The Prion Protein is a Combined Zinc and Copper Binding Protein: Zn^{2+} alters the Distribution of Cu^{2+} Coordination Modes

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Supporting Information:

Experimental

Peptides were synthesized and purified as in previous work (Fmoc solid phase synthesis). The following sequences were used:

PrP 23-28, 57-91: Ac-KKRPKPWGQ$(PHGGGWGQ)_4$-NH_2

PrP 60-91: Ac-$(PHGGGWGQ)_4$-NH_2

PrP 90-115: Ac-GQGGGTHNQWNKPSKPKTNMKHMAG-NH_2

Syrian Hamster PrP (SHaPrP 23-231) was expressed in e. coli (BL21 cells) using the pET101 vector from Invitrogen. Because the eukaryotic signal peptide (1-22) is not included, the placement of the start codon results in an N-terminal methionine. The protein was purified by isolating the inclusion bodies from lysed cells, solubilizing the inclusion bodies in 8 M urea (pH 8) and then passing the solution over a nickel charged IMAC column. The protein was eluted using pH 4.5 8 M urea. The protein was folded by first raising the pH back to 8, then desalting using a G-25 sephadex column (HiPrep, Amersham) with pH 5.5 50 mM sodium acetate buffer as the eluent solvent. The protein was further purified using reverse phase HPLC with a C4 column and water/isopropanol mobile phases. Fractions were analyzed by ESI-MS. Pure fractions were lyophilized. Protein was resolubilized in water and the folding state checked by Circular Dichroism. Concentration of protein stocks was determined as follows: a small aliquot (5-20 µl) was diluted with 6 M guanidine (120-1000 µl) and the absorbance at 280 nm recorded. The concentration was calculated using an absorptivity of 61025 M^{-1} cm^{-1}.

DEPC reactions were performed on peptides by adding 100 µL of 0.5% DEPC in water to 300 µL of 40 µM peptide with 25mM NEM, 25mM MOPS buffer at pH 7.4. The 0.5% DEPC was made immediately before reacting with peptide to prevent hydrolysis. The reaction was run for 1 minute and then quenched by adding 100 µL of 250mM imidizole. The reaction was diluted further, filtered and analyzed using reverse phase HPLC. A Shimadzu SCL-10AVP HPLC system was used with an Alltech analytical C-18 column. A 0.5% per minute gradient was employed with water and acetonitrile (each with 0.65% TFA) as the mobile phases. Fractions were collected and analyzed with a Waters ZMD electrospray mass spectrometer to determine the number of modifications associated with each peak.

EPR spectra were obtained using a Bruker EMX X-band spectrometer equipped with a constant flow cryostat. Buffer for EPR samples was 50 mM MOPS (free acid) and 25% glycerol. Buffer pH was adjusted to 7.4 with KOH. A sample volume of 300 µl was contained in a 4 mm OD quartz tube and flash frozen in liquid nitrogen. Peptide concentrations were between 50 and 100 µM and protein concentrations ranged from 25-50 µM. EPR spectra were recorded at a temperature of 125 K. The field was swept 1200 G in 183 seconds with a time constant of 655 ms. The field was modulated at 100 kHz with an amplitude of 5 G.

EPR Data:

