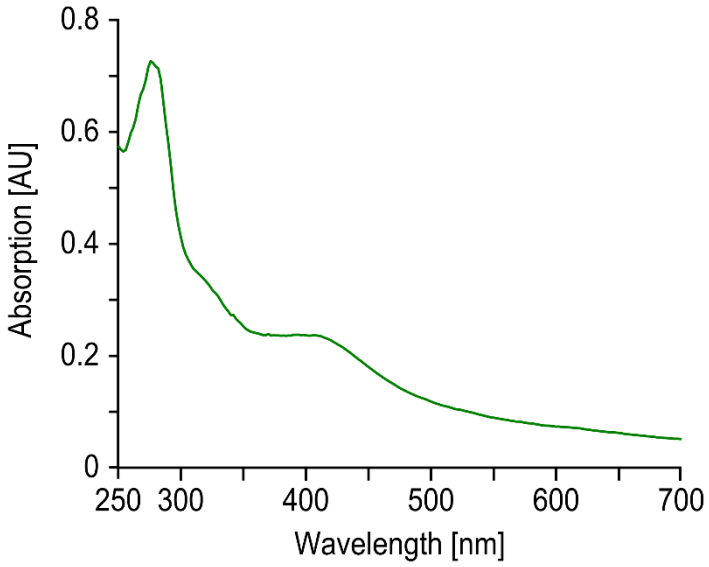
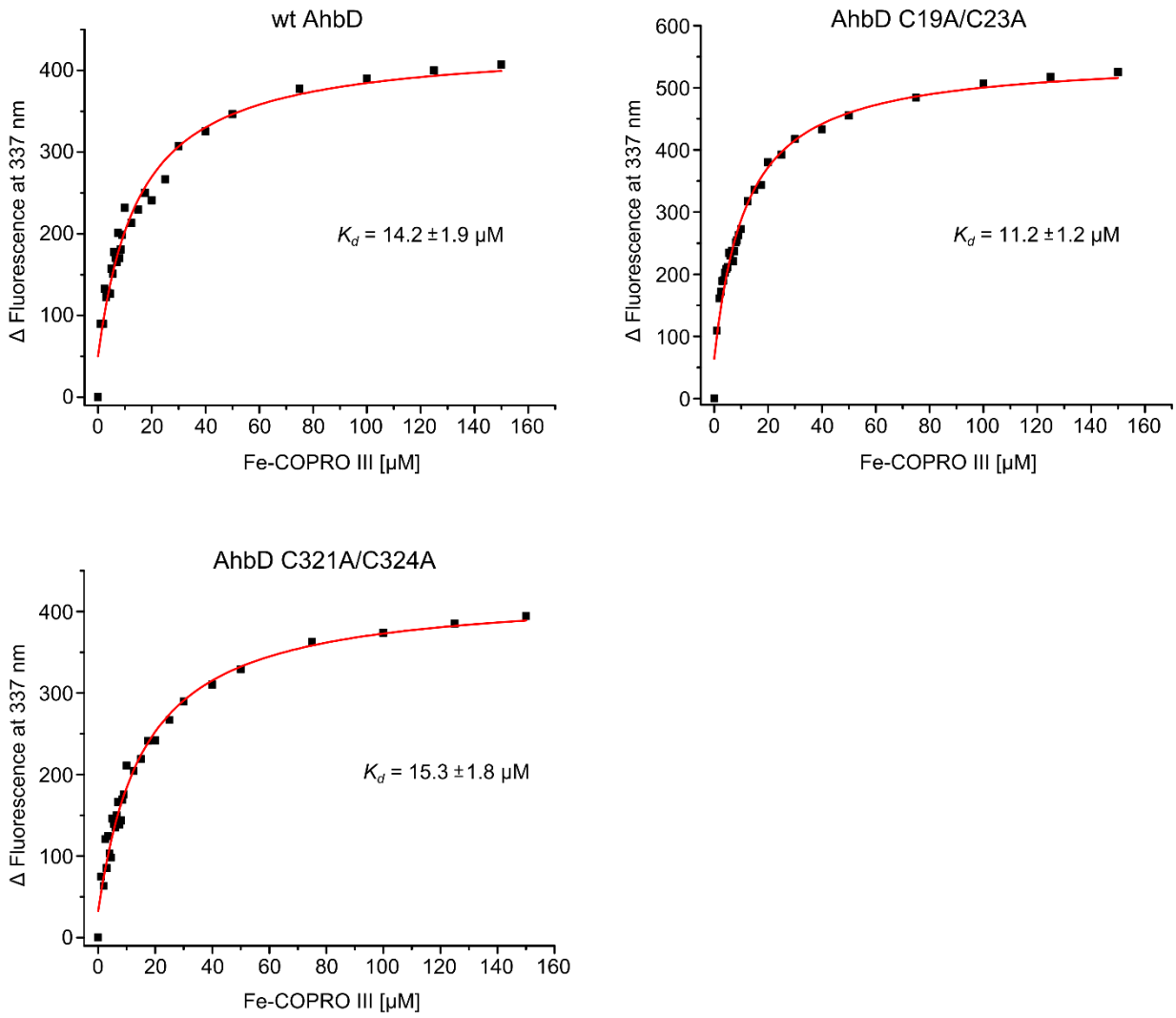
SUPPLEMENTAL DATA

