

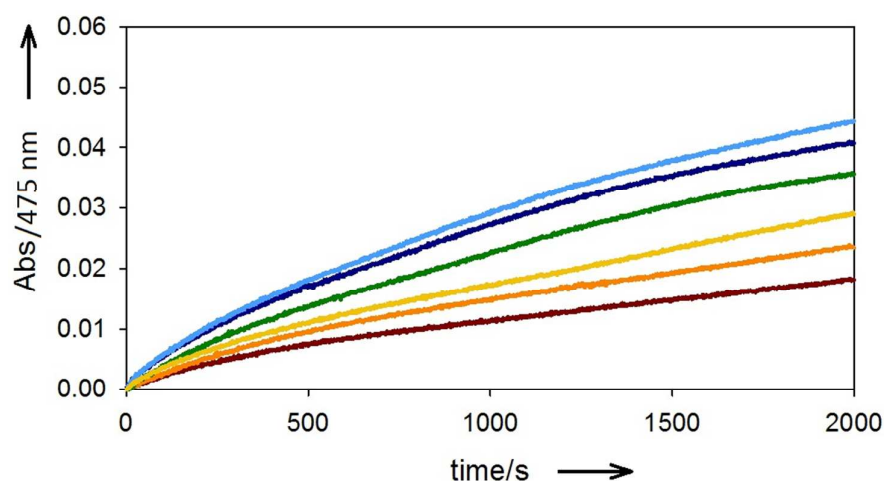
## Supporting Information

### Prion peptides are extremely sensitive to copper induced oxidative stress

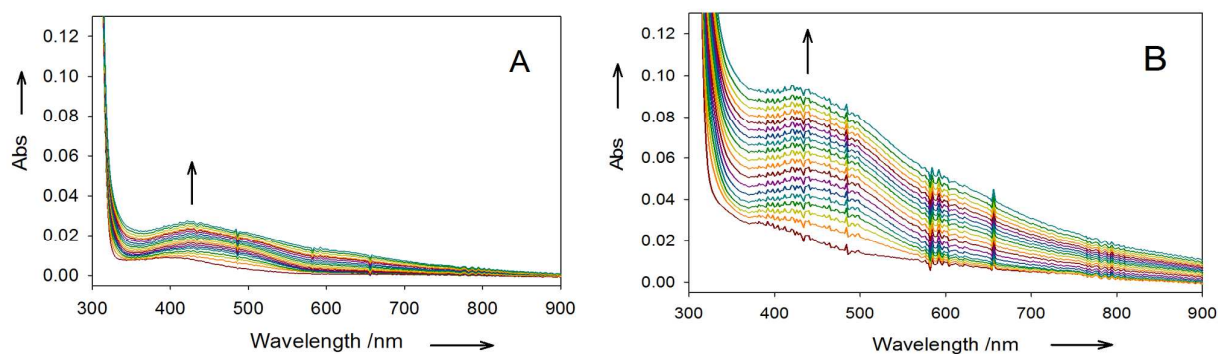
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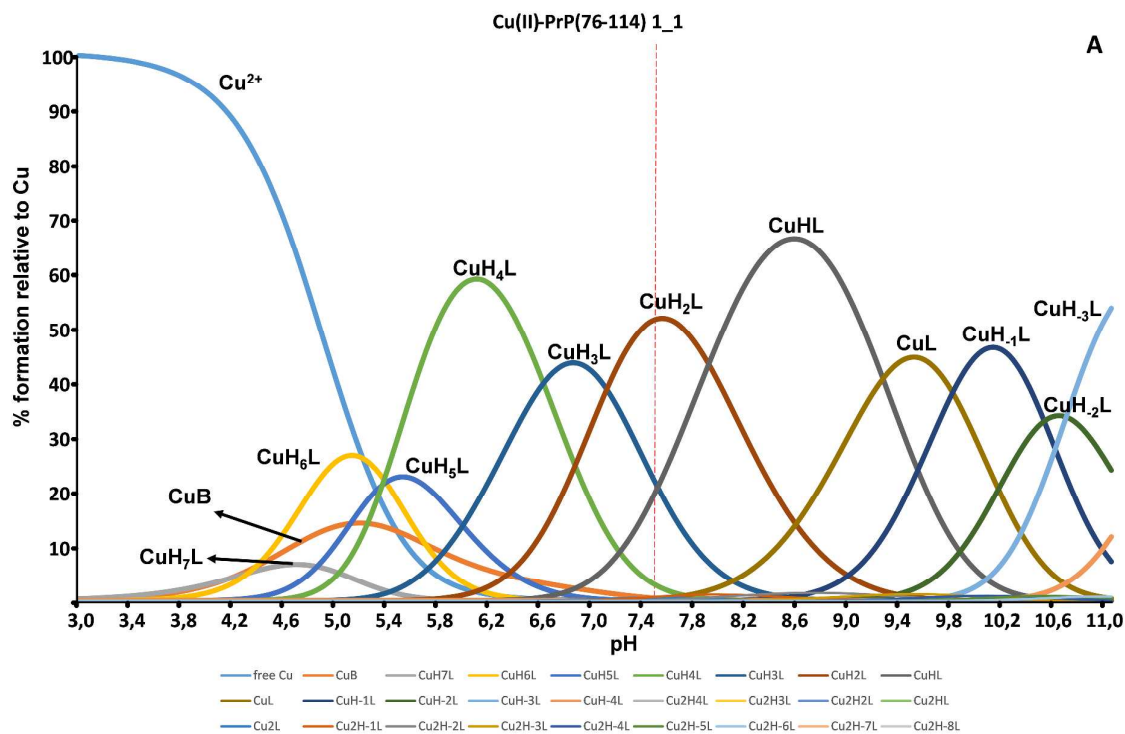
**Figure S1.** Kinetic profiles of DA (3 mM) oxidation with time (DA autoxidation in brown), in 50 mM Hepes buffer solution at pH 7.4 and 20 °C in the presence of Cu<sup>2+</sup> (25 μM) (orange) and with addition of PrP<sub>84-114</sub> (8 μM, yellow; 25 μM, green; 50 μM, blue; 75 μM, light blue). DA oxidation was monitored through the absorption band of dopaminochrome at 475 nm.

