Supporting Information

Banci *et al*. 10.1073/pnas.0800019105

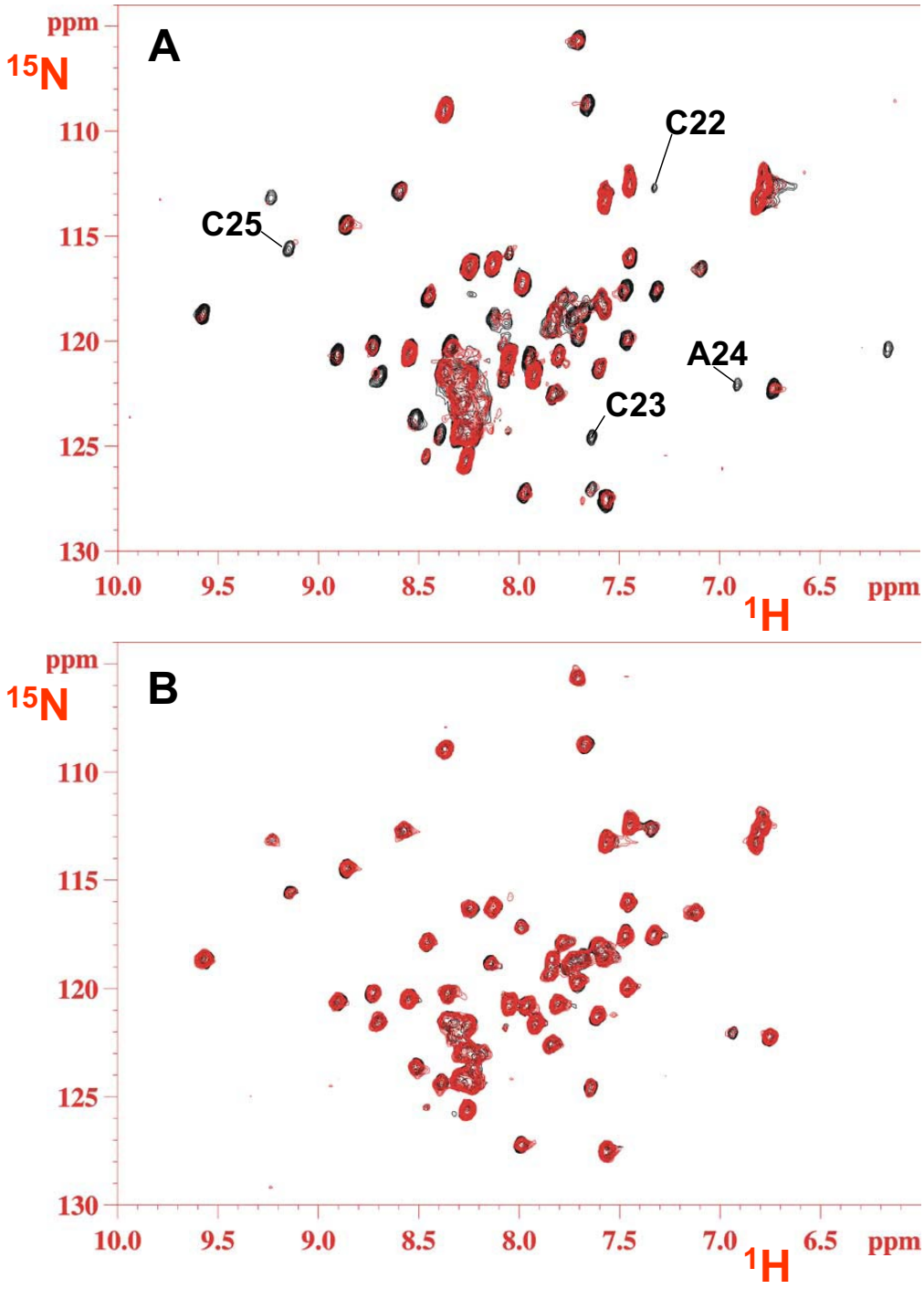

Fig. S1. Titration of ^{15}N-labeled Cu(I)HCox17$_{2S\text{-}S}$ with unlabeled HSco1$_{1S\text{-}S}$ (*A*) or with unlabeled HSco2$_{1S\text{-}S}$ (*B*) followed through ^{1}H–^{15}N HSQC NMR spectra. The ^{1}H–^{15}N HSQC spectrum of Cu(I)HCox17$_{2S\text{-}S}$ (in black) is overlaid with the ^{1}H-^{15}N HSQC spectrum of a 1:1 HSco1$_{1S\text{-}S}$/Cu(I)HCox17$_{2S\text{-}S}$ mixture (*A*, in red) or with that of 1:1 HSco2$_{1S\text{-}S}$/Cu(I)HCox17$_{2S\text{-}S}$ mixture (*B*, in red). Copper(I)-binding ligands, C22 and C23, and the following residues, A24 and C25, broaden beyond detection upon addition of HSco1$_{1S\text{-}S}$. On the contrary, upon addition of HSco2$_{1S\text{-}S}$ the Cu(I)HCox17$_{2S\text{-}S}$ ^{1}H–^{15}N HSQC spectrum does not change.

