Electronic Supplementary Material (ESI) for Metallomics.

Evaluation of Cu(I) Binding to the E2 Domain of the Amyloid Precursor Protein – A Lesson in Quantification of Metal Binding to Proteins via Ligand Competition

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Supplementary Information

Table S1. Modified vector used for recombinant protein expression

Original Vector	Tag	Vector Modification [a]	Protein Modification at C-terminus
pET20b	$(His)_6$ [b]	5'- GGATCCGAAAACCTGTACTTCCAGGGTGGCTGGAGCCACCCGCAGTCCGAAAAAGGC**CTCGAG** - 3'	GSENLYFQ|GGWSHPQFEKGLEHHHHHH [c]

[a] DNA sequences were incorporated at the 5' end of existing *XhoI* restriction enzyme site in pET20b (indicated in **bold**) to create modified vector.

[b] 2.3 kDa C-terminal purification tag includes both **STREP**- and **His**-tag sequences, as well as a **TEV protease** cleavage site to facilitate tag removal. As only the His-tag function was utilised in this work, the tag is referred to as '$(His)_6$' herein.

[c] TEV protease digestion (cleavage position indicated by the sign '|') leaves only the short artificial C-terminal sequence (GSENLYFQ) resulting from BamHI and TEV sites, which is not anticipated to interfere with metal-binding or heparin binding properties.

Table S2. ESI-MS data for recombinant proteins [a]

Protein	Molar Mass (Da)	
	Calc'd	Found
APP E2 [b]	25427	25428
APP E2-qm [b]	25162	25163
APP-D2	6832	6833
Atox1	7402/7270 [c]	7402/7270 [c]

[a] Under denaturing conditions [b] Expressed as N-terminally acetylated forms. [c] Obtained as a mixture of proteins with and without the first methionine, respectively (see ref [2]).

Table S3. Calculated masses of Cu-bound E2 complexes from native MS data

Sample [a]	Species	Molar Mass (Da)	
		Calc'd	Found [b]
(i)	E2	25427	25425 (± 2)
(ii)	Cu-E2	25490	25489 (± 2)
(iii)	E2-Cu-Bca	25834	25833 (± 3)

[a] Refer to Fig. 9. [b] Molar mass calculated from four consecutive charge state peaks (+9 to +12) in MS data.

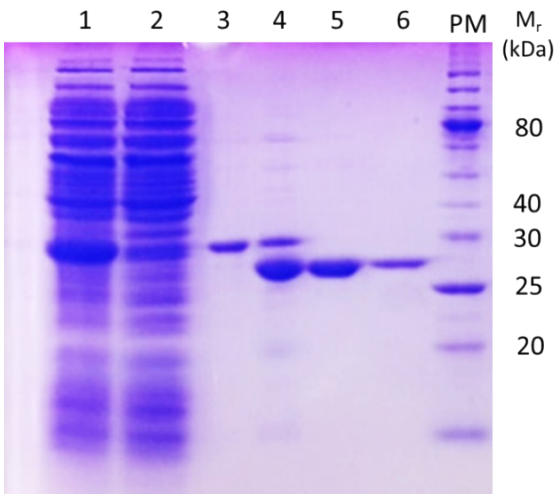

Fig. S1 SDS-PAGE analysis after purification of recombinant APP E2 domain from *E. coli* BL21(DE3) cells. Lanes 1: supernatant of cell lysate; 2: flow through after loading supernatant to IMAC (Ni-NTA) resin; 3: elution of purified APP E2-$(His)_6$ from IMAC resin; 4: after cleavage of hexa-His-tag by TEV protease; 5: after removal of uncleaved protein and protease using IMAC resin; 6: after gel-filtration; PM: protein marker.