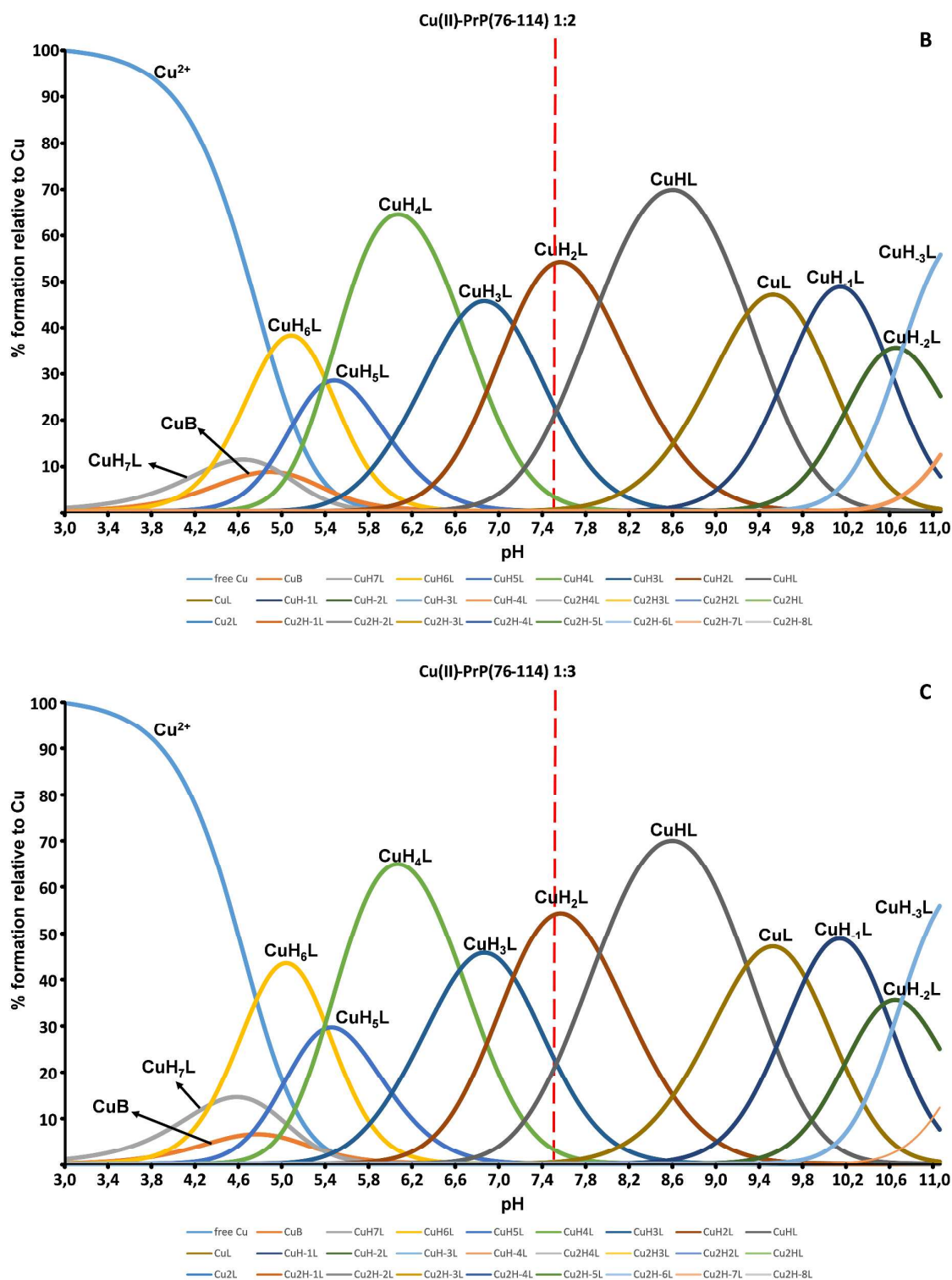


**Figure S2.** Selected absorption spectra recorded during the oxidation of DA (3 mM) in 50 mM Hepes buffer solution at pH 7.4 and 20 °C in the presence of: (A) 25 μM copper(II), and (B) 25 μM copper(II) and 75 μM PrP<sub>76-114</sub>. In (A) and (B), the first spectrum (lower trace) was recorded after 5 s, and the other spectra were recorded every 110 s.

**Table S1.** Computed percentage formation relative to  $\text{Cu}^{2+}$  of complex species formed in the  $\text{Cu}^{2+}$ -PrP<sub>76-114</sub> system in 50 mM Hepes buffer solution at pH 7.4 and different metal to ligand ratios (M/L). Protonation and  $\text{Cu}^{2+}$  complexation constants with PrP<sub>76-114</sub> are from ref. 2S.

M_L	free Cu	CuB	CuH5L	CuH4L	CuH3L	CuH2L	CuHL	CuL	Cu2HL	Cu2L	Cu2H-1L
1_1	0.01	0.59	0.03	3.91	24.70	50.44	16.70	0.25	0.39	0.54	0.61
1_2	0.00	0.01	0.04	4.07	25.70	52.48	17.37	0.26	0.01	0.01	0.02
1_3	0.00	0.01	0.04	4.08	25.71	52.48	17.38	0.26	0.00	0.01	0.01