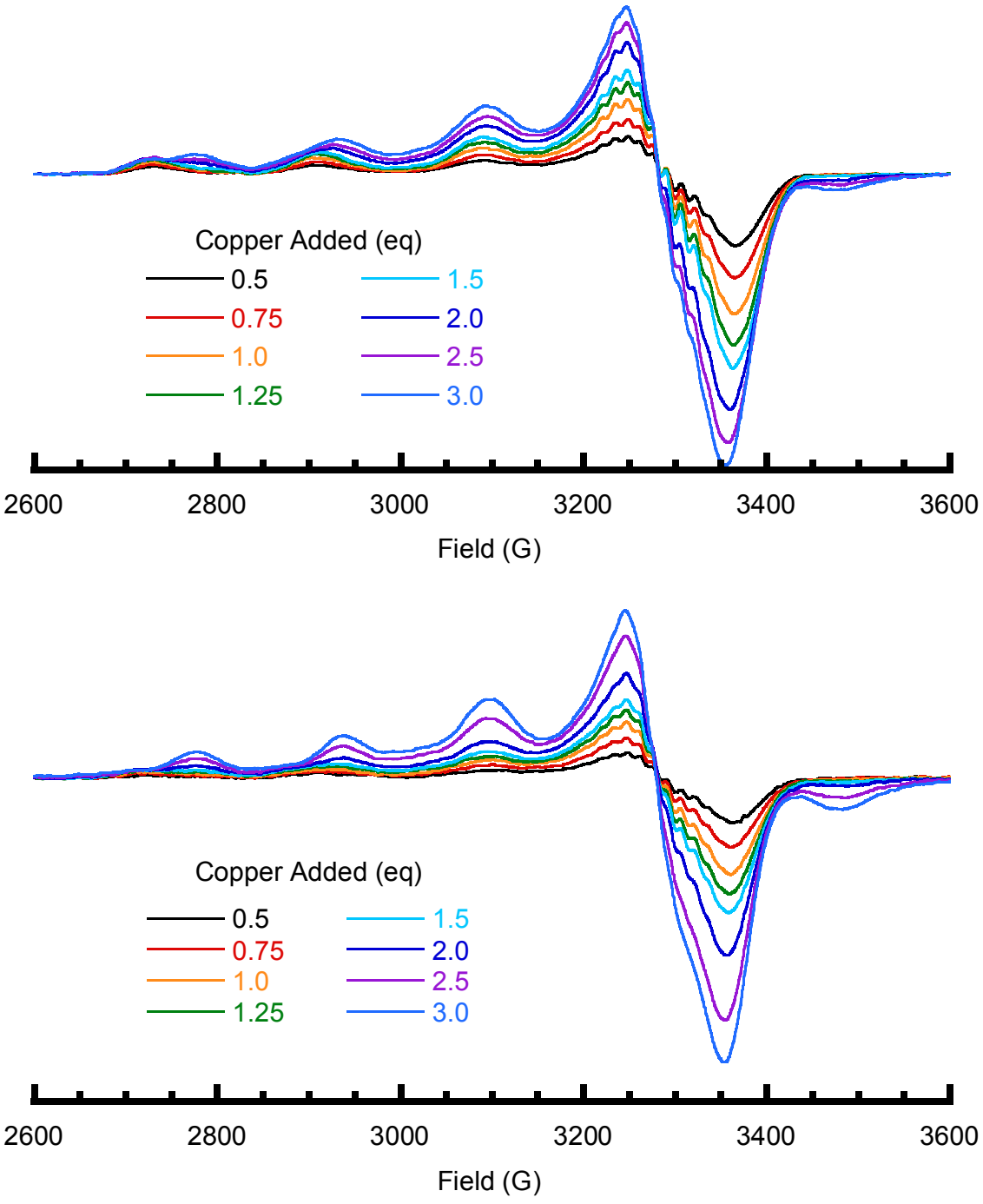
