flexibility of Asn80 is required for high activity. The exchange of Asn80 by alanine led to a dinuclear centre only in the presence of substrate.[75] Also, this mutant showed increased reactivity while its affinity for substrate was greatly decreased. Further studies with GpdQ mutants have been conducted to elucidate the role of the surrounding hydrogen bond network.[79] Specifically, the role of His81 and His217 has been probed by replacing these residues by alanine in site directed mutagenesis experiments.[79] While replacement with alanine did not affect the pH dependence of the enzyme, an increased metal binding affinity resulted, being 2- (His81Ala) and 8-times (His217Ala) higher than in wt-GpdQ.[79] This shows that residues that are not directly interacting with the active site metal ions have an impact on the coordination environment through the hydrogen bond network.

5.   Biomimetics of dinuclear metallohydrolases

The study of biomimetics can be of considerable benefit for the understanding of enzymatic reactions. The term biomimetic refers here to a compound that mimics structural, functional and spectroscopic properties of an enzyme.[33,34] Often only one or two of these aspects are achieved for a model system and they usually display substantially lower activity. There are, however, advantages over the enzyme: model complexes are generally more stable and robust than their enzymatic counterpart, they can be readily crystallised and provide easy accessible structural information on metal ion coordination. Further, the model systems are considerably less complex, kinetic and spectroscopic data interpretation is simplified and – by comparison to data derived for the enzyme – the mechanism of action and structural features can be elucidated and thus related to the metalloenzyme. Ligand and complex design in biomimetic systems is diverse but a few general concepts are normally followed: (i) the metal ions employed are often the same as in the native systems *e.g.* Ni(II) in urease models;[89] and (ii) pyridine or pyrazole residues are often used to mimic the histidine residues in the enzymes; phenol, carboxylate, pyrazolate or water molecules serve as mimics for bridging residues like aspartate, lysine or water/hydroxide; and (iii) dinucleating ligands are used to bring the two metal ions into close proximity. The strategies for ligand and complex design are diverse and have been recently

reviewed.[90–93] A few reported structures of model complexes for dinuclear hydrolytic enzymes are shown in Fig. 6.

As suggested biomimetics have helped to (i) characterise intermediates in enzyme reactions, and (ii) provide structural and mechanistic insights into the native systems. For example, X-ray absorption near-edge spectroscopy data from Cd(II) thiolate and imidazole complexes provided insight into the coordination number and nature of coordinating amino acids in the Cd(II)-containing carbonic anhydrase of a marine diatom, which suggested that the active site of this protein employs a tetrahedral coordination geometry and that Cd(II) is bound to a least one cysteine residue.[94] A dicobalt(II) complex, [Co$_2$(μ-OH)(μ-Ph$_4$DBA)(TMEDA)$_2$(OTf)], (Ph$_4$DBA is the dinucleating ligand dibenzofuran-4,6-bis(diphenylacetate)), reported by Larrabee *et al.* is an excellent spectroscopic model for the 5- and 6-coordinate sites of methionine aminopeptidase and GpdQ.[86–88,95] The magnetic exchange coupling from this μ-OH bridged complex was determined to be ferromagnetic and the results from this study can be used to distinguish μ-aqua and μ-hydroxo bridging motifs in Co(II)Co(II) enzyme systems.[86,87] Neves *et al.* were able to synthesise, and spectroscopically and kinetically characterise a dinuclear Fe(III)-Zn(II) complex with a terminal Fe(III)-bound water molecule at a position equivalent to that of the proposed nucleophile in red kidney bean PAP.[26] The phosphatase activity of this complex yielded a bell-shaped, strongly pH-dependent rate profile with a pH-optimum at 6.5, and pK_a values of 5.3 and 8.1, demonstrating that this complex is an excellent structural and functional model of the active site of PAPs.[26]

In order to generate structurally more relevant biomimetics for dinuclear metallohydrolases much effort has been devoted to the synthesis of asymmetric ligands considered to be more suitable models for the asymmetric coordination environment found in enzymatic systems. Nordlander *et al.* proposed that asymmetric complexes are not only more appropriate functional models for the active site of phosphoesterase enzymes, but also that they exhibit enhanced catalytic rates compared with their symmetric counterparts.[15,19,20] Examples include ligands employed to generate PAP,[19,21,23,24,28,96–100] phosphoesterase,[101] urease,[102,103] catechol oxidase[104] and manganese catalase biomimetics.[105,106] Some ligands generate a hard and a soft coordination site for the generation of heterodinuclear model complexes for PAP metalloenzymes.[24,96] In some cases the ligands have one structural variation in one arm,[23,24,96] in others one donor arm has been omitted.[19,104–106] Often the vacant coordination site is found to be occupied by water or solvent molecules in the complex.[105,106]

5.1   Dinuclear zinc hydrolase mimics

Zn(II) is a metal common in all forms of life; it is not only the second most abundant metal in biological systems after iron, but also occurs in the active site of over 200, mostly hydrolytic, enzymes.[107,108] It is suggested that the high occurrence of Zn(II) in biological systems is due to the abundance of soluble forms of this element in the environment.[108] The advantages of having Zn(II) in an active site of an enzyme include its high

Urease[89]   GpdQ[80]   Alkaline phosphatase[30]

PAP[25]   MβL[14]   Aminopeptidase[203]

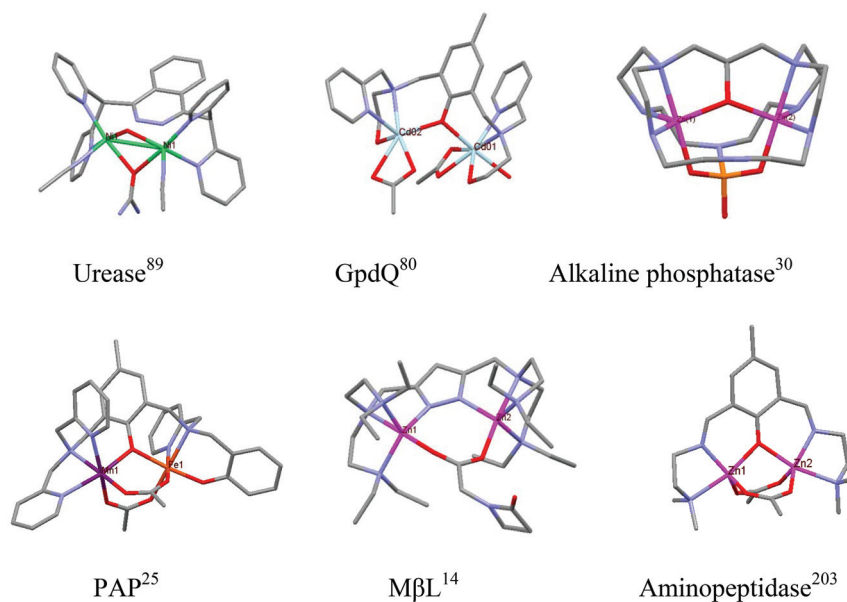**Fig. 6**   Examples of dinuclear complexes mimicking hydrolytic enzymes.

