



# New metal cofactors and recent metallocofactor insights

Robert P Hausinger

A vast array of metal cofactors are associated with the active sites of metalloenzymes. This Opinion describes the most recently discovered metal cofactor, a nickel-pincer nucleotide (NPN) coenzyme that is covalently tethered to lactate racemase from *Lactobacillus plantarum*. The enzymatic function of the NPN cofactor and its pathway for biosynthesis are reviewed. Furthermore, insights are summarized from recent advances involving other selected organometallic and inorganic-cluster cofactors including the lanthanide-pyrroloquinoline quinone found in certain alcohol dehydrogenases, tungsten-pyranopterins or molybdenum-pyranopterins in chosen enzymes, the iron-guanylylpyridinol cofactor of [Fe] hydrogenase, the nickel-tetrapyrrole coenzyme F430 of methyl coenzyme M reductase, the vanadium-iron cofactor of nitrogenase, redox-dependent rearrangements of the nickel-iron-sulfur C-cluster in carbon monoxide dehydrogenase, and light-dependent changes in the multi-manganese cluster of the oxygen-evolving complex

## Address

Departments of Microbiology & Molecular Genetics and Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI 48824, USA

Corresponding author: Hausinger, Robert P ([hausinge@msu.edu](mailto:hausinge@msu.edu))

Current Opinion in Structural Biology 2019, 59:1–8

This review comes from a themed issue on **Catalysis and regulation**

Edited by **Philip Cole** and **Andrea Mattevi**

<https://doi.org/10.1016/j.sbi.2018.12.008>

0959-440X/© 2018 Elsevier Ltd. All rights reserved.

## Introduction

Approximately 30% of enzymes are suggested to possess metallocenters and these metal-containing sites encompass a vast array of structures [1]. Here, I summarize recent studies describing the discovery, properties, and synthesis of a novel metal cofactor in lactate racemase. In addition, I describe fresh insights regarding selected representatives from the broad landscape of previously identified organometallic and inorganic cluster cofactors. Excluded from this synopsis are mononuclear and binuclear metal centers where the metals are bound only to protein side chains.

## Organometallic cofactors

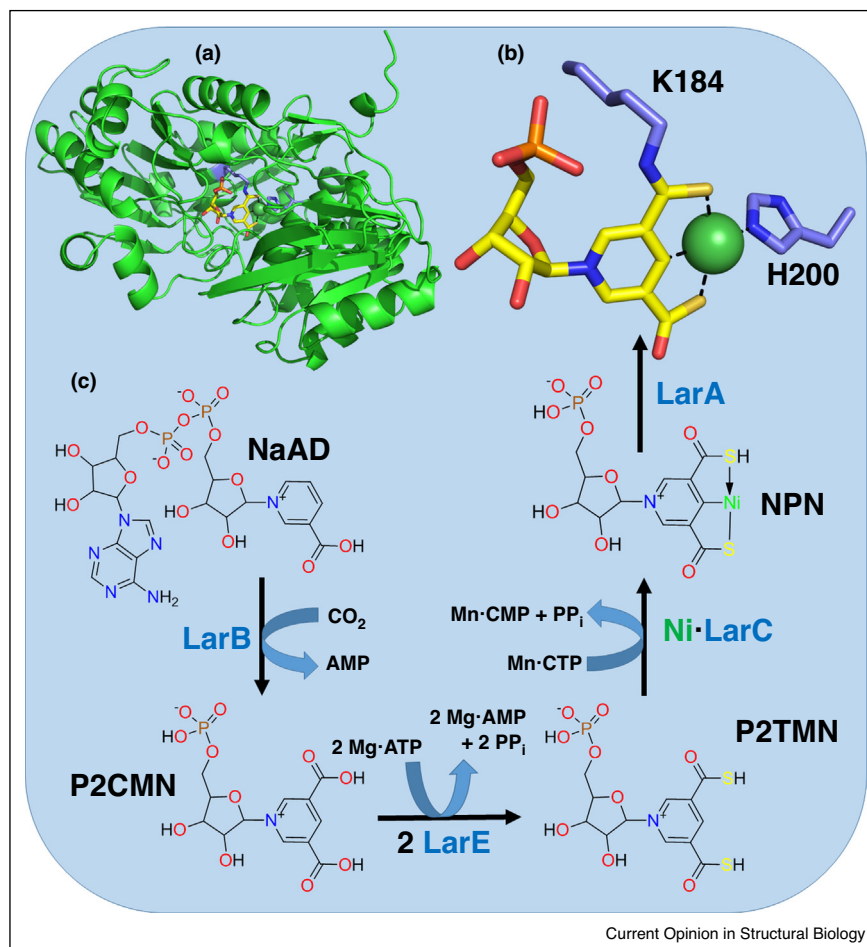
### Nickel-pincer nucleotide cofactor

The most recently identified metal cofactor, discovered in lactate racemase from *Lactobacillus plantarum*, is the nickel-pincer nucleotide (NPN; Figure 1a and b) [2<sup>••</sup>]. The organic portion of this cofactor, pyridinium-3-thioamide-5-thiocarboxylic acid mononucleotide, is covalently tethered to Lys184 of the enzyme and serves as a tridentate ligand to nickel. This molecule, with nickel bound in nearly planar geometry to the pyridinium carbon atom, the two sulfur atoms, and His200, is a biological example of the highly studied pincer complexes in inorganic chemistry [3].

