Electronic Supplementary Material (ESI) for Inorganic Chemistry Frontiers.

Supporting information
for
Cuprous Ions can Disrupt Structure and Functions of the RING Finger Domain of RNF11

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Table S1. Analysis of ESI-MS peaks in Figure 1A

Composition	Formula	m/z (charge)	MW: obsd./cald.
Zn-RNF11	$C_{312}H_{477}N_{81}O_{90}S_{11}Zn_2$	1457.420(+5)	7286.100/7286.081

Table S2. Analysis of ESI-MS peaks in Figure 3D and 3E

Composition	Formula	m/z (charge)	MW: obsd./cald.
apo-RNF11	$C_{312}H_{477}N_{81}O_{90}S_{11}$	1432.049 (+5)	7155.245/7155.226
Cu-RNF11	$C_{312}H_{477}N_{81}O_{90}S_{11}Cu_2$	1457.198 (+5)	7282.990/7283.09

Table S3. ICP-AES to detect the Cu(I) substitution of Zn(II) in Zn-RNF11

Sample	Metallic element	Binding ratio
Zn-RNF11	Zn	1.97
	Cu	/
Cu-RNF11	Zn	0.52
	Cu	2.31

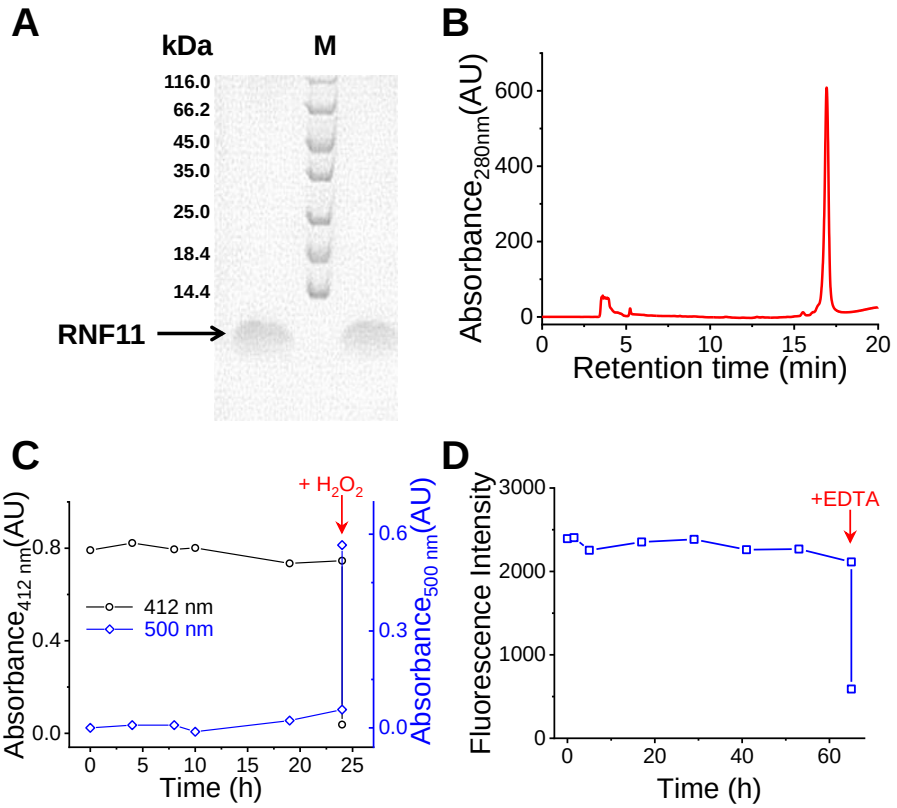

Figure S1. Purification and stability verification of RNF11. (A) Tricine-SDS-PAGE analysis of RNF11; (B) HPLC profile of apo-RNF11. (C) Stability of Zn-RNF11. The content of thiol group in Zn-RNF11 was monitored by Ellman's assay at 412 nm (black curve) with addition of DTNB; the Zn(II) ions released from Zn-RNF11 was monitored by PAR assay at 500 nm (blue curve) with addition of PAR dye. The measurements were performed after incubation for different time at 37 °C. After 24 h incubation, H_2O_2 was added into Zn-RNF11 to fully oxidize the thiol group and release zinc ions in order to further confirm the stability of Zn-RNF11. (D) The fluorescence of Zn-RNF11 measured after incubation at 37 °C for different time. After 65 h incubation, EDTA can added to remove Zn(II) ions from the protein. The steady fluorescence during 65 h and the dramatic drop upon adding EDTA indicates the high stability of Zn-RNF11.