Lewis acidity, which is mostly influenced by the ionic potential Z_{eff}/r (Z_{eff} = effective electrical charge, r = effective radius of the ion),[108] and the coordination flexibility of Zn(II) arising from its d^{10} configuration. However, this closed shell configuration causes problems for the (bio)chemist studying Zn(II) systems as this configuration is spectroscopically silent. The problem is often solved by replacing, *in vitro*, Zn(II) with Co(II) or Cu(II) and utilising the excellent spectroscopic properties of these ions,[109] or with Cd(II) and using ^{113}Cd-NMR spectroscopy as a probe.[110] In addition to dinuclear Zn(II) hydrolases, mono- and trinuclear enzymes are also common in nature.[90] Among the natural occurring Zn$_2$/Zn$_3$-enzymes are the phosphotriesterase from *Pseudomonas diminuta*,[111] the alkaline phosphatase,[112]

phospholipase C,[113] P1 nuclease,[114] the metallo-β-lactamases[115–117] and also some amino peptidases.[118–121] A few examples of the active sites in these enzymes are given in Fig. 7. The structurally related, trinuclear representatives alkaline phosphatase, phospholipase C and P1 nuclease all possess an adjacent M(II) ion in close proximity to the dinuclear Zn(II) site.[90] Phospholipase C and P1 nuclease catalyse the cleavage of phosphodiester bonds, while the alkaline phosphatase hydrolyses monoesters at both alkaline and acidic pH.[90] The active nucleophile for alkaline phosphatases is believed to be the serine residue activated by the second Zn(II) ion.[31,90,112]

Numerous research groups have reported Zn(II)Zn(II) hydrolase mimics.[14–16,18,20,22,90,92,101,122–127] Bringing two Zn(II) ions
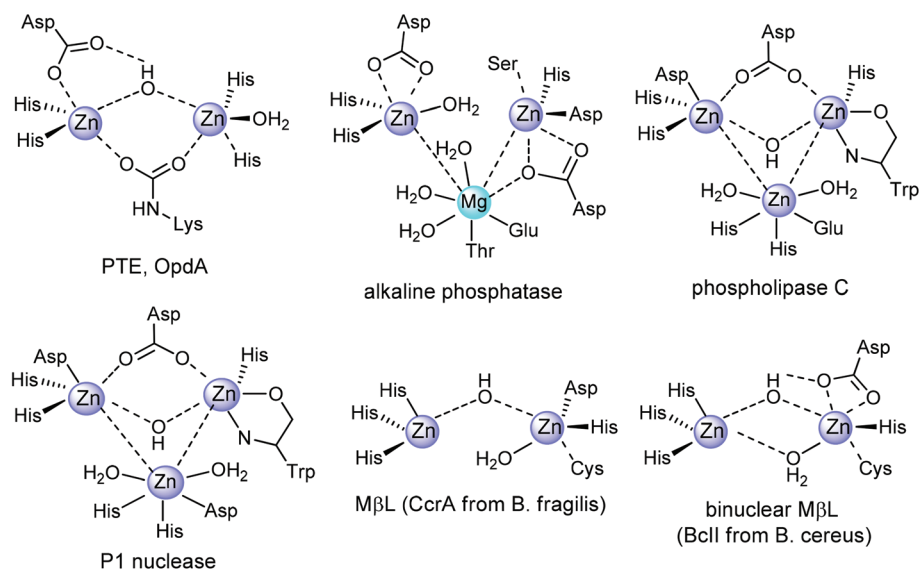

PTE, OpdA   alkaline phosphatase   phospholipase C

P1 nuclease   MβL (CcrA from B. fragilis)   binuclear MβL (BcII from B. cereus)

Fig. 7   Commonly found zinc enzymes in nature.[90,111–115,118]

in close proximity can be achieved with the help of bridging ligands, like phenolate,[101,128,129] carboxylate[130] and pyrazolyl,[17] or by incorporating them into a macrocycle (*i.e.* crown ether), or, finally, by a simple aliphatic linker between two ligands, each of them coordinating one Zn(II) ion.[131] The rate enhancement in phosphate ester hydrolysis by two Zn(II) ions (opposed to catalysis by just one Zn(II) ion) was shown in a study by Bazzicalupi *et al.*[132,133] Employing the macrocyclic ligands L_A and L_B ([[30]aneN$_6$O$_4$] and [15]aneN$_3$O$_2$, respectively, Fig. 8), it was demonstrated that the rate of hydrolysis of BPNPP underwent a 10-fold increase by the dinuclear $[Zn_2L_A(OH)_2]^{2+}$ opposed to the mononuclear $[L_BZn\text{-}OH]^+$ complex.[132] In an attempt to crystallise $[Zn_2L_A(OH)_2]^{2+}$ with diphenyl phosphate a bridging coordination of the phosphate ester to the two Zn(II) ions was found, further substantiating the necessity of two Zn(II) ions during hydrolysis.[132] The bidentate-bridging binding mode of the diphenyl phosphate was also proposed in solution using ^{31}P-NMR measurements because of the rather large shifts observed for the phosphate ester signal.[132]

The separation of the two zinc centres in the biomimetics has been shown to be important. Meyer *et al.* correlated the hydrolytic activity of dizinc phosphodiesterase models with their Zn⋯Zn distances.[16] Here, the length of the ligand side chains determines the Zn⋯Zn separation. The distance of the two Zn(II) ions in the complex with L_G (*N,N'*-((1*H*-pyrazole-3,5-diyl)bis(methylene))bis(2-(pyridin-2-yl)-*N*-(2-(pyridin-2-yl)ethyl)-ethanamine) Fig. 8) is rather short with 3.479(1) Å, while the metal ions are separated by 4.1518(6) Å with an analogous ligand L_F (*N,N'*-((1*H*-pyrazole-3,5-diyl)bis(methylene))bis-(1-(pyridin-2-yl)-*N*-(pyridin-2-ylmethyl)methanamine.[16] The authors proposed that the separation of the Zn(II) centres is significant in terms of the ability of the metal complexes to efficiently catalyse the hydrolysis of the substrate, in this case BPNPP.[16] The k_{cat} for the Zn(II) complex $[Zn_2(L_FH_{-1})(MeOH)\text{-}(OH)](ClO_4)_2$, where the Zn(II) ions have a greater separation, is 2.3×10^{-5} s^{-1}, greater than for the derivative with short intramolecular distances $[Zn_2(L_GH_{-1})(OH)](ClO_4)_2$, k_{cat} 4.9×10^{-6} s^{-1}.[16] Kinetic studies of the hydrolysis of BDNPP and the transesterification with the substrate 2-hydroxypropyl-*p*-nitro-phenyl-phosphate (HPNP) utilising Zn(II) complexes of the asymmetric ligand L_K (2-(*N*-isopropyl-*N*-((2-pyridyl)methyl)amino-methyl)-6-(*N*-(carboxylmethyl)-*N*-((2-pyridyl)methyl)amino-methyl)-4-methylphenol, Fig. 8) and a similar symmetric ligand showed that the more open coordination sphere in the asymmetric ligand led to a five-fold enhancement of k_{cat}.[18,20]