Mechanistic studies of lactate racemase indicate the NPN cofactor participates in a proton-coupled hydride-transfer reaction [4<sup>•</sup>]. Pyruvate was identified as the catalytic intermediate, a substrate isotope effect was observed when using C2-<sup>2</sup>H-lactate, the enzyme chromophore was perturbed in the presence of lactate or sulfite, and the crystal structure of enzyme in the presence of sulfite revealed an adduct of the reagent bound to C4 of the pyridinium group (PDB ID: 6C1W). Density functional theory computations are consistent with hydride addition to the C4 site of the cofactor, but additionally suggest possible formation of a nickel-hydride if His200 dissociates. Thus, the racemase reaction is proposed to involve general base abstraction of the hydroxyl group proton of lactate, hydride transfer to the NPN cofactor forming pyruvate and a nickel-hydride or the C4 reduced species (depending on the starting isomer), interconversion of the two reduced cofactor species, and hydride return to either face of pyruvate [4<sup>•</sup>].

The overall pathway for NPN cofactor biosynthesis has been characterized (Figure 1c), and the responsible enzymes have been studied to varied extents [5<sup>•</sup>]. LarB uses nicotinic acid adenine dinucleotide (NaAD) as its substrate and carries out two reactions, the carboxylation of C5 on the pyridinium ring and hydrolysis of the phosphoanhydride linkage to form AMP and pyridinium-3,5-dicarboxylic acid mononucleotide (P2CMN). LarE is a structurally characterized sulfur transferase that activates a substrate carboxyl group by adenylation (releasing pyrophosphate), forms a thioester to the substrate using Cys176 (releasing AMP), and sacrifices its sulfur atom to the product while forming dehydroalanine in the protein [6]. Two LarE subunits are needed to generate pyridinium-3,5-dithiocarboxylic acid mononucleotide (P2TMN). LarC then installs the nickel atom

Figure 1



Lactate racemase and its nickel-pincer nucleotide (NPN) cofactor. **(a)** Ribbon structure of lactate racemase (green) with selected active site residues and the tethered organic portion of the cofactor shown as sticks (blue and yellow carbon atoms, respectively) with the nickel depicted as a green sphere (PDB ID: 5HUQ). **(b)** Closeup view of the NPN cofactor. **(c)** Biosynthetic pathway of the NPN cofactor in which LarB carboxylates NaAD, LarE catalyzes ATP-dependent sacrificial sulfur insertion into P2CMN, LarC couples CTP hydrolysis to nickel insertion into P2TMN, and the resulting NPN cofactor is incorporated into LarA.

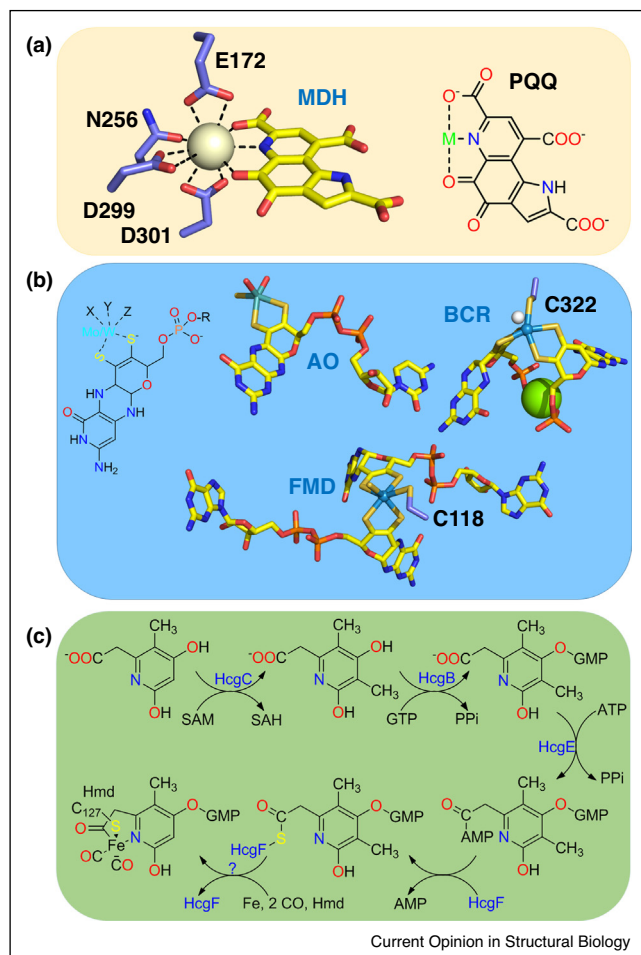
into P2TMN via a CTP-dependent reaction to make the final cofactor that attaches to the lactate racemase [7].

### Metal-pyrroloquinoline quinone cofactors

Early studies of methanol dehydrogenases from methylotrophic bacteria had identified a calcium-pyrroloquinoline quinone (PQQ) cofactor in these enzymes (e.g. PDB ID: 4AAH from *Methylobacterium* W3A1 and PDB ID: 1W6S from *Methylobacterium extorquens*). Thus, it came as a surprise when *Methylobacterium extorquens* SoIV was shown to require a lanthanide metal ion (lanthanum, cerium, neodymium, praseodymium, samarium, europium, or gadolinium) for growth, with the rare earth element incorporated into its methanol dehydrogenase [8<sup>••</sup>]. Structural studies of the enzyme from this organism in the presence of different metals revealed a cerium-PQQ or europium-PQQ cofactor (Figure 2a) [8<sup>••</sup>,9]. A

lanthanide-PQQ methanol dehydrogenase also was structurally characterized from *Methylobacterium buryatense* 5GB1C (PDB ID: 6DAM) and shown to interact with the particulate methane monooxygenase of this organism [10]. By contrast, the structure of methanol dehydrogenase from a marine methylotroph possessed a magnesium-PQQ cofactor (PDB ID: 5XM3) [11]. Studies involving *M. extorquens* AM1 identified a lanthanide-dependent and PQQ-dependent ethanol dehydrogenase, ExaF, along with three methanol dehydrogenases (MxaF with calcium-PQQ, and XoxF1 or XoxF2 with lanthanide-PQQs) [12,13]. Of additional interest, this microorganism possesses a periplasmic lanthanide-binding protein that was named lanmodulin due to the presence of four metal-binding EF hand motifs similar to the motif found in the calcium-binding protein calmodulin [14,15]; the role of lanmodulin is unknown at this time, but it may

