Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary Information

Neuroprotective alpha-cleavage of the human prion protein significantly impacts Cu(II) coordination at its His111 site

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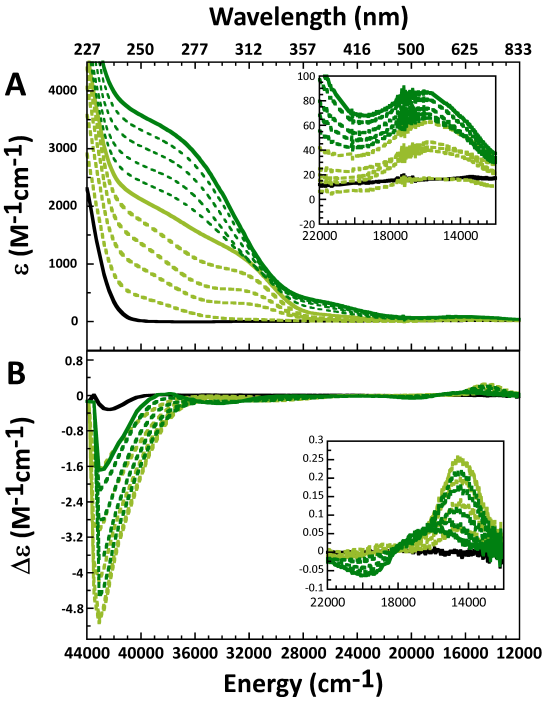


Figure S1. Titration of hPrP(111-115) peptide with Cu(II) as followed by UV-vis absorption (A) and circular dichroism (B) at pH 7.5. Spectra recorded after addition of 0.1, 0.2, 0.3, 0.4 equiv (dashed light green lines), 0.5 equiv of Cu(II) (continuous light green line), 0.6, 0.7, 0.8, 0.9 equiv (dashed dark green lines) and 1.0 equiv of Cu(II) (continuous dark green line) are shown. The negative band at 43000 cm^{-1} corresponds to a NH_2 to Cu(II) LMCT transition.

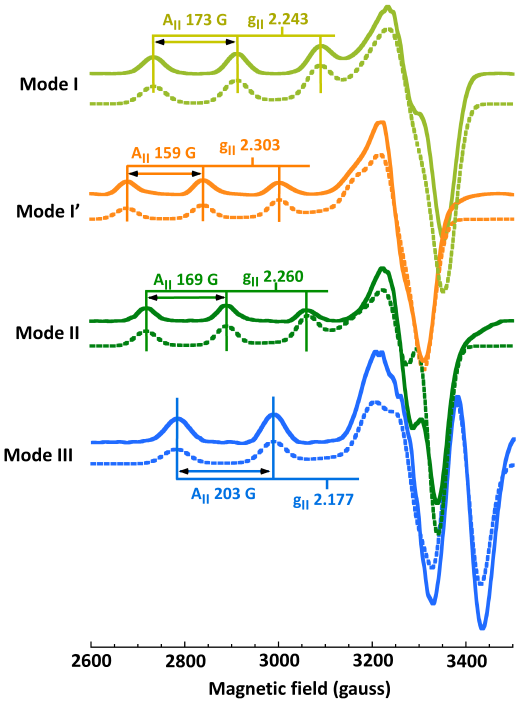


Figure S2. EPR spectra of the Cu(II) complexes with hPrP(111-115): Mode I (light green), Mode I´(orange), Mode II (dark green) and Mode III (blue). Experimental spectra are shown in solid lines, while EPR simulations are shown in dashed lines (using the parameters listed in Table S3).

