

Supplemental Information

Copper alters aggregation behavior of prion protein and induces novel interactions between its N- and C-terminal regions

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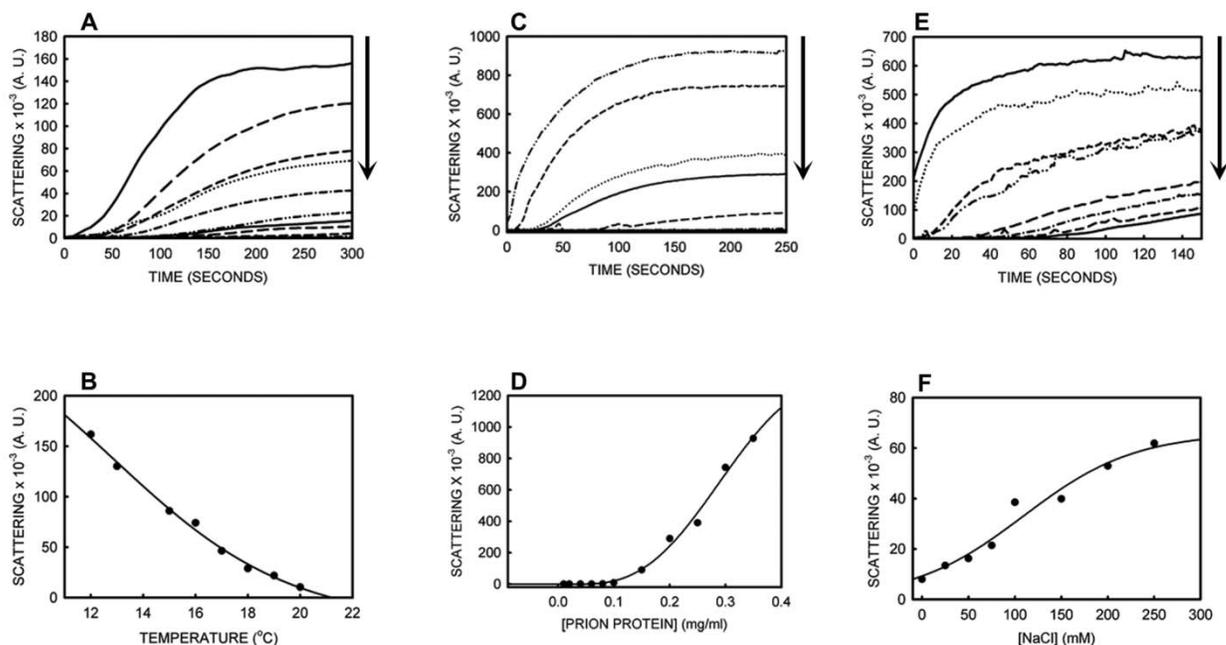


Figure S1: Effect of temperature, protein concentration and salt on reversible aggregation of Cu²⁺-bound PrP. Cu²⁺:glycine complex (6 molar equivalents) was added to protein solution at 37 °C in all experiments and light scattering was monitored. **a)** Cu²⁺-bound PrP (0.15 mg/ml) aggregation was monitored at various temperatures from 12 °C to 20 °C with 1 °C increment after Cu²⁺ addition at 37 °C and incubation of 300 seconds. **(b).** Each circle represents the maximum of scattering intensity monitored in **(a)**. **c)** Aggregation of Cu²⁺-bound PrP was monitored as a function of protein concentration (from 0.01 – 0.35 mg/ml). **d)** The value corresponding to the maximum scattering (circle) recorded in **(c)** was plotted against the protein concentration. **e)** Protein aggregation was monitored by varying salt concentration (0–250 mM) and keeping temperature (15 °C) and protein concentration (0.15 mg/ml) constant. **f)** Maximum scattering intensity recorded in **(e)** was plotted against protein concentration. Each circle represents the maximum scattering intensity monitored in **(f)**.