Figure 2



Selected other organometallic cofactors. **(a)** Active site of methanol dehydrogenase (MDH) with its lanthanide-PQQ cofactor [PDB ID: 4MAE (cerium) or 6FKW (europium)] and a schematic of the metal-PQQ structure. **(b)** Schematic of a generalized Mo/W-pyranopterin site and active sites of the molybdenum-containing or tungsten-containing and pyranopterin-containing enzymes aldehyde oxidase (AO; PDB ID: 5Y6Q), benzoyl-CoA reductase (BCR; PDB ID: 4Z3Y), and formyl-methanofuran dehydrogenase (FMD; PDB ID: 5T5I). **(c)** Biosynthetic pathway for synthesis of the FeGP cofactor.

provide the lanthanide to target alcohol dehydrogenases. The lanthanide-PQQ methanol dehydrogenases are not restricted to methylotrophs as shown by functional and regulatory studies with *Pseudomonas putida* KT2440 [16].

### Metal-pterin cofactors

Molybdenum- or tungsten- and pyranopterin-containing enzymes have long been known [17,18]. Here, the diversity of these enzymes is represented by three recent structurally elucidated examples (Figure 2b). The aldehyde oxidase of *Methylobacillus* sp. KY4400 contains a pyranopterin cytosine dinucleotide with its dithiolene moiety bound to molybdenum, which has two oxygens

and a sulfide to complete its coordination sphere [19]. This architecture is typical of members in the xanthine oxidase family. The second example is benzoyl-coenzyme A (CoA) reductase of *Geobacter metallireducens*, BamBC [20]. In this case, a tungsten is coordinated by two pyranopterin monophosphate molecules and Cys322. A sixth ligand is present, but its identity remains unknown, and the bispyranopterins bind a magnesium ion between them. Finally, formyl-methanofuran dehydrogenase is a CO<sub>2</sub>-fixing enzyme in methanogens, with the structure determined within the FwdABCDG complex from *Methanothermobacter wolfeii* [21]. This enzyme possesses a tungsten bound to two pyranopterin guanine dinucleotides, Cys118, and a sulfide ligand. Humans possess four molybdenum-containing enzymes and inborn errors of metabolism affecting the pyranopterin biosynthesis, and metal insertion are associated with disease states [22].

### Iron-guanylylpyridinol cofactor

The pathway for biosynthesis of the iron-guanylylpyridinol (FeGP) cofactor (Figure 2c), a coenzyme that was identified over 10 years ago in [Fe] hydrogenase HmdI from *Methanocaldococcus jannaschii* (PDB ID: 3DAG), has been the subject of intense recent investigation. Biochemical and structural (e.g. PDB ID: 5D4H) results indicate HcgC is an *S*-adenosylmethionine (SAM)-dependent methyltransferase that converts 6-carboxymethyl-5-methyl-4-hydroxy-2-pyridinol to the dimethyl derivative with release of *S*-adenosylhomocysteine (SAH) [23]. HcgB also was structurally elucidated (e.g. PDB ID: 5D5Q) and shown to be a guanylyl transferase that adds GMP to the 4-hydroxy position of the cofactor, presumably with release of pyrophosphate [24]. HcgE, crystallized from *Methanothermobacter marburgensis* (e.g. PDB ID: 3WV8) [25], is an adenyl transferase that activates the carboxymethyl group of the cofactor with release of pyrophosphate. Cys9 of HcgF, structurally defined from *M. jannaschii* (e.g. PDB ID: 3WVA), then forms a thioester as AMP is released [25]. The process for incorporating the iron atom and two carbon monoxide molecules into the FeGP cofactor remains unclear; however, the structure of *M. jannaschii* HcgD (e.g. PDB ID: 3WSD) reveals a dinuclear iron center, hinting at a possible role in iron delivery [26]. Some hydrogenase activity was observed when an FeGP mimic was reconstituted into the recombinant apoprotein generated in *Escherichia coli* [27]. By contrast, no activity was detected when FeGP was reconstituted into the HmdII apoprotein from *M. jannaschii*, perhaps suggesting an alternative role such as a H<sub>2</sub> sensor [28].

### Metal-tetrapyrrole cofactors

An important family of organometallic cofactors are the metal tetrapyrroles. Many redox enzymes contain red heme pigments, with selected enzymes possessing alternative versions such as siroheme and heme *d*<sub>1</sub> (Figure 3a) [29,30]. Additional modifications of these

