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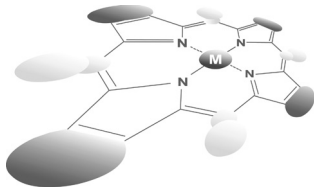

A siloxyl bis-pocket thiolate-tailed Fe(III) porphyrin complex

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ABSTRACT: We report the synthesis and characterization of a bis-pocket iron(III) porphyrin complex that has a covalently attached siloxy thiolate group as the axial ligand. As a new cytochrome P450 model compound, this siloxyl bis-pocket thiolate-tailed iron(III) porphyrin is easily synthesized and is surprisingly stable under air due to the extreme steric protection of the siloxy pockets on both faces of the porphyrin. A single-crystal XRD structure has been determined; the Fe–S bond distance is 2.237 (7) Å with Fe–N bond distances of 2.100 (8) and the Fe is 0.5 Å out of the mean porphyrin plane. The Fe–S bond distance in the siloxy thiolate-tailed iron(III) porphyrin is very similar to that in cytochrome P450 and this structure represents a very rare crystallographically-characterized five-coordinate high spin alkylthiolate-tailed ferric porphyrin. EPR spectrum of this compound showed g values and an E/D ratio very similar to those of cytochrome P450 (CYP450) enzyme, demonstrating the importance of using a very basic thiolate group as the axial ligand in P450 model studies. UV-vis studies of its reduced form with carbon monoxide shows a hyper spectrum, which is characteristic of carbonyl complexes of Fe(II)porphyrin thiolates.

KEYWORDS: cytochrome P450, bis-pocket porphyrin, iron porphyrinate, iron(III)-thiolate, EPR, XRD.

INTRODUCTION

Cytochrome P450 (CYP450) catalyzes the oxidation of organic substrates using O_2 as the oxidant at ambient conditions [1–3]. As an essential enzyme in numerous organisms, CYP450 has many important functions from decomposition of xenobiotics to synthesis of important biomolecules in the cell [4–6]. The active site of CYP450 is a prototype IX heme coordinated by the cysteine thiolate group at the axial position [7]. This thiolate ligation is unusual in heme enzymes because most heme enzymes have a histidine imidazole as the axial ligand. The mechanism of CYP450 catalyzed reactions has been studied extensively since the 1970s, and many intermediates in its catalytic cycle have been spectroscopically and even crystallographically characterized [4, 6, 8–11]. There remain, however, many questions in CYP450's catalytic cycle yet to be answered [7, 12, 13].

To help elucidate the mechanism of CYP450 catalyzed reactions, a few synthetic thiolate-coordinated iron porphyrin complexes have been designed and synthesized as synthetic analogs, and studies of these model compounds have provided many insights into mechanisms of CYP450 catalyzed reactions [14–18]. Synthetic analogs of CYP450 with thiolate axial coordination are generally more difficult to synthesize and characterize than other heme enzyme models in which imidazole or its derivatives is the axial ligand. This is because the electron rich thiolate ligands are typically much less stable and generally decompose quickly when exposed to water and oxygen. As a result, relatively few CYP450 models have been reported in the last 20 years [13, 18, 19], in stark contrast to the well-developed model compounds of myoglobin, hemoglobin, and cytochrome C oxidase [20–23].

Here we report a high-yield, straightforward synthesis of a new P450 model compound that is stable under ambient conditions due to steric protection on both faces of the metalloporphyrin by bulky siloxy subsituents (Scheme 1). Most importantly, this new model compound showed a rhombic EPR spectrum that closely resembles that of the CYP450 enzyme.

