Copper(I)-binding properties of de-coppering drugs for the treatment of Wilson disease

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SUPPLEMENTARY INFORMATION

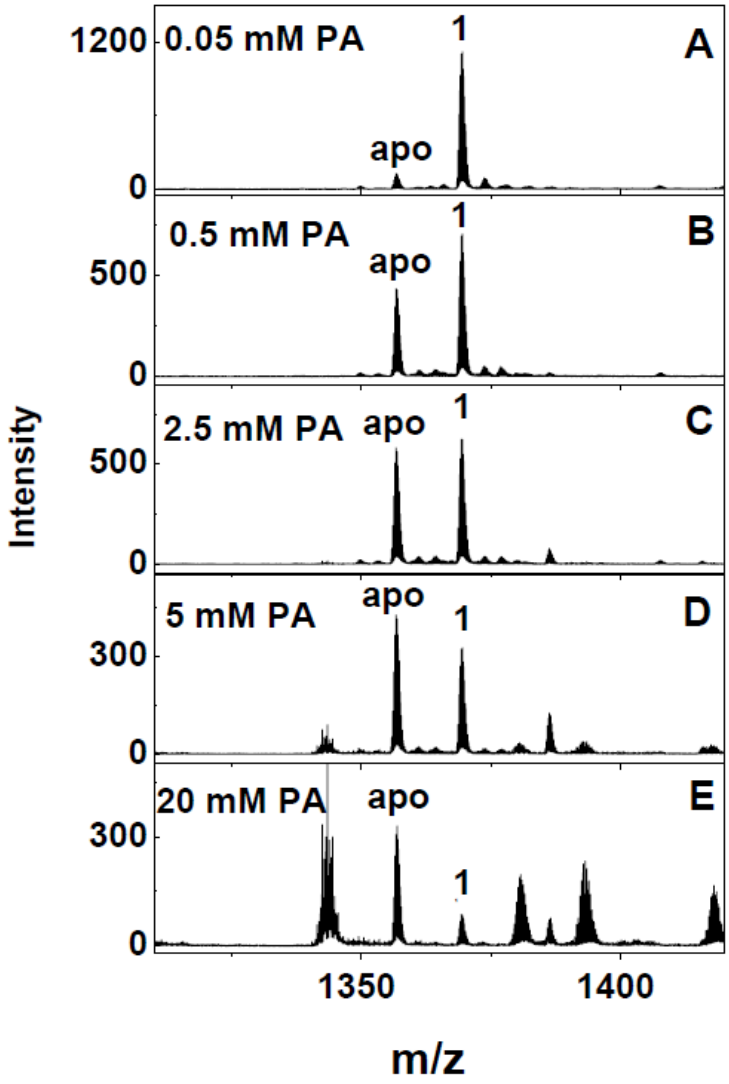

Fig. S1. Determination of the relative Cu(I)-binding affinity of PA in competition with Cu_1Cox17.

ESI-MS spectra of Cu_1Cox17 in the presence of 1 μM – 20 mM PA. Conditions: Cox17 1 μM; 20 mM ammonium acetate, pH=7.3, DTT 50 μM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex.