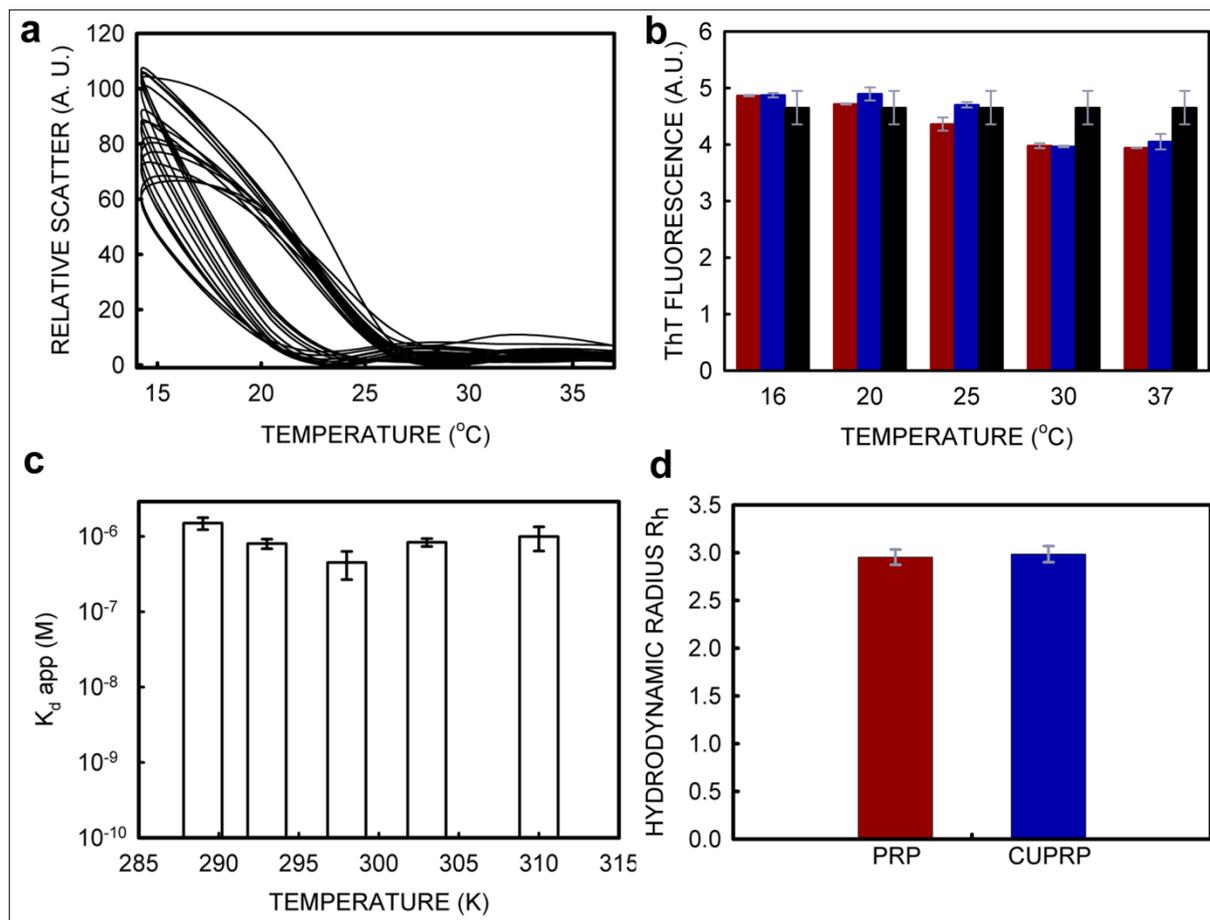


Figure S2: Aggregation Cu²⁺-bound PrP . a) Reversible aggregation of Cu²⁺-bound PrP during 14 continuous heating-cooling cycles in the range of 15-37 °C. **b)** Fluorescence of amyloid-specific probe ThT alone (black bar) and in the presence of PrP 23-231 (red bar) and Cu²⁺-bound PrP 23-231 (blue bar). Error bar represents n=3. **c)** Apparent dissociation constants (K_d app) of copper-binding to PrP at temperatures ranging from 37 °C to 16 °C. **d)** Hydrodynamic radius (R_h) measured using DLS of PrP 23-231 with (CUPRP, blue bar) and without Cu²⁺ (PRP, red bar) at 37 °C. Error bars show SE from 30 values.