The synthesis of the siloxy thiolate-tailed iron(III) porphyrin, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P, was based on the "bis-pocket" siloxy porphyrins developed in our

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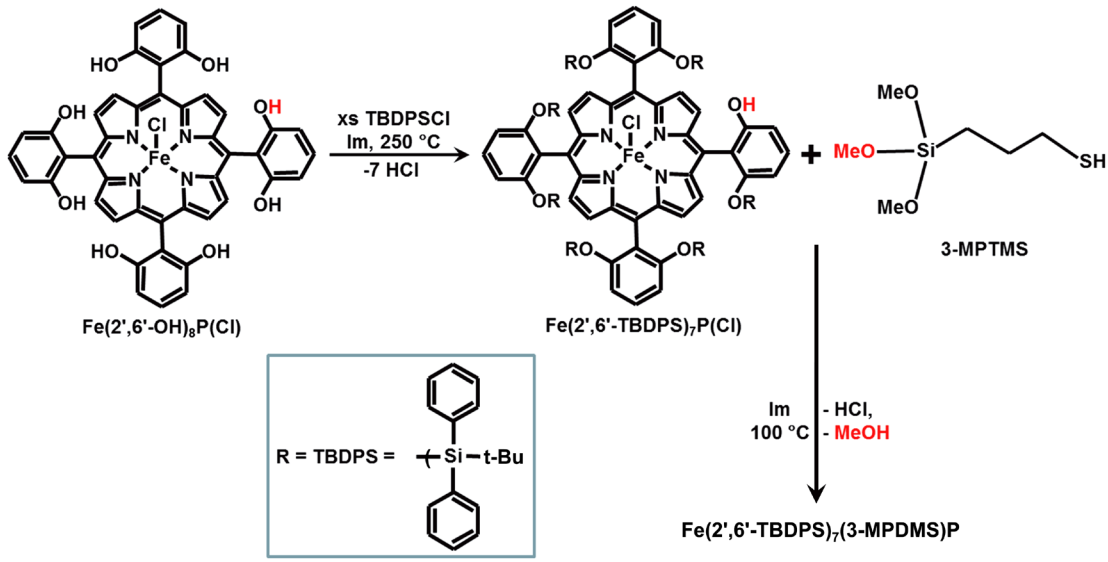

Scheme 1. Synthesis of a thiolate-tailed iron(III) porphyrin, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P. 3-MPDMS = mercaptapropyl-dimethoxysiloxyl tail

group [24, 25]. Silylation reaction of an octahydroxy iron tetra-arylporphyrin, Fe(2′,6′-OH)$_8$P(Cl), with excess *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of imidazole as a base at 250 °C afforded the hepta-substituted siloxyporphyrin, Fe(2′,6′-TBDPS)$_7$P(Cl), in 31% yield (Scheme 1). To install the thiolate tail, a bifunctional silane, mercaptapropyltrimethoxylsilane (3-MPTMS), was chosen to derivatise Fe(2′,6′-TBDPS)$_7$P(Cl). 3-MPTMS was chosen because we reasoned that one of the methoxy groups on one end of 3-MPTMS could react with the one remaining hydroxyl group on Fe(2′,6′-TBDPS)$_7$P(Cl) to covalently attach the tail to the porphyrin moiety. At the same time, the thiol group at the other end of 3-MPTMS could be deprotonated by imidazole to afford the needed thiolate axial ligand (Scheme 1). To our delight, the reaction of 3-MPTMS with Fe(2′,6′-TBDPS)$_7$P(Cl) in the presence of imidazole at 100 °C afforded the thiolate tailed porphyrin, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P, in 48% yield.

EXPERIMENTAL

Pyrrole was purchased from Aldrich and distilled over CaH_2 before use. All other reagents were purchased from Aldrich and used without further purification. ^{1}H spectra were recorded on either a Varian Unity 400 MHz or 500 MHz spectrometer at 293 K. UV-visible spectra were recorded on a Hitachi U-3300 spectrophotometer or an EPP2000-HR high resolution fiber optic spectrophotometer. The University of Illinois Microanalytical Laboratory carried out the elemental analysis. Matrix-assisted laser desorption time-of-flight mass spectra (MALDI-TOFMS) data were collected on an Applied Biosystems Voyager-DE STR Biospectrometry Workstation.

