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ARTICLE

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A Biomimetic Copper Corrole – Preparation, Characterization, and Reconstitution with Horse Heart Apomyoglobin

Martin Bröring,* Frédérique Brégier, Olaf Burghaus and Christian Kleeberg

*Dedicated to Bernd Harbrecht on the Occasion of his 60th Birthday***Keywords:** Biohybrids; Porphyrinoids; Copper; Corrole; Bioinorganic Chemistry

A copper corrole with biomimetic propionate side chains was prepared as a novel heme analog by metalation of the respective ligand as the dimethylester with copper(II) acetate hydrate and subsequent saponification with LiOH. The metalated dimethylester was characterized by spectroscopic and crystallographic means and shown by comparison to contain a divalent copper ion, antiferromagnetically coupled to the radical-dianionic organic ligand. The molecular structure of this complex is characterized by a saddled macrocycle conformation. In addition, the methylpropionate side chains are not stretched out but bent to the same side of the mean copper corrole plane. In buffered solution,

the saponified propionate complex slowly forms an equilibrium mixture with small, insoluble aggregates. The compound cleanly binds in 1:1 stoichiometry to horse heart apomyoglobin without a support by proximal histidin binding. The binding in the protein pocket is rather weak, and the biohybrid compound tends to decompose under the slightly acidic conditions of mass spectrometry. Reduction of the copper corrole was achieved with sodium dithionite inside the protein pocket and yielded an air- and buffer stable, monoanionic, and protein bound copper corrolate complex which could be identified by its EPR spectrum.

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Introduction

The exchange of non-covalently bound cofactors in proteins against synthetic metal complexes is currently in the focus of many bioinorganic research activities aiming towards the preparation and investigation of biohybrid compounds and bioconjugated catalysts.[1] Figure 1 provides a general graphical sketch to illustrate this approach which makes use of the properties of the natural proteinogenic material, i.e., site isolation, as well as chirality and micropolarity of the active site. The most successful approach so far has been the exchange of heme in simple heme proteins, like myoglobin or horseradish peroxidase, for modified hemes or other porphyrins.[2] As the modified metal porphyrins are structurally related to the natural heme group, novel reactivities may be introduced to the protein without significant changes at the active, heme binding pocket. In most cases, the propionate side chains have been employed for the modification by substitution with different organic moieties, and new functionalities like protein-protein or protein-molecule recognition, electron transfer, or increased chemical reactivity have been gained. Another series of heme protein-hybrid compounds has been constructed using different metal porphyrinoids like phthalocyanines,[3] chlorins,[4] porphyrin isomers,[5] and other.[6] In particular iron chelates of the above macrocycles often have a profound impact on the affinity of

the reconstituted proteins towards small molecules, and drastically change these binding interactions, especially with O₂. Therefore, such hybrids are not only interesting for an understanding of the physiological properties of heme proteins in general, but may also be applied for their regulation.

Figure 1. General sketch illustrating the cofactor exchange strategy for the preparation of biohybrid compounds.

Very recently a first cofactor exchange study using an iron corrole was reported.[7] Metal corroles are artificial one-carbon short relatives of the ubiquitous metal porphyrins and have attracted considerable attention over the last decade.[8] The unique properties of metal corroles have now advanced into topical fields like catalysis, photophysics, surface science, and others.[9] These metal chelates are special with respect to their electronic structures[10] and – often – the non-innocent behaviour of the organic ligands which tend to form radicals, stabilized by magnetic coupling to a coordinated metal centre.[11] During the course of our studies on metal corroles[12] and on corrole-based biohybrid systems the question arose whether the binding of the metal corrole to histidine inside a heme apoprotein is important for the stability of the compound. To probe this question we thought to prepare a copper corrole **1** with biomimetic propionate substitution (figure 2), which is devoid of any axial ligand binding capability, and study the conjugation of such a copper corrole to apomyoglobin. This paper reports the results of our study.

Figure 2. Propionate substituted copper corrole **1** in the standard notation (left) and in the ground state resonance form (right) The arrows indicate the magnetic ordering of the unpaired spins.

Results and Discussion

Preparation and characterization of copper corroles. The copper ion was introduced by heating the known ligand **2**[7] in the presence of excess copper(II)acetate hydrate in DMF solution in air (scheme 1). The reaction can be followed by naked eye as the reaction mixture gradually turns from violet to red. The product complex **3** is isolated after aqueous work-up and column chromatography on silica in the stable, oxidized form (51% yield). The expected composition of **3** $C_{37}H_{43}CuN_4O_4$ was confirmed by elemental analysis and high resolution APCI mass spectrometry. In the solid state, **3** forms fibers with a violet, metallic sheen.

Scheme 1. Synthesis of the copper corrole dimethylester **3**; a) $Cu(OAc)_2 \times H_2O$, DMF, O_2 , Δ 3hrs.

3 is an open-shell compound but rendered effectively diamagnetic by the strong antiferromagnetic coupling between the spins in the metal $3d(x^2-y^2)$ and the ligand b_1 -type orbital.[13] Therefore, **3** may simply be analyzed by standard 1H and ^{13}C NMR techniques. As for other copper corroles the 1H NMR spectrum of **3** is characterized by relatively broad resonance lines (figure 3 top). This line broadening is caused by a small contribution of a paramagnetic, thermally excited state of the compound. The protons of the meso CH bridges are isochronic and give a broad signal at 6.15 ppm. The signal groups of the alkyl protons are found at 3.64 ($-OCH_3$) and in the range of 2.99-2.38 ppm ($-CH_2CH_2$, $-CH_2CH_3$, $-CH_3$) and of 1.25-1.20 ppm ($-CH_2CH_3$), and display some of the anticipated fine structure. Line broadening is also an issue in the ^{13}C NMR spectra of **3**. In particular, the signals of the eight pyrrolic $^{13}C(sp^2)$ nuclei are broadened to a very divers extent (figure 3 bottom, inset). All signals could be assigned to single carbon atoms by 2D spectroscopic methods, and apparently the carbon nuclei which cause the most broadened signals are situated at the β -position of the bipyrrrole moiety. The signals for the meso carbon atoms C5,15 and C10 are detected at 103.3 and 101.8 ppm, respectively, and thus shifted to high field with respect to those of the free ligand **2**. Such a shift has been observed earlier for other other transition metal corroles with a diamagnetic ground state.[1]

Figure 3. Top: 1H -NMR spectrum of copper corrole **3** (400 MHz, CD_2Cl_2 , r.t.); bottom: ^{13}C -NMR spectrum of **3** (100.6 MHz, CD_2Cl_2 , r.t.) with insets for details of the $C(sp^2)$ signals.

The optical spectrum of copper corrole **3** was measured in dichloromethane. The spectral habitus is indistinguishable

from those reported for other copper octaalkylcorroles in the literature.[11c,14] Basically, this spectrum consists of two major, broadened absorptions, a high-energy, so-called Soret band at 398 nm ($\epsilon = 94500 L mol^{-1} cm^{-1}$), and a Q-band at 546 nm ($\epsilon = 10600 L mol^{-1} cm^{-1}$).

Single crystals suitable for X-ray diffraction were grown from complex **3** by the diffusion method (dichloromethane, *n*-hexane, 4°C). **3** crystallizes as shiny, violet needles in the triclinic system, space group *P*-1, with two molecules per unit cell (figure 4). Two conformers are present and statistically disordered in a ratio of 73:27. Only the major form will be discussed below.

