Electronic Supplementary Information

Kinetic analysis of copper transfer from a chaperone to its target protein mediated by complex formation

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Experimental Procedures

Construction of an expression vector for the overproduction of $(His)_6$-tagged CopZ. A 200 bp fragment containing the *copZ* gene was amplified by PCR, using pMKNC1 (which contains *copZ/yvgZ*) as template with primers 5'-TTCATATGGAACAAAAAACATTGC (YvgY1) and 5'-TTCTCGAGCGCTAC GTCATAGCCC (YvgY2), which introduced a *Nde*I site at the *copZ* translational start site and a 3' *Xho*I site in place of the TGA stop codon. The PCR product was ligated into *Sma*I-cut pUC18 generating pMKNC2. The *NdeI/XhoI* fragment was ligated into pET21a (Promega) cut with the same enzymes, generating pMKNC3 from which CopZ is expressed with a C-terminal $(His)_6$-tag. The *copZ* gene was confirmed by sequencing (MWG Biotech).

Purification of His_6CopZ, CopZ and CopAab. CopZ and unlabelled and ^{15}N-labelled CopAab were purified as previously described.[1, 2] For His_6CopZ, typically, 2.5 l (10 x 250 ml) of LB medium was inoculated with 10 x 1.5 ml of an overnight culture of *E. coli* BL21(DE3)pLysS containing pMKNC3, and incubated at 37 °C, 200 rpm until the OD_{600} was ~0.6. IPTG (1 mM) was then added and the cultures were incubated with shaking for a further 3 hr. Cells were harvested by centrifugation at 5000 × g for 20 min at 4 °C and re-suspended in 0.1 culture volume of buffer A (20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, pH 7.5). Lysosyme (Sigma) was added to a final concentration of 0.1 mg/ml and the cells incubated at 30 °C for 15 min with gentle shaking. DNase I and RNase A (Sigma) were added to final concentrations of 6 µg/ml and 80 µg/ml, respectively, and the cells were sonicated for 2 x 8 min 20 s using a Status US200 ultrasonicator (Novara) in pulse mode (0.2 s per s) set at 50% power, while on ice. The lysate was centrifuged at 39000 × g for 20 min at 4 °C and the supernatant was heated slowly to 75 °C, stirred continuously for 15 min and placed on ice for a further 15 min. The suspension was centrifuged at 39000 × g for 20 min at 4 °C. DTT was added to the supernatant to a final concentration of 15 mM, and the solution passed through a 0.45 µM filter (Sartorius) before loading onto a 5 ml HiTrap affinity column (GE Healthcare), previously charged with Ni(II), according to the manufacturer's instructions. The column was washed consecutively with 50 ml buffer A and 100 ml buffer B (20 mM Tris-HCl, 3 M NaCl, 60 mM imidazole, pH 7.5) to remove nucleic acid contamination. A 50 ml gradient of 0.06 to 1 M imidazole in the same buffer was applied with $(His)_6$-CopZ eluting at a concentration of ~300 mM imidazole. CopZ containing fractions (as determined by SDS/PAGE analysis) were concentrated to < 5 ml using an ultrafiltration cell fitted with a YM3 membrane (Amicon; Millipore) operating at a pressure of 50 psi and then desalted using a G25 Sephadex column (PD-10; GE Healthcare) into 100 mM HEPES, pH 7.0. Protein concentrations were calculated from UV-visible absorbance spectra, recorded on a Jasco V-550 spectrophotometer, using an extinction coefficient, $\varepsilon_{276\ nm}$, of 1450 $M^{-1}\ cm^{-1}$ for CopZ[3] and 5800 $M^{-1}\ cm^{-1}$ for CopAab.[1]

Preparation of Cu(I)-loaded samples. Prior to the addition of Cu(I), protein samples were treated with 5 mM DTT and excess reductant was removed by passage down a G25 Sephadex column (PD10, GE Healthcare) in an anaerobic glovebox (Faircrest), in which the oxygen concentration was kept below 2 ppm. A 1 mM acidified Cu(I)Cl solution[3, 4] was added to pre-reduced CopZ, His_6CopZ or CopAab protein using a microsyringe (Hamilton) in an anaerobic glovebox. Unbound Cu(I) was removed by passage of the sample down a G25 Sephadex column (PD10, GE Healthcare) equilibrated with working buffer. For solution-phase experiments, buffer was 100 mM MOPS, 100 mM NaCl, pH 7.5. For ESI-MS experiments, buffer was 20 mM ammonium acetate, pH 7.4 and the protein sample was diluted with 20 mM ammonium acetate to a working sample concentration of 15 µM.

Copper dissociation and transfer experiments. For on-column thermodynamic transfer experiments, apo or Cu(I)-loaded His_6CopZ was loaded onto the 1 ml Hitrap Ni(II)-affinity column that had previously been equilibrated with buffer A. Both apo-and Cu(I)-His_6CopZ were found to bind tightly to the column. Cu(I)-loaded or apo-CopAab (depending on the CopZ sample) was then loaded onto the column and eluted with 3 column volumes of buffer A, and 1.0 or 1.5 ml fractions collected. His_6CopZ was subsequently eluted with 3 column volumes of 20 mM Tris-HCl, 0.5 M NaCl, 500 mM imidazole, pH 7.5. Protein contents of each fraction were determined using the method of Bradford[5] (BioRad), with bovine serum albumin as the standard, and protein-containing fractions were further analysed by LC-MS. Fractions were also analysed for copper, using ICP-AE (Varian Vista), for which samples were diluted 16-fold in a final volume of 2% v/v nitric acid.