EPR spectra were recorded at X-band (~9.05 GHz) on a Varian E-122 spectrometer. The data were acquired from frozen glasses at ~15 K using an Air Products Helitran cryostat with liquid helium. The magnetic fields were calibrated with a Varian NMR Gauss meter, and the microwave frequency was measured with an EIP frequency meter.

Synthesis of Fe(2′,6′-TBDPS)$_7$P(Cl), a hepta-substituted bis-pocket porphyrin complex

5,10,15,20-Tetrakis(2′,6′-dihydroxyphenyl)porphyrinatoiron(III) chloride (0.5 g, 0.6 mmol), previously described [24, 25], *tert*-butyldiphenylsilyl chloride (5.0 g, 18 mmol), and imidazole (5.0 g, 74 mmol) were placed in a 50 mL round-bottom flask equipped with a magnetic stir bar. The reaction mixture was heated at 250 °C for 1.5 h under Ar. After removal of the high boiling point starting material and byproducts by vacuum distillation, the resulting solid was dissolved in dichloromethane, washed with a 3 M HCl aqueous solution and then water. The combined organic phase was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure. The resulting crude product mixture was purified by silica gel column chromatography (dichloromethane:hexane = 6.5:10) to give the final product as a dark brown solid (360 mg, yield 31%). MALDI-TOF calcd. for $C_{172}H_{172}N_4ClO_8Si_8Fe$ = 2500.9. Found 2499.3.

Synthesis of Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P, the Fe(III) bis-pocket porphyrin complex with a thiolate tail

5,10,15-Trikis(2′,6′-bis(*tert*-butyldiphenylsiloxy)-phenyl)-20-(2-hydroxy-6-(*tert*-butyldiphenylsiloxy)-phenyl) porphyrinatoiron(III) chloride, Fe(2′,6′-TBDPS)$_7$P(Cl), (1.2 g, 0.46 mmol), (3-mercaptopropyl)trimethoxysilane,

3-MPTMS, (10.0 mL, d = 1.057, 95%, 51 mmol), and imidazole (1.75 g, 26 mmol) were placed in a 50 mL round-bottom flask equipped with a magnetic stir bar. The reaction mixture was heated at 100 °C for 2 h under Ar. After removal of the high boiling point starting material and byproducts by vacuum distillation, the resulting solid was dissolved in dichloromethane, and then washed with water. The combined organic phase was evaporated under reduced pressure. The resulting crude product was purified by normal phase thin layer chromatography (CH_2Cl_2:hexane = 1:1) and then several times of recrystallization with dry CH_3CN/CH_2Cl_2 to give the final product as a dark brown solid (450 mg, yield 48%). Elemental analysis, calcd. for $FeC_{161}H_{165}N_4O_{10}Si_7S$: C = 73.6, H = 6.33, N = 2.13%. Found C = 73.8, H = 6.26, N = 2.29%. MALDI-TOF calcd. for $FeC_{161}H_{165}N_4O_{10}Si_8S$ = 2629. Found 2630.

RESULTS AND DISCUSSION

Methanol vapor was diffused into a dichloromethane solution of Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P at room temperature to give X-ray quality crystals with a plate-like morphology. As shown in Fig. 1, single crystal X-ray crystallography showed that the sulfur atom was bonded to the iron with an Fe–S bond distance of 2.237 (7) Å with Fe–N bond distances of 2.100 (8) (cf. Supplemental information CIF file and Table S1). This Fe–S bond distance is similar to the 2.20 Å value reported for CYP450cam when at its substrate-bound ferric high spin state [26]. The iron was pulled out of the porphyrin plane by 0.50 Å, indicating the iron is in the high spin state. The structure reported here is one of very few thiolate-coordinated iron(III) porphyrin structure (non-protein) reported in the literature and is especially unusual in that it contains an alkylthiolate rather than an aromatic thiolate (which are more stable) [18]. This complex also shows the unusual feature of steric protection on both faces of the metalloporphyrin, a "bis-pocket porphyrin" [25, 27–33], and demonstrates the utility of bulky siloxy substituents to stabilize reactive iron porphyrin species [25].