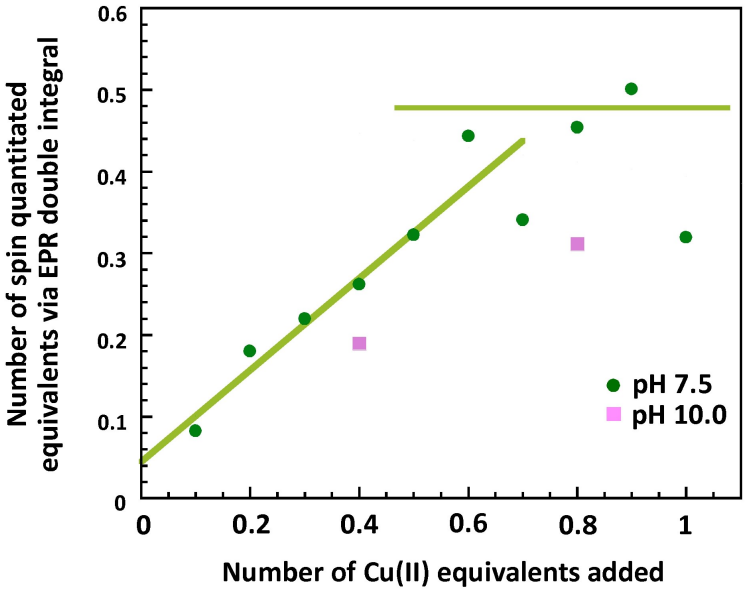
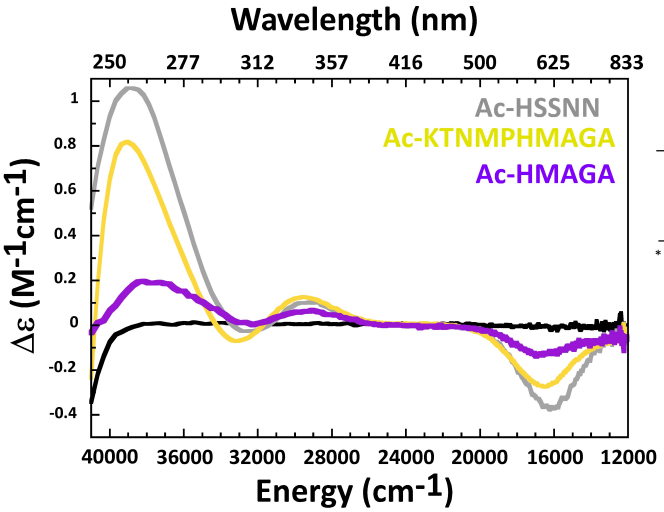


Figure S3. Number of spin quantitated equivalents via EPR double integral as a function of the actual number of Cu(II) equivalents added to hPrP(111-115) fragment at pH 7.5 (green circles) and 10.0 (pink squares). The maximum spin quantitation observed was 0.5 equivalents, even when 1 equivalent of Cu(II) was added.



EPR values

	g_{II}	A_{II}*
Ac-HSSNN	2.299	153
Ac-KTNMPHMAGA	2.244	155
Ac-HMAGA	2.243	156

* x 10^{-4} cm^{-1}

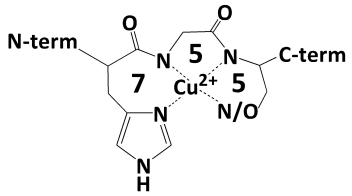


Figure S4. CD spectra and EPR parameters for Cu(II)-peptide complexes with Ac-HSSNN and Ac-KTNMPHMAGA (references 27 and 20, respectively) (in grey and yellow, respectively) at pH 8.5 and Ac-HMAGA (in purple) at pH 7.5. These two complexes involve a His residue as the anchoring site for Cu(II), and the equatorial coordination shell is completed by deprotonated backbone amide groups that follow the His in the sequence, i.e., towards the C-terminal. These coordination modes involve the formation of 3 chelate rings: one 7-membered ring and two 5-membered rings, as shown above, yielding a distinct pattern of Cotton effects (sign) of the CD spectrum. Clearly, Cu(II) coordination to Ac-HMAGA yields a very similar CD spectrum as those observed for the well-characterized Cu(II) complexes with Ac-HSSNN and Ac-KTNMPHMAGA, supporting the conclusion that this complex involves a coordination mode as shown in this figure.