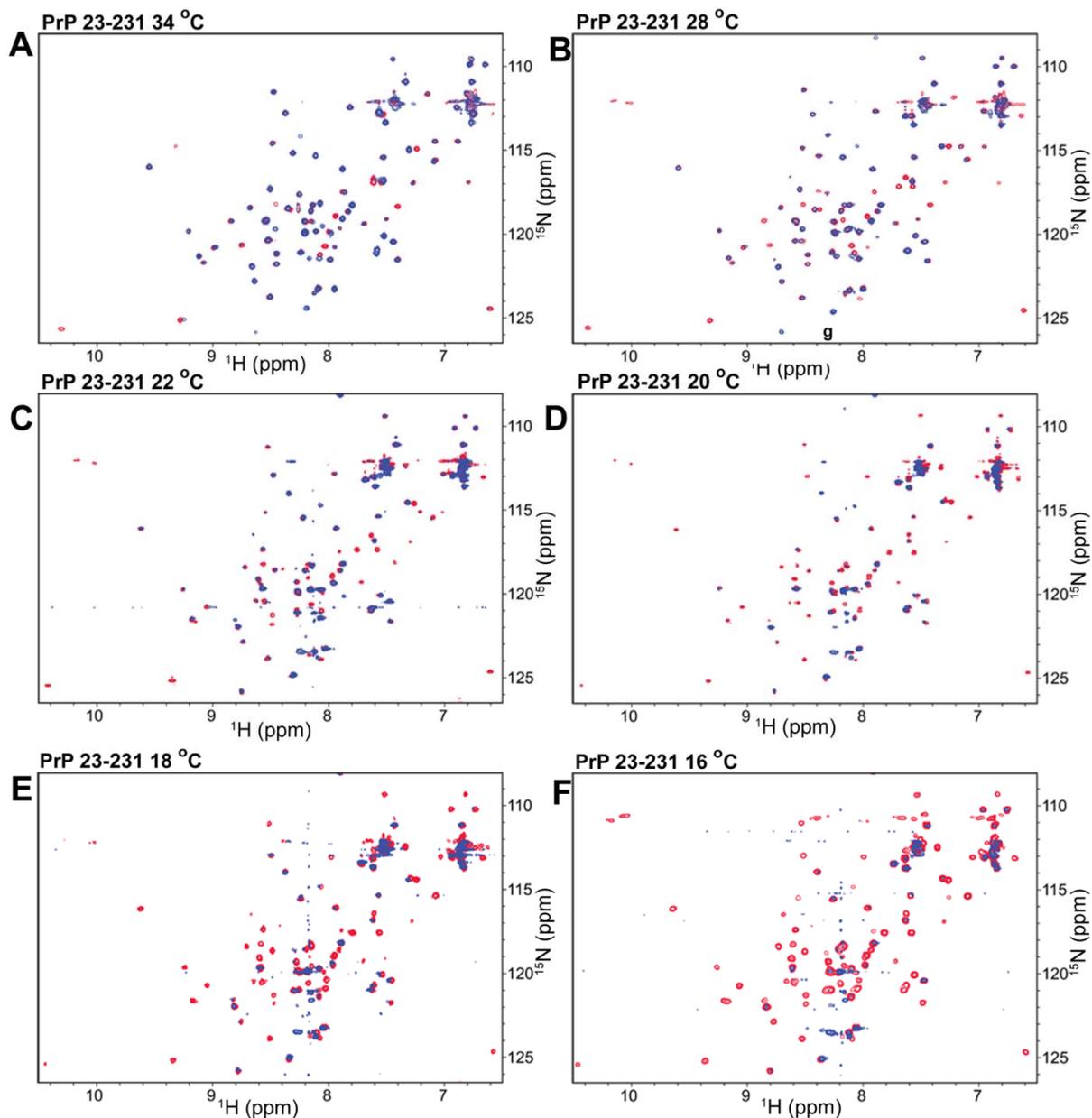


Figure S3: ^{15}N HSQC of PrP 23-231. HSQC of Cu^{2+} -bound PrP 23-231 (blue cross-peaks) is overlaid onto that of PrP 23-231 (red cross-peaks) at temperatures 34 (a), 28 (b), 22 (c), 20 (d), 18 (e) and 16 °C (f).