Figure 4. View of the unit cell with two molecules of **3** (only major conformer). Ellipsoids are set to 50% probability. Hydrogen atoms have been removed for the sake of clarity. Selected bond lengths /Å and angles /°: Cu-N1 1.882(2), Cu-N2 1.897(2), Cu-N3 1.895(2), Cu-N4 1.888(3), N1-Cu-N3 166.8(1), N2-Cu-N4 168.5(1).

The copper atom of the molecular unit of **3** is seated in a distorted square-planar environment with Cu-N distances between 1.882 and 1.897 Å and a displacement of the nitrogen atoms from a mean squares CuN_4 plane of 0.142-0.172 Å. The short Cu-N bond lengths are characteristic for copper corroles and have been found in earlier occasions.[11c,14a,15] In comparison, the Cu-N distances in copper(II) porphyrins and copper(II) porphyrin radical cations are typically in the range of 1.95-2.06 Å[16] while for comparable Cu(III)-N-donor chelates values of 1.80-1.91 Å have been reported.[17] Obviously, the Cu-N bond lengths do not necessarily reflect the oxidation state of the copper ion in a corrole ligand but are rather governed by the restricted size of the macrocycle cavity. A second structural characteristic of copper corroles is the saddle deformation of the tetrapyrrole ligand. The C_4N rings of the corrole are alternately tilted up- and downwards while the meso situated methine groups keep an almost coplanar arrangement. The non-planarity may be quantified by the incline of the mean least-squares planes of the opposite C_4N rings which is measured to 7.75 and 22.69° in this unsymmetric case. The non-planar binding mode allows for an efficient interaction of a metal-centered σ - and a ligand centered π -orbital as stated above, and results in a strong and productive intramolecular antiferromagnetic coupling.[13,18]

Interestingly, the methyl propionate side chains are not stretched out in a parallel conformation but tilted away from the plane of the macrocycle to the same side. This behaviour can be understood from the crystal packing. An investigation of the crystal structure reveals the presence of slipped π -stacked copper corrole interactions with an interplanar distance of 3.439 Å. The observed conformation of the propionate groups therefore appears as a simple steric necessity in order to allow these stacking interactions.

Both methyl ester side chains of **3** could be cleaved at ambient temperature with $LiOH \times H_2O$ in methanol. The free acid **1** is obtained after suitable workup and recrystallization from dichloromethane/*n*-hexane in 61% yield as a violet powder. The proper composition $C_{35}H_{39}CuN_4O_4$ of **1** was verified by an APCI-HRMS

1
2 experiment. As for **3**, spectroscopic evidence for **1** could be
3 obtained from resonance spectroscopy. DMSO-*d*₆ solutions
4 of **1** gave ¹H- and ¹³C-NMR spectra very similar to **3**, but
5 slightly broadened and devoid of the signals of the split-off
6 methyl groups. Optical spectra taken in DMSO and in
7 freshly prepared buffered aqueous solution (0.1 mol/L Tris-
8 HCl, pH 7.2) are almost indistinguishable from each other
9 and very similar to those of **3** in non-polar or halogenated
10 organic solvents. Therefore we assume that **1** contains a
11 four-coordinate copper(II) ion not only in non-polar solvents,
12 but also in donor solvents and in water. As a further proof,
13 imidazole was added in excess (2000 eq.) to a dilute solution
14 of **1** (10⁻⁵ mol/L) in the buffer, without any marked change
15 in the optical spectrum.

16
17
18
19 Figure 5. Time-dependent spectral changes of copper corrole diacid
20 **1** (10⁻⁵ mol/L starting concentration) in Tris-HCl buffer (pH 7.2)
21 over 15 hrs at 20°C; inset: development of the intensity of the
22 strong Soret band at 387 nm.

23
24 In buffered aqueous solution, the absorption bands of **1** in
25 the UV/Vis spectra slowly decrease in intensity. This
26 process can easily be followed photometrically and stops
27 after about 15 hrs at about 50% of the starting intensity
28 (figure 5). The decrease, however is not exponential in time
29 but follows a complex curvature. As no new bands of
30 chemically modified copper corroles (demetalation,
31 oxygenation, donor addition) appear, this observation points
32 to a complex equilibrium of dissolved molecular entities
33 with small, disperse and nonpolar aggregates, presumably
34 with micellar or π-stacked superstructures.

35 *Hybridization of 1 with apomyoglobin.*

36
37 Horse heart apomyoglobin was prepared from the
38 commercial myoglobin by application of the modified
39 TEALE method.[19] A cold solution of the holoprotein is
40 treated with hydrochloric acid (0.1 mol/L) until pH 2 and the
41 released heme group extracted with 2-butanone. An
42 apomyoglobin solution is then obtained from the acidic,
43 aqueous solution by several neutralization and dialysis steps.
44 The concentration of the apoprotein was determined
45 photometrically at 280 nm, and by the BRADFORD test.[20]
46 Reconstitution with **1** was achieved using a concentrated
47 DMSO solution of the cofactor analog to avoid the above-
48 mentioned aggregation in aqueous buffer. This solution is
49 given in small aliquots to the buffered apomyoglobin
50 solution (0.1 mol/L Tris-HCl, pH 7.2) and stirred for one
51 hour with ice cooling. The mixture is then dialyzed for 12
52 hrs against potassium phosphate buffer before purification
53 and separation from excess copper corrole **1**, remaining
54 apoprotein, and denaturation products is accomplished
55 chromatographically by FPLC on a cation exchange CM-52
56 column, equilibrated first with potassium phosphate buffer
57 at pH 6.3 to remove free copper corrole **1**, than increased to
58 pH 7.0 to eluate the biohybrid species. Free apoprotein as
59 well as denaturation products remain on the column under
60 these conditions. The new biohybrid material was
characterized by optical and mass spectrometrical means
(figure 6). The UV/Vis spectra of **1**@ApoMb and **1** in
aqueous buffer are only gradually distinct by a slightly
reduced line broadening in the former case. Photometric

measurements are therefore not useful as an analytical tool
to monitor the cofactor-analog insertion into the protein.

The mass spectrum of **1**@ApoMb provides for some
instructive peculiarities. The molecular weight of the
reconstituted horse heart myoglobin was determined by ESI
mass spectrometry to 17592.2 u (figure 6). Thus, within the
accuracy of the measurement, the loss of two protons to the
expected mass of 17594.7 u is apparent. This deviation may
be interpreted in terms of the deprotonation of the
propionate side chains of **1**. In general, the preparation and
purification results in a solution of the reconstituted protein
at pH 7 when both acidic protons should be absent. Prior to
the mass spectrometric analysis, however, a more volatile
buffer is required, and the potassium phosphate is
exchanged for an ammonium acetate buffer at pH 6.4 by
dialysis. At this pH reprotonation should occur. This is not
observed in the spectrum.

Figure 6. Comparison of optical spectra of copper corrole **1** (grey
line), and of biohybrid **1**@ApoMb (black line; both spectra taken
as fresh solutions in potassium phosphate buffer, pH 7.0), and
deconvoluted ESI-MS of **1**@ApoMb (inset; ammonium acetate
buffer, pH 6.4).

The second peculiarity addresses the high degree of
decomplexation of the biohybrid **1**@ApoMb under the
forcing and acidic conditions of the measurement, as visible
from two additional and intense signal groups in the mass
spectrum. One signal group is detected a higher masses
around 18233.7 u, presumably indicating a protein hybrid
with two fully deprotonated copper corrole molecules. The
most prominent signal in the spectrum occurs at 16951.1 u
and can be assigned to the apoprotein alone. A dynamic
exchange of cofactors and disproportionation of the original
material during concentration and evaporation appears as a
feasible explanation for this phenomenon. Such a process
occurs also with other, histidine bound myoglobin based
biohybrids, but to a very much minor extent. We therefore
interpret our finding as a qualitative indication of the
expected reduced stability of copper corrole binding within
the heme pocket.