Because of the rather bulky size of the siloxy group, the porphyrin can pack either face up or face down, which causes disorder in the structure, in addition to solvate disorder. While the quality of the single crystal diffraction data is somewhat limited (Supporting information Table S1 and CIF file), the structure is still useful. Many highly solvated solids (*e.g.* zeolites, MOFs, and porphyrins) inherently give limited quality overall refinements

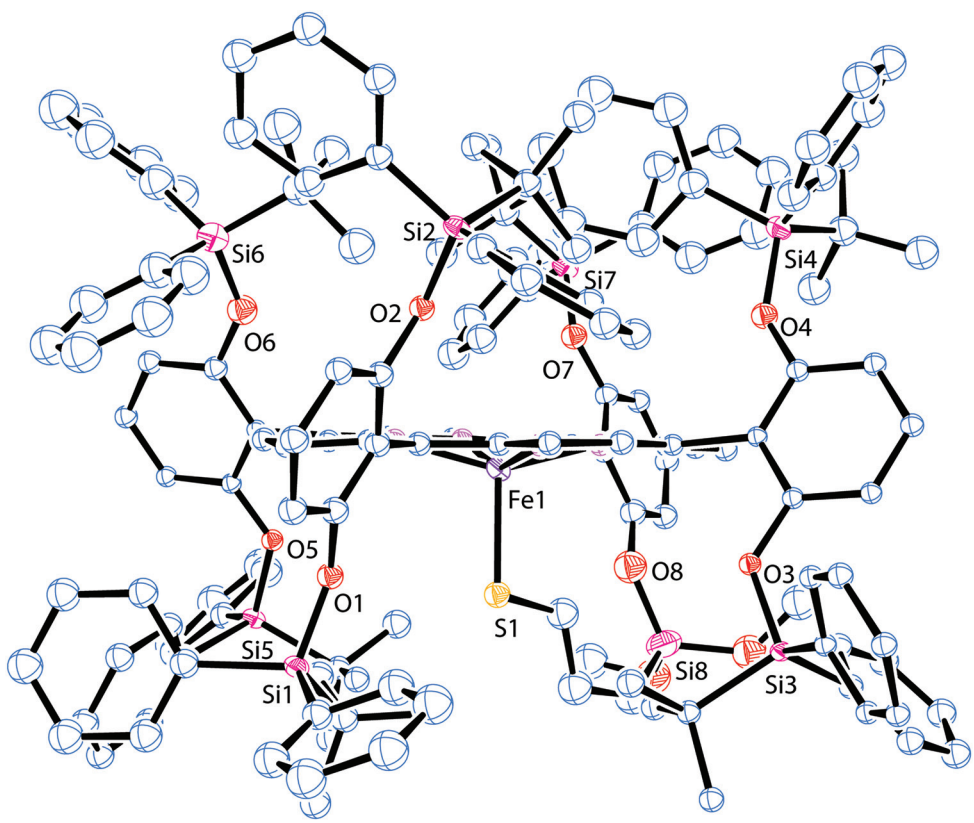

Fig. 1. Crystal structure of the high-spin five-coordinate thiolate Fe(III)porphyrin complex, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P; thermal ellipsoids drawn at the 10% probability level. Hydrogen atoms and disordered CH_2Cl_2 have been omitted for clarity

