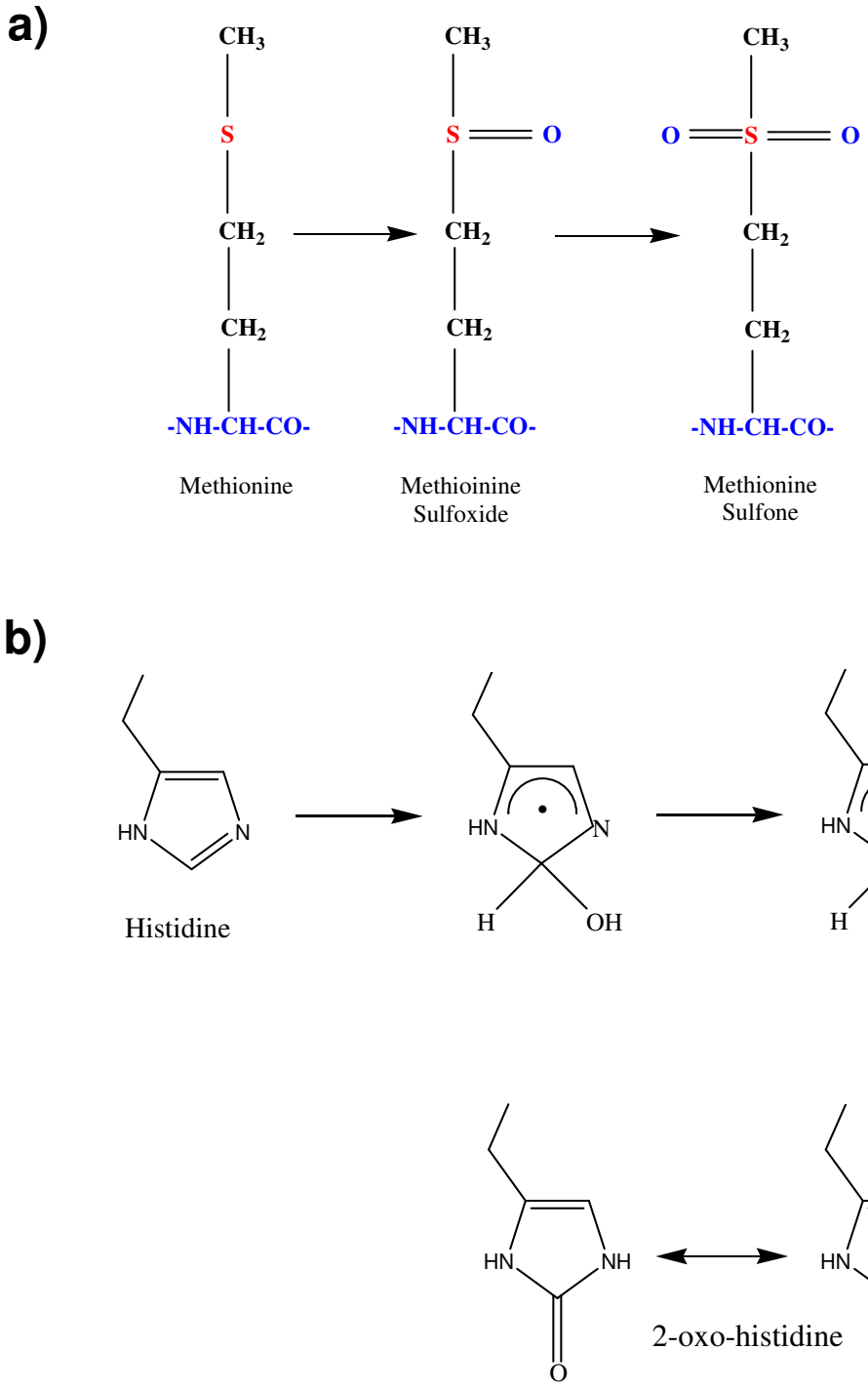
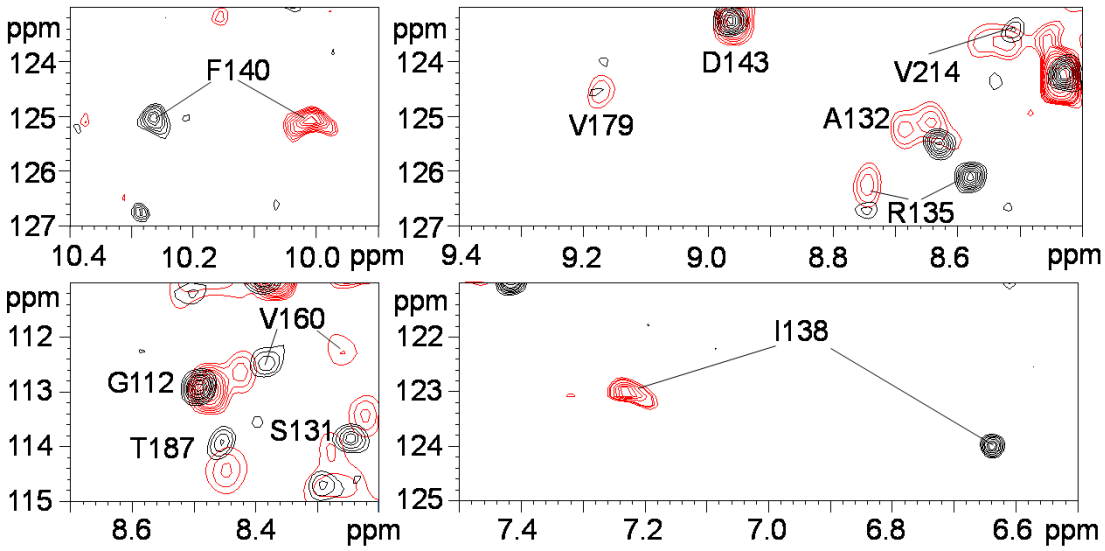
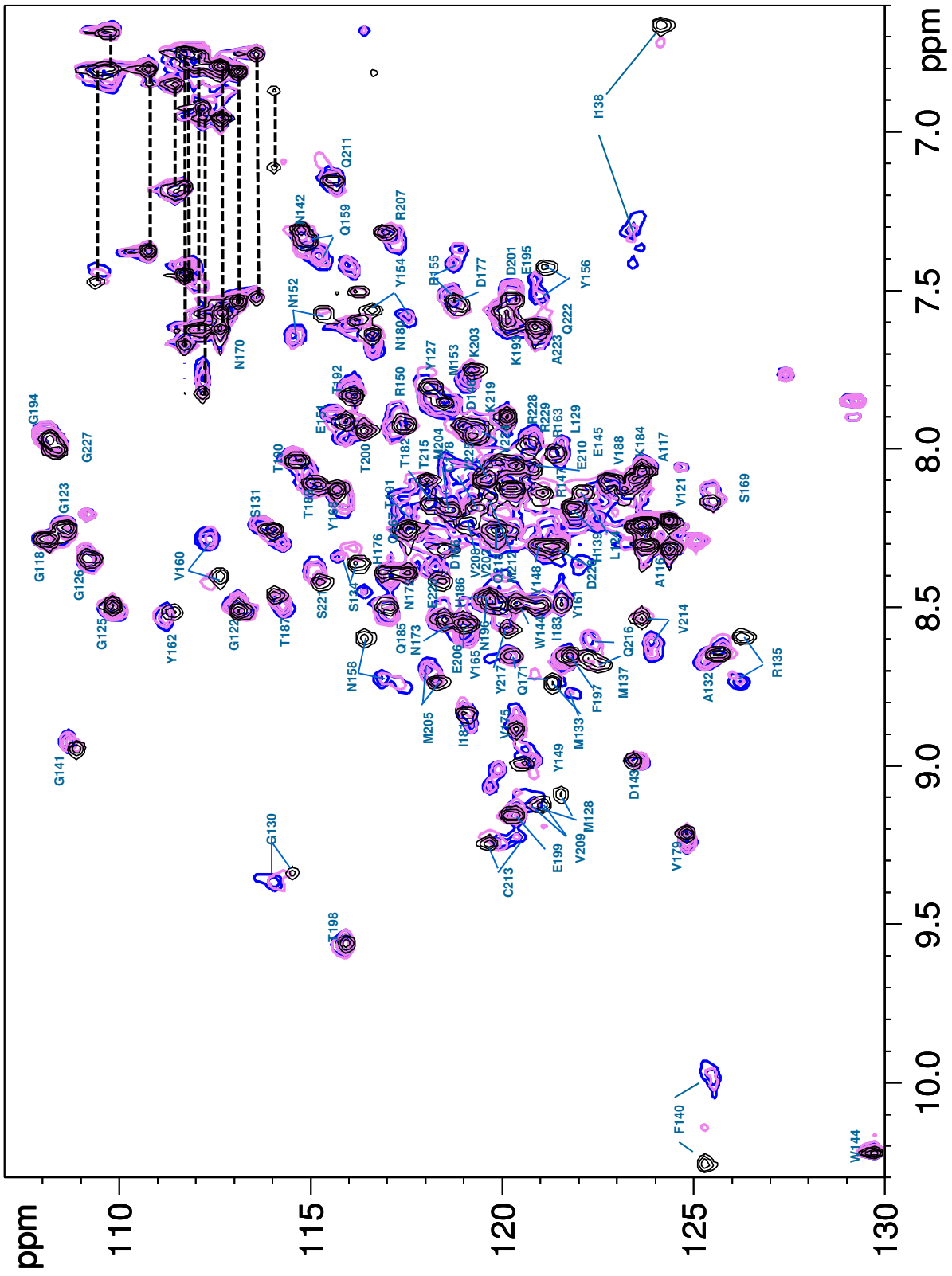