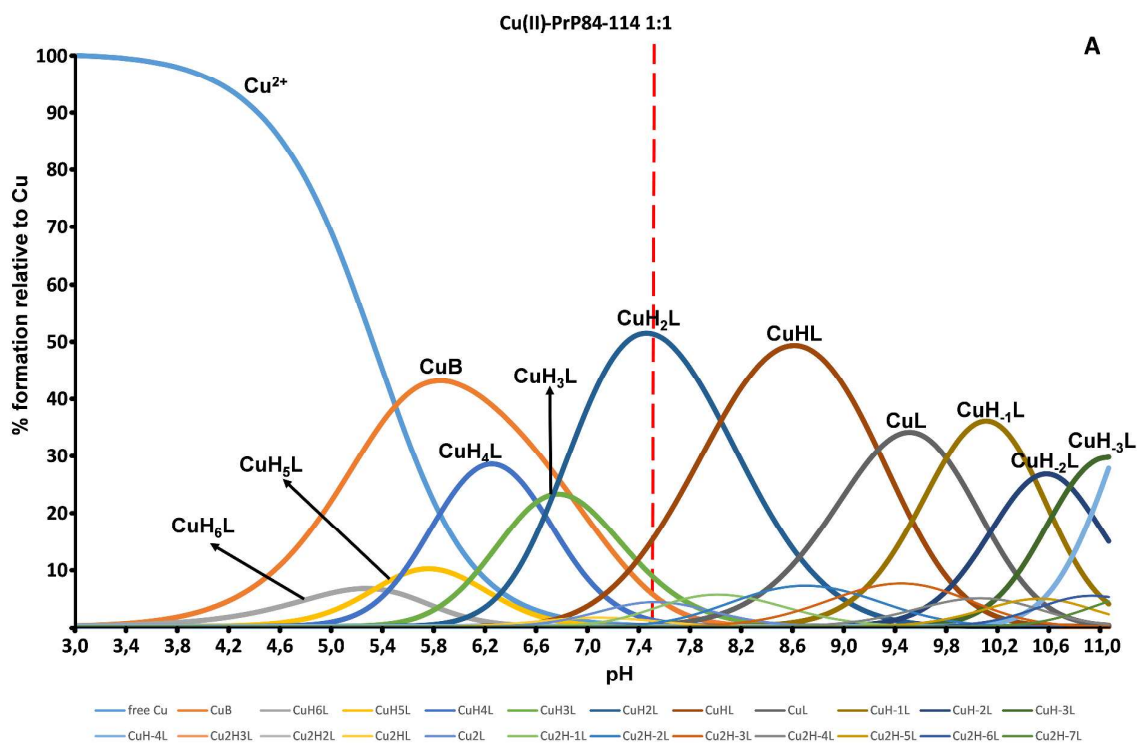


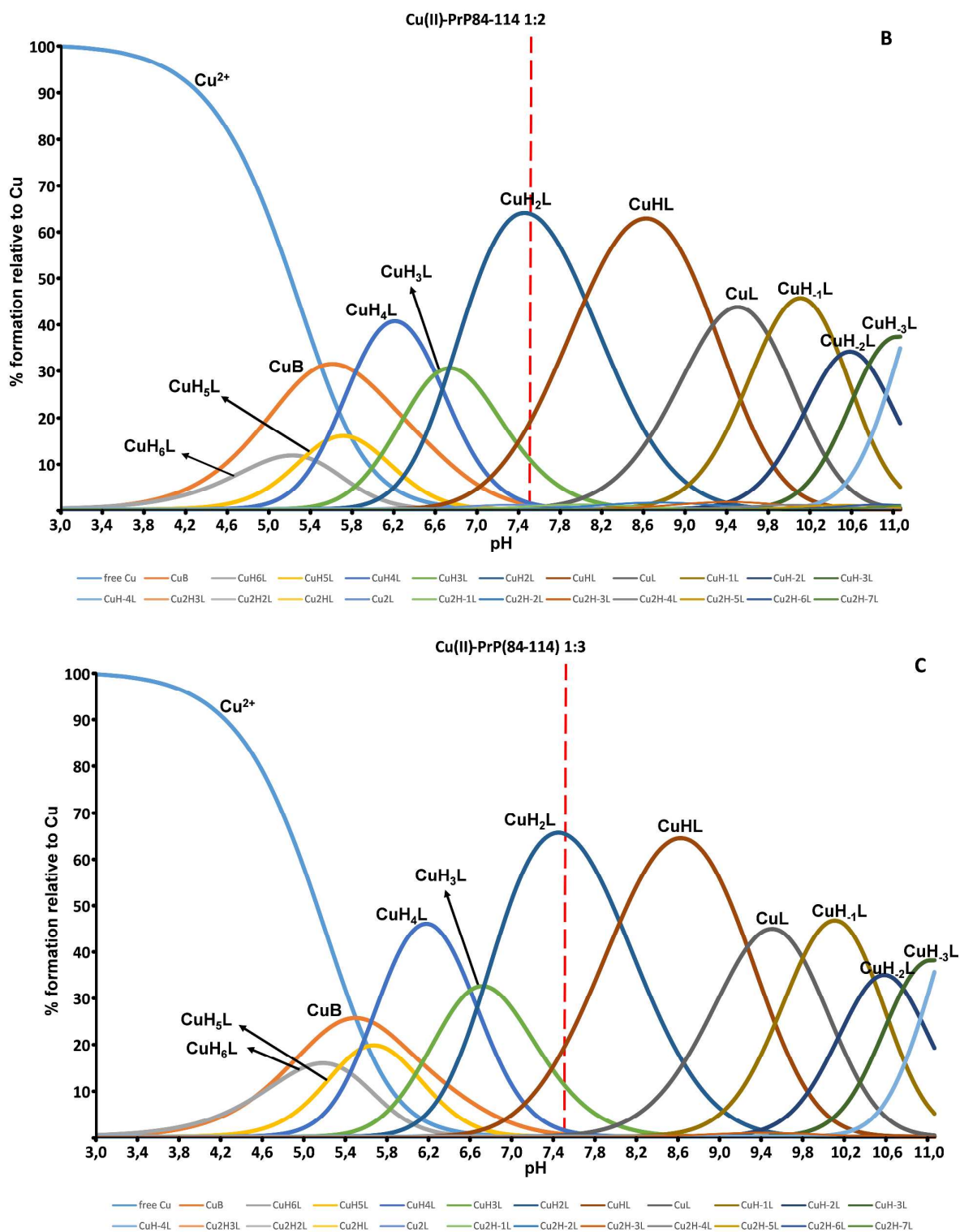


**Figure S3.** Computed species distribution of the complexes formed in the presence of  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ ) and (A) 25  $\mu\text{M}$  PrP<sub>76-114</sub>, (B) 50  $\mu\text{M}$  PrP<sub>76-114</sub> and (C) 75  $\mu\text{M}$  PrP<sub>76-114</sub>, in 50 mM Hepes buffer solution at pH 7.4. Protonation and  $\text{Cu}^{2+}$  complexation constants with PrP<sub>76-114</sub> are from ref. 2S.

**Table S2.** Computed percentage formation relative to  $\text{Cu}^{2+}$  of complex species formed in the  $\text{Cu}^{2+}$ -PrP<sub>84-114</sub> system in 50 mM Hepes buffer solution at pH 7.4 and different metal to ligand ratios (M/L). Protonation and  $\text{Cu}^{2+}$ -complexation constants with PrP<sub>84-114</sub> are from ref. 1S.

.	free Cu	CuB	CuH 5L	CuH4 L	CuH3 L	CuH2 L	CuHL	CuL	Cu2H 3L	Cu2H2 L	Cu2H L	Cu2 L	Cu2H -1L	Cu2H -2L	Cu2H -3L
1_1	0.16	6.79	0.02	1.44	9.99	51.20	13.16	0.20	0.01	0.12	1.10	4.17	2.63	0.44	0.01
1_2	0.03	1.27	0.02	1.80	12.44	63.80	16.40	0.25	0.00	0.03	0.26	0.97	0.61	0.10	0.00
1_3	0.02	0.67	0.02	1.85	12.76	65.48	16.83	0.26	0.00	0.02	0.14	0.52	0.33	0.05	0.00

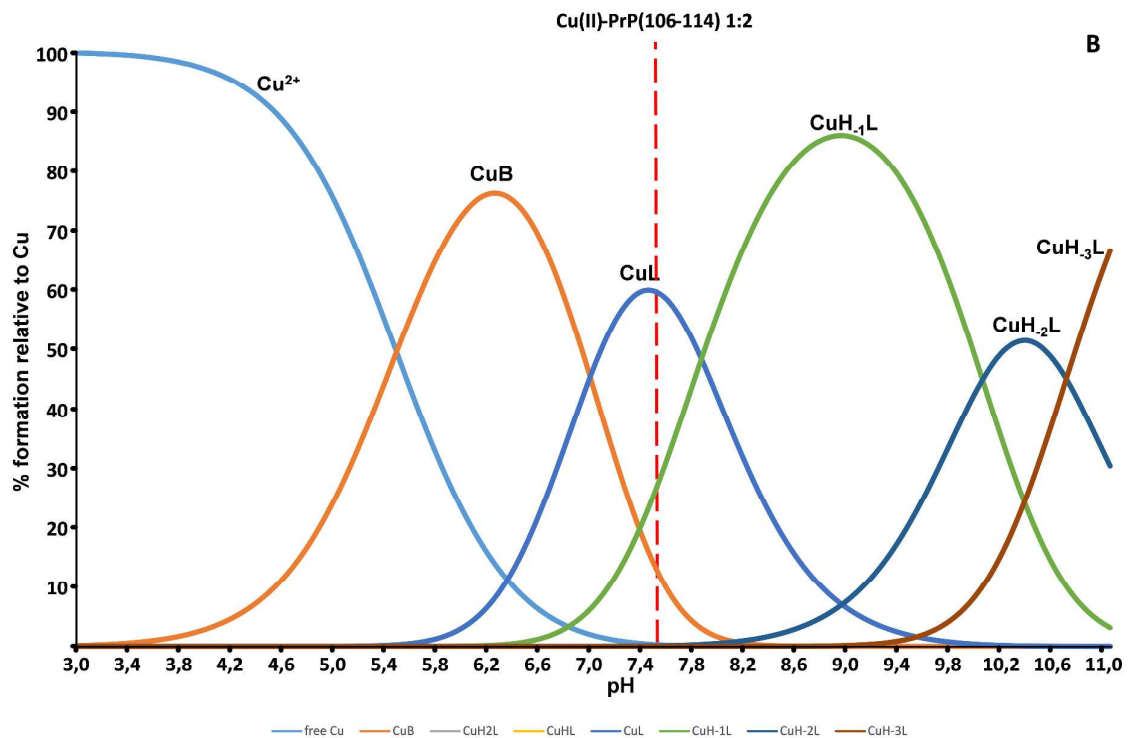
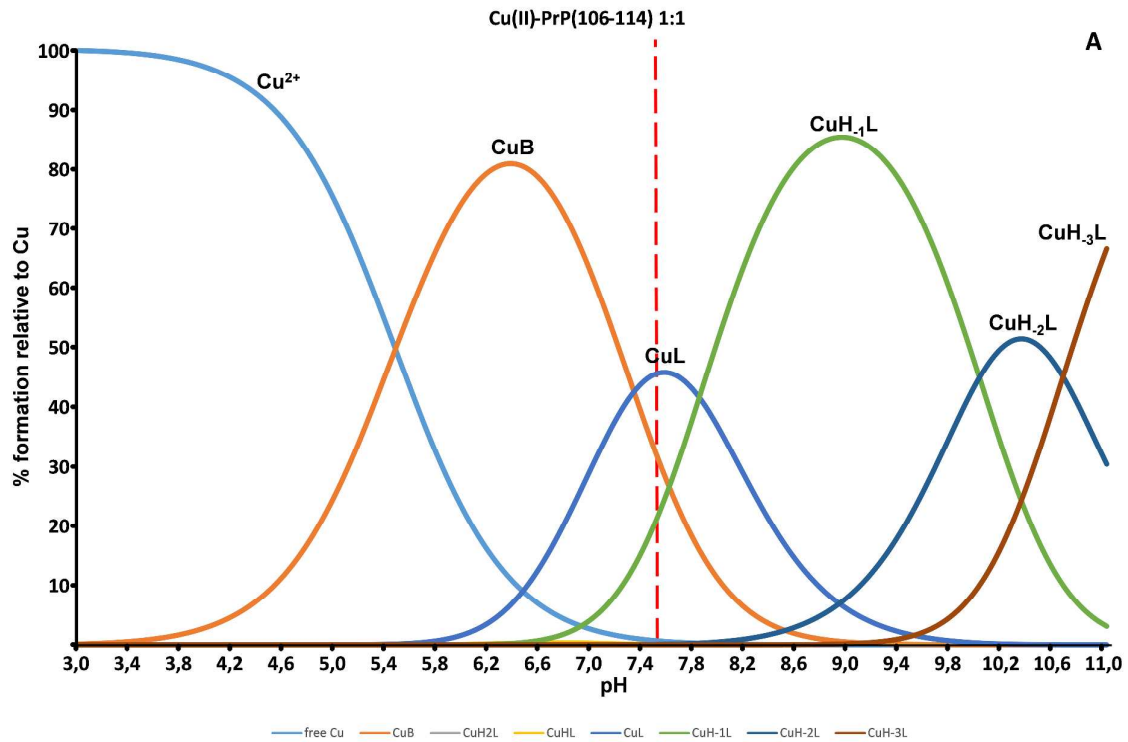




