Electronic Supplementary Material (ESI) for Dalton Transactions.

Novel insights into the metal binding ability of ZinT periplasmic protein from *Escherichia coli* and *Salmonella enterica*

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Electronic Supplementary Information (ESI)

Table S1. Spectroscopic parameters for Cu(II)-**L1** (Ac-HGHHSH-NH_2) system at *T*=298 K, *I*=0.1 mol dm^{-3} ($NaClO_4$) and M:L molar ratio = 0.9:1; $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

	UV-Vis		CD		EPR		Suggested coordination mode
Species	λ (nm)	Δε (M^{-1} cm^{-1})	λ (nm)	Δε (M^{-1} cm^{-1})	$A_{//}$ (G)	$g_{//}$	
$[CuH_2L]^{4+}$	676	12.5	233 592	-1.33 -0.16	163.7	2.285	$2N_{Im}$
$[CuHL]^{3+}$	637	34.3	248 229	1.43 -1.50	-	-	$3N_{Im}$
$[CuL]^{2+}$	608	43.1	566 247 231	-0.17 2.23 -1.04	180.4	2.245	$2N_{Im}$, N^-
$[CuH_{-1}L]^{+}$	582	56.8	625 489 327 249 230	0.20 0.17 -0.38 2.17 -1.63	-	-	
$[CuH_{-2}L]$	560	76.1	614 489 372 327 257 231	0.43 0.16 0.22 -0.58 2.52 -1.05	185.1	2.205	$2N_{Im}$, $2N^-$
$[CuH_{-3}L]^{-}$	523	74.7	620 484 323 292 258 231	0.77 -0.64 0.46 -0.58 3.91 6.20	191.2	2.194	N_{Im}, $3N^-$

Table S2. Spectroscopic parameters for Cu(II)-**L2** (Ac-HGHHAH-NH_2) system at *T*=298 K, *I*=0.1 mol dm^{-3} ($NaClO_4$) and M:L molar ratio = 0.9:1; $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

	UV-Vis		CD		EPR		Suggested coordination mode
Species	λ (nm)	Δε (M^{-1} cm^{-1})	λ (nm)	Δε (M^{-1} cm^{-1})	$A_{//}$ (G)	$g_{//}$	
$[CuH_2L]^{4+}$	653	29.8	233	-1.50	161.3	2.282	$2N_{Im}$
$[CuHL]^{3+}$	626	51.2	575 315 246 231	-0.23 0.19 1.47 -1.47	182.5	2.250	$3N_{Im}$
$[CuL]^{2+}$	600	55.8	571 354 246 230	-0.23 0.16 1.69 -1.74	175.3	2.218	$2N_{Im}$, N^-
$[CuH_{-1}L]^{+}$	586	72.0	618 548 492 355 314 254 232	0.10 -0.09 0.08 0.36 -0.17 1.24 -3.33	-	-	
$[CuH_{-2}L]$	565	97.6	618 492 356 311 264 232	0.26 0.12 0.61 -0.56 1.20 -4.85	175.5	2.200	$2N_{Im}$, $2N^-$
$[CuH_{-3}L]^{-}$	540	104.1	639 540 477 336 300 256 232	0.28 0.04 -0.17 0.14 -0.52 2.12 2.82	195.1	2.188	N_{Im}, $3N^-$ (apical N_{Im})

