Spectroscopic and Electronic Structure Studies of Copper (II) Binding to His111 in the Prion Protein Fragment 106-115: Evaluating the Role of Protons and Methionine Residues

Lina Rivillas-Acevedo, Rafael Grande-Aztatzi, Italia Lomelí, Javier E. García, Erika Barrios, Sarai Teloxa, Alberto Vela, Liliana Quintanar**

Departamento de Química,

Centro de Investigación y de Estudios Avanzados (Cinvestav)

* To whom correspondence should be addressed:

Liliana Quintanar
Departamento de Química, Cinvestav
Av. Instituto Politécnico Nacional No. 2508,
Col. San Pedro Zacatenco,
México, 07360, D.F.
Mexico City
Phone: +52-55-57473723
Fax: +52-55-57473389
lilianaq@cinvestav.mx

SUPPORTING INFORMATION

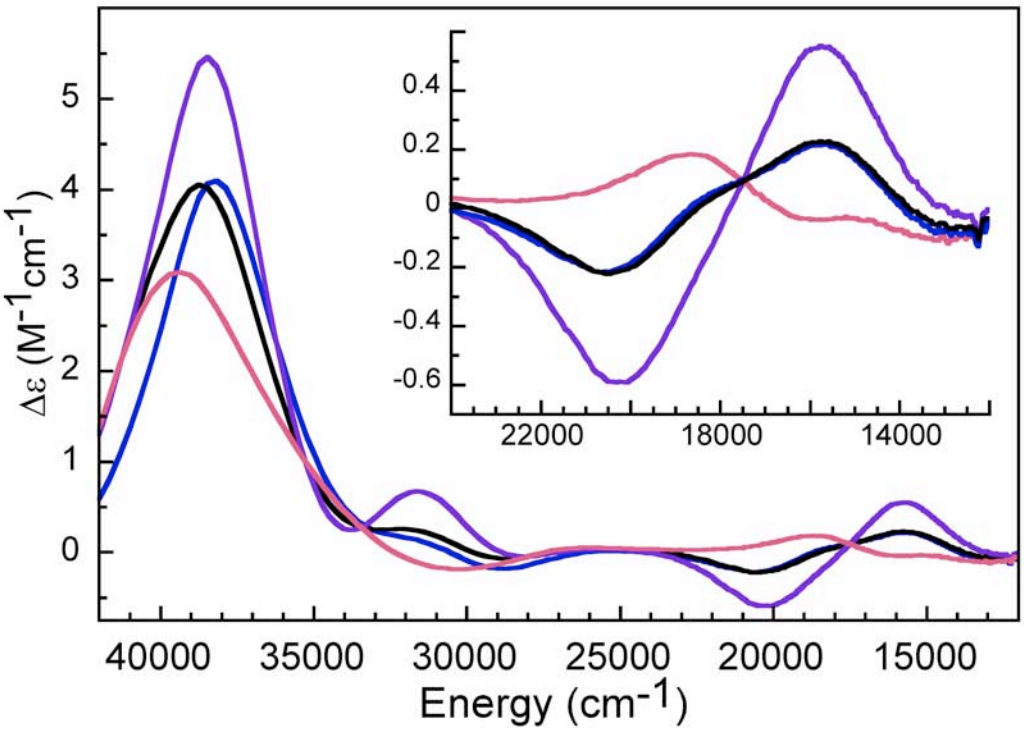

Figure S1. The experimental CD spectrum of Cu(II)-PrP(106-115) complex at pH 7.5 (blue), can be simulated by using a 50/50 mixture (black) of the experimental spectra at pH 6.5 (pink), and at pH 8.5 (purple) of the peptide with one equivalent of Cu(II).