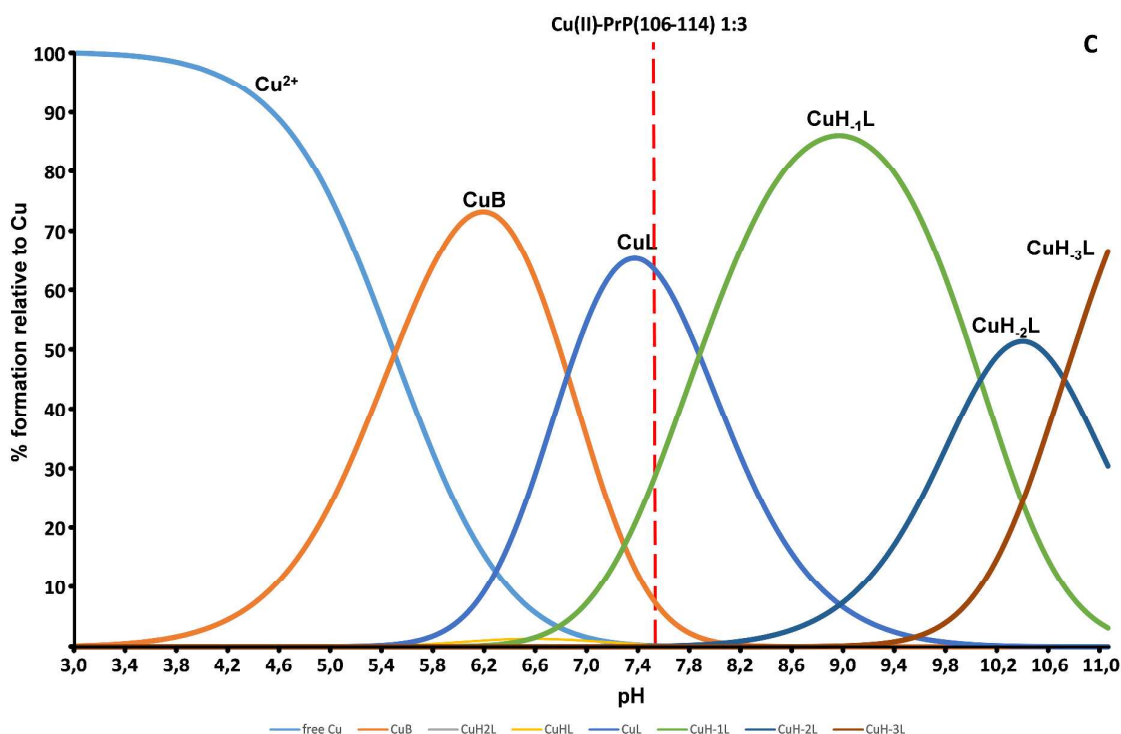
**Figure S4.** Computed species distribution of the complexes formed in the presence of  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ ) and (A) 25  $\mu\text{M}$  PrP<sub>84-114</sub>, (B) 50  $\mu\text{M}$  PrP<sub>84-114</sub> and (C) 75  $\mu\text{M}$  PrP<sub>84-114</sub>, in 50 mM Hepes buffer solution at pH 7.4. Protonation and  $\text{Cu}^{2+}$  complexation constants with PrP<sub>84-114</sub> are from ref. 1S.

**Table S3.** Computed percentage formation relative to  $\text{Cu}^{2+}$  of complex species formed in the  $\text{Cu}^{2+}$ -PrP<sub>106-114</sub> system in 50 mM Hepes buffer solution at pH 7.4 and different metal to ligand ratios (M/L). Protonation and  $\text{Cu}^{2+}$  complexation constants with PrP<sub>106-114</sub> are from ref. 4S.

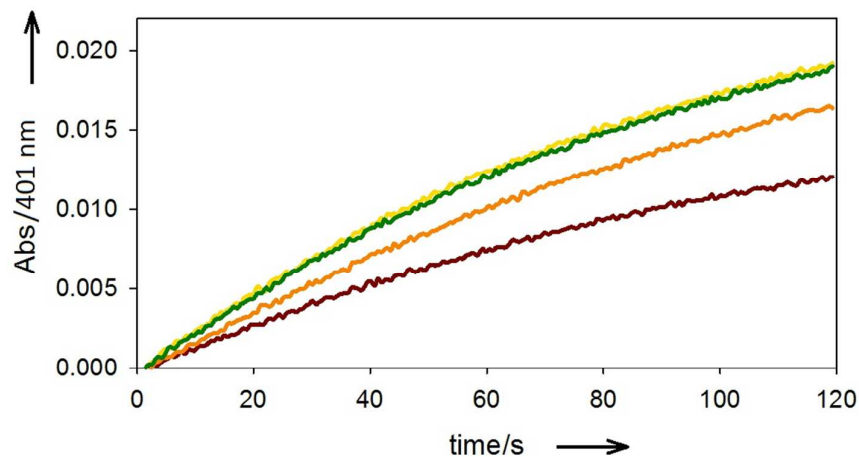
M/L	free Cu	CuB	CuH2L	CuHL	CuL	CuH-1L	CuH-2L	CuH-3L
1_1	0.89	37.69	0.01	0.29	44.23	16.43	0.04	0.00
1_2	0.40	16.92	0.01	0.40	59.80	22.22	0.05	0.00
1_3	0.24	10.23	0.01	0.43	64.84	24.08	0.06	0.00



