Supplementary Information

The non-octarepeat copper binding site of the prion protein is a key regulator of prion conversion

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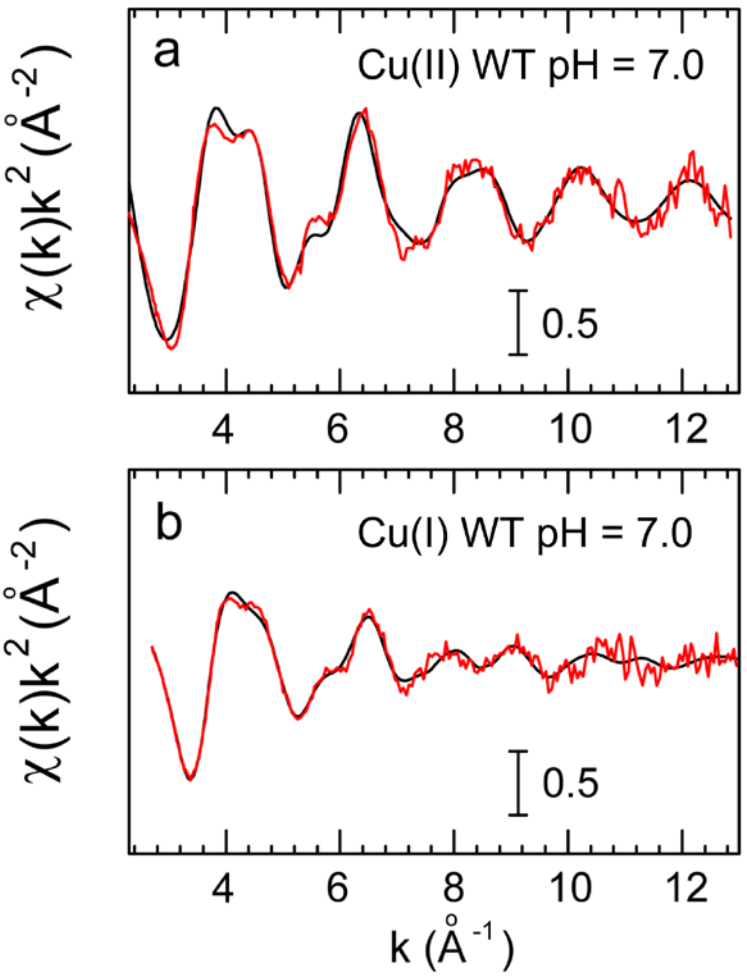


Figure S1. Analysis of the Cu K-edge X-ray absorption spectra. (**a**) Cu K-edge EXAFS experimental data (red) and best fit theoretical signal (black) of Cu(II) WT HuPrP(90-231) protein at pH 7.0. The EXAFS fit has been carried out in the *k* range 2.4-13.0 Å^{-1}. The Cu(II) ion is coordinated with a single histidine residue with a Cu-N distance of 1.99 Å and with a sulphur atom at 3.37 Å. (**b**) Cu K-edge EXAFS experimental data (red) and best fit theoretical signal (black) of Cu(I) WT HuPrP(90-231) protein at pH 7.0. The EXAFS fit has been carried out in the *k* range 2.5-13.0 Å^{-1}. The Cu(I) ion first coordination shell is formed by an histidine residue with a Cu-N distance of 1.99 Å and a methionine residue with a Cu-S distance of 2.27 Å.