Supplemental Figure A



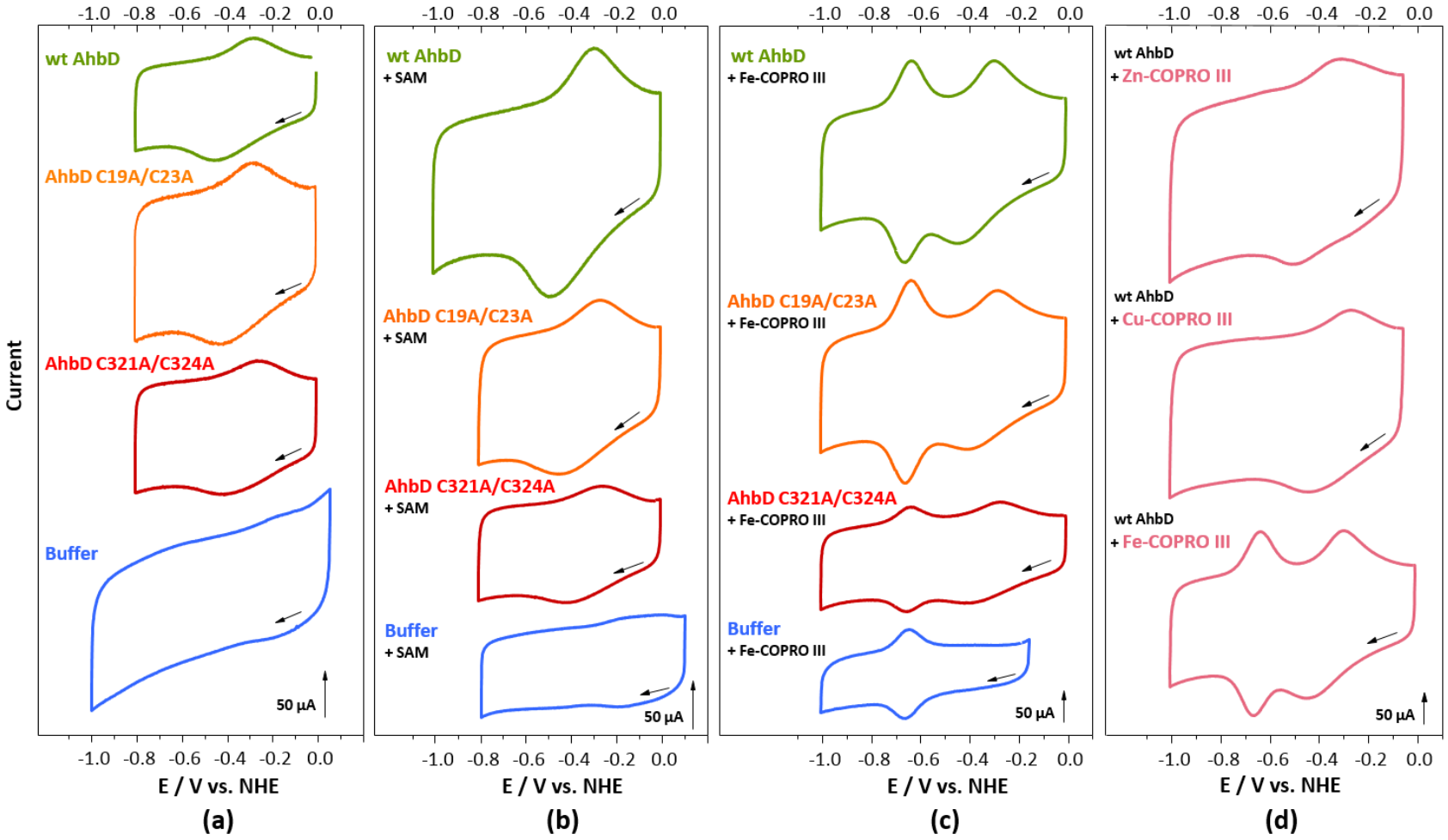
UV-Visible absorption spectrum of purified wt AhbD.

Supplemental Figure B



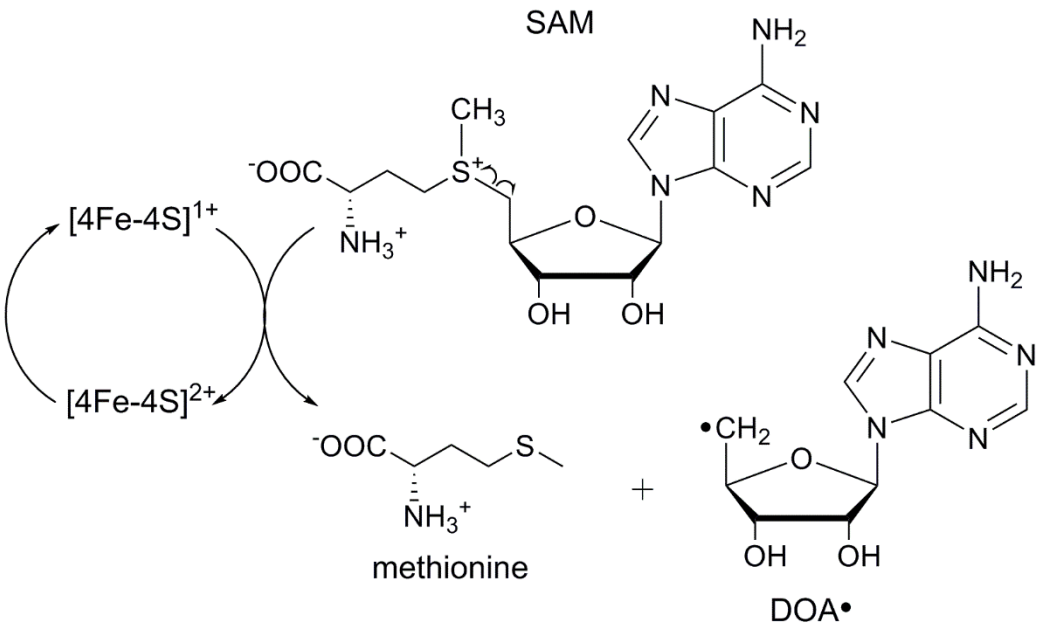
Determination of K_d values for Fe-COPRO III and wt AhbD and the AhbD cluster variants C19A/C23A and C321A/C324A. The difference in fluorescence at 337 nm (excitation at 295 nm) between the sample without substrate and the samples containing increasing amounts of substrate is plotted against the substrate concentration. K_d values were calculated using the equation given in the Experimental Section with the OriginPro 8G (Originlab Corporation) software.

