Supporting Information

Morgada et al. 10.1073/pnas.1505056112

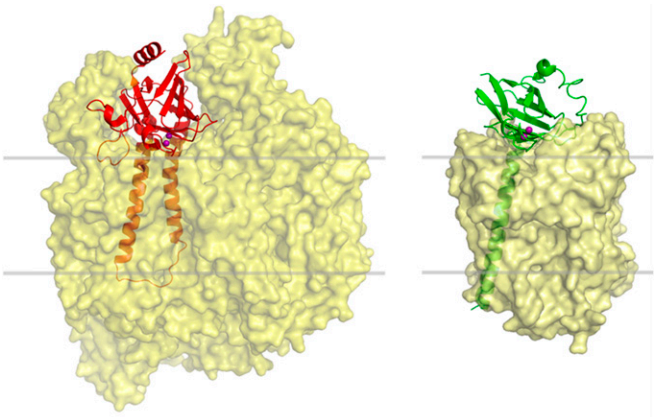

Fig. S1. Arrangement of the COX II subunit within the structure of different oxidases. Eukaryotic COX II (*Left*; PDB ID code 1OCC for bovine COX) (9) displays several interactions with other subunits of the complex, whereas prokaryotic COX II (*Right*; PDB ID code 1EHK for *Tt* COX) (47) displays a small region of interaction with the rest of the complex. Gray lines define approximate limits of the lipid membrane surrounding the complexes.

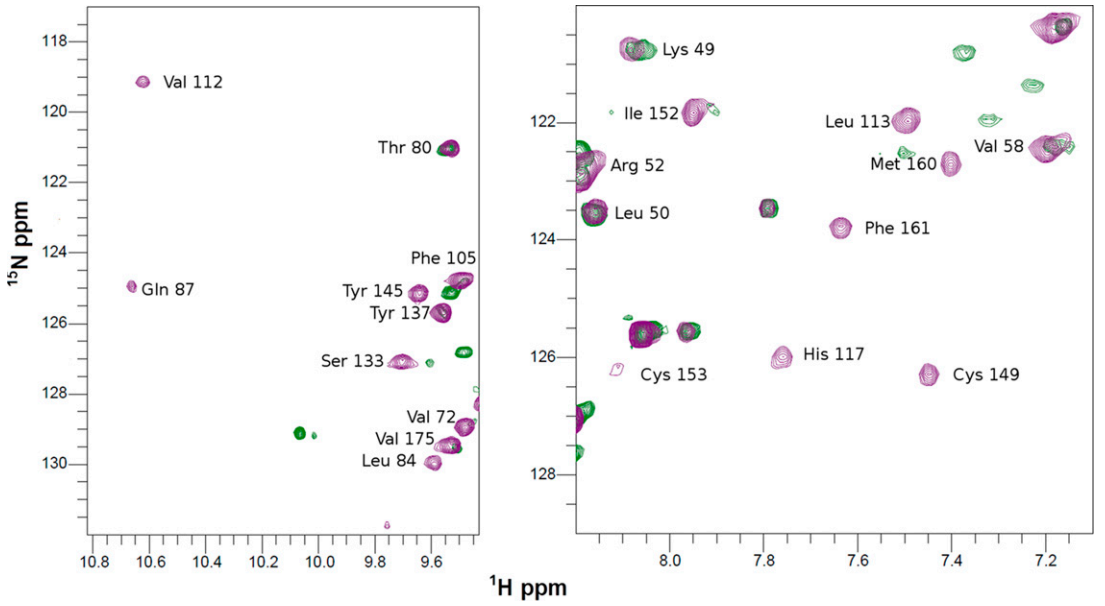

Fig. S2. Cu(I) uptake of Apo-COX II* followed by NMR. ^{1}H-^{15}N HSQC spectra of apo-COX II*$_{2HS}$ (green) and upon addition of two equivalents of Cu(I) (purple). The spectrum of the metallated form has been assigned, showing that the main difference is the appearance of cross peaks belonging to the ligands loops (*Right*) or to the two other loops surrounding the metal site (*Left*).