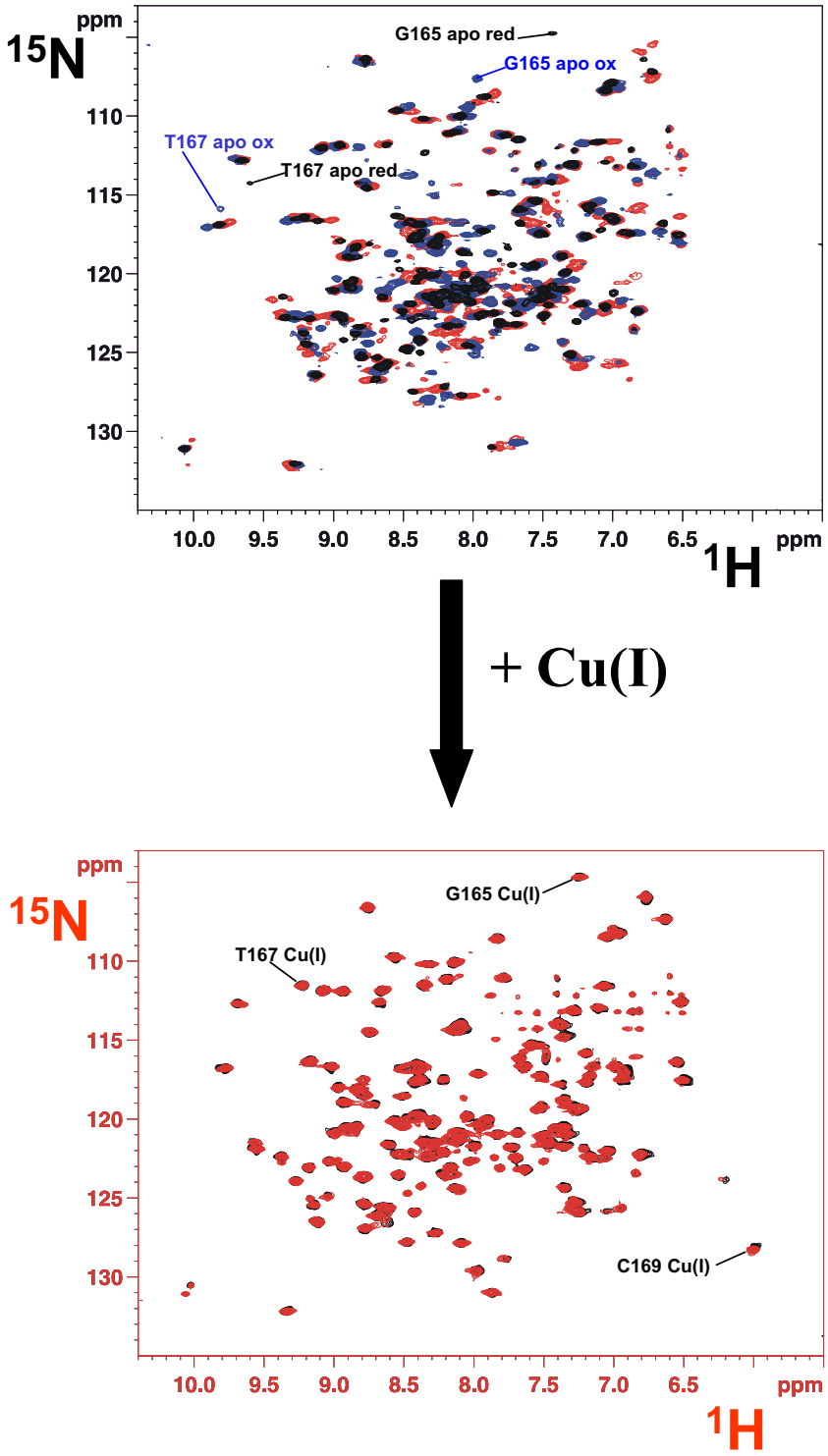

Fig. S2. Titration of ^{15}N-labeled apoHSco1$_{1S\text{-}S}$ with unlabeled apoHCox17$_{2S\text{-}S}$ followed through ^{1}H–^{15}N HSQC NMR spectra. ^{1}H–^{15}N HSQC spectrum of ^{15}N-labeled apoHSco1$_{1S\text{-}S}$ (in blue) is overlaid with the ^{1}H–^{15}N HSQC spectrum of a 1:1 ^{15}N-labeled apoHSco1$_{1S\text{-}S}$/unlabeled Cu(I)HCox17$_{2S\text{-}S}$ mixture (in red) and with ^{1}H-^{15}N HSQC spectrum of ^{15}N-labeled HSco1$_{2SH}$ (in black). Upon addition of 1 eq of Cu(I), the ^{1}H–^{15}N HSQC spectrum of the previous protein mixture drastically changes, becoming completely superimposable with the ^{1}H–^{15}N HSQC spectrum obtained by mixing ^{15}N-labeled apoHSco1$_{1S\text{-}S}$ with unlabeled Cu(I)HCox17$_{2S\text{-}S}$. The assignment of the NH resonances of G165 and T167 in Cu(I)HSco1, oxidized and reduced apoHSco1 forms, is reported. The NH resonance of C169 is detected only in the copper(I)-bound form.