Despite the missing attractive interaction between the
metal atom and the distal imidazole the reconstitution of
horse heart apomyoglobin with copper corrole **1** is
successful. The anchoring of the cofactor analog in the
proteinogenic matrix can be understood in the first place by
specific hydrophobic interactions in the protein pocket. In
addition, stabilizing hydrogen bonds between the
deprotonated propionate side chains of **1** and water, as well
as several well-positioned amino acid residues like His97,
Ser92 or Lys45, ought to stabilize this biohybrid compound
further.[21] For the detailed analysis of such a non-natural
hydrogen bonding network, a protein crystallographic
analysis would be necessary. Despite numerous attempts to
grow suitable protein crystals from **1**@ApoMb, however,
these efforts did not yet succeed.

Figure 7. Reduction of **1**@ApoMb by sodium dithionite: UV/Vis absorption before and after addition of the reductant, and EPR spectrum of the reduced form (inset; 0.1 M Tris-HCl (pH 7.0) with 30% glycerol; 46 dB, 9.21085 GHz, 30 K).

While an chloridoiron corrole, another corrole complex with an oxidized corrole radical ligand, [10c] has been reported to undergo immediate autoreduction to iron(III)corrole upon binding to the histidine ligand of apomyoglobin,[7] such a process was not observed for the copper corrole **1**. Reduction could be induced chemically on **1**@ApoMb by the addition of a 100fold excess of sodium dithionite in potassium phosphate buffer at pH 7.0. Within seconds the optical spectrum changes significantly, with red shifts of the Soret and Q bands to 414 and 573 nm, respectively. Even more noticeable, the intensity and line sharpness of these ligand-derived bands increase dramatically, indicating a closed shell electronic configuration of the corrole π -system in the reduced form. The reduced biohybrid compound [**1**@ApoMb]_{red} is EPR active, and an *in situ* measurement of dithionite reduction was achieved in X-band at 30 K after dilution with glycerol (figure 7). The observed spectrum is axial (presumably with a very small rhombic distortion), with $g_{\parallel} = 2.035$ und $g_{\perp} = 2.127$, and shows couplings with the $^{63/65}\text{Cu}$ ($I = 3/2$; $A_{\perp} = 22.7$ mT) and ^{14}N nuclei ($I = 1$; $a_{\perp} = 1.66$ mT, $a_{\parallel} = 1.83$ mT). Such a spectrum is typical of a Cu(II) ion in a porphyrinoid environment.[22] Hints for a second species are absent, and the presence of a uniform species with an apoprotein-to-cofactor analog ratio of 1:1 is indicated.

The habitus of the optical spectrum of the reduced biohybrid [**1**@ApoMb]_{red} as well as the EPR data point to a one-electron reduction of the artificial cofactor analog by sodium dithionite and the formation of an anionic copper(II) corrolate complex inside the protein pocket. A similar result has earlier been reported on β -alkylsubstituted copper corroles which are reduced electrochemically or with hydrazine in organic solvents.[22] Other than in solution, however, the protein bound copper corrole anion stays stable in air and in the presence of protic solvents for days.

Conclusions

Copper corrole with biomimetic propionate substitution can be introduced to horse heart apomyoglobin by simply mixing suitably prepared solutions of both components, followed by chromatographic purification on a cation exchange resin. The binding of the copper corrole inside the protein pocket is sufficiently stable for investigations on the new biohybrid compounds despite the incapability of the cofactor analog to bind coordinatively to the distal histidine residue. Mass spectrometrical observations as well as the high acid lability of the hybrid suggest that the copper corrole binds as the twofold deprotonated dianion, and that additional stability is gained by hydrogen bonding interactions. The new compound displays a high propensity to undergo a one-electron reduction step. The resulting anionic copper corrole complex remains stable in solution, indicating a protective property of the non-polar myoglobin pocket. The results show that novel functionality may be introduced to myoglobin by exchange of the heme moiety by metal complexes, and that binding to the distal histidine is not a necessary requirement for the formation of biohybrid compounds in this system. This opens the way

towards a multitude of novel opportunities for an in-protein coordination chemistry which we are currently exploring.

Experimental Section

Materials and Methods: Corrole **2** [7] was prepared according to a published procedure. All other chemicals were purchased from Acros, Aldrich, or Merck for chemical, and Fluka, Merck Biosciences, Riedel de Haen, Roth, or Sigma-Aldrich for biochemical experiments, and used without further purification. Deionized water was used except for the buffers, for which bidistilled quality water is required. Horse heart myoglobin (90%, lyophilized, PhastGel, M-1882) was obtained from Sigma-Aldrich and used for apoprotein preparation as received. Solvents were dried by standard procedures and distilled from appropriate drying agents. NMR spectra were obtained with a Bruker DRX-400 or a Bruker DRX-500 spectrometer. Chemical shifts (δ) are given in ppm relative to residual protio solvent resonances. The EPR spectrum of [**1**@ApoMb]_{red} was measured on a Bruker Spin R ESP 300 E at X-band. An Oxford 900 cryostat was used. High and low resolution mass spectra (APCI, ESI) were recorded with an IonSpec Ultima, a Finnigan LTQ FT, or a QStarPulsar i. UV/Vis spectra were measured on a Shimadzu UV-1601 PC in concentrations of about 10^{-5} mol L⁻¹. CHN analyses were recorded on a Elementar Vario EL. Gel permeation chromatography (gpc) was conducted on an ÄKTA prime FPLC instrument (Amersham Bioscience) on HiTrap® columns (5 mL).

Preparation of copper (methylpropionate)corrole **3:** A solution of corrole **2** (501.9 mg, 0.82 mmol) in dry DMF (50 mL) ist treated with copper acetate hydrate (821.1 mg, 4.11 mmol) for 3 hrs at 80°C. After cooling of the reaction mixture to ambient temperature all volatiles are removed in vacuo and the remaining residue is redissolved in dichloromethane (50 mL). The red solution is extracted with water (3 × 100 mL), dried with sodium sulfate, and concentrated under reduced pressure. The remaining material is subjected to column chromatography with dichloromethane on silica, and the intense red major fraction is collected. Removal of the solvent in vacuo and recrystallization from dichloromethane/*n*-hexane yielded the title compound as violet fibers. Yield: 279.3 mg (0.42 mmol, 51%). – calcd (%) for C₃₇H₄₃CuN₄O₄: C 66.20, H 6.46, N 8.35; found: C 66.01, H 6.65, N 8.32. – ¹H NMR (400 MHz, CD₂Cl₂): δ = 6.14 (br. s, 3H, *H*_{meso}), 3.64 (s, 6H, -COOCH₃), 2.97 (t, 4H, ³J = 7.4 Hz, CH₂CH₂COOMe), 2.88 (m, 8H, CH₂CH₃), 2.49 (t, 4H, ³J = 7.4 Hz, CH₂CH₂COOMe), 2.38 (s, 6H, CH₃), 1.24, 1.22 (2xt, 12H, ³J = 7.2 Hz, CH₂CH₃). – ¹³C NMR (100.6 MHz, CD₂Cl₂): δ = 173.3 (COOMe), 150.9, 150.7, 147.1, 143.6, 141.1, 138.6, 138.1, 137.5, 103.3 (C-5,15), 101.8 (C-10), 51.5 (OMe), 35.8, 20.2, 20.1, 18.4, 18.1, 16.6, 10.0. – UV/Vis (CH₂Cl₂): λ_{max} (ϵ) = 280 (27800), 397.5 (94500), 500 sh (7400), 545.5 nm (10600 M⁻¹cm⁻¹). – HRMS (APCI): *m/z* 693.2491; calcd. for [C₃₇H₄₃CuN₄O₄Na]⁺: 693.2473.