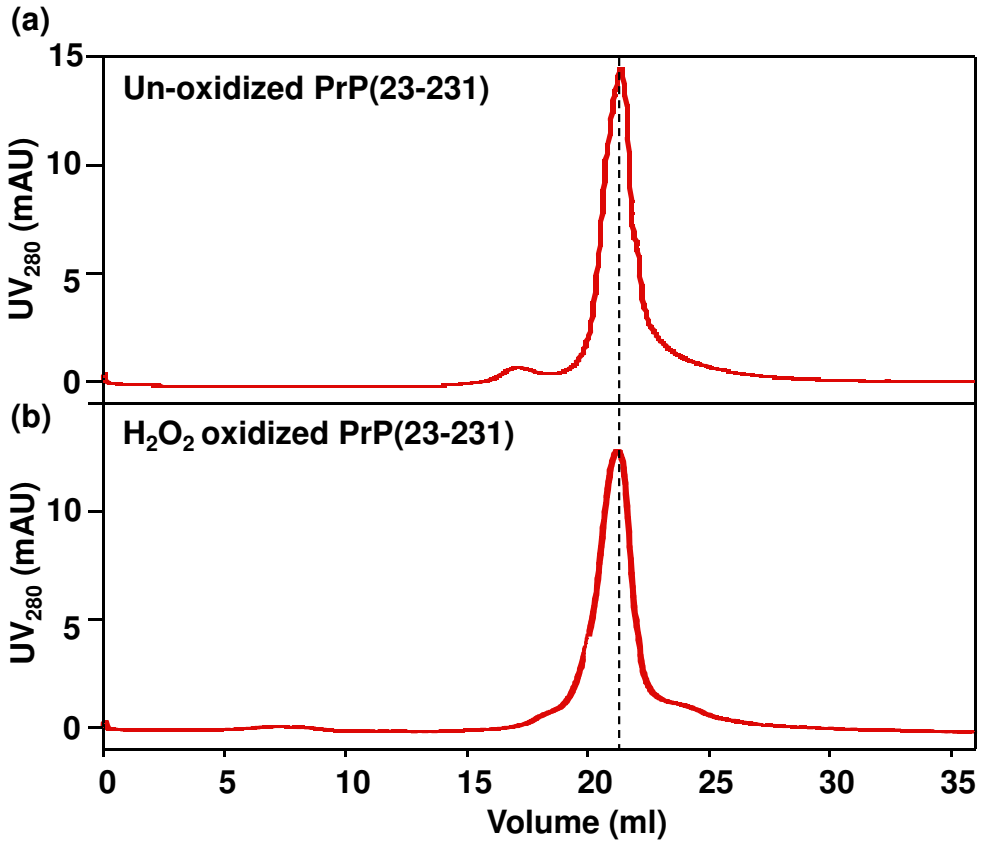
Supplemental Figure S1: (a) Oxidation of methionine to methionine-sulfoxide and methionine-sulfone via hydrogen peroxide. **(b)** Copper catalyzed oxidation of histidine to 2-oxo-histidine.