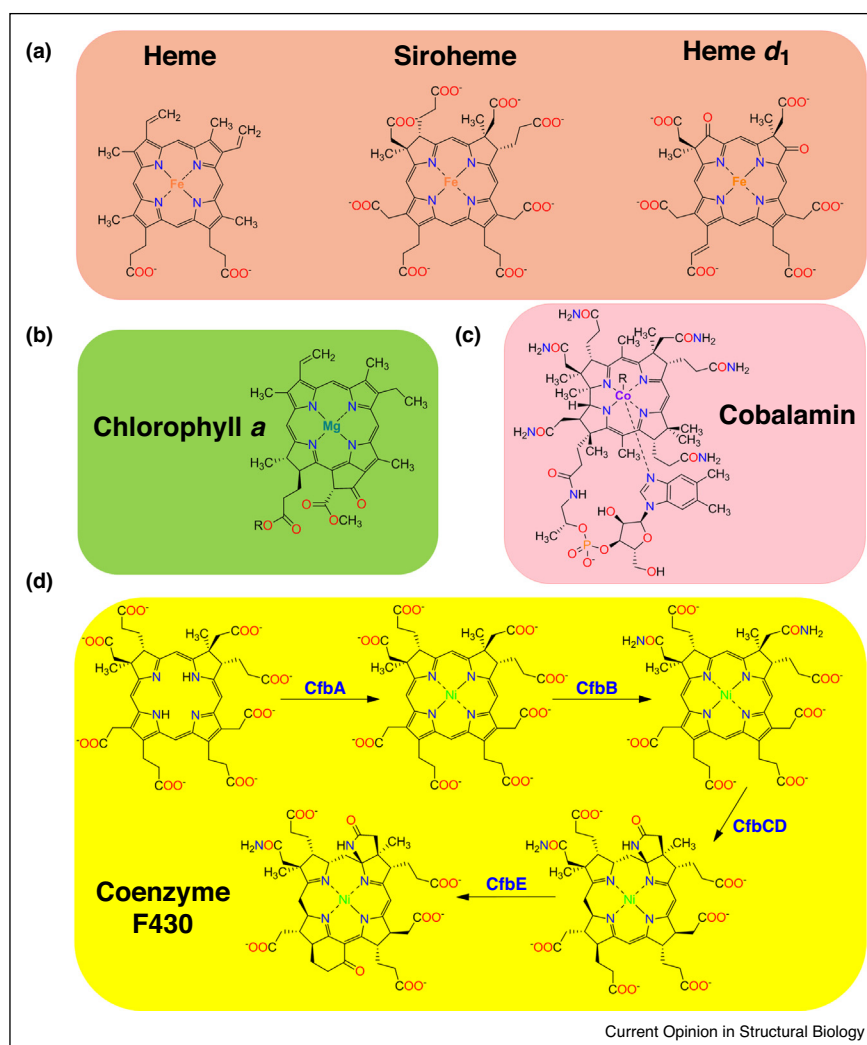
iron-tetrapyrroles also are known, including the lysine-linked version of heme associated with cytochrome P460 [31]. The magnesium-containing tetrapyrrole found at the photosynthetic reaction center and used in antenna proteins in eukaryotes is chlorophyll *a* (Figure 3b), and microbial bacteriochlorophylls encompass a large range of structural derivatives that serve the same roles [32,33]. Cobalamin-containing enzymes use a red cobalt tetrapyrrole (Figure 3c) to promote isomerase, methyl transfer, reductive dehalogenation, and radical reactions [34]. Finally, methanogenic and methanotrophic archaea possess a nickel-containing tetrapyrrole known as coenzyme F430. The biosynthetic pathway of this yellow cofactor was recently uncovered (Figure 3d) [35,36], a number of modified versions of coenzyme F430 were identified [37], and its assembly into methyl coenzyme M reductase is beginning to be clarified [38].

## Inorganic cluster cofactors

### Iron-sulfur clusters

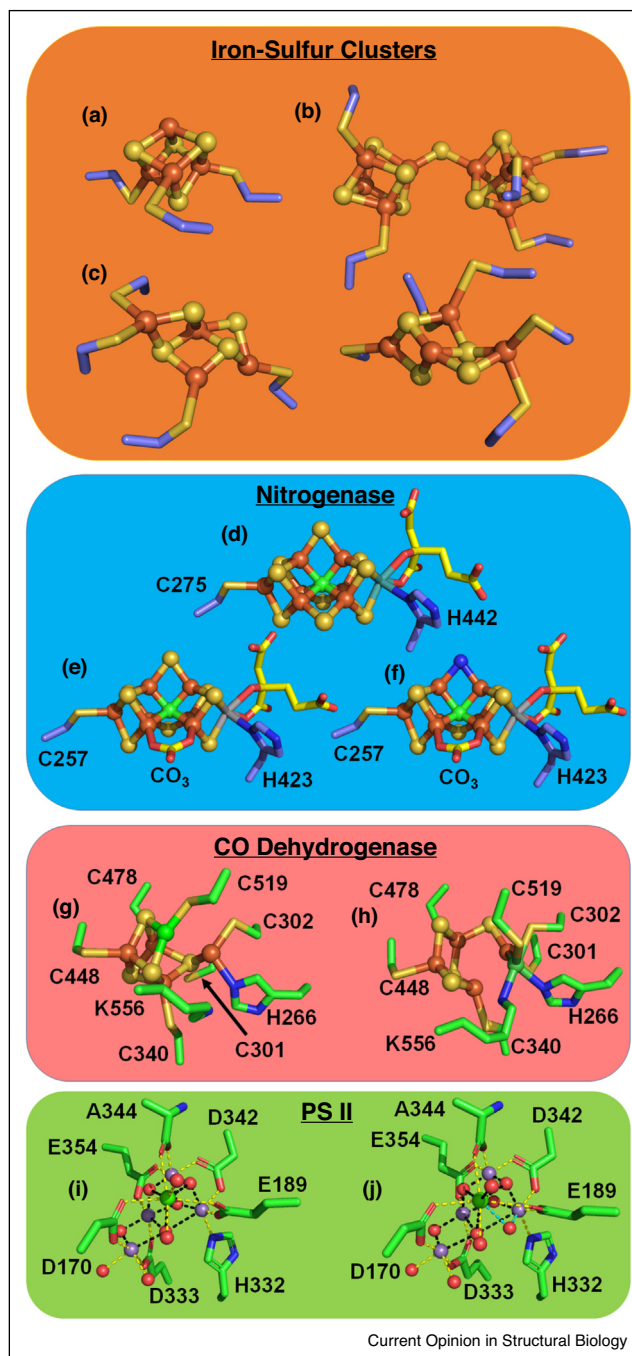
Many enzymes contain cubane-type [4Fe4S] clusters that either participate in electron transfer reactions or facilitate catalysis. When used for electron transfer, amino acid side chains typically coordinate each of the four iron atoms in the cluster; however, the identities of the participating residues vary (e.g. four Cys, three Cys and one Asp/Glu/His/Ser/Met/Tyr, five Cys, six Cys, or other configurations). In contrast, the absence of a protein ligand leaves one iron site available to interact with a substrate so the cluster can then directly function in catalytic activity. A recent example of such a cluster is found in TtuA (Figure 4a), where the open coordination site is thought to bind inorganic sulfide that is used to form the modified tRNA base 2-thiouridine [39,40]. An open coordination site also is found in the vast family of