Collection and Reduction of X-ray Data. Intensity data for **3** was collected from a black plate at 193(2) K, using a Stoe IPDS-1 X-ray diffractometer. Graphite monochromated Mo K_α radiation (0.71073 Å) was used. The structure was solved by direct methods with SIR-92 [23]. Refinements were carried out by full-matrix least-squares techniques against *F*₂ using SHELXL-97 [24]. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to idealized positions. Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data

Centre as supplementary publication no. CCDC-767098. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44(1223) 336-033; E-mail: deposit@cam.ac.uk]. – **Crystal Data for 3:** C₃₇H₄₃CuN₄O₄, 671.29, triclinic, space group *P*-1, *a* = 5.7170(7), *b* = 16.2285(19), *c* = 18.802(2) Å, α = 109.779(13)°, β = 95.761(14)°, γ = 95.180(14)°, *V* = 1618.8(4) Å³, *Z* = 2, ρ_{calc} = 1.377 g cm⁻³, $\mu(\text{Mo-K}\alpha)$ = 0.722 mm⁻¹, θ_{min} = 2.24°, θ_{max} = 26.07°, 16016 reflections measured, 5894 independent, 4609 observed with *I* > 2 σ (*I*), 444 parameters, 9 restraints, *R1* (*I* > 2 σ (*I*)) = 0.0478, *wR2* (all data) = 0.1356, max./min. peak = 1.595/-0.582 e Å⁻³.

Preparation of heme cofactor analog 1: Under a blanket of argon copper corrole **3** (149.6 mg, 0.22 mmol) is dissolved in THF (20 mL) and treated with a degassed solution of LiOH × H₂O (95.0 mg, 2.26 mmol) in water (4 mL). This mixture is stirred for 12 hrs before it is diluted with dichloromethane (20 mL) and hydrochloric acid (20 mL of a 1 mol/L solution). The organic layer is separated, washed with water (50 mL), dried with sodium sulfate and evaporated to dryness in vacuo. Recrystallization from dichloromethane/*n*-hexane (1:1) yields the title compound as a violet powder. Yield: 86.8 mg (0.14 mmol, 61%). – **¹H NMR** (500 MHz, DMSO-*d*₆): δ = 12.10 (br. s, 2H, COOH), 6.28 (br. s, 3H, *H*_{meso}), 2.97 (br. s, 4H, CH₂CH₂COOH), 2.84 (br. s, 8H, CH₂CH₃), 2.38 (br. s, 4H, CH₂CH₂COOH), 2.36 (br. s, 6H, CH₃), 1.17 (br. s, 12H, CH₂CH₃). – **¹³C NMR** (100.6 MHz, CD₂Cl₂): δ = 173.7 (COOH), 150.1, 150.0, 146.1, 142.5, 141.0, 138.5, 136.6, 103.7 (C-5,15), 102.5 (C-10), 35.4, 19.5, 19.4, 18.2, 17.6, 16.5, 9.7. – **UV/Vis** (0.1 M Tris-HCl pH 7.2): λ_{max} (ϵ_{rel}) = 388 (1), 556 nm (0.17). – **HRMS** (APCI): *m/z* 642.2249; calcd. for [C₃₅H₃₉CuN₄O₄]⁺: 642.2262.

Insertion of copper corrole 1 in horse heart apomyoglobin: Copper corrole **1** (5 eq with respect to the protein binding sites) is dissolved in a small amount of DMSO and slowly added to a freshly prepared apomyoglobin solution at 4°C.[19] After 2 hrs stirring at this temperature the reaction mixture is subjected to dialysis against potassium phosphate buffer (10 mmol/L, pH 6.3) for 12 hrs to remove DMSO and most of the excess copper corrole **1**, and to exchange the buffer for the next purification step. The protein from this solution is absorbed on a cation exchange resin (CM-52 column, Whatman, equilibrated with potassium phosphate buffer (10 mmol/L, pH 6.3)), washed extensively with further buffer, and desorbed as a homogenous band by slowly increasing salt concentration and pH. The reconstituted protein **1**@ApoMb is finally concentrated by zentrifugation up to a concentration of 2-15 mg/mL, and this solution used for further experiments.

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[1] a) Y. Lu, S. M. Berry, T. D. Pfister, *Chem. Rev.* **2001**, *101*, 3047-3080; b) D. Qi, C.-M. Tann, D. Haring, M. D. Distefano, *Chem. Rev.* **2001**, *101*, 3081-3112; c) Y. Lu, *Curr. Opin. Chem. Biol.* **2005**, *9*, 118-126; d) C. M. Thomas, T. R. Ward, *Chem. Soc. Rev.* **2005**, *34*, 337-346; e) Y. Lu, *Angew. Chem. Int. Ed.* **2006**, *45*, 5588-5601; f) T. Ueno, S. Abe, N. Yokoi, Y. Watanabe, *Coord. Chem. Rev.* **2007**, *251*, 2717-2731; g) T. Ueno, T. Koshiyama, S. Abe, N. Yokoi, M. Ohashi, H. Nakajima, Y. Watanabe, *J. Organomet. Chem.* **2007**, *692*, 142-147.