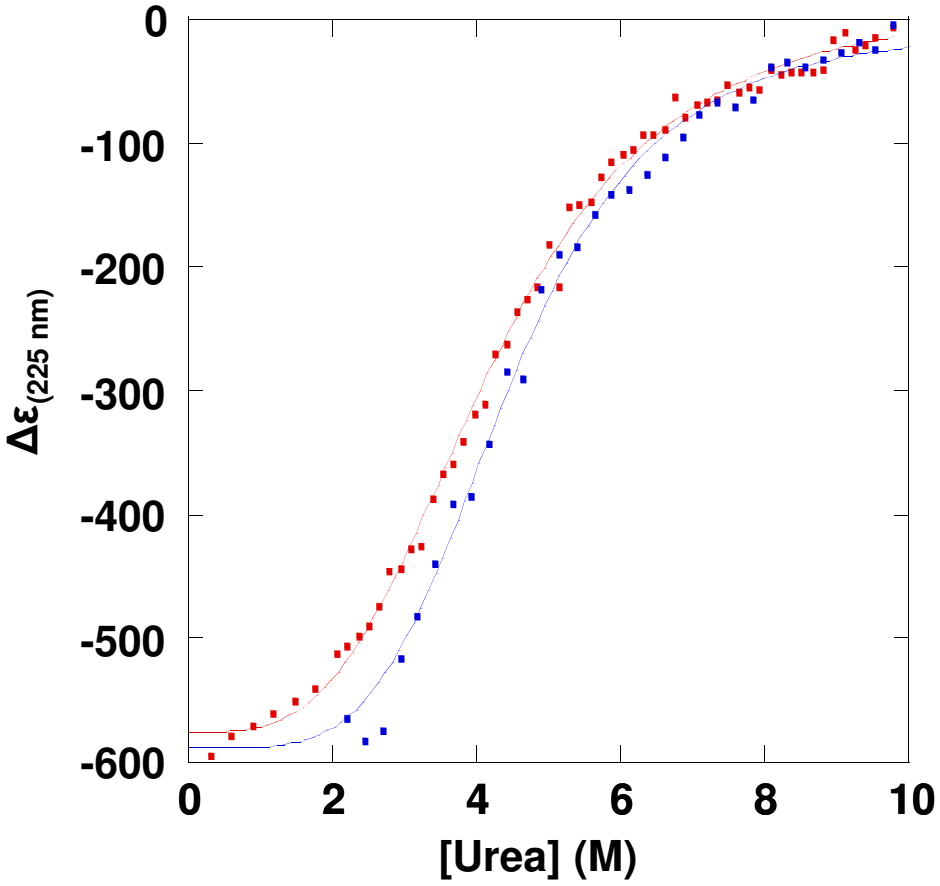
Supplemental Figure S2 : 2D ^{1}H-^{15}N HSQC of H_2O_2 Oxidized PrP(23-231)
Un-oxidized PrP(23-231) spectra (black), oxidized PrP(23-231) after 9 hrs incubation at 37 ºC (red). PrP(23-231) (130 micromolar) oxidized with 10 mM H_2O_2 in 10 mM sodium acetate buffer pH 5.5. Residues perturbed by H_2O_2 oxidation are the same to those observed from PrP(113-231), shown in Figure 1.



