Supplementary Information

Investigation on the metal binding sites of a putative Zn(II) transporter in opportunistic yeast species *Candida albicans*

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Table S1. Overall (log *β*) and step (log *K*) protonation constants for L1 at *T*=298 K, *I*=0.1 mol dm^{-3} ($NaClO_4$) and pH range 3–9. Values in parentheses are standard deviations on the last significant figure.

Species	log *β*	log K_{step}	Residue
HL	7.50 (5)	7.50	His
H_2L^+	14.15 (4)	6.66	His
H_3L^{2+}	20.69 (5)	6.54	His
H_4L^{3+}	26.53 (4)	5.84	His
H_5L^{4+}	32.27 (3)	5.74	His
H_6L^{5+}	36.45 (4)	4.18	Glu

Table S2. Overall (log *β*) and step (log *K*) protonation constants for L2 at *T*=298 K, *I*=0.1 mol dm^{-3} ($NaClO_4$) and pH range 3–9. Values in parentheses are standard deviations on the last significant figure.

Species	log *β*	log K_{step}	Residue
HL^+	7.12 (2)	7.12	His
H_2L^{2+}	13.58 (1)	6.47	His
H_3L^{3+}	19.66 (2)	6.08	His
H_4L^{4+}	25.14 (1)	5.48	His

Table S3. Overall (log *β*) and step (log *K*) protonation constants for L3 at T=298 K, I=0.1 mol dm^{-3} ($NaClO_4$) and pH range 3–9. Values in parentheses are standard deviations on the last significant figure.

Species	log *β*	log K_{step}	Residue
HL^{2-}	10.54	10.54	Lys
H_2L^{-}	18.32 (2)	7.78	His
H_3L	25.47 (4)	7.15	His
H_4L^{+}	32.46 (7)	6.99	His
H_5L^{2+}	39.0 (1)	6.6	His
H_6L^{3+}	45.6 (1)	6.6	His
H_7L^{4+}	51.7 (1)	6.1	His
H_8L^{5+}	57.77 (9)	6.03	His
H_9L^{6+}	63.61 (5)	5.84	His
$H_{10}L^{7+}$	69.07 (3)	5.47	His
$H_{11}L^{8+}$	74.22 (1)	5.14	His
$H_{12}L^{9+}$	78.12 (2)	3.90	Glu
$H_{13}L^{10+}$	81.64 (1)	3.52	Asp

Table S4. Spectroscopic parameters at different pH values for the system Cu(II)/L2; M:L ratio = 1:1.25.

	UV-Vis			**CD**			**EPR**		
Species	***pH***	***λ (nm)***	***ε (M^{-1} cm^{-1})***	***pH***	***λ (nm)***	***Δε (M^{-1} cm^{-1})***	***pH***	***A_{II} (G)***	***g_{II}***
$[CuH_2L]^{4+}$	3.09	-	-	3.09	242.1 232.0	-0.60 1.00	3.08	119.43	2.415
$[CuH_2L]^{4+}$	4.16	732	25.02	4.16	242.6 232.5	-0.65 0.96	4.00	119.28	2.412
$[CuH_2L]^{4+}$ $[CuL]^{2+}$	5.13	660	39.06	5.13	542.6 233.5	-0.54 0.81	5.06	121	2.416
$[CuL]^{2+}$	6.05	593	64.91	6.05	257.6 241.6 236.1	0.07 -0.29 0.06	6.09	215	2.213
$[CuL]^{2+}$ $[CuH_{-2}L]$	7.09	579	76.80	7.09	544.4 324.2 259.1 241.1 236.6	0.11 -0.34 0.59 -0.74 -0.03	7.13	195	2.205
$[CuH_{-2}L]$	8.02	564	80.40	8.02	619.5 329.7 257.1 239.0	0.26 -1.05 2.60 -2.20	8.10	197	2.20
$[CuH_{-2}L]$ $[CuH_{-3}L]^-$	9.14	545	85.80	9.14	622.0 510.2 332.9 256.7 237.1	0.51 -0.18 -1.13 3.81 -1.88	9.17	197	2.20
-	10.09	534	105.54	10.09	623.5 496.2 336.3 307.2 257.7 237.1	0.86 -0.48 -0.42 0.29 4.90 -1.74	10.02	189	2.193
-	11.01	522	102.70	11.01	627.0 494.7 346.9 315.3 292.7 257.7 237.1	1.05 -0.66 -0.16 0.42 -0.29 5.35 -1.79	11.05	188	2.185

Table S5. Spectroscopic parameters at different pH values for the system Cu(II)/L3; M:L ratio = 1:1.25.

	UV-Vis			**CD**		
Species	***pH***	***λ (nm)***	***ε (M^{-1} cm^{-1})***	***pH***	***λ (nm)***	***Δε (M^{-1} cm^{-1})***
$[CuH_{10}L]^{9+}$	3.19	660	47.22	3.02	237.7	-7.16
$[CuH_{10}L]^{9+}$ $[CuH_9L]^{8+}$ $[CuH_8L]^{7+}$	4.09	658	62.35	4.05	240.7	-5.09
$[CuH_8L]^{7+}$ $[CuH_7L]^{6+}$ $[CuH_6L]^{5+}$	5.06	602	104.36	5.23	530.4 309.2 244.0	1.12 -0.23 -3.82
$[CuH_6L]^{5+}$ $[CuH_5L]^{4+}$ $[CuH_4L]^{3+}$	6.11	591	118.20	6.19	549.8 308.6 238.5	0.32 -0.25 -9.95
$[CuH_3L]^{2+}$ $[CuH_2L]^{+}$ [CuHL]	7.05	595	148.62	7.08	549.8 314.7 238.5	0.32 -0.24 -8.84
[CuHL] $[CuH_{-1}L]^{2-}$	8.19	568	123.35	8.01	606.0 333.9 258.7 238.0	0.29 -0.87 2.23 -5.45
$[CuH_{-1}L]^{2-}$ $[CuH_{-3}L]^{4-}$	9.32	539	144.92	9.07	626.5 502.9 337.8 256.6 238.0	0.81 -0.49 -0.80 5.94 -2.42
-	10.34	521	152.49	10.09	624.7 493.3 347.0 314.2 257.2 237.5	1.25 -1.00 -0.23 0.59 7.04 -1.25
-	11.01	521	152.49	11.02	627.5 492.3 353.0 316.2 291.4 257.1 235.9	1.30 -1.12 -0.21 0.72 -0.25 7.13 -1.32

Table S6. Spectroscopic parameters at different pH values for the system Cu(II)/L1; M:L ratio = 1:1.25.

	UV-Vis			CD			EPR		
Species	***pH***	***λ (nm)***	***ε (M^{-1} cm^{-1})***	***pH***	***λ (nm)***	***Δε (M^{-1} cm^{-1})***	***pH***	***A_{II} (G)***	***g_{II}***
$[CuH_4L]^{5+}$ $[CuH_3L]^{4+}$	4.26	-	-	4.26	234.6	-2.68	3.96	122	2.415
$[CuH_3L]^{4+}$ $[CuH_2L]^{3+}$ $[CuHL]^{2+}$	5.50	620	86.30	5.50	253.1 233.6	0.65 -3.22	4.98	119	2.410
$[CuHL]^{2+}$ $[CuL]^{+}$	6.34	593	110.32	6.34	253.4 235.1	0.76 -3.98	5.98	183	2.225
$[CuL]^{+}$ $[CuH_{-2}L]^{-}$	7.27	590	116.16	7.27	335.9 253.4 235.6	-0.26 1.54 -3.27	7.51	193	2.20
$[CuH_{-2}L]^{-}$ $[CuH_{-3}L]^{2-}$	8.04	554	124.34	8.04	627.5 542.9 485.9 333.4 254.0 235.1	0.28 -0.16 0.13 -1.08 4.15 -1.83	8.00	196	2.20
$[CuH_{-3}L]^{2-}$	9.31	530	140.68	9.31	621.3 490.7 340.8 256.2	0.98 -0.59 -0.45 6.31	9.13	189	2.201
-	10.04	525	141.35	10.04	622.4 492.9 348.2 316.1 256.7	1.24 -0.98 -0.20 0.59 7.17	10.09	192	2.192
-	11.03	522	143.85	11.03	622.4 492.4 317.6 352.5 292.3 256.7	1.45 -1.22 0.76 -0.16 -0.19 7.64	10.99	196	2.185

Table S7. Experimental details for the potentiometric titrations of the free ligands; T=298 K and I=0.1 mol·dm^{-3} ($NaClO_4$).

Ligand	Number of titrations	Sample volume (ml)	Ligand concentration (mM)	pH range
L1	5	3.0	0.39-0.53	3-9
L2	3	3.0	0.50	3-9
L3	5	3.0	0.46-0.47	3-9

Table S8. Experimental details for the potentiometric titrations of the metal/ligand sample solutions; T=298 K and I=0.1 mol·dm^{-3} ($NaClO_4$).

Ligand	Metal ion	Number of titrations	Sample volume (ml)	Ligand concentration (mM)	Metal concentration (mM)	pH range
L1	Zn(II)	2	3.0	0.48-0.53	0.41-0.45	3-6
	Cu(II)	1	3.0	0.39	0.31	3-9
L2	Zn(II)	1	3.0	0.50	0.39	3-9
	Cu(II)	1	3.0	0.50	0.39	3-9
L3	Zn(II)	4	3.0-4.6	0.31-0.47	0.19-0.37	3-9
	Cu(II)	2	3.0-4.0	0.35-0.47	0.28-0.37	3-9

Table S9. Experimental details for MS measurements.

Ligand	Metal ion	Ligand concentration (mM)	Metal concentration (mM)	pH
L1	Zn(II)	0.50	0.44	6.38
	Cu(II)	0.51	0.40	5.97
L2	Zn(II)	0.50	0.44	7.04
	Cu(II)	0.54	0.41	5.62
L3	Zn(II)	0.48	0.44	6.76
	Zn(II)	0.47	0.39	5.30
	Cu(II)	0.48	0.40	5.74

Table S10. Experimental details for Vis absorption measurements; T=298 K and I=0.1 mol·dm^{-3} ($NaClO_4$).

Ligand	Metal ion	Ligand concentration (mM)	Metal concentration (mM)	Optical path (cm)	Wavelength range (nm)	pH range
L1	Cu(II)	0.46	0.36	1	200-800	3-11
L2	Cu(II)	0.99	0.80	1	200-800	3-11
L3	Cu(II)	0.98	0.80	1	200-800	3-6
	Cu(II)	0.49	0.40	1	200-800	6-11
	Cu(II)	0.38	0.32	1	200-800	3-11

Table S11. Experimental details for CD measurements; T=298 K and I=0.1 mol·dm^{-3} ($NaClO_4$).

Ligand	Metal ion	Ligand concentration (mM)	Metal concentration (mM)	Optical path (cm)	Wavelength range (nm)	pH range
L1	Cu(II)	0.46	0.36	1	200-800	3-11
L2	Cu(II)	0.99	0.80	1	200-800	3-11
L3	Cu(II)	0.98	0.80	1	200-800	3-6
	Cu(II)	0.49	0.40	1	200-800	6-11
	Cu(II)	0.10	0.08	0.01	180-300	3-11
	Zn(II)	0.10	0.08	0.01	180-300	3-11

Table S12. Experimental details for EPR measurements.

Ligand	Metal	Ligand concentration (mM)	Metal concentration (mM)	pH range
L1	Cu(II)	1.20	1.00	3-11
L2	Cu(II)	1.20	1.00	3-11

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C4YJH2      C4YJH2_CANAW       120  GLVSNGVGLVLFHEHGHSHSHGSGGGGGGSDHSGDSKSHSHSHSHSHGE-ATTFSQGDEE  178
0A1D8PGL8   A0A1D8PGL8_CANAL   120  GLVSNGVGLVLFHEHGHSHSHGGG-GGGGSDHSGDSKSHSHSHSHSHGE-ATTFSQGDEE  177
P20107      ZRC1_YEAST         121  GLISNVVGLFLFHDHGSDSLHSHSHGSVESGNNDLDIESNATHSHSHASLPNDNLAIDED  180
B9WAQ7      B9WAQ7_CANDC       120  GLISNGVGLVLFHEHGHSHSHGTNHSGDSN-----SNSNSTSHSHSHGEVATTFTQGDEE  174
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Scheme S1. Alignment of the C-terminal domain of C4YJH2 from *C. albicans* with a transmembrane Zn(II) transporter from C. *albicans* (A0A1D8PGL8) and with two zinc resistance proteins from *Saccharomyces cerevisiae* (P20107) and *Candida dubliniensis* (B9WAQ7). [UniProt Knowledgebase]

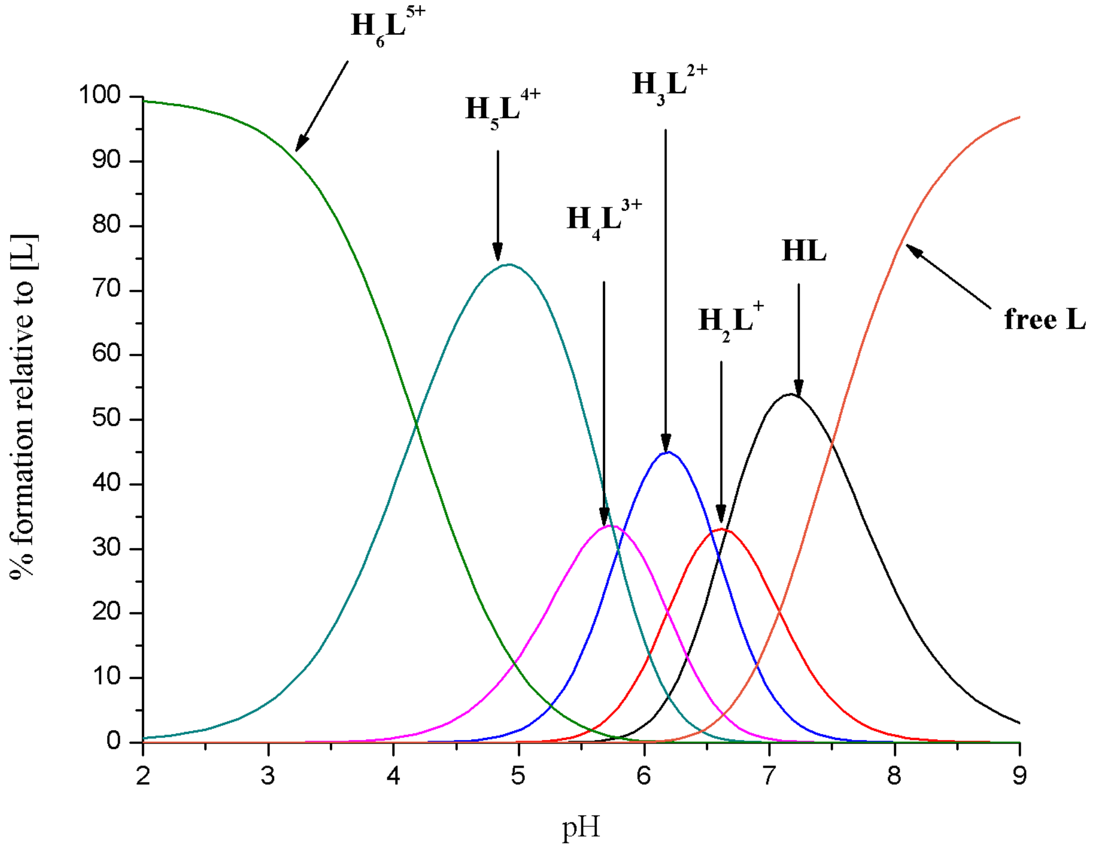

Figure S1. Species distribution diagram for protonation equilibria of the ligand L1.

