Table of Contents

Supporting Methods

Supporting Figures

Figure S1 – Current measurements on ATP7A mutants.

Figure S2 – ESI-MS spectrum of $^{15}N,^{13}C$-Cys *apo*Mnk1.

Figure S3 – 2D $^{1}H,^{13}C$-HSQC spectra of $^{15}N,^{13}C$-Cys *apo*Mnk1 and Cu^{+}-Mnk1 incubated with cisplatin.

Figure S4 – Overlay of 2D $^{1}H,^{13}C$-HSQC spectra of $^{15}N,^{13}C$-Cys Mnk1 in the absence and in the presence of cisplatin.

Figure S5 – 2D $^{1}H,^{15}N$-HSQC spectra of *cis*-$PtCl_2(^{15}NH_3)_2$ incubated with *apo*Mnk1 in the presence of glutathione.

Figure S6 – ESI-MS spectra of $^{15}N,^{13}C$-Cys Cu^{+}-Mnk1 incubated with cisplatin.

Figure S7 – 2D $^{1}H,^{15}N$-HSQC spectra of $^{15}N,^{13}C$-Cys *apo*Mnk1 and Cu^{+}-Mnk1 incubated with cisplatin.

References

Supporting Methods

Synthesis of platinum complexes. Zeise's salt ($K[PtCl_3(\eta^2\text{-}C_2H_4)\cdot H_2O]$),[1] cisplatin (*cis*-$[PtCl_2(NH_3)_2]$),[2] and oxaliplatin ([Pt(*R*-*R*-1,2(DACH)(oxalate)]),[3] were prepared by literature methods.

For the synthesis of *cis*-$[Pt(NH_3)_2(H_2O)(SO_4)]$ and [Pt(1,2-DACH)$(H_2O)(SO_4)$], a water suspension of *cis*-$[Pt(NH_3)_2I_2]$ or [Pt(1,2-DACH)Cl_2] was treated with a stoichiometric amount of $AgSO_4$, and the reaction mixture kept stirring in the dark at room temperature for 24 h. The suspension was filtered through celite, and the mother liquor taken to dryness under reduced pressure. The oily residue, upon addition of EtOH, afforded a white solid, which was filtered off, washed with EtOH, and dried under vacuum. A similar procedure was adopted for preparing ^{15}N-labeled *cis*-$[Pt(^{15}NH_3)_2(H_2O)(SO_4)]$.

Protein production. Wild-type and mutant recombinant Cu-ATPases (ATP7A and ATP7B) were obtained from COS-1 cells infected with adenovirus vectors containing CMV promoter-driven cDNA fused with a 3' c-Myc tag as previously described.[4] The content of the specific expressed protein was evaluated in the microsomal fraction of the infected COS-1 cells by SDS gel electrophoresis and Western blotting.

Electrical measurements. Charge movements were measured by adsorbing microsomes from COS-1 cells containing the Cu-ATPase (ATP7A or ATP7B) onto an alkanethiol/phospholipid bilayer anchored to a gold electrode (the SSM).[5] Then, Cu-ATPases were activated by ATP concentration jumps. If the ATP jump induces charge displacement into the microsomes, a current transient is recorded. Kinetic information can be obtained by fitting with exponential terms the decaying current versus time curves.

In ATP concentration-jump experiments, the buffer solution contained 300 mM KCl, 50 mM MES-triethanolamine (pH 6.0), 5 mM $MgCl_2$, 0.1 mM $CaCl_2$, 10 mM DTT, and 5 μM metallic species under investigation ($CuCl_2$ or Pt complex). In the case of Pt, the 5 μM concentration was obtained by addition of the required amount of *cis*-$[Pt(NH_3)_2(H_2O)(SO_4)]$ or *cis*-[Pt(R,R-DACH)$(H_2O)(SO_4)$] taken from a 10 mM water solution. In the latter case, before performing the experiment, the buffer solution was allowed to equilibrate for ~ 30 min. The ATP7A/B incorporating microsomes adsorbed on the SSM were first incubated with the buffer solution for ~ 30 min, then the buffer

solution was replaced by an identical solution containing 5 μM metallic species and, in addition, 100 μM ATP for ATPase activation.

Charge measurements were performed by the SURFE^{2}R^{One} device (Nanion Technologies, Munich, Germany). The temperature was maintained at 22-23 °C for all the experiment time.