In our work we have prepared a series of symmetric and unsymmetric ligands and their di-Zn(II) complexes (Fig. 9).[101,134–139] These ligands were designed to study the influence of groups in the *para*-position to the bridging phenolic oxygen (CO$_2$Et**HL2**, CH$_3$**HL2**, NO$_2$**HL2** and Br**HL2**) on magnetism in the case of Co(II) complexes, and phosphoesterase activity or to compare mechanistic implications of directly
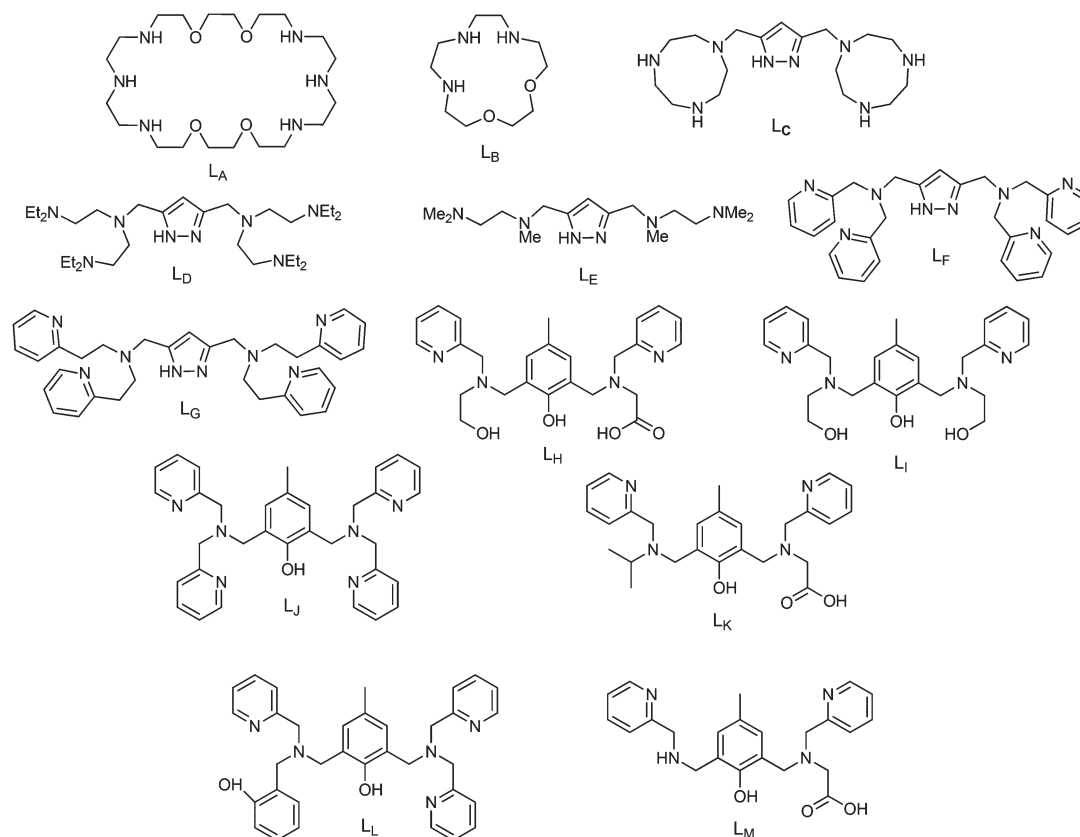

Fig. 8 Overview of ligands for dinuclear phosphatase mimics: L_A, L_B[132,133]:L_C[188]: L_D[189]:L_E[189]:L_F[16]:L_G[189]:L_H[101]:L_I[126]:L_J[21–24]:L_K[15,19,20]; L_L[28,96]:L_M.[190]
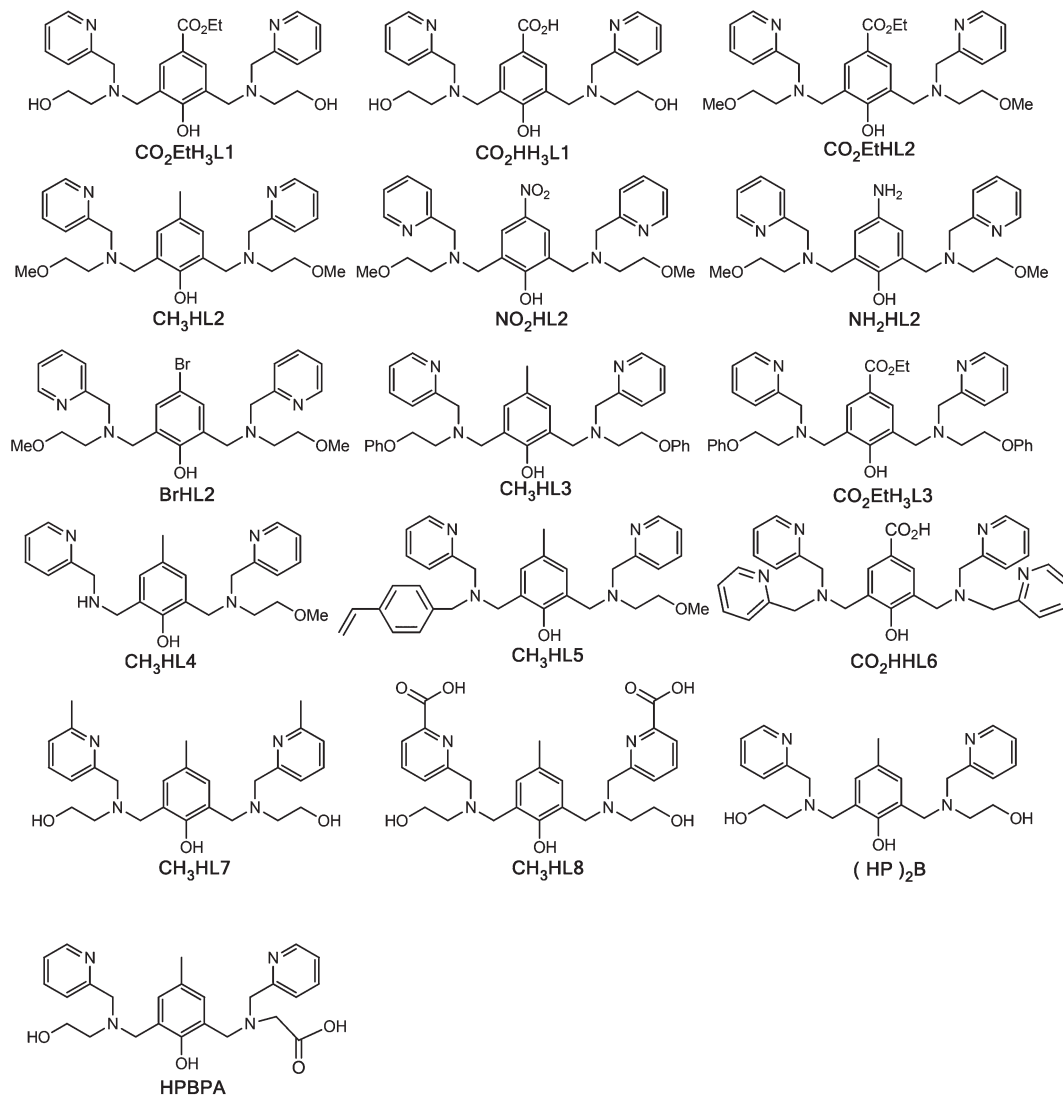
Fig. 9 Overview of the ligands synthesized in our work.[101,134–139]

bound groups like methyl ether and alcohol ($CO_2Et H_3$**L1** and $CO_2Et H$**L2**), in addition to a study of steric influences of bulky ligands arms by comparing complexes of CH_3H**L2** and CH_3H**L3**.[134–139] The pyridine residues are mimics for histidine, and the alcohol and ether-arms are mimicking asparagine and aspartate residues, present in enzyme active sites. The bridging phenol mimics a bridging aspartate or hydroxide. The coordination sphere in the metal complexes is predominantly completed by bridging acetato ligands. Some ligands feature functional groups ($CO_2H H_3$**L1**, NH_2H**L2**, $CO_2H H$**L6** and CH_3H_2**L4**) which allow attachment of the ligands to resins, inorganic supports such as silica, Merrifield resin and magnetite nanoparticles.[134,140] Mass spectrometry in the presence and absence of substrates, ^{1}H, ^{13}C and ^{31}P NMR including ^{18}O-labeling studies, X-ray crystallography and kinetic assays were employed to address questions like substrate binding mode, nucleophiles in OP hydrolysis and in-solution structure of the Zn(II) complexes.