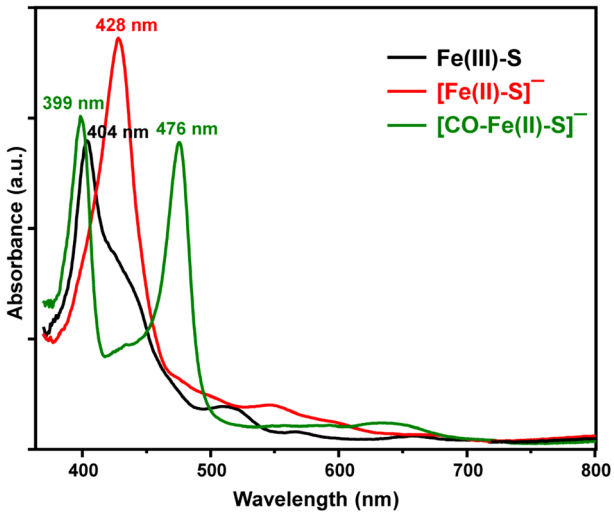

Fig. 2. UV-visible spectrum of (1) the five-coordinate thiolate-tailed Fe(III) complex, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P, in black, (2) its reduced form [Fe^{II}(2′,6′-TBDPS)$_7$(3-MPDMS)P]$^-$ in red, and (3) its CO adduct [(CO)Fe^{II}(2′,6′-TBDPS)$_7$(3-MPDMS)P]$^-$ in green. All spectra collected in THF at -78 °C

due to the disorder of the solvates; nonetheless, useful framework information can be obtained even from a structure with high R values [34], especially when a large fraction of the components are relative rigid groups (*i.e.* in this case, phenyl rings and porphyrins).

The Fe(II) carbon monoxide complex in cytochrome P450 has a characteristic visible spectrum with split Soret bands at 380 and 450 nm due to charge transfer from the lone pair electrons of sulfur to the porphyrin π* orbitals [35]. An accurate synthetic analog of CYP450 should be able to reproduce this hyper porphyrin spectrum. Indeed, split Soret absorption bands were observed at 399 and 470 nm when the Fe(III) complex with thiolate tail, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P was reduced to Fe(II) by CoCp*$_2$ under a CO atmosphere in THF at -78 °C (Fig. 2). The split Soret bands are red-shifted ~20 nm compared to those of CYP450cam, possibly due to the higher polarity of the solvent (THF) that was used. It has been noted [36] that the visible spectra of heme carbonyl complexes are very sensitive to the nature of the solvent. In polar solvents such as THF, the split Soret bands are blue shifted compared to the ones in nonpolar solvents such as toluene. The split Soret bands also show equal intensities in polar solvents [36]. In addition, we find that the stability of the CO complex of the thiolate-tailed porphyrin is solvent dependent. It is stable at -78 °C in THF for an hour, but for only seconds in dichloromethane. When toluene, an even less polar solvent, was used, the CO complex could not be observed at -78 °C. This indicates that polar solvents are essential to stabilize the charge separation in reduced carbonyl complexes with thiolate axial coordination.

The EPR spectrum of CYP450 in its resting state shows significant rhombic character [37]. Although previous P450 synthetic analogs show a certain degree of rhombic character in their EPR spectra, most of them have E/D ratios that are much smaller than the 0.087 value reported for CYP450cam [37]. This is likely related to the fact that the cysteine thiolate in CYP450 is more basic than the thiolate groups used in previous model compounds, which are typically aromatic thiolates [15–17, 19]. Two rhombic conformers were observed in the EPR spectrum of Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P recorded at 15K, Fig. 3. Simulation studies of this EPR spectrum showed that the first rhombic species (36%) has E/D = 0.088 and gives rises to the peaks at g = 7.65, 3.91, and 1.76. In comparison, EPR values of P450cam(substrate bound) were reported to be at 7.81, 3.93 and 1.77 with an E/D ratio of 0.087 [38]. Hence, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)Fe appears to be unusual among CYP450 model complexes in that is has such similar high spin g values and E/D ratios to those of the CYP450 enzyme. This similarity emphasizes the importance of using a more basic thiolate ligand in model studies. The second

