Electronic Supplementary Material (ESI) for Dalton Transactions

Supporting Information

The influence of protein folding on the copper affinities of trafficking and target sites

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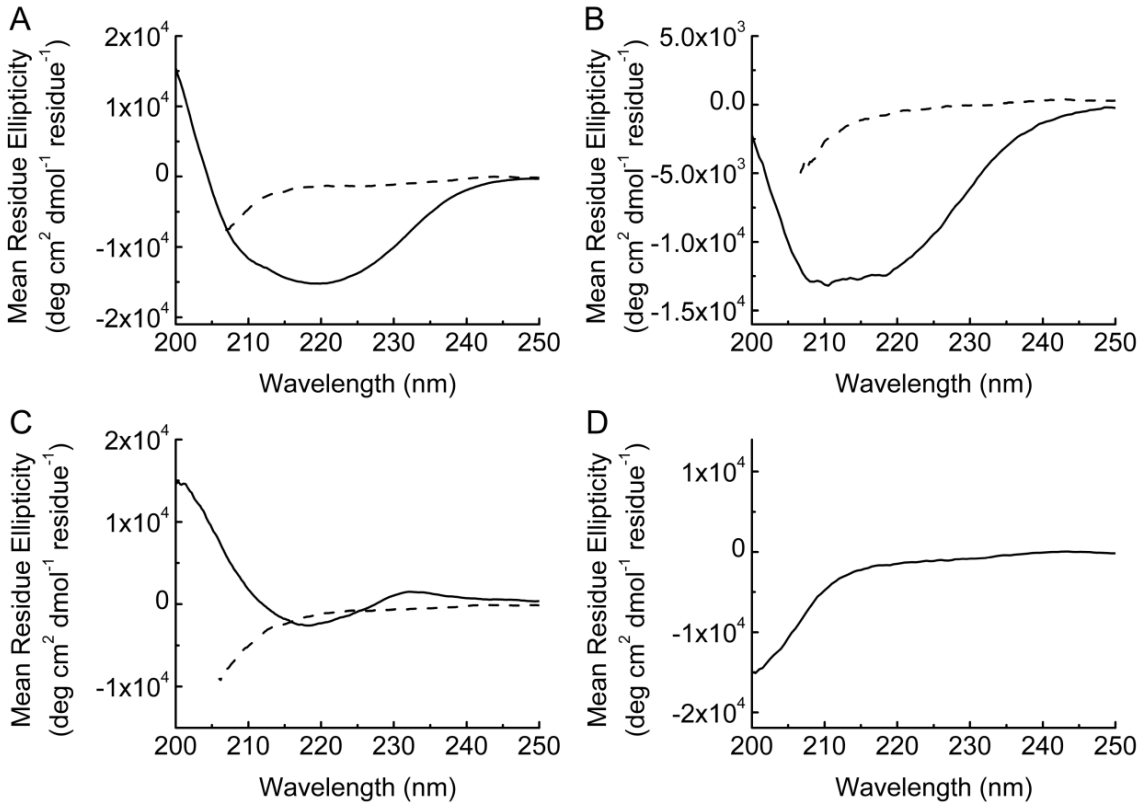

Fig. S1 Far-UV circular dichroism (CD) spectra of apo-HAH1 (A), *S. cerevisiae* apo-Atx1 (B), apo-plastocyanin from *Synechocystis* PCC 6803 (C) and human β-secretase C-terminal domain peptide (BACE1-CTD) (D) in the absence (solid line) and presence (dashed line) of 7 M urea. Protein concentrations were 0.5 mg/ml, except for BACE1-CTD (0.4 mg/ml), and the spectra measured in 20 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (Hepes) containing 200 mM NaCl (200 mM KCl for BACE1-CTD) at pH 7.0.

