Supplementary Material (ESI) for New Journal of Chemistry

The complex-formation behaviour of His residues in the fifth Cu^{2+} binding site of human Prion protein: a close look

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SUPPLEMENTARY DATA

Table S1.

Thermodynamic parameters for protonation of PrP_{91-114}, PrP_{92-100}, $PrP_{106-113}$, in aqueous solution. T = 298 K, I = 0.1 mol dm^{-3} (KCl or KNO_3). Standard deviations are given in parentheses. Charges are omitted for clarity.
Data from F. Berti *et al.*, Chem. Eur. J. 13 (2007) 1991 and M. Remelli *et al.*, Dalton Trans. (2005) 2876.

Species	log β	logK_{step}	$-\Delta H°$ kJ mol^{-1}	$\Delta S°$ J K^{-1} mol^{-1}
		PrP_{91-114}		
HL	11.15	11.15	-	-
H_2L	21.46	10.31	-	-
H_3L	31.49	10.04	-	-
H_4L	40.85	9.35	-	-
H_5L	47.39	6.54	-	-
H_6L	53.11	5.73	-	-
		PrP_{92-100}		
HL	6.38	6.38	25	38
		$PrP_{106-113}$		
HL	10.52	10.52	81	-69
H_2L	20.32	9.80	111	16
H_3L	26.48	6.16	135	53

Table S2.

Thermodynamic parameters for Cu^{2+} complex-formation of PrP_{91-114}, PrP_{92-100}, $PrP_{106-113}$, in aqueous solution. T = 298 K, I = 0.1 mol dm^{-3} (KCl or KNO_3). Standard deviations are given in parentheses. Charges are omitted for clarity.
Data from F. Berti *et al.*, Chem. Eur. J. 13 (2007) 1991 and M. Remelli *et al.*, Dalton Trans. (2005) 2876.

Species	log β	logK_{step}	-$\Delta H°$ kJ mol^{-1}	$\Delta S°$ J K^{-1} mol^{-1}
		PrP_{91-114}		
CuH_5L	51.35	5.29	-	-
CuH_4L	46.06	-	-	-
CuH_2L	34.18	6.56	-	-
CuHL	27.62	7.40	-	-
CuL	20.22	8.78	-	-
$CuH_{-1}L$	11.44	-	-	-
$CuH_{-3}L$	-8.66		-	-
		PrP_{92-100}		
CuL	3.85	6.25	20	6
$CuH_{-1}L$	-2.40	5.82	8	-72
$CuH_{-2}L$	-8.22	7.55	-39	-27
$CuH_{-3}L$	-15.77	9.51	-65	-85
$CuH_{-4}L$	-25.28		-88	-187
		$PrP_{106-113}$		
CuH_2L	23.84	6.19	161	-82
CuHL	17.65	5.04	93	27
CuL	12.61	7.71	82	-33
$CuH_{-1}L$	4.90	9.85	53	-84
$CuH_{-2}L$	-4.95	10.64	20	-162
$CuH_{-3}L$	-15.59	11.81	-48	-138
$CuH_{-4}L$	-27.40		-	-

Table S3.

Spectroscopic parameters for Cu^{2+} complex-formation of PrP_{92-113}, PrP_{92-100}, $PrP_{106-113}$ analogues, in aqueous solution. Molar absorptivity is always referred to the total molar concentration of the Cu^{2+} ion. T = 298 K, I = 0.1 mol dm^{-3} (KCl). Charges are omitted for clarity.

(H96A)PrP$_{92-113}$						**(H111A)PrP$_{92-113}$**					
Species (pH)	**UV-Vis** λ/nm	ε/M^{-1} cm^{-1}	**CD** λ/nm	$\Delta\varepsilon$/$M^{-1}cm^{-1}$	**EPR** A_{II}/G	g_{II}	**Species**	**UV-Vis** λ/nm	ε/M^{-1} cm^{-1}	**CD** λ/nm	$\Delta\varepsilon$/$M^{-1}cm^{-1}$	**EPR** A_{II}/G	g_{II}
CuH_4L (pH 5.0)	785	31	292 444	-0.17 0.11	123	2.52	**CuH_4L (pH 5.5)**	795	15	264 297 340 526	0.13 -0.06 0.02 0.01	142	2.44
CuH_2L (pH 6.5)	617	118	253 326 385 532	5.19 -0.44 0.14 0.31	168	2.23	**CuH_3L (pH 6.0)**	780	16	258 305 343 514 626	0.62 -0.08 0.13 0.05 -0.05	142	2.45
CuHL (pH 7.5)	574	133	254 317 345 390 493 644	7.05 0.23 -0.06 0.13 -0.39 0.57	178	2.22	**CuH_2L (pH 7.0)**	628	55	271 322 379 489 616	1.46 -0.09 0.11 0.04 -0.44	n.d.	
CuL (pH 9.0)	534	163	257 293 318 359 404 497 634	7.30 -0.05 1.20 -0.06 0.06 -1.11 1.10	192	2.20	**CuHL (pH 8.5)**	576	97	276 335 391 612	2.22 -0.32 0.16 -0.84	175	2.22
$CuH_{-1}L$ (pH 10.0)	530	175	256 293 318 363 401 497 635	7.58 -0.11 1.32 -0.06 0.07 -1.22 1.16	192	2.20	**CuL (pH 9.5)**	570	105	275 339 393 601	2.57 -0.28 0.16 -0.95	204	2.19
$CuH_{-2}L$ (pH 10.5)	n.d.		n.d.		n.d.		**$CuH_{-1}L$ (pH 10.0)**	566	113	259 336 393 586	2.59 -0.18 0.10 -0.93	209	2.19
$CuH_{-3}L$ (pH 11.0)	528	175	257 293 319 399 498 637	7.81 -0.28 1.32 0.07 -1.31 1.19	192	2.20	**$CuH_{-2}L$ (pH 11.0)**	556	121	253 316 393 581	3.27 -0.36 0.05 -0.95	212	2.19
							$CuH_{-3}L$	n.d.		n.d.		n.d.	

