Electronic Supplementary Material (ESI) for Metallomics.

Supporting information

for

Copper Finger Protein of Sp1: the Molecular Basis of Copper Sensing

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Contents

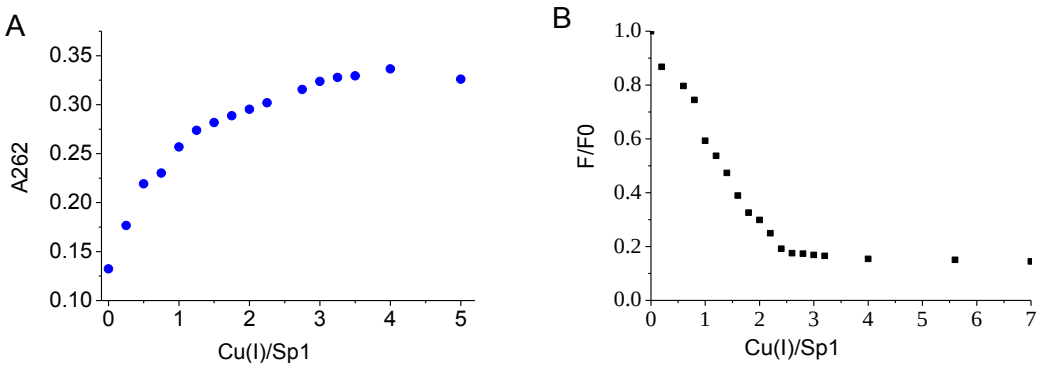

Figure S1. Characterization of Cu(I) binding to Sp1. (A) UV titration of Cu(I) to apo-Sp1; (B) Fluorescence titration of Cu(I) to apo-Sp1. All experiments were conducted on 10 μM protein at 25°C in HEPES buffer containing 100 mM NaCl in the presence of 5 molar equivalents of TCEP.

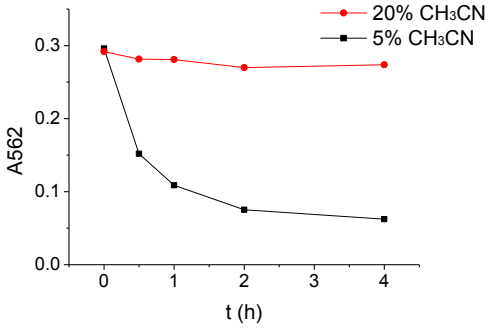

Figure S2. The stability of $[Cu(CH_3CN)_4]^+$ in CH_3CN solution. 50 μM $[Cu(CH_3CN)_4]\cdot PF_6$ was dissolved in 5% or 20% CH_3CN (V/V), and samples were open to air in eppendorf tubes at 37 °C. After different incubation time, 200 μM Cu(I) dye BCA was added and the UV absorption at 562 nm measured. The result showed that Cu(I) content gradually decreased in 5% CH_3CN, indicating the oxidation of $[Cu(CH_3CN)_4]^+$. However, $[Cu(CH_3CN)_4]^+$ was rather stable in 20% CH_3CN; ~ 93% copper remained Cu(I) state in 4 h.

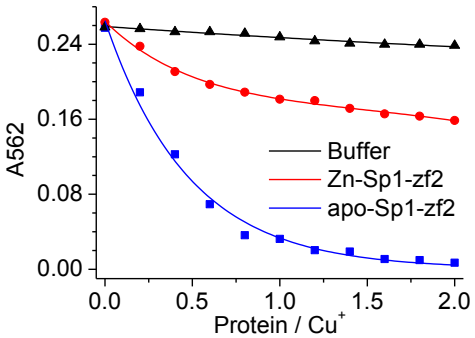

Figure S3. The substitution of Cu(I) coordination in $[Cu(BCA)_2]^{3-}$ complex by Sp1-zf2. The color of curves denotes the apo-Sp1-zf2 (blue), Zn-Sp1-zf2 (red) and buffer control (black). Experiments were carried out on 40 μM $[Cu(BCA)_2]^{3-}$ in 20 mM HEPES buffer containing 100 mM $NaNO_3$. 0.2 molar equivalent of protein was titrated in each aliquot and the UV absorbance at 562 nm was recorded.