Supplemental Figure C



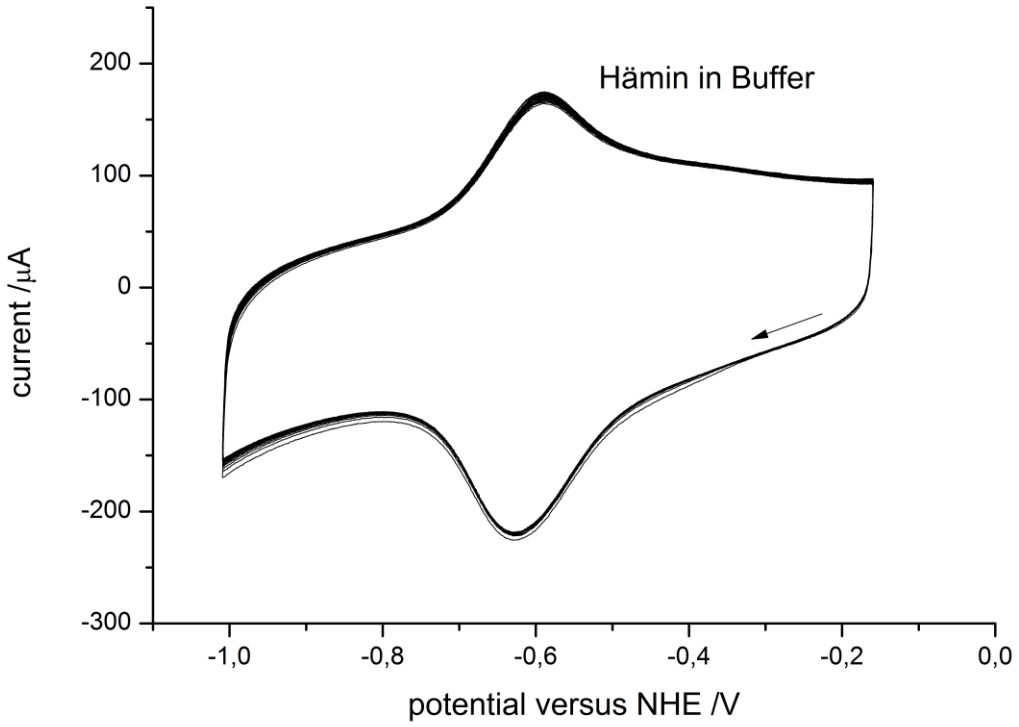
Cyclic voltammograms of (a) wt AhbD and the AhbD variants, (b) of wt AhbD and the AhbD variants with added SAM, (c) of wt AhbD and the AhbD variants with added Fe-COPRO III and (d) of wt AhbD with added Zn-COPRO III, Cu-COPRO III or Fe-COPRO III.