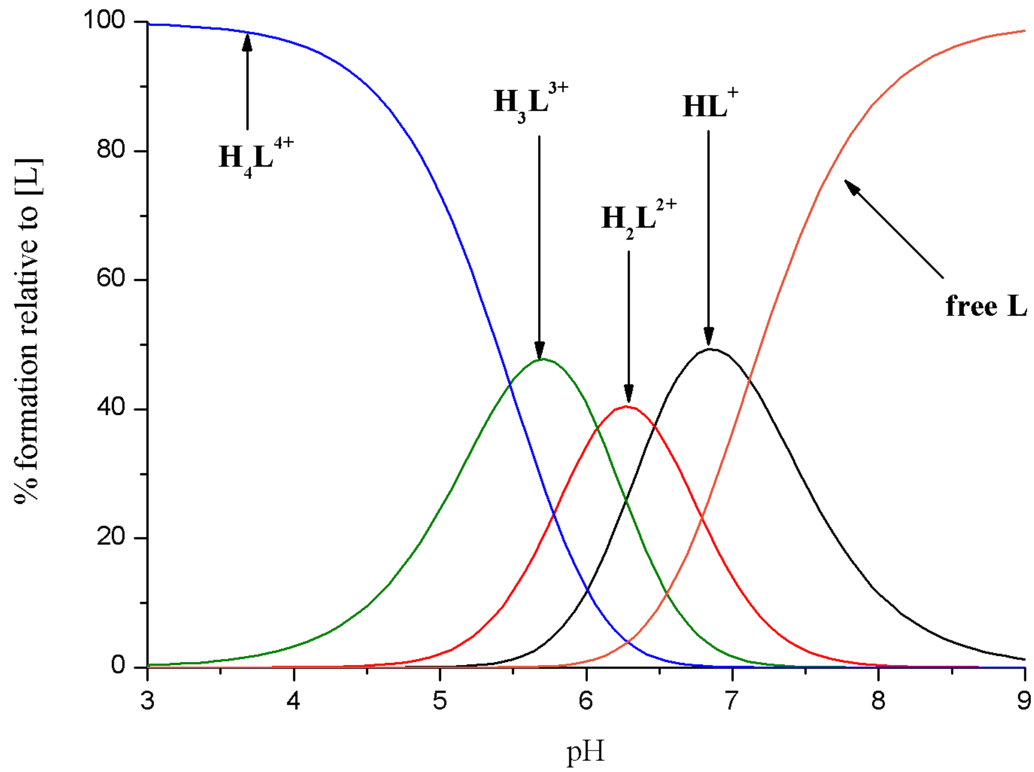

Figure S2. Species distribution diagram for protonation equilibria of the ligand L2.

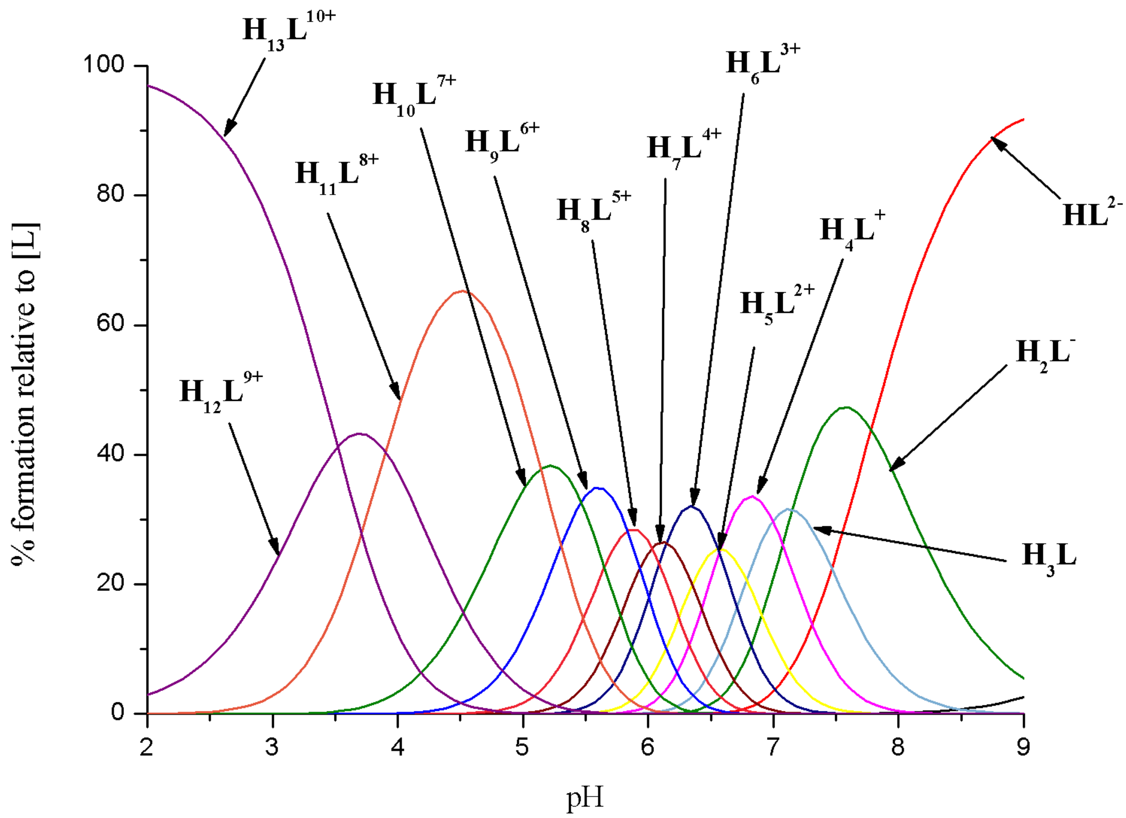

Figure S3. Species distribution diagram for protonation equilibria of the ligand L3.

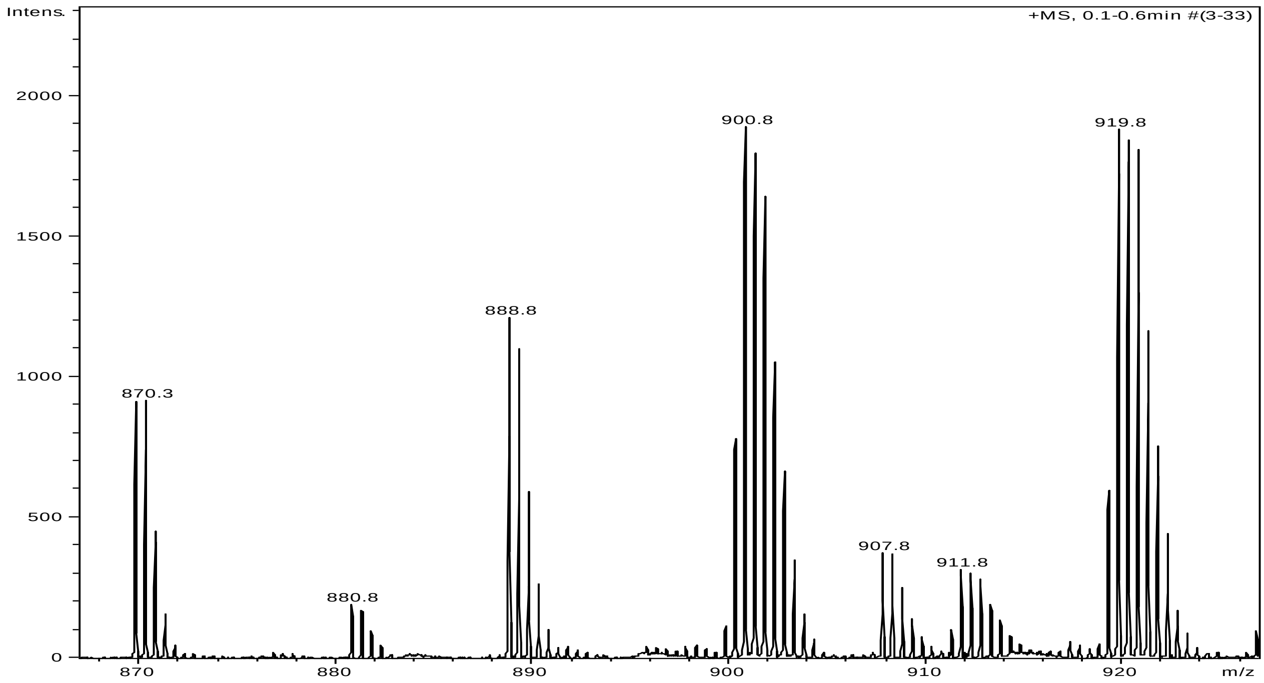

Figure S4. ESI-MS spectrum for the Zn(II)/L1 system at M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=6.38.

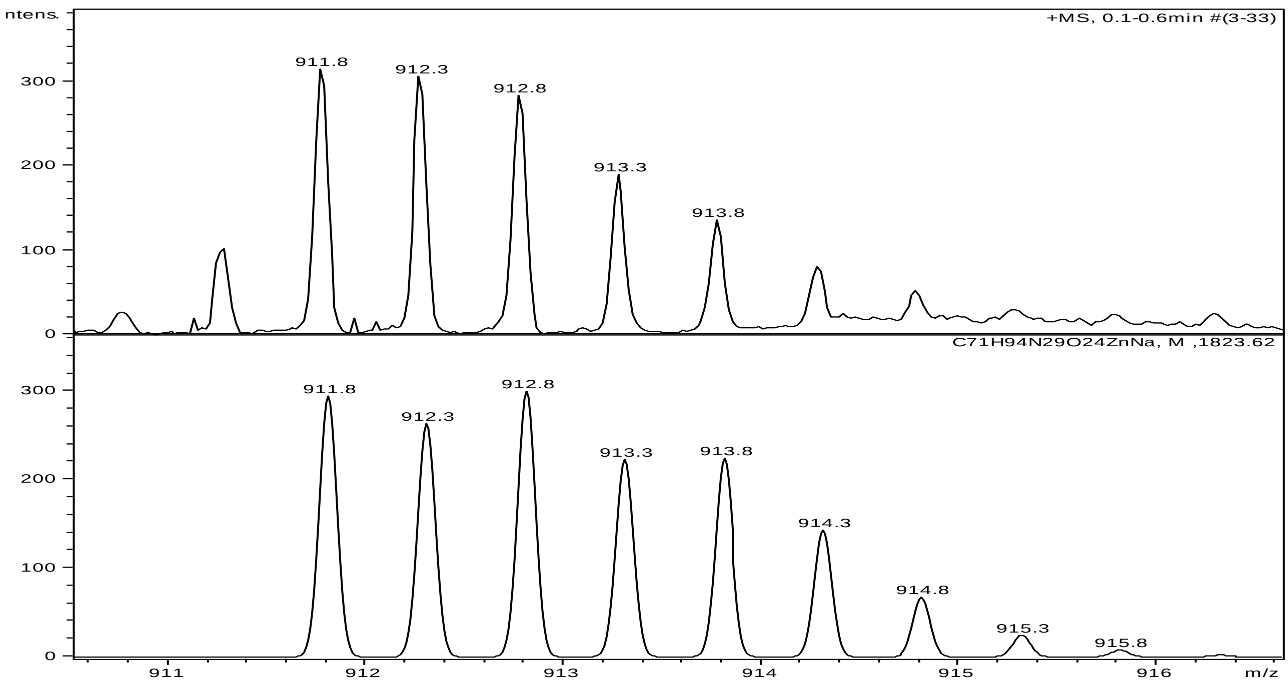

Figure S5. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ([ZnL] · $Na)^{2+}$ in the system Zn(II)/L1; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=6.38.

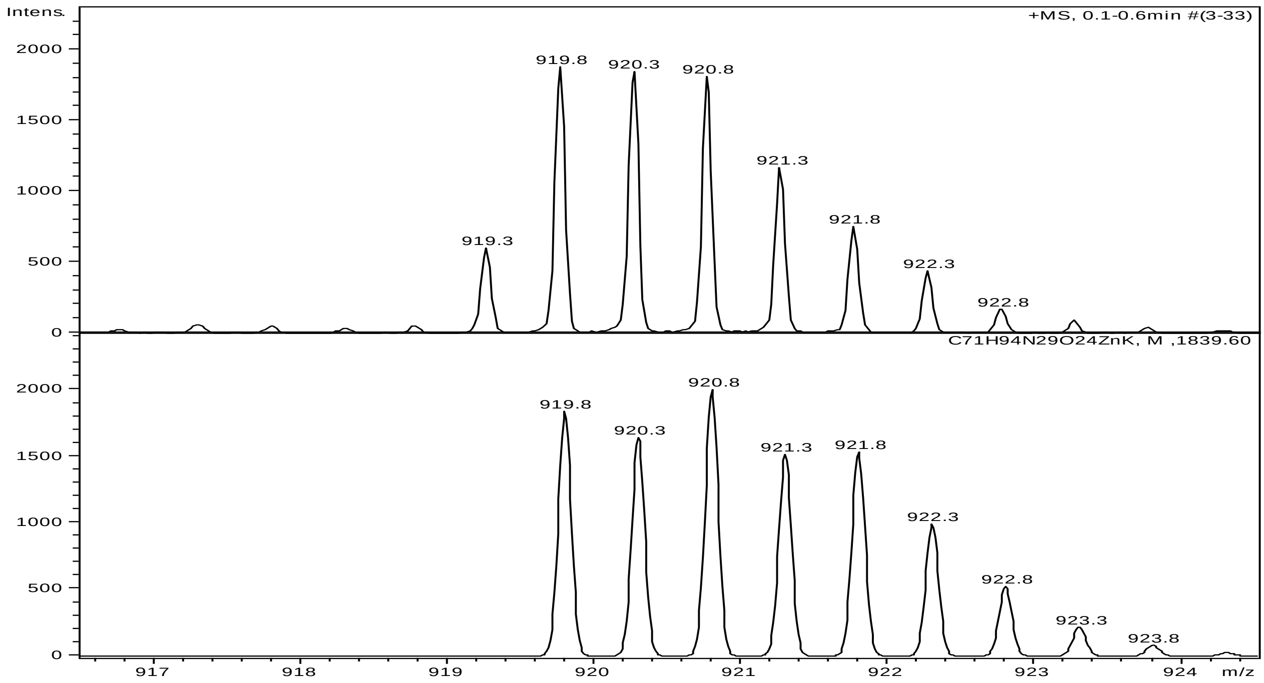

Figure S6. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ([ZnL] · K)$^{2+}$ in the system Zn(II)/L1; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=6.38.

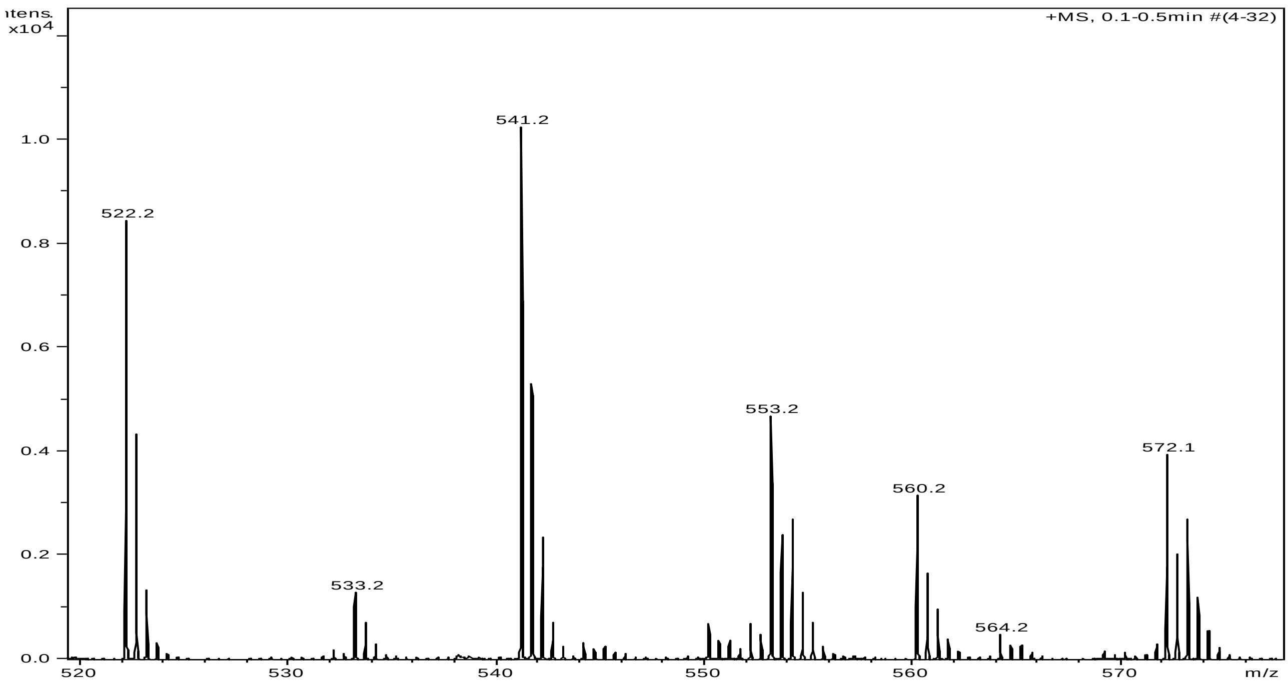

Figure S7. ESI-MS spectrum for the Zn(II)/L2 system at M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=7.04.