para-Substituents to the bridging phenolic oxygen were found to influence the Zn···Zn distances in these complexes to a very small extent.[139] The Zn···Zn distance in the most strongly electron withdrawing NO_2**L2** ligand was slightly elongated (3.341 Å) compared to the Br**L2** (3.304 Å) and CH_3**L2** (3.296 Å) analogues. The Zn–O–Zn angle of the **L2** series ligands is similar and close to a perfect tetrahedral angle while for the **L3** ligand with the bulky phenyl ether arms enforces a larger deviation from 109° (107.49°).[139]

Phosphatase-like activities of the di-Zn(II) complexes were measured using the activated substrate BDNPP over a pH range 5–10.5.[139] The data are shown in Table 2. The rate (ν_0) *vs.* pH profiles for three of the Zn(II) complexes, [$Zn_2(CH_3$**L2**)-(CH_3COO)$_2$](PF_6), [$Zn_2(CH_3$**L3**)(CH_3COO)$_2$](PF_6), and [$Zn_2(Br$**L2**)-(CH_3COO)$_2$](PF_6), were consistent with the presence of two protonation equilibria and were fitted to bell-shaped curves. [$Zn_2(CH_3$**L2**)(CH_3COO)$_2$](PF_6) and [$Zn_2(CH_3$**L3**)(CH_3COO)$_2$](PF_6) exhibited similar catalytic behaviour; the least efficient

Table 2 Kinetic properties of some Zn(II)Co(II) and Cd(II) phosphoesterase mimics

Complex[b]	k_{cat} [s^{-1}]	K_m [mM]	Substrate	pH optimum	T [K]	Solvent system	Kinetic pK_a
[Zn$_2$**L**$_{A-2H}$(OH)$_2$]$^{2+132,133}$	1.15×10^{-4a}	—	BPNPP	10.5	308	Water	~9.5
[Zn**L**$_B$(OH)$_2$]$^{2+132,133}$	1.31×10^{-5a}	—	BPNPP	10	308	Water	~8.9
[Zn$_2$(**L**$_C$H$_{-2}$)]^{188}	2.24×10^{-6}	12	BPNPP	9.73	308	Water	—
[Zn$_2$(**L**$_D$H$_{-1}$)(MeOH)(OH)](ClO$_4$)$_2$^{189}	1.9×10^{-6}	42	BPNPP	8.28	323	DMSO–water 1:1	7.57
[Zn$_2$(**L**$_E$H$_{-1}$)]^{189}	4.2×10^{-5}	55	BPNPP	8.28	323	DMSO–water 1:1	7.66
[Zn$_2$(**L**$_F$H$_{-1}$)(MeOH)(OH)](ClO$_4$)$_2$^{16}	2.3×10^{-5}	56	BPNPP	8.28	323	DMSO–water 1:1	7.60
[Zn$_2$(**L**$_G$H$_{-1}$)(OH)](ClO$_4$)^{16}	4.9×10^{-6}	51	BPNPP	8.28	323	DMSO–water 1:1	7.96
[Zn$_2$(**L**$_H$H$_{-2}$)(OAc)](PF$_6$)^{101}	1.26×10^{-6}	1.96	BPNPP	9.0	323	CH$_3$CN–water 1:1	7.87
[Zn$_2$(**L**$_I$H$_{-2}$)(OAc)(H$_2$O)](PF$_6$)^{126}	4.6×10^{-6}	61.5	BDNPP	7.2	323	DMSO–water 3:7	7.31
[Zn$_2$(**L**$_J$H$_{-1}$)(OH)]$^{2+22}$	6.4×10^{-4}	13.5	HPNP	—	298	DMSO–water 3:7	7.4
[(Zn$_2$(**L**$_K$H$_{-2}$)(OAc))$_2$](PF$_6$)$_2$^{20}	1.2×10^{-4}	3.6	HPNP	8.5	298	CH$_3$CN–water 1:1	~8.5
[(Zn$_2$(**L**$_K$H$_{-2}$)(OAc))$_2$](PF$_6$)$_2$^{20}	6.4×10^{-4}	16	BDNPP	8.5	298	CH$_3$CN–water 1:1	6.63
[Zn$_2$(CH$_3$**L2**)(CH$_3$COO)$_2$](PF$_6$)^{138}	5.70×10^{-3}	20.8	BDNPP	8.8	298	CH$_3$CN–water 1:1	6.76, 11.00
[Zn$_2$(Br**L2**)(CH$_3$COO)$_2$](PF$_6$)^{138}	0.76×10^{-3}	6.5	BDNPP	8.8	298	CH$_3$CN–water 1:1	6.55
[Zn$_2$(NO$_2$**L2**)(CH$_3$COO)$_2$](PF$_6$)^{138}	1.90×10^{-3}	7.1	BDNPP	9.5	298	CH$_3$CN–water 1:1	6.56, 10.60
[Zn$_2$(CH$_3$**L3**)(CH$_3$COO)$_2$](PF$_6$)^{138}	3.60×10^{-3}	18.9	BDNPP	8.5	298	CH$_3$CN–water 1:1	7.72, 10.83
[Zn$_2$(CH$_3$**L4**)(CH$_3$COO)$_2$](PF$_6$)^{138}	2.45×10^{-3}	9.48	BDNPP	7.7	298	CH$_3$CN–water 1:1	7.39
[Zn$_2$(CH$_3$**L5**)(CH$_3$COO)$_2$](PF$_6$)^{138}	0.97×10^{-3}	7.01	BDNPP	7.7	298	CH$_3$CN–water 1:1	7.50
[Co$_2$(CO$_2$Et**H$_2$L1**)(CH$_3$COO)$_2$](PF$_6$)^{135}	9.12×10^{-3}	3.26	BDNPP	10.70	298	CH$_3$CN–water 1:1	10.54
[Co$_2$(CO$_2$Et**H$_2$L1**)(CH$_3$COO)$_2$](PF$_6$)^{135}	9.12×10^{-3}	3.26	BDNPP	10.70	298	CH$_3$CN–water 1:1	10.54
[Co$_2$(CO$_2$Et**L2**)(CH$_3$COO)$_2$](PF$_6$)^{135}	11.40×10^{-3}	4.31	BDNPP	10.40	298	CH$_3$CN–water 1:1	8.34
[Co$_2$(CH$_3$**L2**)(CH$_3$COO)$_2$](PF$_6$)^{135}	5.48×10^{-3}	4.48	BDNPP	11.00	298	CH$_3$CN–water 1:1	8.53
[Co$_2$(NO$_2$**L2**)(CH$_3$COO)$_2$](PF$_6$)^{135}	9.23×10^{-3}	6.83	BDNPP	9.55	298	CH$_3$CN–water 1:1	8.12
[Co$_2$(Br**L2**)(CH$_3$COO)$_2$](PF$_6$)^{135}	19.10×10^{-3}	4.62	BDNPP	10.00	298	CH$_3$CN–water 1:1	8.75
[Cd$_2$((HP)$_2$B)(CH$_3$COO)$_2$(OH$_2$)](PF$_6$)^{138}	3.8×10^{-3}	8.4	BDNPP	10.5	298	CH$_3$CN–water 1:1	8.9
[Cd$_2$(CO$_2$Et**H$_2$L1**)(CH$_3$COO)$_2$](PF$_6$)^{138}	9.4×10^{-3}	9.4	DNPP	10.4	298	CH$_3$CN–water 1:1	10.11