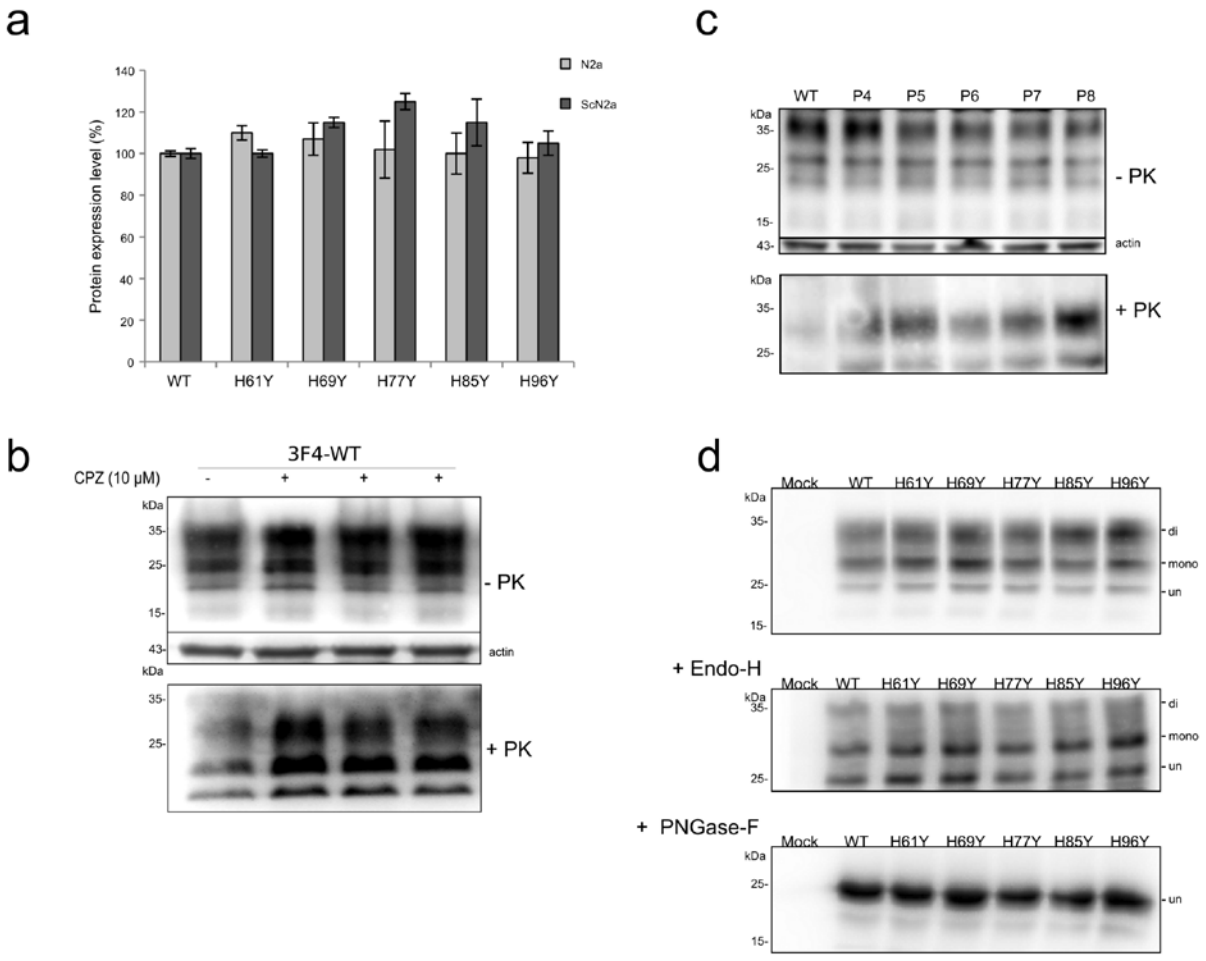


Figure S2. (**a**) Quantitative analysis of actin-normalized total PrP expression levels in both N2a and ScN2a cells transfected with 3F4-tagged MoPrP constructs (n = 4). (**b**) Ten μM cuprizone (CPZ) treatments (n=3) on ScN2a cells transfected with 3F4-WT MoPrP promoted increased PrP^{Sc} levels. (**c**) N2a cells were transiently transfected with 3F4-H96Y MoPrP and regularly passaged every 7 days up to passage (P) 8. The PrP^{res} levels were monitored upon PK digestion and detected by anti-PrP 3F4 antibody. β-actin is used as internal control. (**d**) The OR and non-OR mutations share the same glycosylation patterns and proteolytic features when treated with Endo-H and PNGase-F as the WT $MoPrP^{C}$. The positions of diglycosylated, monoglycosylated and unglycosylated forms (denoted as di, mono and un) of PrP^{C} are on the right of each WB. PrPs were detected by anti-PrP 3F4 antibody.