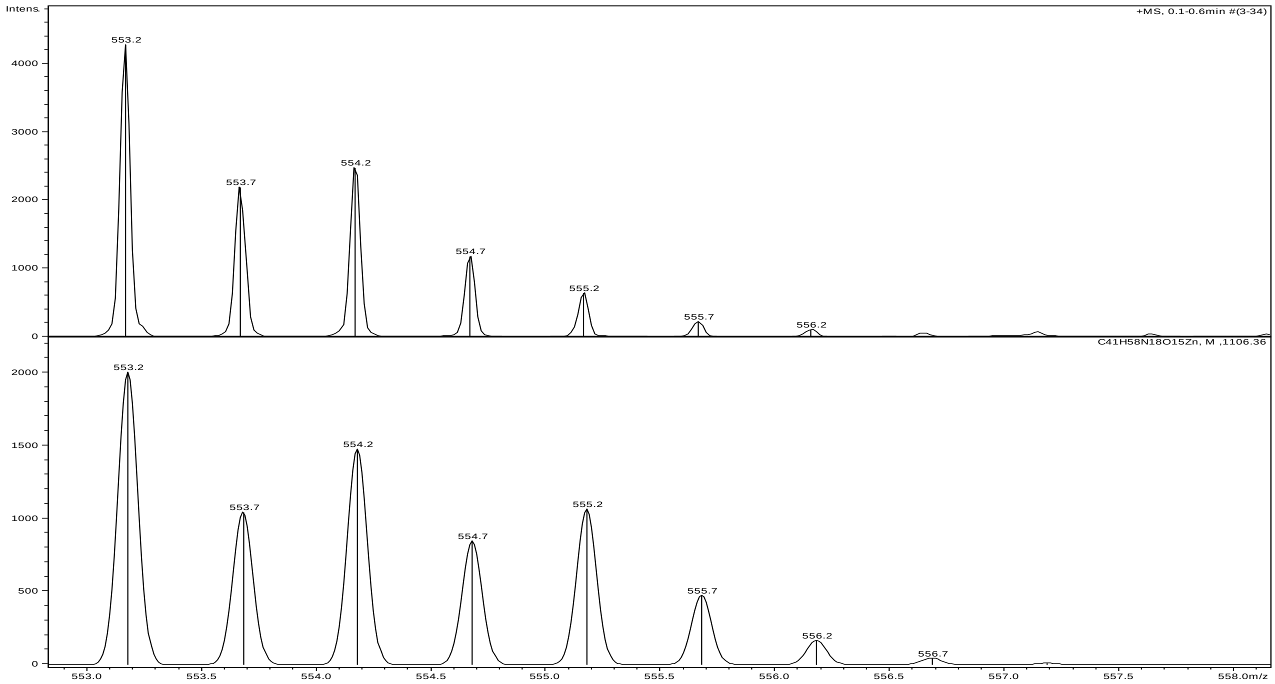

Figure S8. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species $[ZnL]^{2+}$ in the system Zn(II)/L2; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=7.04.

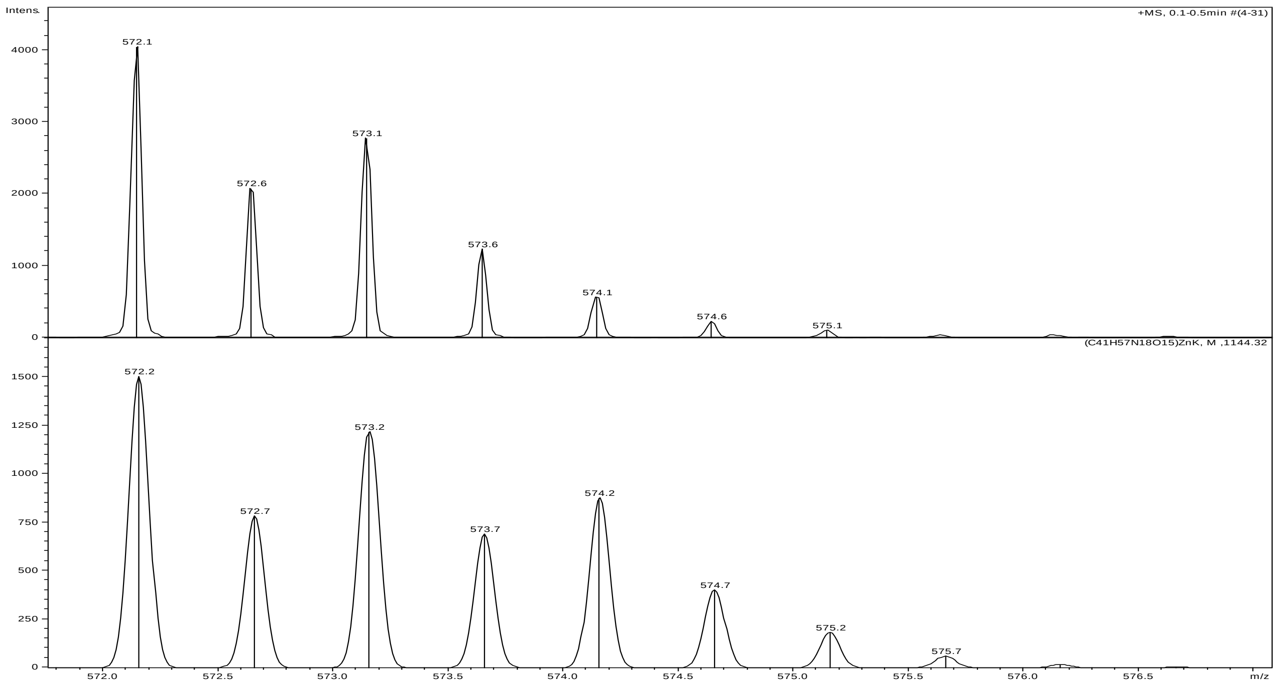

Figure S9. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species $([ZnH_{-1}L]\cdot K)^{2+}$ in the system Zn(II)/L2; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=7.04.

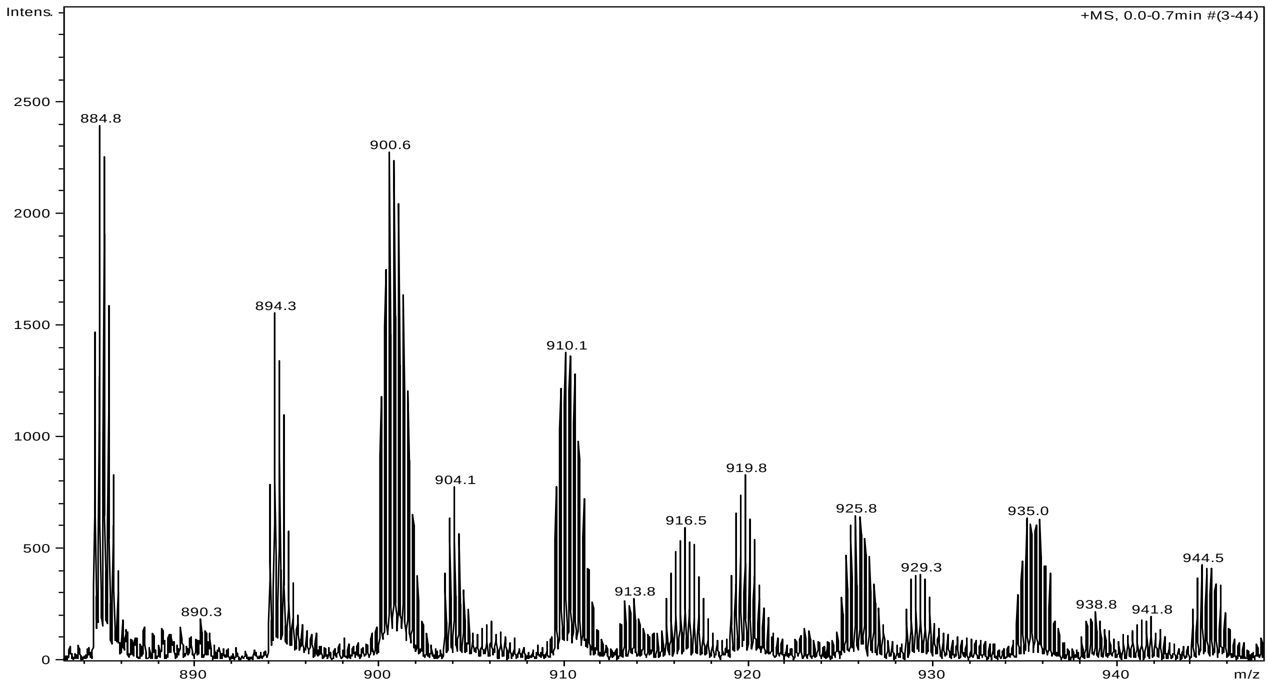

Figure S10. ESI-MS spectrum for the Zn(II)/L3 system at M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.30.

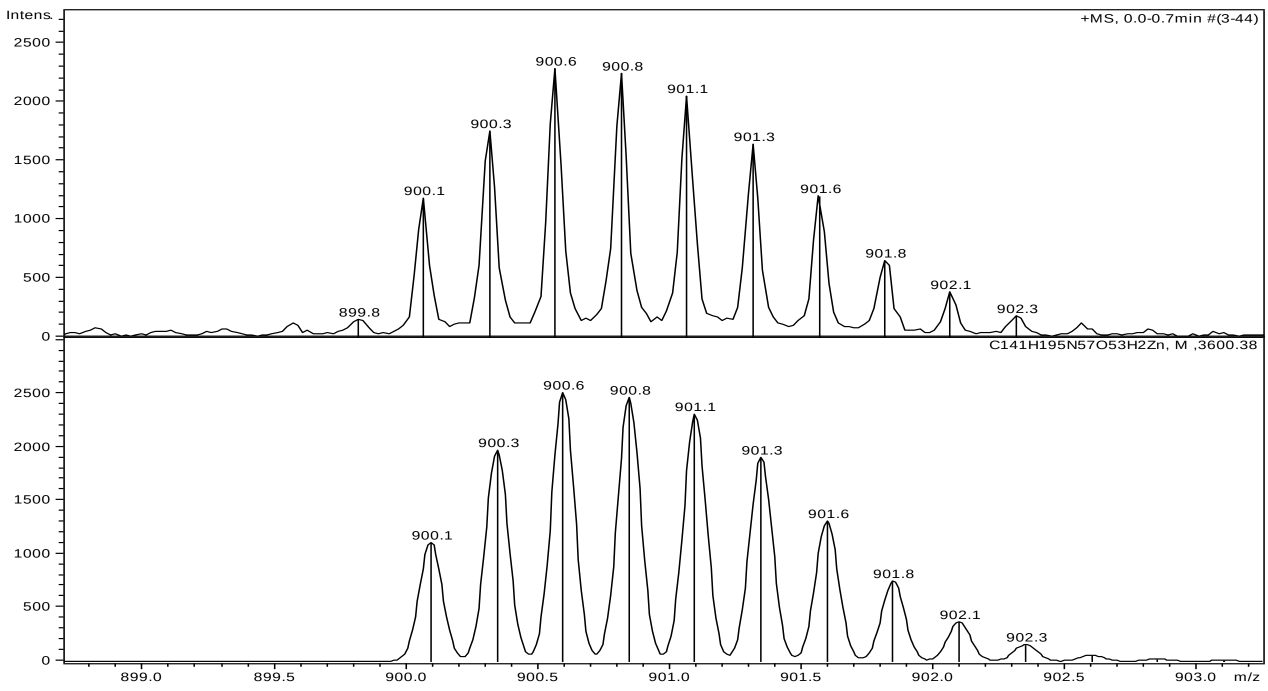

Figure S11. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species $[ZnH_5L]^{4+}$ in the system Zn(II)/L3; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.30.

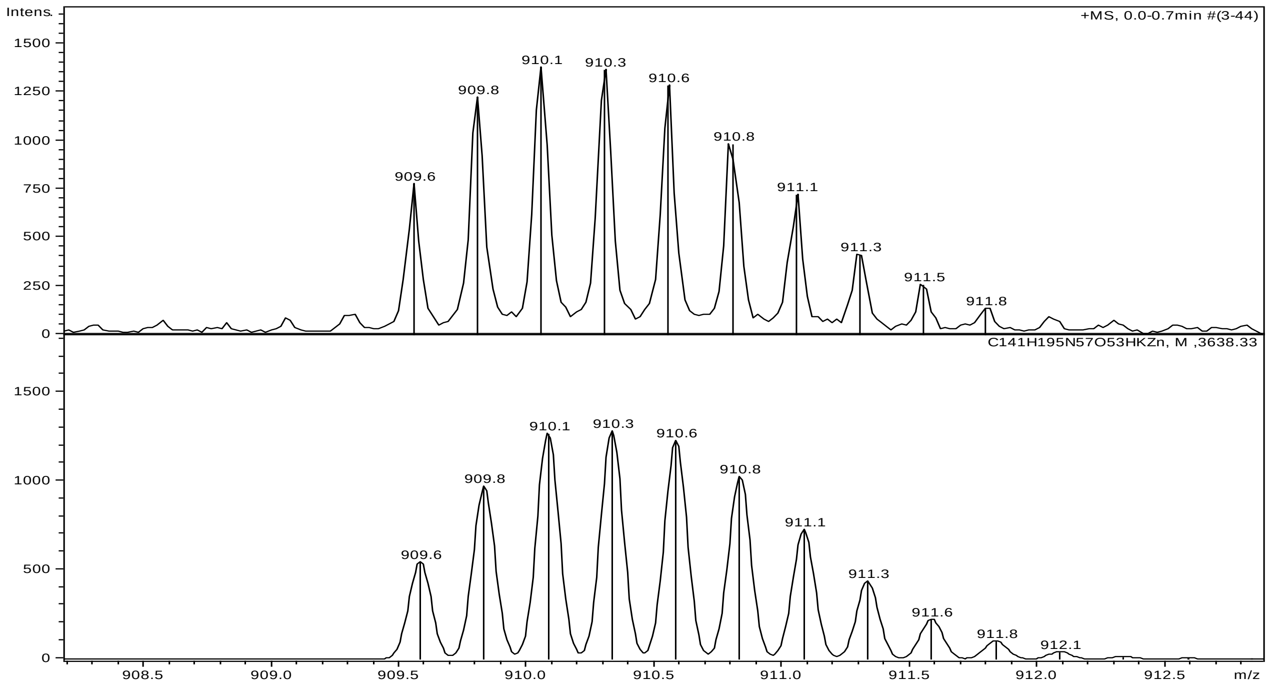

Figure S12. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ([ZnH_4L] · K)$^{4+}$ in the system Zn(II)/L3; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.30.

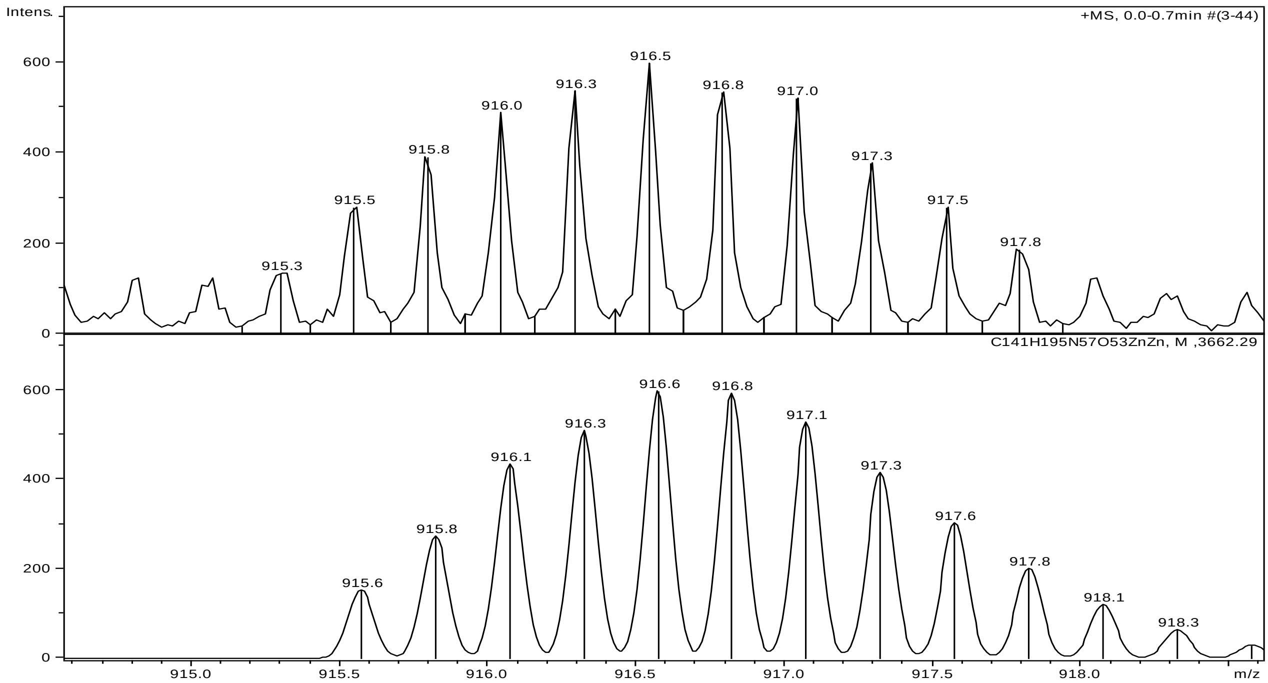

Figure S13. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species [Zn_2H_3L]$^{4+}$ in the system Zn(II)/L3; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.30.