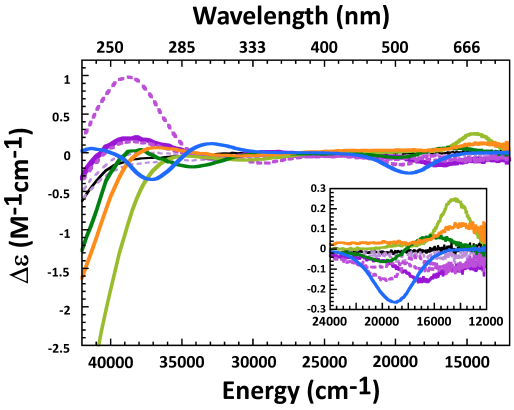


Figure S5. Comparison of CD spectra of Cu(II) complex with hPrP(111-115) in Mode I (light green), Mode I´ (orange), Mode II (dark green) and Mode III (blue); and hPrP(Ac-111-115) at different pH values 5.0, 6.5, 8.5, 10.0 (dashed purple lines) and pH 7.5 (continuous purple line).

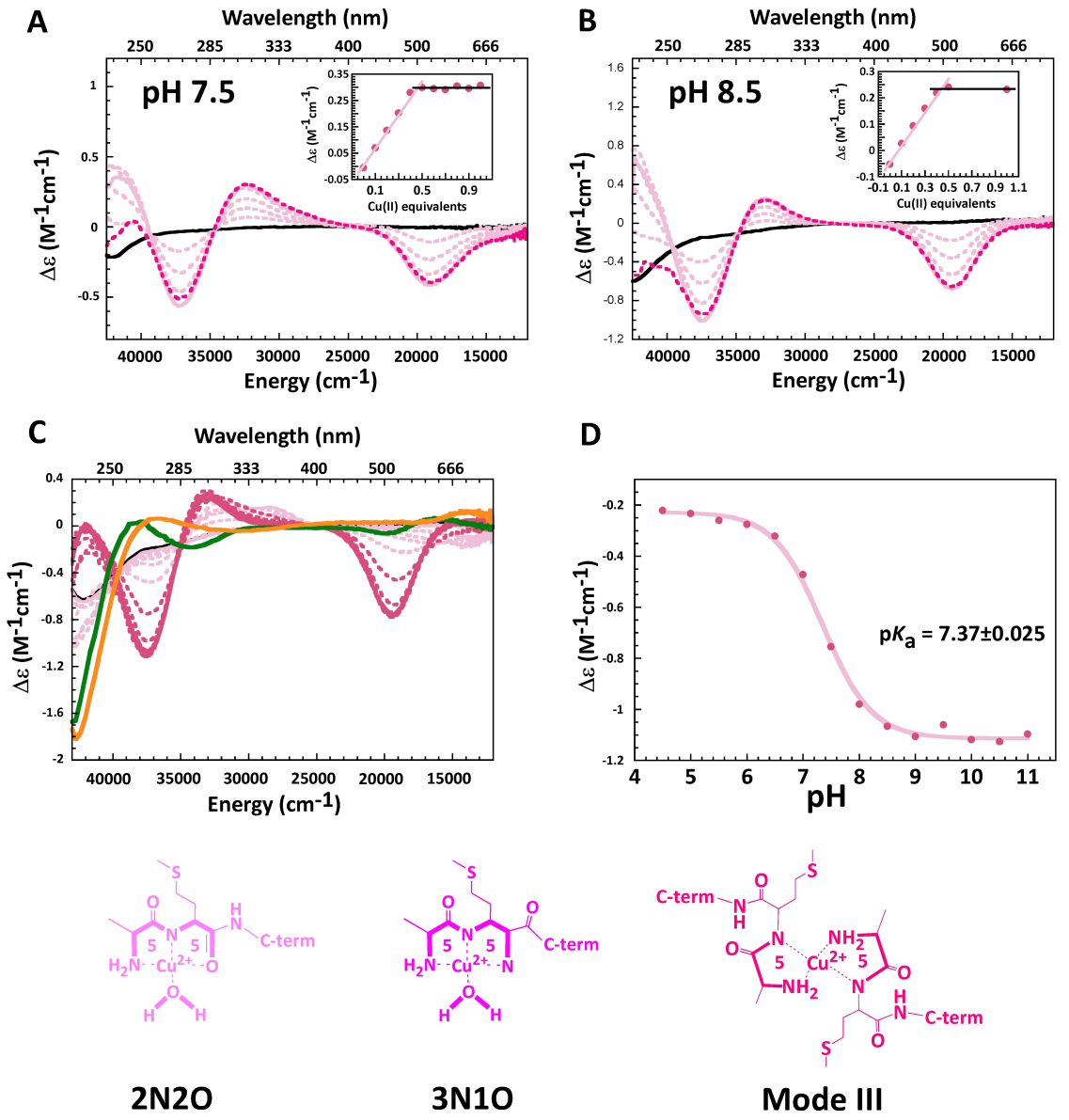


Figure S6. Titration of hPrP(111-115 H111A) fragment with Cu(II) as followed by circular dichroism at pH 7.5 (A) and pH 8.5 (B); spectra were recorded after addition of 0.1, 0.2, 0.3, 0.4 equiv (dashed light pink lines), 0.5 equiv of Cu(II) (continuous light pink line) and 1.0 equiv of Cu(II) (dashed dark pink line) are shown. Insets in (A) and (B) shows the growth of the CD signal intensity at 32468 cm^{-1} plotted as a function of the number of equivalents of Cu(II), extracted from each titration of the H111A variant. (C) pH titration of the Cu(II)-hPrP(111-115 H111A) complex as followed by CD, from pH 4.5 (light color lines) to pH 11.0 (dark color lines); spectra for intermediate pH values are shown in dashed lines. The spectra in orange and green, correspond to those associated to Mode I' and Mode II, respectively; this comparison clearly indicates that modes I´ and II are not formed in the absence of

His111. The trace for the CD signal intensity changes at 37594 cm^{-1} was fit to the model of one protonation equilibrium to determine the pK_a value (D). Schemes at the bottom represent proposed models for the protonated species 2N2O, 3N1O that derive from Mode III for the Cu(II)-hPrP(111-115 H111A) complex.