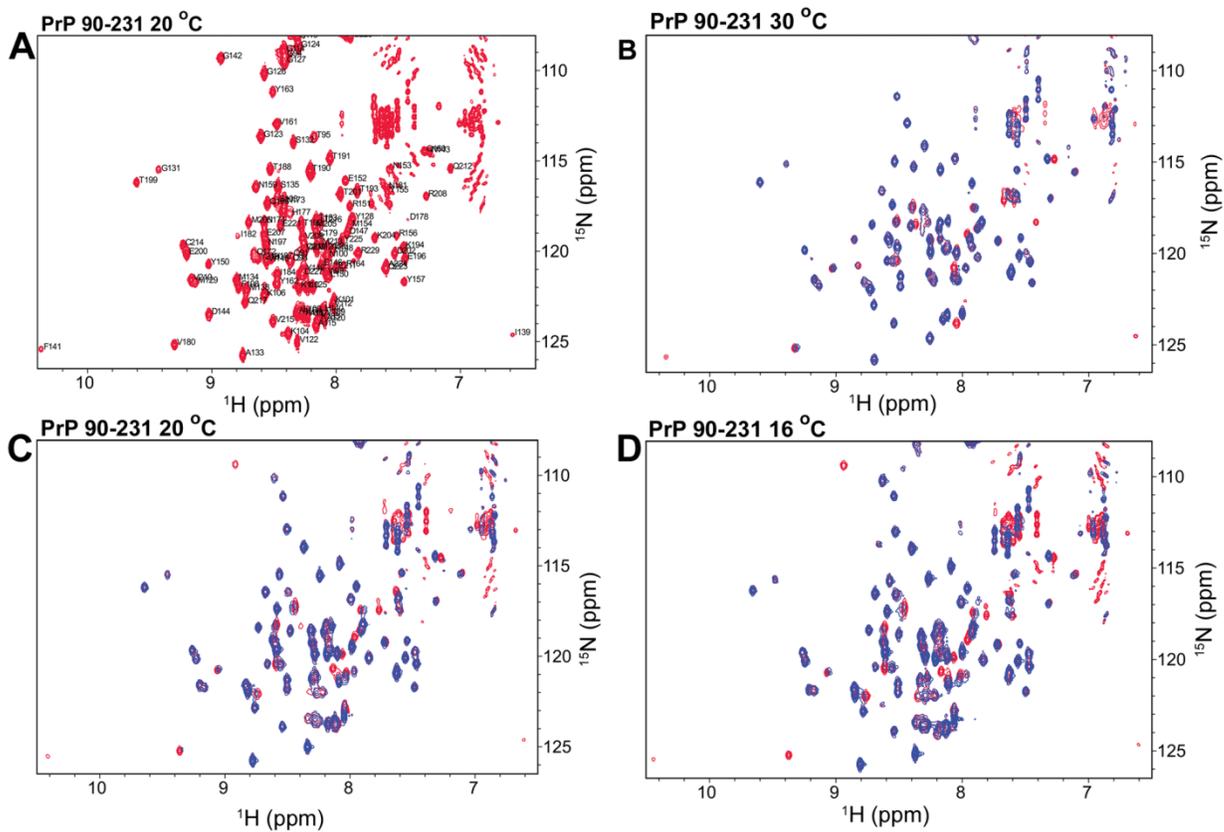


Figure S4: ^{15}N HSQC of PrP 90-231. a) HSQC spectra of PrP 90-231 recorded in 5 mM sodium phosphate buffer, pH 7.0 at 20 °C. Peaks were assigned using BMRB file 16071. HSQC of Cu^{2+} -bound PrP 90-231 (blue cross-peaks) is overlaid onto that of PrP 90-231 (red cross-peaks) at temperatures 30 (b), 20 (c) and 16 °C (d).