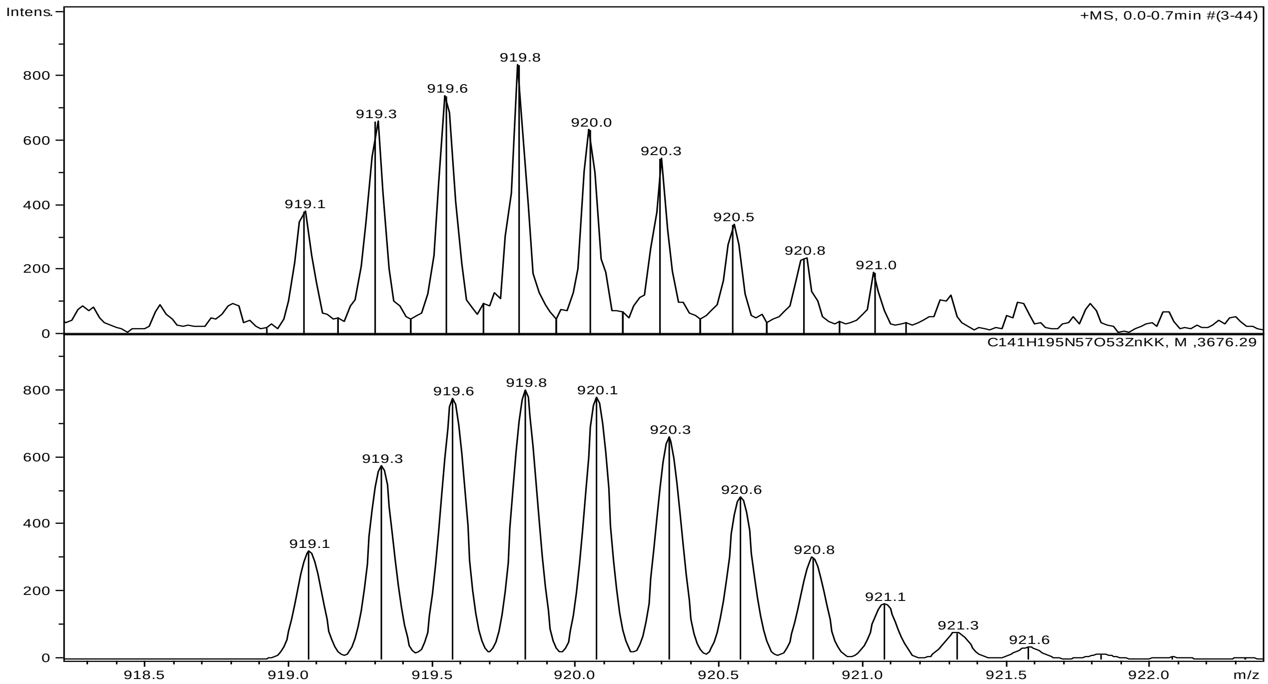

Figure S14. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ($[ZnH_3L] \cdot K_2)^{4+}$ in the system Zn(II)/L3; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.30.

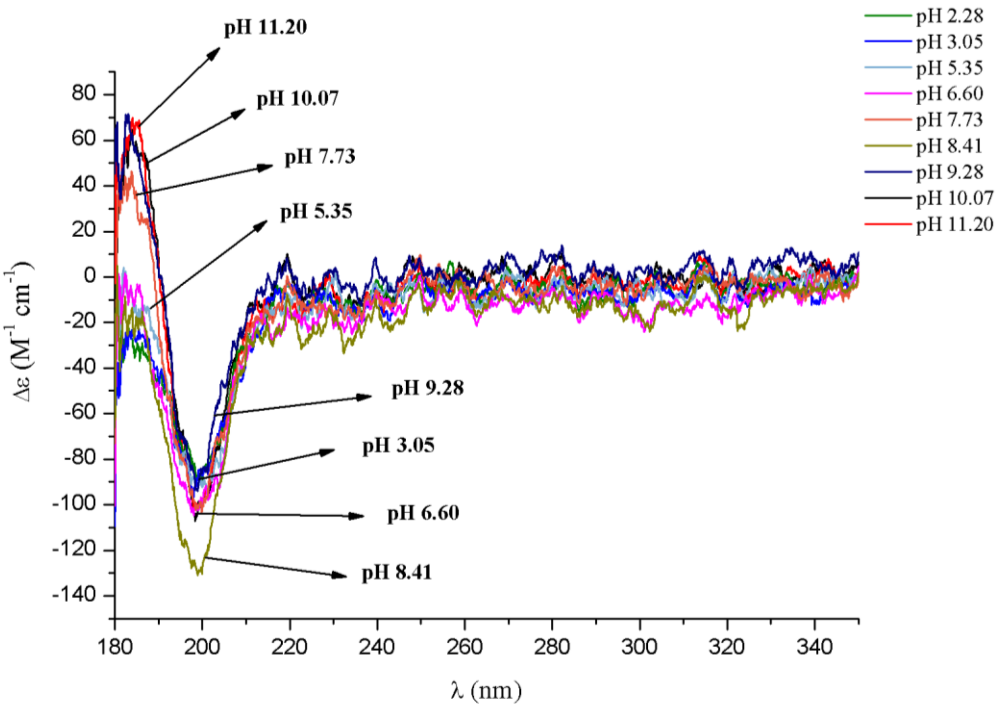

Figure S15. CD spectra [180 – 350 nm; optical path 0.01 cm] for the Zn(II)/L3 system. M:L ratio=1:1.25.

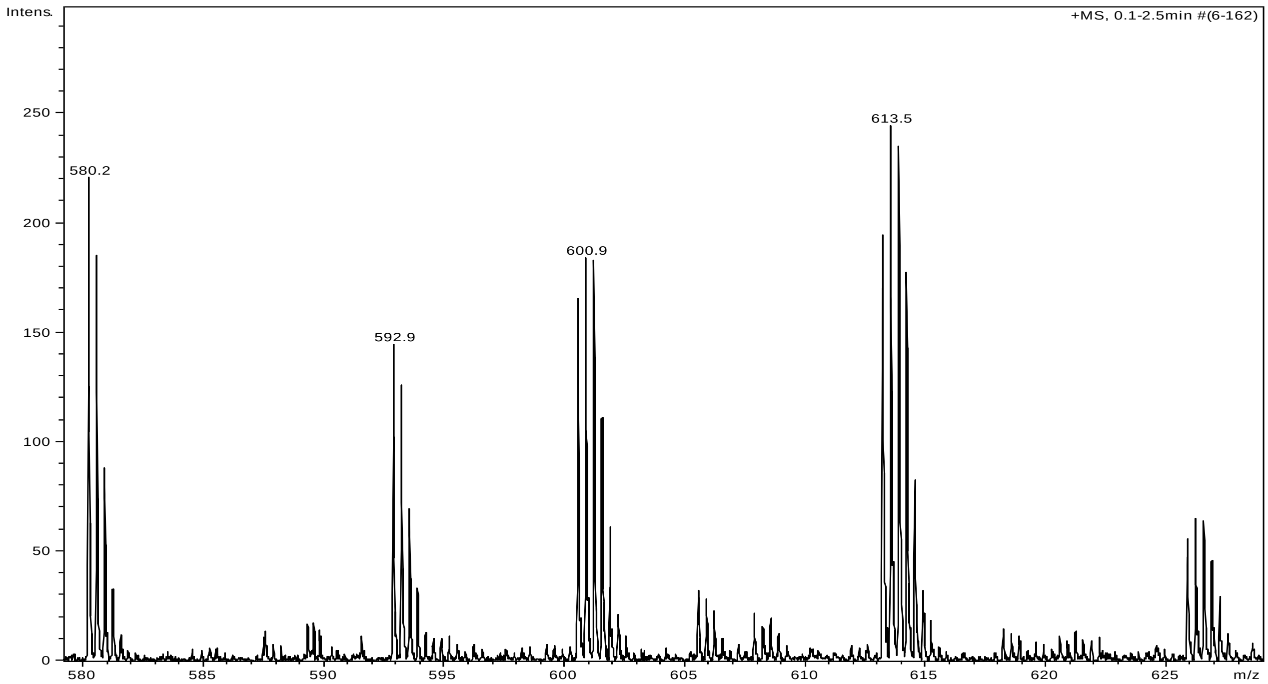

Figure S16. ESI-MS spectrum for the Cu(II)/L1 system at M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.97.

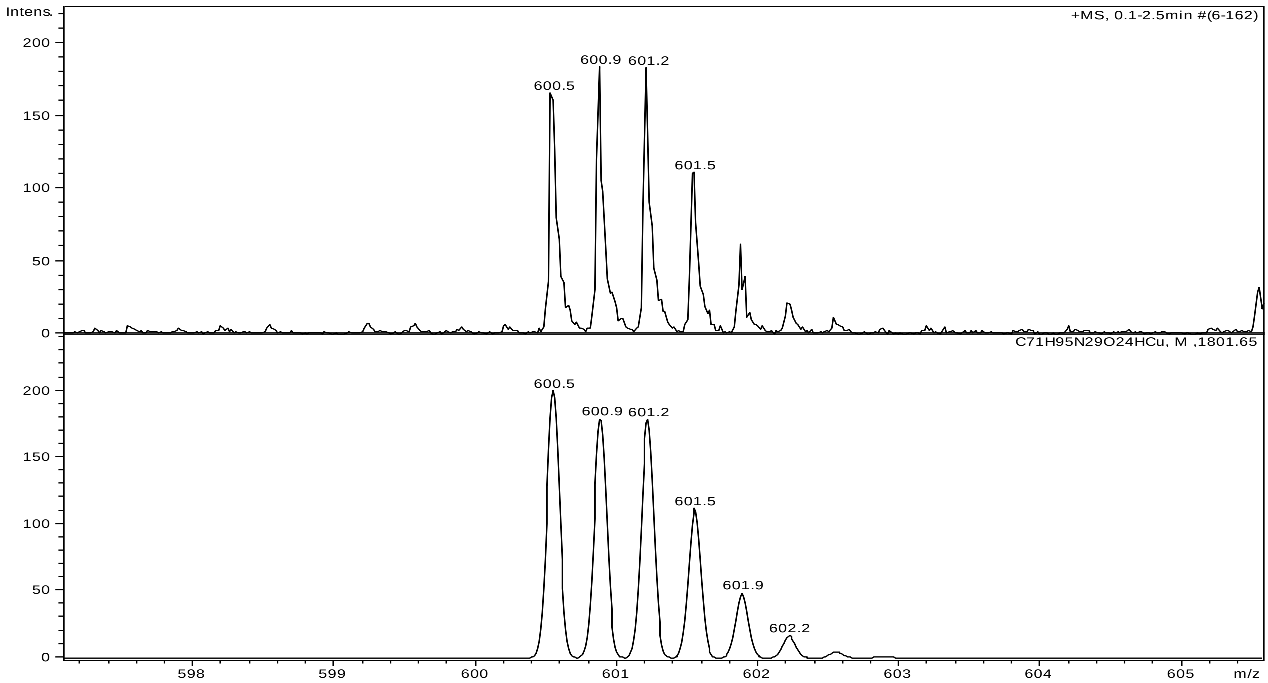

Figure S17. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species $[CuH_2L]^{3+}$ in the system Cu(II)/L1; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.97.

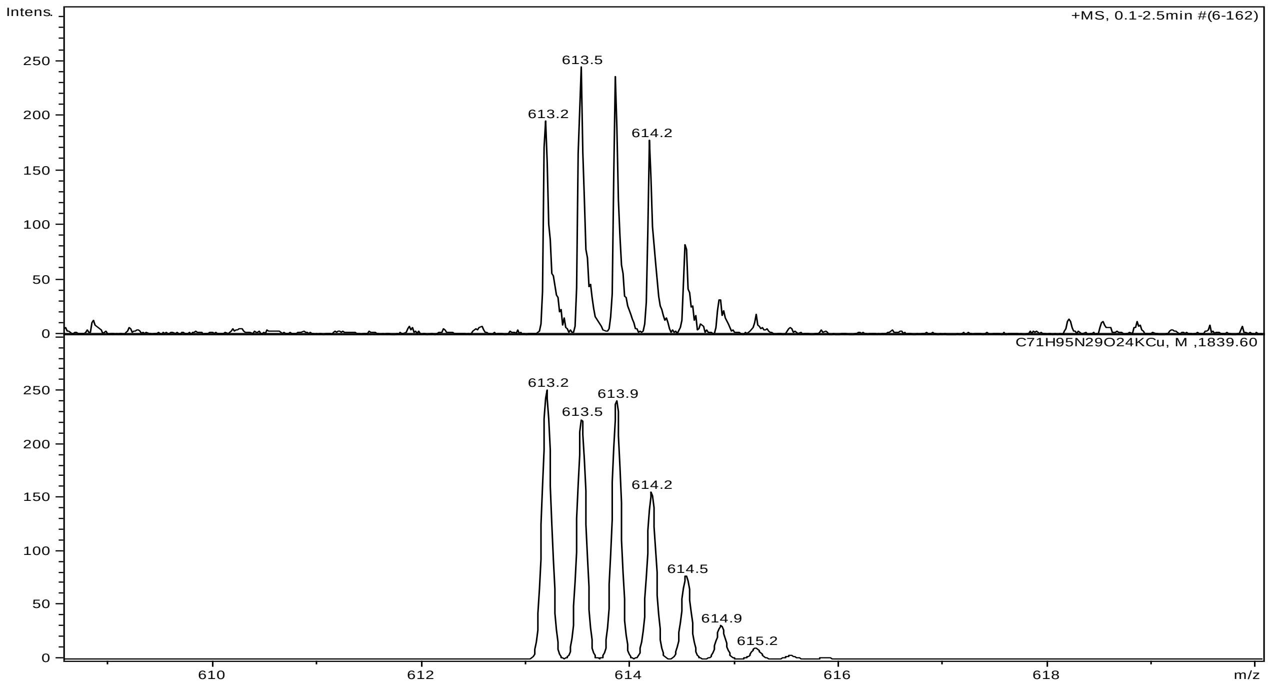

Figure S18. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ([CuHL] · K)$^{3+}$ in the system Cu(II)/L1; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.97.