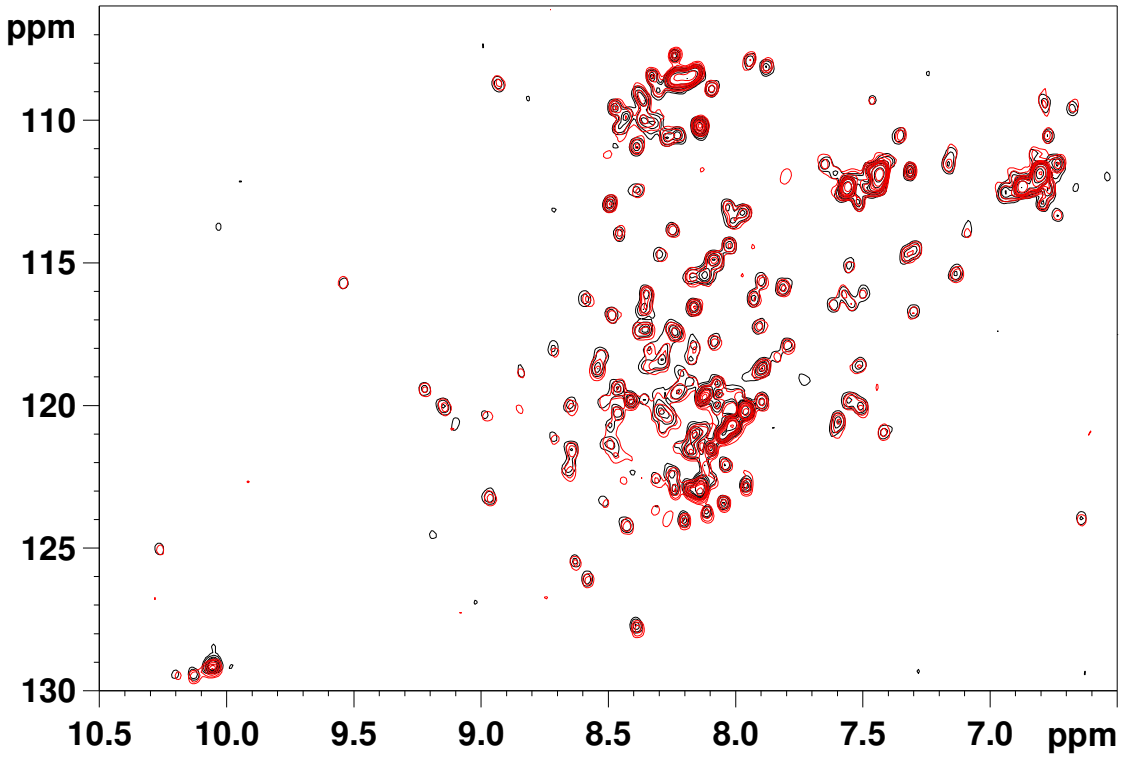
Supplemental Figure S3: Methionine oxidation of PrP(113-231). 2D ^{1}H-^{15}N HSQC spectra of PrP(113-231) (130 micromolar) in 10 mM sodium acetate buffer, pH 5.6 at 37 ^{o}C, showing un-oxidized spectra (black) and H_2O_2 (10 mM) incubated sample at 3 hrs (pink) and 9 hrs (blue) incubation times. Selected regions of HSQC are shown in Figure 1 together with chemical shift perturbations.