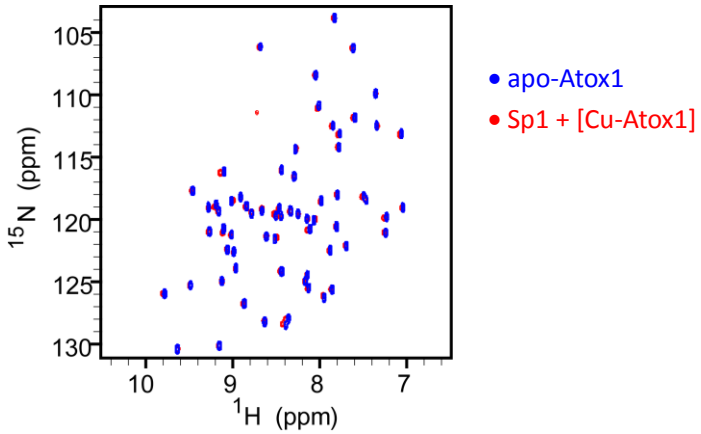

Figure S4. Superposition of 2D ^{1}H-^{15}N HSQC NMR spectra of apo-Atox1 (blue) and Cu-Atox1 with equimolar apo-Sp1 addition (red). NMR spectra were recorded at 298 K in 50 mM HEPES (pH=7.40) containing 100 mM NaCl. Two spectra are nearly identical, indicating the generation of apo-Atox1 in the reaction of Cu-Atox1 and Sp1.

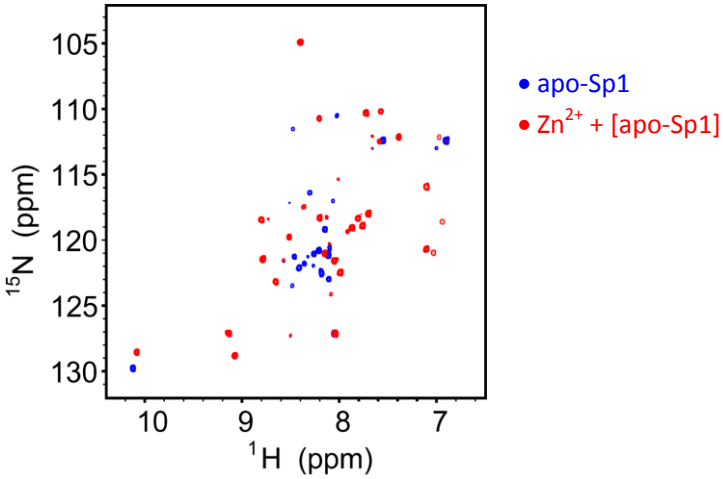

Figure S5. Superposition of 2D ^{1}H-^{15}N HSQC NMR spectra of apo-Sp1-zf2 before (blue) and after (red) the incubation with equimolar of Zn(II) ions.

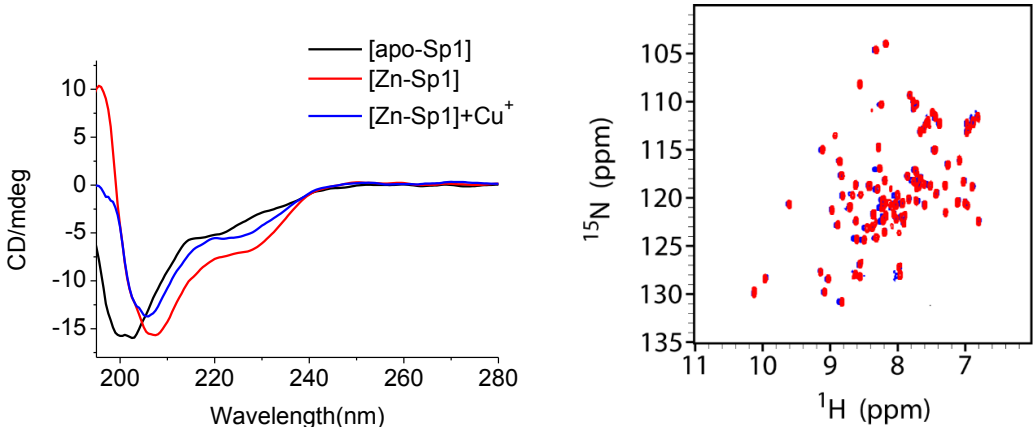

Figure S6. (A) Far-UV CD spectra of Sp1. Curves denote apo-Sp1 (black line), Zn-Sp1 (solid red line) and Zn-Sp1 with 3 equimolar Cu^{+} ions (blue line). (B) Superposition of 2D ^{1}H-^{15}N HSQC NMR spectra of Zn-Sp1 before (blue) and after (red) the incubation with 3 molar equivalents of Cu(I) ions.