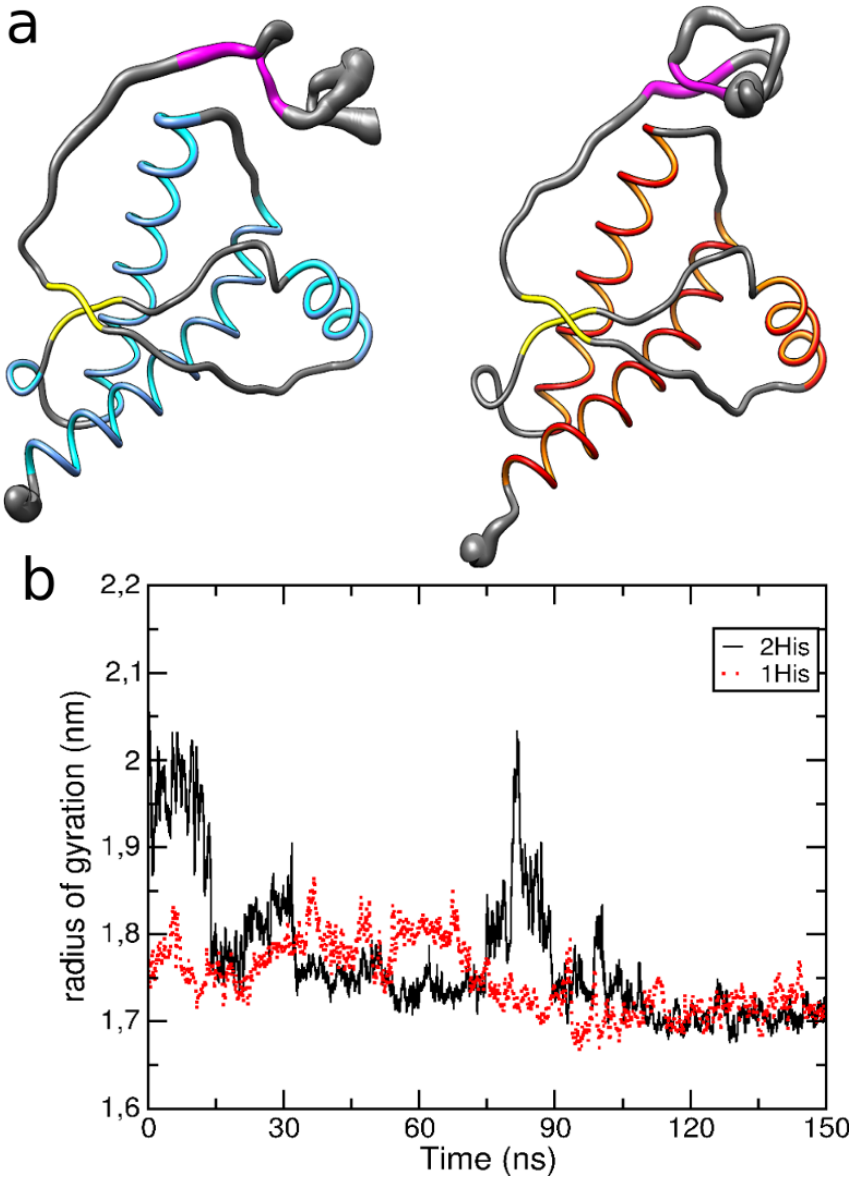
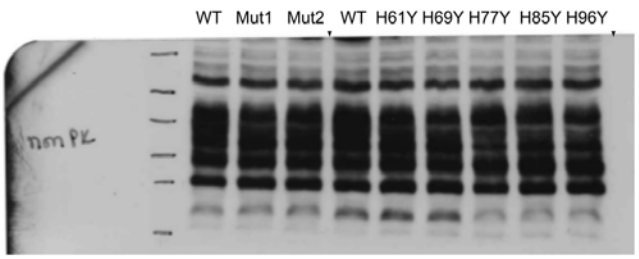
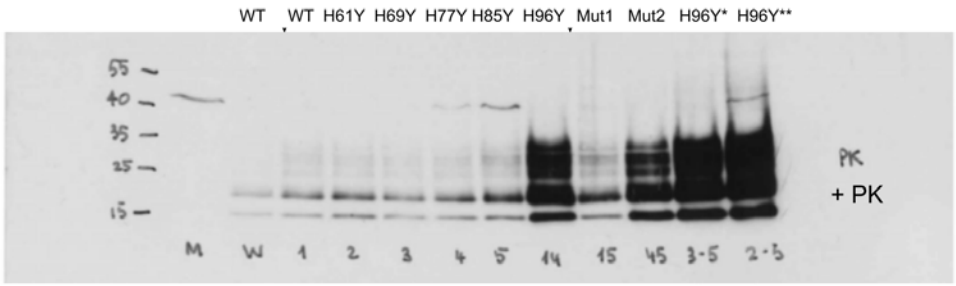