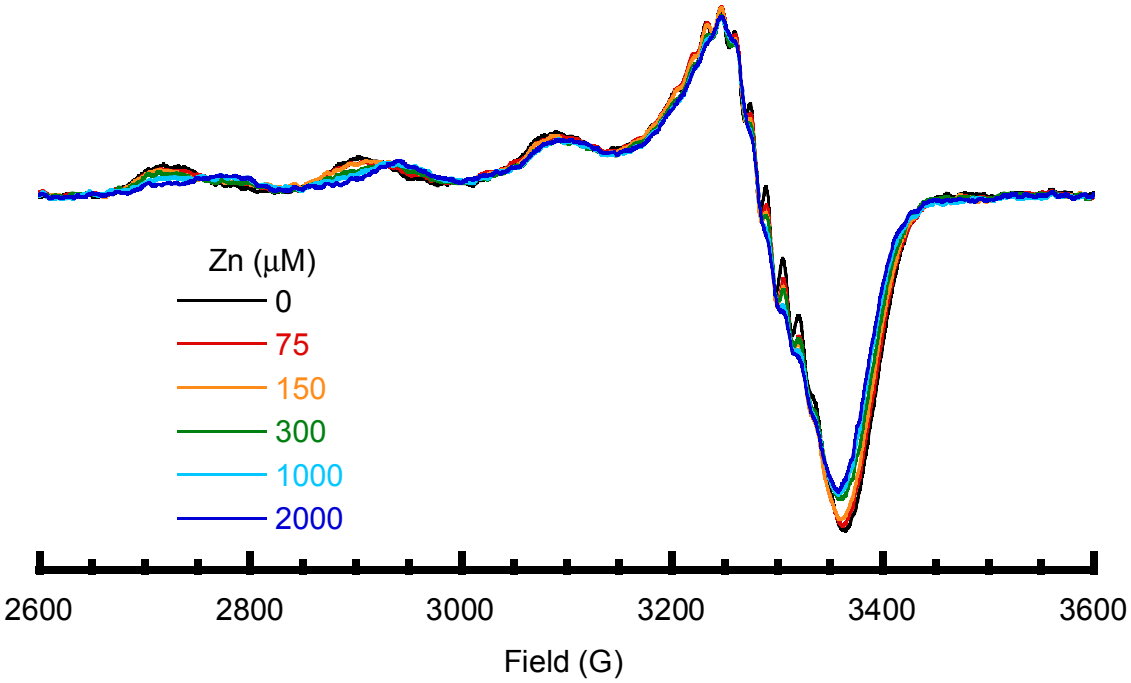