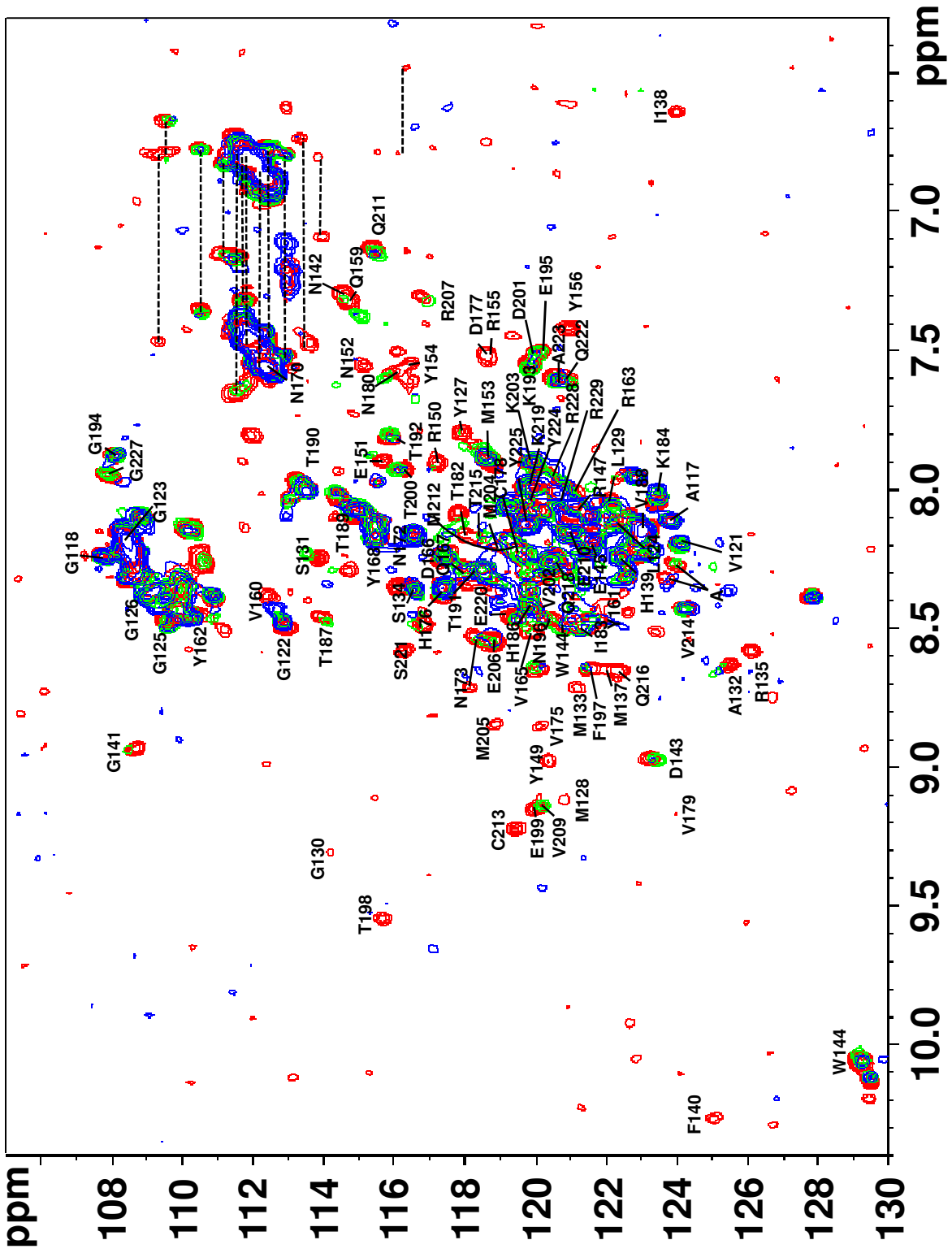
Supplemental Figure S4: Size Exclusion Chromatogram of oxidized PrP(23-231) by H_2O_2. (a) Un-oxidized PrP(23-231), elution volume typical of a monomer. (b) PrP(23-231) incubated with 10 mM H_2O_2, elution volume also typical of a monomer. Samples were incubated at 37 ^oC, in 10 mM sodium acetate buffer at pH 5.5, for 7 hours, at 3mg/ml PrP(23-231) concentration and diluted to 0.1 mg/ml for injection onto size-exclusion Superdex-200 column.



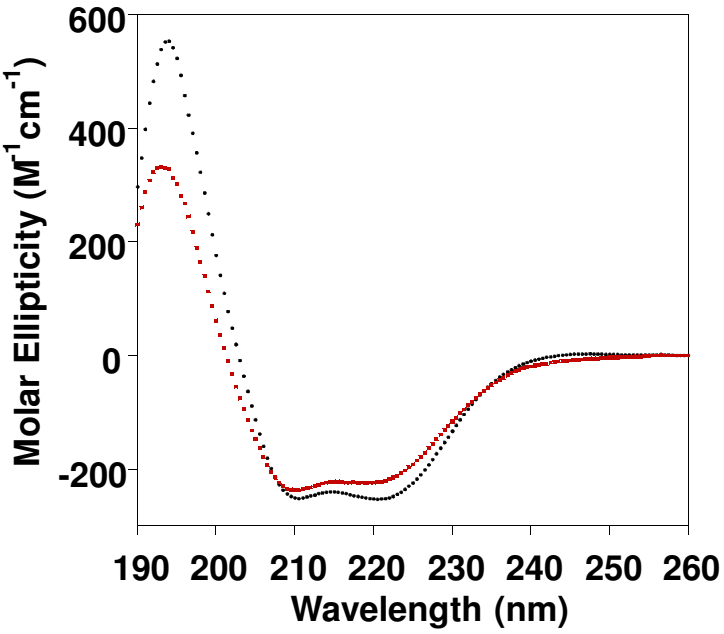
Supplemental Figure S5: Reversible unfolding of H_2O_2 oxidized PrP(23-231). Circular dichroism of chemical denaturation (using urea) on oxidized PrP(23-231) under the mild conditions; the unfolding trace (red) and refolding trace (blue) show the change at 225 nm. All carried out at pH 5.5, using 10 mM sodium acetate buffer, with a protein concentration of 4.3 μM. Refolding data obtained by dilution of 10 M urea sample. Very similar curves, with the $[D]^{50\%}$ for the unfolding is 4.2 M and for the refolding is 4.4 M, indicating urea unfolding is reversible.