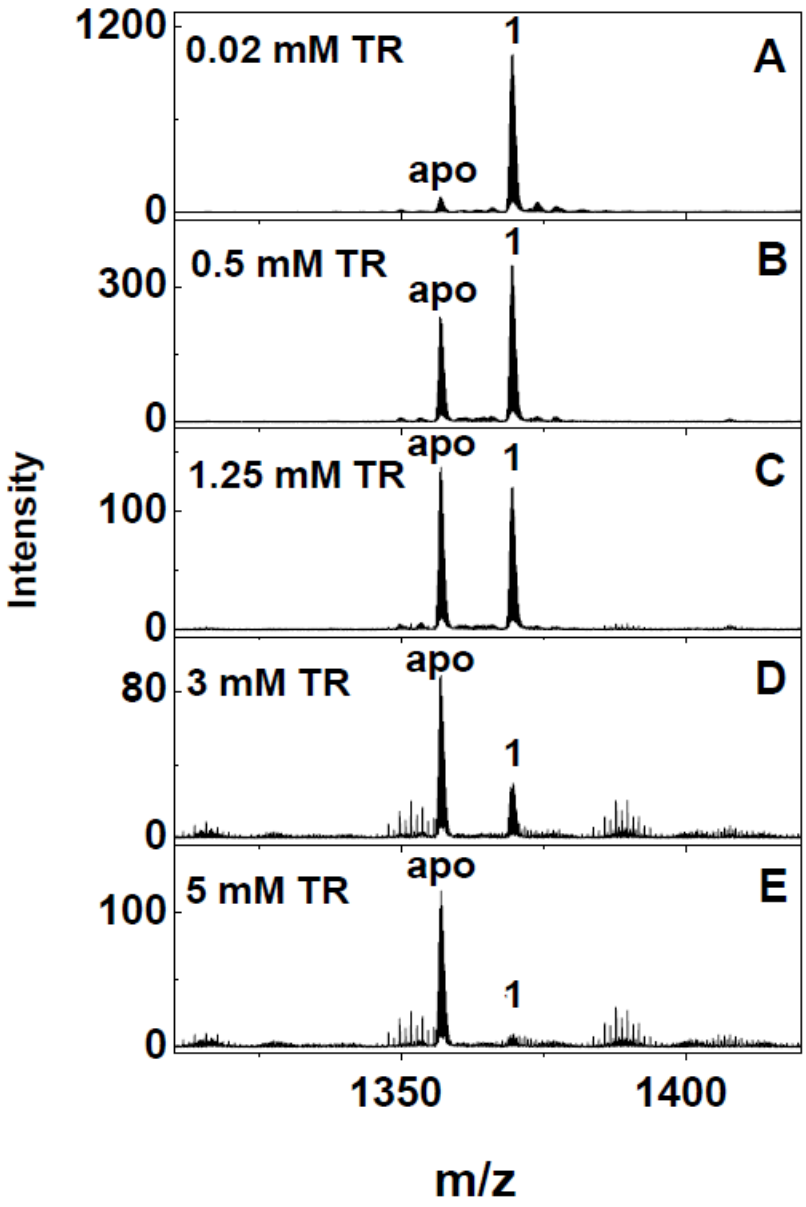

Fig. S2. Determination of the relative Cu(I)-binding affinity of TR in competition with Cu_1Cox17.

ESI-MS spectra of Cu1Cox17 in the presence of 1 μM – 5 mM TR. Conditions: Cox17 1 μM; 20 mM ammonium acetate, pH=7.3, DTT 50 μM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex.

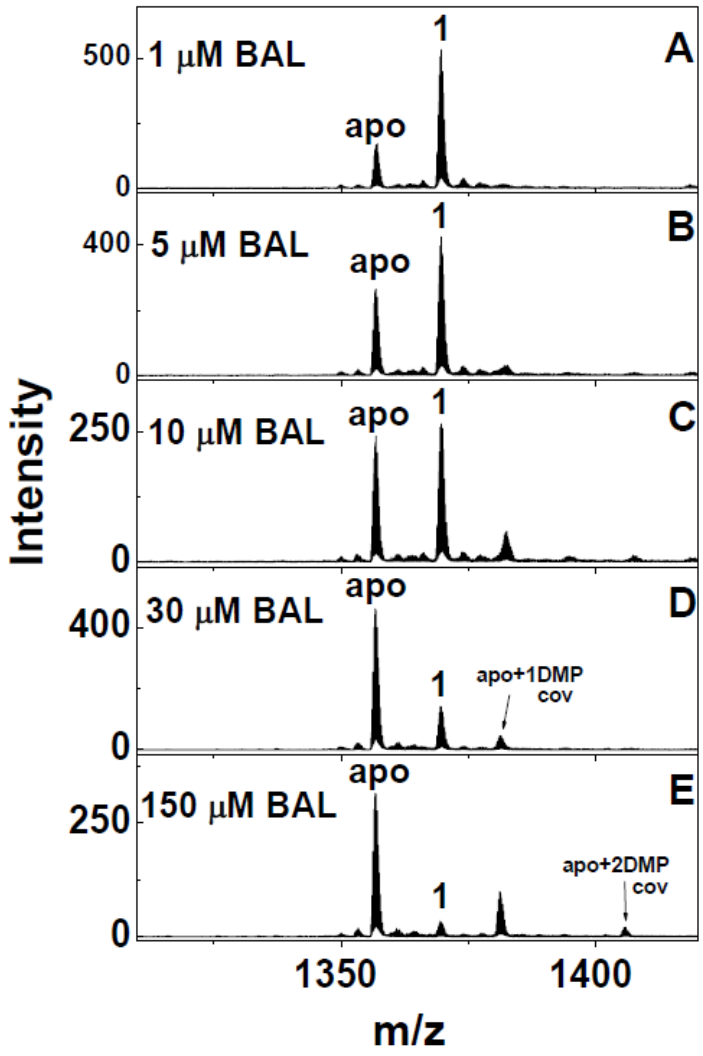

Fig. S3. Determination of the relative Cu(I)-binding affinity of BAL in competition with Cu1Cox17.