- [2] a) T. Hayashi, A. Tomokuni, T. Mizutani, Y. Hisaeda, T. Matsuo, H. Ogoshi, *Chem. Lett.* **1998**, 1229; b) T. Hayashi, Y. Hitomi, T. Ando, T. Mizutani, Y. Hisaeda, S. Kitagawa, H. Ogoshi, *J. Am. Chem. Soc.* **1999**, *121*, 7747-7750; c) T. Hayashi, T. Matsuda, Y. Hisaeda, *Chem. Lett.* **2003**, *32*, 496-497; d) T. Hayashi, T. Nakagawa, K. Harada, T. Matsuo, Y. Hitomi, Y. Hisaeda, *Chem. Lett.* **2004**, *33*, 1512; e) H. Sato, M. Watanabe, Y. Hisaeda, T. Hayashi, *J. Am. Chem. Soc.* **2005**, *127*, 56-57; f) T. Hayashi, Y. Hitomi, T. Takimura, A. Tomokuni, T. Mizutani, Y. Hisaeda, H. Ogoshi, *Coord. Chem. Rev.* **1999**, *190-192*, 961-974; g) T. Hayashi, Y. Hisaeda, *Acc. Chem. Res.* **2002**, *35*, 35-43; h) I. Hamachi, S. Shinkai, *Eur. J. Org. Chem.* **1999**, 539-549; i) H. Sato, T. Hayashi, T. Ando, Y. Hisaeda, T. Ueno, Y. Watanabe, *J. Am. Chem. Soc.* **2004**, *126*, 436-437.
- [3] D. V. Stynes, S. Liu, H. Marcus, *Inorg. Chem.* **1985**, *24*, 4335-4338.
- [4] C. Sotiriou-Leventis, C. K. Chang, *Inorg. Chim. Acta* **2000**, *311*, 113-118.
- [5] a) S. Neya, M. Tsubaki, H. Hori, T. Yonetani, N. Funasaki, *Inorg. Chem.* **2001**, *40*, 1220-1225; b) B. Hager, M. Alva-Astudillo, H. Falk, *Monatsh. Chem.* **2003**, *134*, 1499-1507; c) S. Neya, K. Imai, Y. Hiramatsu, T. Kitagawa, T. Hoshino, M. Hata, N. Funasaki, *Inorg. Chem.* **2006**, *45*, 4238-4242; d) S. Neya, N. Funasaki, H. Imai, S. Nagatomo, T. Iwase, T. Yonetani, *Chem. Lett.* **1999**, 989; e) S. Neya, K. Imai, H. Hori, H. Ishikawa, K. Ishimori, D. Okuno, S. Nagatomo, T. Hoshino, M. Hata, N. Funasaki, *Inorg. Chem.* **2003**, *42*, 1456-1461; f) T. Matsuo, H. Dejima, S. Hirota, D. Murata, H. Sato, T. Ikegami, H. Hori, T. Hayashi, *J. Am. Chem. Soc.* **2004**, *126*, 16007-16017; g) T. Hayashi, H. Dejima, T. Matsuo, H. Sato, D. Murata, Y. Hisaeda, *J. Am. Chem. Soc.* **2002**, *124*, 11226-11227; h) T. Matsuo, T. Tsuruta, K. Maehara, H. Sato, Y. Hisaeda, T. Hayashi, *Inorg. Chem.* **2005**, *44*, 9391-9396; i) T. Matsuo, D. Murata, Y. Hisaeda, H. Hori, T. Hayashi, *J. Am. Chem. Soc.* **2007**, *129*, 12906-12907; j) T. Matsuo, K. Ito, Y. Nakashima, Y. Hisaeda, T. Hayashi, *J. Inorg. Biochem.* **2008**, *102*, 166-173; k) T. Matsuo, T. Ikegami, H. Sato, Y. Hisaeda, T. Hayashi, *J. Inorg. Biochem.* **2006**, *100*, 1265-1271; l) T. Hayashi, D. Murata, M. Makino, H. Sugimoto, T. Matsuo, H. Sato, Y. Shiro, Y. Hisaeda, *Inorg. Chem.* **2006**, *45*, 10530-10536.
- [6] S. Neya, H. Hori, K. Imai, Y. Kawamura-Konishi, H. Suzuk, Y. Shiro, T. Iizuka, N. Funasaki, *J. Biochem.* **1997**, *121*, 654-660.
- [7] T. Matsuo, A. Hayashi, M. Abe, T. Matsuda, Y. Hisaeda, T. Hayashi, *J. Am. Chem. Soc.* **2009**, *131*, 15124-15125.
- [8] a) R. Paolesse in *The Porphyrin Handbook* (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, San Diego, **2000**, vol. 2, pp. 201-233; b) C. Erben, S. Will, K. M. Kadish in *The Porphyrin Handbook* (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, San Diego, **2000**, vol. 2, pp. 233-300.
- [9] a) L. Flamigni, D. T. Gryko, *Chem. Soc. Rev.* **2009**, *38*, 1635-1646; b) I. Aviv-Harel, *Z. Gross, Chem.-Eur. J.* **2009**, *15*, 8382-8394; c) S. Kuck, G. Hoffmann, M. Bröring, M. Fichtel, M. Funk, R. Wiesendanger, *J. Am. Chem. Soc.* **2008**, *130*, 14072-14073; d) I. Aviv, *Z. Gross, Chem. Commun.* **2007**, 1987-1999.
- [10] a) I. Wasbotten, A. Ghosh, *Inorg. Chem.* **2006**, *45*, 4910-4913; b) R. K. Hocking, S. DeBeer George, Z. Gross, F. A. Walker, K. O. Hodgson, B. Hedman, E. I. Solomon, *Inorg. Chem.* **2009**, *48*, 1678-1688; c) S. Ye, T. Tuttle, E. Bill, L. Simkhovich, Z. Gross, W. Thiel, F. Neese, *Chem.-Eur. J.* **2008**, *14*, 10839-10851.
- [11] a) B. O. Roos, V. Varyazov, J. Conradie, P. R. Taylor, A. Ghosh, *J. Phys. Chem. B* **2008**, *112*, 14099-14102; b) S. Nardis, R. Paolesse, S. Licocchia, F. R. Fronczek, M. G. H. Vicente, T. K. Shokhireva, S. Cai, F. A. Walker, *Inorg. Chem.* **2005**, *44*, 7030-7046; c) M. Bröring, F. Brégier, E. Cónsul Tejero, C. Hell, M. C. Holthausen, *Angew. Chem. Int. Ed.* **2007**, *46*, 445-448.
- [12] a) M. Bröring, C. Milsman, S. Ruck, S. Köhler, *J. Organomet. Chem.* **2009**, *694*, 1011-1015; b) M. Bröring, M.