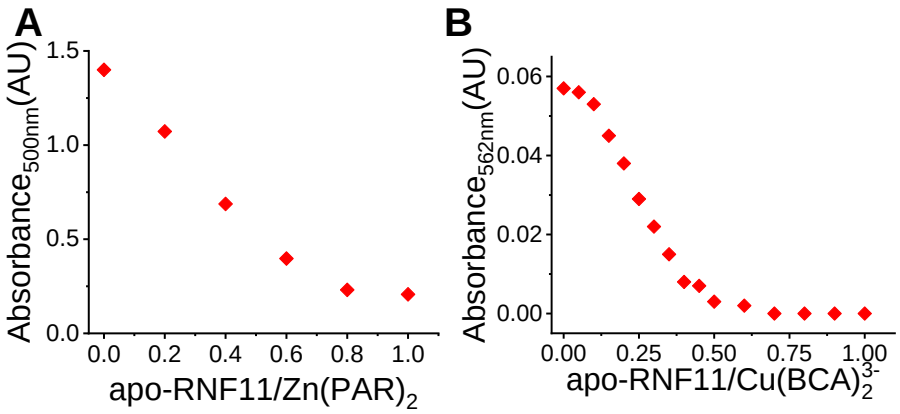

Figure S2. The UV-vis analyses of titration of apo-RNF11 into (A) $Zn(PAR)_2$ or (B) $Cu(BCA)_2$. (A) $Zn(PAR)_2$ measured at 500 nm; (B) $Cu(BCA)_2$ measured at 562 nm.

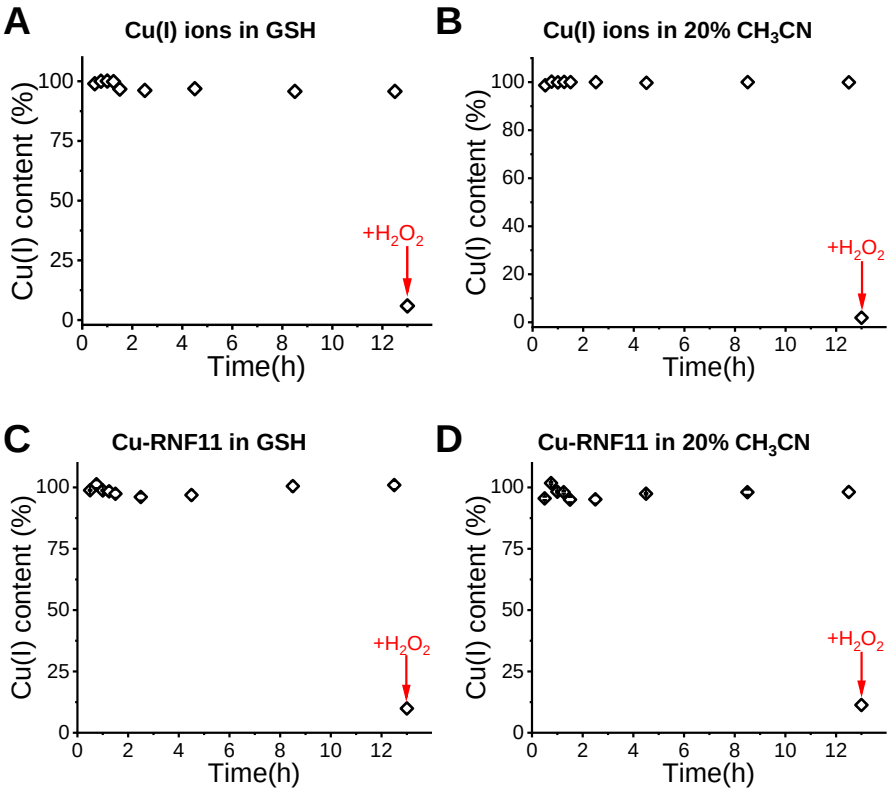

Figure S3. Redox stability of Cu(I) ions under air atmosphere. (A-B) Content of cuprous state ions in the free Cu(I) ions in the presence of 6 molar equivalents of GSH (A) or 20% acetonitrile (v/v) (B). The measurements were conducted on 40 μM $[Cu(CH_3CN)_4]ClO_4$ solution in 50 mM HEPES (pH 7.4), and the amount of Cu(I) at different time was measured using BCS assay. (C-D) Content of cuprous state ions in the Cu-RNF11 protein in the presence of 6 molar equivalents of GSH (C) or 20% acetonitrile (v/v) (D). The measurements were conducted on 20 μM Cu-RNF11 protein in 50 mM HEPES (pH 7.4). After 12 hours measurements, H_2O_2 was added into the samples to fully oxidize Cu(I) to Cu(II), confirming that the oxidation Cu(I) can be effectively detected by this method.