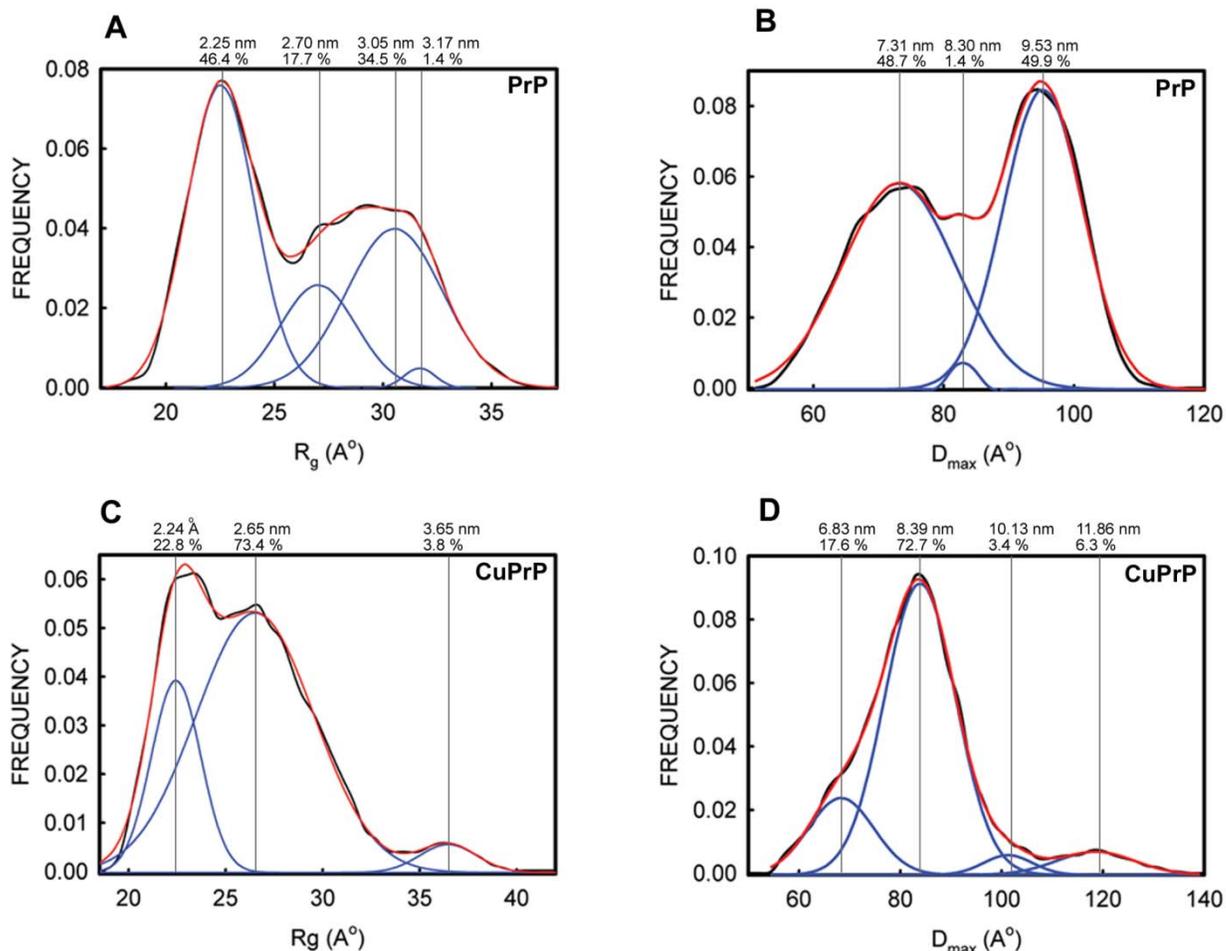


Figure S5: Deconvolution of R_g and D_{max} distributions. In each panel black trace is obtained from the selected conformers using EOM program. Blue traces correspond to the deconvoluted curves and sum of these curves are represented in red trace. Gray lines indicate the peak positions of each of the deconvoluted curves. The peak position and % contribution in terms of area is shown above each gray line. Top two panels (a and b) are R_g and D_{max} distribution of PrP and the bottom two (c and d) correspond to that of Cu^{2+} -bound PrP, respectively.