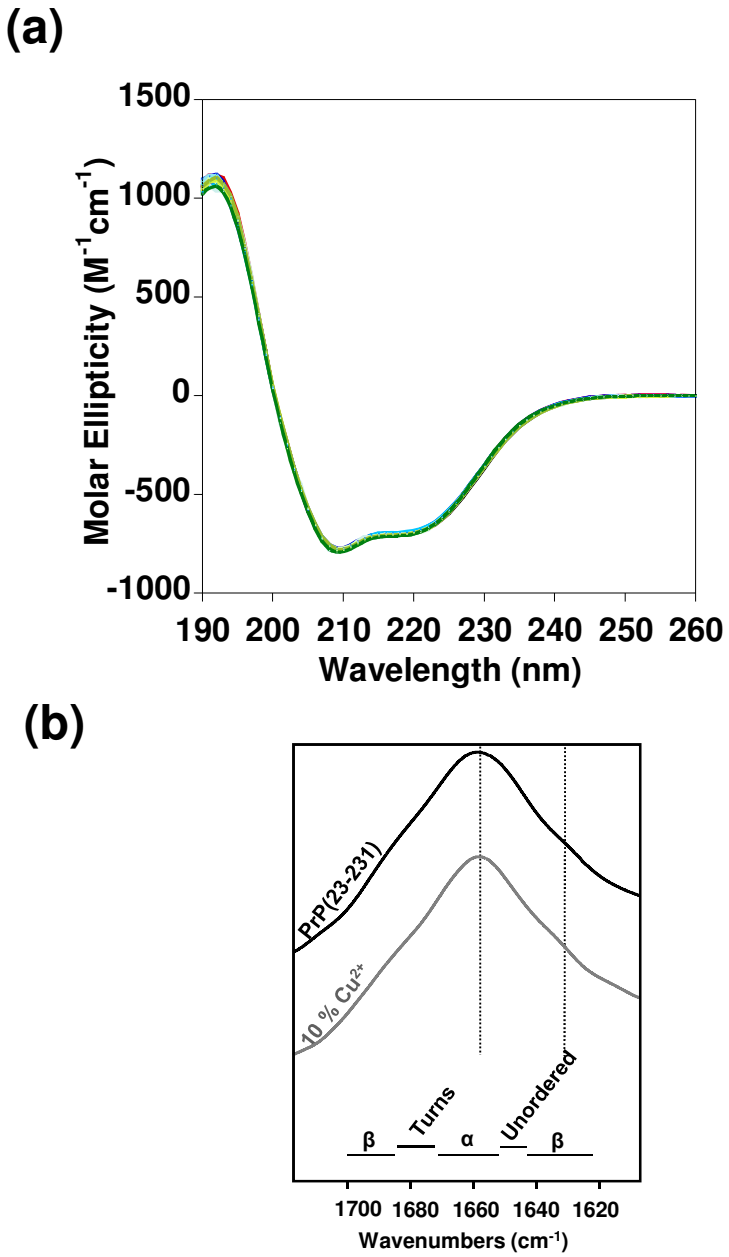
Supplemental Figure S6: 2D ^{1}H-^{15}N HSQC of PrP(23-231) with and without 0.1 mole equivalents Cu^{2+} ions. PrP(23-231) apo (black) and PrP(23-231) with 0.1 mole equivalents Cu^{2+} ions (red). Only very minor perturbations in the ^{1}H-^{15}N HSQC spectra with 0.1 mole equivalents of paramagnetic Cu(II) present. There was no change in the spectra recorded over time.



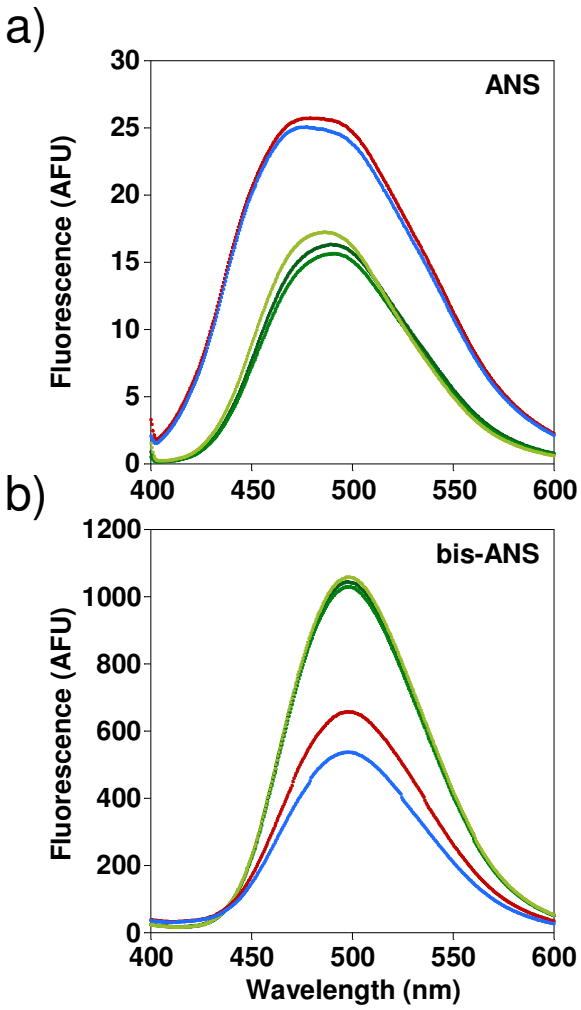
Supplemental Figure S7: 2D ^{1}H-^{15}N HSQC of copper catalyzed oxidation of PrP(23-231). (Same data as shown in Figure 3) Un-oxidized PrP(23-231) (red), Cu^{2+}catalyzed oxidation of PrP(23-231) after 4 hrs of incubation (green) and 16 hrs of incubation (blue). PrP(23-231) (3 mg/ml) oxidized with 10 mM H_2O_2 and 0.1 mole equivalents Cu^{2+} ions in 10 mM sodium acetate pH 5.5. After 4 hours some resonances from helix-C retain the signal and chemical shift values while many other signal from the structured domain lose their signal intensity due exchange broadening of a molten-globule fold.