Kinetic transfer experiments were carried out using both CopZ and His_6CopZ (equivalent data were obtained for each; the His-tagged protein was easier to purify and so was used for the majority of the stopped-flow experiments), along with CopAab in 100 mM MOPS, 100 mM NaCl, pH 7.5, at variable temperature using an Applied Photophysics Bio-Sequential DX.17MV stopped-flow instrument. The flow path was flushed with deoxygenated buffer prior to each experimental run. For each experiment, 2000 data points were collected over 0.5 s on a logarithmic time base, and three measurements were averaged for each experiment. Absorbance kinetic data were fitted using Origin 8 software (OriginLab).

For off rate determination experiments, samples of CopZ loaded with 0.5 Cu(I) per protein and apo-His_6CopZ, each at 60 µM in 100 mM MOPS, 100 mM NaCl, pH 7.5, were loaded into separate dialysis cassettes (5 ml, Spectra/Por® Float-A-Lyzer G2 Biotech) and submerged in the same buffer solution with gentle stirring. UV-vis spectra were acquired at several time points over the course of 46 hr.

Electrospray ionisation mass spectrometry of CopZ and CopAab mixtures. LC-MS experiments were performed using an UltiMate 3000 HPLC system (Dionex, Sunnyvale, CA, USA) coupled to a Bruker micrOTOF-QIII electrospray ionisation (ESI) time-of-flight (TOF) mass spectrometer (Bruker Daltonics, Coventry, UK) operating in positive ion mode and calibrated using ESI-L Low Concentration Tuning Mix (Agilent Technologies, San Diego, CA). A 1 µL injection volume of protein in 2% acetonitrile was applied to a ProSwift® reversed phase RP-1S column (4.6 x 50mm; Dionex) at 25 °C. Gradient elution was performed at a flow rate of 200 µL/min using solvents A (0.1% formic acid) and B (acetonitrile, 0.1% formic acid), with the following chromatographic method: isocratic wash (2% B, 0–2 min), linear gradient from 2–100% B (2–12 min), followed by an isocratic wash (100% B, 12–14 min) and column re-equilibration (2% B, 14–15 min). MS acquisition parameters were as follows: dry gas flow 8 L/min, nebuliser gas pressure 0.8 Bar, dry gas 240 °C, capillary voltage 4500 V, offset 500 V, collision RF 650 Vpp. Processing and analysis of MS experimental data was carried out using Compass DataAnalysis version 4.1 (Bruker Daltonik, Bremen, Germany).

Samples for non-denaturing MS were prepared by first adding DTT (15 mM, Formedium) and removing excess reductant by passage down a G25 Sephadex column (PD10, GE Healthcare) in an anaerobic glovebox (Faircrest Engineering, O_2 concentration <2 ppm) using 20 mM ammonium acetate, pH 7.4 (Sigma) as the elution buffer. The protein sample was diluted with 20 mM ammonium acetate to a working sample concentration of 15 µM. To prepare Cu(I)-bound protein samples, a deoxygenated solution of Cu(I)Cl prepared in 100 mM HCl and 1 M NaCl was added to anaerobic, reduced CopZ or CopAab using a microsyringe (Hamilton) in an anaerobic glovebox. Unbound Cu(I) was removed by passage of the sample down a G25 Sephadex column (PD10, GE Healthcare) equilibrated with 20 mM ammonium acetate pH 7.4. Mass spectra were acquired using a Bruker micrOTOF-QIII electrospray ionisation (ESI) time-of-flight (TOF) mass spectrometer (Bruker Daltonics, Coventry, UK), in positive ion mode and calibrated as above. Native protein samples were introduced to the ESI source via a syringe pump (Cole-Parmer) at 5 µL/min, and data acquired for 3 min, with ion scans between 50 – 3000 m/z. MS acquisition was controlled using Bruker oTOF Control software, with parameters as follows: dry gas flow 5 L/min, nebuliser gas pressure 0.8 Bar, dry gas temperature 180 °C, quadrupole RF set at 2000 Vpp (25%) and 3200 Vpp (75%). Processing and analysis of MS experimental data was carried out using Compass DataAnalysis version 4.1 (Bruker Daltonik, Bremen, Germany). Neutral mass spectra were generated using the ESI Compass version 1.3 Maximum Entropy deconvolution routine over a mass range of 7200 – 35000 Da. Exact masses are reported from peak centroids representing the isotope average neutral mass. Predicted masses are given as the isotope average of the neutral protein or protein complex, in which Cu(I)-binding is expected to be charge compensated.[2]

NMR Titration of apo-CopAab with Cu(I)-CopZ. NMR spectra were collected at 298 K on Avance 900 Bruker spectrometers operating at proton nominal frequencies of 900.13 MHz. Spectra were processed using the standard Bruker software (XWINNMR), and analyzed through the CARA software.[6] Titrations of ^{15}N-labelled apo-CopAab with Cu(I)-CopZ in the presence of 1 mM DTT were performed by monitoring the ^{1}H-^{15}N HSQC spectral changes upon the addition of increasing amounts of the titrant. Aliquots were added in a Coy chamber under a nitrogen atmosphere at 25 °C.

Supplementary Tables

Table S1. $^{1}H,^{15}N$ chemical shifts of isolated apo-CopAab and in the presence of 2 equivalents of Cu(I)CopZ.

