Both N-Terminal and C-Terminal Histidine Residues of the Prion Protein are Essential for Copper Coordination and Neuroprotective Self-Regulation.

Kevin M. Schilling[a], Lizhi Tao[b], Bei Wu[c], Joseph T.M. Kiblen[a], Natalia C. Ubilla-Rodriguez[a], M. Jake Pushie[d], R. David Britt[b], Graham P. Roseman[a], David A. Harris*[c], and Glenn L. Millhauser*[a]

[a] 1156 High Street. Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA, 95064, United States
[b] 1 Shields Ave. Department of Chemistry, University of California, Davis, CA, 95616, United States
[c] 72 E. Concord St Silvio Conte. Department of Biochemistry, Boston University School of Medicine, Boston, MA, 02118, United States
[d] 107 Wiggins Rd B419. Department of Surgery, College of Medicine, University of Saskatchewan, Saskatoon, SK S7N 5E5, Canada

Correspondence: DA Harris: daharris@bu.edu, or GL Millhauser: glennm@ucsc.edu
+1 617-358-4280 (phone) +1 831 459 2176 (phone)
+1 617-358-4353 (fax) +1 831 459 2935 (fax)

Supplemental Data

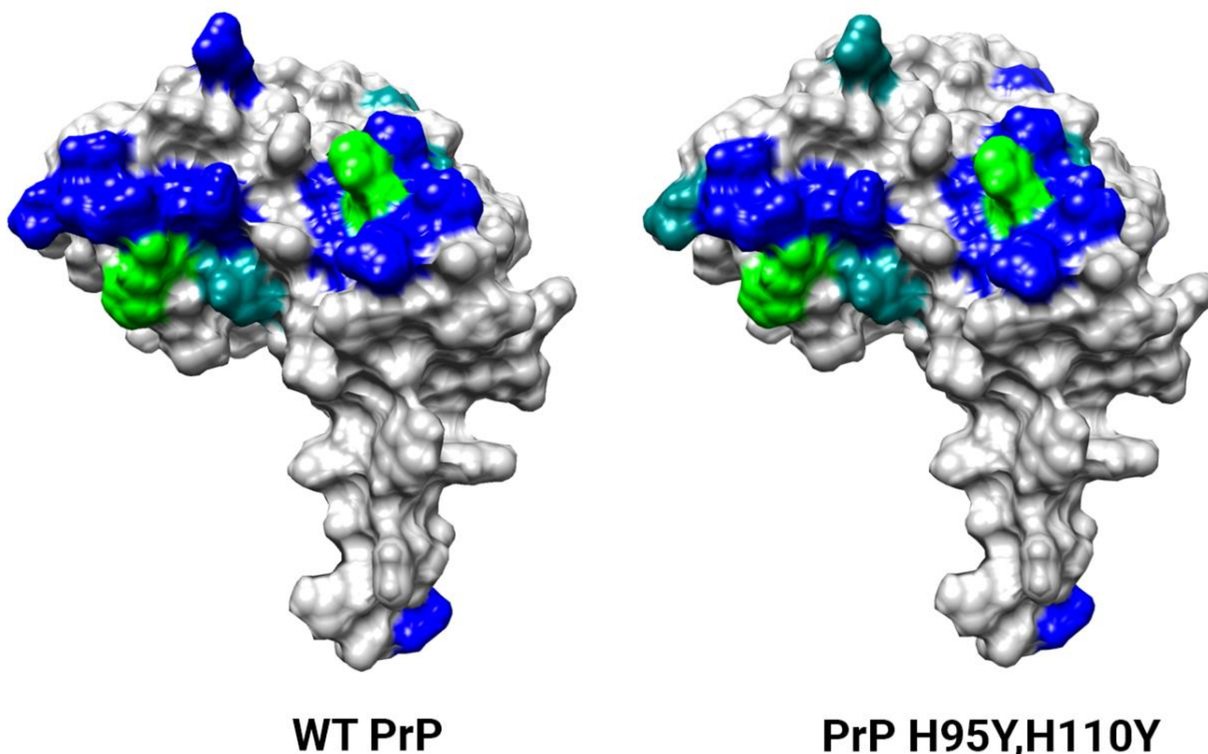

Figure S1. A comparison of peak intensity reduction in WT PrP^C vs. PrP^C (H95Y,H110Y) shows that histidines 95 and 110 are not driving the interaction between the protein's N and C termini, ruling out the possibility that the C-terminus of PrP^C is interacting with copper bound solely to a (H95,H110) complex. Samples were recorded with 300 μM protein, 10 mM MES buffer pH 6.0, 10% D_20, at 37 °C, both with and without 300 μM $CuCl_2$. Residues that broadened strongly in the presence of copper (dark blue), broadened weakly in the presence of copper (light blue) and histidines (green) are colored. Coordinates for the C-terminal PrP^C structure are from PDB:1XYX.