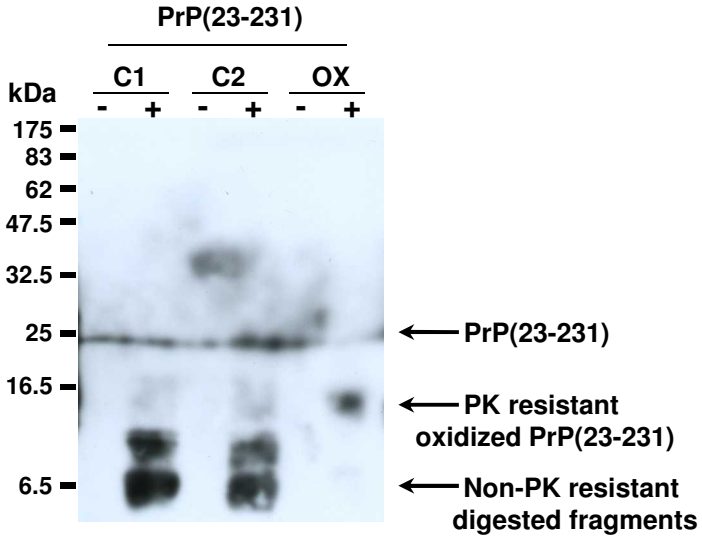
Supplemental Figure S8: UV-CD spectra with PrP(113-231) H_2O_2 oxidized under milder conditions. Red spectra are un-oxidized PrP and Black is oxidized PrP. Oxidizing conditions were PrP(113-231) 130 micromolar with 10 mM H_2O_2 (no Cu^{2+}) and incubated for 24 hrs. 10 mM sodium acetate buffer at pH 5.5. Little change in total helical content when oxidized by H_2O_2 under these mild conditions.



Supplemental Figure S9: **(a)** UV-CD spectrum of PrP^C with 0.1 mole equivalent Cu^{2+} added no H_2O_2. PrP(23-231), 0.2 mg/ml, pH 7.4 . Spectra recorded every 2 hrs up to 20 hrs. **(b)** FT-IR spectra of full-length PrP^C with 0.1 mole equivalents Cu^{2+} added (no H_2O_2) no change in α-helical amide-I band is observed. Low levels of Cu^{2+} ions alone (0.1 mole equivalents) have no effect on the structure of PrP^C and structure remained unchanged over 20 hours.

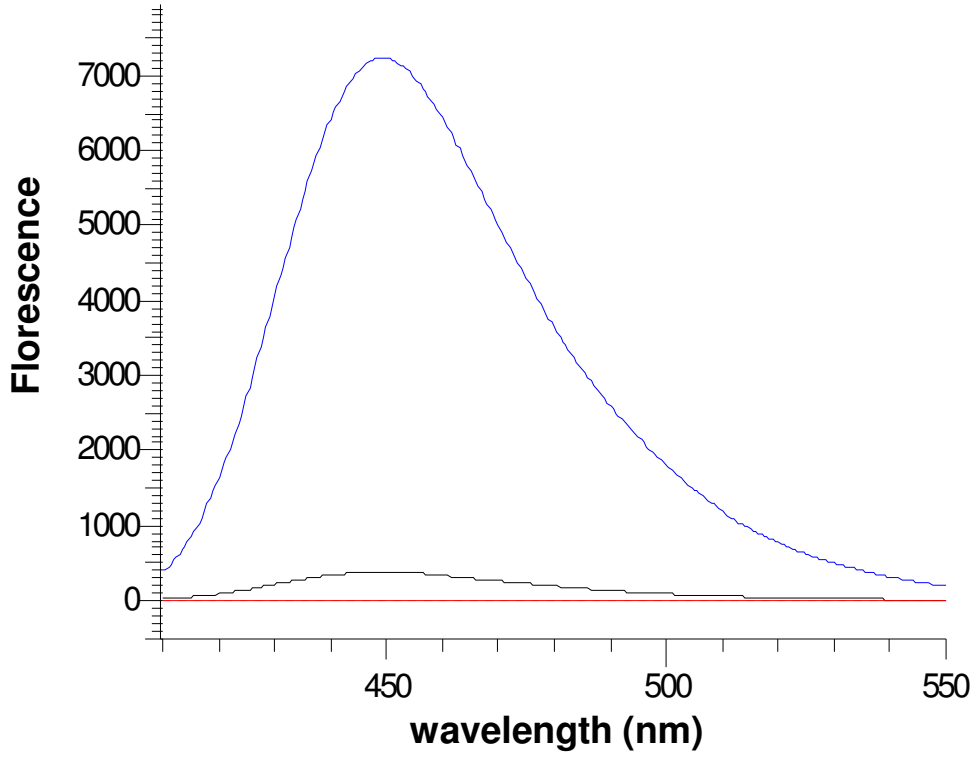


Supplemental Figure S10: ANS (a) and bis-ANS (b) fluorescence for oxidized (H_2O_2) and copper oxidized PrP using harsh oxidizing conditions.
PrP(23-231) 4 µM was oxidized by incubation with 10 mM H_2O_2 (Red) or with 10 mM H_2O_2 plus 0.1 mol equivalents Cu^{2+} ions (blue). Sample were incubated for 22 hours at 37 ˚C, Un-oxidized PrP(23-231) 4 µM is also shown (green). In contrast, to the mildly oxidizing conditions little ANS fluorescence and no bis-ANS fluorescence relative to un-oxidized PrP^C is not observed. This suggests that the strongly oxidized PrP does not have molten-globule like properties.