To verify the reproducibility of the current transients on the same SSM, each measurement was repeated six times and then averaged to improve the signal-to-noise ratio. Standard deviations did not exceed 5%. Each set of measurements was usually reproduced using 3-4 different SSM electrodes.

Sample preparation for ESI-MS and NMR experiments. Cisplatin and oxaliplatin were dissolved immediately prior to use in pure deoxygenated water at 2 mM final concentration. The complex solutions were extensively vortexed and sonicated and the exact Pt concentration was determined by atomic absorption spectroscopy using a Varian 880Z instrument.

The preparation of the protein samples was carried out in an anaerobic chamber.

The ^{15}N,^{13}C-Cys Mnk1, purchased from Giotto Biotech S.r.l. (Sesto Fiorentino, Italy), contained 1 mM DTT and 50 mM phosphate buffer (pH 7.0). To completely remove the DTT reducing agent, the Mnk1 samples were washed with deoxygenated water by using Amicon Ultra centrifugal filters (3kDa MWCO) under inert N_2 atmosphere and immediately used for the incubation with the metallic species.

Cu^+-Mnk1 was obtained by addition of one equivalent of $[Cu^I(CH_3CN)_4]PF_6$ directly to *apo*Mnk1.

Electrospray Mass Spectrometry. ESI-MS was performed with an electrospray interface and an ion trap mass spectrometer (1100 Series LC/MSD Trap system, Agilent, USA).

The reaction between Mnk1 (100 μM) and the Pt complex (100 μM) was carried out with stirring under anaerobic conditions at 25 °C. From time to time, aliquots of the reaction mixture were removed, diluted five-fold with deoxygenated water, treated with 1% acetic acid (1:1, v/v) to ensure a good volatilization, and then injected at a rate of 10 μL/min. Ionization was achieved in the positive ion mode by application of +4 kV at the entrance of the capillary; the pressure of the nebulizer gas was 15 psi.

The drying gas was heated to 350 °C at a flow rate of 5 l/min. Full-scan mass spectra were recorded in the mass/charge (m/z) range of 50-2200.

Nuclear Magnetic Resonance Spectroscopy. NMR experiments were recorded on a ^{15}N,^{13}C-Cys Mnk1 sample (100 μM) in 50 mM phosphate buffer (pH 7.0) at 25 °C. Resonance assignment of

apo- and Cu^{+}-Mnk1 was carried out by using the available ^{1}H, ^{15}N, and ^{13}C chemical shift data[6,7] and with the aid of 2D $^{1}H,^{13}C$ HSQC-TOCSY spectra.

The ^{15}N-labeled complex, *cis*-$[PtCl_2(^{15}NH_3)_2]$, was added to the protein (*apo* or Cu^{+}-Mnk1) to obtain an equimolar mixture and the reaction was monitored through $^{1}H,^{15}N$ and $^{1}H,^{13}C$ HSQC spectra, acquired using a gradient-enhanced sequence in which coherence selection and water suppression were achieved via gradient pulses. 16 transients were acquired over an F2 (^{1}H) spectral width of 14 ppm into 1024 complex data points for each of 256 t_1 increments with an F1 spectral width of 40 ppm centered at 120 ppm (for amide ^{15}N) or 100 ppm centered at -50 ppm (for $^{15}NH_3$), or 70 ppm centered at 40 ppm (for ^{13}C). The sequence was optimized with a delay $1/(4J_{XH})$ of 2.78 ms (X = ^{15}N) or 1.72 ms (X = ^{13}C), and a recycle delay of 1.0 s.

All spectra were collected on a Bruker Avance 600 UltraShield Plus magnet using a triple-resonance (TXI) probe equipped with pulsed-field gradients along the z axis, processed using the standard Bruker software (TOPSPIN), and analyzed with the programs CARA, developed at ETH-Zürich (www.cara.nmr.ch).