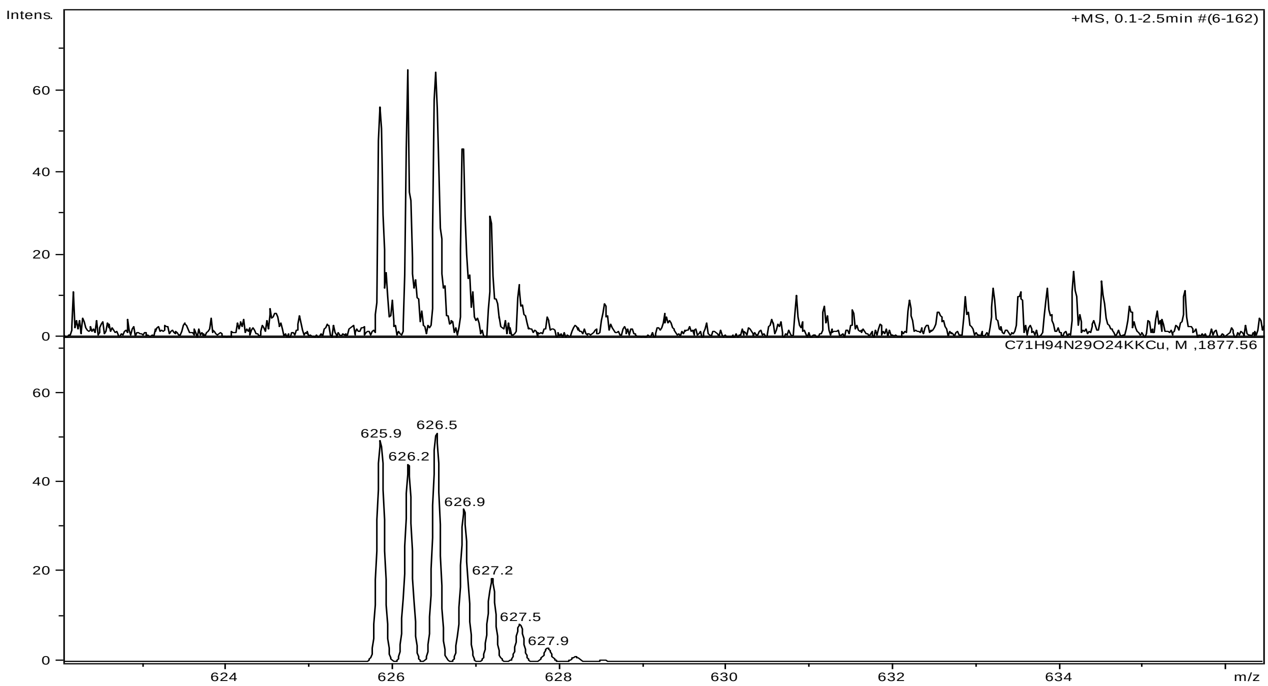

Figure S19. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ([CuL] · K$_2$)$^{3+}$ in the system Cu(II)/L1; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.97.

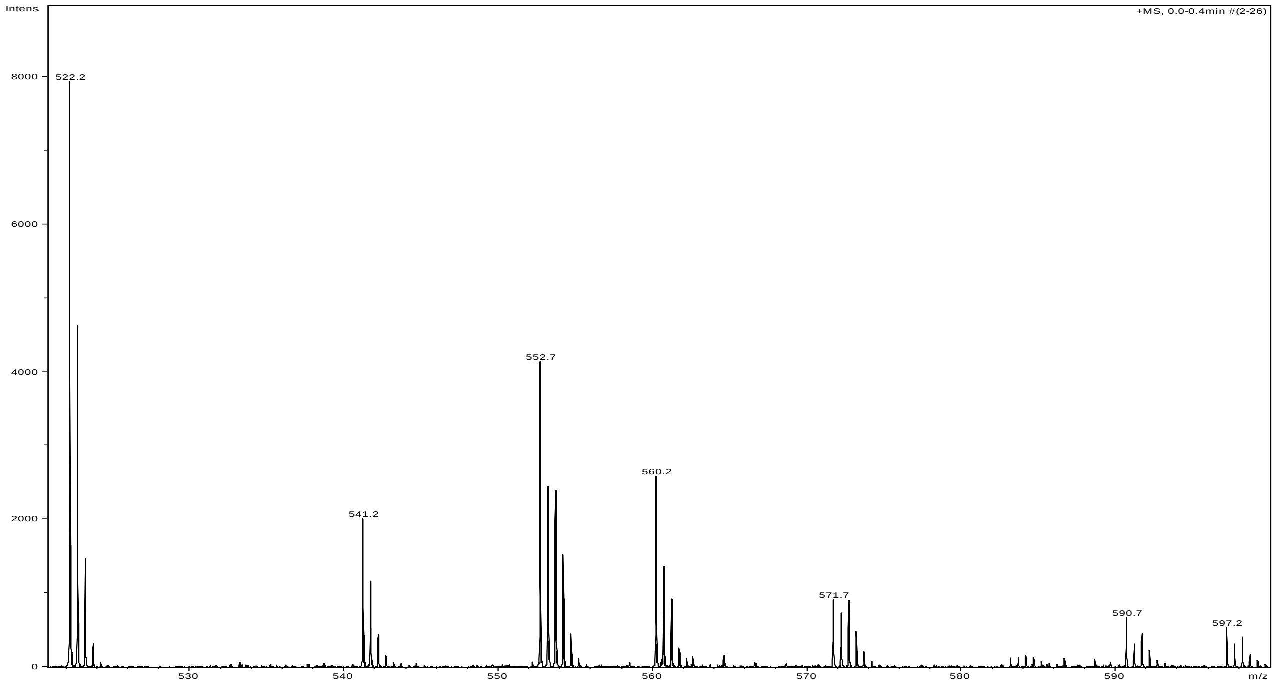

Figure S20. ESI-MS spectrum for the Cu(II)/L2 system at M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.62.

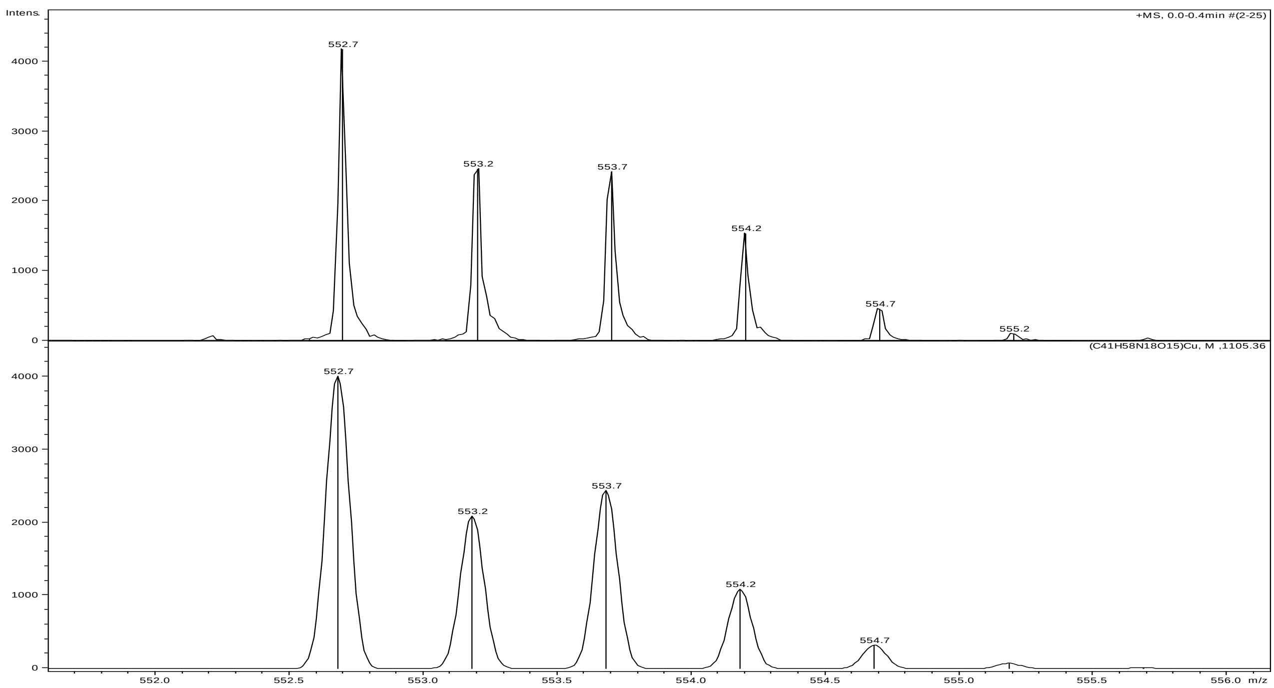

Figure S21. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species $[CuL]^{2+}$ in the system Cu(II)/L2; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.62.

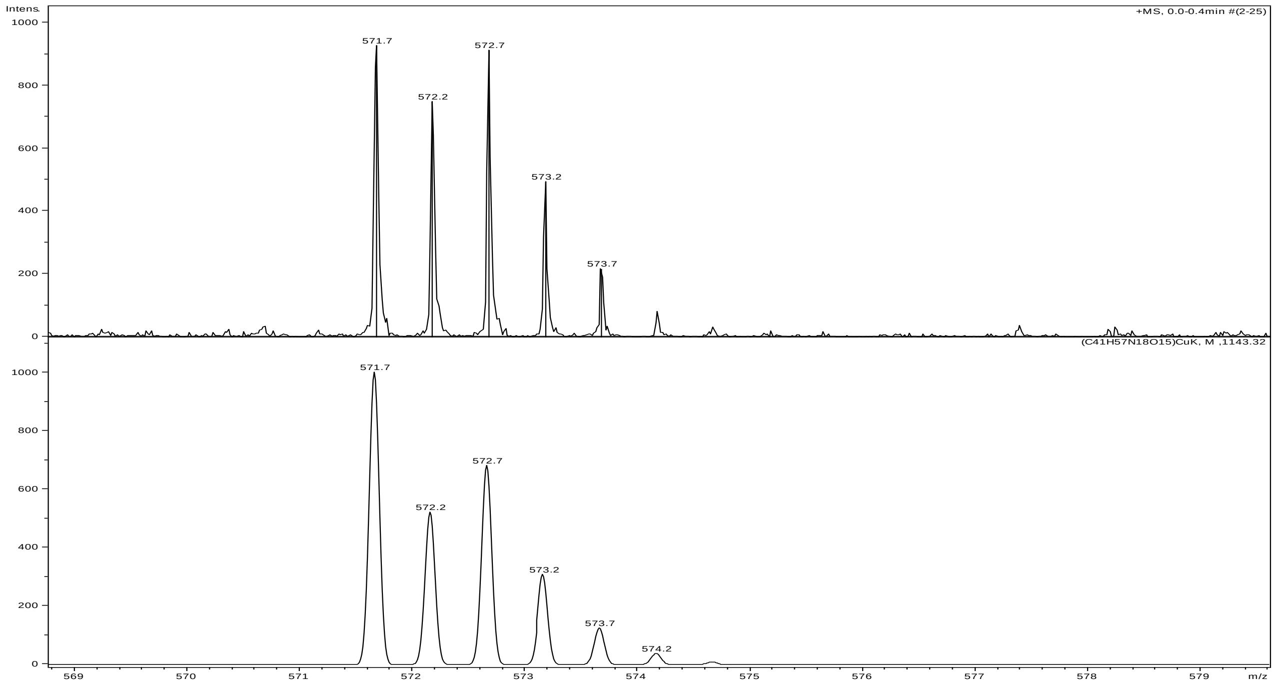

Figure S22. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ($[CuH_{-1}L] \cdot K)^{2+}$ in the system Cu(II)/L2; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.62.

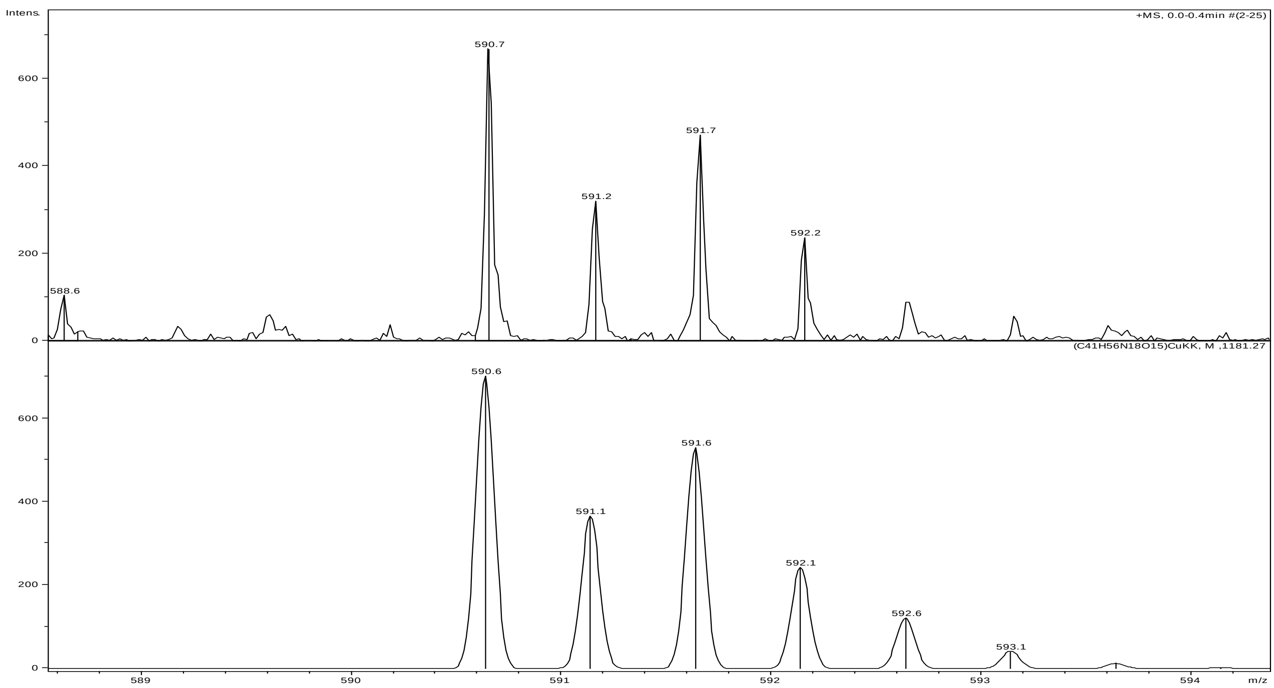

Figure S23. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species ($[CuH_{-2}L] \cdot K_2)^{2+}$ in the system Cu(II)/L2; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.62.

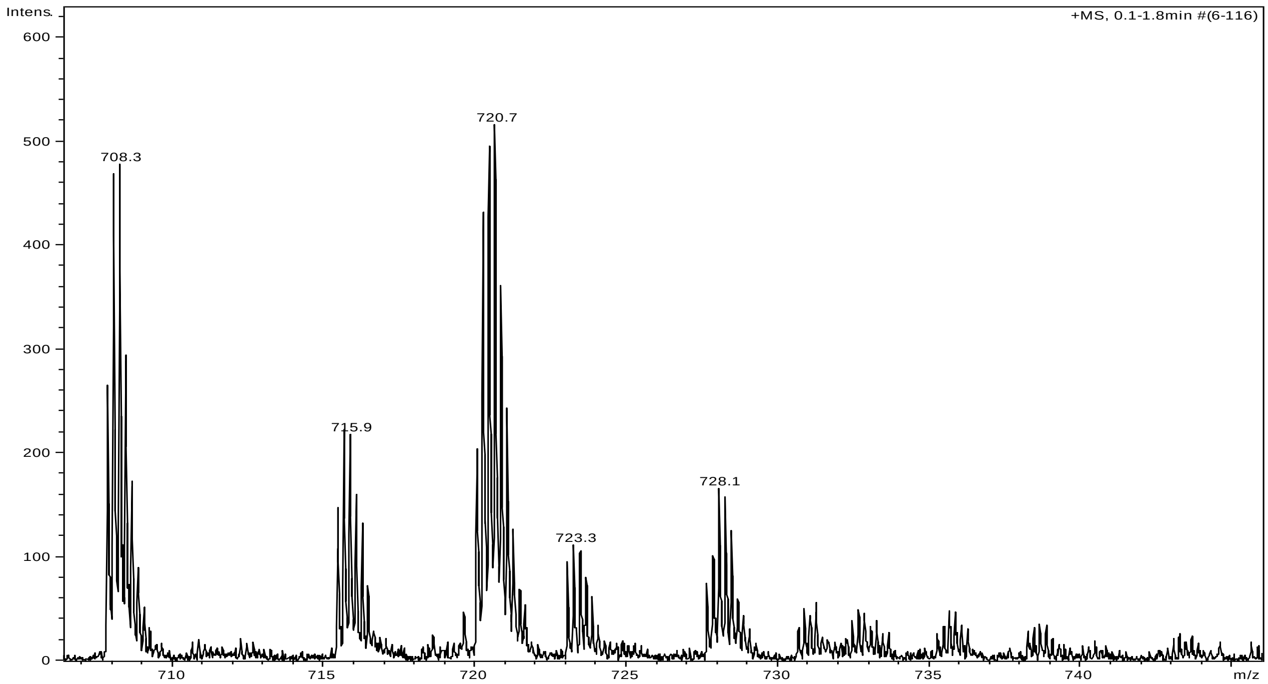

Figure S24. ESI-MS spectrum for the Cu(II)/L3 system at M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.74.