Figure S3. (**a**) Cartoon representation of RMSF. Ribbon width is proportional to fluctuations; the coordinates correspond to those of the first centroid obtained for each trajectory by cluster analysis of the C-terminal domain. The ratio between radius and RMSF is the same in the two models. The average relative orientation of residues 106-109 and 114-117 (colored in magenta) in the 1His system is also clearly observable. (**b**) Radius of gyration (R_g) calculated for the 2His (black) and 1His (red) trajectories. The R_g of the 2His trajectory features transitions between different conformation with R_g up to 2 nm with a lifetime of several nanoseconds each while the R_g of the 1His simulated is stably oscillates around 1.74 nm.

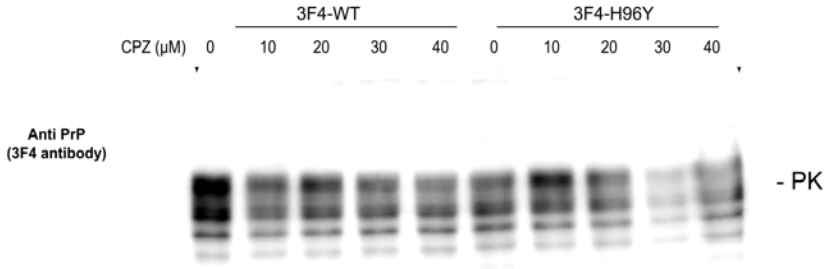
a


WT Mut1 Mut2 WT H61Y H69Y H77Y H85Y H96Y
- PK

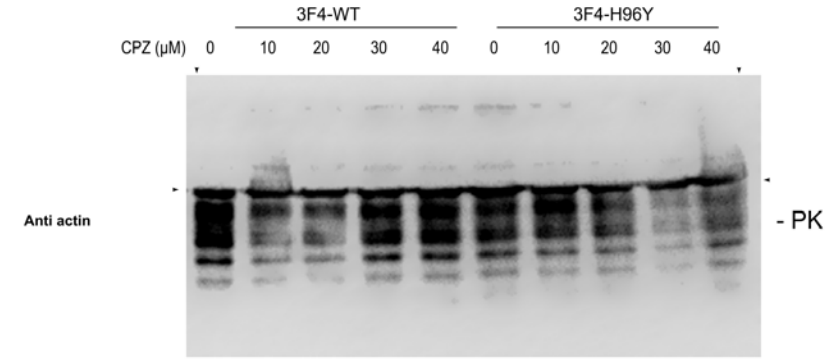
b


WT WT H61Y H69Y H77Y H85Y H96Y Mut1 Mut2 H96Y* H96Y**
55
40
35
25
15
M W 1 2 3 4 5 14 15 45 3-5 2-5
PK
+ PK

c


3F4-WT
3F4-H96Y
CPZ (μM) 0 10 20 30 40 0 10 20 30 40
Anti PrP
(3F4 antibody)
- PK