Figure 3



Metal-tetrapyrrole cofactors. (a) Heme, siroheme, and heme *d*<sub>1</sub>. (b) Chlorophyll *a*. (c) Cobalamin. (d) The F430 biosynthetic pathway.

Figure 4



Inorganic cluster cofactors. (a) Cubane-type [4Fe-4S] cluster with an open coordination site on one iron atom (TtuA, PDB ID: 5MKQ). (b) [8Fe-9S] cluster of a reductase (PDB ID: 6ENO). (c) Non-cubane [4Fe-4S] clusters of methanogenic heterodisulfide reductase (PDB ID: 5ODH). (d) Iron-molybdenum cofactor of nitrogenase (PDB ID: 3U7Q). (e) Iron-vanadium cofactor of nitrogenase with a belt sulfide replaced by a carbonate (PDB ID: 5N6Y). (f) Possible reaction intermediate of nitrogenase containing the iron-vanadium cofactor (PDB ID: 6FEA). (g) and (h) Reduced (PDB ID: 6B6X) and oxidized (PDB ID: 6B6W) forms of the C-cluster of carbon monoxide dehydrogenase. (i) and (j) The multi-manganese cofactor of the photosystem II (PS II) oxygen-evolving complex is shown in the  $S_1$  and  $S_3$  states, revealing a [Ca-4Mn-O5] to [Ca-4Mn-6O] transition (PDB ID: 6DHE and 6DHH; manganese, calcium, and solvent atoms are depicted as purple, green, and red spheres; the newly incorporated solvent atom is indicated by cyan bonding).

radical SAM enzymes; SAM coordinates to one iron atom of the [4Fe–4S] cluster that generates a reactive 5'-deoxyadenosyl radical via formation of an Fe–C5' bond, denoted intermediate  $\Omega$  [41]. Within this group of enzymes is the interesting example of TYW1 containing two cubane [4Fe–4S] clusters with three coordinating Cys residues; one cluster functions to catalyze typical radical SAM chemistry while an auxiliary cluster coordinates a Schiff base intermediate formed between a lysine side chain and the substrate pyruvate, which condenses with *N*-methylguanosine to form 4-demethyl-tyrosine in tRNA [42].

Not all iron–sulfur clusters in enzymes are simple cubanes. A protein with an intriguing [8Fe–9S] double cubane cluster (Figure 4b) was recently identified in *Carboxydotherrmus hydrogengenoformans* and appears to operate with a co-expressed ATPase as a very low potential reductase [43]. Furthermore, a pair of noncubane [4Fe–4S] clusters (Figure 4c) are found in heterodisulfide reductase of methanogenic archaea, an enzyme that forms coenzyme M (CoM) and coenzyme B (CoB) by catalyzing a flavin-based electron bifurcation reaction to reduce both ferredoxin and the CoM–CoB heterodisulfide [44]. The CoM–CoB disulfide is proposed to bind between the two clusters.

### Nitrogenase

Molybdenum-containing nitrogenase of *Azotobacter vinelandii* possesses a [1Mo–7Fe–9S–1C] cofactor (Figure 4d) with homocitrate and a His residue coordinating the molybdenum, a Cys residue ligating the distal iron, and three sulfides forming a 'belt' around the cluster [45]. The vanadium nitrogenase of this microorganism has a very similar [1V–7Fe–8S–1C–CO<sub>3</sub>] cofactor (Figure 4e), in which vanadium replaces the molybdenum and one belt sulfide is replaced by a bridging carbonate ligand [46]. Additional studies with this enzyme revealed the possible structure of a bound reaction intermediate (Figure 4f), in which another belt sulfide is replaced by a probable bridging protonated nitrogen atom [47].

### Carbon monoxide dehydrogenase

Nickel-containing carbon monoxide dehydrogenases contain standard electron-transferring iron–sulfur clusters as well as the distinct C-cluster that has been described as a [Ni–3Fe–4S] cluster connected through a linking sulfide to another Fe atom. Recent studies have shown a dramatic redox-dependent cluster rearrangement for the C-cluster of the enzyme from *Desulfovibrio vulgaris* [48]. Whereas the reduced C-cluster has four Cys residues (Cys340, Cys448, Cys478, and Cys519) binding a [Ni–3Fe–4S] unit with one of the sulfides bridging to Fe that is further coordinated by Cys302 and His266 (Figure 4g), the oxidized C-cluster possesses a [4Fe–4S] unit bound by five Cys (Cys301, Cys302, Cys340, Cys448, and Cys478) with Cys302 also binding Ni that is additionally

coordinated by His266, Cys519, and Lys556. This cluster rearrangement with its shift in nickel coordination environment was suggested to provide stability of the cluster against oxygen inactivation [48].