ESI-MS spectra of Cu_1Cox17 in the presence of 1 – 150 μM BAL. Conditions: Cox17 1 μM; 20 mM ammonium acetate, pH=7.3, DTT 50 μM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex.

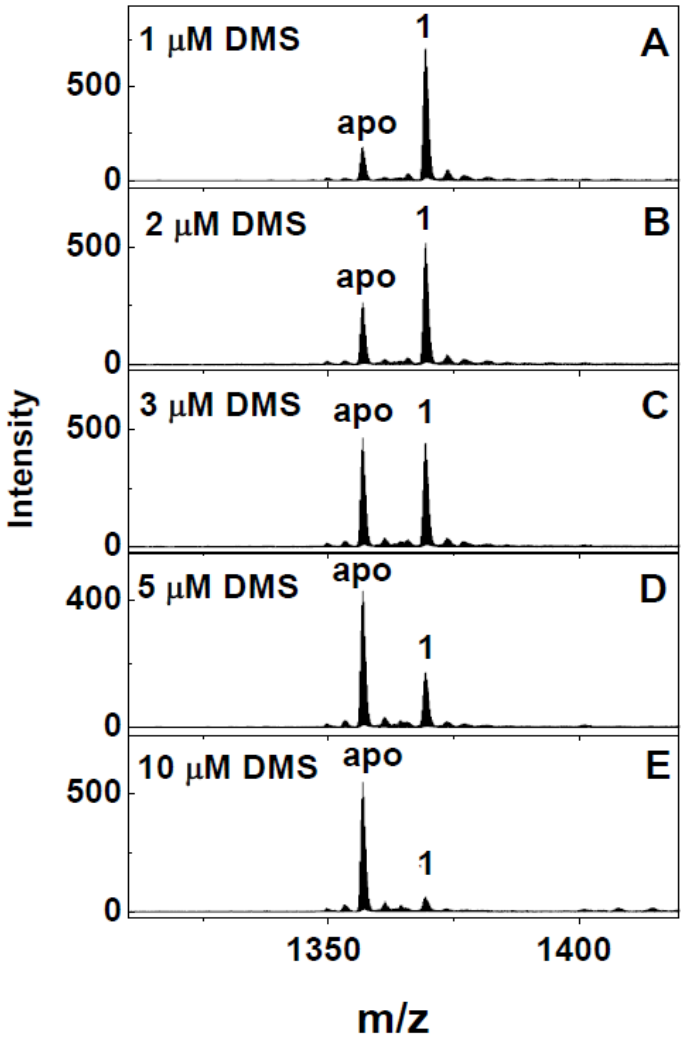

Fig. S4. Determination of the relative Cu(I)-binding affinity of DMS in competition with Cu_1Cox17.

ESI-MS spectra of Cu_1Cox17 in the presence of 1 μM – 15 μM DMS. Conditions: Cox17 1 μM; 20 mM ammonium acetate, pH=7.3, DTT 50 μM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex.