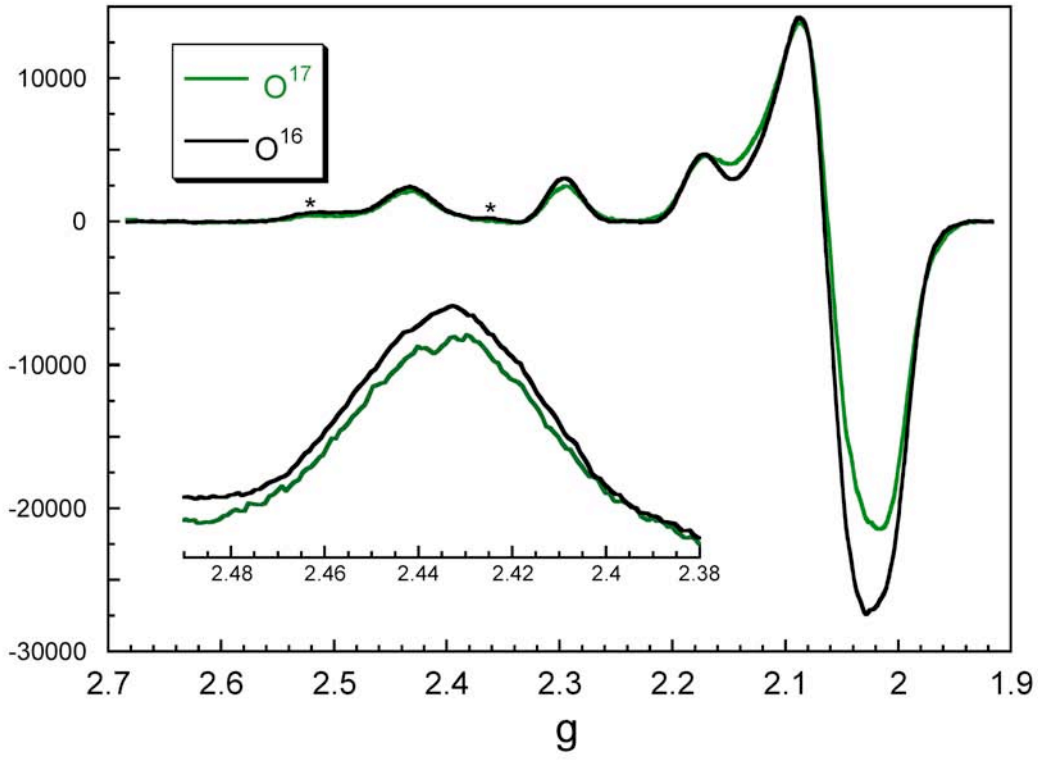

Figure S2. Comparison of X-band EPR spectra of the Cu(II) complex with PrP(106-115) at pH 6.5, prepared in ^{16}O (black) and ^{17}O water (green). Spectra were collected under the conditions specified in the experimental section at 20 K. Asterisks indicate signals that are associated to free Cu(II) in the buffer solution.