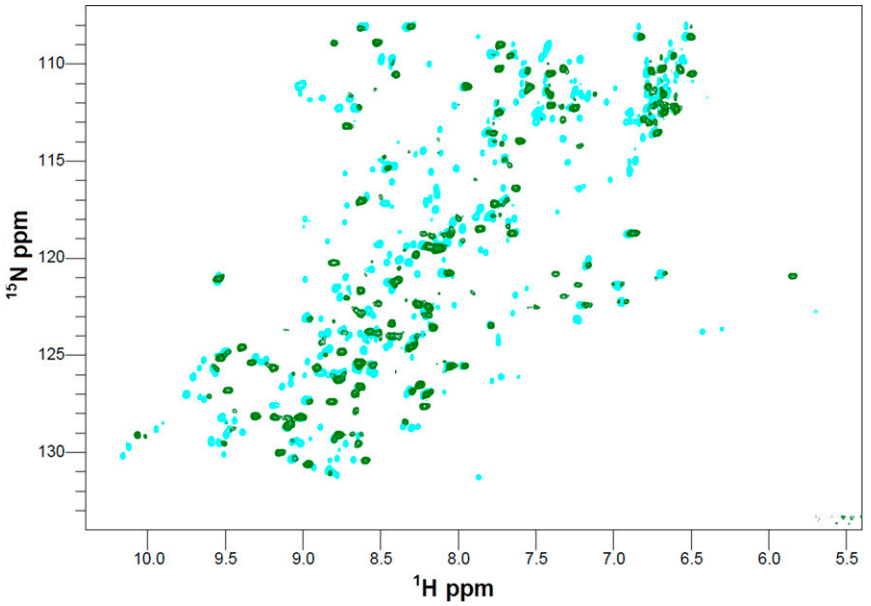

Fig. S3. Reduction of Apo-COX II*$_{S\text{-}S}$ to Apo-COX II*$_{2SH}$ followed by NMR. ^{1}H-^{15}N HSQC spectra of Apo-COX II*$_{S\text{-}S}$ (cyan) and Apo-COX II*$_{2SH}$ (green).