^{a} Second order rate constants in [M^{-1} s^{-1}]. ^{b} The structures of these ligands are shown in Fig. 8 and 9.

complex was [Zn$_2$(NO$_2$L2)(CH$_3$COO)$_2$](PF$_6$). Overall the k_{cat} and catalytic efficiency of these complexes were comparable to similar systems (Table 2). The most interesting result of the change of *para*-substituent was the effect on rate of hydrolysis of the substrate. A linear correlation between the Hammett parameter σ for the substituent (CH$_3$–, NO$_2$– and Br–) and log (k_{cat}) was found suggesting that for these complexes the catalytic efficiency could be manipulated by judicious choices of substituent.[139] A similar effect has been reported in a PAP model with a Fe(III)Zn(II) centre.[141] In neither case, however, were similar correlations found with K_m suggesting that the substituent in *para*-position is primarily affecting nucleophilic activation.

The nucleophile in zinc complex-catalysed hydrolysis reactions is in most cases proposed to be a terminal water molecule (pK_a = 6.60–9.5; (A) and (B) in Fig. 10).[101,142] If the complex contains an additional alcohol moiety the assignment of the hydrolysis-initiating nucleophile can be

ambiguous.[126,142] In monomeric Zn(II) systems it was proposed, based on DFT calculations and reactivity studies, that a metal bound alkoxide is more reactive than a water molecule.[31,143,144] Deprotonation constants of water bound to free Zn(II) have been observed to range from 8.2–9.2,[145] whereas the pK_a of a zinc-bound alcohol was shown to be around 7.2.[126,142] A bridging hydroxide was initially proposed in the zinc complex (Fig. 10C). The authors, however, proposed that the bridging hydroxide acts as a general base to activate an external water molecule.[146] The pK_a of 7.31 was assigned to the zinc-bound alcohol-arm in complex (Fig. 10D). However, the authors did not indicate how the catalyst is recovered after nucleophilic attack of the ligand arm.[126] The di-Zn(II) complexes in our work were also probed to determine the active nucleophile. Use of 50/50 mixture of ^{18}O–^{16}O water and ^{31}P NMR were employed to investigate the mechanism of phosphoesterase activity of these complexes, specifically the role of the
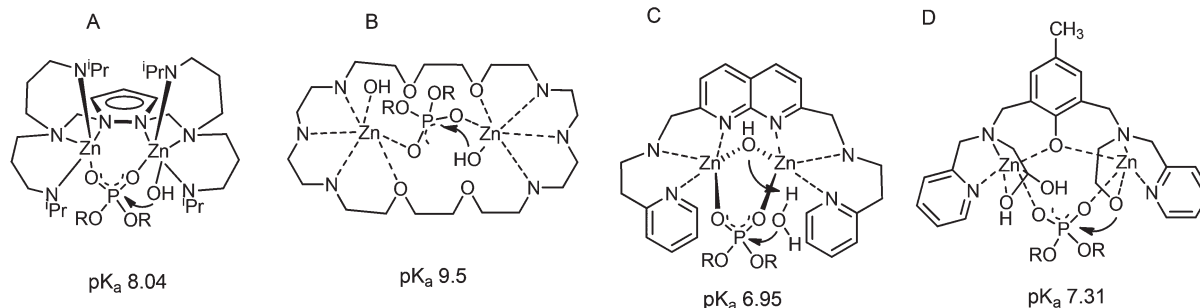

Fig. 10 Different hydrolysis initiation nucleophiles found in dinuclear Zinc(II) complexes.

potential alkoxide nucleophiles in the reaction. ^{31}P-chemical shifts are very sensitive to structural changes arising from an ^{18}O bound to the phosphorus instead of an ^{16}O; the signal arising from ^{18}O is thus shifted to lower frequency.[147–149] For complexes utilising a nucleophile arising from a coordinated aqua ligand in a 50 : 50 ^{18}O–^{16}O environment is it expected that the product of the hydrolysis reaction will be found with 50% ^{18}O incorporated from the nucleophile.[147–149] The observation in the ^{31}P spectrum of a splitting of the product peak into a doublet (peak separation of approximately 0.02 ppm) thus reflecting the ^{16}O/^{18}O substitution suggests that for complexes such as for [Zn$_2$(CH$_3$**L2**)(CH$_3$COO)$_2$](PF$_6$) and [Zn$_2$(CH$_3$H**L1**)(CH$_3$COO)-(H$_2$O)](PF$_6$) the nucleophile is a Zn(II) bound water/hydroxide from the solvent. The proposed mechanism is shown in Fig. 11. However, the dual action of both the alkoxide and hydroxide nucleophiles cannot be dismissed as a possible mechanistic pathway for these complexes.

5.2 Dinuclear cadmium hydrolase mimics

Cadmium gained its name from the Greek word "kadmeia", an ancient name for Zn(II) oxide.[150] Cadmium is similar in some ways to Zn(II) and the most common oxidation state is Cd(II). In coordination compounds Cd(II) is often found hepta-coordinate[80,151,152] and it is often used as a probe for structural and mechanistic studies of proteins in which Zn(II) is substituted by Cd(II) in their active centres. Often a higher hydrolytic activity is found than in the corresponding Zn(II) enzymes and complexes.[80] The natural abundance of ^{113}Cd is 12.22%[153] and it is very sensitive to environment changes due to the large surrounding electron cloud. It is also a spin 1/2 nucleus which generally yields sharp NMR signals, making ^{113}Cd NMR spectroscopy a useful method for structure elucidation in biological, inorganic and organometallic cadmium-containing samples.[110,154] Cd(II) is known to be extremely toxic to mammals, hence it is generally viewed as an element that is

not used by nature. However, in 2005 Morel and co-workers reported an investigation of a metalloenzyme from *Thalassiosira weissflogii*, a marine phytoplankton, which specifically uses Cd(II) to achieve its biological function.[155,156] Following X-ray absorption near-edge spectroscopy and comparison with Cd(II) thiolate and imidazole complexes the authors suggested that the Cd(II) containing active site of this protein employed a geometry close to the tetrahedral coordination found in a Zn(II)-containing class of carbonic anhydrases in higher plants and, moreover, that Cd(II) was bound to a least one cysteine residue.[156,157] A subsequent crystal structure of this enzyme showed that indeed Cd(II) was coordinated by two cysteine and one histidine residues.[156] The tetrahedral coordination of Cd(II) was completed by a water molecule which is connected to an extensive and well-ordered water hydrogen bond network around the active site.