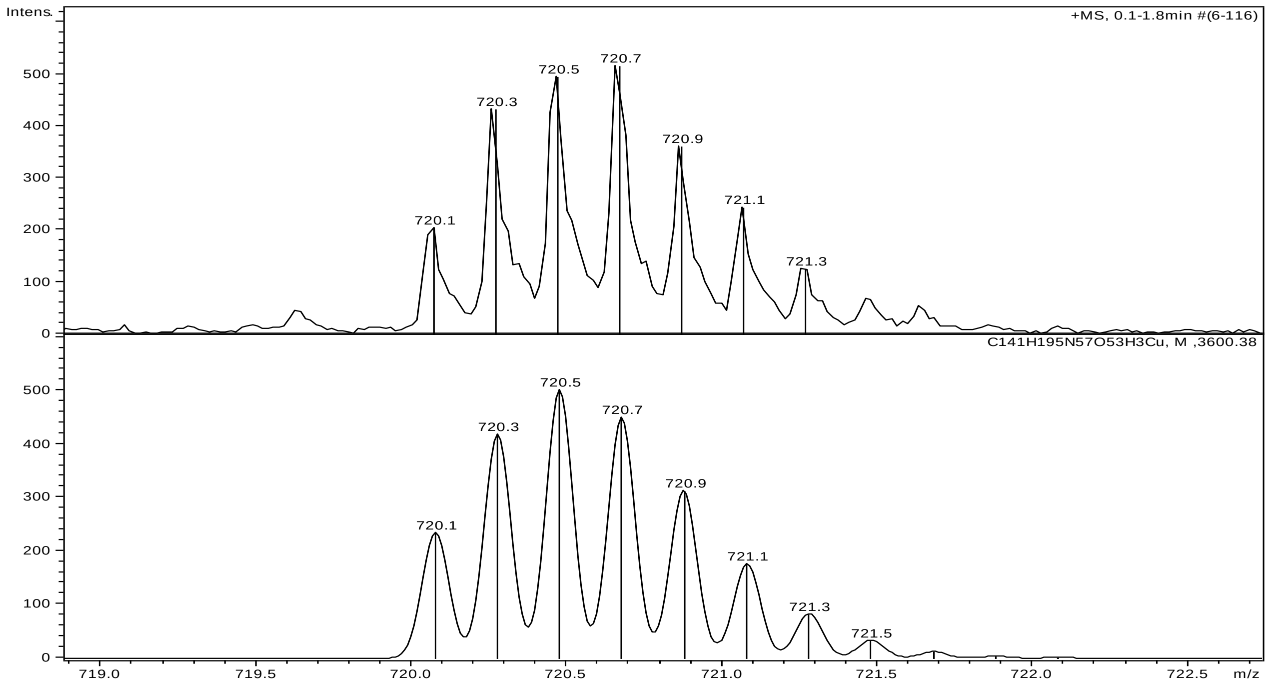

Figure S25. Experimental ESI-MS spectrum (upper trace) and simulated pattern (lower trace) for the species $[CuH_6L]^{5+}$ in the system Cu(II)/L3; M:L molar ratio=1:1.25 in water/acetonitrile 50:50 solution at pH=5.74.

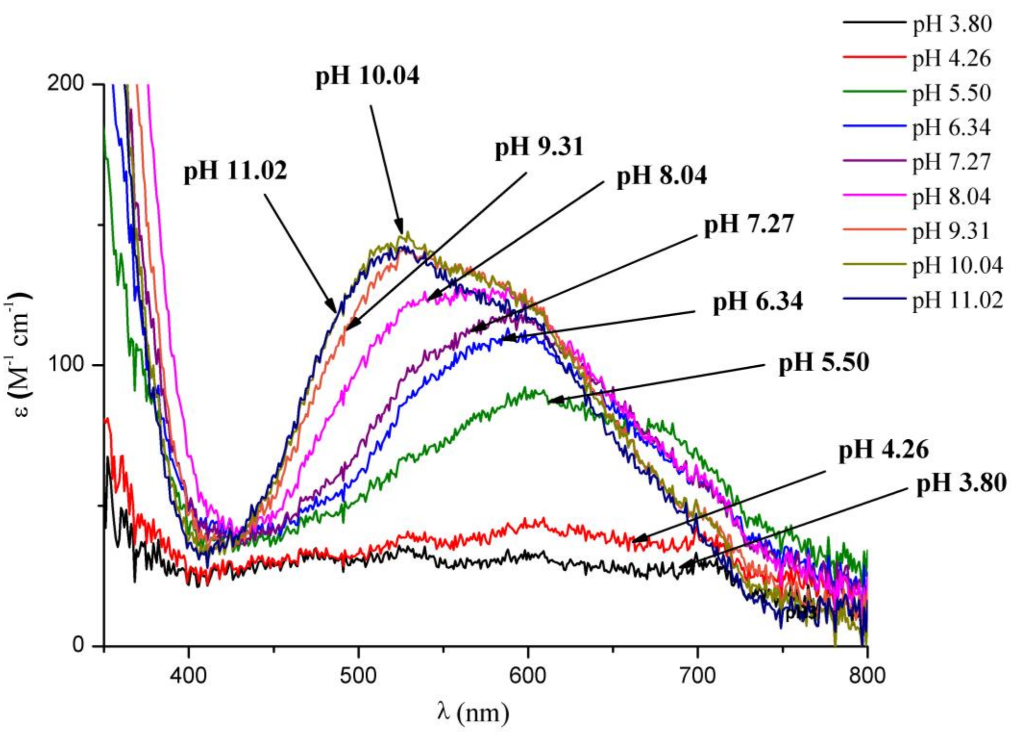

Figure S26. Vis absorption spectra at variable pH for Cu(II) complexes with L1, at *T*=298 K and *I*=0.1 mol dm^{-3} ($NaClO_4$); M:L ratio = 1:1.25.

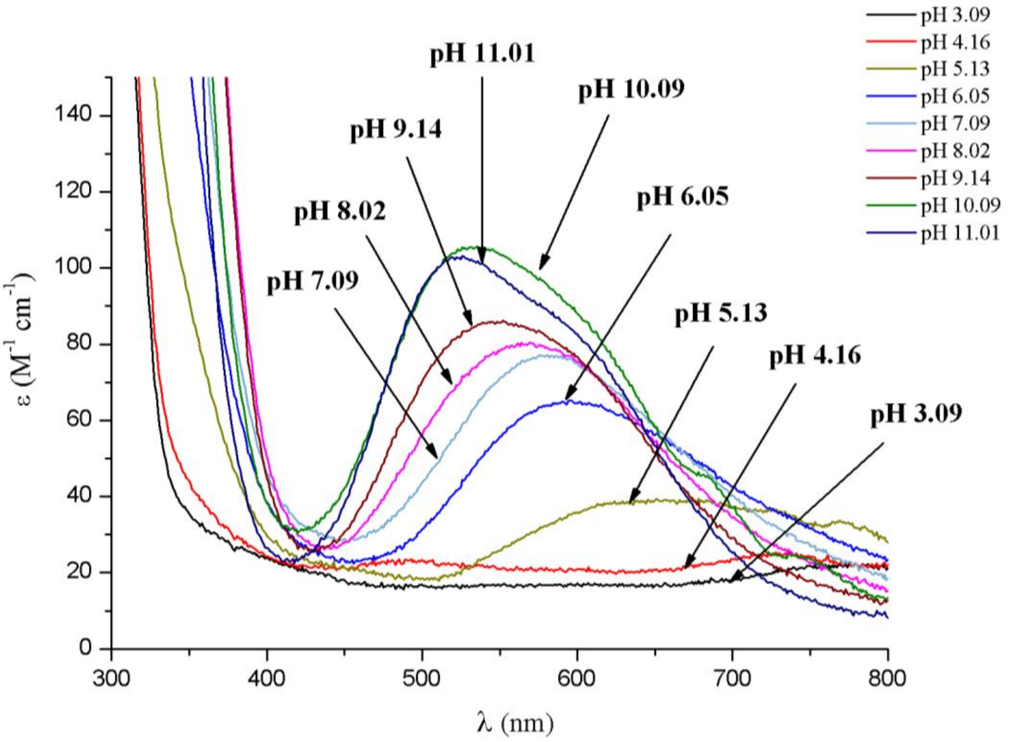

Figure S27. Vis absorption spectra at variable pH for Cu(II) complexes with L2, at *T*=298 K and *I*=0.1 mol dm^{-3} ($NaClO_4$); M:L ratio = 1:1.25.

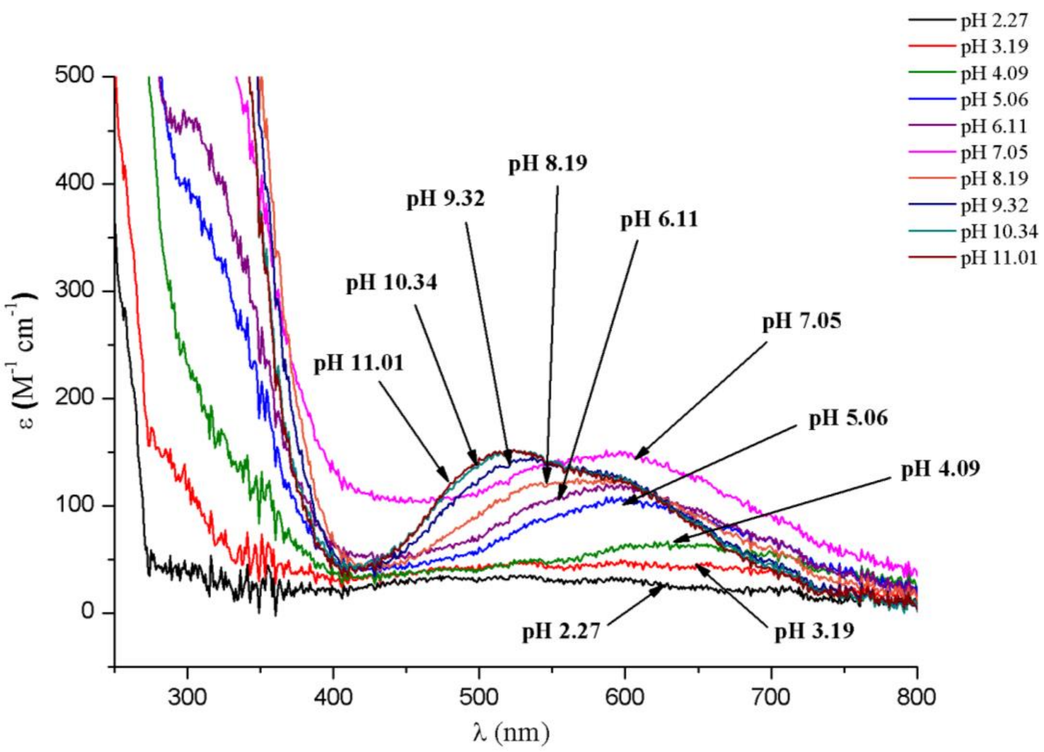

Figure S28. Vis absorption spectra at variable pH for Cu(II) complexes with L3, at T=298 K and I=0.1 mol dm^{-3} ($NaClO_4$); M:L ratio = 1:1.25.

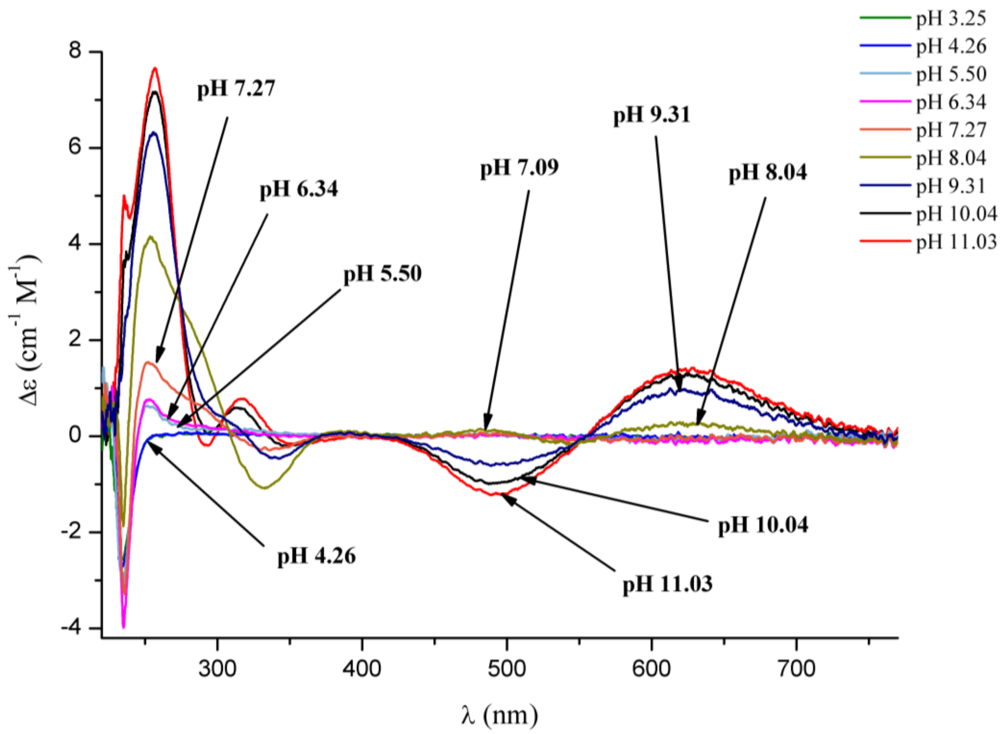

Figure S29. CD spectra [220 – 800 nm; optical path 1 cm] at variable pH for Cu(II) complexes with L1, at T=298 K and I=0.1 mol dm^{-3} ($NaClO_4$); M:L ratio = 1:1.25.

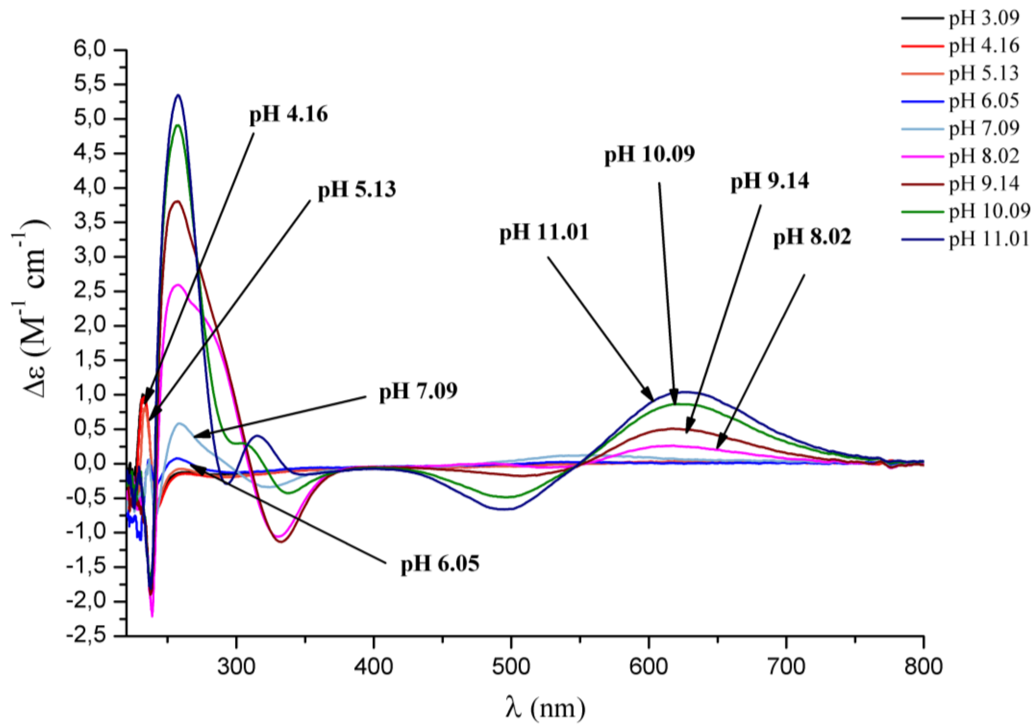

Figure S30. CD spectra [220 – 800 nm; optical path 1 cm] at variable pH for Cu(II) complexes with L2, at *T*=298 K and *I*=0.1 mol dm^{-3} ($NaClO_4$); M:L ratio = 1:1.25.