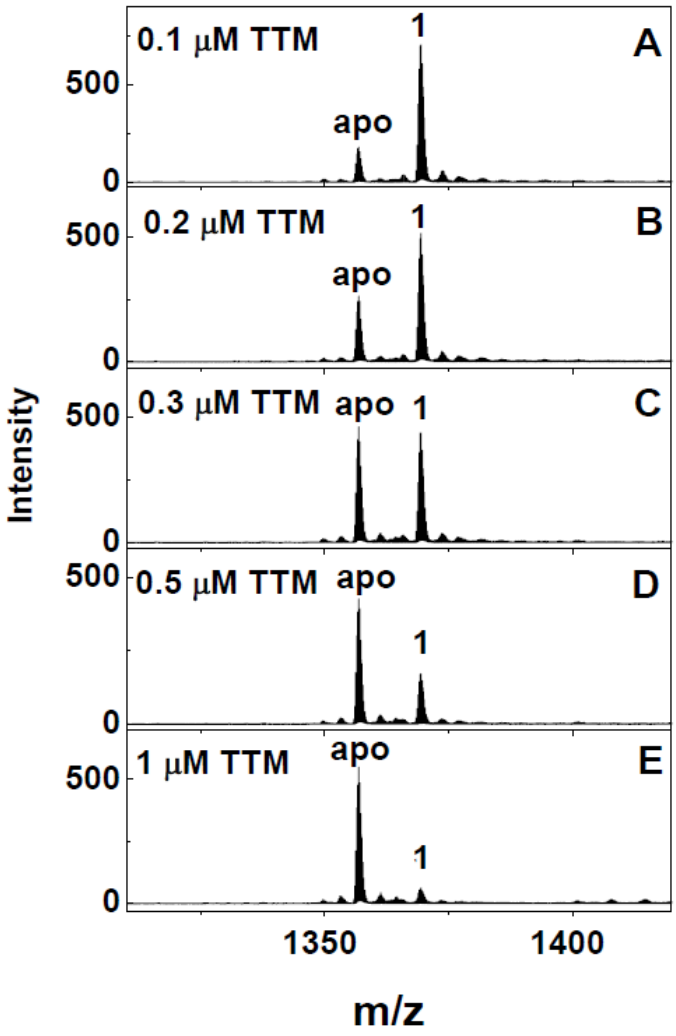

Fig. S5. Determination of the relative Cu(I)-binding affinity of ammonium TTM and bis-choline TTM in competition with Cu_1Cox17.

ESI-MS spectra of Cu_1Cox17 in the presence of 0.1 – 2.5 μM ammonium TTM. Conditions: Cox17 1 μM; 20 mM ammonium acetate, pH=7.3, DTT 50 μM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex.

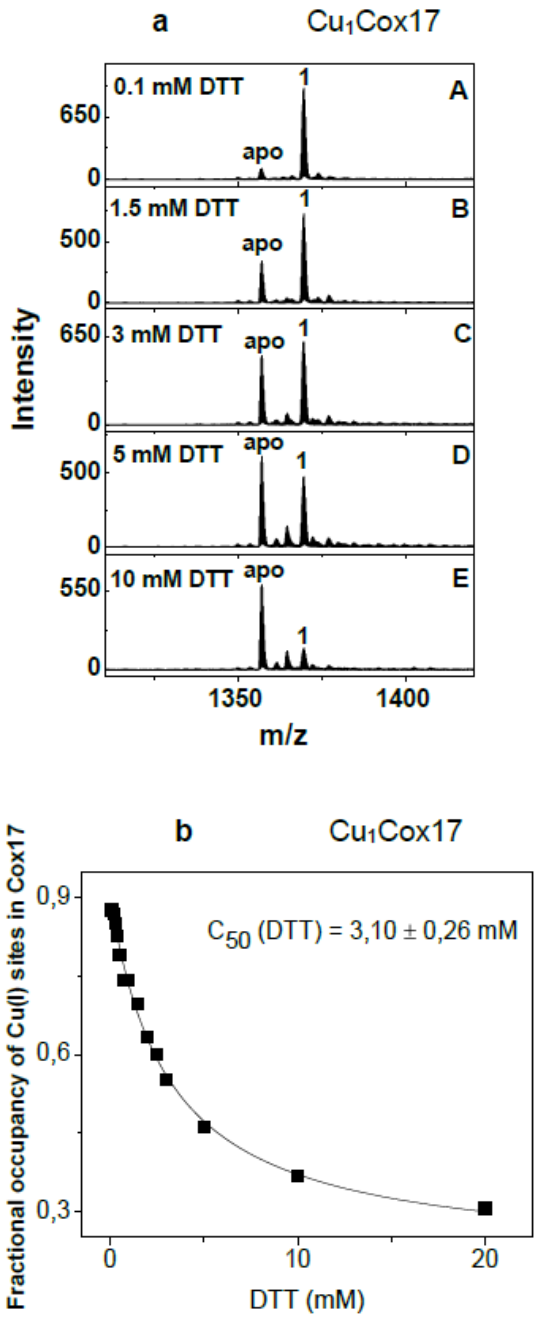

Fig. S6. Determination of the relative Cu(I)-binding affinity of DTT in competition with Cu_1Cox17.