Supporting Figures

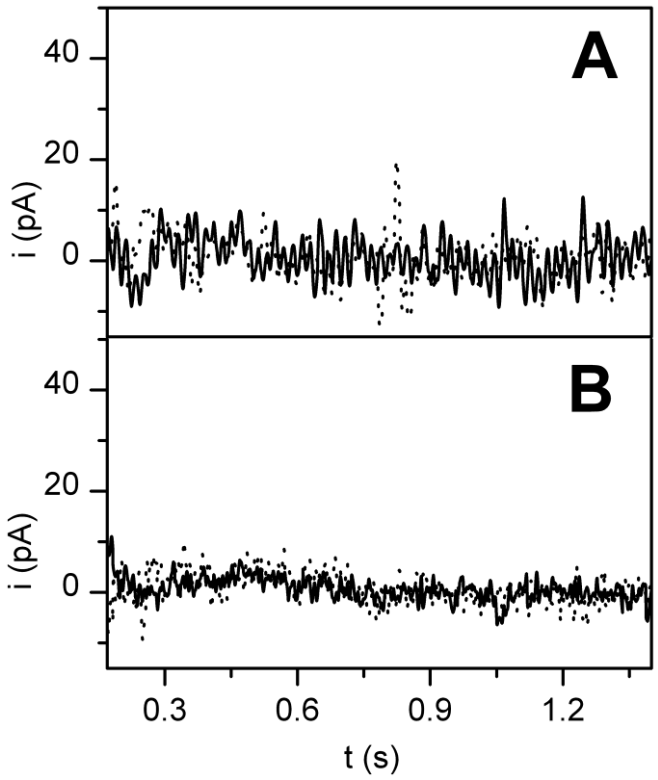

Figure S1 – Current measurements after 100 µM ATP concentration jumps in the presence of 5 µM $CuCl_2$ (*dotted lines*) or 5 µM aquated cisplatin (*solid lines*) on D1044A ATP7A mutant (A), and C575A/C578A ATP7B double mutant (B).

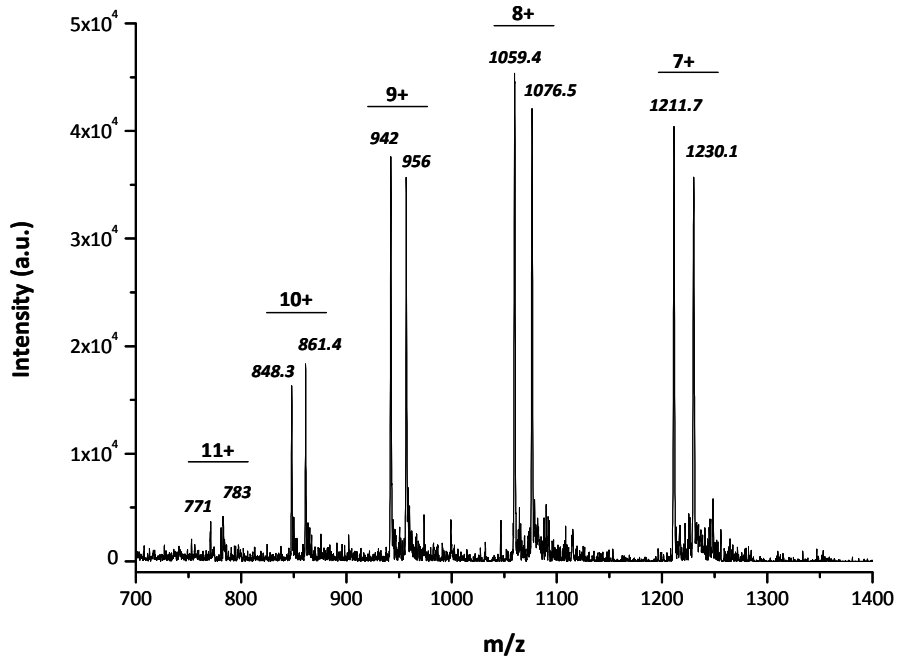

Figure S2 – ESI-MS spectrum of $^{15}N,^{13}C$-Cys Mnk1 in its *apo* state.