Supplemental Figure D



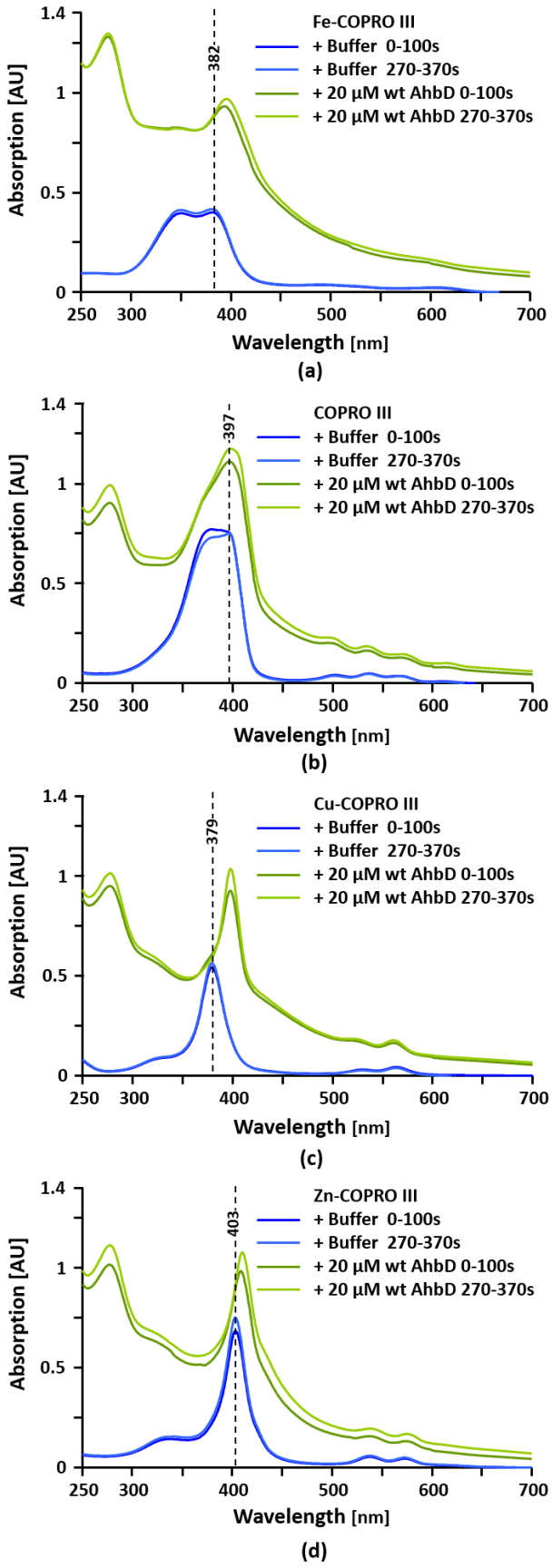
Mechanism of the SAM cleavage reaction. The reduced N-terminal FeS-cluster of AhbD transfers an electron to the bound SAM, which is thereby cleaved into methionine and the 5'-desoxyadenosyl radical (DOA•).

Supplemental Figure E



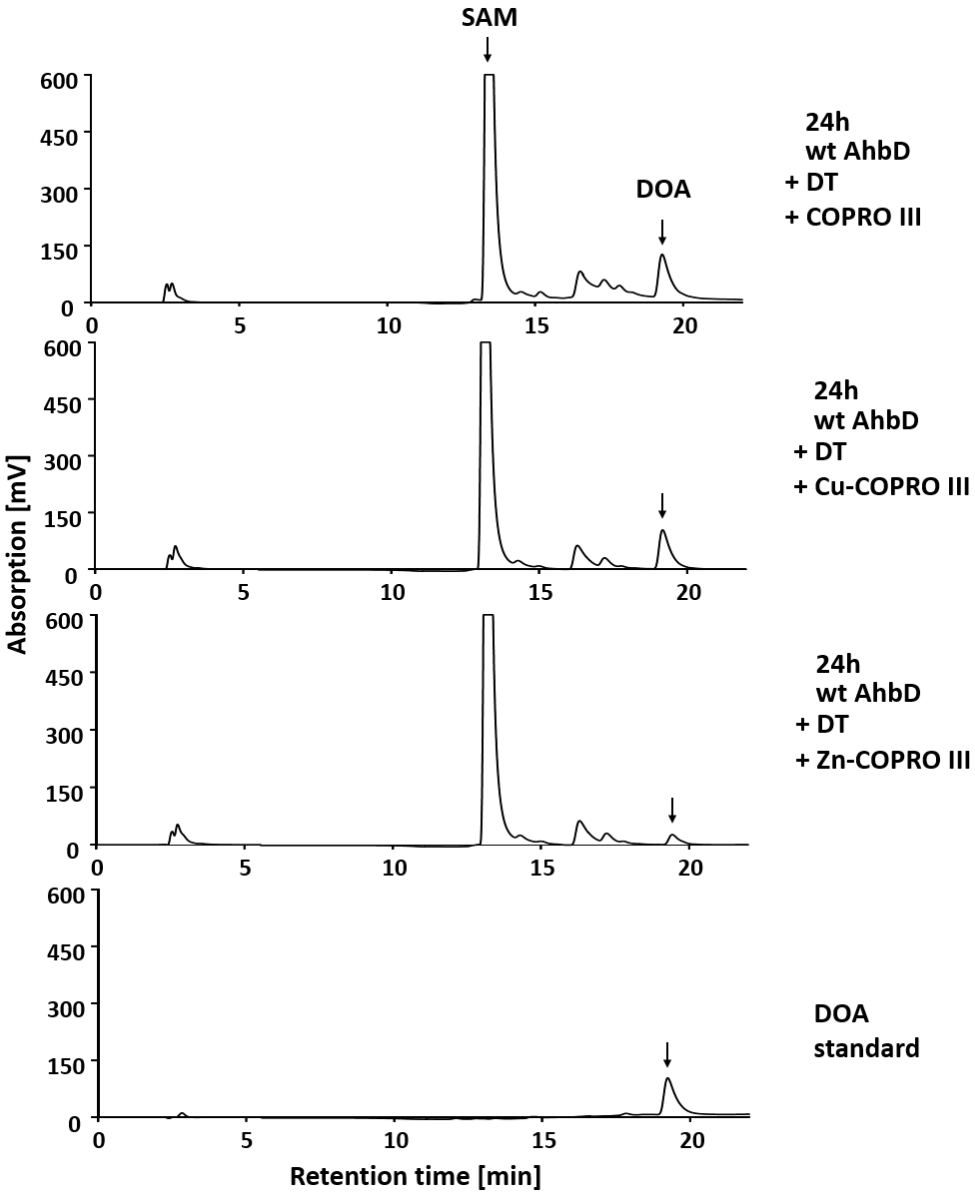
Cyclic voltammogram of heme (haemin) in buffer B.