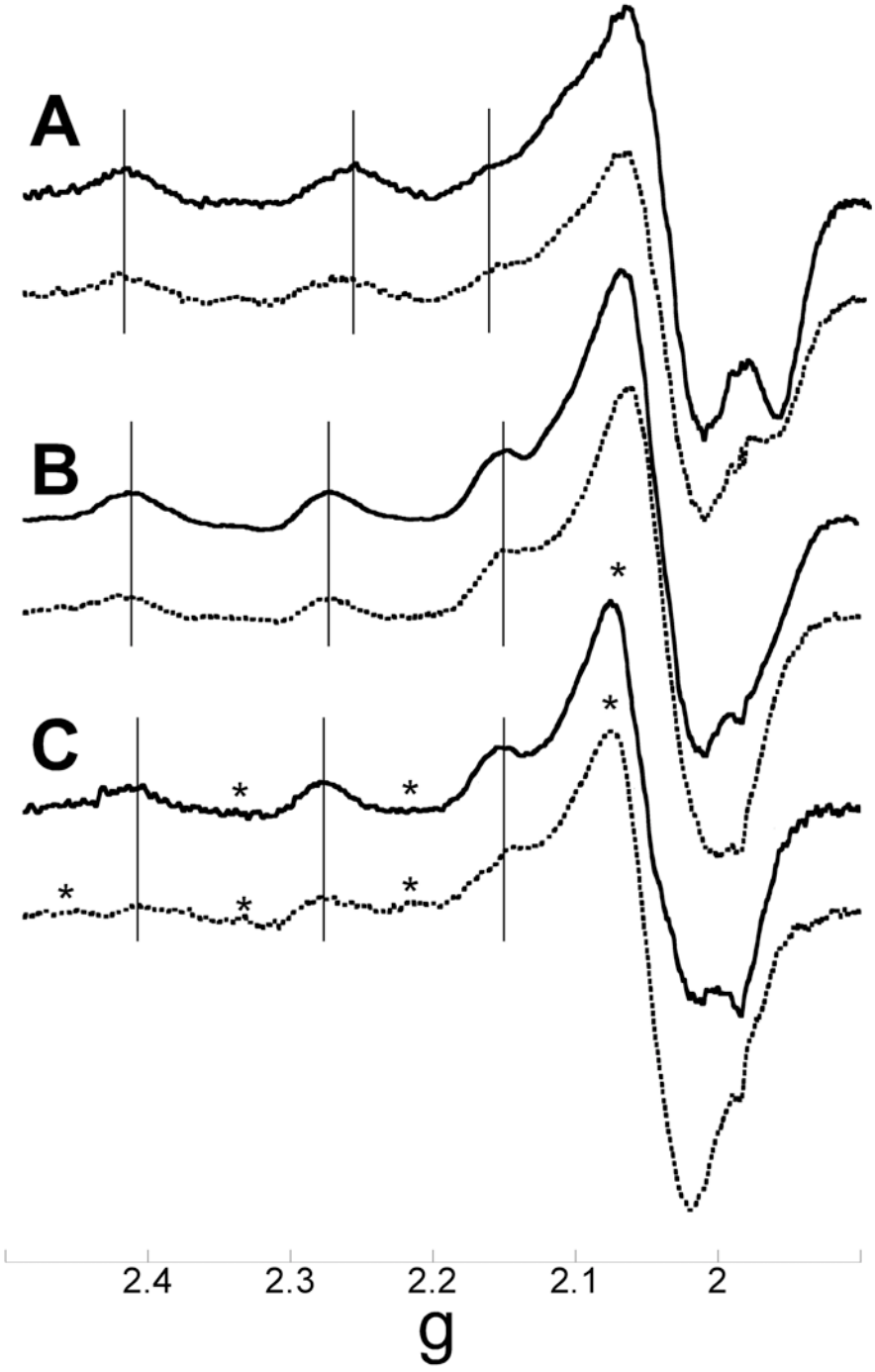

Figure S3. Comparison of X-band EPR spectra of PrP(106-115) (solid line) and KTNMKH (dashed line) at pH 8.5 (**A**) and 7.5 (**B**) with one equivalent of Cu(II), and at pH 6.5 (**C**) with 0.5 equivalents of Cu(II). Spectra were collected under the conditions specified in the experimental section at 77K.