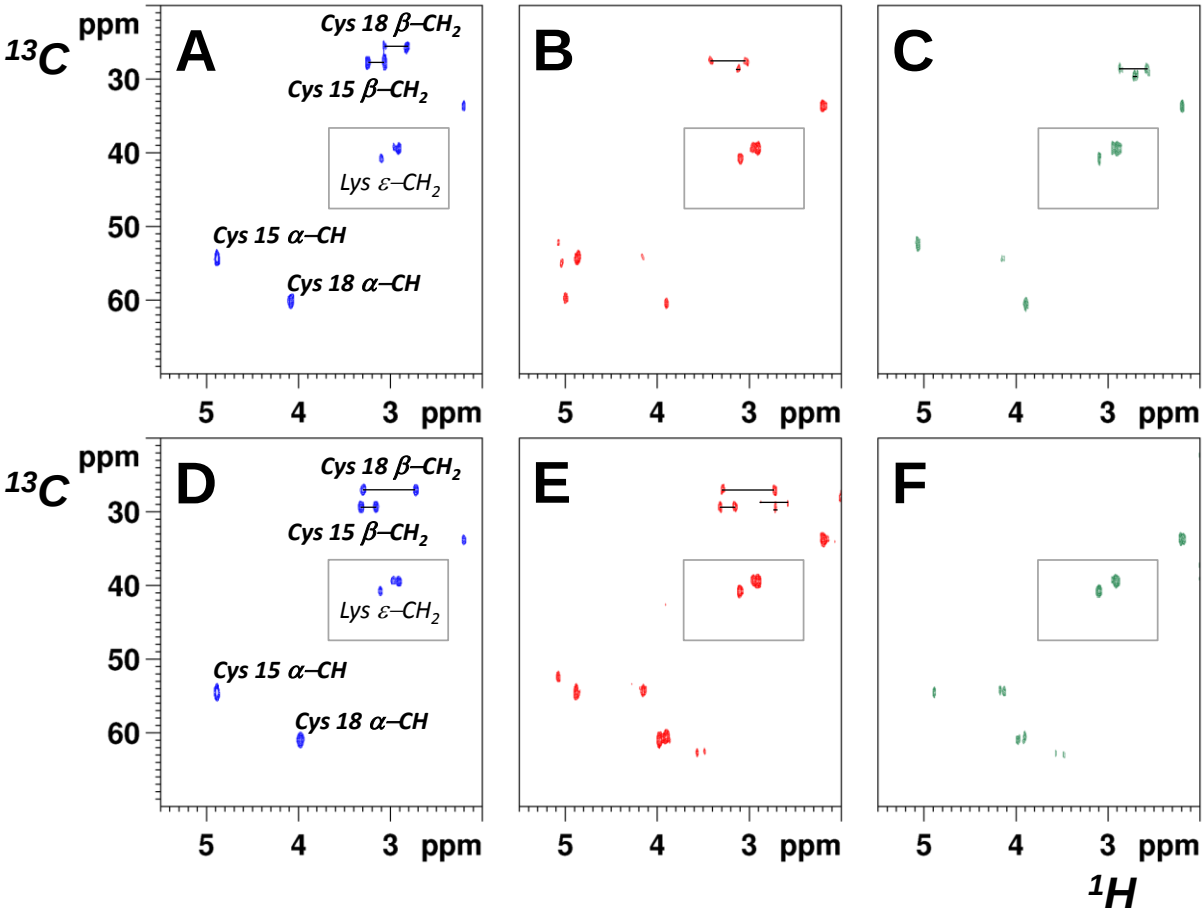

Figure S3 – 2D $^{1}H,^{13}C$-HSQC spectra of cisplatin-incubated $^{15}N,^{13}C$-Cys *apo*Mnk1 (A-C) and Cu^{+}-Mnk1 (D-F) at equimolar ratio, recorded at 0 (A and D, *blue contours*), 3 (B and E, *red contours*), and 24 h (C and F, *green contours*) after mixing. The assignment of Cys signals of apoMnk1 and Cu^{+}-Mnk1 is shown in panels A and D, respectively.

