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The  $d^9$ -Cu(II)-corrin cupribyrinate (**Cuby**) was synthesized in 93% crystalline yield by rapid chelation of Cu<sup>2+</sup>-ions by the metal-free corrin-ligand of vitamin B<sub>12</sub>. Single crystals of the EPR-active **Cuby** allowed for the first X-ray structure determination of a Cu-corrin. SCF-calculations provided insights complementary to the experimental data of **Cuby** and indicated an out-of-plane displacement of the reduced  $d^{10}$ -Cu(I)-ion, consistent with the observed reductive activation of **Cuby** towards loss of its Cu-center.

The ring-contracted natural corrin ligand of the B<sub>12</sub>-derivatives is a uniquely skewed, helical environment<sup>1,2</sup> that binds cobalt-ions very tightly.<sup>3,4</sup> This biosynthetically costly ligand for cobalt<sup>5,6</sup> represents a precisely evolved entatic state module,<sup>2</sup> giving B<sub>12</sub>-cofactors the unique capacity for their exceptional bio-organometallic catalysis.<sup>7-9</sup> The complementary fundamental question, why cobalt? in B<sub>12</sub>-cofactors,<sup>1,3,9,10</sup> has generated the long-standing experimental quest for non-cobalt analogues of the B<sub>12</sub>-derivatives,<sup>11,12</sup> a challenge met by newly developed synthetic approaches.<sup>2,13</sup> We have, thus, prepared Rh(III),<sup>13-17</sup> Ni(II),<sup>18</sup> and Zn(II)-complexes<sup>19</sup> of natural corrin ligands for studies of their structures and reactivity. Here, we report on cupribyrinate (**Cuby**) (Scheme 1), the Cu(II)-complex of hydrogenobyrinic acid (**Hby**),<sup>2</sup> including the first Cu-corrin X-ray crystal structure.

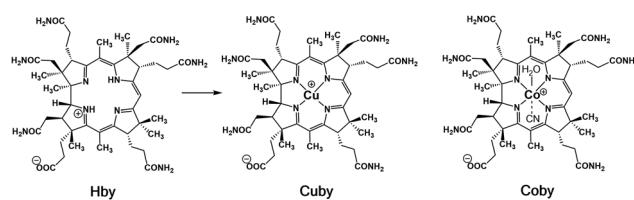
The complexation of metal-free **Hby** with Cu(II)-ions occurred readily at room temperature (RT) in a 0.25 M aqueous solution of Cu(II)-acetate at pH 6 and was practically quantitative within

90 min (see the ESI<sup>‡</sup>). It did not require the reported strong heating ('brief boiling').<sup>11,20</sup> Crystallization of the raw **Cuby**-isolate from water/acetonitrile mixtures furnished **Cuby** in >93% yield.

The UV/Vis-spectrum (Fig. 1) of an aqueous solution of **Cuby** exhibits a corrin-type and is comparable to the earlier reported spectra of partially characterized Cu(II)-corrins.<sup>20,21</sup> UV/Vis- and CD-spectra of **Cuby** show remarkably similar features to the corresponding spectra of the Zn(II)-complex<sup>19</sup> of **Hby**, consistent with the dominating role of the corrin chromophore for the spectral signature in the UV- and Vis-range. A HR-ESI mass spectrum of **Cuby** confirmed the calculated molecular formula of C<sub>45</sub>H<sub>64</sub>CuN<sub>10</sub>O<sub>8</sub> (see the ESI<sup>‡</sup> Fig. S1).

Glassy frozen solutions of the paramagnetic Cu(II)-corrin **Cuby** in 20% glycerol in H<sub>2</sub>O showed the typical EPR-signature (see Fig. 2, for a spectrum at T = 148 K) of a roughly square-planar 4-coordinate Cu(II)-N<sub>4</sub>-complex with an index<sup>22,23</sup> g<sup>II</sup>/A<sup>II</sup> = 98.2 cm, assigning an exceptionally low value to the encasement of the Cu(II)-ion by the corrin ligand (see the ESI<sup>‡</sup> for further details).