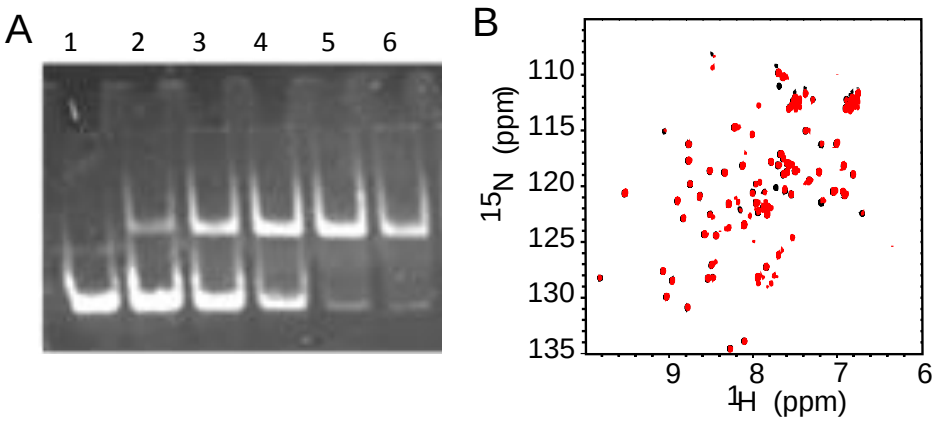

Figure S7. (A) The formation of Sp1/DNA complex. Lane 1: DNA control; Lanes 2-6: DNA was incubated with different molar equivalents of Zn-Sp1 (1, 2, 3, 4 and 5, respectively). (B) The superposition of 2D ^{1}H-^{15}N HSQC NMR spectra of Zn-Sp1 in the absence (black) and presence (red) of DNA.

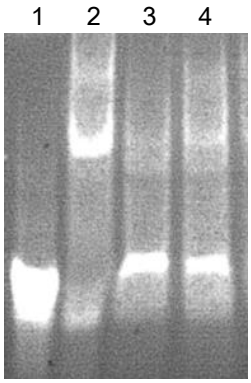

Figure S8. Cu(I) inhibits the binding of Sp1 to *hCtr1* promoter. Lane 1: DNA control; Lane 2: DNA/Sp1 complex; Lane 3: DNA/Sp1 complex incubated with 3 molar equivalents of Cu(I); Lane 4: Cu(I)-Sp1 incubated with DNA. Reactions were conducted in 50 mM HEPES buffer (pH 7.4) containing 100 mM NaCl in the presence of 0.2 mM GSH.

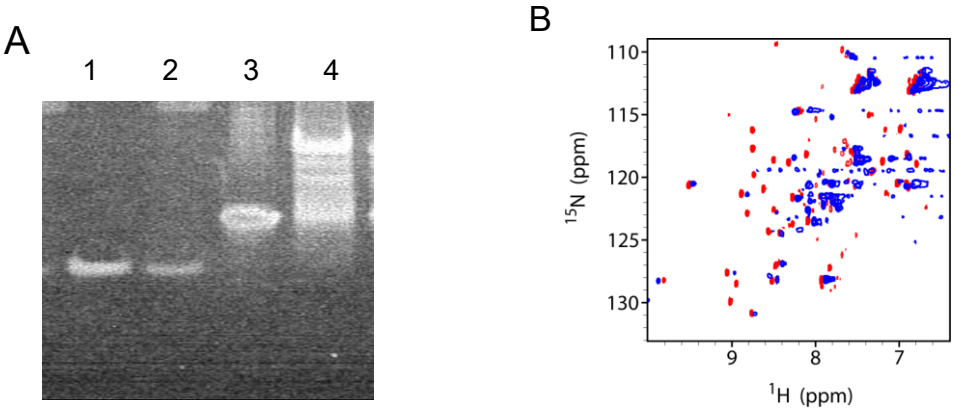

Figure S9. (A) The influence of DNA sequence to Zn-Sp1/DNA complexes. Lane 1: a scramble DNA; Lane 2: a scramble DNA with Zn-Sp1 addition; Lane 3: *hCtr1* promoter; Lane 4: *hCtr1* promoter with Zn-Sp1 addition. (B) Superposition of 2D ^{1}H-^{15}N HSQC NMR spectra of Zn-Sp1with the addition of *hCtr1* promoter (red) and a scramble DNA sequence (blue). The scramble DNA sequence is AATTAGCTAATT. Samples were prepared with 30 μM Zn-Sp1 and 10 μM DNA in 50 mM HEPES (pH=7.40) containing 100 mM NaCl. NMR spectra were recorded at 298 K.