d


3F4-WT
3F4-H96Y
CPZ (μM) 0 10 20 30 40 0 10 20 30 40
Anti actin
- PK

e

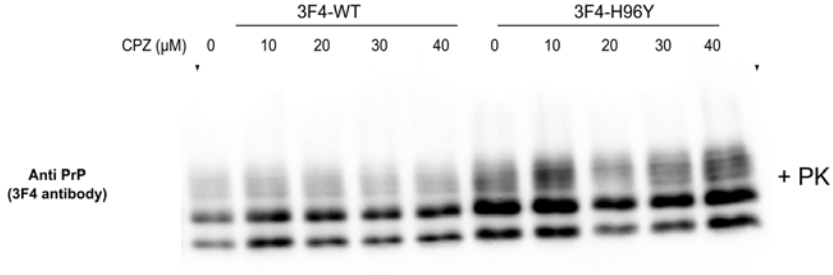
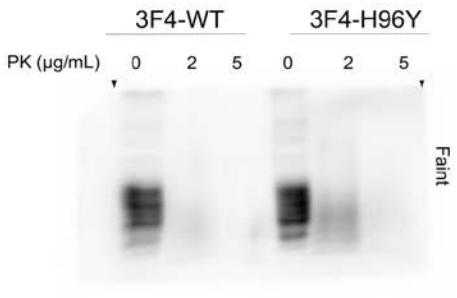
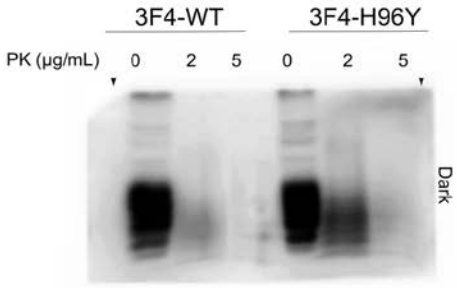

3F4-WT
3F4-H96Y
CPZ (μM) 0 10 20 30 40 0 10 20 30 40
Anti PrP
(3F4 antibody)
+ PK

Figure S4. Full-length western-blots (WB) presented in Figure 4a and 4c in the main manuscript. Arrows (▼, ► and ◄) indicate positions on the gels where the blots have been cropped. **(a)**, fifty μg of undigested lysates from ScN2a cells expressing 3F4-tagged WT and mutated MoPrPs was applied to each lane. Mut1 and Mut2 are ScN2a cell lysates expressing 3F4-tagged constructs pcDNA3.1::MoPrP(1-254, H61Y, H69Y, H77Y, H85Y) and pcDNA3.1::MoPrP(1-254, H61Y, H69Y, H77Y, H85Y, H96Y), respectively (not considered in this work). **(b)**, five hundred μg of cell lysate was digested with PK (20 μg/mL) at 37ºC for 1 hour. H96Y* and H96Y** are ScN2a cell lysates expressing 3F4-H96Y mutant digested with 10 and 5 μg/mL PK, respectively. Mut1 and Mut2 constructs have not been considered in this work. **(c)** and **(e)**, full-length WB presented in Figure 4c. **(d)**, β-actin signals (arrows ► and ◄) on WB membrane previously probed with 3F4 antibody.