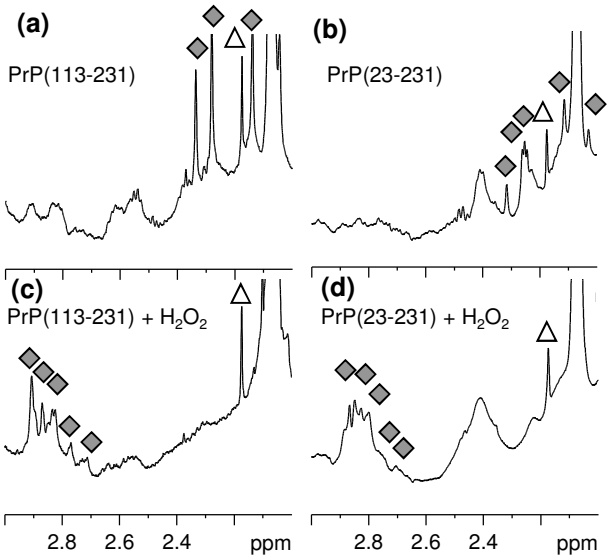


Supplemental Figure S11: Proteinase K treatment of PrP^C under harsh H_2O_2 oxidizing conditions

A western blot of proteinase K treatment of 50 ng of PrP(23-231) was prepared following SDS–PAGE (14% gel). In the absence of proteinase K "-" and in the presence of 20 mole equivalents of proteinase K "+" . Two controls were used the first was PrP(23-231) with 10 mM NaOAc, pH 5.5, incubated at 4 ˚C for 47 hours (C1) and the second was PrP(23-231) with 10 mM NaOAc, pH 5.5, incubated at 37 ˚C for 47 hours (C2). PrP(23-231) with 10 mM H_2O_2 in the presence of 10 mM NaOAc, pH 5.5, incubated at 37 ˚C for 47 hours (OX). A primary monoclonal antibody (ICMS-18), detects mouse PrP^C, was used when immunostaning. Mouse HRP-conjugated secondary antibody was used for detection. The highlighted band corresponds to a ~13 kDa fragment which is PK resistant, this is formed by the harsh H_2O_2 treatment typical of digestion into PrP(90-231). Both of the controls of un-oxidized PrP^C are not PK resistant producing smaller 6-10 kDa fragments form the PK digest.



Supplemental Figure S12: Detection of hydroxyl radical by 3-CCA assay. Fluorescence signal at 450 nm indicates the presence of hydroxyl radicals. Spectra confirm that H_2O_2 and Cu^{2+} will generate hydroxyl radicals. Cu^{2+} with H_2O_2 after 1 hour (black) and 20 hours (blue) at 37 ^{o}C. Incubation of H_2O_2 in absence of Cu^{2+} ions will not generate an appreciable fluorescence signal. H_2O_2 (2.5 mM) and Cu^{2+} ions (25 μM) in phosphate buffer at pH 7.4. Hydroxyl radical detection was carried out using 3-Coumarin Carboxylic Acid (3-CCA) assay (King, M., et al. 2003). The product, 7-OH-CCA fluoresces at 450 nm with an excitation maxima at 388 nm. Fluorescence emission spectra were obtained with a Hitachi F-2500 fluorescence spectrophotometer using a 1 cm path-length quartz cuvette (Hellma) and 1 mM 3-CCA.



Supplemental Figure S13: 1D ^{1}H NMR spectra of full-length and fragment PrP when incubated with hydrogen peroxide. Panel (a) and (c) are PrP(113-231) (140 micromolar) in sodium acetate buffer (10 mM) at pH 5.6 at 37 ^{o}C. Panel (b) and (d) are PrP(23-231) (130 micromolar) in sodium acetate buffer (10 mM) at pH 5.4 at 37 ^{o}C (c) and (d) after 24 hours incubated with H_2O_2 (10 mM). The singlets assigned to εH of Met (diamonds) are lost upon oxidation, new resonance's assigned to Met-sulphoxide εH are apparent. Triangle is a sodium acetate peak.

It has been possible to directly monitor oxidation of the Met residues using simple 1D ^{1}H NMR methods. Even for full-length PrP it is possible to observe the singlet signals for the εH methyl proton of Met residues in the 1D ^{1}H NMR spectra. Addition of H_2O_2 causes the gradual loss of the signals at ~ 2.5 ppm and appearance of new signals downfield at ~2.9 ppm for the sulphoxide for of εH of Met^{OX}. Complete loss of un-oxidized methionine εCH_3 NMR signals are apparent within 24 hours, while, other resonances are unaffected in particular the His εH singlets remain unaffected. In contrast, a mixture of Cu^{2+} with H_2O_2 is sufficient to generate hydroxyl radicals. The hydroxyl radicals is highly reactive, in particular, the His residues which chelate the Cu^{2+} ions are oxidized to generate 2-oxo-His species. ^{1}H NMR indicates loss of His εH signals, although the oxidized side-chain is difficult to observe.