Table S1. Electronic transitions for Cu(II) complexes with the hPrP(111-115) fragment in the Modes I, I´, II and III; hPrP(Ac-111-115) and hPrP(111-115 H111A), as observed by CD.

	Energy (cm^{-1})	$\Delta\varepsilon$ (M^{-1} cm^{-1})
Mode I	43000	-5.08
	30600	-0.09
	14400	+0.24
Mode I´	42700	-1.83
	36600	+0.06
	30500	-0.04
	13700	+0.12
Mode II	43000	-1.67
	34100	-0.17
	19500	-0.06
	16200	+0.06
Mode III	37100	-1.38
	32900	+0.47
	19100	-1.06
hPrP(Ac-111-115)	38400	+0.25
	32300	-0.03
	28900	+0.03
	16300	-0.09
hPrP(111-115 H111A)	37200	-1.12
	32400	+0.59
	19100	-0.82

Table S2. Parameters from 1D-IR experiment of the protons Hε and Hδ of His111.

	T_{1free}	T_{1bound}	R_{1bfree}	R_{1bound}	R_{1p}	R_{1M}	Distance (nm)
Hε	5.55	0.46	0.18	2.17	1.99	8823.37	0.30 (fixed)
Hδ	3.95	0.52	0.25	1.92	1.67	490.08	0.49

Table S3. EPR simulation parameters for the Cu(II) complexes with the hPrP(111-115) fragment in the Modes I, I´, II and III.

	Mode I	**Mode I´**	**Mode II**	**Mode III**
$\mathbf{g_x}$	2.043	2.072	2.055	2.030
$\mathbf{g_y}$	2.070	2.060	2.070	2.068
$\mathbf{g_z}$	2.243	2.303	2.260	2.177
$\mathbf{A_x}$	13	15	17	14
$\mathbf{A_y}$	19	4	32	20
$\mathbf{A_z}$	181	171	178	206
$\mathbf{A_z}$ **(gauss)**	173	159	169	203
Line Width x	25	23	22	18
Line Width y	30	28	30	25
Line Width z	30	26	26	30

A values and line widths are given in x 10^{-4} cm^{-1}, unless otherwise stated. The simulations were performed using the XSophe Program.