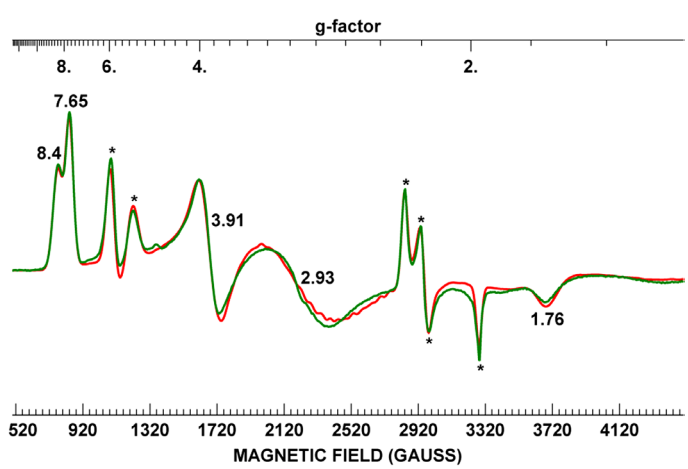

Fig. 3. EPR spectrum of the Fe(III) thiolate-tailed bis-pocket porphyrin complex, Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P in frozen CH_2Cl_2 at 15K (green) and the simulated spectrum (red). *starred peaks originate from less than 6% of high spin and low spin impurity peaks

rhombic species (58%) has E/D = 0.142 and gives rise to the peaks at $g = 8.41$ and 2.93, which may originate from further distortion of the porphyrin plane.

When an excess amount of methanol was added to the dichloromethane solution of Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P, the intensity of the rhombic high spin signal in the EPR decreased, accompanied by an increase of the low spin signal intensity. This is consistent with a high spin to low spin conversion caused by methanol coordination to the iron complex, analogs to the effect of water coordination in CYTP450. It is interesting to note that addition of *tert*-butyl alcohol did not induce the high spin to low spin transition, presumably because the sterically hindered siloxy pocket prevents the larger *tert*-butyl alcohol from binding to iron. Addition of water to the dichloromethane solution also did not change the high spin EPR spectrum of Fe(2′,6′-TBDPS)$_7$(3-MPDMS)P, likely due to the strong hydrophobicity of the siloxy porphyrin pockets, which would deter water from entering the pocket and thus destablized aquo ligation.

CONCLUSIONS

In summary, a new synthetic route for a bis-pocket alkylthiolate-tailed CYP450 model compound was developed. The high stability of this compound is likely due to the protection from the siloxy pockets on both faces of the metalloporphyrin. The rhombic, high spin EPR spectra of this model compound and the P450 enzyme are very similar, suggesting that the use of more basic alkylthiolate ligation is essential to reproduce the electronic structures of cytochrome P450. Upon reduction under CO, the typical hyper spectrum characteristic of CYP450 with a split Soret band is observed.

Acknowledgments

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Supporting information

Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Center (CCDC) under number CCDC 1816459. Copies can be obtained on request, free of charge, *via* www.ccdc.cam.ac.uk/data_request/cif or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223-336-033 or email: data_request@ccdc.cam.ac.uk).