Supplemental Figure F



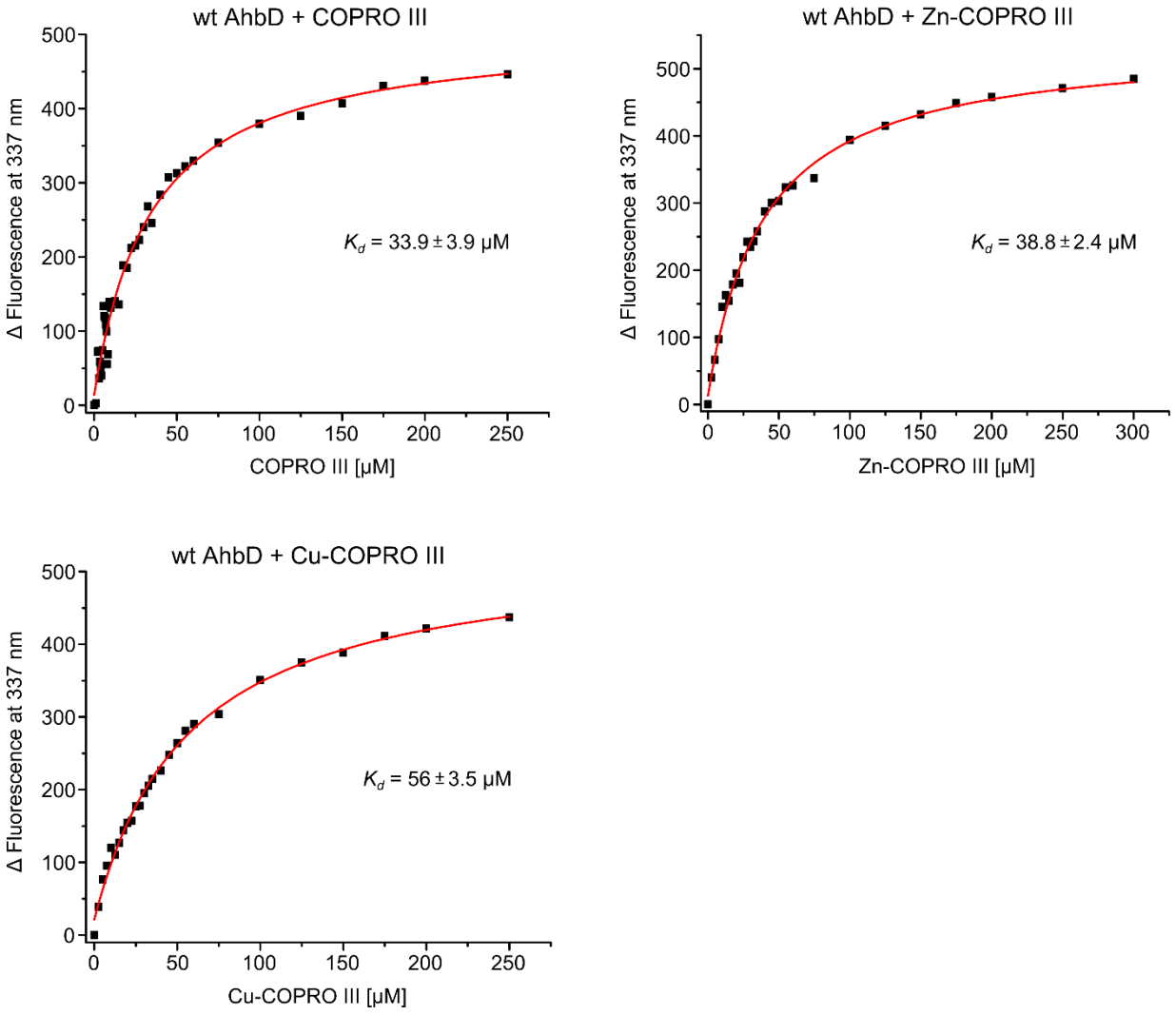
Binding assay for wt AhbD using different substrate analogs. The binding of Fe-COPRO III, COPRO III, Cu-COPRO III and Zn-COPRO III to wt AhbD was followed by UV-Visible absorption spectroscopy. The spectra were recorded directly after mixing the protein with the respective substrate analog (0-100 s) and after 270 s of incubation (270-370 s). The maxima of the Soret bands of the substrate and substrate analogs in the unbound form are indicated.