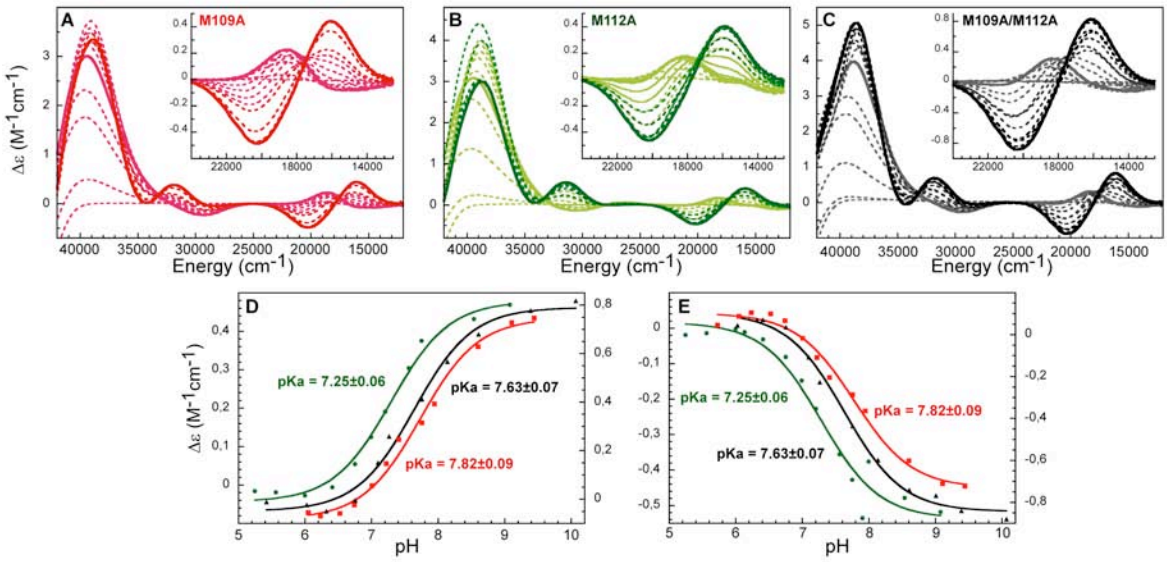

Figure S4. pH titration of the Cu(II) complexes with PrP(106-115)M109A (**A**), PrP(106-115)M112A (**B**), and PrP(106-115)M109A/M112A (**C**), as followed by circular dichroism. The Cu(II) complex solutions were titrated from pH ~ 5.5 (thick light color line in each figure) to pH ~ 9.5-10 (thick dark line in each figure); spectra for intermediate pH values are shown in thin lines. The traces for the CD signal intensity changes at 15800 cm^{-1} (**D**) and 20800 cm^{-1} (**E**) for the Cu(II) complexes with PrP(106-115)M109A (red), PrP(106-115)M112A (green), and PrP(106-115)M109A/M112A (black) were fit to the model described in the text (solid lines) to determine the associated pKa.