### Photosynthetic Mn cluster

The 20-subunit crystal structure of photosystem II (PS II) with its [1Ca–4Mn–5O] oxygen-evolving complex from *Thermosynechococcus vulcanus* was solved at 1.9 Å resolution in 2011 (PDB ID: 3WU2) [49]. Concerns about potential photodamage by the X-ray source led to recent studies to obtain a damage-free structure by using a free-electron laser to generate femtosecond pulses with this [50] and the *Thermosynechococcus elongatus* PS II [51]; for similar reasons, the structure also was evaluated using very low doses of conventional X-rays [52]. Of additional interest are the structural effects of light flashes on the site of dioxygen formation [51,53]. In particular, structures of intermediates associated with Kok's oxidation clock reveal the conversion of the [1Ca–4Mn–5O] species in the S<sub>1</sub> state to a [1Ca–4Mn–6O] species in the S<sub>3</sub> state (Figure 4i and j) [54].

### Conclusions and outlook

Despite the wealth of knowledge already available on metalloenzymes, it is still possible to identify additional new metal cofactors as shown by the discovery of the NPN cofactor. Some microorganisms possess multiple copies of genes homologous to that of lactate racemase and other microorganisms possess genes for NPN cofactor biosynthesis while lacking an obvious lactate racemase. These findings suggest the NPN cofactor may function in enzymatic reactions beyond the racemization of lactic acid, and this research area is certain to be an area of future investigation. New features of established metal cofactors also continue to be uncovered, and additional insights into the properties of these metallocenters are sure to be revealed, especially related to their biosynthetic pathways and catalytic intermediates.

### Acknowledgement

Studies conducted in the Hausinger laboratory were supported by the National Science Foundation (CHE-1516126 and CHE-1807073).

### References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Bertini I, Gray HB, Stiefel EI, Valentine JS: *Biological Inorganic Chemistry*. Sausalito, CA: University Science Books; 2007.
2. Desguin B, Zhang T, Soumilion P, Hols P, Hu J, Hausinger RP: **A tethered niacin-derived pincer complex with a nickel-carbon bond in lactate racemase**. *Science* 2015, **349**:66–69.
3. Peris E, Crabtree RH: **Key factors in pincer ligand design**. *Chem Soc Rev* 2018, **47**:1959–1968.