Supplemental Figure G



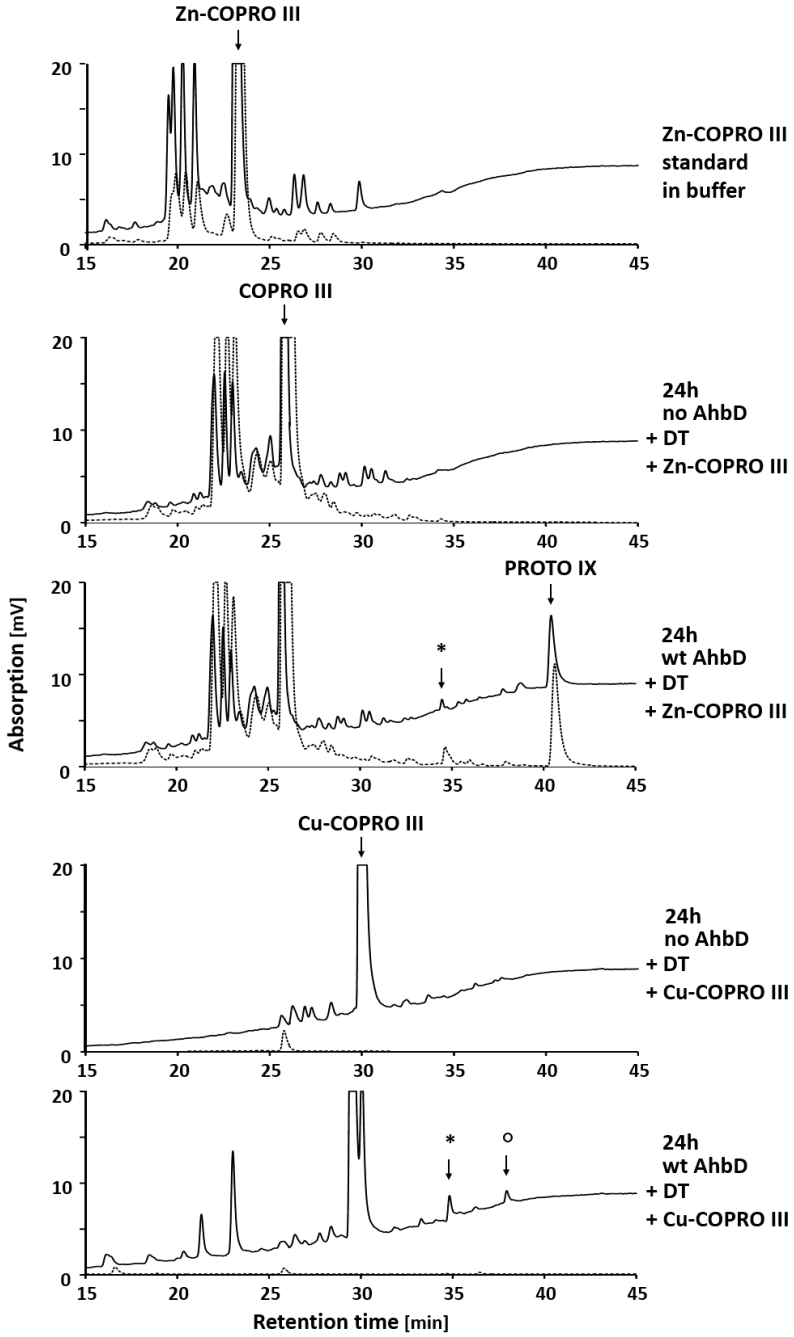
SAM cleavage activity of wt AhbD in the presence of the substrate analogs COPRO III, Cu-COPRO III and Zn-COPRO III. The formation of the SAM cleavage product DOA was followed by HPLC analysis. The elution of SAM and DOA was monitored by measuring the absorption at 254 nm. SAM eluted at 12.3 min and DOA at 18.6 min.

Supplemental Figure H



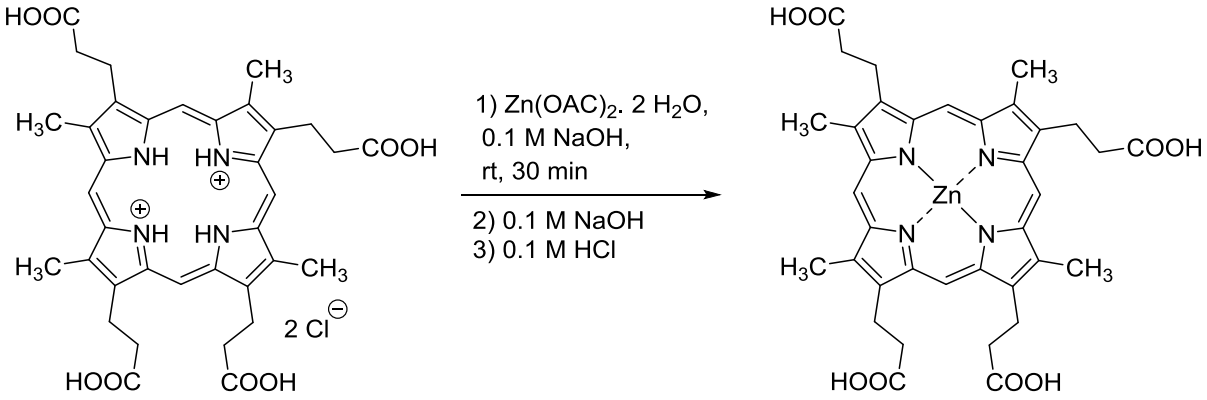
Determination of K_d values for COPRO III, Zn-COPRO III and Cu-COPRO III and wt AhbD. The difference in fluorescence at 337 nm (excitation at 295 nm) between the sample without substrate analog and the samples containing increasing amounts of substrate analog is plotted against the substrate analog concentration. K_d values were calculated using the equation given in the Experimental Section with the OriginPro 8G (Originlab Corporation) software.