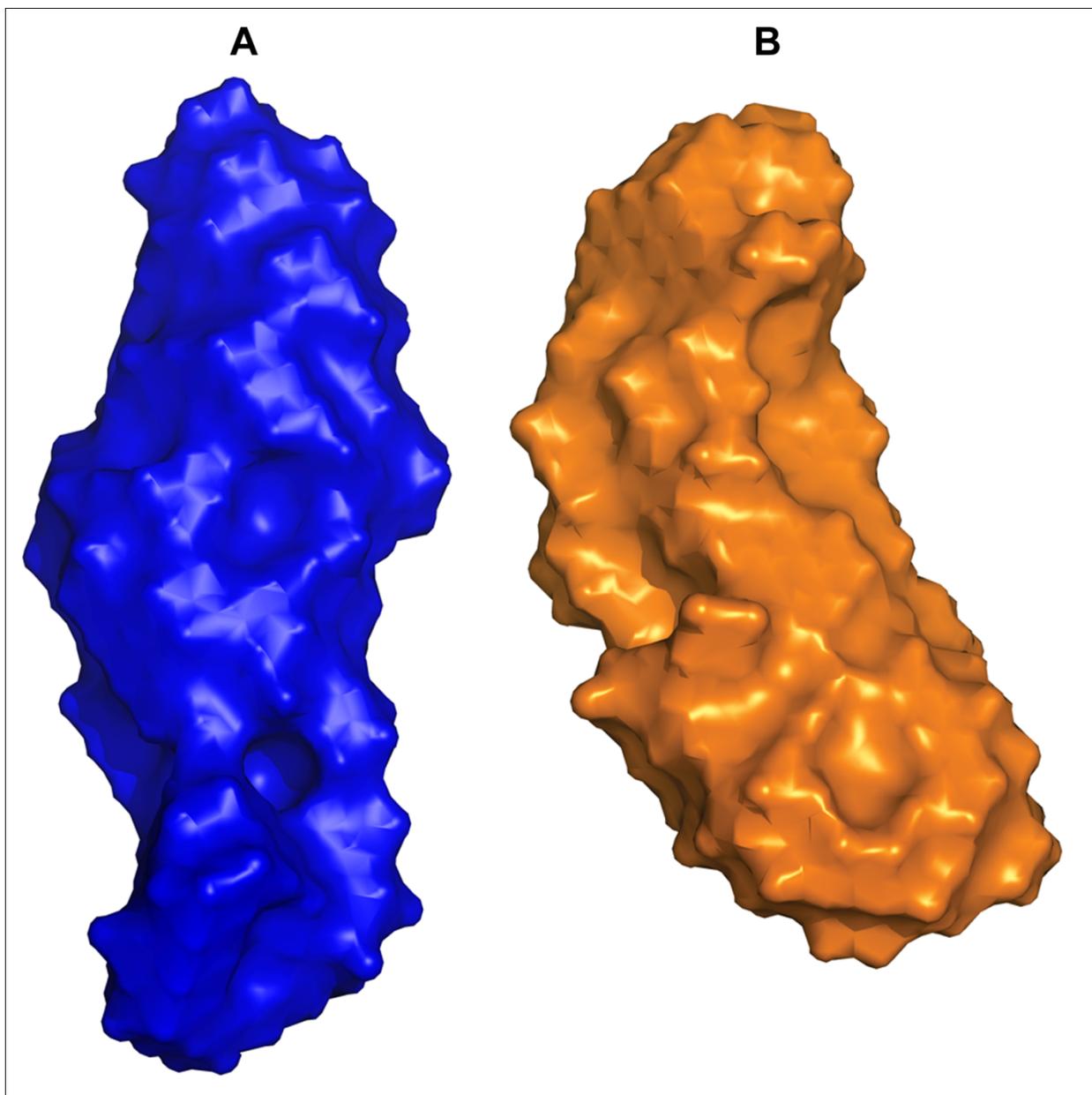


Figure S6: SAXS derived *ab initio* models. Envelope of SAXS-derived *ab initio* model was visualised using PyMol. **a)** Envelope of PrP (blue) and **b)** Cu^{2+} -bound PrP (orange).

37 °C	34 °C	31 °C	28 °C	24 °C	22 °C**	20 °C	18 °C	16 °C
A120	E146	I139*	C170		M129	T188	M154	Q223
F141	N174	C180	I182		W145	V215	T199	A224
N143	D178	N181	R208		Y150	M206	E200	D202
D147			K185		Q212	E207	Y157	Y149
					Y162	N100	E207	Q160
					I184	R148	V209	Y225
					H187	Y163	Q212	Y226
					Y218	Q172	Q217	Y155
					K204	N159	E221	E152
					E211		M134	T191
					M213		M205	V161
					T183		C214	
							Q186	
							T190	
							V210	
							T216	
							Y157	
							Q219	

Table S1: Lists of residues whose cross-peaks are missing upon addition of Cu²⁺ at various temperatures, related to Figure 4 and 5. In the HSQC spectra of Cu²⁺-bound PrP 23-231 cross-peaks of these residues are broadened at particular temperature compared to that of PrP 23-231. Cross peaks of the residues (red) are also found to be missing in the HSQC spectra of Cu²⁺-bound PrP 90-231. In the case of PrP 90-231, cross peak arising from I139 disappeared at 25 °C in contrast to that of PrP 23-231 where it disappeared at Below 24 °C, cross-peaks are broadening due to the aggregation process which is prevalent at or below 22 °C. Except D178, residues in red color belong to the helix-1 region and those till 24 °C (black) and D178 correspond to the helix-2 region. The broadened peaks shown in the table at each temperature are progressive in nature. Peaks broadened at each temperature include broadened peaks at higher temperature and at the current temperature. For example, peaks which were broadened at 28 °C included broadened peaks from 37 and 34 °C and those at 28 °C.

* In the case of PrP 90-231, cross-peak arising from I139 was found to be disappeared at 25 °C.

** In the case of prP 90-231 no further disappearance of cross-peaks were observed below 25 °C.