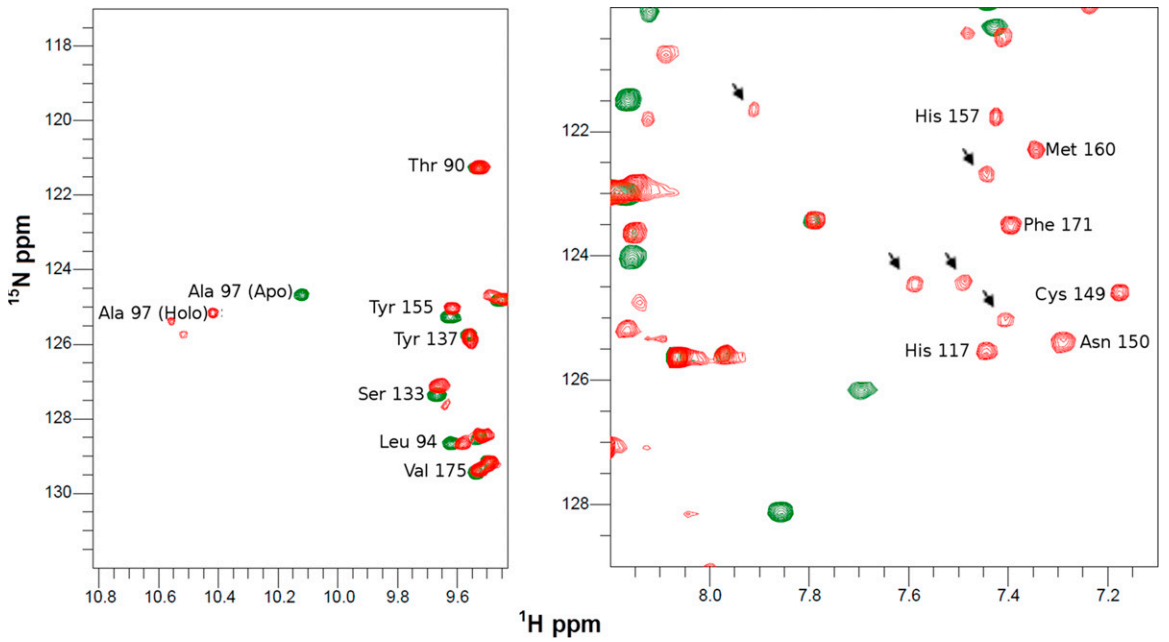

Fig. S4. Cu(I) transfer from Cu(I)-Sco1 to Apo-*Tt*COX II$_{2SH}$ followed by NMR. ^{1}H-^{15}N HSQC spectra of *Tt*COX II$_{2SH}$ (green) and upon addition of two equivalents of Cu(I)-Sco1 (red). Arrows indicate cross peaks that do not correspond neither to apo nor to the metallated form of the protein.

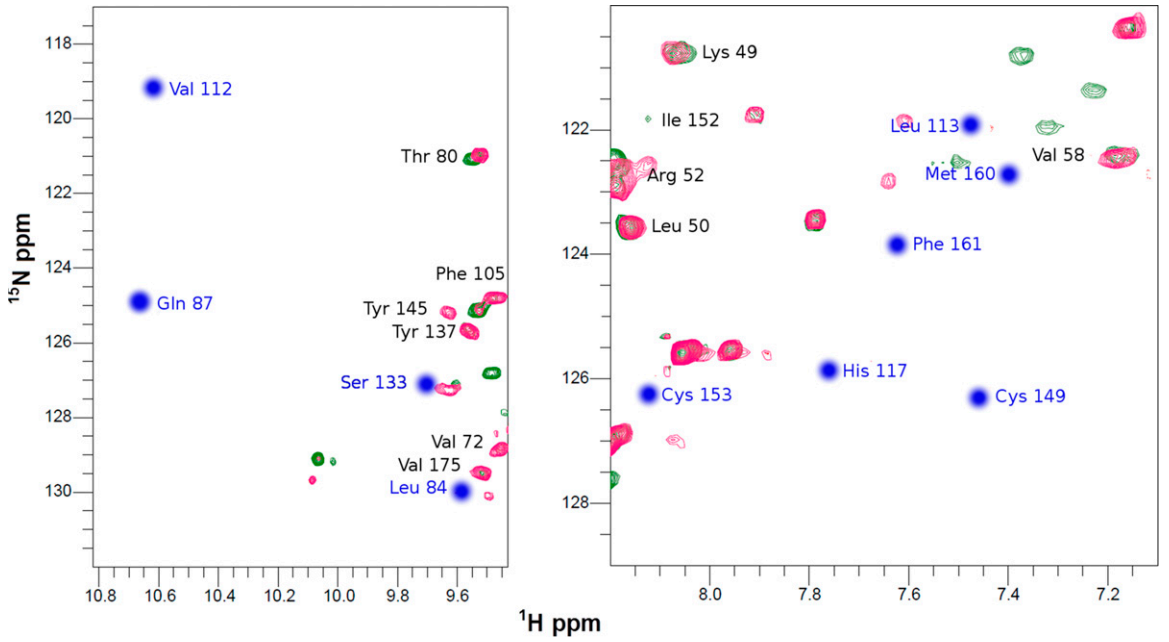

Fig. S5. Cu(I) transfer from Cu(I)-*Tt*Sco1 to Apo-COX II*$_{2SH}$ followed by NMR. ^{1}H-^{15}N HSQC spectra of COX II*$_{2SH}$ (green) and upon addition of two equivalents of Cu(I)-*Tt*Sco1 (pink), showing that *Tt*Sco1 is not able to transfer Cu(I) ions to the oxidase. The position of the expected cross peaks corresponding to the holo form protein absent in the final spectrum are indicated in blue.