Supplemental Figure I



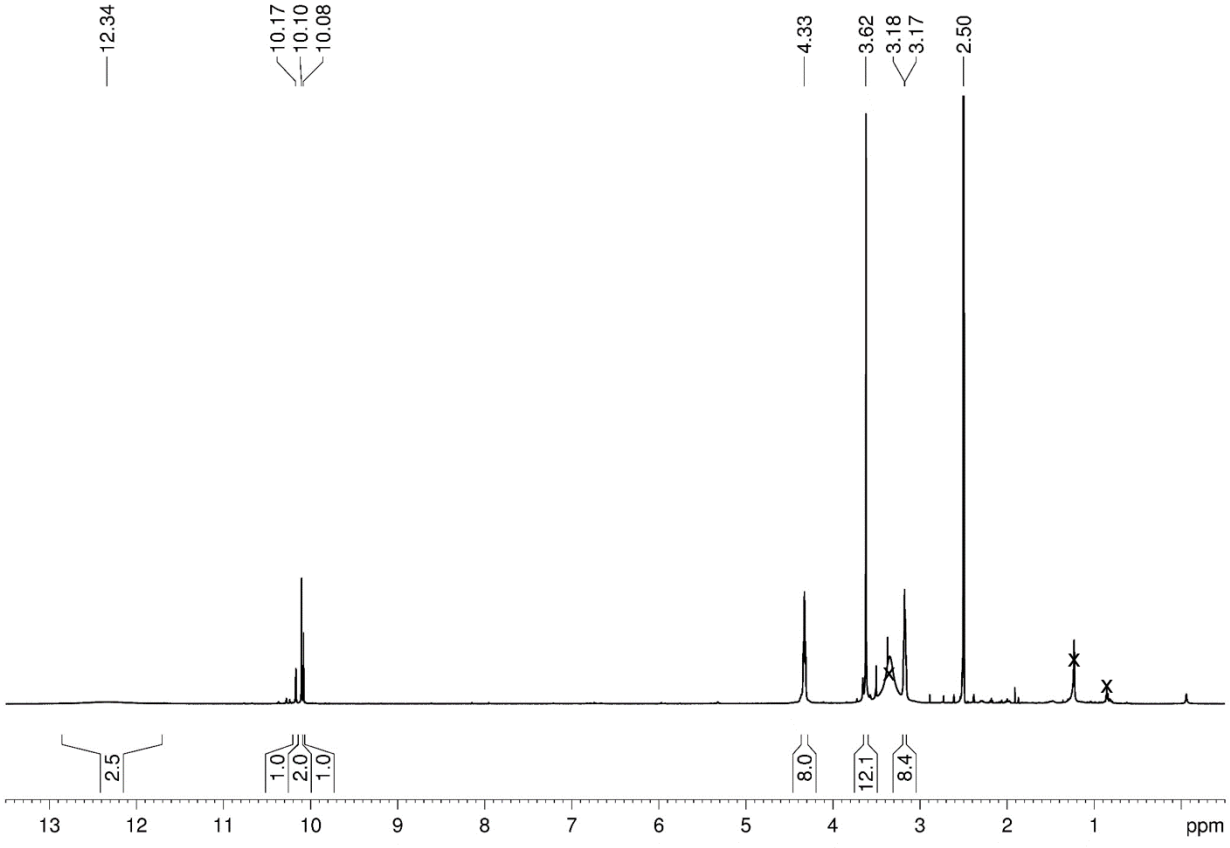
Decarboxylase activity of wt AhbD with either Zn-COPRO III or Cu-COPRO III as the substrate. The tetrapyrrole content in the assay mixtures was analyzed after 24 h of incubation at 17 °C by HPLC. The elution of the tetrapyrroles was monitored by measuring the absorption at 400 nm (solid line) and the fluorescence (dotted line, excitation at 409 nm, emission at 630 nm). Zn-COPRO III eluted at 23.2 min. Under the acidic extraction conditions the Zn-COPRO III was demetallated during the tetrapyrrole extraction after the assay and therefore the remaining substrate and the reaction products were detected in their Zn-free forms. COPRO III eluted at 25.7 min, the corresponding monovinyl intermediate at 34.5 min and PROTO IX at 40.5 min (lower panels). Cu-COPRO III eluted at 29.9 min, the Cu-monovinyl intermediate at 34.8 min and Cu-PROTO IX at 38.0 min. The respective monovinyl intermediates are highlighted by an asterix.