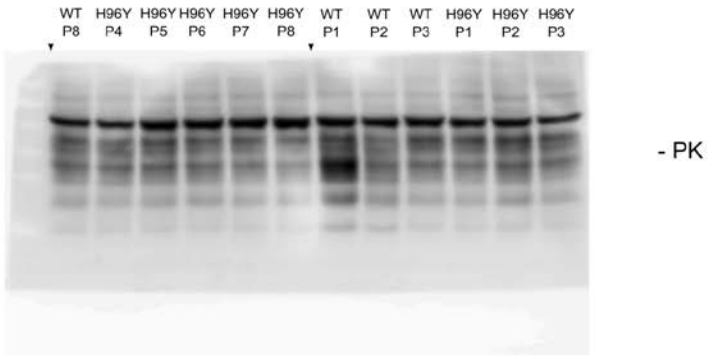
a


3F4-WT
3F4-H96Y
PK (µg/mL)
0
2
5
0
2
5
Faint

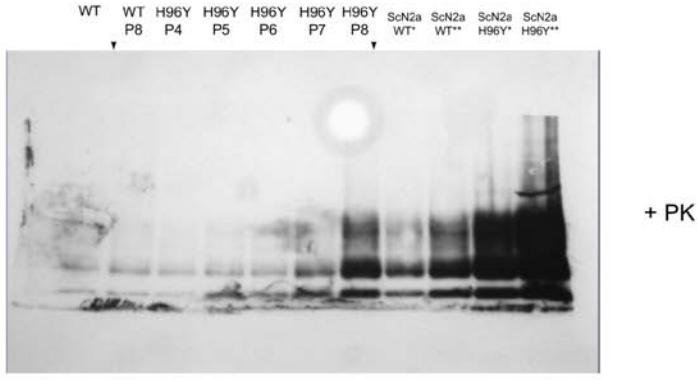
b


3F4-WT
3F4-H96Y
PK (µg/mL)
0
2
5
0
2
5
Dark

c


WT P8
H96Y P4
H96Y P5
H96Y P6
H96Y P7
H96Y P8
WT P1
WT P2
WT P3
H96Y P1
H96Y P2
H96Y P3
- PK

d


WT
WT P8
H96Y P4
H96Y P5
H96Y P6
H96Y P7
H96Y P8
ScN2a WT*
ScN2a WT**
ScN2a H96Y*
ScN2a H96Y**
+ PK

72

73

Figure S5. Full-length western-blots (WB) presented in Figure 5a and 5c in the main manuscript. Arrows (▼) indicate positions on the gels where the blots have been cropped. The H96Y mutant displays PK-resistance when expressed in N2a cells regularly passaged every 7 days up to passage (P) 8. Cell lysates were treated with 2 or 5 µg/mL of PK. Two exposures of the same blot are shown: **(a)** faint, 30 sec exposure; **(b)** dark, 6 min exposure. PTA-extracted PrP^{Sc} from N2a cells transfected with 3F4-H96Y MoPrP were inoculated into N2a cells and regularly passaged every 7 days up to P8. **(c)**, fifty µg of undigested lysates from N2a cells expressing 3F4-tagged WT and H96Y MoPrPs at different passages. **(d)**, the PrP^{res} detection was assessed by PK digestion (5 µg/mL) through passages (lines 1-7). ScN2a WT* and ScN2a WT** (lines 8-9) are ScN2a cell lysates expressing 3F4-WT MoPrP digested with 10 and 5 µg/mL PK, respectively. ScN2a H96Y* and ScN2a H96Y** (lines 10-11) are ScN2a cell lysates expressing 3F4-H96Y MoPrP digested with 10 and 5 µg/mL PK, respectively.