	Apo-CopAab		**Apo-CopAab:Cu(I)CopZ 1:2**	
	H	N	H	N
S3	8.254	117.238	8.248	117.271
E4	8.42	123.1	8.411	123.036
Q5	7.978	119.346	7.973	119.251
K6	8.55	122.986	8.519	122.755
E7	8.127	120.121	8.134	120.21
I8	9.08	122.386	9.072	122.394
A9	8.583	129.38	8.628	129.6
Q11	9.826	126.553	9.834	126.485
S13	9.578	123.405	9.507	123.157
G14	8.529	109.668	8.519	109.611
M15	9.085	120.631		Not detected
T16	10.532	118.888		Not detected
C20	7.674	122.392		Not detected
A21	6.507	119.178		Not detected
A22	7.703	118.084	7.703	118.084
R23	8.327	117.231	8.327	117.231
I24	7.792	119.592	7.792	119.592
G27	7.781	105.029		Not detected
L28	8.296	120.737	8.296	120.737
K29	7.977	115.053		Not detected
R30	6.946	114.545	6.946	114.545
G33	8.514	110.975	8.496	110.978
V34	7.522	120.887	7.534	120.199
T35	9.139	125.456	9.112	125.087
D36	8.359	124.911	8.365	124.846
A37	8.209	121.119	8.234	121.115
N38	8.634	117.895	8.611	118.06
V39	9.713	126.737		Not detected
N40	8.552	126.988		Not detected
L41	8.793	126.451	8.793	126.451
A42	8.324	114.78	8.324	114.78
T43	7.314	104.294	7.338	104.328
E44	8.163	122.923	8.163	122.923
T45	7.538	110.199	7.517	110.415
S46	8.192	125.561	8.192	125.561
V48	9.043	124.865	9.053	125.058
I49	8.481	127.887	8.471	127.936
Y50	9.33	124.667	9.304	124.572
D51	8.75	119.413	8.767	119.416
A53	8.542	121.071	8.53	121.052
E54	7.772	115.958	7.761	115.931
T55	8.013	114.414	8	114.08
G56	7.866	105.269	7.87	105.287
T57	8.324	110.545	8.304	110.533
A59	7.665	121.189	7.647	121.04
I60	7.457	120.92	7.457	120.92

Q61	8.133	118.593	8.133	118.593
E62	8.03	117.791	8.052	117.723
K63	7.633	120.185	7.66	120.325
I64	7.457	118.36	7.446	118.395
E65	7.854	118.182	7.864	118.307
G68	7.641	104.494		Not detected
Y69	7.249	120.184	7.214	119.959
H70	7.75	114.645	7.754	114.633
V71	8.902	125.118	8.868	125.152
V72	8.462	129.265	8.447	129.192
T73	7.814	117.267	7.804	117.217
E74	8.589	120.931	8.575	120.866
K75	8.281	118.451	8.281	118.451
A76	8.911	126.311	8.91	126.238
E77	8.348	122.942	8.339	122.976
F78	8.943	119.809	8.935	119.736
D79	9.123	121.112	9.123	121.112
I80	8.133	118.994	8.135	119.058
E81	9.413	129.507	9.4	129.564
G82	8.743	110.296	8.751	110.345
M83	9.026	120.017	9.072	119.641
T84	10.601	119.587		Not detected
C85	8.501	118.834		Not detected
A86	8.281	114.78	8.281	114.78
C88	7.773	122.271		Not detected
A89	6.453	119.169		Not detected
N90	7.373	115.28	7.41	114.528
E93	8.279	119.588	8.279	119.588
K94	8.17	116.073		Not detected
R95	7.555	117.373	7.543	117.167
L96	8.425	119.307	8.433	119.318
N97	7.734	113.261	7.682	113.317
K98	7.054	116.498	7.057	116.539
I99	7.338	123.3	7.328	123.191
E100	8.783	131.215	8.783	131.215
G101	8.57	110.64	8.57	110.64
V102	7.92	123.393	7.9	123.284
A103	9.099	131.313	9.099	131.313
N104	7.55	112.623	7.55	112.623
A105	8.867	124.111	8.849	124.047
V107	8.262	129.797	8.245	129.83
N108	9.061	127.577	9.058	127.49
F109	8.768	125.296	8.772	125.307
L111	7.229	113.586	7.229	113.586
E112	7.699	116.954	7.717	117.017
T113	7.716	108.367	7.716	108.367
V114	8.582	116.983	8.582	116.983
T115	7.838	125.842	7.814	125.808
V116	9.316	127.12	9.308	127.071
E117	8.698	128.792	8.706	128.765
Y118	9.459	124.323	9.453	124.259
N119	8.727	118.995	8.739	119.024
K121	7.737	116.192	7.731	116.09
E122	7.655	116.546	7.655	116.546

A123	8.058	121.787	8.058	121.787
S124	8.397	112.335	8.39	112.293
V125	8.639	120.272	8.627	120.245
S126	8.174	114.109	8.155	114.127
D127	7.528	121.825	7.528	121.825
L128	7.332	118.771	7.332	118.771
K129	7.872	115.56	7.865	115.661
E130	8.247	119.274	8.247	119.274
A131	7.374	118.981	7.384	119.044
V132	7.141	115.76	7.153	115.756
K134	7.57	116.991	7.595	117.167
L135	7.351	117.201	7.315	117.092
G136	7.616	103.46	7.59	103.366
Y137	6.984	118.786	6.964	118.759
K138	8.025	117.089	7.984	117.025
L139	8.805	123.771	8.799	123.984
K140	8.913	122.512	8.908	122.598
L141	9.01	128.362	9.006	128.358
K142	8.435	126.697	8.435	126.697
G143	8.587	111.521	8.586	111.562
E144	7.892	120.477	7.892	120.477
Q145	8.469	121.481	8.469	121.481
D146	8.335	122.234	8.335	122.234
S147	7.812	121.018	7.812	121.018