Figure 1. EPR spectra of a copper titration of PrP 23-28, 57-91. Top, 0 µM zinc; Bottom, 300 µM zinc. These spectra were decomposed into component spectra using non-negative least squares fitting. Those results were used to construct Figure 1b in the main article.



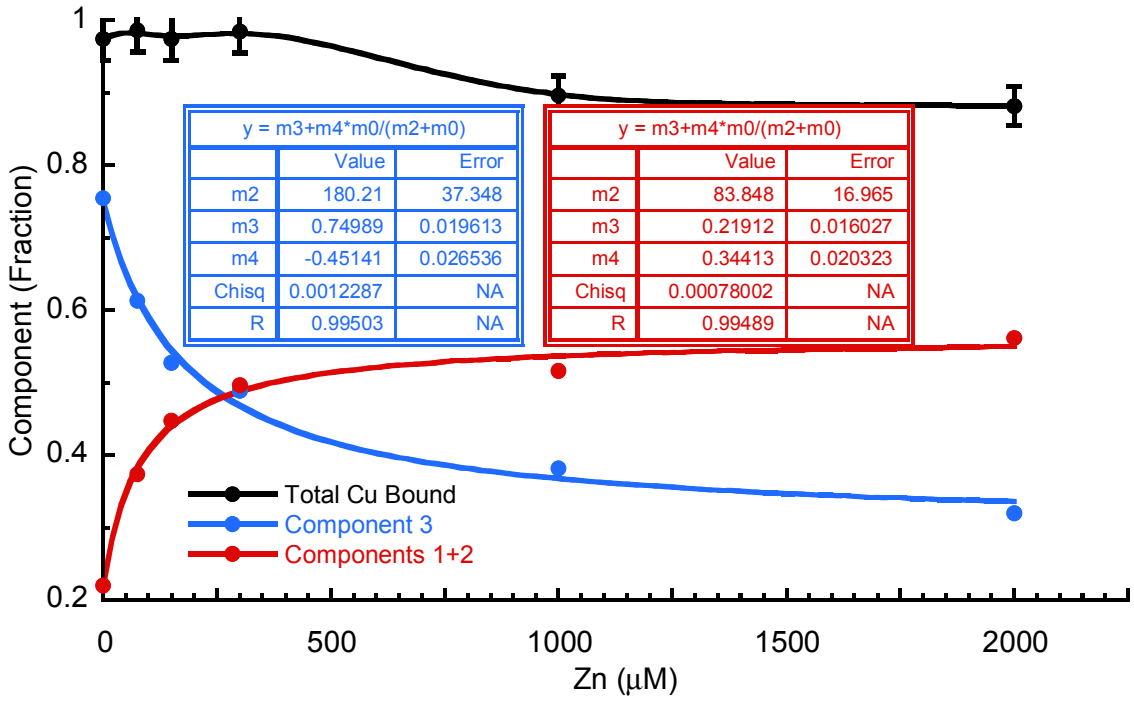


Figure 2. Zinc titration of PrP 23-28, 57-91 loaded with 1 eq of copper. Top, EPR spectra; Bottom, Components of the EPR spectra as determined by Non-negative Least Squares fitting (dots), Total Cu Bound shown with a smooth line to guide the eye (solid black line), error bars are derived from residual of fit, Best fit saturation curves (solid lines) for Components 3 and 1+2. The bottom figure is an expansion of Figure 1a in the main article.