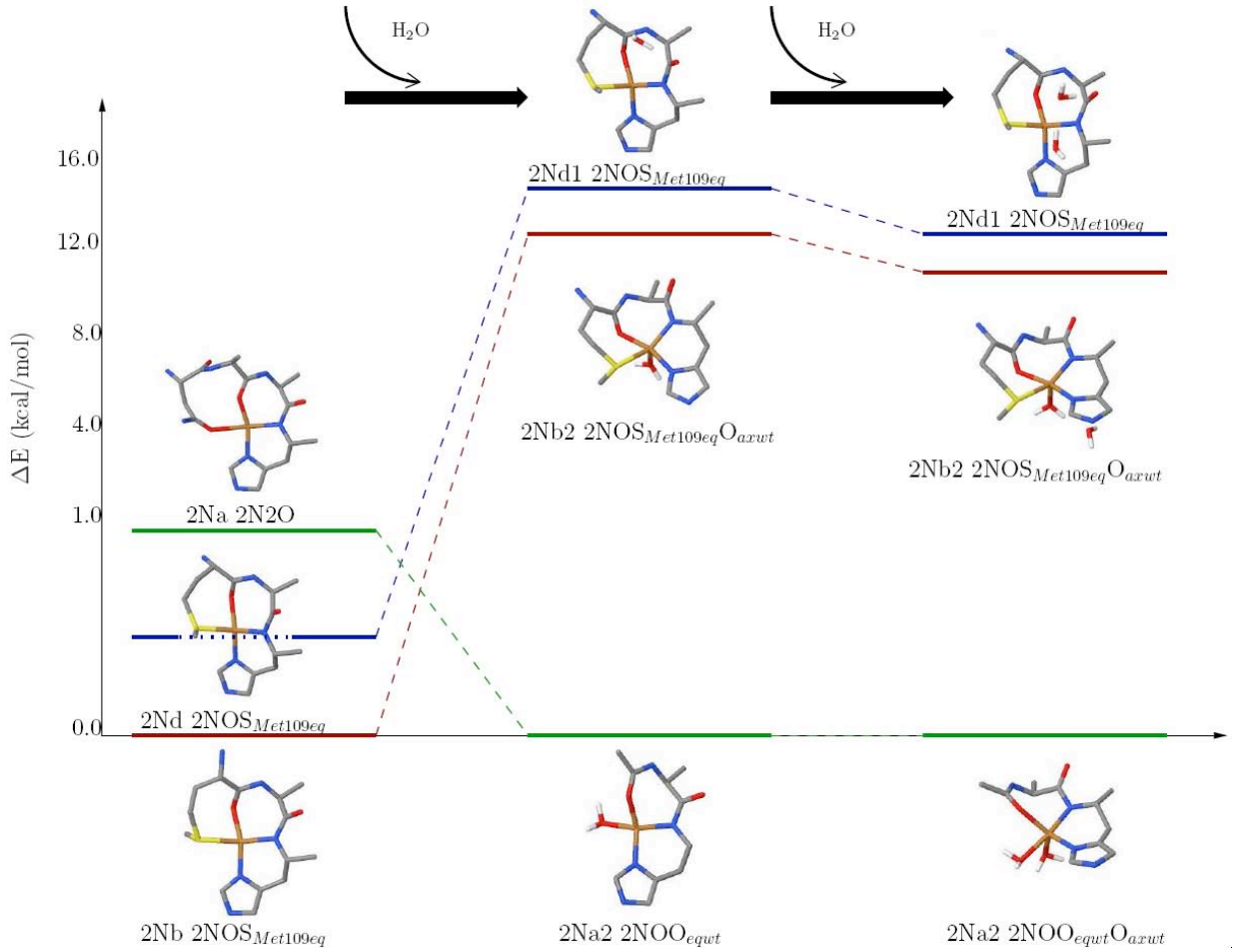

Figure S5. Energy diagram for selected structures of the 2N models of the Cu(II)-PrP(106-113) complex without water (left) and after the successive addition of two explicit water molecules. Most side chains are not shown for clarity; however, the geometry optimizations were done with the complete Cu(II)-PrP(106-113) complex.

Table S1. EPR simulation parameters for the Cu(II) complexes with the PrP(106-115) fragment and the M109A/M112A variant at pH 8.5 and 6.5 (as used for the EPR simulations shown in Figure 4)

	pH 8.5		**pH 6.5**	
	PrP(106-115)	**PrP(106-115) M109A/M112A**	**PrP(106-115)**	**PrP(106-115) M109A/M112A**
g_x	2.035	2.038	2.030	2.035
g_y	2.063	2.048	2.075	2.078
g_z	2.199	2.193	2.226	2.227
A_x	19	17	19	20
A_y	13	17	10	10
A_z	191	195	167	167
$^{N\,Im}A_x$	11	14	12	13
$^{N\,Im}A_y$	9	12	10	10
$^{N\,Im}A_z$	9	12	10	10
$^{N\,amideHis}A_x$	11	12	10	12
$^{N\,amideHis}A_y$	13	14	20	20
$^{N\,amideHis}A_z$	11	12	10	12
$^{N\,amideLys}A_x$	18	19	12	12
$^{N\,amideLys}A_y$	16	17	10	10
$^{N\,amideLys}A_z$	16	17	10	10
$^{N\,amideMet}A_x$	11	12	-	-
$^{N\,amideMet}A_y$	13	14	-	-
$^{N\,amideMet}A_z$	11	12	-	-
L_x	6	6	6	6
L_y	4	5	7	9
L_z	28	24	20	24

[a] Hyperfine splittings and line-widths are given in x 10^{-4} cm^{-1}. The line shape of the signal was modeled as Gaussians, using a model that considers an angular dependence of the g value, as implemented in the EPR program XSophe.

Table S2: Structural parameters of the optimized geometries at LDA (Dirac-VWN) and GGA (PBE) levels for the 4N set.*

ID	Bond (Å)	LDA	PBE	Angle (degrees)	LDA	PBE
4Na	Cu-N1 Im	1.96	2.06	N1-Cu-N2	94.68	91.55
	Cu-N2 H111	1.95	2.02	N2-Cu-N3	84.57	83.34
	Cu-N3 K110	1.89	1.93	N3-Cu-N4	83.83	83.13
	Cu-N4 M109	1.92	1.97	N4-Cu-N1	109.44	113.83
	Cu-S1 M109	5.99	6.05	N1-Cu-N3	144.69	137.11
	Cu-S2 M112	8.29	8.61	N2-Cu-N4	151.85	153.28
4Nb	Cu-N1 Im	1.95	2.05	N1-Cu-N2	95.50	92.69
	Cu-N2 H111	1.98	2.02	N2-Cu-N3	83.91	83.31
	Cu-N3 K110	1.88	1.93	N3-Cu-N4	82.72	82.46
	Cu-N4 M109	1.93	1.97	N4-Cu-N1	107.03	110.39
	Cu-S1 M109	3.02	4.85	N1-Cu-N3	158.82	140.78
	Cu-S2 M112	8.69	8.73	N2-Cu-N4	148.40	156.13
4Nc	Cu-N1 Im	1.96	2.05	N1-Cu-N2	93.64	91.61
	Cu-N2 H111	1.95	2.01	N2-Cu-N3	83.71	82.24
	Cu-N3 K110	1.89	1.95	N3-Cu-N4	84.47	83.99
	Cu-N4 M109	1.94	1.99	N4-Cu-N1	109.58	113.55
	Cu-S1 M109	6.02	6.18	N1-Cu-N3	146.06	144.95
	Cu-S2 M112	4.50	4.71	N2-Cu-N4	152.82	151.52