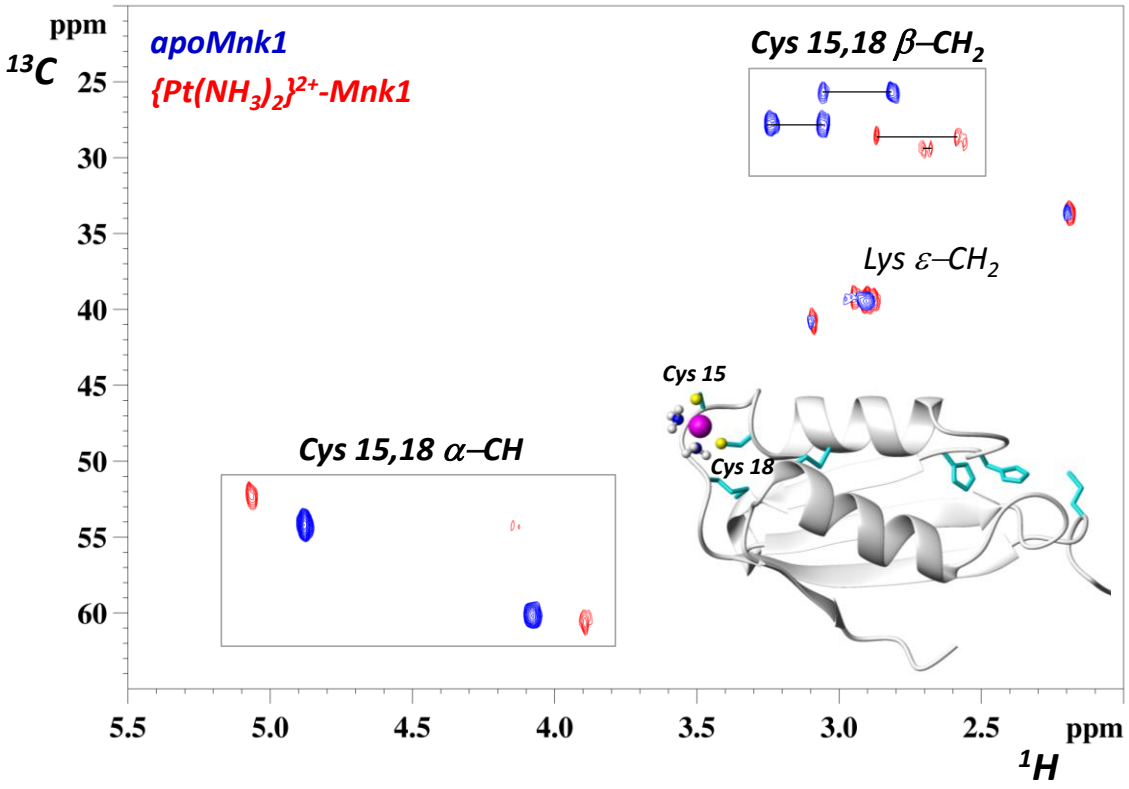

Figure S4 – Overlay of 2D $^{1}H,^{13}C$-HSQC spectra of $^{15}N,^{13}C$-Cys Mnk1 in the absence (blue contours) and in the presence (red contours) of 1 mol equiv of cisplatin (after 24 h incubation). The inset shows a structural model of the cisplatin-Mnk1 adduct. The Pt atom is shown in magenta, the N atoms of the ammine ligands are in blue, and the S atoms of Cys15 and Cys18 are in yellow. The side chains of Cys and other potential Pt binding residues (His and Met) are shown in cyan.