4. Rankin JA, Mauban RC, Fellner M, Desguin B, McCracken J, Hu J, Varganov SA, Hausinger RP: **Lactate racemase nickel-pincer cofactor operates by a proton-coupled hydride transfer mechanism.** *Biochemistry* 2018, **57**:3244-3251.
- This study defines features of the catalytic mechanism of lactate racemase and its NPN cofactor by a combination of studies that trap the pyruvate intermediate, measure the deuterated substrate kinetic isotope effect, monitor the cofactor absorption changes upon adding substrate, determine the protein structure in the presence of sulfite, and use computational methods to identify hydride placement.
5. Desguin B, Soumilion P, Hols P, Hausinger RP: **Nickel-pincer cofactor biosynthesis involves LarB-catalyzed pyridinium carboxylation and LarE-dependent sacrificial sulfur insertion.** *Proc Natl Acad Sci U S A* 2016, **113**:5598-5603.
- This study identifies the overall pathway for NPN biosynthesis involving the sequence action of LarB, LarE, and LarC, and identifies LarE as a sacrificial sulfur transferase.
6. Fellner M, Desguin B, Hausinger RP, Hu J: **Structural insights into the catalytic mechanism of a sacrificial sulfur insertase of the N-type ATP pyrophosphatase family, LarE.** *Proc Natl Acad Sci U S A* 2017, **114**:9074-9079.
7. Desguin B, Fellner M, Riant O, Hu J, Hausinger RP, Hols P, Soumilion P: **Biosynthesis of the nickel-pincer nucleotide cofactor of lactate racemase requires a CTP-dependent cyclometallase.** *J Biol Chem* 2018, **293**:12303-12317.
8. Pol A, Barends TRM, Dietl A, Khadem AF, Eygensteyn J, Jetten MSM, Op den Camp HJM: **Rare earth metals are essential for methanotrophic life in volcanic mudpots.** *Environ Microbiol* 2014, **16**:255-264.
- This study reports the first use of lanthanides in an enzyme and defines the structure of a lanthanide-PQQ cofactor in a methanol dehydrogenase.
9. Jahn B, Pol A, Lumpe H, Barends TRM, Dietl A, Hogendoorn C, Op den Camp HJM, Daumann LJ: **Similar but not the same: first kinetic and structural analyses of a methanol dehydrogenase containing a europium ion in the active site.** *ChemBioChem* 2018, **19**:1147-1153.
10. Deng YW, Ro SY, Rosenzweig AC: **Structure and function of the lanthanide-dependent methanol dehydrogenase XoxF from the methanotroph *Methylobacterium buryatense* 5GB1C.** *J Biol Inorg Chem* 2018, **23**:1037-1047.
11. Cao T-P, Choi JM, Kim SW, Lee SH: **The crystal structure of methanol dehydrogenase, a quinoprotein from the marine methylotrophic bacterium *Methylophaga aminisulfidivorans* MP.** *J Microbiol* 2018, **56**:246-254.
12. Good NM, Vu HN, Suriano CJ, Subuyuj GA, Skovran E, Martinez-Gomez NC: **Pyrroloquinoline quinone ethanol dehydrogenase in *Methylobacterium extorquens* AM1 extends lanthanide-dependent metabolism to multicarbon substrates.** *J Bacteriol* 2016, **198**:3109-3118.
13. Vu HN, Subuyuj GA, Vijayakumar S, Good NM, Martinez-Gomez NC, Skovran E: **Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth.** *J Bacteriol* 2016, **198**:1250-1259.
14. Cotruvo JA Jr, Featherston ER, Mattocks JA, Ho JV, Laremore TN: **Lanmodulin: a highly selective lanthanide-binding protein from a lanthanide-utilizing bacterium.** *J Am Chem Soc* 2018, **140**:15056-15061.
15. Cook EC, Featherston ER, Showalter SA, Cotruvo JA Jr: **Structural basis for rare earth element recognition by *Methylobacterium extorquens* lanmodulin.** *Biochemistry* 2019, **58**:120-125.
16. Wehrmann M, Billard P, Martin-Meriadec A, Zegeye A, Klebensberger J: **Functional role of lanthanides in enzymatic activity and transcriptional regulation of pyrroloquinoline quinone-dependent alcohol dehydrogenases in *Pseudomonas putida* KT 2440.** *MBio* 2017, **8**:e00570-00517.
17. Romão MJ: **Molybdenum and tungsten enzymes: a crystallographic and mechanistic overview.** *Dalton Trans* 2009:4053-4068.
18. Hille R, Hall J, Basu P: **The mononuclear molybdenum enzymes.** *Chem Rev* 2014, **114**:3963-4038.
19. Uchida H, Mikami B, Yamane-Tanabe A, Ito A, Hirano K, Oki M: **Crystal structure of an aldehyde oxidase from *Methylobacillus* sp. KY4400.** *J Biochem* 2018, **163**:321-328.
20. Weinert T, Huwiler SG, Kung JW, Weidenweber S, Hellwig P, Stärk H-J, Biskup T, Weber S, Cotelesage JJH, George GN et al.: **Structural basis of enzymatic benzene ring reduction.** *Nat Chem Biol* 2015, **11**:586-591.
21. Wagner T, Ermler U, Shima S: **The methanogenic CO<sub>2</sub> reducing- and-fixing enzyme is bifunctional and contains 46 [4Fe-4S] clusters.** *Science* 2016, **354**:114-117.
22. Schwarz G: **Molybdenum cofactor and human disease.** *Curr Opin Chem Biol* 2016, **31**:179-187.
23. Fujishiro T, Bai L, Xu T, Xie X, Schick M, Kahnt J, Rother M, Hu X, Ermler U, Shima S: **Identification of HcgC as a SAM-dependent pyridinol methyltransferase in [Fe]-hydrogenase cofactor biosynthesis.** *Angew Chem Int Ed* 2016, **55**:9648-9651.
24. Fujishiro T, Tamura H, Schick M, Kahnt J, Xie X, Ermler U, Shima S: **Identification of the HcgB enzyme in [Fe]-hydrogenase cofactor biosynthesis.** *Angew Chem Int Ed* 2013, **52**:12555-12558.
25. Fujishiro T, Kahnt J, Ermler U, Shima S: **Protein-pyridinol thioester precursor for biosynthesis of the organometallic acyl-iron ligand in [Fe]-hydrogenase cofactor.** *Nat Commun* 2015, **6**:6895.
26. Fujishiro T, Ermler U, Shima S: **A possible iron delivery function of the dinuclear iron center of HcgD in [Fe]-hydrogenase cofactor biosynthesis.** *FEBS Lett* 2014, **588**:2789-2793.
27. Shima S, Chen D, Xu T, Wodrich MD, Fujishiro T, Schultz KM, Kahnt J, Ataka K, Hu X: **Reconstitution of [Fe]-hydrogenase using model complexes.** *Nat Chem* 2015, **7**:995-1002.
28. Fujishiro T, Ataka K, Ermler U, Shima S: **Towards a functional identification of catalytically inactive [Fe]-hydrogenase paralogs.** *FEBS J* 2015, **282**:3412-3423.
29. Poulos TL: **Heme enzyme structure and function.** *Chem Rev* 2014, **114**:3919-3962.
30. Dailey HA, Dailey TA, Gerdes S, Jahn D, Jahn M, O'Brian MR, Warren MJ: **Prokaryotic heme biosynthesis: multiple pathways to a common essential product.** *Microbiol Mol Biol Rev* 2017, **81**:e00048-00016.
31. Smith MA, Lancaster KM: **The eponymous cofactors in cytochrome P460s from ammonia-oxidizing bacteria are iron porphyrinoids whose macrocycles are bibasic.** *Biochemistry* 2017, **57**:334-343.
32. Gisriel C, Sarrou I, Ferlez B, Goldbeck JH, Redding KE, Fromme R: **Structure of a symmetric photosynthetic reaction center-photosystem.** *Science* 2017, **357**:1021-1025.
33. Saer RG, Blankenship RE: **Light harvesting in phototrophic bacteria: structure and function.** *Biochem J* 2017, **474**.
34. Bridwell-Rabb J, Grell TAJ, Drennan CL: **A rich man, poor man story of S-adenosylmethionine and cobalamin revisited.** *Annu Rev Biochem* 2018, **87**:555-584.
35. Zheng K, Ngo PD, Owens VL, Yang X-p, Mansoorabadi SO: **The biosynthetic pathway of coenzyme F<sub>430</sub> in methanogenic and methanotrophic archaea.** *Science* 2016, **354**:339-342.
- The enzymes and reactions are described for the coenzyme F<sub>430</sub> biosynthetic pathway.
36. Moore SJ, Sowa ST, Schuchardt C, Deery E, Lawrence AD, Ramos JV, Billig S, Birkemeyer C, Chivers PT, Howard MJ et al.: **Elucidation of the biosynthesis of the methane catalyst coenzyme F<sub>430</sub>.** *Nature* 2017, **543**:78-82.
37. Allen KD, Wegener G, White RH: **Discovery of multiple modified F<sub>430</sub> coenzymes in methanogens and anaerobic methanotrophic archaea suggests possible new roles for F<sub>430</sub> in Nature.** *Appl Environ Microbiol* 2014, **80**:6403-6412.
38. Lyu Z, Chou C-W, Shi H, Wang L, Ghebream R, Phillips D, Yan Y, Duin EC, Whitman WB: **Assembly of methyl coenzyme M reductase in the methanogenic archaeon *Methanococcus maripaludis*.** *J Bacteriol* 2018, **200**:e00746-00717.