REFERENCES

1. de Montellano PRO. *Chemical Reviews* 2010; **110**: 932–948.
2. Poulos TL. *Chemical Reviews* 2014; **114**: 3919–3962.
3. Hrycay EG and Bandiera SM. In *Monooxygenase, Peroxidase and Peroxygenase Properties and Mechanisms of Cytochrome P450*, Vol. 851, Hrycay EG, Bandiera SM. (Eds.) Springer–Verlag Berlin: Berlin, 2015; pp. 1–61.
4. Denisov IG, Makris TM, Sligar SG and Schlichting I. *Chem. Rev.* 2005; **105**: 2253–2278.
5. Guengerich FP. *Chemical Research in Toxicology* 2001; **14**: 611–650.
6. Meunier B, de Visser SP and Shaik S. *Chemical Reviews* 2004; **104**: 3947–3980.
7. Yosca TH, Ledray AP, Ngo J and Green MT. *J. Biol. Inorg. Chem.* 2017; **22**: 209–220.
8. Rittle J and Green MT. *Science* 2010; **330**: 933–937.
9. Schlichting I, Berendzen J, Chu K, Stock AM, Maves SA, Benson DE, Sweet RM, Ringe D, Petsko GA and Sligar SG. *Science* 2000; **287**: 1615–1622.
10. Zaragoza JPT, Yosca TH, Siegler MA, Moenne-Loccoz P, Green MT and Goldberg DP. *J. Am. Chem. Soc.* 2017; **139**: 13640–13643.
11. Yosca TH and Green MT. *Isr. J. Chem.* 2016; **56**: 834–840.
12. Lai WZ and Shaik S. *J. Am. Chem. Soc.* 2011; **133**: 5444–5452.
13. Chatterjee S, Sengupta K, Mondal B, Dey S and Dey A. *Accounts Chem. Res.* 2017; **50**: 1744–1753.
14. Collman JP and Groh SE. *J. Am. Chem. Soc.* 1982; **104**: 1391–1403.
15. Suzuki N, Higuchi T, Urano Y, Kikuchi K, Uekusa H, Ohashi Y, Uchida T, Kitagawa T and Nagano T. *J. Am. Chem. Soc.* 1999; **121**: 11571–11572.
16. Tani F, Matsu-ura M, Nakayama S, Ichimura M, Nakamura N and Naruta Y. *J. Am. Chem. Soc.* 2001; **123**: 1133–1142.
17. Matsu-ura M, Tani F and Naruta Y. *J. Am. Chem. Soc.* 2002; **124**: 1941–1950.
18. Suzuki H, Inabe K, Shirakawa Y, Umezawa N, Kato N and Higuchi T. *Inorg. Chem.* 2017; **56**: 4245–4248.
19. Tani F, Matsu-ura M, Nakayama S and Naruta Y. *Coord. Chem. Rev.* 2002; **226**: 219–226.
20. Rehder D. *Bioinorganic Chemistry*; Oxford University Press: Oxford, 2014.
21. Collman JP, Boulatov R, Sunderland CJ and Fu L. *Chem. Rev.* 2004; **104**: 561–588.
22. Collman JP and Fu L. *Accounts Chem. Res.* 1999; **32**: 455–463.
23. Suslick KS and Reinert TJ. *Journal of Chemical Education* 1985; **62**: 974–983.
24. Sen A and Suslick KS. *J. Am. Chem. Soc.* 2000; **122**: 11565–11566.
25. Fang M, Wilson SR and Suslick KS. *J. Am. Chem. Soc.* 2008; **130**: 1134–1135.
26. Poulos TL, Finzel BC and Howard AJ. *J. Molec. Biol.* 1987; **195**: 687–700.
27. Patel BR and Suslick KS. *J. Am. Chem. Soc.* 1998; **120**: 11802–11803.

28. Bhyrappa P, Young JK, Moore JS and Suslick KS. *J. Molec. Catal. A-Chem.* 1996; **113**: 109–116.
29. Suslick KS and Fox MM. *J. Am. Chem. Soc.* 1983; **105**: 3507–3510.
30. Sen J and Suslick KS. *Journal of the American Chemical Society* 2000; **122**: 11565–11566.
31. Bhyrappa P, Vaijayanthimala G and Suslick KS. *J. Am. Chem. Soc.* 1999; **121**: 262–263.
32. Cook BR, Reinert TJ and Suslick KS. *J. Am. Chem. Soc.* 1986; **108**: 7281–7286.
33. Suslick K, Cook B and Fox M. *Chem. Commun.* 1985: 580–582.
34. Eddaoudi M, Moler DB, Li H, Chen B, Reineke TM, O'Keeffe M and Yaghi OM. *Acc. Chem. Res.* 2001; **34**: 319–330.
35. Hanson LK, Eaton WA, Sligar SG, Gunsalus IC, Gouterman M and Connell CR. *J. Am. Chem. Soc.* 1976; **98**: 2672–2674.
36. Chang CK and Dolphin D. *Proc. Natl. Acad. Sci. USA* 1976; **73**: 3338–3342.
37. Tang SC, Koch S, Papaefthymiou GC, Foner S, Frankel RB, Ibers JA and Holm RH. *J. Am. Chem. Soc.* 1976; **98**: 2414–2434.
38. Tsai R, Yu CA, Gunsalus IC, Peisach J, Blumberg W, Orme-Johnson WH and Beinert H. *Proc. Natl. Acad. Sci. USA* 1970; **66**: 1157–1163.