Supplementary Figures

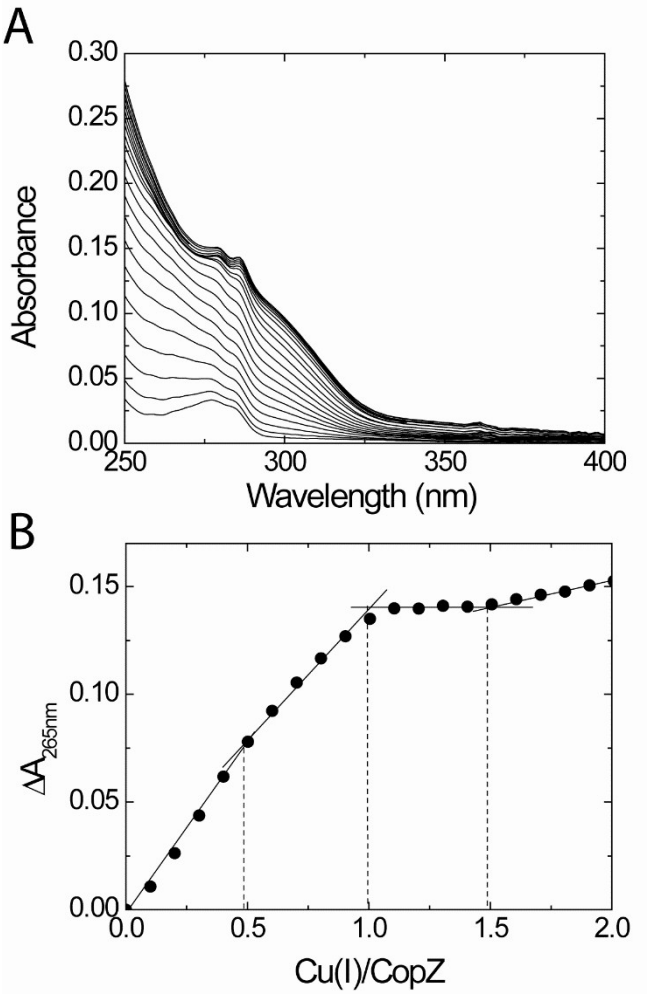

***Figure S1*. UV-visible absorption analysis of Cu(I)-binding to (His)6-CopZ**. **A)** UV-visible absorption anaerobic titration of $(His)_6$-CopZ (25 µM) in 100 mM Mops, 100 mM NaCl, pH 7.5. CopZ was pre-reduced with 15 mM DTT and passed down a G25 Sephadex desalting column (PD-10) in an anaerobic glove box to remove the DTT. Cu(I) was added as a 1 mM Cu(I)Cl solution, as previously described (1-3). **B)** Plot of $\Delta A_{265\,nm}$ as a function of Cu(I)/CopZ. Solid and dotted lines respectively indicate distinct binding phases and the levels of Cu(I) at which they intersect. Like wild type CopZ, Cu(I) binding to $(His)_6$-CopZ occurred in distinct phases: 0 – 0.5, 0.5 – 1.0, 1.0 – 1.5, and > 1.5 Cu(I) ions per protein. The behaviour is essentially identical to that previously reported for the untagged protein (1), demonstrating that the Cu(I)-binding properties of CopZ are not affected by the presence of the C-terminal tag.

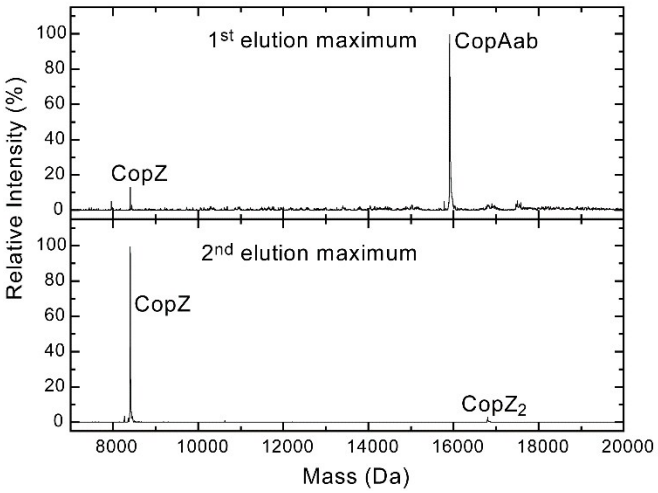

***Figure S2*. LC-ESI-MS analysis of protein containing fractions from thermodynamic Cu(I)-transfer experiment.** Deconvoluted spectra corresponding to the first and second elution peaks from the Ni^{2+}-affinity column as described in Figure 1 of the main paper. The first peak represents protein that did not bind to the column and consists almost entirely of CopAab (observed at 15911 Da, predicted mass 15911 Da). Some $(His)_6$-CopZ (observed at 8403 Da, predicted mass 8402 Da) is present, but because CopZ ionises somewhat more efficiently than CopAab, the relative intensity of the two protein peaks represents an overestimation of the amount of CopZ present in this fraction. The second peak represents protein that eluted from the column only in the presence of imidazole (500 mM). Only $(His)_6$-CopZ was observed, the vast majority as a monomer, with a small amount of disulfide bonded dimer (16802 Da).