The neutral cupribyrinate **Cuby** crystallized from an aqueous solution upon addition of acetonitrile. The monoclinic crystals (space group P2<sub>1</sub>) contain two **Cuby** molecules per unit cell, as well as molecules of water and acetonitrile (ordered near the **Cuby**-carboxylate). The Cu(II)-center of the **Cuby** molecule sits only +0.033 Å above the mean plane of the four 'inner' corrin N-atoms, which span an unsymmetrical and nearly planar coordination pattern (see Fig. 3), as reflected by the value of



**Scheme 1** Structure-based outline of the synthesis of cupribyrinate (**Cuby**) from hydrogenobyrinic acid (**Hby**) (see the ESI<sup>‡</sup>) and the structural formula of Co<sub>2</sub>cyano, Co<sub>3</sub>aquo-cobyric acid (**Coby**).

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† Dedicated to the memory of Albert Eschenmoser on the occasion of his 100th birthday.

‡ Electronic supplementary information (ESI) available. CCDC 2402239. For ESI and crystallographic data in CIF or other electronic format see DOI: <https://doi.org/10.1039/d5cc02129d>



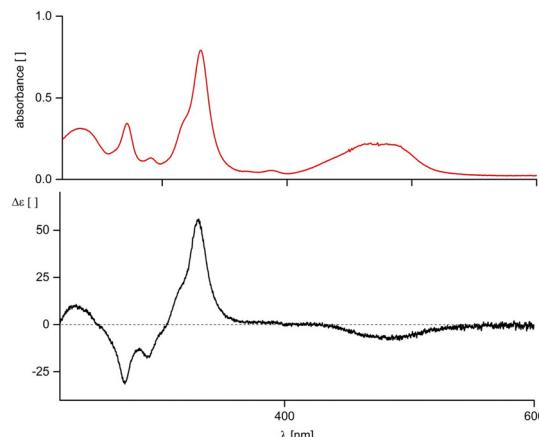


Fig. 1 UV/Vis- and CD-spectra of **Cuby** (19  $\mu$ M in 10 mM aqueous phosphate pH 7).

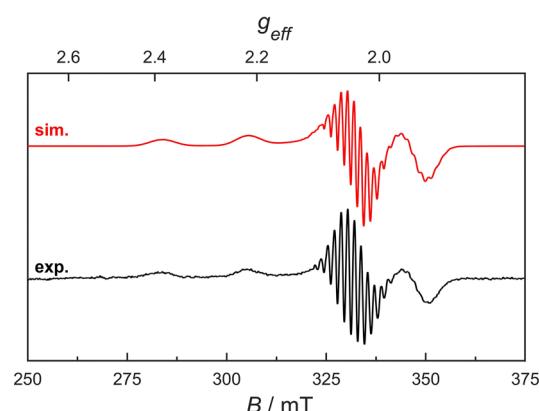
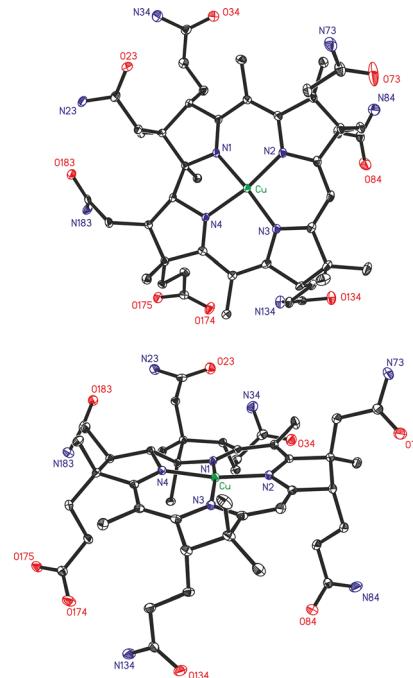


Fig. 2 EPR-spectrum of a 1.34 mM frozen solution (at  $T = 148$  K) of **Cuby** in  $\text{H}_2\text{O}$ :glycerol (4 : 1) and its simulation with key parameters obtained by least square fitting (for details see the ESI,‡ Table S1). The spectra exhibited a significant T-dependence, with a maximum signal intensity of around 200 K and continuous decrease at lower temperatures (see the ESI,‡ Fig. S4).