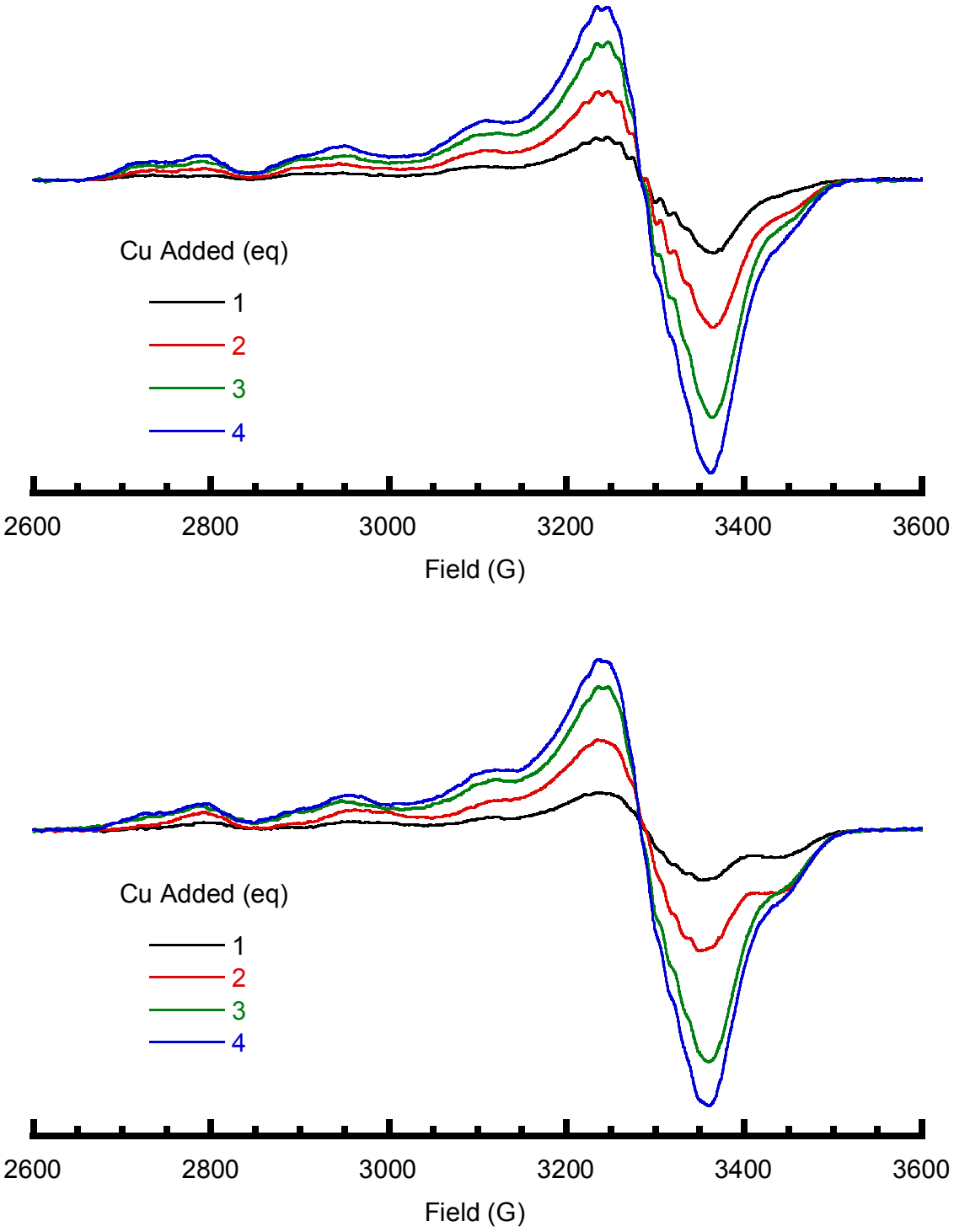
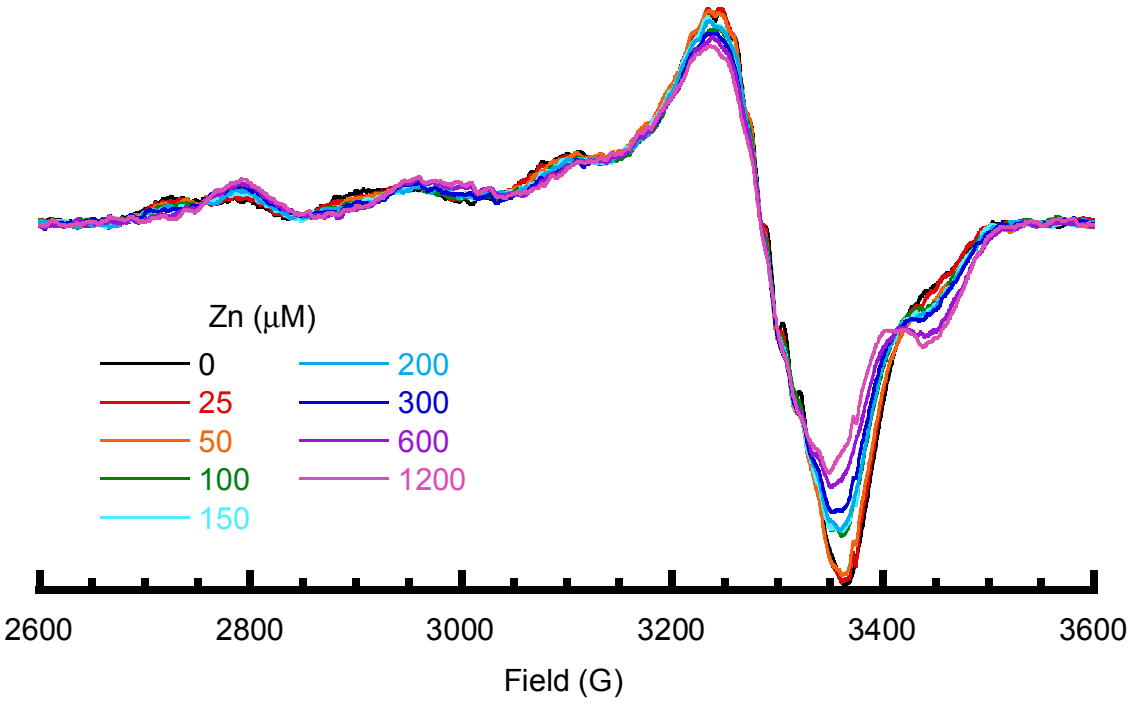