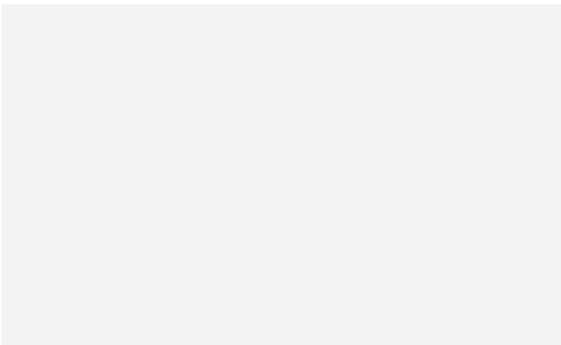
- Funk, C. Milsmann, *J. Porphyrins Phthalocyanines* **2009**, *13*, 107-113; c) M. Bröring, S. Köhler, *J. Porphyrins Phthalocyanines* **2008**, *12*, 1111-1117; d) M. Bröring, F. Brégier, R. Krüger, C. Kleeberg, *Eur. J. Inorg. Chem.* **2008**, 5505-5512; e) M. Bröring, S. Köhler, C. Kleeberg, *Angew. Chem. Int. Ed.* **2008**, *47*, 5658-5661; f) M. Bröring, M. Cordes, S. Köhler, *Z. Anorg. Allg. Chem.* **2008**, *634*, 125-130; g) M. Bröring, C. Hell, F. Brégier, O. Burghaus, E. Cónsul Tejero, *Inorg. Chem.* **2007**, *46*, 5477-5479; h) M. Bröring, C. Hell, M. Steiner, C. D. Brandt, *Z. Anorg. Allg. Chem.* **2007**, *633*, 1082-1086; i) M. Bröring, C. Hell, C. D. Brandt, *Chem. Commun.* **2007**, 1861-1862; j) D. T. Gryko, M. Tasiór, T. Peterle, M. Bröring, *J. Porphyrins Phthalocyanines*, **2006**, *10*, 1360-1370; k) M. Bröring, C. Hell, C. D. Brandt, E. Cónsul Tejero, *J. Porphyrins Phthalocyanines* **2003**, *7*, 214-219; l) J. Harmer, S. van Doorslaer, I. Gromov, M. Bröring, G. Jeschke, A. Schweiger, *J. Phys. Chem. B* **2002**, *106*, 2801-2811; m) M. Bröring, C. Hell, *Chem. Commun.* **2001**, 2336-2337.
- [13] The commonly used C_{2v} notation for metalloporphyrins is used herein; for reference see A. Ghosh, T. Wondimagegn, A. B. J. Parusel, *J. Am. Chem. Soc.* **2000**, *122*, 5100-5104. Since only planar metalloporphyrins can display b_1 -symmetric π orbitals, we use the term "b₁-type" for the corresponding orbitals of nonplanar porphyrins.
- [14] a) S. Will, J. Lex, E. Vogel, H. Schmickler, J.-P. Gisselbrecht, C. Hauptmann, M. Bernard, M. Gross, *Angew. Chem. Int. Ed.* **1997**, *36*, 357-361; b) A. W. Johnson, I. T. Kay, *J. Chem. Soc.* **1965**, 1620-1628; c) Y. Murakami, Y. Matsuda, K. Sakata, S. Yamada, Y. Tanaka, Y. Aoyama, *Bull. Chem. Soc. Jpn.* **1981**, *54*, 163-169; d) R. Grigg, A. W. Johnson, G. Shelton, *J. Chem. Soc. (C)* **1971**, 2287.
- [15] a) C. Brückner, R. P. Briñas, J. A. Krause Bauer, *Inorg. Chem.* **2003**, *42*, 4495-4497; b) I. Luobeznova, L. Simkhovich, I. Goldberg, Z. Gross, *Eur. J. Inorg. Chem.* **2004**, 1724-1732; c) R. Guillard, C. P. Gros, J. M. Barbe, E. Espinosa, F. Jerome, A. Tabard, J. M. Latour, J. Shao, Z. Ou, K. M. Kadish, *Inorg. Chem.* **2004**, *43*, 7441-7455.
- [16] a) K. Heinze, A. Reinhart, *J. Chem. Soc. Dalton Trans.* **2008**, 469-480; b) E. B. Fleischer, C. K. Miller, L. E. Webb, *J. Am. Chem. Soc.* **1964**, *86*, 2342-2347; c) L. M. Henling, W. P. Schaefer, J. A. Hodge, M. E. Hughes, H. B. Gray, J. E. Lyons, P. E. Ellis Jr, *Acta Cryst. C* **1993**, *C49*, 1743-1747; d) I. Moustakali, A. Tulinsky, *J. Am. Chem. Soc.* **1973**, *95*, 6811-6815; e) J. P. Collman, A. O. Chong, G. B. Jameson, R. T. Oakley, E. Rose, E. R. Schmittou, J. A. Ibers, *J. Am. Chem. Soc.* **1981**, *103*, 516-533; f) J. P. Fillers, K. G. Ravichandran, I. Abdalmuhdi, A. Tulinsky, C. K. Chang, *J. Am. Chem. Soc.* **1986**, *108*, 417-424; g) M. W. Renner, K. M. Barkigia, Y. Zhang, C. J. Medforth, K. M. Smith, J. Fajer, *J. Am. Chem. Soc.* **1994**, *116*, 8585-8592; h) R. Pak, W. R. Scheidt, *Acta Cryst. C* **1991**, *C47*, 431-433; i) W. P. Schaefer, J. A. Hodge, M. E. Hughes, H. B. Gray, J. E. Lyons, P. E. Ellis Jr, R. W. Wagner, *Acta Cryst. C* **1993**, *C49*, 1342-1345; j) T. Nagata, A. Osuka, K. Maruyama, K. Toriumi, *Acta Cryst. C* **1990**, *C46*, 1745-1747; k) J.-H. Fuhrhop, L. Witte, W. S. Sheldrick, *Liebigs Ann. Chem.* **1976**, 1537-1559; l) W. S. Sheldrick, *Acta Cryst. B* **1978**, *B34*, 663-665; m) L. K. Woo, M. R. Maurya, R. A. Jacobson, S. Yang, S. L. Ringrose, *Inorg. Chem.* **1992**, *31*, 913-919; n) T. Kaufmann, B. Shamsai, R. S. Lu, R. Bau, G. M. Miskelly, *Inorg. Chem.* **1995**, *34*, 5073-5079; o) M. Li, Z. Xu, X. You, X. Huang, X. Zheng, H. Wang, *Inorg. Chim. Acta* **1997**, *261*, 211-215; p) C.-H. Tsai, J.-Y. Tung, J.-H. Chen, F.-L. Liao, S.-L. Wang, S.-S. Wang, L.-P. Hwang, C.-B. Chen, *Polyhedron* **2000**, *19*, 633-639; q) B. S. Erler, W. F. Scholz, Y. J. Lee, W. R. Scheidt, C. A. Reed, *J. Am. Chem. Soc.* **1987**, *109*, 2644-2652; r) E. M. McGhee, M. R. Godfrey, B. M. Hoffman, J. A. Ibers, *Inorg. Chem.* **1991**, *30*, 803-808; s) W. R. Scheidt, K. E. Brancato-Buentello, H. Song, K. V. Reddy, B. Cheng, *Inorg. Chem.* **1996**, *35*, 7500-7507.
- [17] a) U. Knof, T. Weyhermüller, T. Wolter, K. Wieghardt, *J. Chem. Soc., Chem. Commun.* **1993**, 726-727; b) F. C. Anson, T. J. Collins, T. G. Richmond, B. D. Santarsiero, J. E. Toth, B. G. R. D. Treco, *J. Am. Chem. Soc.* **1987**, *109*, 2974-2979; c) K. J. Olivier, T. N. Waters, *J. Chem. Soc., Chem. Commun.* **1982**, 1111-1112; d) L. L. Diaddario, W. R. Robinson, D. L. Margerum, *Inorg. Chem.* **1983**, *22*, 1021-1025.
- [18] A. B. Alemayehu, E. Gonzalez, L. K. Hansen, A. Ghosh, *Inorg. Chem.* **2009**, *48*, 7794-7799.
- [19] a) F. W. J. Teale, *Biochim. Biophys. Acta* **1959**, *35*, 543; b) E. Breslow, *J. Biol. Chem.* **1964**, *239*, 486-496; c) T. Yonetani, *J. Biol. Chem.* **1967**, *242*, 5008-5013; d) P. A. Adams, *Biochem. J.* **1977**, *163*, 153-158.
- [20] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248-254.
- [21] S. V. Evans, G. D. Brayer, *J. Biol. Chem.* **1988**, *263*, 4263-4268.
- [22] K. M. Kadish, V. A. Adamian, E. Van Caemelbecke, E. Gueletii, S. Will, C. Erben, E. Vogel, *J. Am. Chem. Soc.* **1998**, *120*, 11986-11993.
- [23] A. Altomare, G. Cascarano, C. Giacovazzo, A. Gualardi, *J. Appl. Cryst.* **1993**, *26*, 343-350.
- [24] G. M. Sheldrick, SHELXL-97, Program for Structure Refinement, University of Göttingen, Göttingen, Germany, **1997**.