* In the table
N1 Im is the nitrogen belonging to imidazole ring of the His111
N2 H111 is the nitrogen belonging to deprotonated amide of the His111
N3 K110 is the nitrogen belonging to deprotonated amide of the Lys110
N4 M109 is the nitrogen belonging to deprotonated amide of the Met109
S1 M109 is the sulfur belonging to side chain of the Met109
S2 M112 is the sulfur belonging to side chain of the Met112

Table S3: Lowest harmonic frequencies of the optimized structures without explicit water molecules of the 4N, 3N and 2N series calculated with LDA and PBE functionals.The frequency is reported in wave number (cm^{-1})

ID	LDA	PBE
4N set		
4Na	11.7	8.2
4Nb	6.2	6.1
4Nc	8.0	-30.9
3N set		
3Na	2.3	3.1
3Nb	12.0	6.3
3Nc	14.2	13.2
3Nd	2.0	3.1
3Ne	4.9	-5.6
2N set		
2Na	-11.2	-6.8
2Nb	-6.3	-7.3
2Nc	9.4	6.6
2Nd	-6.5	2.7
2Ne	4.3	6.0

Table S4: Structural parameters of the 3N set optimized at LDA (Dirac-VWN) and GGA (PBE) levels without and with 1 and 2 water molecules.*

ID		No water		1 H_2O		2 H_2O	
		LDA	PBE	1	2	1	2
3Na	Cu-N1 Im	1.92	1.99	1.99	1.99	1.99	2.02
	Cu-N2 H111	1.92	1.99	1.99	2.00	1.99	1.98
	Cu-N3 K110	1.89	1.94	1.94	1.94	1.94	1.96
Bond (Å)	Cu-O1 N108	2.03	2.14	2.14	2.12	2.13	2.13
	Cu-S1 M109	4.43	4.74	4.74	4.73	4.73	4.81
	Cu-S2 M112	8.30	7.41	7.18	7.22	7.39	6.71
	Cu-O H_2O1			9.88	3.52	10.02	3.77
	Cu-O H_2O2					4.86	3.03
Angle (degrees)	N1-Cu-N2	96.06	95.58	95.63	95.69	95.37	93.24
	N2-Cu-N3	85.01	84.71	84.75	84.58	84.62	74.42
	N3-Cu-O1	104.16	102.84	102.62	104.57	102.20	104.06
	O1-Cu-N1	98.32	99.62	99.87	97.41	100.07	95.87
	N1-Cu-N3	149.01	149.74	149.85	148.98	149.86	144.22
	N2-Cu-O1	131.24	131.14	130.63	134.14	131.95	149.62
3Nb	Cu-N1 Im	1.97	2.06	2.05		1.98	
	Cu-N2 H111	1.96	2.00	2.00		1.98	
	Cu-N3 K110	1.97	2.01	2.01		1.94	
Bond (Å)	Cu-S1 M109	2.30	2.40	2.41		4.74	
	Cu-O1 N108	1.97	2.29	2.31		2.13	
	Cu-S2 M112	7.55	7.87	7.92		7.35	
	Cu-O H_2O1			4.45		8.22	
	Cu-O H_2O2					6.84	
	N1-Cu-N2	89.52	88.61	88.44		94.54	
	N2-Cu-N3	80.47	80.08	79.99		84.13	
	N3-Cu-S1	89.34	90.13	88.33			
	S1-Cu-N1	94.74	94.73	96.93			
	N1-Cu-O1	109.93	98.63	100.67		99.83	
	N2-Cu-O1	101.15	101.96	100.91		134.44	
Angle (degrees)	N3-Cu-O1	109.02	104.32	104.15		102.81	
	S1-CU-O1	94.82	94.90	94.27			
	N1-Cu-N3	156.29	156.04	154.17		149.38	
	N2-Cu-S1	163.16	162.13	162.69			