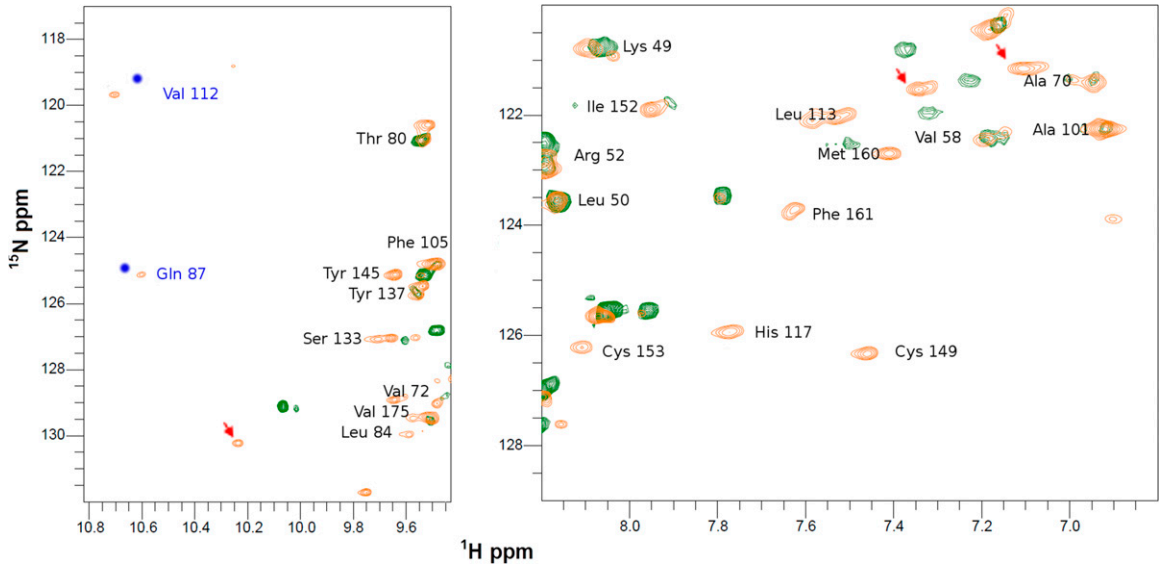

Fig. S6. Cu(I) transfer from Cu(I)-Sco2 to Apo-COX II*$_{2SH}$ followed by NMR. ^{1}H-^{15}N HSQC spectra of COX II*$_{2SH}$ (green) and upon addition of two equivalents of Cu(I)-Sco2 (orange). The position of the expected cross peaks corresponding to the holo form protein shifted in the final spectrum are indicated in blue.

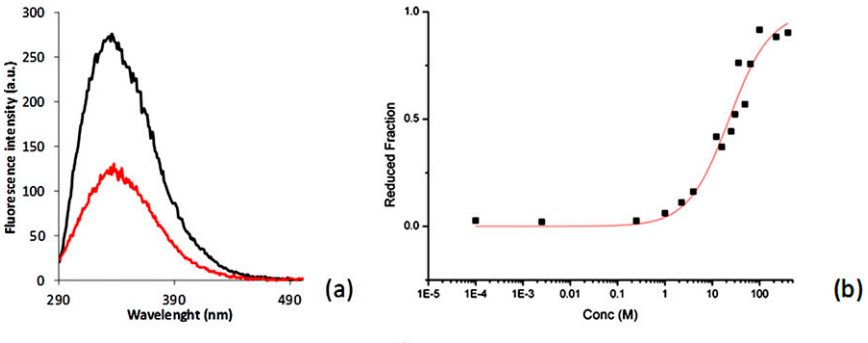

Fig. S7. Redox potential of the CX_3C motif of Apo-COX II*. (*A*) Fluorescence emission spectra of oxidized (50 mM phosphate buffer, pH 7.0, 0.01 mM GSSG; black line) and reduced (50 mM phosphate buffer, pH 7.0, 200 mM GSH; red line) Apo-COX II* upon excitation at 280 nm. (*B*) The redox equilibrium of Apo-COX II* with different $[GSH]_2$/GSSG ratios is shown. Data processing and determination of the equilibrium constant was previously described (3). After nonlinear regression, a value of $K_{eq} = 23 \pm 2$ mM was determined for the Apo-COX II*/glutathione equilibrium, corresponding to a redox potential of −288 ±3 mV for the Apo-COX II* S-S –2SH redox couple.