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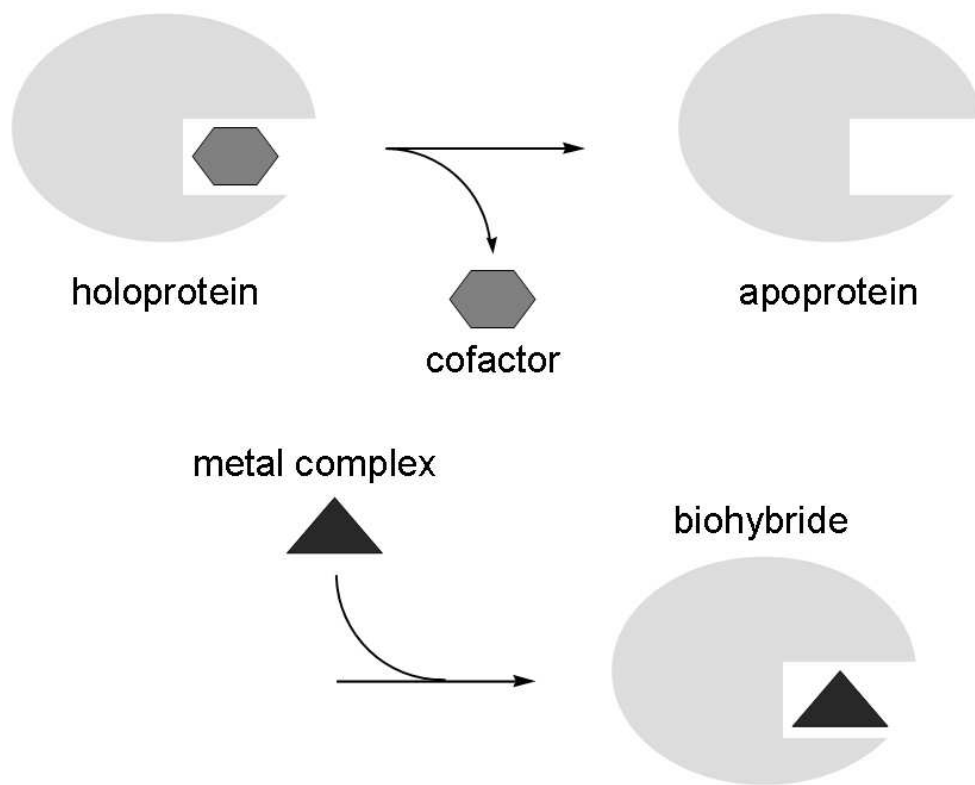
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Entry for the Table of Contents

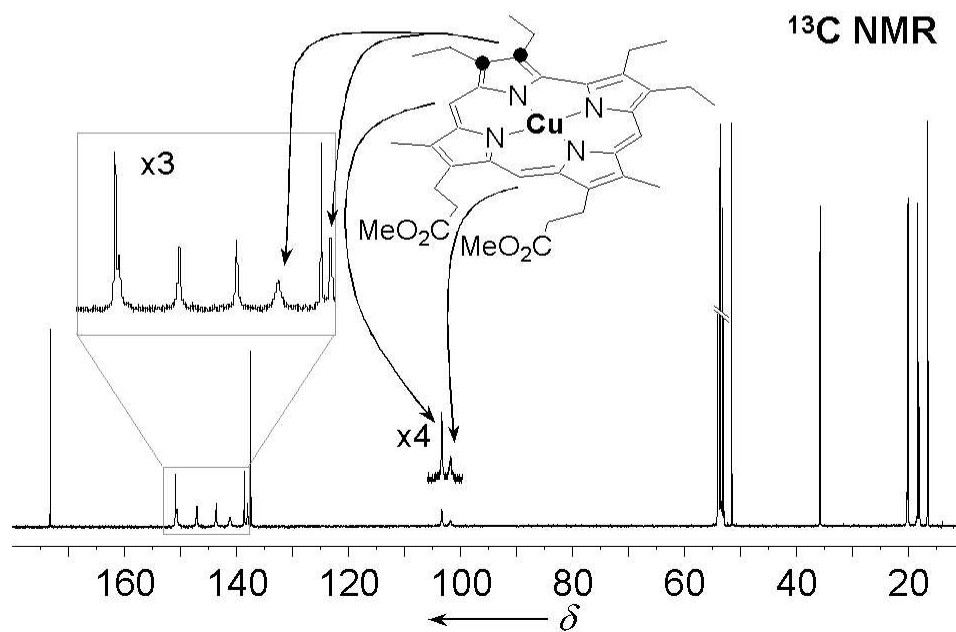
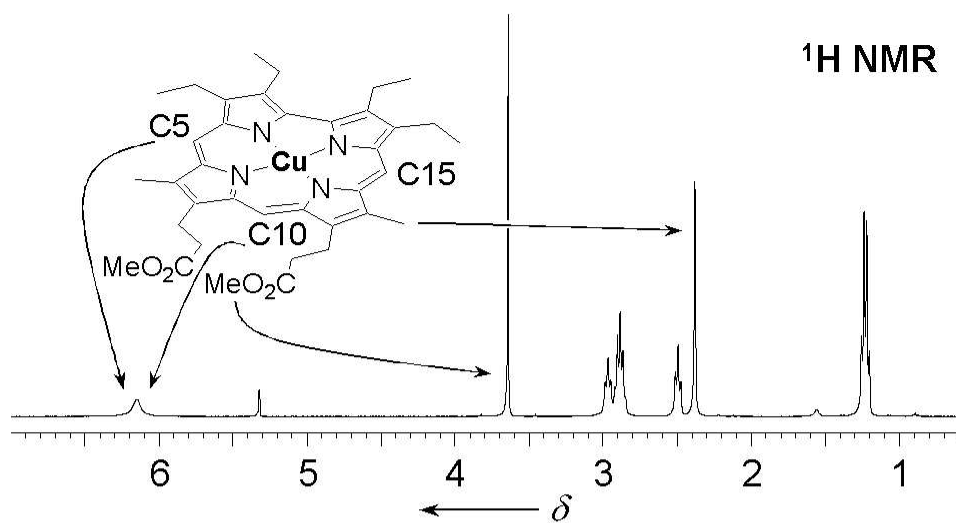


Martin Bröring,* Frédérique Brégier, Olaf Burghaus, and Christian Kleeberg **Page No. – Page No.**

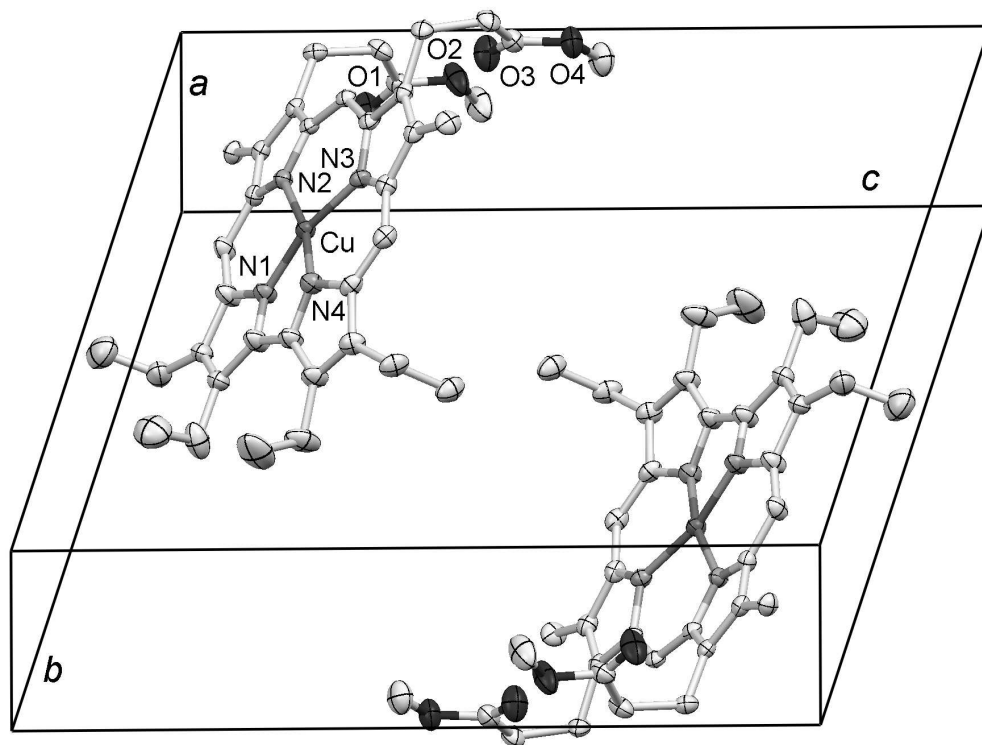
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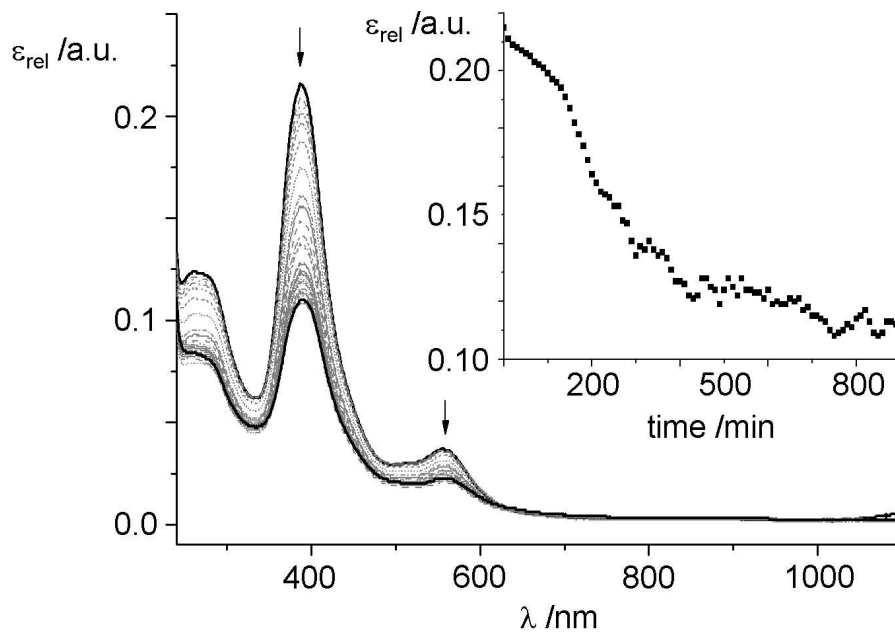