Cu(II) WT pH=5.5 **(1)**			Cu(II) WT pH=7.0 **(2)**			Cu(II) Q212P pH=5.5 **(3)**			Cu(II) Q212P pH=7.0 **(4)**		
N	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**
2 N_{His}	1.98(2)	0.006(3)	1 N_{His}	1.99(2)	0.006(3)	1 N_{His}	2.00(2)	0.007(3)	1 N_{His}	1.99(2)	0.007(3)
2 O/N	1.98(2)	0.008(3)	3 O/N	1.99(4)	0.009(3)	3 O/N	1.99(2)	0.009(3)	3 O/N	1.99(3)	0.009(3)
1 O	2.31(3)	0.013(4)	1 O	2.38(4)	0.012(4)	1 O	2.40(4)	0.013(4)	1 O	2.39(3)	0.012(4)
1 S	3.25(4)	0.013(4)	1 S	3.37(4)	0.013(4)	1 S	3.47(4)	0.014(4)	1 S	3.45(4)	0.012(4)
Cu(II) P102L pH=5.5 **(5)**			Cu(II) P102L pH=7.0 **(6)**			Cu(II) H96Y pH=5.5 **(7)**			Cu(II) H96Y pH=7.0 **(8)**		
N	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**
1 N_{His}	2.00(3)	0.006(3)	1 N_{His}	1.99(2)	0.006(3)	1 N_{His}	2.00(2)	0.007(3)	1 N_{His}	2.00(2)	0.008(3)
O/N	1.99(3)	0.008(3)	3 O/N	1.99(3)	0.009(3)	3 O/N	1.99(2)	0.009(3)	3 O/N	1.99(2)	0.008(3)
1 O	2.34(4)	0.013(4)	1 O	2.38(3)	0.012(4)	1 O	2.40(3)	0.013(4)	1 O	2.39(3)	0.012(4)
1 S	3.32(4)	0.013(4)	1 S	3.38(4)	0.013(4)	1 S	3.47(4)	0.014(4)	1 S	3.45(4)	0.013(4)
Cu(I) WT pH=5.5 **(9)**			Cu(I) WT pH=7.0 **(10)**			Cu(I) Q212P pH=5.5 **(11)**			Cu(I) Q212P pH=7.0 **(12)**		
N	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**
2 N_{His}	1.98(2)	0.006(3)	1 N_{His}	1.98(2)	0.007(3)	1 N_{His}	1.99(2)	0.007(3)	1 N_{His}	1.99(2)	0.007(3)
2 O/N	1.99(3)	0.009(3)	1 O/N	2.00(2)	0.009(3)	1 O/N	1.99(2)	0.009(3)	3 O/N	1.99(3)	0.009(3)
1 O	2.32(4)	0.014(4)	1 S	2.27(4)	0.009(4)	1 S	2.28(4)	0.008(3)	1 S	2.27(3)	0.009(4)
1 S	3.26(5)	0.013(4)									
Cu(I) P102L pH=5.5 **(13)**			Cu(I) P102L pH=7.0 **(14)**								
N	**R (Å)**	**σ^2 (Å^2)**	**N**	**R (Å)**	**σ^2 (Å^2)**						
1 N_{His}	2.00(2)	0.009(3)	1 N_{His}	1.99(2)	0.008(3)						
1 O/N	1.99(2)	0.009(3)	3 O/N	1.99(3)	0.009(3)						
1 S	2.28(4)	0.009(3)	1 O	2.29(3)	0.010(4)						

Table S1. Structural parameters derived from the EXAFS analysis. Structural parameters detemined from the fit of the EXAFS data at the Cu K-edge of samples 1 to 14. N is the coordination number, R is the distance between the copper ion and the ligand, σ^2 is the Debye-Waller factor. Statistical errors are reported in parentheses. Codes 1 to 14 correspond to the

following samples: Cu(II) WT HuPrP(90-231) pH5.5 **(1)**, Cu(II) WT HuPrP(90-231) pH 7.0 **(2)**, Cu(II) HuPrP(90-231, Q212P) pH 5.5 **(3)**, Cu(II) HuPrP(90-231, Q212P) pH 7.0 **(4)**, Cu(II) HuPrP(90-231, P102L) pH 5.5 **(5)**, Cu(II) HuPrP(90-231, P102L) pH 7.0 **(6)**, Cu(II) HuPrP(90-231, H96Y) pH 5.5 **(7)**, Cu(II) HuPrP(90-231, H96Y) pH 7.0 **(8)**, Cu(I) WT HuPrP(90-231) pH 5.5 **(9)**, Cu(I) WT HuPrP(90-231) pH 7.0 **(10)**, Cu(I) HuPrP(90-231, Q212P) pH 5.5 **(11)**, Cu(I) HuPrP(90-231, Q212P) pH 7.0 **(12)**, Cu(I) HuPrP(90-231, P102L) pH 5.5 **(13)**, Cu(I) HuPrP(90-231, P102L) pH 7.0 **(14)**