the geometry index  $\tau_4 = 0.17$ .<sup>24</sup> However, **Cuby** exhibits a less planar arrangement around its 4-coordinate  $d^9$  Cu(II)-center, than in the Ni(II)-corrin nibyrate (**Niby**),<sup>18</sup> which experiences a better fit of its 4-coordinate low-spin  $d^8$  Ni(II)-ion (see the ESI,‡ Table S3). The average Cu–N distance in **Cuby** amounts to 1.91 Å, merely 0.05 Å longer than in **Niby**, in which the 0.08 Å smaller low spin  $d^8$ -ion Ni(II)<sup>25</sup> induced a slight contraction.<sup>18</sup> In fact, binding of the  $d^9$  Cu(II)-ion largely retains the architecture of the coordination hole of the metal-free corrin ligand **Hby**, expanded by two ‘inner’ protons.<sup>2</sup> In **Cuby**, the critical angle parameters corrin-fold<sup>26</sup> (10.0°) and corrin helicity<sup>2</sup> (12.4°) are also similar to those of the ligand **Hby**,<sup>2</sup> but remarkably larger than in **Niby**. Likewise, the angle between the planes N1–Cu–N2 and N3–Cu–N4 (roughly 13.6°) relating to the inner coordination-sphere around the Cu(II)-center (see the ESI,‡ Table S3) is close to the value derived for **Hby**.<sup>2</sup> Interestingly, the N1–M–N3 pseudo-diagonal in **Cuby** was roughly 0.07 Å shorter than the N2–M–N4 counterpart, thus



insights into the bonding interactions of a  $d^9$ -Cu(II)-corrin were fully consistent with separately located calculated frontier molecular orbitals, either as  $\pi$ -type corrin ligand MOs or as the singly occupied  $d_{x^2-y^2}$ -type orbital on the Cu(II)-center (see Fig. 4 and ESI; Fig. S13). We also tested computed models of cuprobyric acid (Cu(I)by) to shed light on the difficult<sup>32</sup> one-electron reduction to a  $d^{10}$ -Cu(I)-corrin (for details, see the ESI; Fig. S14). The calculations suggest a large upper axial out of plane movement of the Cu(I)-ion, comparable to the position of the rather weakly bound Zn(II)-center in zincobyrinic acid (**Znby**).<sup>19</sup> Indeed, the iso-electronic nature of the closed shell  $d^{10}$ -ions Cu(I) and Zn(II) suggested the likelihood of the complete removal of a Cu(I)-ion from reduced **Cuby** in a weakly acidic aqueous medium. In an exploratory experiment, **Cuby** was treated with Zn-powder in an aqueous NH<sub>4</sub>Cl solution, leading to the effective replacement of the Cu-center of **Cuby** by Zn(II), furnishing **Znby**,<sup>19</sup> and its tentatively (by mass- and UV/Vis-spectroscopy) characterized dihydro-form **H<sub>2</sub>-Znby**, an unprecedented ring-reduced yellow corrinoid<sup>33,34</sup> (see ESI; Scheme S1). We ascribe the observed formation of **Znby** from **Cuby** to a transient generation of an exchange-labile  $d^{10}$ -Cu(I)-center by the Zn-reduction, thus strategically circumventing Eschenmoser's postulate that a B-type transition metal could not be removed without destruction of the corrin-ligand.<sup>35</sup>

The replacement, by copper, of the biologically selected cobalt-center of a corrinoid B<sub>12</sub>-derivative<sup>1,36,37</sup> erases its fundamental organometallic redox-reactivity.<sup>7</sup> The single unpaired electron of the paramagnetic **Cuby** does not contribute any (cobalt-mimetic) radical reactivity, but is 'buried' in a  $d_{x^2-y^2}$  orbital of its  $d^9$  Cu(II)-center. Consistent with the EPR-spectral fingerprint of **Cuby** and its large <sup>14</sup>N-hfcs with the four inner corrin N-atoms, in particular, the unpaired spin is located in a  $d_{x^2-y^2}$  orbital of an effectively antibonding type with respect to the coordinating corrin N-atoms (Fig. 4). Compared to the  $d^8$  Ni(II)-ion in **Niby**, the Cu(II)-N bonds in **Cuby** are, indeed, longer. Copper complexes of the superficially similar corroles represent a remarkably more complex situation:<sup>38,39</sup> there 'non-innocence' of the corrole ligand is caused by its extended  $\pi$ -system, assisting an intramolecular electron-shift and stabilizing the copper center in a higher oxidation state.<sup>40</sup>