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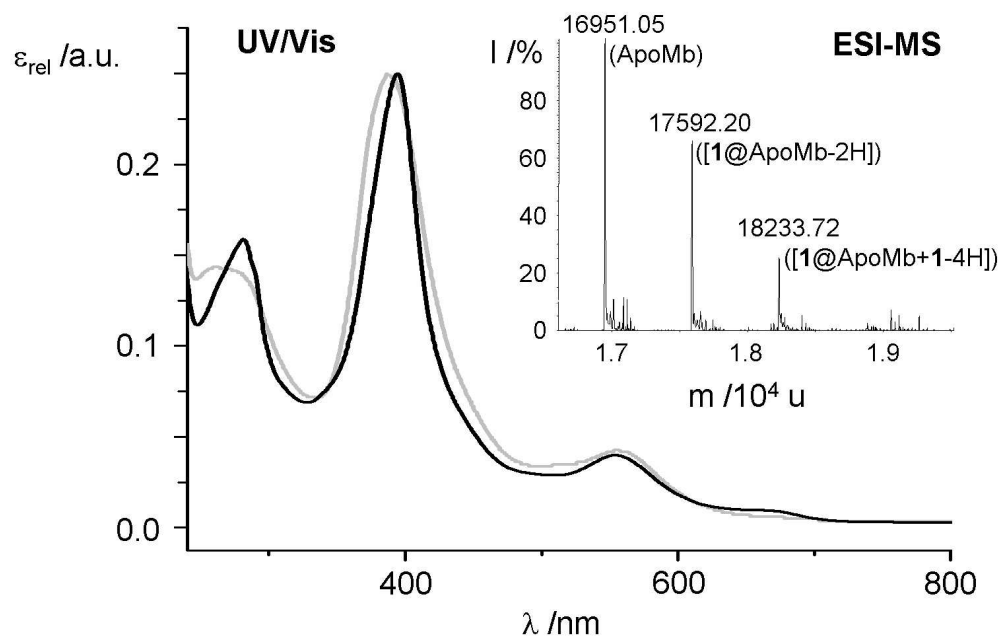


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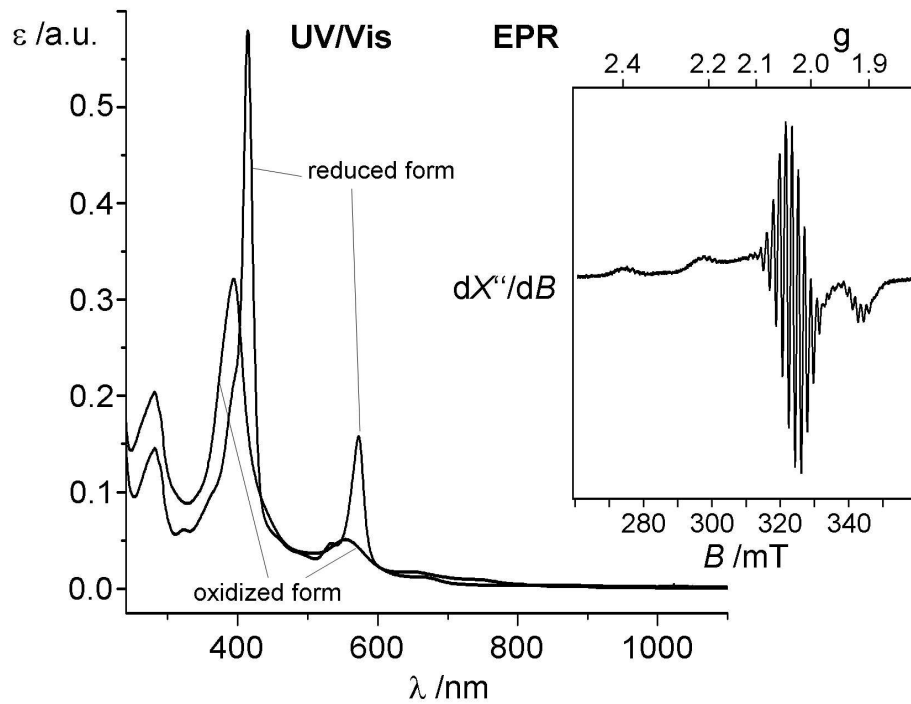
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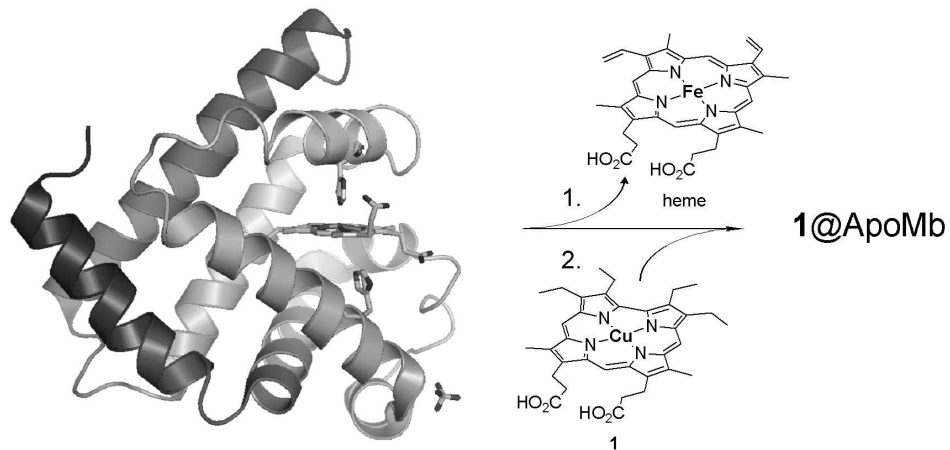
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