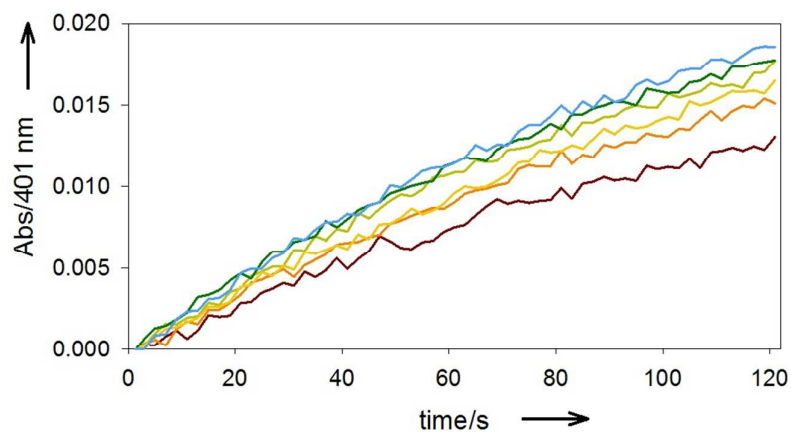




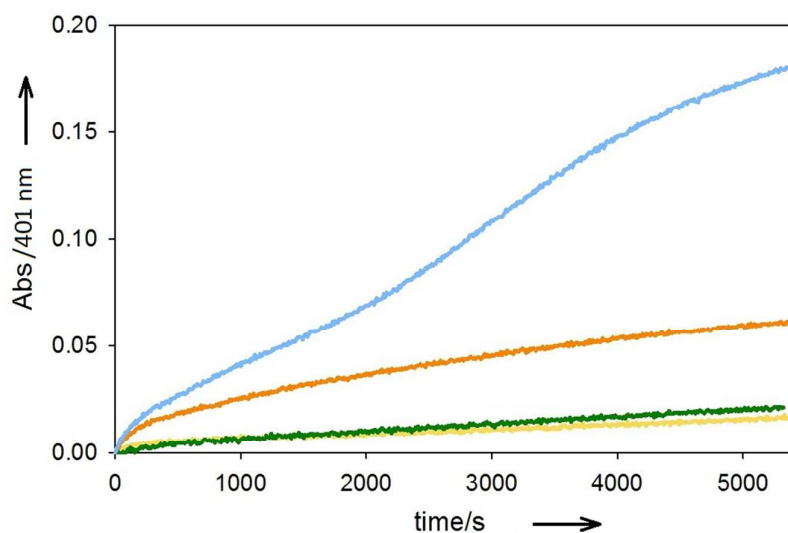
**Figure S5.** Computed species distribution of the complexes formed in the presence of  $\text{Cu}^{2+}$  ( $25 \mu\text{M}$ ) and (A)  $25 \mu\text{M}$  PrP<sub>106-114</sub>, (B)  $50 \mu\text{M}$  PrP<sub>106-114</sub> and (C)  $75 \mu\text{M}$  PrP<sub>106-114</sub>, in 50 mM Hepes buffer solution at pH 7.4. Protonation and  $\text{Cu}^{2+}$  complexation constants with PrP<sub>106-114</sub> are from ref. 4S.



**Figure S6.** Kinetic profiles of MC (3 mM) oxidation with time, in 50 mM Hepes buffer solution at pH 7.4 and 20 °C in the presence of  $\text{Cu}^{2+}$  ( $25 \mu\text{M}$ ) (brown) and with addition of PrP<sub>84-114</sub> ( $25 \mu\text{M}$ , orange;  $50 \mu\text{M}$ , yellow;  $75 \mu\text{M}$ , green).

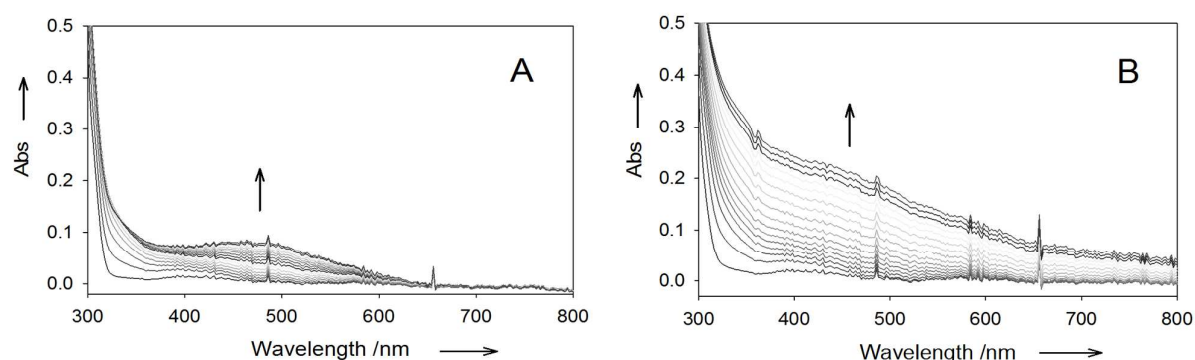


**Figure S7.** Kinetic profiles of MC (3 mM) oxidation with time, in 50 mM Hepes buffer solution at pH 7.4 and 20 °C in the presence of  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ ) (brown) and with addition of PrP<sub>106-114</sub> (15  $\mu\text{M}$ , orange; 25  $\mu\text{M}$ , light green; 35  $\mu\text{M}$ , yellow; 50  $\mu\text{M}$ , green; 75  $\mu\text{M}$ , light blue).

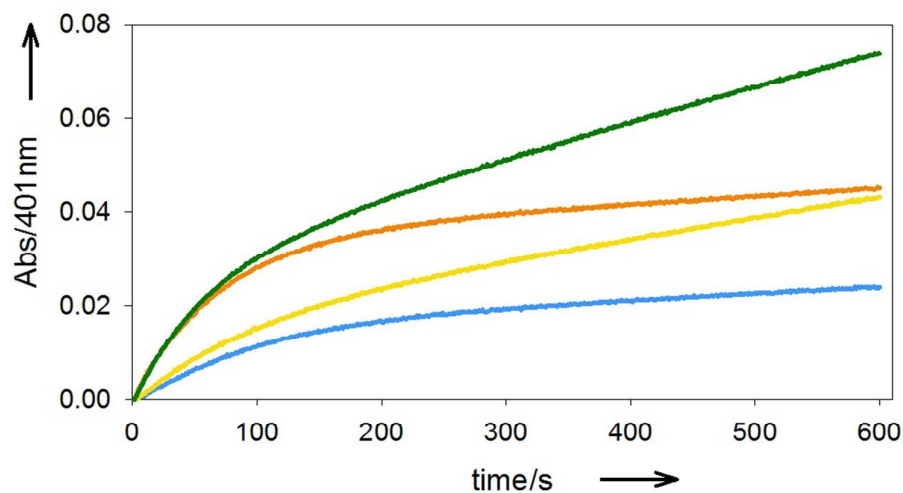


**Figure S8.** Kinetic traces of absorbance at 401 nm vs. time for the oxidation of MC (3 mM) at pH 7.4 and 20 °C in the presence of free  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ ) (orange trace), PrP<sub>76-114</sub> (25  $\mu\text{M}$ ) (green trace),  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ ) and PrP<sub>76-114</sub> (25  $\mu\text{M}$ ) (light blue trace) in Hepes buffer (5 mM). MC autoxidation is shown by the yellow trace.