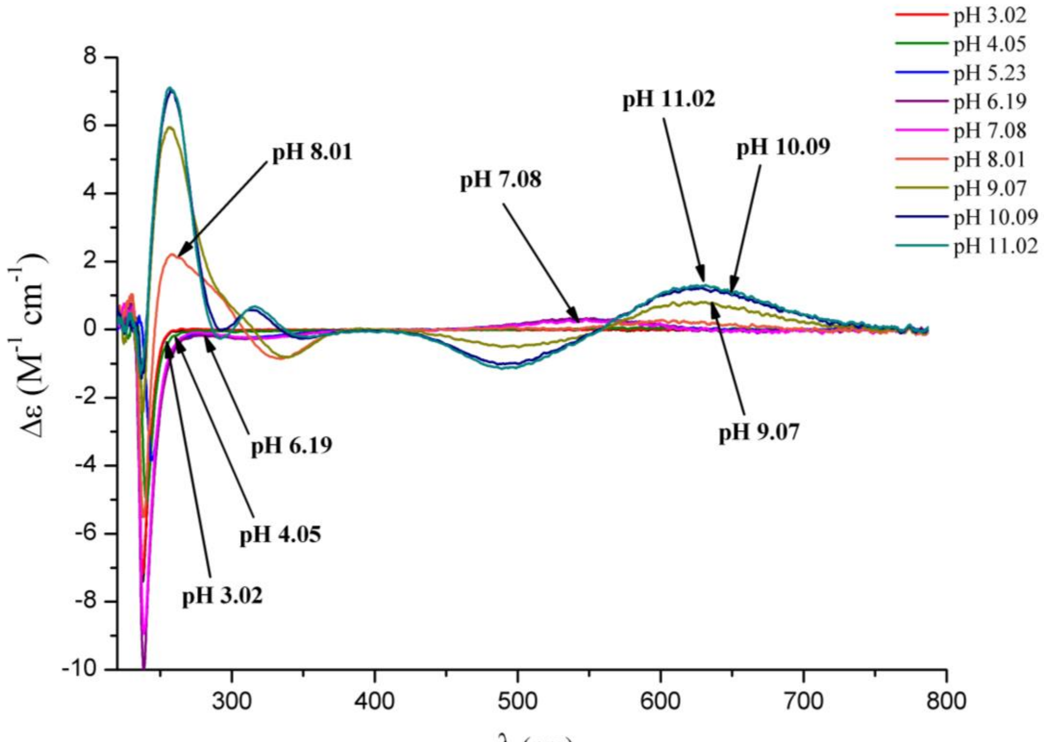

Figure S31. CD spectra [220 – 800 nm; optical path 1 cm] at variable pH for Cu(II) complexes with L3, at *T*=298 K and *I*=0.1 mol dm^{-3} ($NaClO_4$); M:L ratio = 1:1.25.

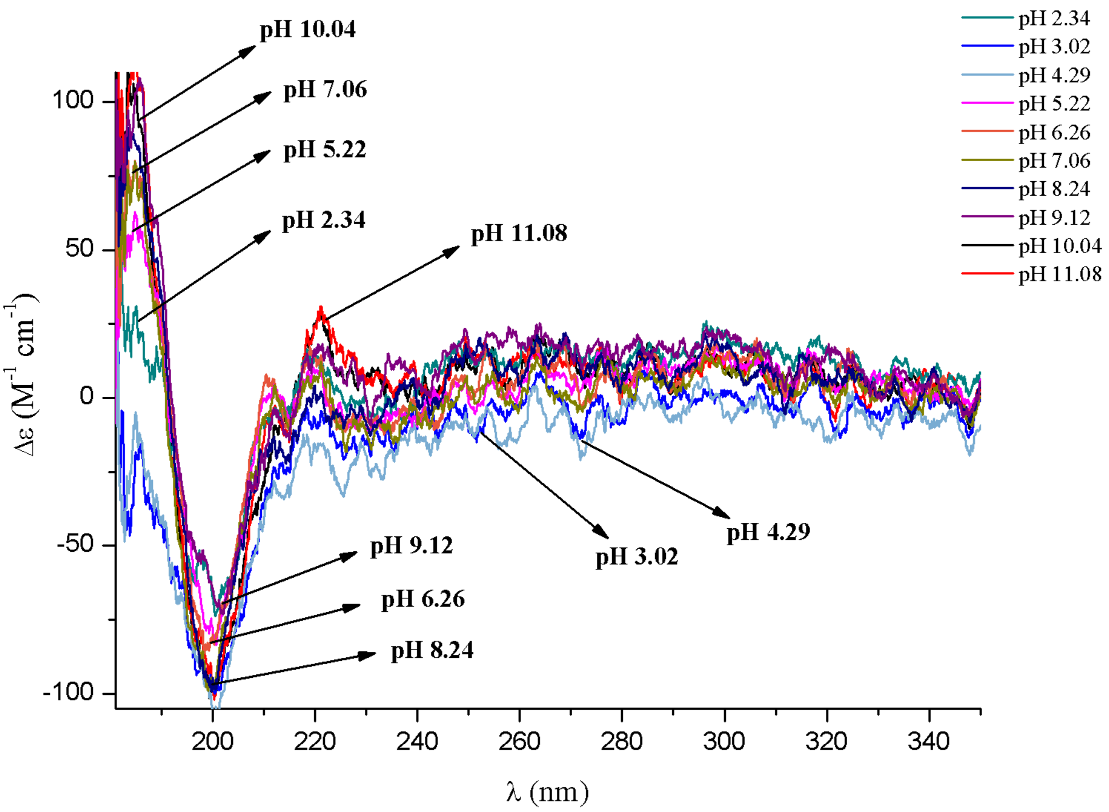

Figure S32. CD spectra [180 – 350 nm; optical path 0.01 cm] at variable pH for Cu(II) complexes with L3, at T=298 K and I=0.1 mol dm^{-3} ($NaClO_4$); M:L ratio = 1:1.25.

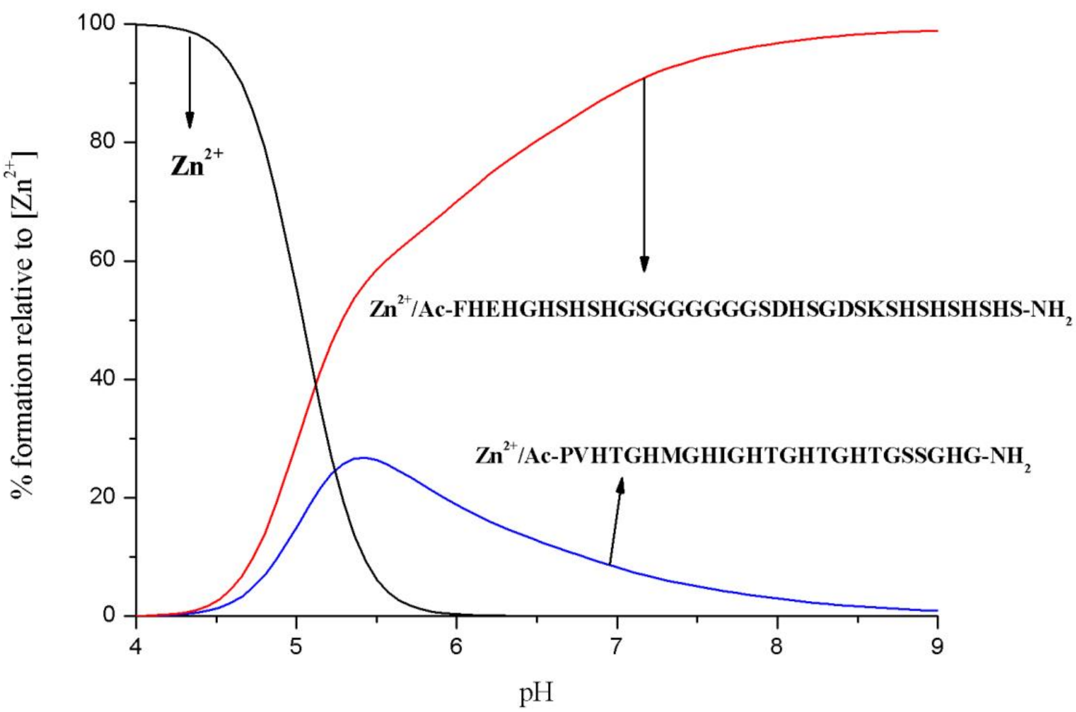

Figure S33. Competition plot for a ternary solution containing: Zn(II), L3 and Ac-PVHTGHMGHIGHTGHTGHTGS SGHG-NH_2 (zp-PrP63-87).

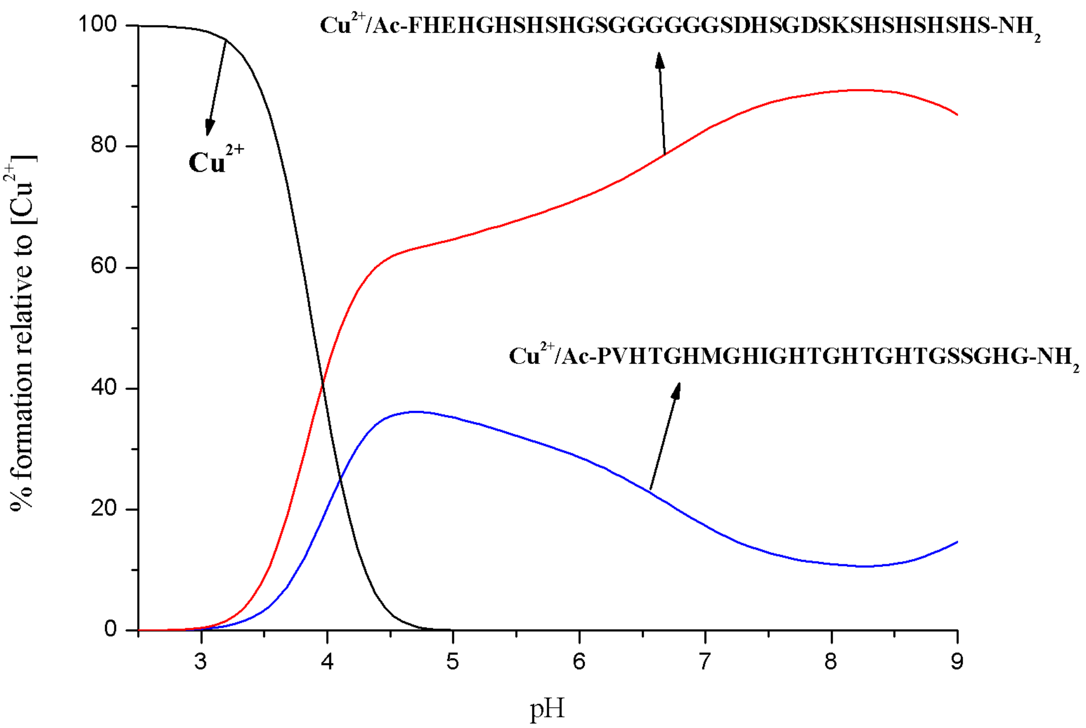

Figure S34. Competition plot for a ternary solution containing: Cu(II), L3 and Ac-PVHTGHMGHIGHTGHTGHTGS SGHG-NH_2 (zp-PrP63-87).

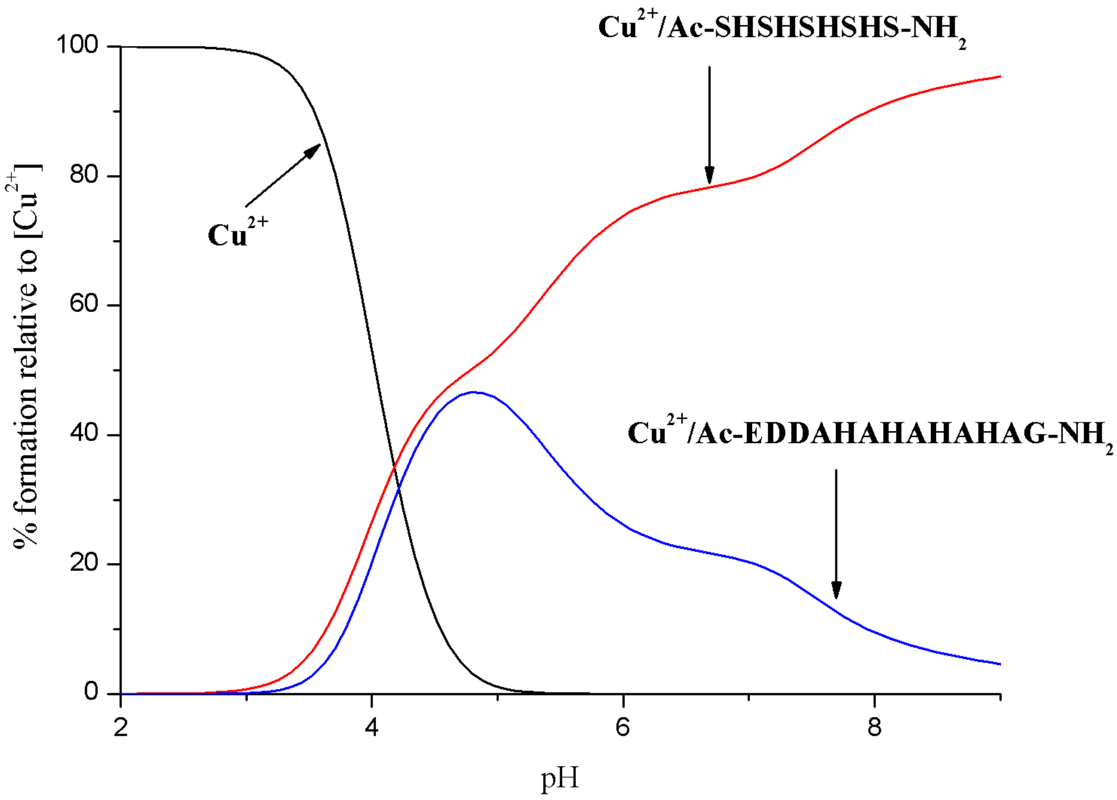

Figure S35. Competition plot for a ternary solution containing: Cu(II), L2 and Ac-EDDAHAHAHAHAG-NH_2.

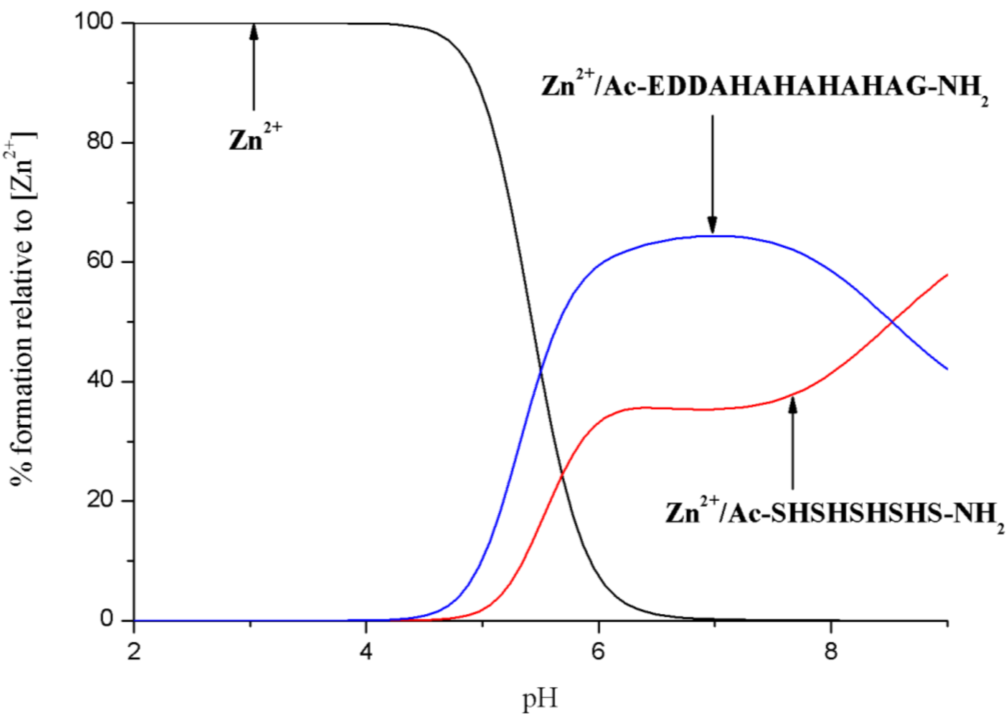

Figure S36. Competition plot for a ternary solution containing: Zn(II), L2 and Ac-EDDAHAHAHAHAG-NH_2.