Supplemental Figure J



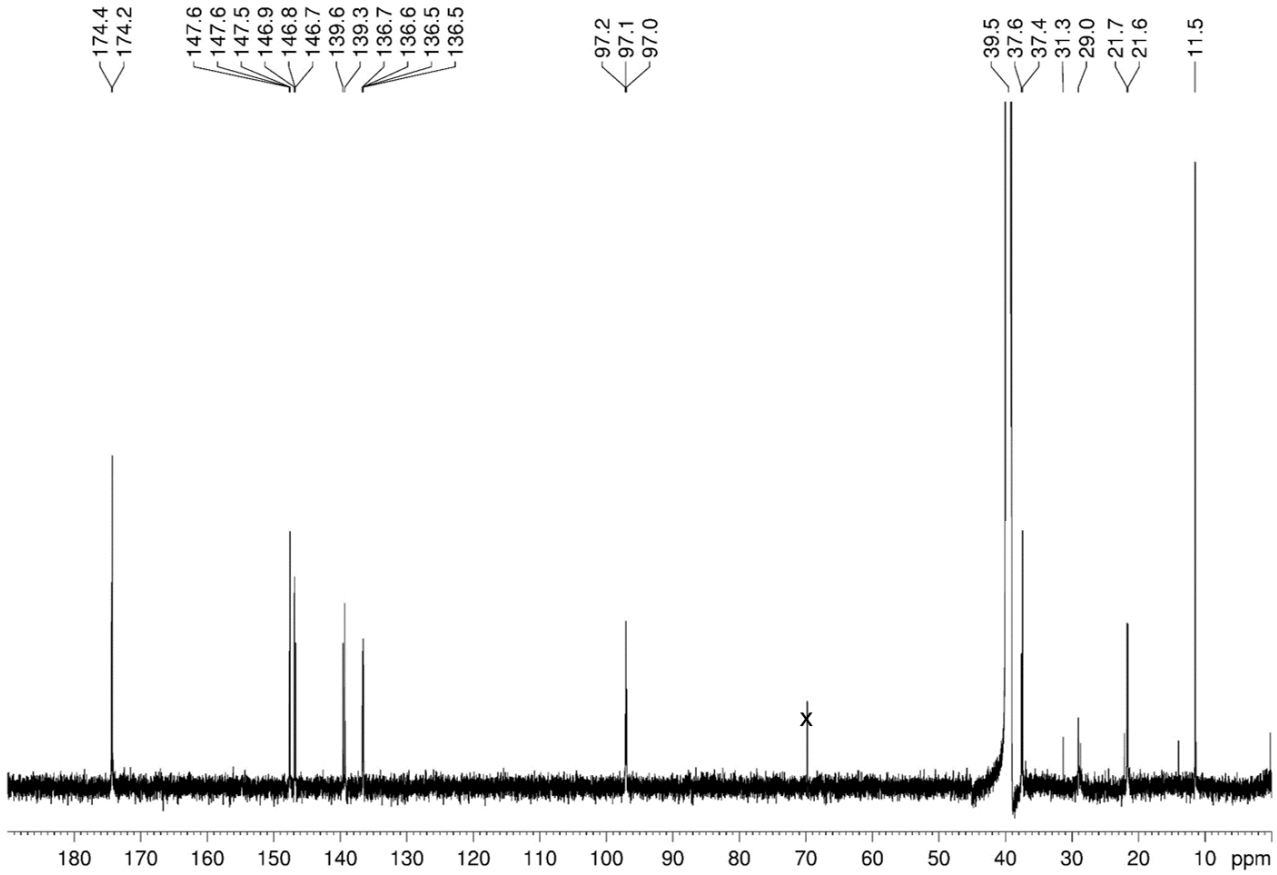
Synthesis of Zn-COPRO III.

Supplemental Figure K



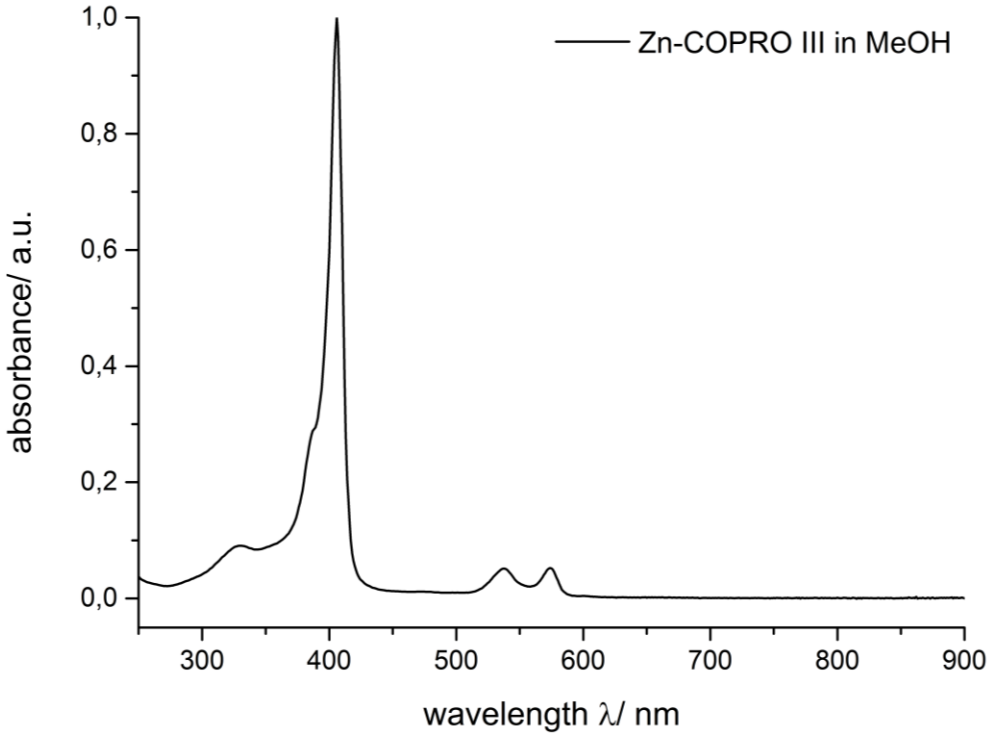
^{1}H NMR spectrum (600 MHz) of Zn-COPRO III in d_6-DMSO at 298 K.

Supplemental Figure L



^{13}C NMR spectrum (150 MHz) of Zn-COPRO III in d_6-DMSO at 298 K.

Supplemental Figure M



UV-Visible absorption spectrum of Zn-COPRO III in MeOH.