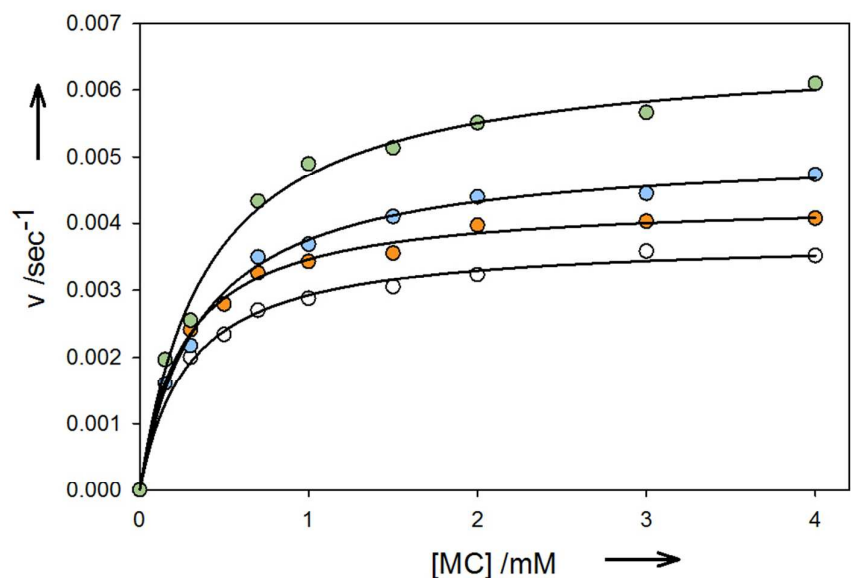




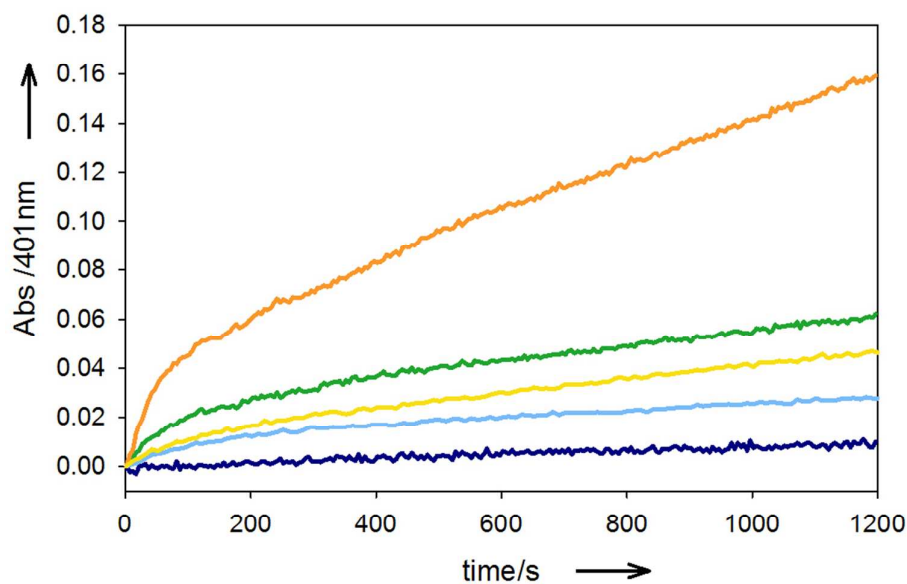
**Figure S9.** Selected absorption spectra recorded during the oxidation of MC (3 mM) in 5 mM Hepes buffer solution at pH 7.4 and 20 °C in the presence of : (A) 25  $\mu\text{M}$  copper(II), and (B) 25  $\mu\text{M}$  copper(II) and 25  $\mu\text{M}$  PrP<sub>76-114</sub>. In (A) and (B), the first spectrum (lower trace) was recorded after 5 s, and the other spectra were recorded every 360 s.



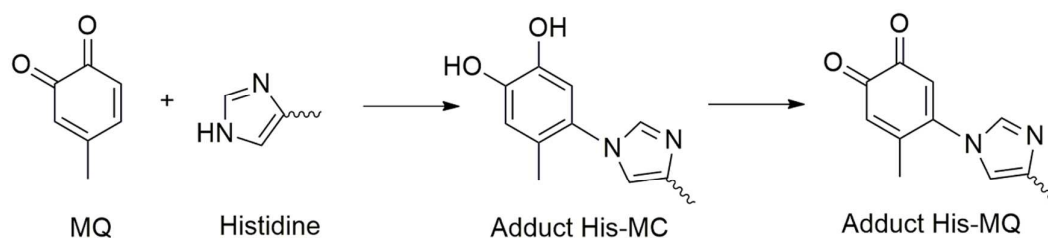
**Figure S10.** Kinetic traces of MC (3 mM) oxidation with time, in 50 mM Hepes buffer solution at pH 7.4 and 20 °C in the presence of  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ , light blue trace) and with addition of PrP<sub>76-114</sub> (50  $\mu\text{M}$ , yellow trace). A similar experiment was carried out with the solvent saturated with pure oxygen with  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ , orange trace) and with addition of PrP<sub>76-114</sub> (50  $\mu\text{M}$ , green trace).



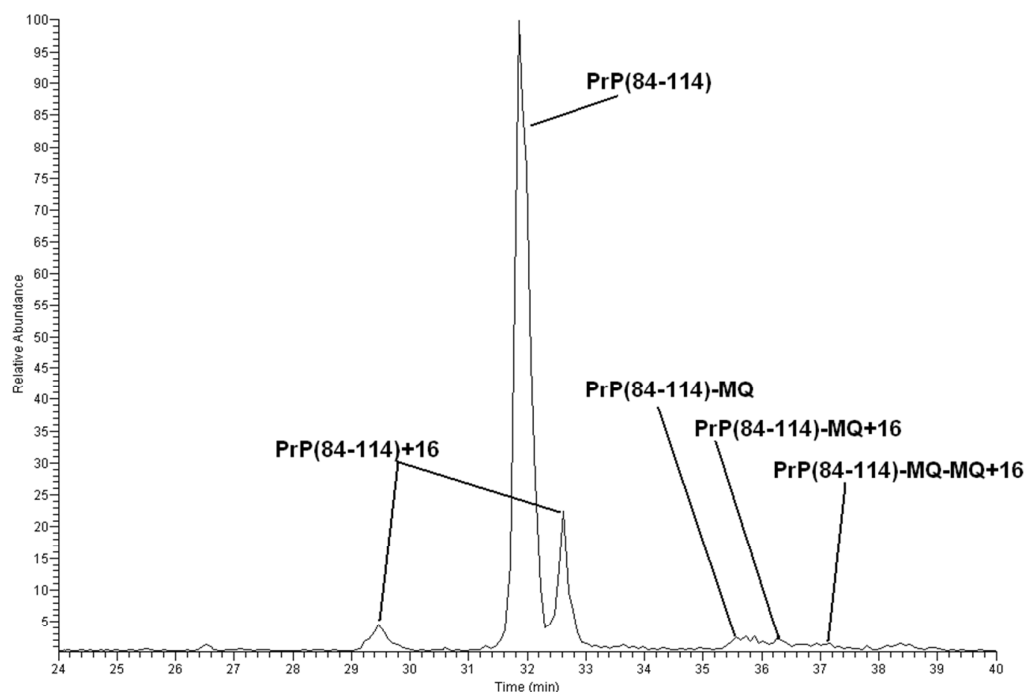
**Figure S11.** Dependence of the reaction rates of 4-methylquinone formation on the concentration of MC. The reactions were performed in Hepes buffer (50 mM) pH 7.4, at 20°C, in the presence of free copper(II) (white circles),  $[\text{Cu}^{2+}\text{-PrP}_{106-114}]$  complex (25  $\mu\text{M}$ ) (orange circles),  $[\text{Cu}^{2+}\text{-PrP}_{84-114}]$  complex (25  $\mu\text{M}$ ) (light blue circles), and  $[\text{Cu}^{2+}\text{-PrP}_{76-114}]$  complex (25  $\mu\text{M}$ ) (green circles). Solid lines correspond to fitting of experimental data with Michaelis-Menten equation.



**Figure S12.** Kinetic traces of MC (3 mM) oxidation with time, in 50 mM Hepes buffer solution at pH 7.4 and 20 °C in the presence of  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ , light blue trace) and with addition of  $\text{PrP}_{76-114}$  (25  $\mu\text{M}$ , yellow trace). Similar experiments were carried out with 1 mM  $\text{H}_2\text{O}_2$  with both  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ , green trace) and  $\text{Cu}^{2+}$  (25  $\mu\text{M}$ ) with addition of  $\text{PrP}_{76-114}$  (25  $\mu\text{M}$ , orange trace). Blank experiment in the presence of MC and  $\text{H}_2\text{O}_2$  is shown by the blue trace.