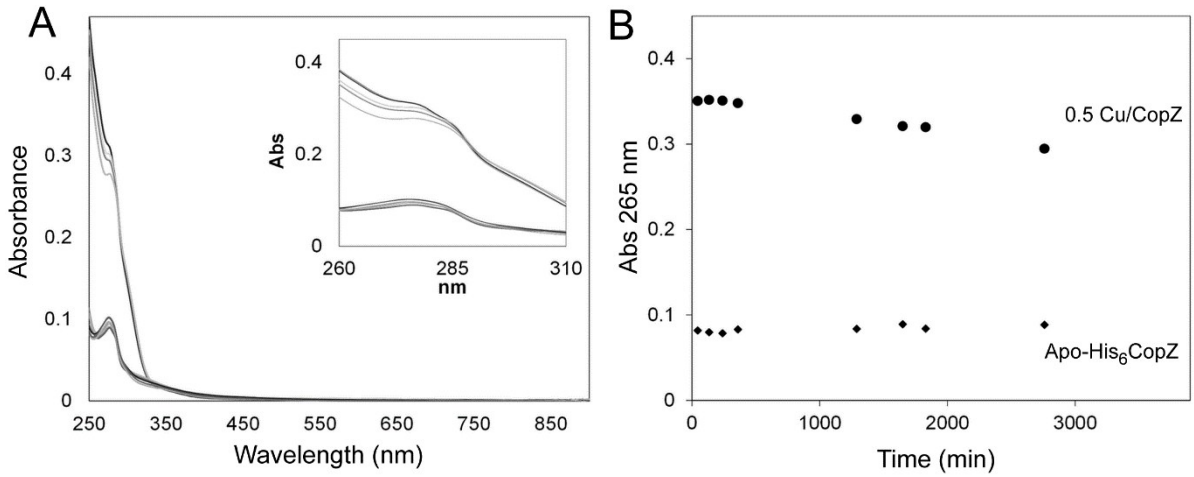

***Figure S3*. Measurements of Cu(I) dissociation from CopZ.** CopZ, prepared at 0.5 Cu/protein, and apo-His_6CopZ were placed into separate dialysis cassettes and both submerged in the same buffer solution. Here, the two proteins are unable to interact, and so transfer of Cu(I) from Cu-CopZ to apo-CopZ can only result from the dissociation of Cu(I) from CopZ into bulk solution. Because association of Cu(I) with CopZ occurs rapidly, the rate limiting step of Cu(I) transfer is dissociation, and so the rate of transfer reports directly on the dissociation rate constant. **A)** Overlaid UV-visible absorbance spectra of 0.5 Cu(I)/CopZ and apo-His_6CopZ acquired over 46 hr. **B**) Absorbance intensity at 265 nm monitored over 46 hr for 0.5 Cu/CopZ and apo-His_6CopZ. Proteins were 60 µM in 100 mM MOPS, 100 mM NaCl, pH 7.5. Only small changes in absorbance were observed. At longer time periods, scattering due to protein precipitation occured and so Cu(I)-transfer could not be followed further, but it is clear that little or no transfer of copper occurred during the experiment. If it is assumed that the loss of $A_{265\ nm}$ intensity of the Cu(I)-CopZ sample was due to Cu(I) transfer, extrapolation of the decay as a first order process gave an estimate of the dissociation rate constant of $\sim 6 \times 10^{-5}\ s^{-1}$. This represents an upper limit because the decrease in Cu(I)-CopZ $A_{265\ nm}$ is very likely due to a combination of some Cu(I) dissociation and loss of protein due to, for example, adsorption onto the dialysis cassette membrane.

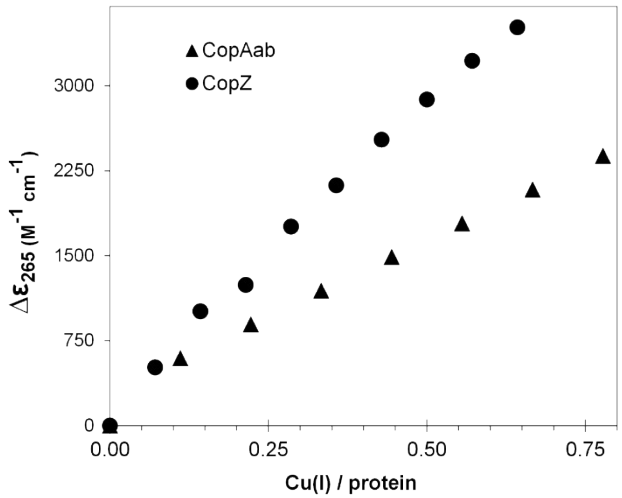

***Figure S4*. Distinct UV-visible 265 nm absorbance intensity responses of CopZ and CopAab during titration with Cu(I).** Plots of absorbance intensity at 265 nm against Cu(I)/protein ratio during titration of CopZ (40 µM) and CopAab (40 µM) with Cu(I). The different responses indicate the direction of absorbance change when apo- and Cu-loaded protein samples are mixed together.

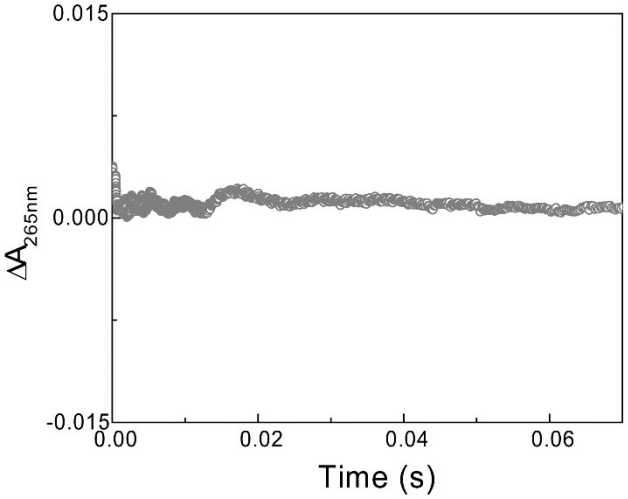

Figure S5. **Absorbance changes at 265 nm upon mixing of apo-CopZ with apo-CopAab.** Control experiment in which apo-CopZ (60 µM) was mixed with apo-CopAab (40 µM) and changes in absorbance at 265 nm measured over the first 70 ms by stopped-flow. Proteins were in 100 mM MOPS, pH 7.5, temperature was 15 °C. Virtually identical data were obtained at 25 °C.