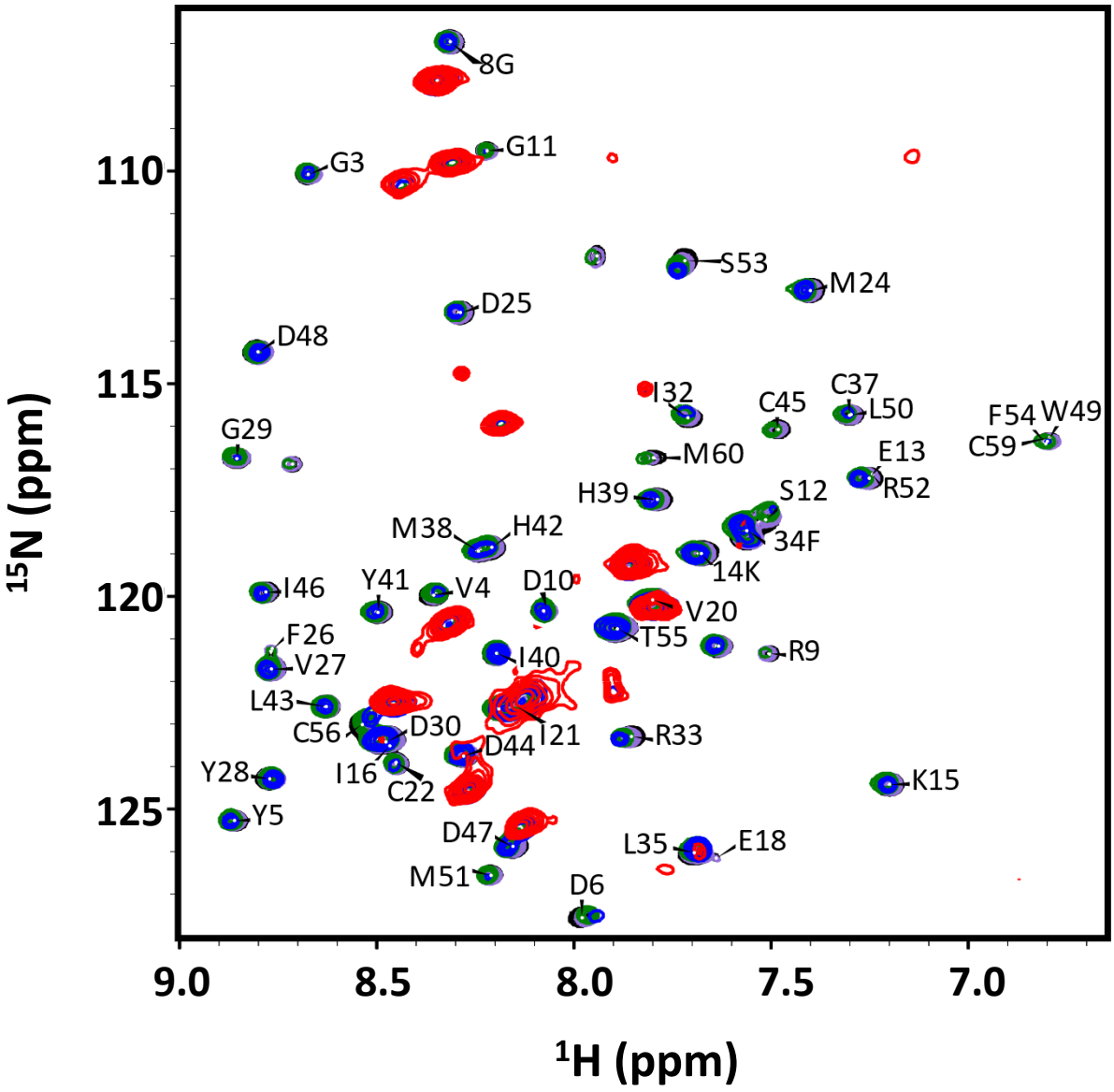

Figure S4. Superposition of ^{1}H-^{15}N HSQC NMR spectra of 0.28 mM Zn-RNF11 before (black) and after incubation with 0.5 (purple), 1 (green), 1.5 (blue), 2 (red) molar equivalents of Cu(I). The reactions were performed in the presence of 6 molar ratios of GSH in 50 mM HEPES buffer (pH 7.4) at 25 °C.