39. Chen M, Asai S-i, Narai S, Nambu S, Omura N, Sakaguchi Y, Suzuki T, Ikeda-Saito M, Watanabe K, Yao M *et al.*: **Biochemical and structural characterization of oxygen-sensitive 2-thiouridine synthesis catalyzed by an iron-sulfur protein TtuA.** *Proc Natl Acad Sci U S A* 2017, **114**:4954-4959.
40. Arragain S, Bimai O, Legrand P, Caillat S, Ravanat J-L, Touati N, Binet L, Atta M, Fontecave M, Golinelli-Pimpaneau B: **Nonredox thiolation in tRNA occurring via sulfur activation by a [4Fe-4S] cluster.** *Proc Natl Acad Sci U S A* 2017, **114**:7355-7360.
41. Byer AS, Yang H, McDaniel EC, Kathiresan V, Impano S, Pagnier A, Watts H, Denler C, Vagstad AL, Piel J *et al.*: **Paradigm shift for radical S-adenosyl-L-methionine reactions: the organometallic intermediate  $\Omega$  is central to catalysis.** *J Am Chem Soc* 2018, **140**:8634-8638.
42. Grell TAJ, Young AP, Drennan CL, Bandarian V: **Biochemical and structural characterization of a Schiff base in the radical-mediated biosynthesis of 4-demethylwyosine by TYW1.** *J Am Chem Soc* 2018, **140**:6842-6852.
43. Jeoung J-H, Dobbek H: **ATP-dependent substrate reduction at an  $[\text{Fe}_8\text{S}_9]$  double-cubane cluster.** *Proc Natl Acad Sci U S A* 2018, **115**:2994-2999.
44. Wagner T, Koch J, Ermiler U, Shima S: **Methanogenic heterodisulfide reductase (HdrABC-MvhAGD) uses two noncubane [4Fe-4S] clusters for reduction.** *Science* 2017, **357**:699-703.
45. Spatzal T, Aksoyoglu M, Zhang L, Andrade SLA, Schleicher E, Weber S, Rees DC, Einsle O: **Evidence for interstitial carbon in nitrogenase FeMo cofactor.** *Science* 2011, **334**:940.
46. Sippl D, Einsle O: **The structure of vanadium nitrogenase reveals an unusual bridging ligand.** *Nat Chem Biol* 2017, **13**:956-960.  
A surprising twist was uncovered in the composition of the cofactor of vanadium nitrogenase by protein structural studies.
47. Sippl D, Rohde M, Netzer J, Trncik C, Gies J, Grunau K, Djurdjevic I, Decamps L, Andrade SLA, Einsle O: **A bound reaction intermediate sheds light on the mechanism of nitrogenase.** *Science* 2018, **359**:1484-1489.  
Structural studies were used to uncover a likely intermediate in nitrogenase catalysis.
48. Wittenborn EC, Merrouch M, Ueda C, Fradale L, Léger C, Fourmond V, Pandelia M-E, Dementin S, Drennan CL: **Redox-dependent rearrangements of the NiFeS cluster of carbon monoxide dehydrogenase.** *eLife* 2018, **7**:e39451.
49. Umena Y, Kawakami K, Shen J-R, Kamiya N: **Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å.** *Nature* 2011, **473**:55-60.
50. Suga M, Akita F, Hirata K, Ueno G, Murakami H, Nakajima Y, Shimizu T, Yamashita K, Yamamoto M, Ago H *et al.*: **Native structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses.** *Nature* 2015, **517**:99-103.
51. Young ID, Ibrahim M, Chatterjee R, Gul S, Fuller F, Koroidov S, Brewster AS, Tran R, Alonso-Mori R, Kroll T *et al.*: **Structure of photosystem II and substrate binding at room temperature.** *Nature* 2016, **540**:453-457.
52. Tanaka A, Fukushima Y, Kamiya N: **Two different structures of the oxygen-evolving complex in the same polypeptide frameworks of photosystem II.** *J Am Chem Soc* 2017, **139**:1718-1721.
53. Suga M, Akita F, Sugahara M, Kubo M, Nakajima Y, Nakane T, Yamashita K, Umena Y, Nakabayashi M, Yamane T *et al.*: **Light-induced structural changes and the site of O=O bond formation in PSII caught by XFEL.** *Nature* 2017, **543**:131-135.
54. Kern J, Chatterjee R, Young ID, Fuller FD, Lassalle L, Ibrahim M, Gul S, Fransson T, Brewster AS, Alonso-Mori R *et al.*: **Structures of the intermediates of Kok's photosynthetic water oxidation clock.** *Nature* 2018, **563**:421-425.  
Structural changes during oxidation of the multi-manganese cluster of the oxygen evolving complex for photosystem II are revealed.