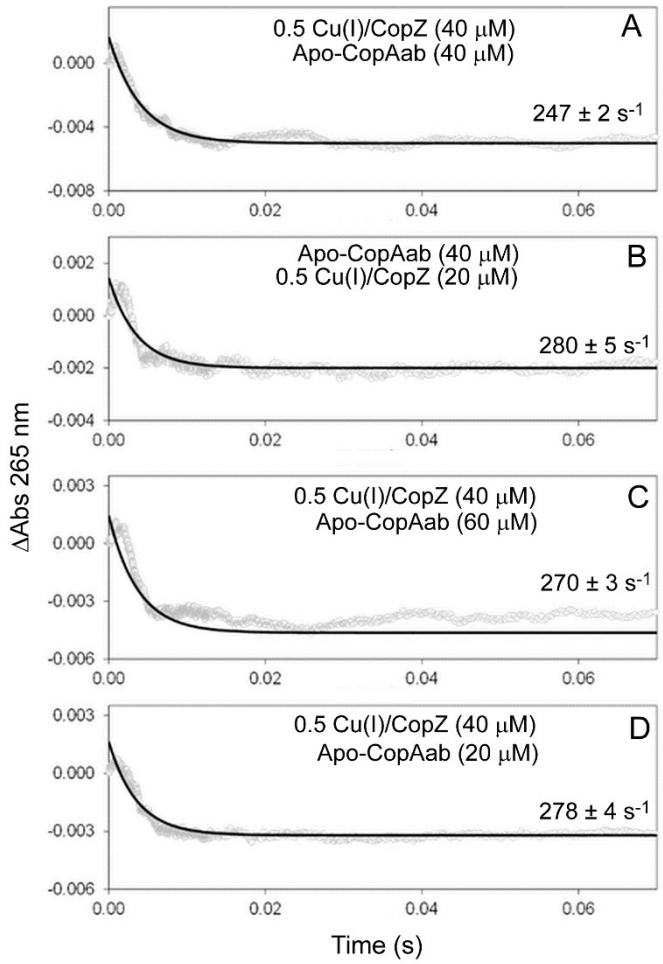

***Figure S6*. Kinetics of Cu(I) transfer between 0.5 Cu/His_6CopZ and apo-CopAab at 25 °C.** Change in absorbance intensity at 265 nm plotted against time after rapid mixing of: apo-CopAab (40 μM) mixed with: **A)** 0.5 Cu(I)/His_6CopZ (40 μM); **B)** 0.5 Cu(I)/His_6CopZ (20 μM); or 0.5 Cu(I)/His_6CopZ (40 μM) mixed with **C)** apo-CopAab (60 μM); **D)** apo-CopAab (20 μM). Proteins were in 100 mM MOPS, pH 7.5. The data were fitted with a single exponential function from which an observed rate constant was derived, as indicated.

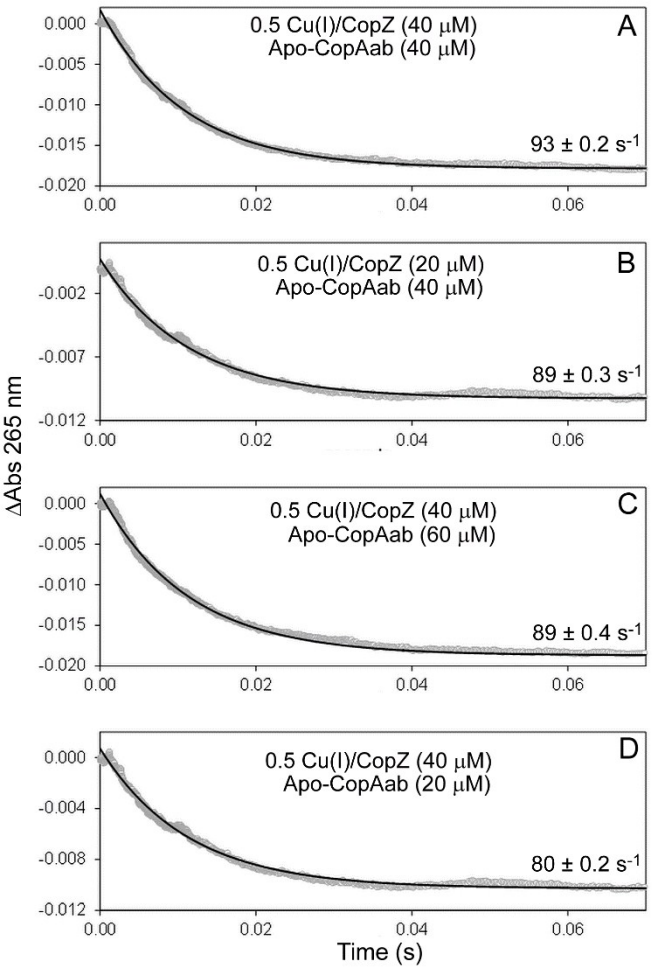

Figure S7. **Kinetics of Cu(I) transfer between 0.5 Cu/His_6CopZ and apo-CopAab at 15 °C.** Change in absorbance intensity at 265 nm plotted against time after rapid mixing of: apo-CopAab (40 μM) mixed with: **A)** 0.5 Cu(I)/His_6CopZ (40 μM); **B)** 0.5 Cu(I)/His_6CopZ (20 μM); or 0.5 Cu(I)/His_6CopZ (40 μM) mixed with **C)** apo-CopAab (60 μM); **D)** apo-CopAab (20 μM). Proteins were in 100 mM MOPS, pH 7.5. The data were fitted with a single exponential function from which an observed rate constant was derived, as indicated. The average rate constant was 89 ±6 s^{-1}.