(*a*) ESI-MS spectra of Cu_1Cox17 in the presence of 0.1 – 10 mM DTT. Conditions: Cox17 1 μM; 20 mM ammonium acetate, pH=7.3, DTT 50 μM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex. (*b*) Fractional occupancy of Cu(I)-binding sites in Cox17 at different concentrations of DTT in a metal competition experiment. The solid line shows the fitting curve with hyperbolic equation (y=P1*[1-(x/[P2+x])]+P3), where P2 = C_{50}.

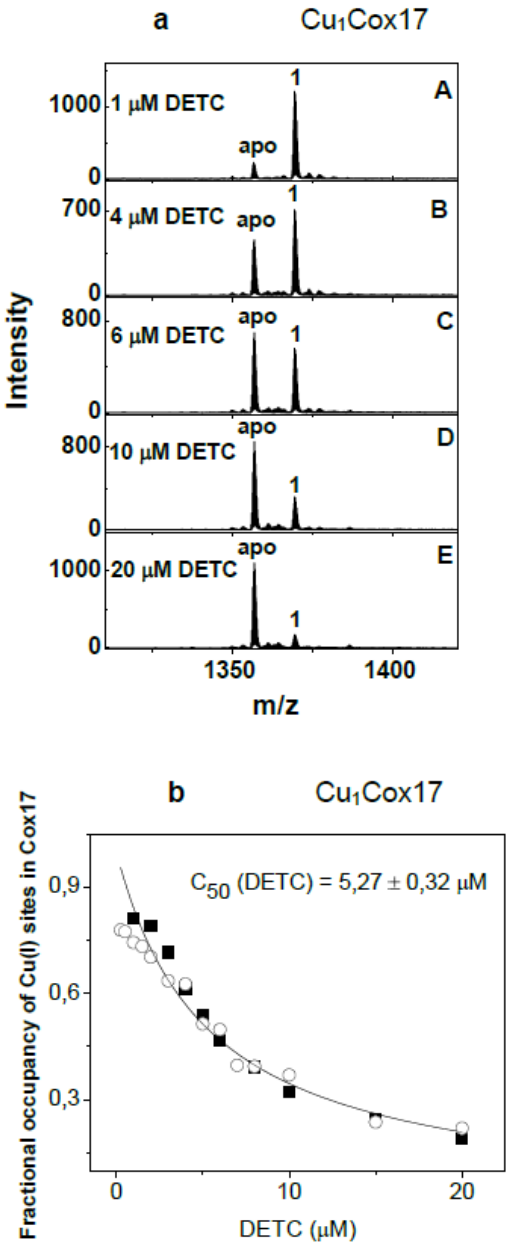

Fig. S7. Determination of the relative Cu(I)-binding affinity of DETC in competition with Cu1Cox17.

(a) ESI-MS spectra of Cu_1Cox17 in the presence of 1 – 20 µM DETC. Conditions: Cox17 1 µM; 20 mM ammonium acetate, pH=7.3, DTT 50 µM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex. (*b*) Fractional occupancy of Cu(I)-binding sites in Cox17 at different concentrations of DETC in a metal competition experiment. Results of duplicate experiments are presented with different symbols. The solid line shows the fitting curve with hyperbolic equation (y= P1*[1-(x/[P2+x])]+P3), where P2 = C_{50}.