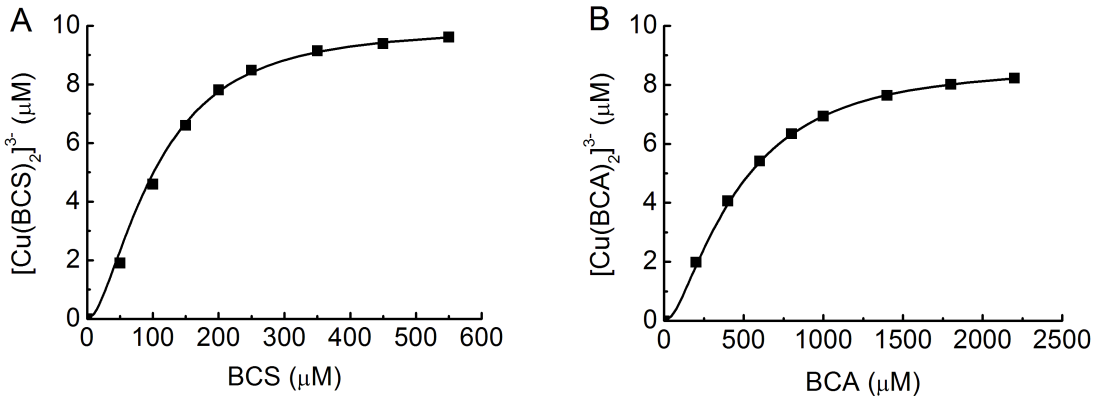

Fig. S2 Titrations of (A) bathocuproine disulfonate (BCS) into Cu(I)-BACE1-CTD (10 μM) plus an excess of apo-peptide (10 μM apo-BACE1-CTD) in 20 mM Hepes containing 200 mM KCl at pH 7.0, and of (B) bicinchoninic acid (BCA) into Cu(I)-thioredoxin (9 μM) plus an excess of apo-protein (5 μM) in 20 mM Hepes containing 200 mM NaCl at pH 7.0. The lines show fits of the data to equation S1 giving K_b values of $(3.1 \pm 0.2) \times 10^{16}$ (A) and $(3.1 \pm 0.1) \times 10^{15}$ M^{-1} (B).

Table S1 Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) and Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) results for *Synechocystis* His61Lys Atx1 and *S. cerevisiae* Atx1

Protein	MS Analysis	Experimental mass (Da)	Theoretical mass (Da)
Synechocystis His61Lys Atx1	FT-ICR	6542	6545 (-Met)
S. cerevisiae Atx1	MALDI-TOF	8089	8091 (-Met)

Table S2 Elution volumes and corresponding calculated apparent molecular weights for wild type (WT) *Synechocystis* apo-Atx1, His61Lys *Synechocystis* apo- and Cu(I)-Atx1 and *S. cerevisiae* apo-Atx1 determined from analytical gel filtration chromatography

Protein	Elution Volume (ml)	Apparent Mass (kDa)
Synechocystis WT apo-Atx1[a]	13.90	13.2
Synechocystis His61Lys apo-Atx1	12.86	21.2
Synechocystis His61Lys Cu(I)-Atx1	13.92	13.8
S. cerevisiae apo-Atx1	14.55	10.6

[a] Data from reference 1.

Table S3 Comparison of secondary structure content compositions determined from available structures using STRIDE[a] with those calculated from far-UV CD spectra of mainly apo-proteins using Dichroweb[b]

Protein[c]	Data	Secondary structure element (%)			
		α-helix	β-sheet	Turn	Random
Synechocystis WT Atx1[d]	Crystal structure	40	21	21	19
	Far-UV CD[e]	48	11	17	25
Synechocystis His61Lys apo-Atx1	Far-UV CD	21	24	19	36
Synechocystis His61Lys Cu(I)-Atx1	Far-UV CD	42	6	30	22
S. cerevisiae Atx1	Crystal structure	32	36	11	21
	Far-UV CD[e]	21	33	22	24
HAH1	Crystal structure	36	36	6	22
	Far-UV CD[e]	42	15	17	26
Synechocystis plastocyanin	Crystal structure	7	53	17	23
	Far-UV CD[e]	7	44	19	30

[a]Reference 2. [b]Reference 3. [c]The following PDB files were used for the determination of protein secondary structure compositions using STRIDE: 2XMT (WT *Synechocystis* Atx1), 1FEE (HAH1), 1CC8 (*S. cerevisiae* Atx1) and 1PCS (*Synechocystis* plastocyanin). [d]Data from reference 1. [e] For reduced apo-proteins.

Determination of Cu(I) affinities

Cu(I) affinities (K_b values) were obtained by fitting data from titrations of BCS or BCA into a mixture of Cu(I)-protein and excess apo-protein to equation S1, where [L], [P] and [Cu] represent the total concentrations of BCS or BCA, protein and Cu(I) respectively, and β is the overall association constant of $[Cu(BCS)_2]^{3-}$ or $[Cu(BCA)_2]^{3-}$.[4,5]

$$[L] = 2[CuL_2] + \sqrt{\frac{K_b([P]-[Cu]+[CuL_2])[CuL_2]}{([Cu]-[CuL_2])\beta}} \qquad \text{(S1)}$$

Supporting References

1 A. Badarau, S. J. Firbank, A. A. McCarthy, M. J. Banfield and C. Dennison, *Biochemistry*, 2010, **49**, 7798.

2 M. Heinig and D. Frishman, *Nucleic Acids Res.*, 2004, **32**, W500.

3 L. Whitemore and B. A. Wallace, *Nucleic Acids Res.*, 2004, **32**, W668.

4 A. Badarau and C. Dennison, *J. Am. Chem. Soc.*, 2011, **133**, 2983.

5 A. Badarau and C. Dennison, *Proc. Natl. Acad. Sci. U.S.A.*, 2011, **108**, 13007.