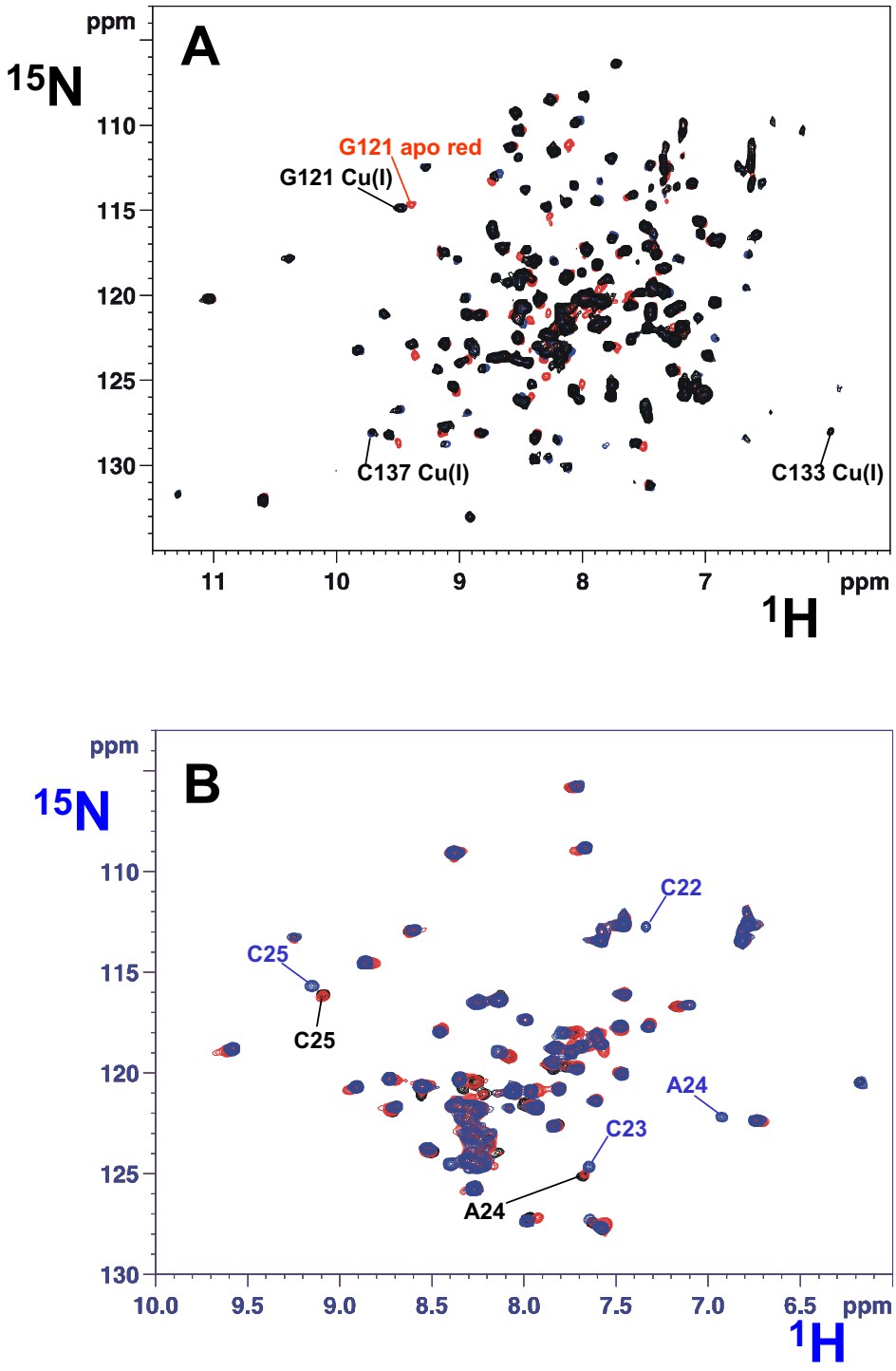

Fig. S3. Titration of ^{15}N-labeled apoHSco2$_{2SH}$ with unlabeled Cu(I)HCox17$_{2S\text{-}S}$ (*A*) and of ^{15}N-labeled Cu(I)HCox17$_{2S\text{-}S}$ with unlabeled apoHSco2$_{2SH}$ (*B*), followed by ^{1}H–^{15}N HSQC NMR spectra. The ^{1}H–^{15}N HSQC spectrum of apoHSco2$_{2SH}$ (in red) is superimposed with the ^{1}H–^{15}N HSQC spectrum of a 1:1 ^{15}N-labeled HSco2$_{2SH}$/unlabeled Cu(I)HCox17$_{2S\text{-}S}$ mixture (in blue) and with the ^{1}H-^{15}N HSQC spectrum of ^{15}N-labeled Cu(I)HSco2 (in black). The assignment of the NH resonances of C137, C133 and G121 in Cu(I)HSco2 and apoHSco2$_{2SH}$ forms, when detectable, is reported. In panel (B), ^{1}H-^{15}N HSQC spectrum of Cu(I)HCox17$_{2S\text{-}S}$ (in blue) is superimposed with ^{1}H-^{15}N HSQC spectrum of a 1:1 mixture of ^{15}N-labeled Cu(I)HCox17$_{2S\text{-}S}$/unlabeled HSco2$_{2SH}$ (in red) and with the ^{1}H–^{15}N HSQC spectrum of apoHCox17$_{2S\text{-}S}$ (in black). The assignment of the NH resonances of A24 and C25 in Cu(I)HCox17$_{2S\text{-}S}$ and apoHCox17$_{2S\text{-}S}$ forms is reported. The NH resonances of C22 and C23 are detected only in the copper(I)-bound form.