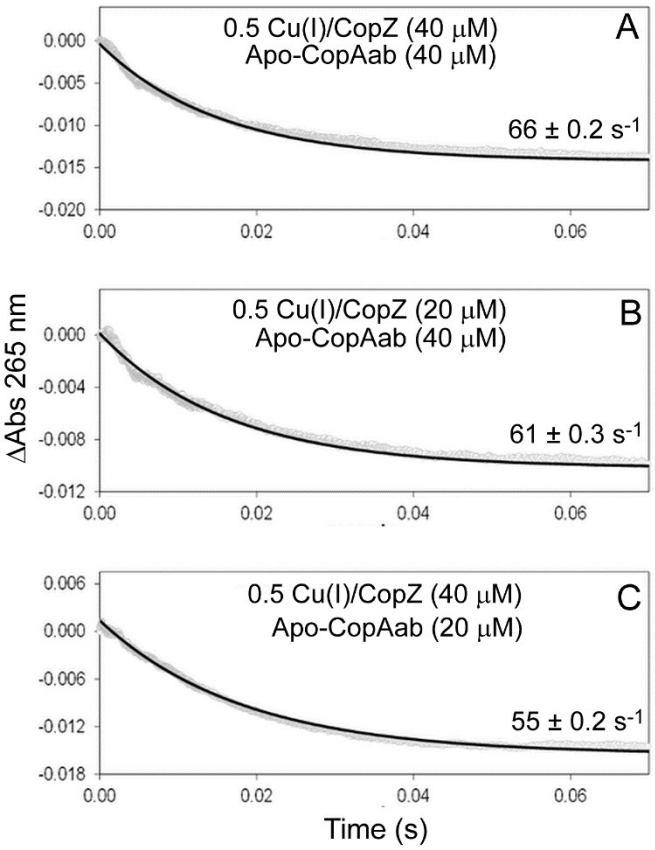

***Figure S8*. Kinetics of Cu(I) transfer between 0.5 Cu/His_6CopZ and apo-CopAab at 10 °C.** Change in absorbance intensity at 265 nm plotted against time after rapid mixing of: apo-CopAab (40 μM) mixed with: **A)** 0.5 Cu(I)/His_6CopZ (40 μM); **B)** 0.5 Cu(I)/His_6CopZ (20 μM); or 0.5 Cu(I)/His_6CopZ (40 μM) mixed with **C)** apo-CopAab (20 μM). Proteins were in 100 mM MOPS, pH 7.5. The data were fitted with a single exponential function from which an observed rate constant was derived, as indicated. The average rate constant was 59 ±6 s^{-1}.

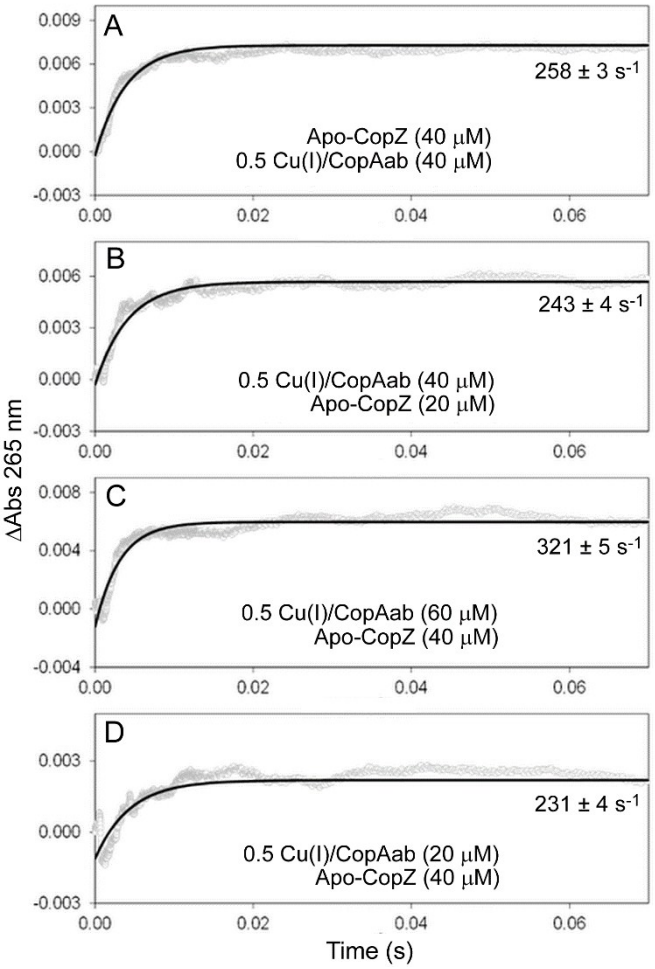

***Figure S9*. Kinetics of Cu(I) transfer between apo-His_6CopZ and 0.5 Cu/CopAab at 25 °C.** Change in absorbance intensity at 265 nm plotted against time after rapid mixing of: 0.5 Cu(I)/CopAab (40 μM) mixed with: **A)** apo-His_6CopZ (40 μM); **B)** apo-His_6CopZ (20 μM); or apo-His_6CopZ (40 μM) mixed with **C)** 0.5 Cu(I)/CopAab (60 μM); **D)** 0.5 Cu(I)/CopAab (20 μM). Proteins were in 100 mM MOPS, pH 7.5. The data were fitted with a single exponential function from which an observed rate constant was derived, as indicated.