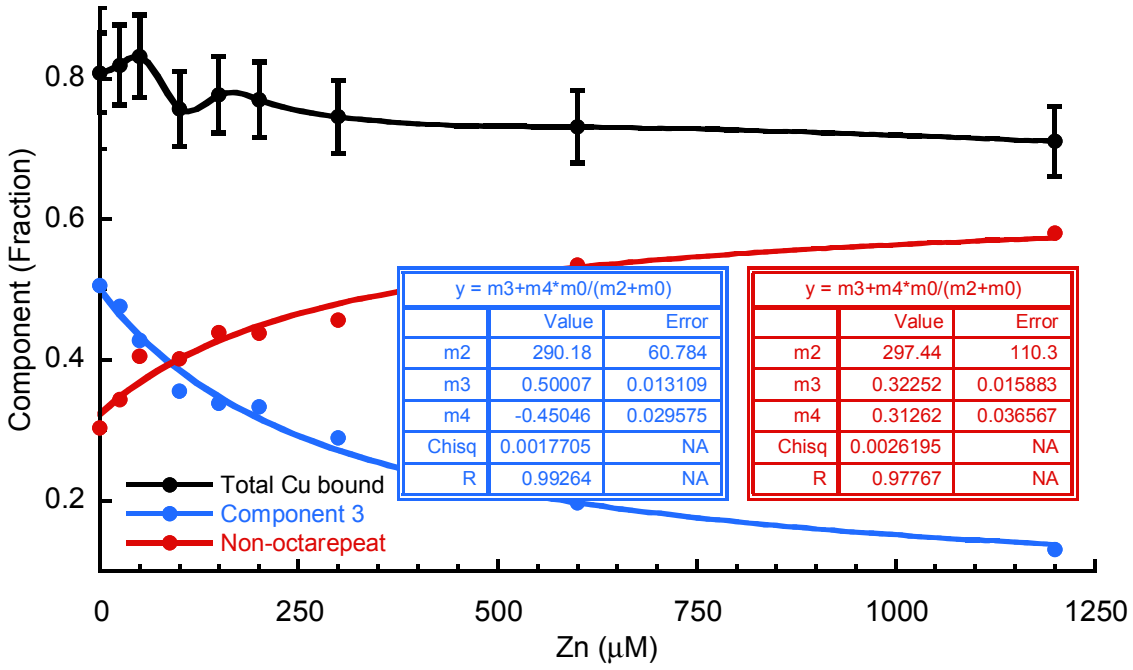