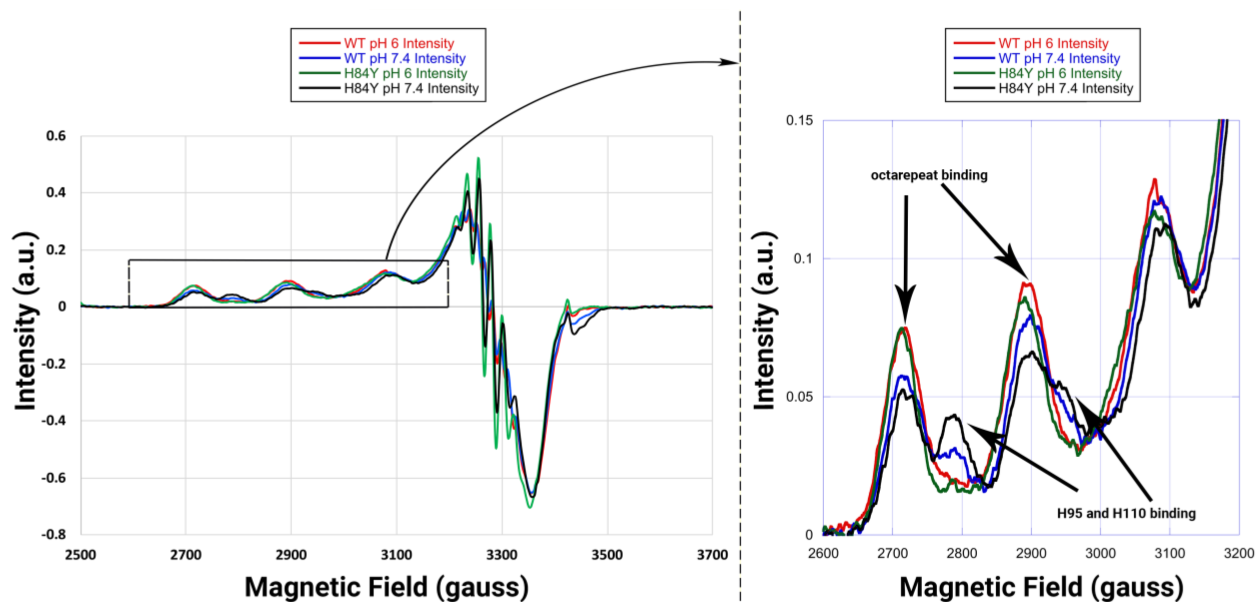

Figure S2. Continuous wave EPR of WT PrP^{C} and PrP^{C} H84Y shows that the copper binding to a central region histidine complex (H95,H110) that is observed at pH 7.4 is not observed at pH 6.0. Samples were recorded with 100 μM PrP, 50 mM MES (pH 6.0) or MOPS (pH 7.4) buffer, 25% glycerol, and 100 μM $CuCl_2$. Noise reduction was applied post sample collection. The 4-histidine splitting has an a-parallel of 185 G and a g-parallel of 2.249. The two histidine splitting (H95,H110) has an a-parallel of 159 G and a g-parallel of 2.226.