ID		No water		1 H_2O		2 H_2O	
		LDA	PBE	1	2	1	2
3Nc	Cu-N1 Im	1.93	2.00	2.00	1.98	1.99	1.97
	Cu-N2 H111	1.94	2.00	2.00	2.01	2.00	2.01
	Cu-N3 K110	1.89	1.94	1.94	1.94	1.94	1.93
Bond (Å)	Cu-O1 N108	2.03	2.14	2.13	2.16	2.12	2.23
	Cu-S1 M109	4.40	4.73	4.83	4.52	6.04	4.50
	Cu-S2 M112	4.13	4.66	4.56	5.96	4.51	5.97
	Cu-O H_2O1			6.35	3.43	6.31	3.66
	Cu-O H_2O2					6.57	3.46
Angle (degrees)	N1-Cu-N2	95.24	95.12	95.69	95.78	95.45	96.47
	N2-Cu-N3	84.30	84.40	84.24	83.25	84.22	83.31
	N3-Cu-O1	100.66	136.10	100.65	96.22	100.66	94.65
	O1-Cu-N1	93.96	99.60	96.32	91.23	95.42	92.67
	N1-Cu-N3	156.99	157.50	153.94	169.79	154.74	169.61
	N2-Cu-O1	141.60	136.10	138.25	138.59	140.70	136.17
3Nd	Cu-N1 Im	1.96	2.04	2.01	2.05	2.00	2.04
	Cu-N2 H111	1.95	2.01	1.98	1.99	2.00	2.01
	Cu-N3 K110	1.93	1.99	1.99	2.00	1.97	2.01
Bond (Å)	Cu-S1 M109	2.32	2.44	2.39	2.39	2.41	2.39
	Cu-S2 M112	8.81	8.68	8.32	8.45	8.48	8.76
	Cu-O H_2O1			3.45	3.55	4.89	3.90
	Cu-O H_2O2					3.57	4.10
Angle (degrees)	N1-Cu-N2	91.89	90.89	91.78	89.03	90.79	88.73
	N2-Cu-N3	84.17	82.98	83.37	82.28	83.44	81.33
	N3-Cu-S1	113.99	113.97	114.44	111.72	114.29	102.71
	S1-Cu-N1	88.35	90.37	103.61	91.73	96.13	95.14
	N1-Cu-N3	144.62	144.77	136.44	143.17	146.12	148.52
	N2-Cu-S1	146.01	145.43	125.31	153.13	125.87	163.80
3Ne	Cu-N1 Im	1.93	1.99	1.99	2.00	1.98	2.00
	Cu-N2 H111	1.95	2.01	2.00	2.02	2.00	2.00
	Cu-N3 K110	1.91	1.96	1.97	1.95	1.95	1.96
Bond (Å)	Cu-S1 M112	2.28	2.40	2.42	2.40	2.45	2.43
	Cu-S2 M109	6.68	7.01	7.11	7.04	6.94	7.03
	Cu-O H_2O1			3.46	4.64	3.41	6.99
	Cu-O H_2O2					3.49	4.25

ID		No water		1 H_2O		2 H_2O	
		LDA	PBE	1	2	1	2
Angle (degrees)	N1-Cu-N2	94.69	93.33	92.67	92.73	93.49	93.31
	N2-Cu-N3	83.79	83.17	83.66	83.48	84.31	83.19
	N3-Cu-S1	93.23	95.68	98.05	95.99	92.20	95.64
	S1-Cu-N1	111.52	115.48	112.84	116.74	113.71	119.16
	N1-Cu-N3	147.97	143.62	143.83	142.14	149.86	140.42
	N2-Cu-S1	129.65	124.30	125.54	123.61	122.74	120.82

* In the table
N1 Im is the nitrogen belonging to imidazole ring of the H111
N2 H111 is the nitrogen belonging to deprotonated amide of the His111
N3 K110 is the nitrogen belonging to deprotonated amide of the Lys110
O1 N108 is the oxygen belonging to the carbonyl of the Asn108
S1 M109 is the sulfur belonging to side chain of the Met109
S2 M112 is the sulfur belonging to side chain of the Met112

Table S5: Ordering of structures of 3N set with the GGA (PBE) functional, without and with one and two explicit water molecules. Δ E is the relative energy in kcal/mol.