(H96His-N$^{\tau}$-Me)PrP$_{92-113}$						(H111His-N$^{\tau}$-Me)PrP$_{92-113}$					
pH	UV-Vis λ/nm	ε/M^{-1} cm^{-1}	CD λ/nm	$\Delta\varepsilon$/M^{-1}cm^{-1}	EPR A_{II}/G g_{II}	pH	UV-Vis λ/nm	ε/M^{-1} cm^{-1}	CD λ/nm	$\Delta\varepsilon$/M^{-1}cm^{-1}	EPR A_{II}/G g_{II}
pH 4.0	803	29	266	0.24	n.d.	**pH 4.0**	809	29	267	0.18	120 2.42
			292	-0.26					305	-0.10	
pH 5.0	800	33	265	0.28	n.d.	**pH 5.0**	798	23	264	0.24	120 2.42
			293	-0.20					305	-0.10	
pH 5.5	795	30	255	1.60	n.d.	**pH 5.5**	768	31	258	1.74	120 2.42
			314	-0.14					317	-0.17	
			425	-0.02					386	0.01	
			527	0.07					523	0.08	
			611	-0.05					610	-0.04	
pH 6.0	632	59	255	4.48	n.d.	**pH 6.0**	635	51	255	4.74	120 2.42
			317	-0.35					321	-0.31	
			376	0.04					386	0.05	
			435	-0.02					526	0.23	
			525	0.23					613	-0.11	
			616	-0.15							
pH 6.5	619	100	254	6.65	n.d.	**pH 6.5**	621	101	254	6.23	171 2.22
			316	-0.46					316	-0.34	
			377	0.06					383	0.06	
			434	-0.01					525	0.28	
			524	0.33					606	-0.15	
			609	-0.22							
pH 7.0	611	120	253	6.79	n.d.	**pH 7.0**	613	115	254	6.43	175 2.22
			313	-0.47					315	-0.29	
			377	0.05					381	0.04	
			433	-0.01					440	-0.02	
			521	0.30					523	0.22	
			602	-0.24					595	-0.16	
									677	-0.01	
pH 7.5	602	124	254	6.30	n.d.	**pH 7.5**	601	121	255	6.11	175 2.22
			310	-0.46					307	-0.23	
			379	0.024					330	-0.09	
			436	-0.02					385	0.02	
			518	0.22					472	-0.06	
			591	-0.28					521	0.11	
			664	0.03					585	-0.19	
									662	0.06	
pH 8.0	587	130	254	5.90	n.d.	**pH 8.0**	578	126	255	5.65	175 2.22
			302	-0.47					301	-0.23	
			350	-0.12					327	0.03	
			472	-0.07					353	-0.08	
			517	0.07					478	-0.13	
			579	-0.36					574	-0.27	
			659	0.16					657	0.17	
pH 8.5	562	138	255	5.63	180 2.20	**pH 8.5**	556	144	256	5.27	179 2.20
			300	-0.40					300	-0.31	
			354	-0.13					325	0.14	
			475	-0.12					357	-0.10	
			574	-0.42					481	-0.21	
			659	0.25					568	-0.36	
									655	0.24	
pH 9.0	549	147	255	5.43	182 2.20	**pH 9.0**	547	153	256	5.11	186 2.20
			299	-0.50					298	-0.31	
			327	0.05					325	0.21	
			356	-0.15					358	-0.11	
			475	-0.17					485	-0.25	
			570	-0.47					566	-0.43	
			658	0.32					656	0.29	
pH 10.0	541	170	256	5.23	180 2.18	**pH 10.0**	539	167	257	5.05	195 2.19
			298	-0.59					297	-0.40	
			326	0.10					324	0.25	
			356	-0.14					361	-0.11	
			481	-0.20					484	-0.26	
			569	-0.53					566	0.49	
			658	0.35					658	0.31	
pH 11.0	542	173	255	5.38	192 2.20	**pH 11.0**	536	177	257	5.15	196 2.19
			297	-0.87					297	-0.55	
			330	0.08					326	0.33	
			361	-0.11					365	-0.08	
			480	-0.17					487	-0.31	
			574	-0.63					563	-0.53	
			661	0.31					658	0.33	