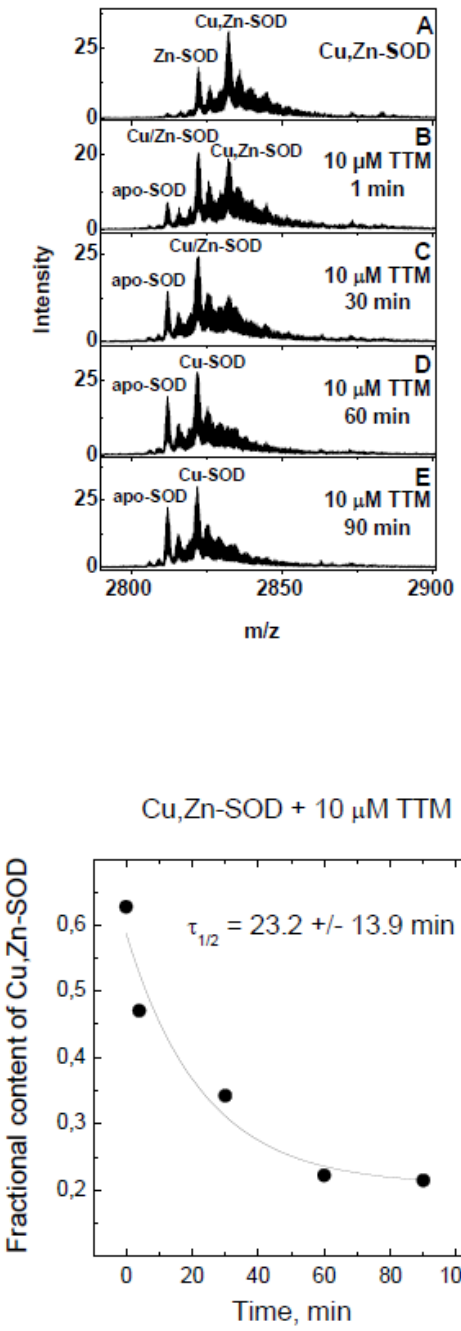

Fig. S8. Dissociation of Cu(I) ions from Cu,Zn-SOD by the influence of TTM.

(*a*) ESI-MS spectra of 5 μM Cu,Zn-SOD 2a in the presence of 10 μM TTM. Conditions: 20 mM ammonium acetate, pH=7.3, DTT 10 mM; T=25°C. Ions with a charge state +6 are shown; (*b*) Demetallation kinetics of 5 μM Cu,Zn-SOD by the influence of 10 μM TTM.

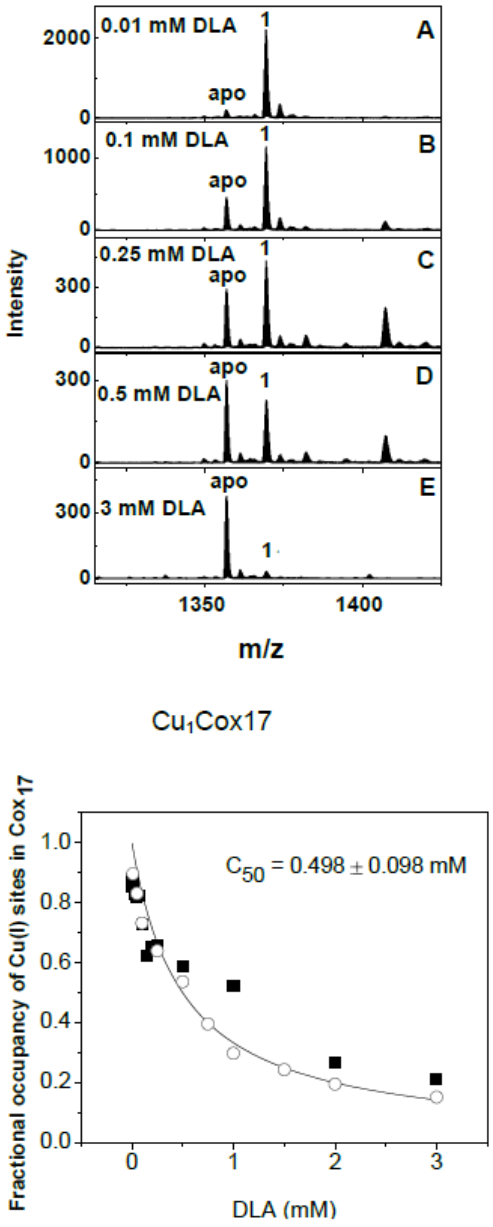

Fig. S9. Determination of the relative Cu(I)-binding affinity of DLA in competition with Cu_1Cox17.

(*a*) ESI-MS spectra of Cu_1Cox17 in the presence of 1 μM – 3 mM DLA. Conditions: Cox17 1 μM; 20 mM ammonium acetate, pH=7.3, DTT 50 μM; T=25°C. Ions with a charge state +5 are shown; numbers on the peaks denote the metal stoichiometry of the complex. (*b*) Fractional occupancy of Cu(I)-binding sites in Cox17 at different concentrations of DMS in a metal competition experiment. Results of duplicate experiments are presented with different symbols. The solid line shows the fitting curve with hyperbolic equation (y= P1*[1-(x/[P2+x])]+P3), where P2 = C_{50}.