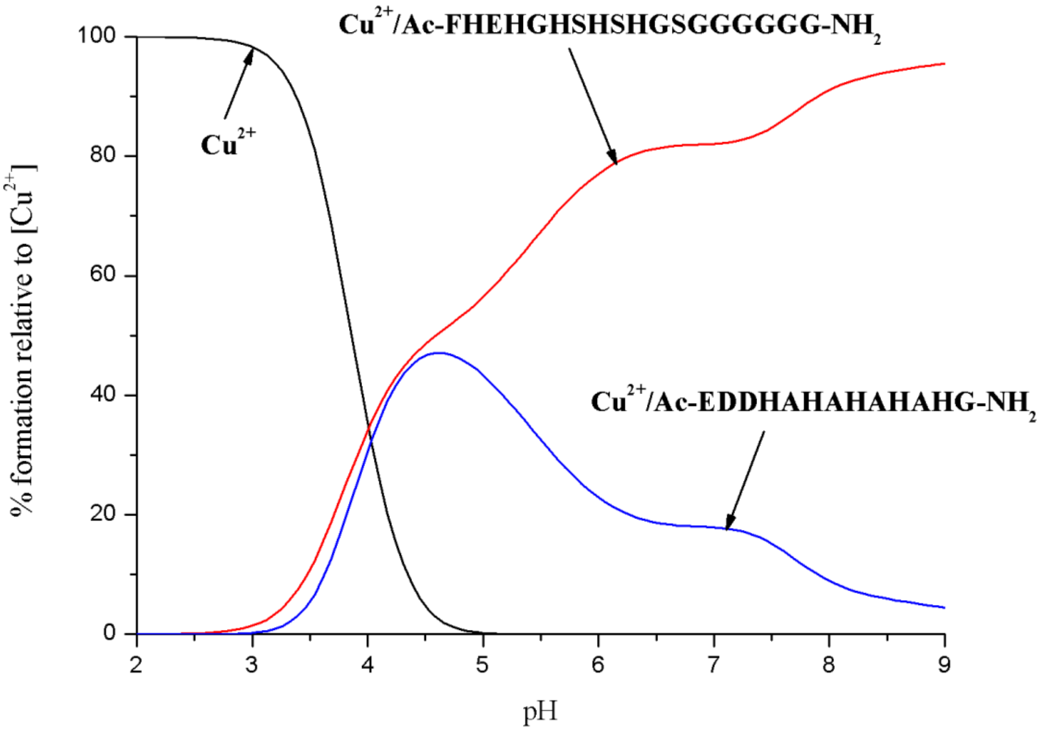

Figure S37. Competition plot for a ternary solution containing: Cu(II), L1 and Ac-EDDHAHAHAHAHG-NH_2.

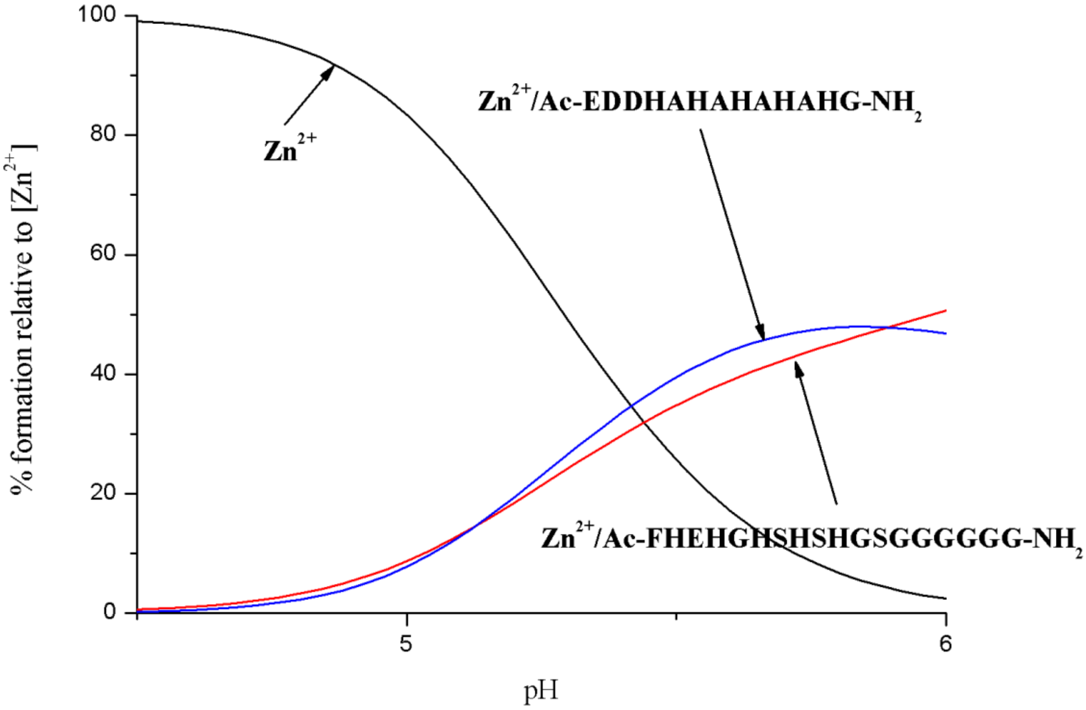

Figure S38. Competition plot for a ternary solution containing: Zn(II), L1 and Ac-EDDHAHAHAHAHG-NH_2.

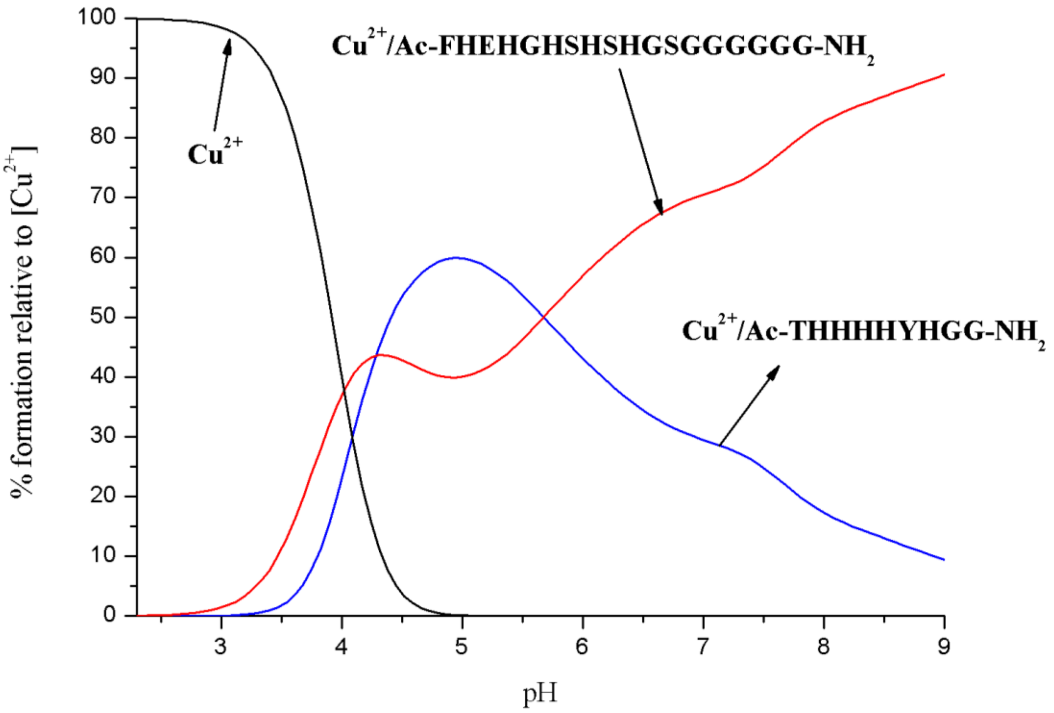

Figure S39. Competition plot for a ternary solution containing: Cu(II), L1 and Ac-THHHHYHGG-NH_2.

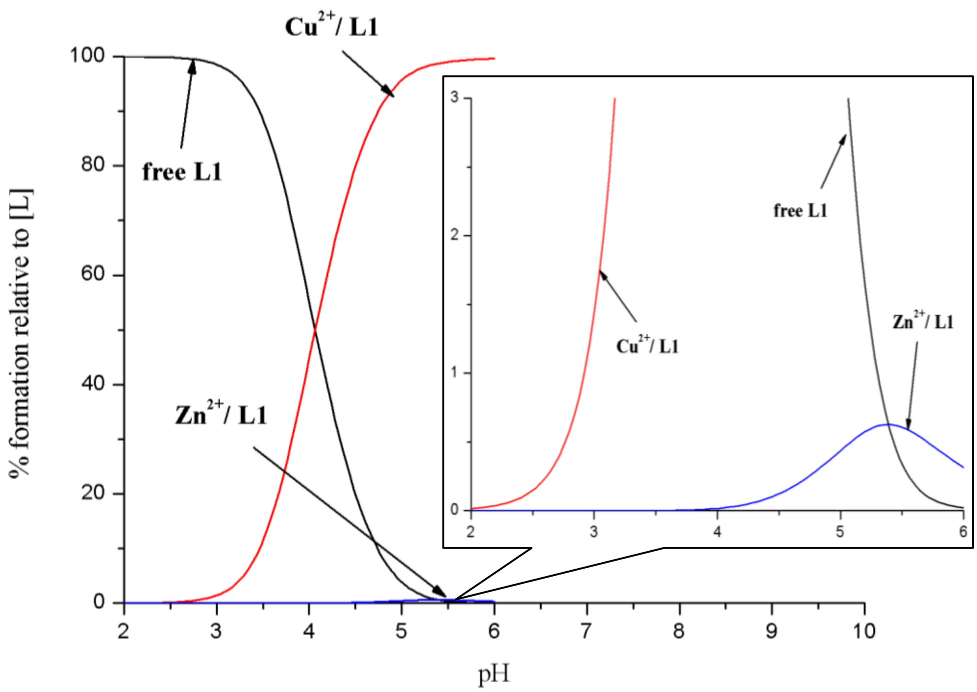

Figure S40. Competition plot for the formation of L1 complexes with Cu(II) or Zn(II).

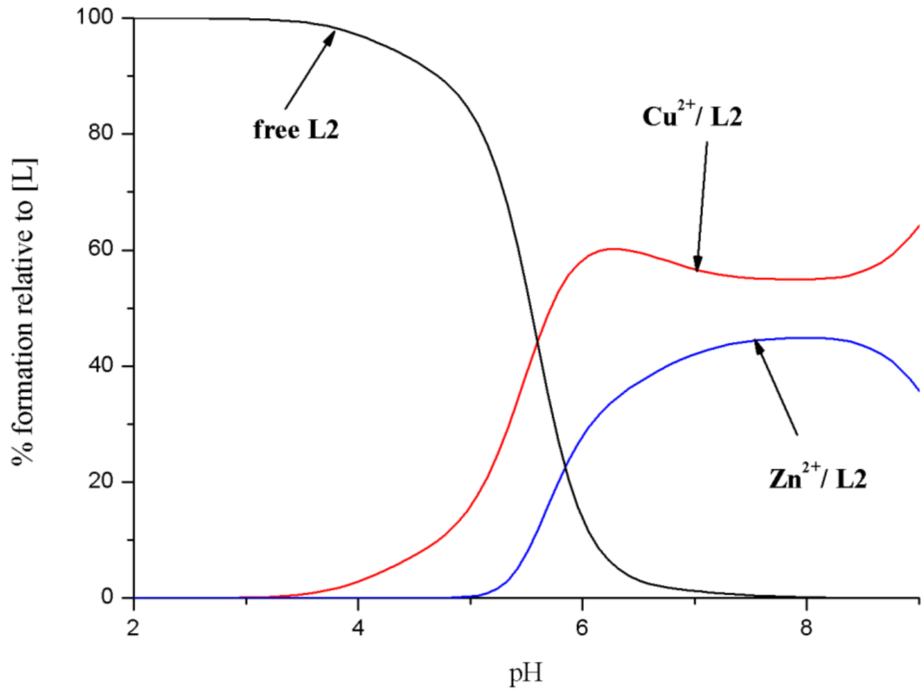

Figure S41. Competition plot for the formation of L2 complexes with Cu(II) or Zn(II).

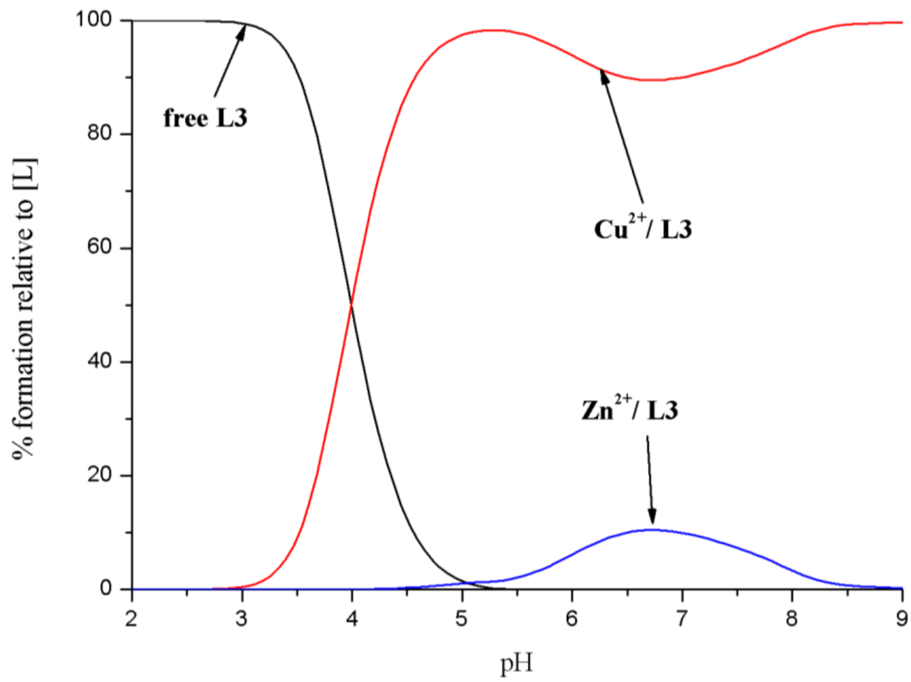

Figure S42. Competition plot for the formation of L3 complexes with Cu(II) or Zn(II).

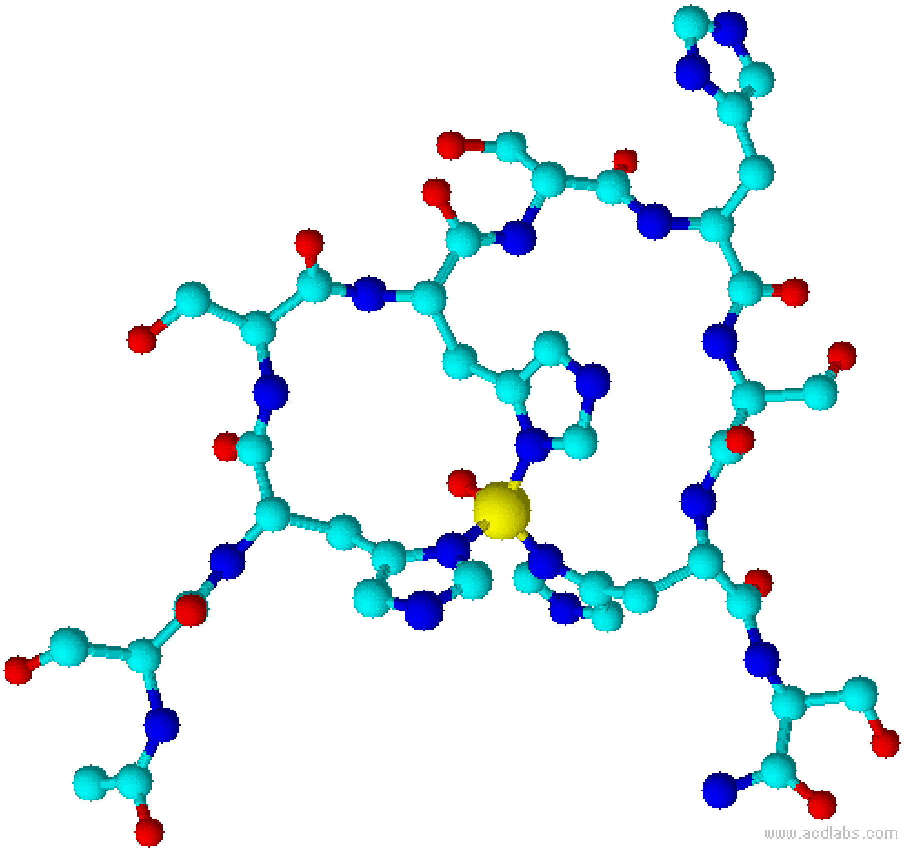

Figure S43. Proposed molecular structure of Zn(II)/Ac-SHSHSHSHS-NH_2 (L2) complexes at physiological pH. Explicit hydrogen atoms are omitted for clarity. Color code: red=oxygen, blue=nitrogen, yellow=zinc, light blue=carbon.