No water			1 H_2O			2 H_2O		
ID	Coordination Mode	Δ E	ID	Coordination Mode	Δ E	ID	Coordination Mode	Δ E
3Na	3NO	0.00	3Na2	3NO	0.00	3Nb1	3NO	0.00
3Nd	$3NS_{Met109eq}$	0.33	3Na1	3NO	0.76	3Nc1	3NO	1.05
3Nc	3NO	3.56	3Nc1	3NO	2.81	3Na2	3NO	2.51
3Nb	$3NS_{Met109eq}O_{ax}$	7.15	3Nd2	$3NS_{Met109eq}$	4.85	3Nd2	$3NS_{Met109eq}$	6.41
3Ne	$3NS_{Met112eq}$	13.70	3Nd1	$3NS_{Met109eq}$	7.42	3Na1	3NO	6.45
			3Nc2	3NO	7.43	3Nd1	$3NS_{Met109eq}$	10.28
			3Nb1	$3NS_{Met109eq}O_{ax}$	11.36	3Nc2	3NO	10.33
			3Ne1	$3NS_{Met112eq}$	14.15	3Ne1	$3NS_{Met112eq}$	19.48
			3Ne2	$3NS_{Met112eq}$	17.30	3Ne2	$3NS_{Met112eq}$	21.54

Table S6: Ordering of structures of 2N set with the GGA (PBE) functional, without and with one and two explicit water molecules. Δ E is the relative energy in kcal/mol.

No water			1 H_2O			2 H_2O		
ID	Coordination Mode	Δ E	ID	Coordination Mode	Δ E	ID	Coordination Mode	Δ E
2Nb	$2NOS_{Met109eq}$	0.00	2Na2	$2NOO_{eqwt}$	0.00	2Na2	$2NOO_{eqwt}O_{axwt}$	0.00
2Nd	$2NOS_{Met109eq}$	0.55	2Na1	$2NOO_{eqwt}$	6.36	2Na1	$2NOO_{eqwt}$	9.32
2Na	2N2O	0.93	2Nb2	$2NOS_{Met109eq}O_{axwt}$	12.79	2Nb2	$2NOS_{Met109eq}O_{axwt}$	10.80
2Nc	$2NOS_{Met112eq}$	4.77	2Nb1	$2NOS_{Met109eq}$	12.94	2Nb1	$2NOS_{Met109eq}$	11.33
2Ne	$2NOS_{Met112eq}$	6.96	2Nc2	$2NOS_{Met112eq}O_{axwt}$	13.44	2Nd1	$2NOS_{Met109eq}$	12.66
			2Nd1	$2NOS_{Met109eq}$	14.65	2Nc2	$2NOS_{Met112eq}O_{axwt}$	14.75
			2Nd2	$2NOS_{Met109eq}$	16.13	2Nd2	$2NOS_{Met109eq}$	16.71
			2Ne1	$2NOS_{Met112eq}$	20.72	2Ne1	$2NOS_{Met112eq}$	19.47
			2Ne2	$2NOS_{Met112eq}$	24.83	2Ne2	$2NOS_{Met112eq}$	23.44
			2Nc1	$2NOS_{Met112eq}$	29.81	2Nc1	$2NOS_{Met112eq}$	29.09

Table S7: EPR parameters of nitrogen atoms coordinated to copper in the structures shown in Table 5. The **A** tensor was calculated with the non-empirical hybrid functional PBE0 and the EPR-III basis set. The **A** tensor components are reported in MHz

ID		A_x	A_y	A_z
4Na	N1-Im	24.96	19.27	20.07
	N2-H111	25.95	36.82	26.52
	N3-K110	56.57	33.61	34.43
	N4-M109	37.11	47.53	36.04
4Nb	N1-Im	28.04	21.79	22.62
	N2-H111	27.12	38.07	27.73
	N3-K110	61.06	39.09	40.27
	N4-M109	38.96	50.51	37.87
3Nb1	N1-Im	28.48	21.62	22.25
+ 2 H_2O	N2-H111	34.15	56.56	34.95
	N3-K110	38.55	26.32	27.03
3Na2	N1-Im	29.39	22.55	23.19
+ 2 H_2O	N2-H111	38.58	58.22	39.66
	N3-K110	37.68	24.82	25.18