Figure 3. EPR spectra of a copper titration of SHaPrP 23-231. Top, 0 μM zinc; Bottom, 300 μM zinc. These spectra were used to construct Figure 1d in the main article.





y = m3+m4*m0/(m2+m0)		
	Value	Error
m2	290.18	60.784
m3	0.50007	0.013109
m4	-0.45046	0.029575
Chisq	0.0017705	NA
R	0.99264	NA

y = m3+m4*m0/(m2+m0)		
	Value	Error
m2	297.44	110.3
m3	0.32252	0.015883
m4	0.31262	0.036567
Chisq	0.0026195	NA
R	0.97767	NA

Figure 4. Zinc titration of SHaPrP 23-231 loaded with 2 eq of copper. Top, EPR spectra; Bottom, Components of the EPR spectra as determined by Non-negative Least Squares fitting (dots), Total Cu Bound shown with a smooth line to guide the eye (solid black line), error bars are derived from residual from fit. Best fit saturation curves (solid lines) for Components 3 and 1+2. The bottom figure is an expansion of Figure 1c in the main article.

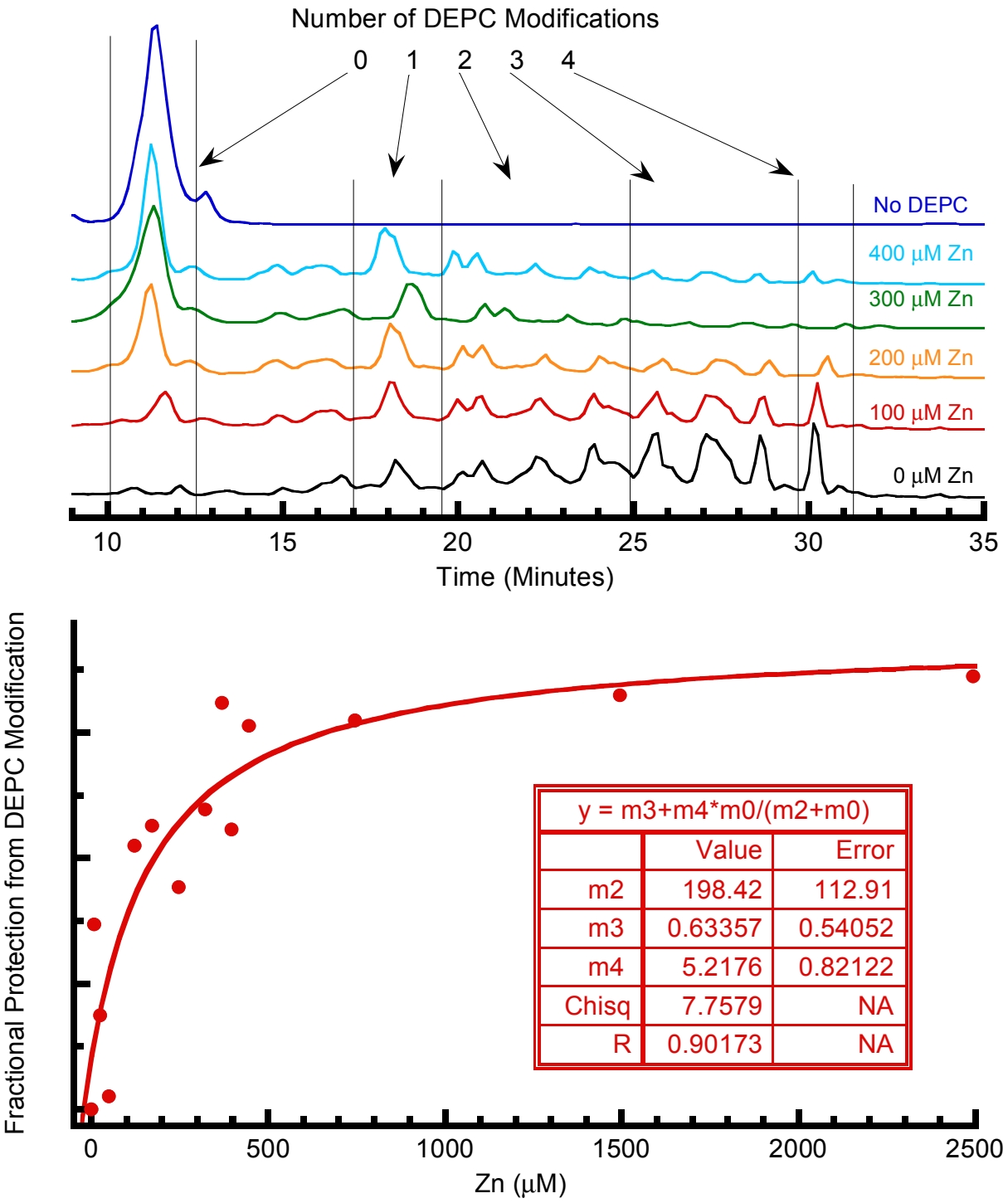


Figure 5. DEPC Modification of PrP 60-91. Top, HPLC chromatographs for selected concentrations of Zn. Bottom, Fractional Protection from DEPC Modificationas monitored by the change in the HPLC peak at 30-31 min (4 modifications) as a fraction of the total integration of all HPLC peaks (dots) fit with a saturation curve (solid line).