Biomimetic Cd(II) complexes which mimic either structural or functional aspects of their enzymatic counterpart are rare in the literature and mostly based on mimicking mononuclear enzymes like alcohol dehydrogenase and carbonic anhydrase.[124,152,157–162] Di- and multinuclear Cd(II) complexes are mostly studied in terms of their NMR-shift, structure and coordination number.[151,152,163–165] For GpdQ we reported a comparative study of its Cd(II) derivative and a biomimetic.[80] The structure of the model complex [Cd$_2$((HP)$_2$B)-(CH$_3$COO)$_2$(OH$_2$)]PF$_6$ ((HP)$_2$B = 2,6-bis([(2-pyridylmethyl)-(2-hydroxyethyl)amino]methyl)-4-methylphenol) is depicted in Fig. 12; the ligand mimics the N,O donor atoms of the active site of the enzyme GpdQ (Fig. 12, right). The X-ray structure of [Cd$_2$((HP)$_2$B)(CH$_3$COO)$_2$(OH$_2$)]PF$_6$ shows that one Cd(II) ion is seven-coordinate 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.[80] This is an important feature, as a terminal
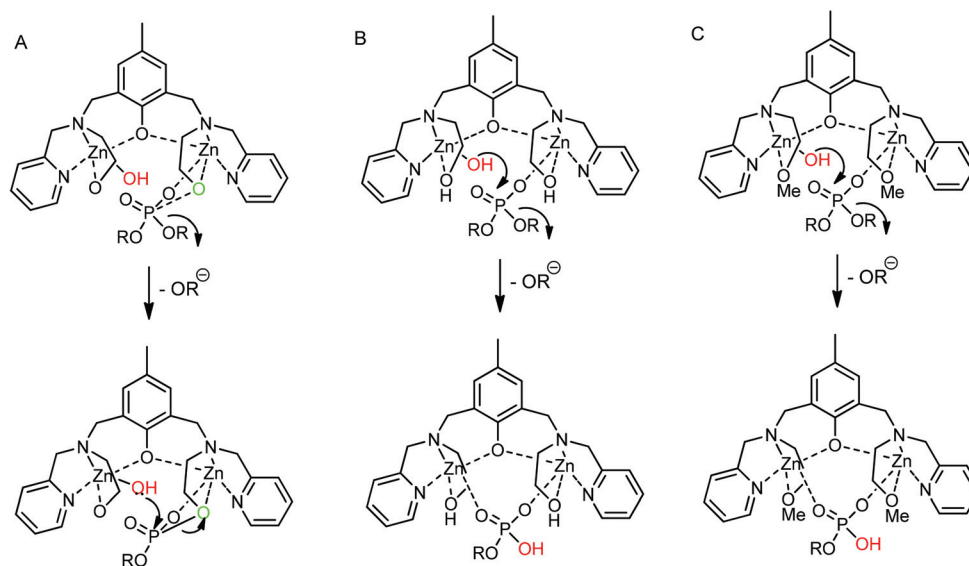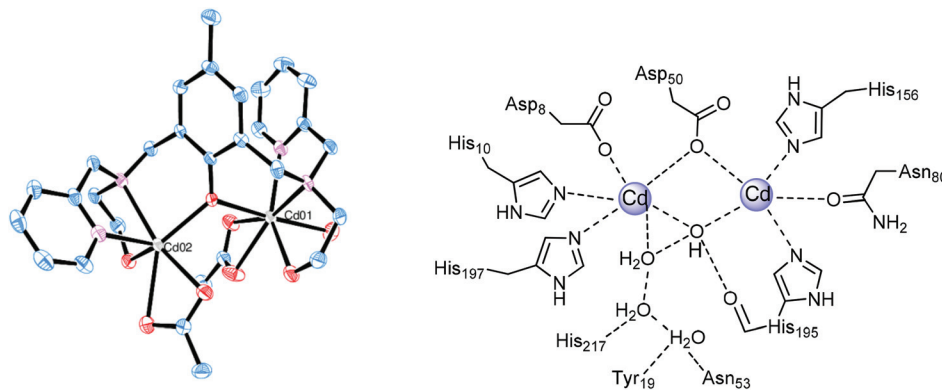

Fig. 11 The different nucleophiles involved in phosphoester cleavage in [Zn$_2$(CH$_3$H**L1**)(CH$_3$COO)(H$_2$O)](PF$_6$) and Zn$_2$(CH$_3$**L2**)(CH$_3$COO)$_2$](PF$_6$).

Fig. 12 The structure of $[Cd_2((HP)_2B)(CH_3COO)_2(OH_2)]PF_6$[80] Counter ion and hydrogen atoms have been omitted for clarity. Left: the dinuclear active site of GpdQ.[38]

water molecule is the likely nucleophile in GpdQ.[38,77] Remarkably, potentiometric studies showed that the two Cd(II) ions show different binding affinities, with $\log K_1 = 13.6$ and $\log K_2 = 3.2$, mimicking the different affinities found for the native enzyme.[38,77,80] The phosphoesterase-like activity of the biomimetic was studied utilising the substrate BDNPP and yielded a kinetic pK_a of 8.9, with $k_{cat} = 0.004$ s^{-1}. In the same work, studies of Cd$_2$-GpdQ and the phosphodiester substrate bis-(4-nitrophenyl)phosphate (BPNPP) resulted in a k_{cat} of 15 s^{-1} and a catalytically relevant pK_a of 9.4.[80] For both the biomimetic and the enzyme, a terminal hydroxide is implicated as the catalytic nucleophile. Another mechanistic study of a Cd(II)-substituted phosphoesterase has been reported by Ely et al.[71] The kinetically relevant pK_a (~4–5) for Cd$_2$-OpdA (organophosphate-degrading agent) in the hydrolysis of ethyl paraoxon suggested a bridging hydroxide as the nucleophile.[71]

We have investigated the cadmium(II) complexes of other ligands. The absence of a metal bound alcohol in $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ (an "ether-arm" complex) allows the assignment of the kinetically relevant pK_a (8.7) to a metal-bound terminal water molecule as found in related Zn(II) complexes.[138,142] The higher pK_a opposed to the Zn(II) systems is attributed to the less acidic character of the Cd(II) ion.[32] Interestingly, previous studies of similar Zn(II) complexes have shown that the pK_as of the ether- and alcohol-ligand catalysed reaction are often found to be similar within error suggesting that the same nucleophile is present.[138] It is known that for $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ the ether-arms are only partially coordinated in solution whereas the donors in $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ (an "alcohol-arm" complex) should be more tightly bound.[126,138] Thus, in the mechanism proposed for $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$, a nucleophile (terminal water/hydroxide) or substrate molecule could replace an ether-arm, whereas in the mechanism with $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ the alcohol-arms are bound more tightly restricting the geometry of the complex somewhat so that the pK_a of terminal water molecule is shifted to a more alkaline region. Another scenario is that the alcohol-arm of the complex with EtCO$_2$H$_3$\mathbf{L1}$ is acting as general base. Lippard and coworkers reported a Zn(II) complex with a bridging hydroxide where the