The chelation of the fluorescent metal-free corrin **Hby**<sup>2,41</sup> by Cu(II)-ions in aqueous solution occurs cleanly at ambient temperature at pH 5. The Cu(II)-ions chelate **Hby** with a rate  $k^{Cu(II)} = 0.54 \pm 0.04 \text{ L mol}^{-1} \text{ min}^{-1}$ , remarkably quicker by about  $2 \times 10^2$  times than the binding of the biologically crucial Co(II)-ions ( $k^{Co(II)}$  of about  $3 \times 10^{-3} \text{ L mol}^{-1} \text{ min}^{-1}$ ), and five times faster than Zn(II)-ions ( $k^{Zn(II)} = 0.111 \pm 0.002 \text{ L mol}^{-1} \text{ min}^{-1}$ , see the ESI). The chelation rates of these metal ions follow the trend established with Eschenmoser's model corrin<sup>35</sup> and with a water-soluble tetra-mesopyridyl-porphyrin.<sup>42</sup>

Obviously, the biological roles of Co<sup>7,8,43</sup> and Cu<sup>44-47</sup> do not match. However, the 4-coordinate Cu(II)-complexes of natural corrin ligands may serve as structural mimics of reduced B<sub>12</sub>-derivatives. In concert with the divergent reactivity profiles of copper- and cobalt-corrins, biologically interesting applications are likely. **Cuby** is structured similar to the corrin-core of

enzyme-activated 4-coordinate Co(II)-cobamides, first characterized in an ATP:Co(I)-corrinoid adenosyltransferase that generates **AdoCbl** from 4-coordinate Co(II)-Cbl.<sup>48</sup> With their largely inert 4-coordinate  $d^9$ - and  $d^8$ -metal-centers, respectively, Cu(II)- and Ni(II)-corrins<sup>18</sup> may effectively mimic the structures of the highly activated 4-coordinate Co(II)- and Co(I)-corrins. Indeed, nibalamin (**Nibl**), the diamagnetic Ni(II)-analogue of 'base-off' Co(II)-Cbl, was shown to be an effective inhibitor of the corrinoid adenosyltransferase BtuR from *Brucella melitensis*.<sup>18</sup> The crystal structure of **Cuby** qualifies Cu(II)-containing B<sub>12</sub>-derivatives, such as cupribalamin (**Cubl**), for similar inhibitory effects.

Transition metal analogues of vitamin B<sub>12</sub> and other cobalamins (Cbls), also classified as metbalamins (Metbls),<sup>49,50</sup> lack the precise cobalt-dependent reactivity of Cbls<sup>8,43</sup> and, when mimicking Cbl-structures, may represent genuine antivitamins B<sub>12</sub>.<sup>50,51</sup> This is the case for rhodibalamins (Rhbls), the Rh(III)-homologues of Cbls. Surprisingly, their Rh(III)-center has even been revealed to experience a slightly better fit to the corrin ligand than the naturally selected Co(III)-ions.<sup>13-15</sup> Whereas uptake and physiological activity of Metbls with stable 4-coordinate corrin-bound metal centers are still unknown in humans and animals, microorganisms are typically more structure-promiscuous for B<sub>12</sub>-import, satisfying their supply with cobamides by *de novo* biosynthesis<sup>5</sup> or by partial assembly from salvaged natural corrinoids.<sup>52,53</sup> As deduced for some Rhbls<sup>13,15</sup> and for **Nibl**,<sup>18</sup> transition metal-based structural mimics of B<sub>12</sub>-cofactors or of corrinoid B<sub>12</sub>-biosynthesis intermediates<sup>17</sup> may selectively inhibit bacterial growth. As mimics of enzyme-bound Cbl-structures in B<sub>12</sub>-dependent enzymes at intermediate stages of catalysis, Metbls may specifically act as very effective enzyme inhibitors. The Cu(II)-analogues of natural B<sub>12</sub>-derivatives are, hence, EPR-active candidates for their applications as B<sub>12</sub>-antimetabolites for B<sub>12</sub>-dependent microorganisms, an expansion of the toolbox of Cu-coordinating natural products<sup>47</sup> as antimicrobial agents.

Synthetic, analytical and spectroscopic work: C. K. and M. W.; crystallography: C. K. and K. W.; theoretical and computational study: L. P. P., D. F. D., and K. R. L.; EPR-spectroscopy – data acquisition, supervision and data curation: D. L., M.-K. Z., M. B., and S. H.; and research conceptualization and conduction and original draft: B. K.; all authors have reviewed and contributed to the final draft.

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## Conflicts of interest

There are no conflicts to declare.

## Data availability

See the ESI. Crystallographic data for cupribyrate (**Cuby**) have been deposited at the Cambridge Crystallographic Data Center



(CCDC) and are available under accession number CCDC-2402239.