(H96His-N^{τ}-Me)PrP_{92-100}						(H111His-N^{τ}-Me)$PrP_{106-113}$					
Species	**UV-Vis** λ/nm	ε/M^{-1} cm^{-1}	**CD** λ/nm	$\Delta\varepsilon$/$M^{-1}cm^{-1}$	**EPR** A_{II}/G	g_{II}	**Species**	**UV-Vis** λ/nm	ε/M^{-1} cm^{-1}	**CD** λ/nm	$\Delta\varepsilon$/$M^{-1}cm^{-1}$	**EPR** A_{II}/G	g_{II}
$CuH_{-1}L$	617	35	259	0.64	119	2.41	**CuHL**	n.d.		258	2.83	119	2.41
			306	-0.04						332	-0.34		
			341	0.05						530	0.20		
										617	-0.08		
$CuH_{-2}L$	600	70	251	3.68	170	2.23	**CuL**	617	79	249	8.32	170	2.22
			306	-0.63						337	-0.61		
			346	0.48						388	0.09		
			503	0.40						459	-0.04		
			601	-0.60						536	0.30		
										613	-0.06		
										779	-0.19		
$CuH_{-3}L$	555	110	247	2.46	180	2.22	**$CuH_{-1}L$**	568	100	256	7.47	182	2.21
			309	-1.48						316	1.15		
			494	0.91						359	-0.11		
			589	-1.46						494	-1.03		
										629	0.75		
$CuH_{-4}L$	550	112	246	2.79	196	2.20	**$CuH_{-2}L$**	549	108	257	7.65	192	2.20
			278	0.17						317	1.46		
			310	-1.77						363	-0.10		
			363	-0.04						495	-1.27		
			493	0.80						627	0.94		
			587	-1.54									
							$CuH_{-3}L$	543	111	257	7.94	195	2.19
										319	1.56		
										365	-0.07		
										496	-1.44		
										627	1.03		

Fig. S1

Proton paramagnetic relaxation contributions (R_{1p}) of (H96A)$PrP_{92\text{-}113}$ α protons calculated in presence of 0.1 Cu^{2+} equivalents, T=298 K, pH 6.5 and by using $p_f = 1$ (solid squares) or $p_f = 0.9$ (open circles).

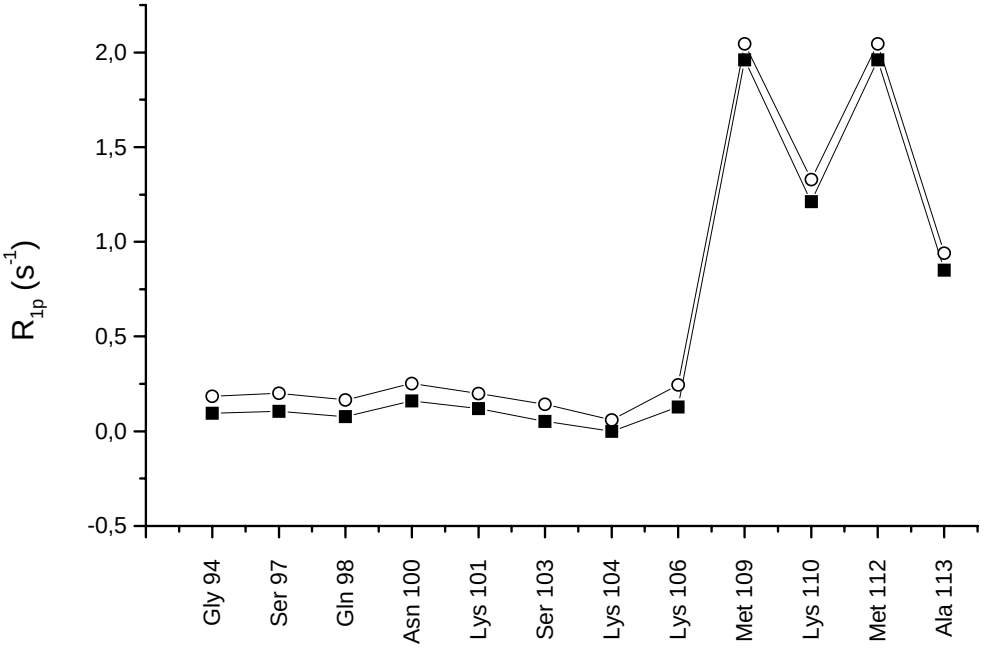

Fig. S2

Comparison between proton paramagnetic relaxation contributions (R_{1p}) of (A) (H96His-N^{τ}-Me)PrP_{92-113} and (H96His-N^{τ}-Me)PrP_{92-100}; (B) (H111His-N^{τ}-Me)PrP_{92-113} and (H111His-N^{τ}-Me)$PrP_{106-113}$ calculated in presence of 0.1 Cu^{2+} equivalents, T=298 K, pH 6.5.