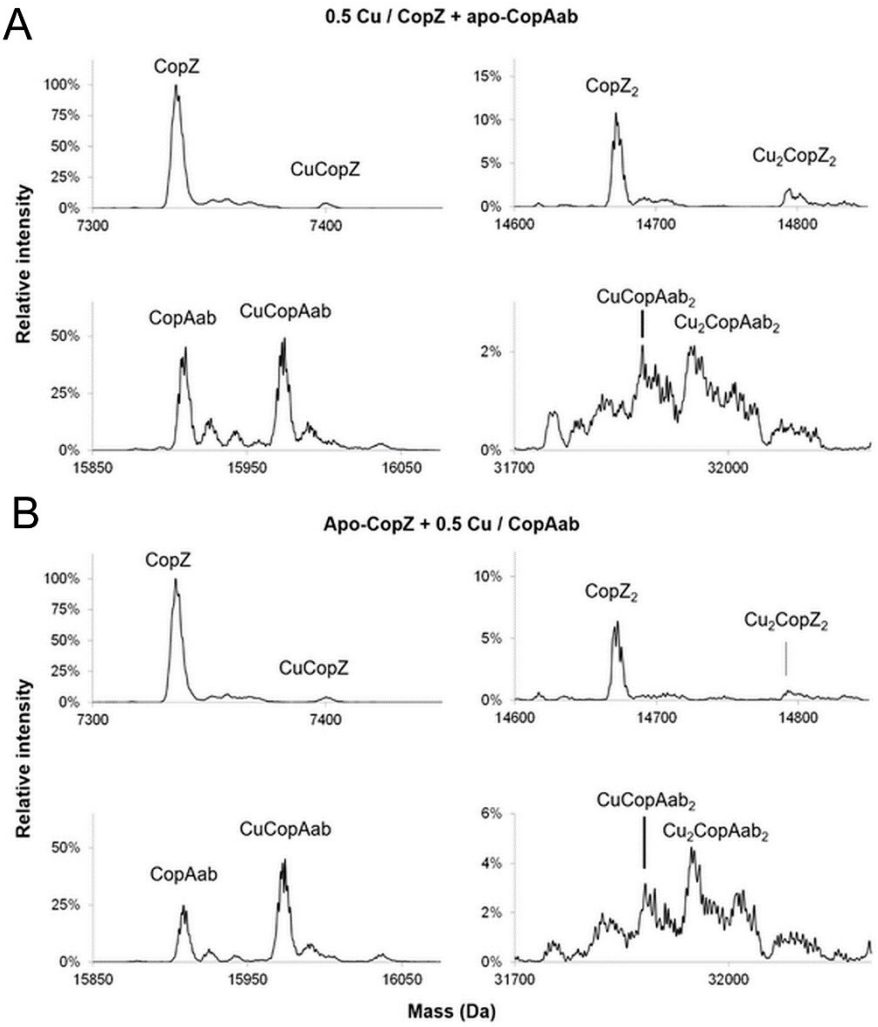

***Figure S10*. ESI-MS of CopZ/CopAab mixtures: CopZ and CopAab species.** **A**) Deconvoluted mass spectra of samples following mixing of 0.5 Cu/CopZ with apo-CopAab. CopZ monomer and dimer, and CopAab monomer and dimer regions are shown, as indicated. **B**) As in A) except that apo-CopZ was mixed with 0.5 Cu/CopAab. Proteins were in 20 mM ammonium acetate, pH 7.4.

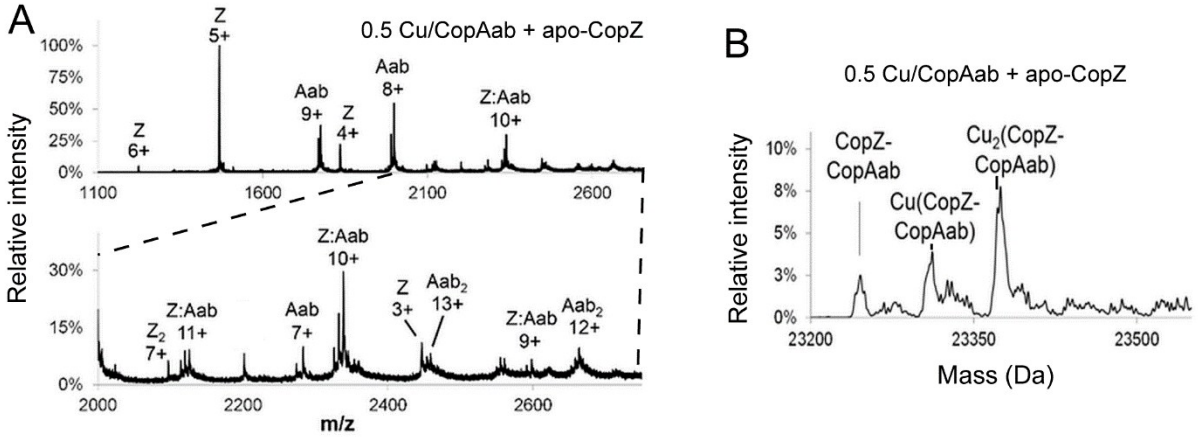

***Figure S11*. ESI-MS of CopZ/CopAab mixtures: CopZ-CopAab complexes. A)** ESI-MS mass spectrum following mixing of 0.5 Cu/CopAab with apo-CopZ. An expanded view of the m/z = 2000 – 2750 region is shown. Each protein envelope is labelled with the corresponding charge state of each peak, as follows: Aab, CopAab monomer; Aab_2, CopAab dimer; Z, CopZ monomer; Z_2, CopZ dimer; Z:Aab, CopZ-CopAab complex. **B**) Deconvoluted mass spectrum of the CopZ-CopAab complex region for the m/z data shown in A). Proteins were in 20 mM ammonium acetate, pH 7.4.

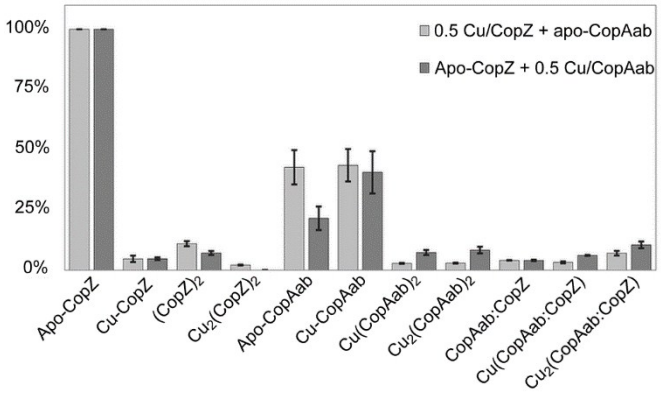

***Figure S12*. Species present in the mass spectra after copper transfer between CopZ and CopAab.** Bar graph illustrating the relative intensity of each species present in the deconvoluted mass spectra of samples, as indicated. Data from Figures 3, S8 and S9.

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