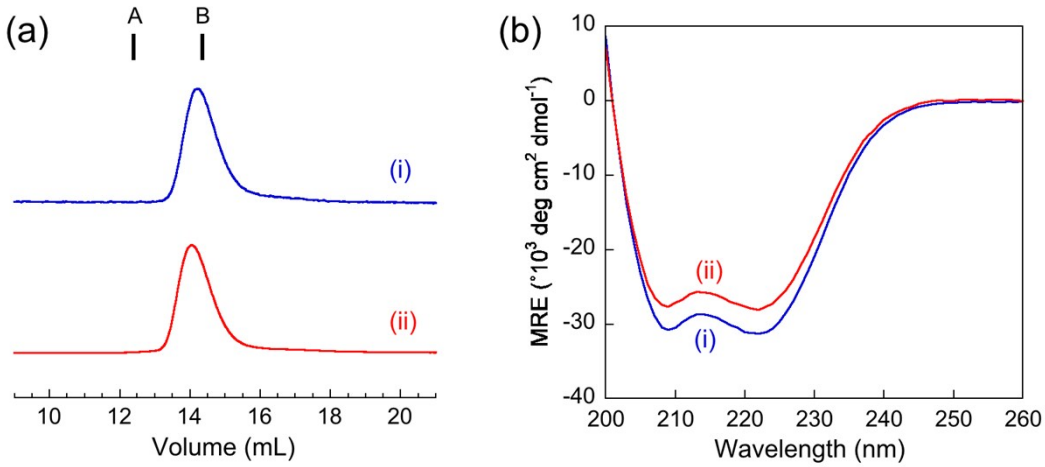

Fig. S2 Biophysical characterization of recombinant APP E2 domains. (a) Size exclusion chromatography for (i) E2 and (ii) E2-qm; elution positions of molecular weight standards are indicated by solid lines above chromatogram: A - ovalbumin (42.7 kDa), B chymotrypsinogen (25.6 kDa). (b) Circular dichroism spectra for (i) E2 and (ii) E2-qm.

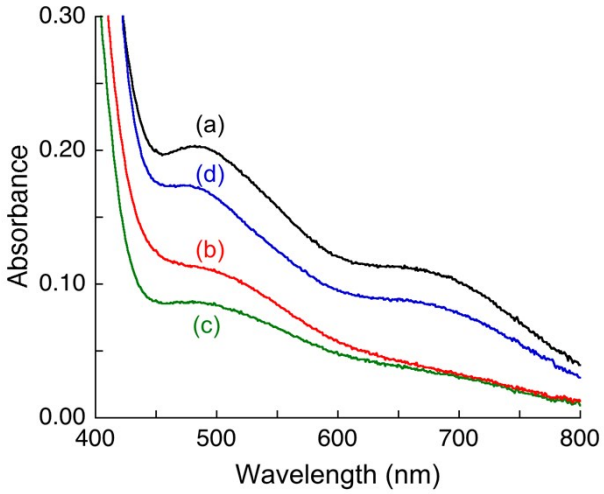

Fig. S3 Changes in solution spectrum of $[Cu^{I}(Fs)_2]^{3-}$ under various conditions: (a) the solutioin spetrum of $[Cu^{I}(Fs)_2]^{3-}$ (compositions: $[Cu]_{tot}$ = 30 μM, $[Fs]_{tot}$ = 70 μM, $[NH_2OH]_{tot}$ = 1.0 mM) in Mops (50 mM, pH 7.4); (b) as (a) but with inclusion of APP E2 (6.0 μM); (c) as (a) but with inclusion of APP E2 (6.0 μM) in the presence of one equivalent of heparin H3393 (6.0 μM); (d) as (a) but with inclusion of APP-D2 (20 μM). Controls showed that addition of the same amount of heparin H3393 (6.0 μM) into solutions (a) and (d) caused little changes for their spectra.

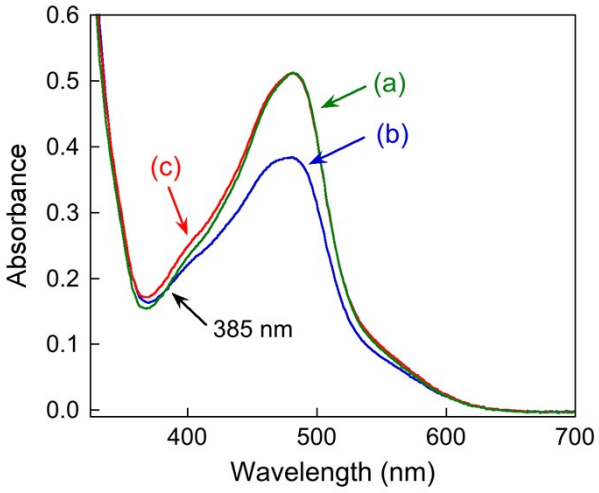

Fig. S4 Solution spectra in Mops buffer (50 mM, pH 7.4, 100 mM NaCl, 1.0 mM NH_2OH) containing: (a) $[Cu]_{tot}$ = 40 μM; $[Bcs]_{tot}$ = 100 μM; (b) as (a) but with added APP *apo*-E2 (50 μM); (c) as (b) but with added heparin (50 μM). An isosbestic point at 385 nm is apparent.