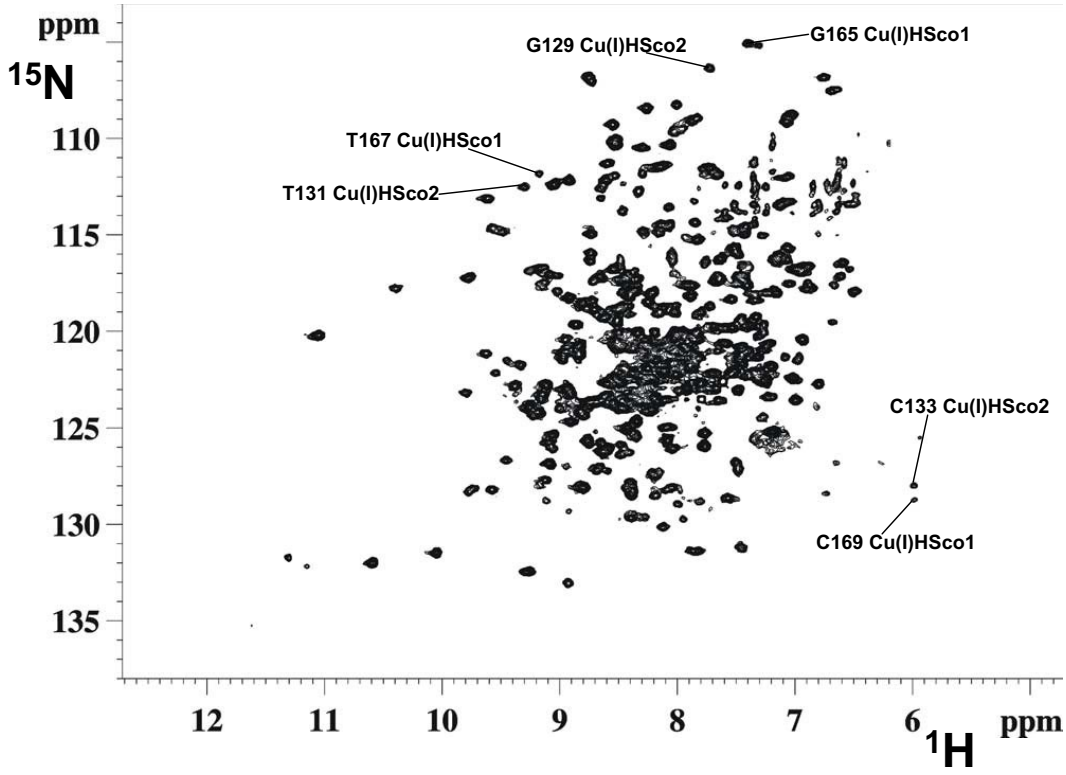

Fig. S4. Titration of a 1:1 ^{15}N apoHSco1$_{2SH}$/^{15}N apoHSco2$_{2SH}$ mixture with copper(I) ion followed through ^{1}H–^{15}N HSQC NMR spectra. The ^{1}H–^{15}N HSQC spectrum of the protein mixture after addition of 1 eq of copper(I) acetonitrile complex is shown. NH resonances of some residues whose chemical shift is typical of the copper forms of HSco1 and HSco2 are indicated.