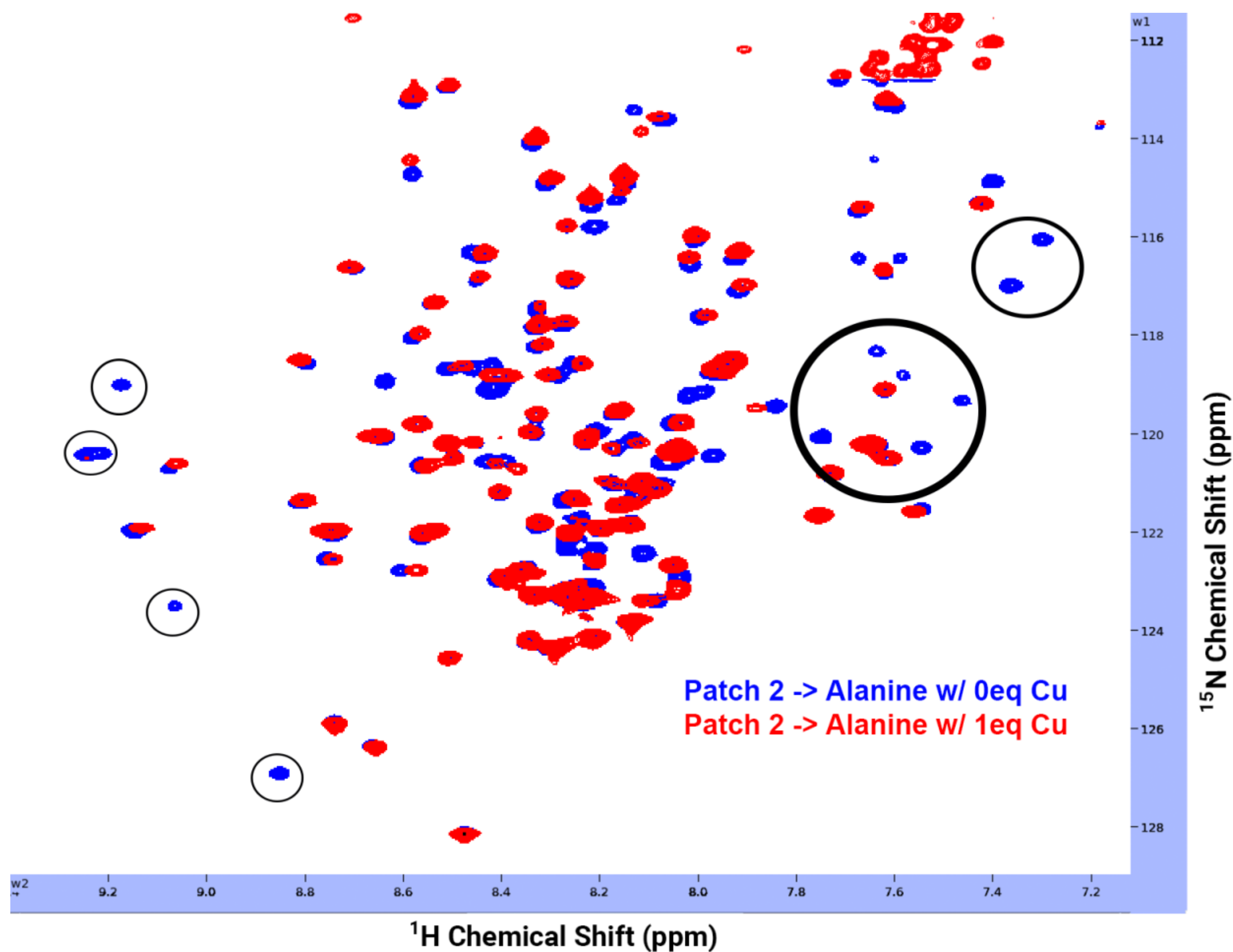

Figure S3. PrP^C (172A,173A,175A,179A,180A) with and without copper shows that residues near His176 do not play a role in the *cis* interaction, highlighting the importance of His176. Samples were recorded with 300 μM protein, 10 mM MES buffer pH 6.0, 10% D_2O, at 37 °C, both with and without 300 μM $CuCl_2$. Because most of the strongly broadened peaks in the protein have been changed in this mutant, we do not have assignments for them. However, we know that they are the peaks that are new to this mutant (inside the big circle). These peaks disappear with copper, indicating that the *cis* interaction is still occurring in this mutant.