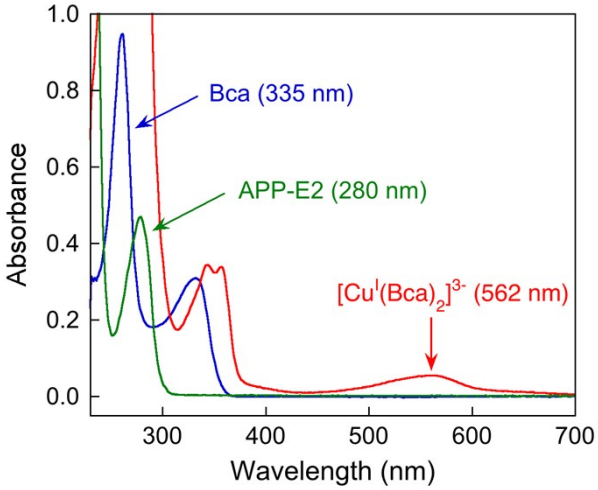

Fig. S5 Solution spectra in Mops buffer (50 mM, pH 7.4, 100 mM NaCl) of recombinant protein APP E2 (black trace), ligand Bca (blue trace) and $[Cu^{I}(Bca)_2]^{3-}$ complex (red trace).

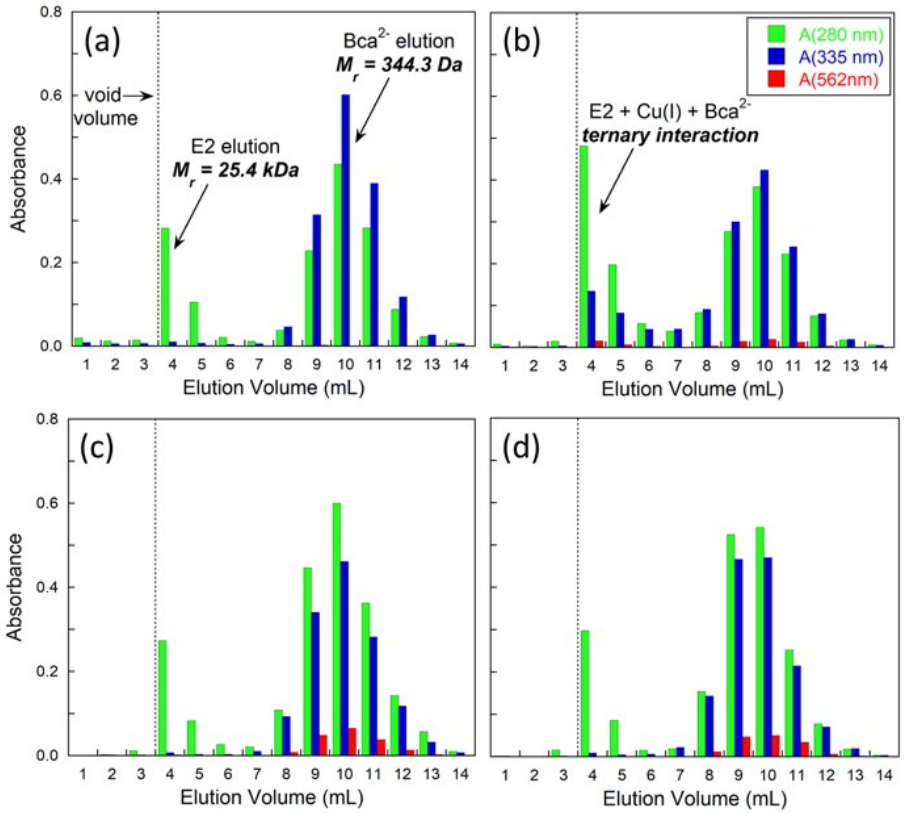

Fig. S6 Analysis of the solution absorbance at 280, 335 and 562 nm for the fractions eluted from a desalting column separating a mixture containing: (a) APP E2 (30 μM), Bca (80 μM) and NH_2OH (1.0 mM); (b) as (a) plus $CuSO_4$ (30 μM, reduced to Cu^{I} *in situ*); (c) as (b) plus heparin (30 μM); (d) as (b) but the APP E2 was replaced with APP E2-qm.

References

(1) Ciccotosto, G. D.; Tew, D. J.; Drew, S. C.; Smith, D. G.; Johanssen, T.; Lal, V.; Lau, T.-L.; Perez, K.; Curtain, C. C.; Wade, J. D. *Neurobiol. Aging* **2011**, *32*, 235.

(2) Xiao, Z.; Brose, J.; Schimo, S.; Ackland, S. M.; La Fontaine, S.; Wedd, A. G. *J. Biol. Chem.* **2011**, *286*, 11047.