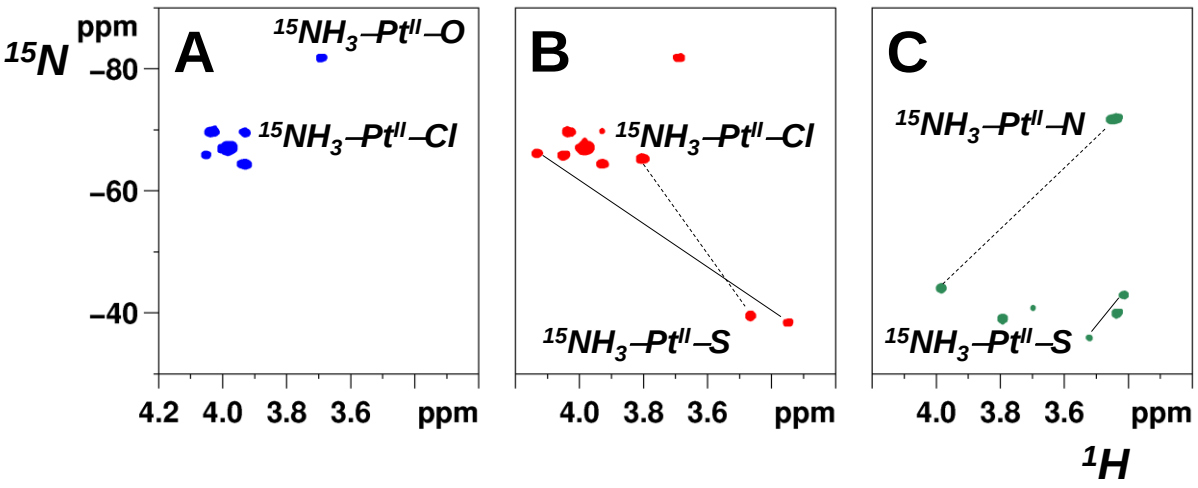

Figure S5 – 2D ^{1}H,^{15}N-HSQC spectra of *cis*-$PtCl_2(^{15}NH_3)_2$ incubated with 1 mol equiv of *apo*Mnk1 in 1 mM reduced glutathione (GSH), at 0 (A, *blue contours*), 3 (B, *red contours*), and 24 h (C, *green contours*) after mixing. Cross-peaks are assigned to $^{15}NH_3$ trans to N, Cl, or S donor atoms, respectively. Cross-peaks belonging to Mnk1 and to GSH adducts are connected by straight lines and by dashed lines, respectively. There is perfect coincidence with cisplatin-Mnk1 cross-peaks detected in the absence of GSH.

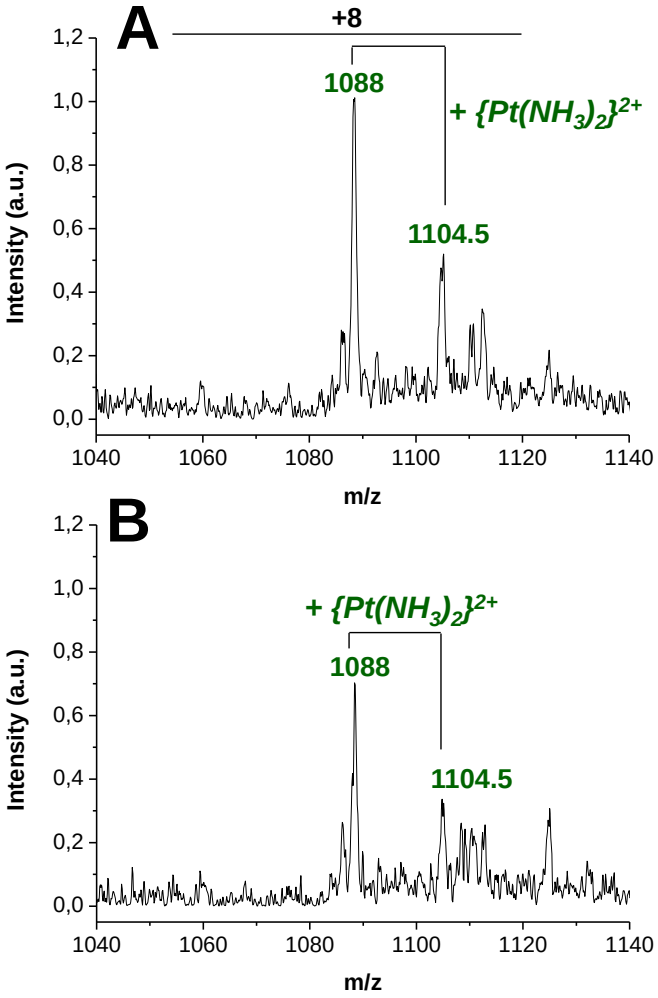

Figure S6 – ESI-MS spectra of $^{15}N,^{13}C$-Cys Cu^{+}-Mnk1 treated with 1 mol equiv of cisplatin after 3 (A) and 24 h (B) of incubation. Peaks corresponding to +8 charged state are shown. Each species comprises two pairs of signals corresponding to Mnk1 with and without Met1 as first residue.