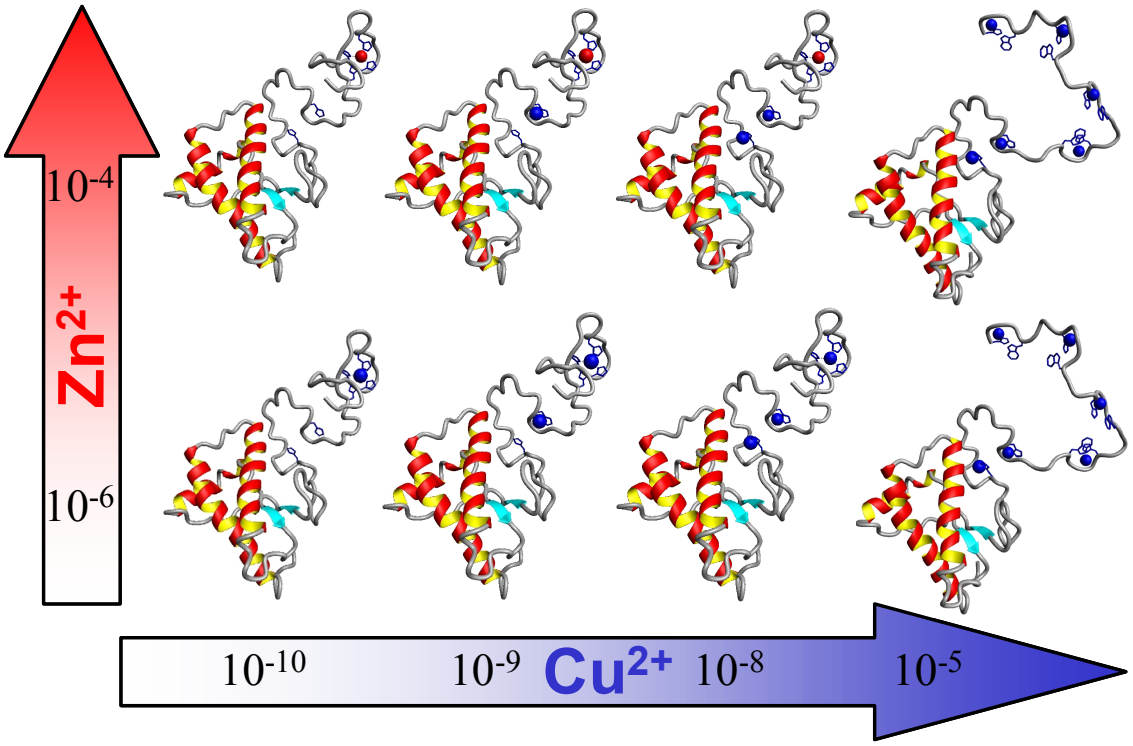


Figure 6. Models representing metal binding in the N-terminal domain of PrP. Top row (High Zinc); Zinc (red) is bound by the octarepeat region (left) while non-octarepeat sites (H96 and H111) are available for copper binding (blue, middle). Copper at high concentration will displace zinc from octarepeats to form up to 4 eq of Component 1 (right). Bottom row (Low Zinc); Copper (blue) is bound by the octarepeats in Component 3 when copper is low (left), with increasing copper loads the non-octarepeat sites (middle). High copper (right column) results in Component 1 copper binding by the octarepeats. Approximate molar metal concentrations are shown in the arrows. Octarepeat structures based on data from Chattopadhyay et al. This figure is an expansion of Figure 2 in the main article.