nucleophile was an external water ($pK_a = 7.06$) activated by the bridging hydroxide as base.[146] The pK_a of 10.1, however, would be unusually high for a Cd(II)-bound alcohol. An ^{18}O-labeling experiment suggests that the nucleophile in OP hydrolysis by $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ is not a ligand centred alkoxide but rather a water molecule which is terminally bound to one Cd(II) ion.[138] It is thus proposed that in both complexes the acting nucleophile is terminally bound water (Fig. 13A and B). The difference in pH is attributed to subtle structural and electronic differences in the complexes. In $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ a terminally Cd(II)-bound water ($pK_a = 8.7$) is replacing one of the ether-arms, in $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$, however, the alcohol-arm is bound tightly to the Cd(II) ions and thus the terminal water has to compete with the ligand donor atoms for coordination. Other mechanistic scenarios are possible. One would be where the alcohol-arm of the ligand is actively involved in the mechanism. In this case it could be envisaged that the alcohol-arm is deprotonated at pH 10.1 and acts as the primary nucleophile to hydrolyse the substrate BDNPP. The ligand is then recovered by an external water molecule and the substrate is released. A second scenario is possible where, upon deprotonation, the alkoxide acts as a general base to activate an external water molecule which then hydrolyses the substrate. Both scenarios would be consistent with the finding of a 50% ^{18}O labeled DNPP (Fig. 13C and D).[147–149] Data related to the activity of these Cd(II) complexes is presented in Table 2.

The higher activity and the difference in pH dependence of $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ opposed to the complex $[Cd_2((HP)_2B)(CH_3COO)_2(OH_2)]PF_6$ may be attributed to the ethyl ester group in para-position of the bridging phenolic oxygen.[80,138] This effect is analogous to the effect of the para-substituents seen in the di-Zn(II) complexes. The latter complex with the same donor atom set as the former but a methyl group in para-position of the bridging phenolic oxygen, exhibited a pK_a around 9,[80] suggesting that the ethyl ester has an effect on the polarization of the nucleophile, shifting the catalytically relevant pK_a. Peralta et al. reported a linear correlation between the pK_a values attributed to deprotonation of the Fe(III)-bound water for complexes with different substituents, methyl, H, Br and NO$_2$ respectively.[141] These studies
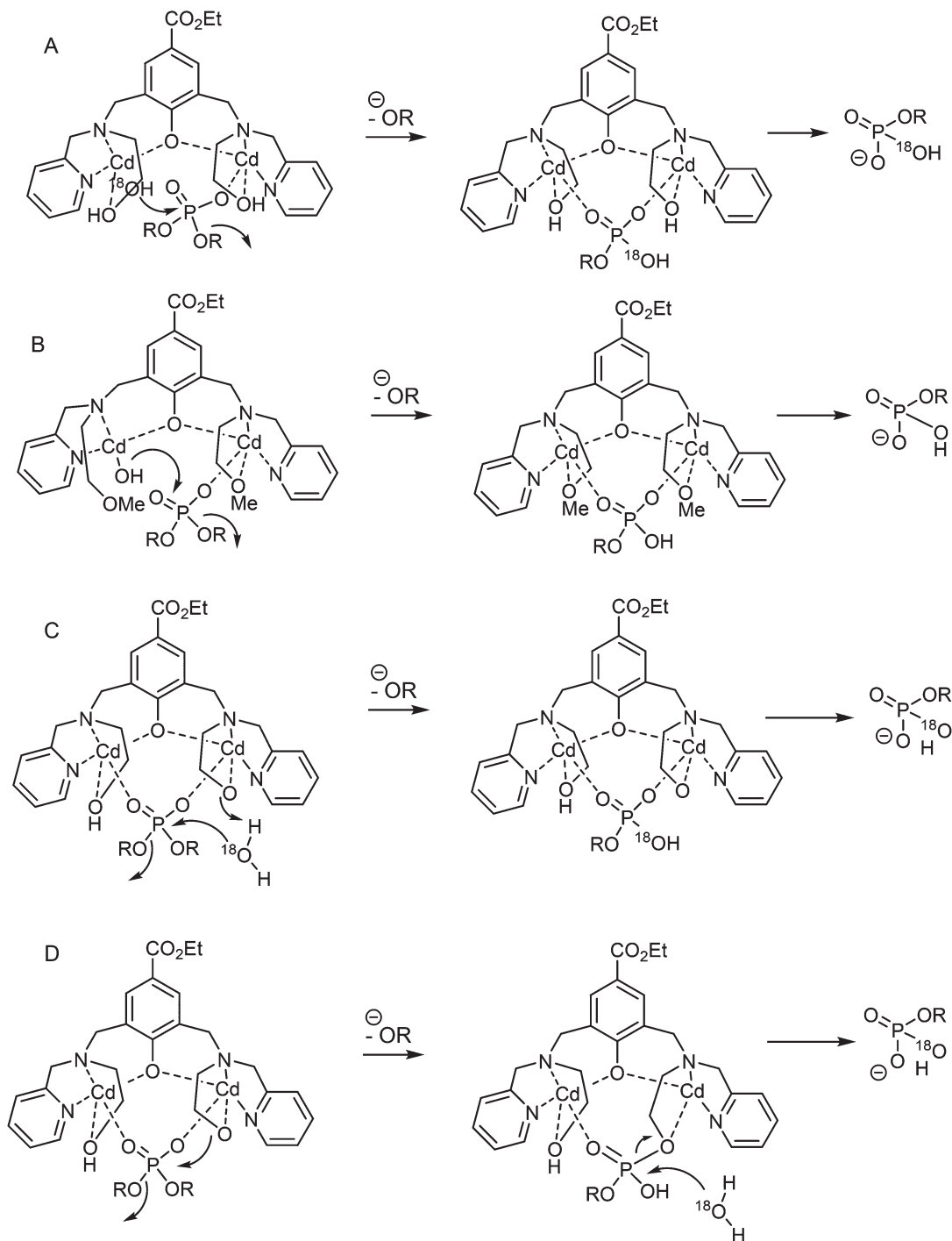
Fig. 13 Proposed mechanism for the BDNPP hydrolysis by $[Cd_2(CO_2EtH_2\textbf{L1})(CH_3COO)_2]^+$ (A) and $[Cd_2(CO_2Et\textbf{L2})(CH_3COO)_2]^+$ (B). Possible alternative mechanisms involving the alkoxide as a nucleophile that could lead ^{18}O being incorporated in the hydrolysis product (C and D).

suggest that subtle structural changes in the ligand can influence catalytic activity and the acidity of the Cd(II) ions in nucleophilic activation.