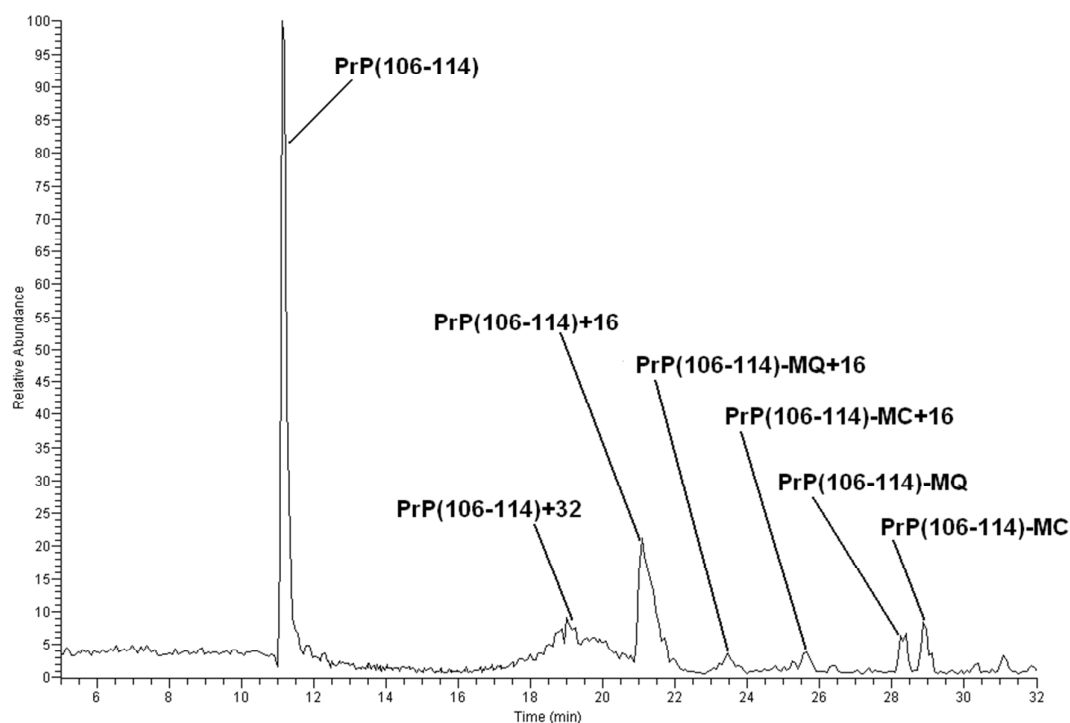
**Scheme S1.** Reaction of histidine residues of PrP peptides with MQ and subsequent oxidation of the resulting adduct to quinone.



**Figure S13.** HPLC-MS elution profiles of peptides resulting from oxidation of MC (3 mM) by Cu-PrP<sub>84-114</sub> (25 μM) at 60 min reaction time in 50 mM HEPES buffer at pH 7.4 and 20 °C. The assignment of the peaks is shown (MQ indicates a mass increment of 120 Da corresponding to the formation of a covalent adduct with 4-methylquinone).

**Table S4.** Modifications of PrP<sub>84-114</sub> peptide (25 μM) detected by LC-MS analysis, in the presence of 25 μM copper(II) nitrate and 3 mM MC in HEPES buffer (50 mM) pH 7.4 at 20 °C.

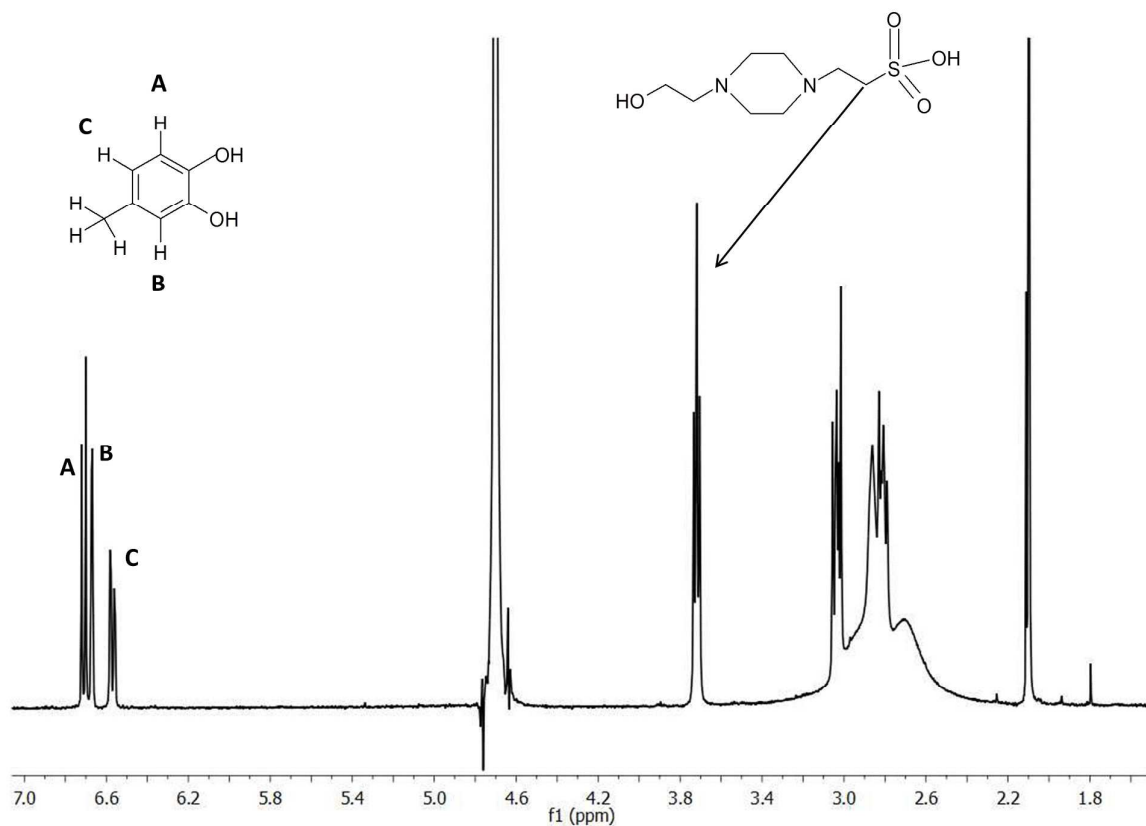
Time (min)	PrP <sub>84-114</sub> (%)	+ 16 (%)	+120 (%)	+136 (%)	+152 (%)	
	(R <sub>t</sub> 31')	(R <sub>t</sub> 32')	(R <sub>t</sub> 29')	(R <sub>t</sub> 35')	(R <sub>t</sub> 36')	(R <sub>t</sub> 37')
1	95.6	1.2	0.4	1.8	0.4	0.6
10	80.0	3.4	3.5	5.8	5.4	1.9
15	72.7	8.9	2.4	7.6	6.2	2.2
60	59.0	7.7	8.3	6.9	11.7	6.4
90	44.9	8.9	1.7	9.0	21.7	13.8
100	11.0	14.5	4.1	13.7	28.0	28.7