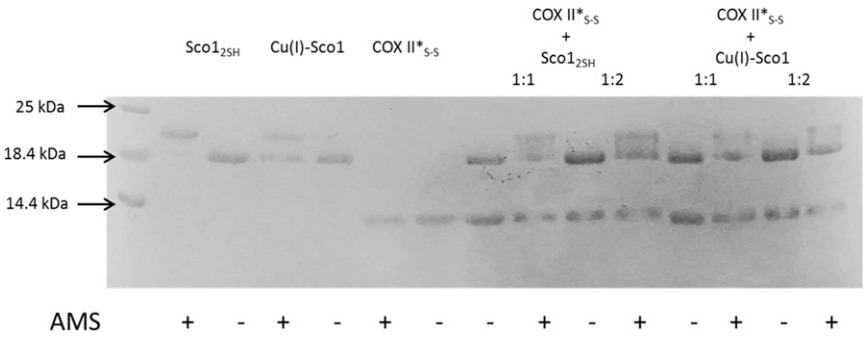

Fig. S8. Electron transfer followed by AMS-reacted, nonreducing SDS/PAGE. Sco1$_{2SH}$ and Cu(I)-Sco1 mixed with COX II*$_{S-S}$. Mixtures were done in a 1:1 and 2:1 ratio Sco1:COX II*. The proteins in their different states are also reported as reference. Signs + and – indicate if the AMS reactive was added to the mixture.