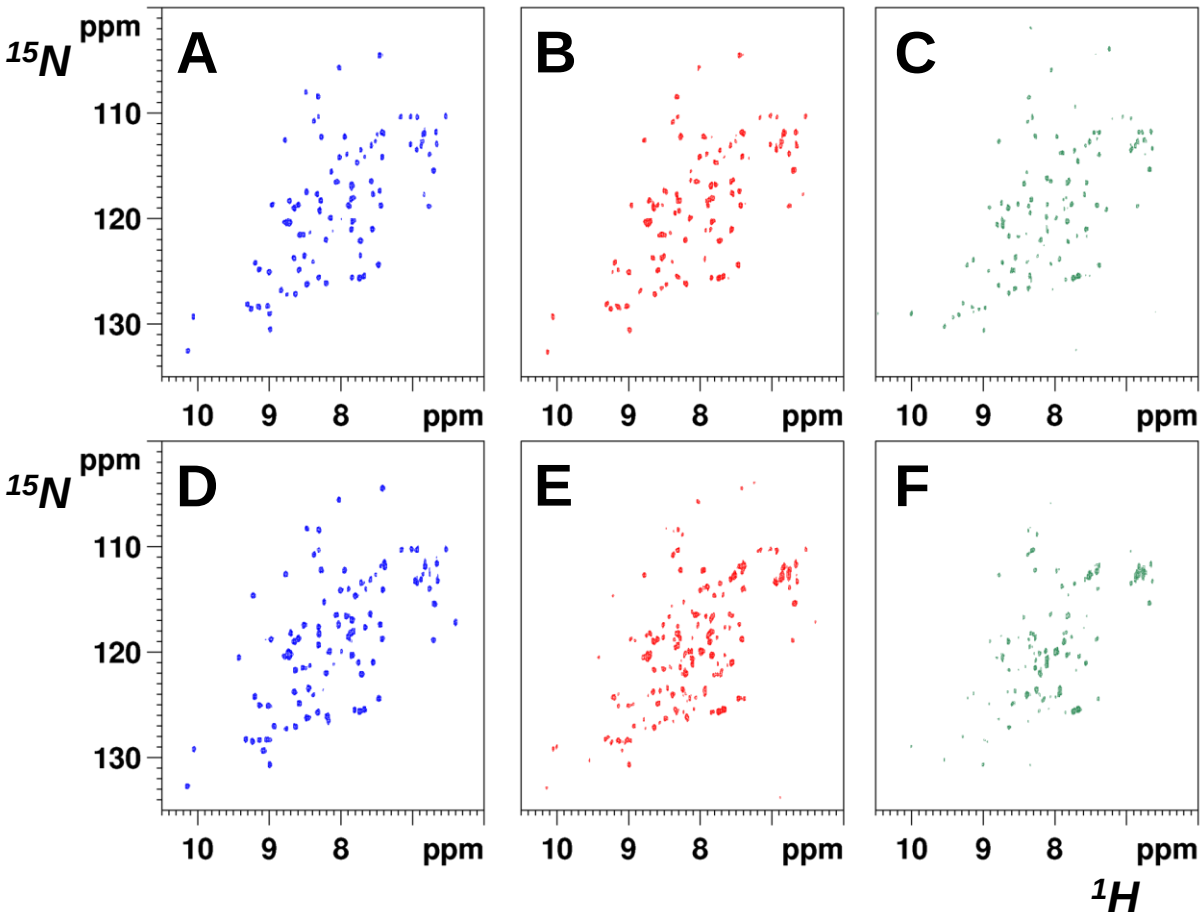

Figure S7 – 2D $^{1}H,^{15}N$-HSQC spectra of $^{15}N,^{13}C$-Cys *apo*Mnk1 (A-C) and Cu^{+}-Mnk1 (D-F) incubated with 1 mol equiv of cisplatin, recorded at 0 (A and D, *blue contours*), 3 (B and E, *red contours*), and 24 h (C and F, *green contours*) after mixing.

---

[1] C. R. Barone, M. Benedetti, V. M. Vecchio, F. P. Fanizzi, L. Maresca, G. Natile, *Dalton Trans.* **2008**, 5313-5322.
[2] S. Dhara, *Indian J. Chem.* **1970**, *8*, 193-194.
[3] Y. Kidani, M. Noji, T. Tashiro, *Gann* **1980**, *71*, 637-643.
[4] R. Pilankatta, D. Lewis, C. M. Adams, G. Inesi, *J. Biol. Chem.* **2009**, *284*, 21307-21316.
[5] F. Tadini-Buoninsegni, G. Bartolommei, M. R. Moncelli, K. Fendler, *Arch. Biochem. Biophys.* **2008**, *476*, 75-86.
[6] T. M. DeSilva, G. Veglia, S. J. Opella, *Proteins* **2005**, *61*, 1038-1049.
[7] L. Banci, I. Bertini, F. Cantini, N. Della Malva, M. Migliardi, A. Rosato, *J. Biol. Chem.* **2007**, *282*, 23140-23146.