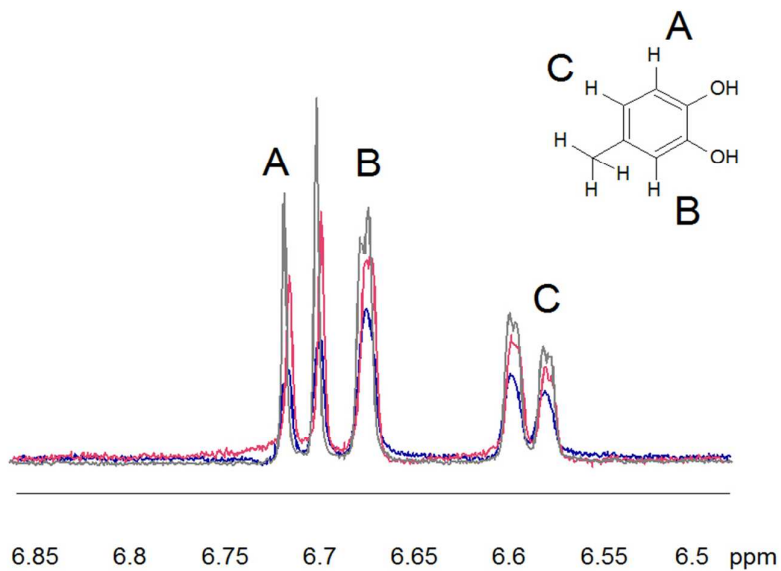
**Figure S14.** HPLC-MS elution profiles of peptides resulting from the oxidation of MC (3 mM) by Cu-PrP<sub>106-114</sub> (25 μM) at 100 min reaction time in 50 mM HEPES buffer at pH 7.4 and 20 °C. The assignment of the peaks is shown (MC and MQ indicate mass increments of 122 and 120 Da, corresponding to the formation of covalent adducts with 4-methylcatechol and 4-methylquinone, respectively).

**Table S5.** Modifications of PrP<sub>106-114</sub> peptide (25 μM) detected by LC-MS analysis, in the presence of 25 μM copper(II) nitrate and 3 mM MC in HEPES buffer (50 mM) pH 7.4 at 20 °C.

Time (min)	PrP <sub>106-114</sub> (%) (R <sub>t</sub> 11')	+16 (%) (R <sub>t</sub> 21')	+32 (%) (R <sub>t</sub> 19')	+120 (%) (R <sub>t</sub> 28')	+122 (%) (R <sub>t</sub> 29')	+136 (%) (R <sub>t</sub> 23')	+138 (%) (R <sub>t</sub> 25')
10	82.7	2.8	0.2	6.5	6.4	0.5	0.9
20	67.7	9.0	1.7	10.2	3.9	1.5	6.0
30	60.6	9.5	0.3	13.7	11.8	2.7	1.4
55	47.0	23.2	3.6	7.4	8.1	7.4	3.3
100	35.1	34.9	5.1	4.8	7.8	7.9	4.4
120	21.1	36.8	8.3	4.9	7.5	14.2	7.2



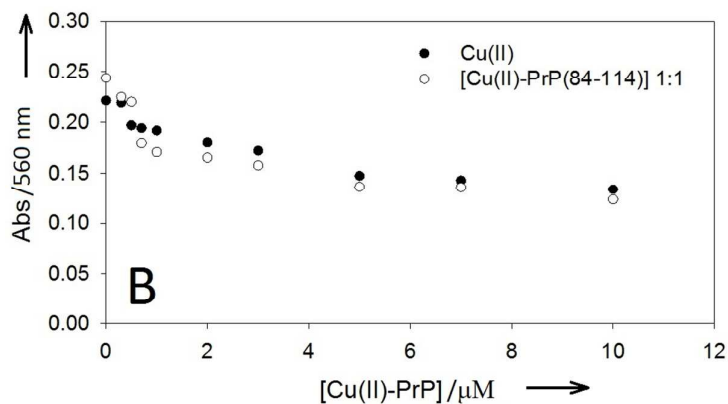
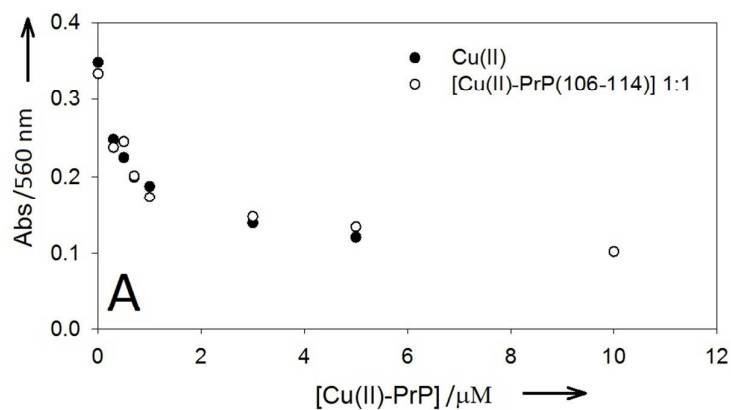
**Figure S15.** Proton NMR spectrum of Hepes buffer (5 mM) pH 7.4 and MC (3 mM) in D<sub>2</sub>O at 20 °C. The Hepes buffer signal of the -CH<sub>2</sub>- group bound to sulfonic acid group at 3.7 ppm was used as internal standard.



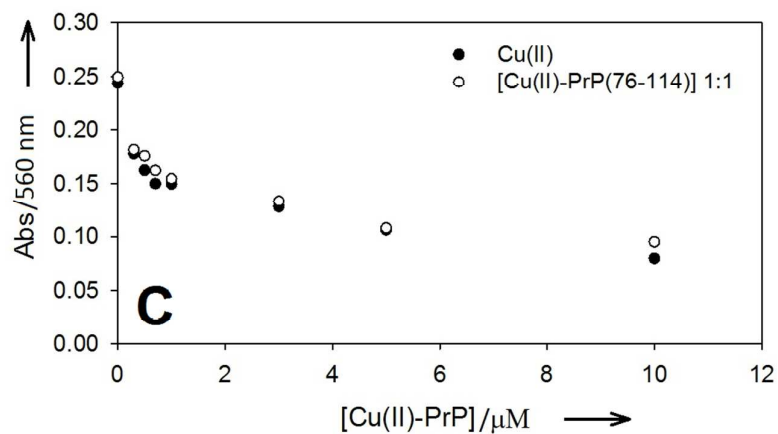
**Figure S16.** Enlargement of the aromatic region of the proton NMR spectra of MC (3 mM) and Hepes buffer (5 mM) pH 7.4 in D<sub>2</sub>O at 20 °C (gray trace), and after 2 h of reaction in the presence of copper(II) nitrate (25 μM) (red trace) and Cu<sup>2+</sup>-PrP<sub>76-114</sub> (25 μM) (blue trace).

**Table S6.** Determination of residual MC concentration by NMR upon oxidation of MC (3 mM) by Cu<sup>2+</sup> and Cu<sup>2+</sup>-PrP<sub>76-114</sub> (25 μM) in 5 mM Hepes deuterated buffer at pH 7.4 and 20 °C.

Time (min)	[MC] (mM)	
	Cu <sup>2+</sup>	Cu <sup>2+</sup> -PrP <sub>76-114</sub>
0	3.00	3.00
5	2.94 ± 0.08	2.55 ± 0.02
30	2.82 ± 0.09	2.36 ± 0.06
60	2.68 ± 0.10	2.17 ± 0.10
90	2.53 ± 0.06	1.88 ± 0.04
120	2.44 ± 0.02	1.55 ± 0.07
150	2.34 ± 0.04	1.32 ± 0.04







**Figure S17.** Plots of UV-Vis absorbance at 560 nm for the NBT reduction to  $\text{MF}^+$  by  $\text{O}_2^-$  in the presence of  $\text{Cu}^{2+}$  (black circles) and  $\text{Cu}^{2+}$  complexes (white circles) with PrP<sub>106-114</sub> (A), PrP<sub>84-114</sub> (B) and PrP<sub>76-114</sub> (C). Spectra were taken at 20 °C, in 50 mM phosphate buffer at pH 7.4.