5.3 Dinuclear cobalt hydrolase mimics

Cobalt is a transition metal that is less abundant in nature than most other first row transition metals.[166] However, as a cofactor for vitamin B_{12} it is an essential trace element for humans.[167] Cobalt is usually found to be coordinated by a corrin ligand backbone in natural systems and one of the few true organometallic compounds in nature is the Co(III)-alkyl group in vitamin B_{12}.[168] A few Co(II)-containing enzymes such as prolidase, nitrile hydratase, bromoperoxidase or glucose isomerase have been isolated from bacteria.[167] Co(II) is, however, extensively used as spectroscopic probe for Zn(II)-containing enzyme active sites due to its unique spectroscopic and

magnetic properties. Often the catalytic activity in the cobalt substituted protein is retained,[109] and the active site geometry employed by Co(II) is frequently found to be virtually identical to the Zn(II) enzymes.[109] Octahedral or tetrahedral geometries are easily distinguished in the electronic absorption spectra, with the absorption band of the octahedral Co(II) displaying a characteristic splitting from spin–orbit coupling.[88]

As for Co(II)-metalloenzymes the MCD of Co(II)-containing model complexes is extremely useful. A number of dinuclear Co(II) complexes have been studied by MCD,[86,87,95] and with the aid of VTVH MCD magnetic coupling parameters have been obtained,[86] complementing magnetic susceptibility measurements. Distinct transitions for 4- (negative band a 600 ± 50 nm and less intense positive band at 525 ± 50 nm), 5- (negative bands between 650 and 450 nm) and 6-coordinate Co(II) (sharp negative band around 500 ± 50 nm) can be assigned.[88] Band intensities can also be an indicator for the coordination number of a Co(II) ion.[88] While 4- and 5-coordinate Co(II) displays bands of substantial intensity at room temperature the transitions from 6-coordinate Co(II) are up to ten times less intense. At very low temperature, however, the latter are often more intense than 5-coordinate transitions.

Fitting of experimental susceptibility data and explaining the magnetic properties for Co(II) systems is a challenging endeavor.[86,169–173] High spin (perfect) octahedral Co(II) displays $^4T_{1g}$ symmetry in the ground state and the susceptibility often shows considerable temperature dependence; the interpretation of data is difficult due to a partial quenching of the angular orbital momentum and to large spin orbit coupling resulting in substantial zero field splitting causing high magnetic anisotropy.[169,174] Zero field splitting parameters (D) can be measured by VTVH MCD and their magnitude provides valuable information about the coordination number and geometry of paramagnetic metal ions.[88] An important parameter in dinuclear Co(II) systems is the magnetic exchange coupling (J). Larrabee et al. reported a range of dinuclear Co(II)Co(II), Co(II)Co(III) and Co(III)Co(III) complexes to provide insight into the electronic structure and reactivity of cobalt substituted EcMetAp (methionine aminopeptidase from Escherichia coli).[86,95] By studying these models the authors were able to develop a method to distinguish between μ-aqua and μ-hydroxo species in related enzymes. By comparing the J values obtained by VTVH-MCD measurements the authors found that μ-aqua complexes display, in general, a greater J value and can thus be distinguished from μ-hydroxo complexes.[87,95] A Co(II) complex employing a mixed 5- and 6-coordination sphere which proved to be an excellent spectroscopic model not only for Co(II)-substituted methionine aminopeptidase but also for the enzyme GpdQ and other dinuclear metalloenzymes was reported by Larrabee and co-workers.[87] The authors gained insight about the potential nucleophile in hydrolysis of phosphate esters by comparing the J values.[87] The trend that μ-hydroxo structures have positive J values and are ferromagnetically coupled and μ-aqua bridged derivatives exhibit weak antiferromagnetic (negative J values) or no coupling, was observed.[87] Susceptibility studies

of a series of dicobalt(II) complexes based on the ligands shown in Fig. 9 indicate very weak antiferro- or ferro-magnetic coupling between the two Co(II) sites; DFT calculations were unable to explain the differences in the coupling although did suggest that the major pathway for the interaction was through the phenoxo donor and there were no consistent trends based on structural parameters to explain the variations in both the sign and magnitude of the coupling in a range of dinuclear Co(II) complexes.[134]

5.4 Mechanism of phosphodiester hydrolysis for the Co(II) complexes

In a prescient study, one of the first synthetic compounds capable of phosphate ester cleavage via a dinuclear metal centre was reported in 1984 by Sargeson (Fig. 14).[175] The presence of a second Co(III) centre in this complex was suggested to be responsible for a 26-fold increase in the rate of hydrolysis compared to that observed for the corresponding mononuclear complex.[175] It was proposed that the coordinated 4-nitrophenylphosphate (PNPP) was cleaved by an intramolecular attack of a terminal bound hydroxide. Since Sargeson's early work other examples of functional or spectroscopic model complexes for several dicobalt(III)/(II) complexes have been reported.[149,176–185]

For the Co(II) complexes in the present studies the activity towards organophosphoesters using BDNPP, was investigated (Table 2).[136] All complexes are good functional mimics for phosphodiesterases and show one pK_a relevant for hydrolysis. For the complexes with the methyl-ether donor, the absence of an alkoxide nucleophile and the kinetically relevant pK_a in the range 8.12–8.75 suggests that a terminal water molecule bound to Co(II) is the active nucleophile. For $[Co_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2](PF_6)$, fitting of the data resulted in a pK_a of 10.5. For this complex, as with previous studies with this type of ligand, the possibility exists that the alkoxide may be the active nucleophile. However, the pK_a of 10.5 for this complex is still in the range of that for a Co(II)-OH_2,[145] and hence the identity of the nucleophile is uncertain. What is apparent is that the catalytic activity of the Co(II) complexes towards the substrate BDNPP is similar to that displayed by the analogous Zn(II) complexes although it does not approach the efficiency of Co(II)$_2$-GpdQ ($k_{cat} = 1.62$ s^{-1}, $k_{cat}/K_m = 1.16$ mM^{-1} s^{-1}).[38]
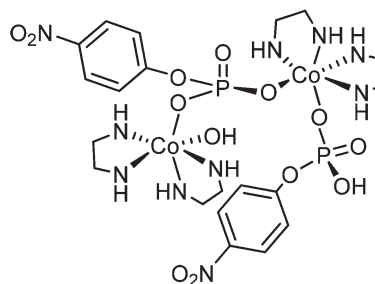

Fig. 14 Phosphate ester cleavage by a dinuclear Co(III) complex.

6. Conclusion

The enzyme GpdQ has shown itself to be a fertile source for the investigation of structure and function but also as an inspiration to develop model systems which have some properties consistent with those displayed by the enzyme itself. In terms of GpdQ itself, future work could include optimization and testing of both mutant and wild type systems on a range of pesticides and nerve agents. Our studies of a number of Zn(II), Cd(II) and Co(II) of variously substituted dinucleating ligands have allowed the investigation of the impacts of ligand modifications on the phosphoesterase mechanism, as well as intensive analysis of the relevant spectroscopic data arising from these complexes. Where appropriate the properties of these model systems have been related back to the enzyme systems. Our most recent work has explored ligands which mimic the asymmetric coordination environment of GpdQ more accurately.[134] The zinc(II) complexes in the crystal structure display a 5,6-coordination sphere, mimicking the coordination arrangement of GpdQ, this is not the case for the solution structure. The complexes were shown to be less active than substances based on symmetric ligands. We have also recently explored the immobilization of both GpdQ and model systems on solid supports, Merrifield resin and magnetite nanoparticles.[140] The complexes were shown to be active towards organophosphates and provide promising systems for the use in filter systems. The challenge is now to enhance activity of GpdQ and biomimetics towards commonly used pesticides. Given the importance of stability, the model complexes could be good alternatives to the enzyme systems. However, their activity needs to be enhanced towards 'real life substrates'. This remains the challenge for the development of true enzyme models.

Acknowledgements

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