## Notes and references

- 1 A. Eschenmoser, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 5–39.
- 2 C. Kieninger, E. Deery, A. D. Lawrence, M. Podewitz, K. Wurst, E. Nemoto-Smith, F. J. Widner, J. A. Baker, S. Jockusch, C. R. Kreutz, K. R. Liedl, K. Gruber, M. J. Warren and B. Kräutler, *Angew. Chem., Int. Ed.*, 2019, **58**, 10756–10760.
- 3 J. M. Pratt, *Inorganic Chemistry of Vitamin B<sub>12</sub>*, Academic Press, New York, 1972.
- 4 N. J. Lewis, R. Nussberger, B. Kräutler and A. Eschenmoser, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 736–737.
- 5 D. A. Bryant, C. N. Hunter and M. J. Warren, *J. Biol. Chem.*, 2020, **295**, 6888–6925.
- 6 E. Raux, H. L. Schubert and M. J. Warren, *Cell. Mol. Life Sci.*, 2000, **57**, 1880–1893.
- 7 B. Kräutler, in *Adv. Bioorganomet. Chem.*, ed. T. Hirao and T. Moriuchi, Elsevier, Cambridge, USA, 2019, pp. 399–429.
- 8 K. L. Brown, *Chem. Rev.*, 2005, **105**, 2075–2149.
- 9 H. M. Marques, *J. Coord. Chem.*, 2024, **77**, 1161–1210.
- 10 E.-I. Ochiai, *J. Chem. Educ.*, 1978, **55**, 631.
- 11 V. B. Koppenhagen, in *B<sub>12</sub>*, ed. D. Dolphin, John Wiley & Sons, 1982, vol. 2, pp. 105–150.
- 12 G. Holze and H. H. Inhoffen, *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 867–869.
- 13 F. J. Widner, A. D. Lawrence, E. Deery, D. Heldt, S. Frank, K. Gruber, K. Wurst, M. J. Warren and B. Kräutler, *Angew. Chem., Int. Ed.*, 2016, **55**, 11281–11286.
- 14 F. J. Widner, C. Kieninger, K. Wurst, E. Deery, M. J. Warren and B. Kräutler, *Synthesis*, 2021, 332–337.
- 15 M. Wiedemair, C. Kieninger, K. Wurst, M. Podewitz, E. Deery, M. D. Paxhia, M. J. Warren and B. Kräutler, *Helv. Chim. Acta*, 2023, **106**, e202200158.
- 16 M. Ruetz, R. Mascarenhas, F. Widner, C. Kieninger, M. Koutmos, B. Kräutler and R. Banerjee, *Biochemistry*, 2024, **63**, 1955–1962.
- 17 F. J. Widner, C. Kieninger and B. Kraeutler, *J. Porph. Phthal.*, 2025, **29**, 408–417.
- 18 C. Kieninger, K. Wurst, M. Podewitz, M. Stanley, E. Deery, A. D. Lawrence, K. R. Liedl, M. J. Warren and B. Kräutler, *Angew. Chem., Int. Ed.*, 2020, **59**, 20129–20136.
- 19 C. Kieninger, J. A. Baker, M. Podewitz, K. Wurst, S. Jockusch, A. D. Lawrence, E. Deery, K. Gruber, K. R. Liedl, M. J. Warren and B. Kräutler, *Angew. Chem., Int. Ed.*, 2019, **58**, 14568–14572.
- 20 V. B. Koppenhagen and J. J. Pfiffner, *J. Biol. Chem.*, 1970, **245**, 5865–5867.
- 21 V. B. Koppenhagen and J. J. Pfiffner, *J. Biol. Chem.*, 1971, **246**, 3075–3077.
- 22 M. Ray, R. Mukherjee, J. F. Richardson, M. S. Mashuta and R. M. Buchanan, *J. Chem. Soc., Dalton Trans.*, 1994, 965–969.
- 23 U. Sakaguchi and A. W. Addison, *J. Chem. Soc., Dalton Trans.*, 1979, 600–608.
- 24 L. Yang, D. R. Powell and R. P. Houser, *Dalton Trans.*, 2007, 955–964.
- 25 B. Cordero, V. Gomez, A. E. Platero-Prats, M. Reves, J. Echeverria, E. Cremades, F. Barragan and S. Alvarez, *Dalton Trans.*, 2008, 2832–2838.