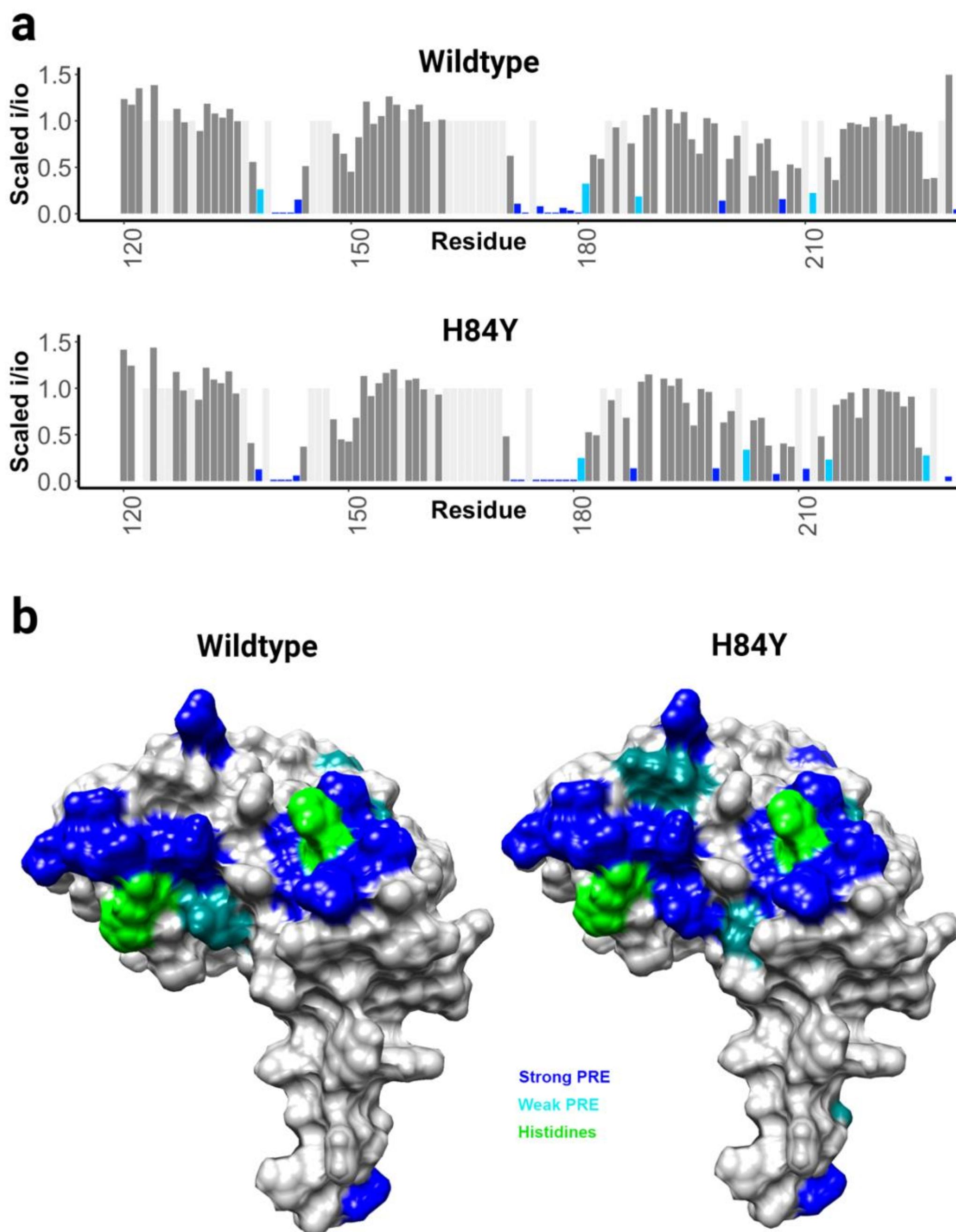

Figure S4. A comparison of peak intensity reduction in WT PrP^C vs. PrP^C H84Y shows that the *cis* interaction is preserved and even strengthened when one octarepeat histidine is deleted. This agrees with the hypothesis that only three out the four octarepeat histidines are involved in the *cis* interaction at any given time. Samples were recorded with 300 μM protein, 10 mM MES buffer pH 6.0, 10% $D_2$0, at 37 °C, both with and without 300 μM $CuCl_2$. a) Bar plots showing the magnitude of the peak intensity reduction with the addition of one equivalent of copper. b) Surface representations showing areas engaged in the cis interaction, as measured by peak intensity reduction. Residues that broadened strongly in the presence of copper (dark blue), broadened weakly in the presence of copper (light blue) and histidines (green) are colored. Coordinates for the C-terminal PrP^C structure are from PDB:1XYX