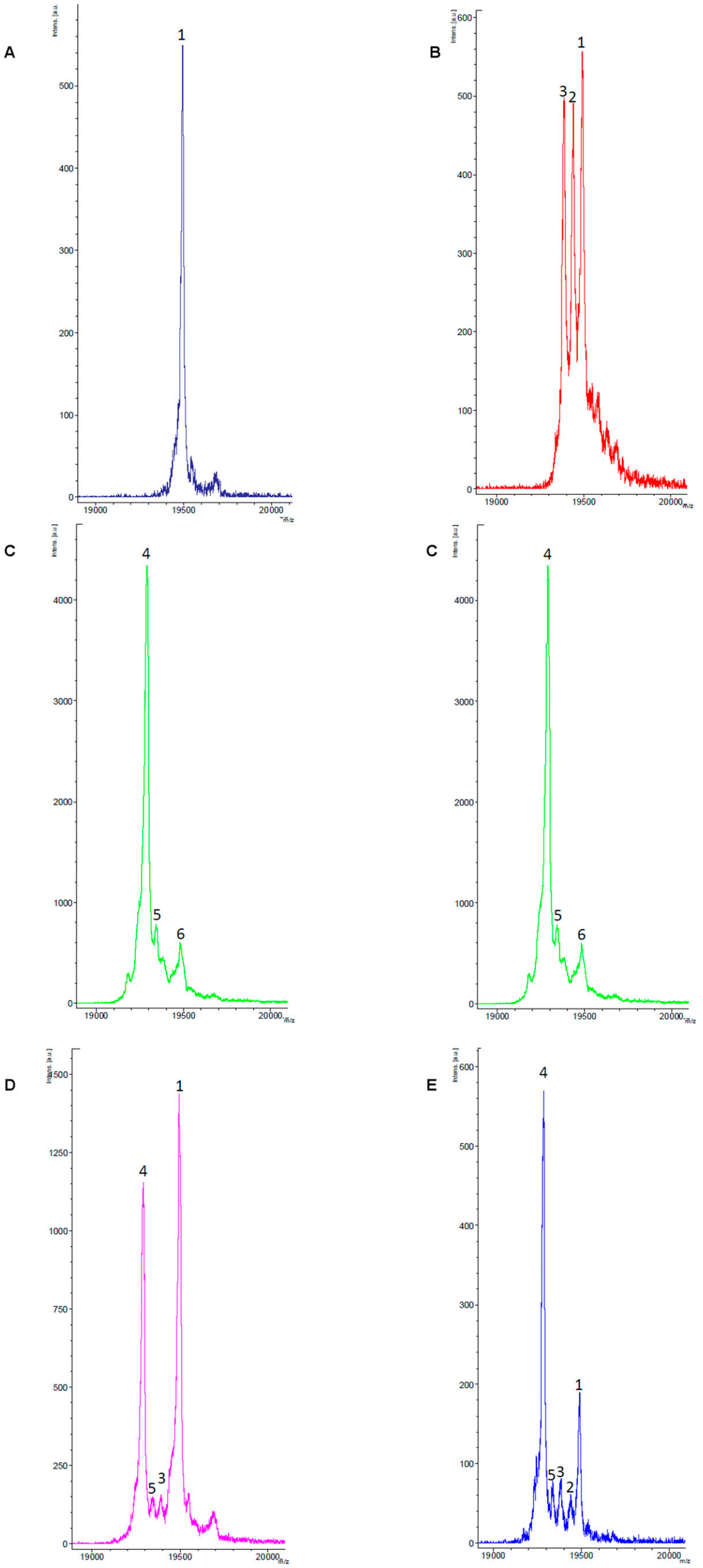

Fig. S9. MALDI-TOF-MS spectra of the iodoacetamide (IAM)-derivatized samples of (*A*) $Sco1_{2SH}$ showing the presence a dominant species labeled as **1**, corresponding to the $Sco1(IAM)_2$ adduct, indicating that the two reduced Cys at the active site are in the reduced form; (*B*) Cu(I)-Sco1 showing the presence of three species corresponding to $Sco1(IAM)_2$, $Sco1(IAM)_1$, and Sco1, labeled as **1**, **2**, and **3**, respectively; (*C*) $Sco2_{S\text{-}S}$ with the presence of the presence a dominant species corresponding to the Sco2(IAM) adduct, where Cys115 (far from the active site) is alkylated, and the two Cys at the active site are oxidized, not being alkylated, labeled as **4**; minority species **5** and **6** correspond to $Sco2(IAM)_2$ and $Sco2(IAM)_3$; (*D*) a 1:1 mixture of $Sco1_{2SH}$ and $Sco2_{S\text{-}S}$, where the peaks of the reference spectra are preserved, showing that there is no redox reaction; (*E*) a 1:1 mixture of Cu(I)-$Sco1_{2SH}$ and $Sco2_{S\text{-}S}$, where the peaks of the reference spectra are preserved, showing that there is no redox reaction.

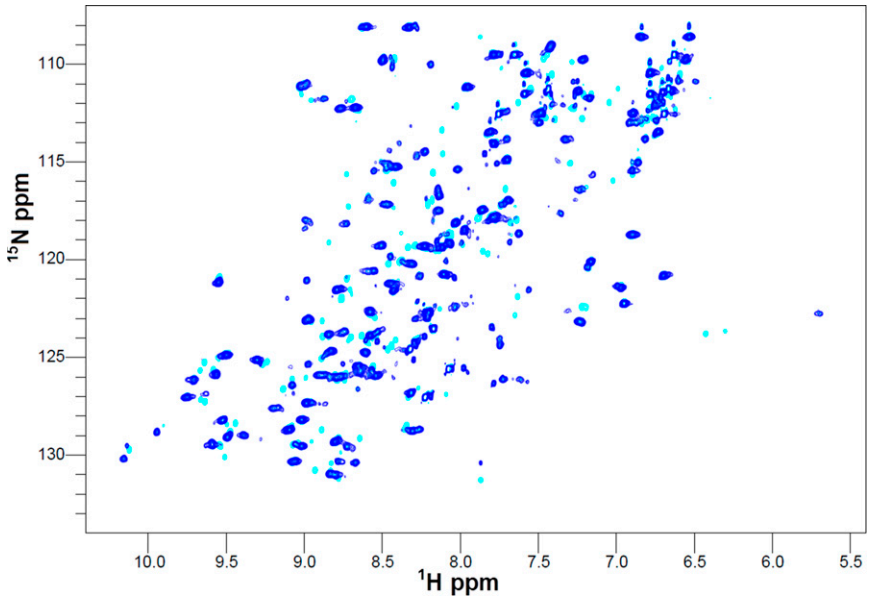

Fig. S10. Electron transfer from Cu(I)-Sco2 to Apo-COX II*$_{S\text{-}S}$ followed by NMR. ^{1}H-^{15}N HSQC spectra of Apo-COX II*$_{S\text{-}S}$ (cyan) and upon addition of one equivalent of Cu(I)-Sco2 (blue). The disappearance of several cross peaks compared with the oxidized form of the protein is typical of spectra of COX II*$_{2SH}$.