- 26 V. B. Pett, M. N. Liebman, P. Murray-Rust, K. Prasad and J. P. Glusker, *J. Am. Chem. Soc.*, 1987, **109**, 3207–3215.
- 27 B. Kräutler, W. Keller and C. Kratky, *J. Am. Chem. Soc.*, 1989, **111**, 8936–8938.
- 28 B. Kräutler, W. Keller, M. Hughes, C. Caderas and C. Kratky, *J. Chem. Soc., Chem. Commun.*, 1987, 1678–1680.
- 29 L. Ouyang, P. Rulis, W. Y. Ching, G. Nardin and L. Randaccio, *Inorg. Chem.*, 2004, **43**, 1235–1241.
- 30 C. Kratky and B. Kräutler, in *Chemistry and Biochemistry of B<sub>12</sub>*, ed. R. Banerjee, John Wiley & Sons, New York, Chichester, 1999, pp. 9–41.
- 31 B. Kräutler, R. Konrat, E. Stupperich, G. Färber, K. Gruber and C. Kratky, *Inorg. Chem.*, 1994, **33**, 4128–4139.
- 32 K. A. Robinson, J. Caja, R. W. Hurst, E. Itabashi, T. M. Kenyhercz, W. R. Heineman and H. B. Mark, *J. Chem. Soc., Chem. Commun.*, 1980, 47–48.
- 33 G. Schlingmann and V. B. Koppenhagen, Zürich Switzerland, 1979.
- 34 G. Schlingmann, B. Dresow, L. Ernst and V. B. Koppenhagen, *Liebigs Ann. Chem.*, 1981, 2061–2066.
- 35 H. U. Blaser, E. L. Winnacker, A. Fischli, B. Hardegger, D. Bormann, N. Hashimoto, J. Schossig, R. Keese and A. Eschenmoser, *Helv. Chim. Acta*, 2015, **98**, 1845–1920.
- 36 G. A. Holliday, J. A. Thornton, A. Marquet, A. G. Smith, F. Fabrice Rebeille, R. Mendel, H. L. Schubert, A. D. Lawrence and M. J. Warren, *Nat. Prod. Rep.*, 2007, **24**, 972–1087.
- 37 A. Eschenmoser, *Angew. Chem., Int. Ed.*, 2011, **50**, 12412–12472.
- 38 A. Ghosh, *Chem. Rev.*, 2017, **117**, 3798–3881.
- 39 M. Bröring, F. Brégier, E. C. Tejero, C. Hell and M. C. Holthausen, *Angew. Chem., Int. Ed.*, 2007, **46**, 445–448.
- 40 C. M. Lemon, M. Huynh, A. G. Maher, B. L. Anderson, E. D. Bloch, D. C. Powers and D. G. Nocera, *Angew. Chem., Int. Ed.*, 2016, **55**, 2176–2180.
- 41 S. Jockusch and B. Kräutler, *Chem. Commun.*, 2025, **61**, 3904–3907.
- 42 P. Hambright, in *The Porphyrin Handbook*, Academic Press, 2000, vol. 3, pp. 129–210.
- 43 B. Kräutler, in *Compreh. Organomet. Chem. IV*, ed. G. Parkin, K. Meyer and D. O'Hare, Elsevier, Oxford, 2022, pp. 73–95.
- 44 E. I. Solomon, R. K. Szilagyi, S. D. George and L. Basumallick, *Chem. Rev.*, 2004, **104**, 419–458.
- 45 E. Kim, E. E. Chufan, K. Kamaraj and K. D. Karlin, *Chem. Rev.*, 2004, **104**, 1077–1133.
- 46 G. E. Kenney and A. C. Rosenzweig, *Ann. Rev. Biochem.*, 2018, **87**, 645–676.
- 47 O. M. Manley and A. C. Rosenzweig, *J. Biol. Inorg. Chem.*, 2025, **30**, 111–124.
- 48 M. S. S. Maurice, P. Mera, K. Park, T. C. Brunold, J. C. Escalante-Semerena and I. Rayment, *Biochemistry*, 2008, **47**, 5755–5766.
- 49 F. Zelder, M. Sonnay and L. Prieto, *ChemBioChem*, 2015, **16**, 1264–1278.
- 50 B. Kräutler, *Chem. – Eur. J.*, 2020, **26**, 15438–15445.
- 51 B. Kräutler, *Chem. Eur. J.*, 2015, **21**, 11280–11287.
- 52 S. Gude, G. J. Pherribo and M. E. Taga, *Msystems*, 2022, **7**, 00288.
- 53 J. C. Escalante-Semerena, *J. Bacteriol.*, 2007, **189**, 4555–4560.