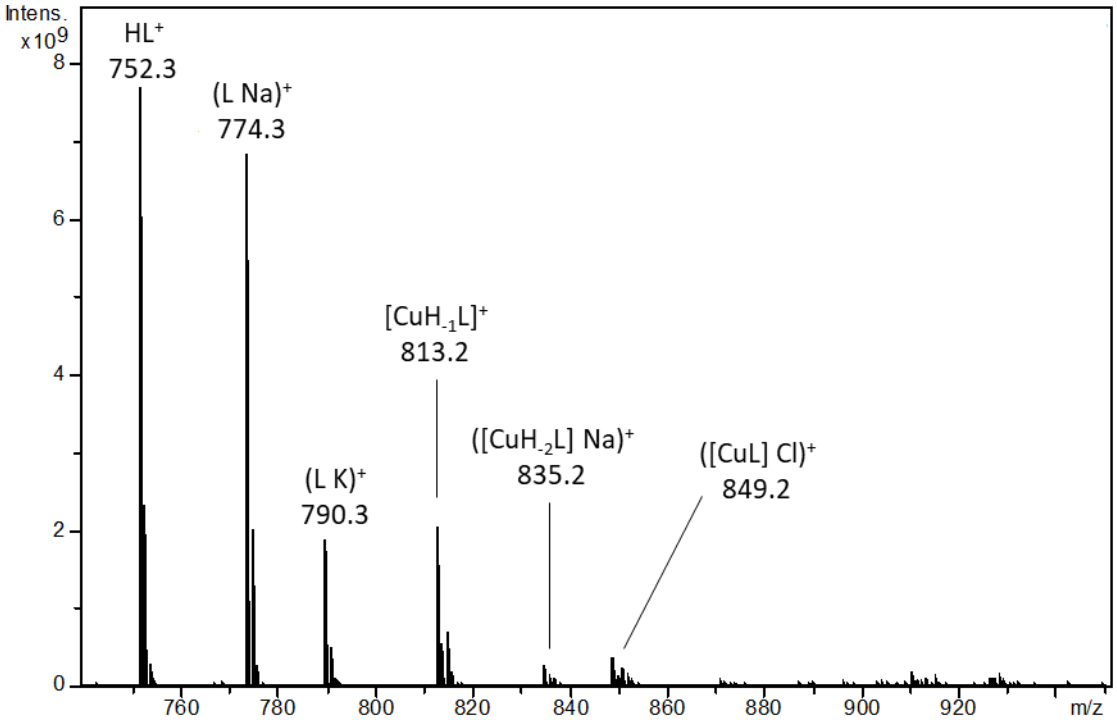
Table S3. Spectroscopic parameters for Cu(II)-**L3** (Ac-DHIIAPRKSSHFH-NH_2) system at *T*=298 K, *I*=0.1 mol dm^{-3} ($NaClO_4$) and M:L molar ratio = 0.9:1; $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

	UV-Vis		CD		EPR		Suggested coordination mode
Species	λ (nm)	Δε (M^{-1} cm^{-1})	λ (nm)	Δε (M^{-1} cm^{-1})	$A_{//}$ (G)	$g_{//}$	
$[CuH_2L]^{4+}$	654	20.87	232	-7.34	163.3	2.278	$2N_{Im}$, COO^-
$[CuHL]^{3+}$	603	46.0	232 254 339	-5.78 1.29 -0.47	-	-	$3N_{Im}$, COO^-
$[CuL]^{2+}$	543	73.67	233 251 340 485 549 647	-2.31 3.82 -1.29 0.35 -0.32 0.31	194.4	2.197	$3N_{Im}$, N^-
$[CuH_{-1}L]^{+}$	530	93.1	233 251 340 488 550 647	-2.31 4.61 -1.53 0.42 -0.40 0.39	194.4	2.200	$2N_{Im}$, $2N^-$
$[CuH_{-2}L]$	544	87.7	233 263 341 487 550 658	-4.69 3.27 -0.86 0.21 -0.38 0.29	201.4	2.200	$2N_{Im}$, $2N^-$ (apical ε-NH_2)
$[CuH_{-3}L]^{-}$	548	82.8	233 268 343 556	-8.47 2.59 -0.40 -0.40	201.4	2.200	$2N_{Im}$, $2N^-$ (apical ε-NH_2)

Table S4. Spectroscopic parameters for Cu(II)-**L4** (Ac-DHIIAPRKSAHFH-NH_2) system at *T*=298 K, *I*=0.1 mol dm^{-3} ($NaClO_4$) and M:L molar ratio = 0.9:1; $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

	UV-Vis		CD		EPR		Suggested coordination mode
Species	λ (nm)	Δε (M^{-1} cm^{-1})	λ (nm)	Δε (M^{-1} cm^{-1})	$A_{//}$ (G)	$g_{//}$	
$[CuH_2L]^{4+}$	650	26.58	233	-6.65	173.3	2.280	$2N_{Im}$, COO^-
$[CuHL]^{3+}$	610	40.94	233 254 342	-2.31 1.07 -0.61	-	-	$3N_{Im}$, COO^-
$[CuL]^{2+}$	608	68.48	233 249 342 489 549 635	-2.26 3.71 -1.47 0.31 -0.36 0.38	194.2	2.200	$3N_{Im}$, N^-
$[CuH_{-1}L]^{+}$	531	93.52	232 249 342 488 549 635	-0.78 5.64 -2.13 0.44 -0.51 0.44	194.2	2.197	$2N_{Im}$, $2N^-$
$[CuH_{-2}L]$	531	93.52	232 250 341 488 544 635	-1.29 4.84 -1.40 0.28 -0.51 0.44	194.2	2.197	$2N_{Im}$, $2N^-$
$[CuH_{-3}L]^{-}$	531	93.52	233 261 338 538 635	-1.07 4.23 -0.43 -0.58 0.44	194.2	2.197	$2N_{Im}$, $2N^-$

A)



B)

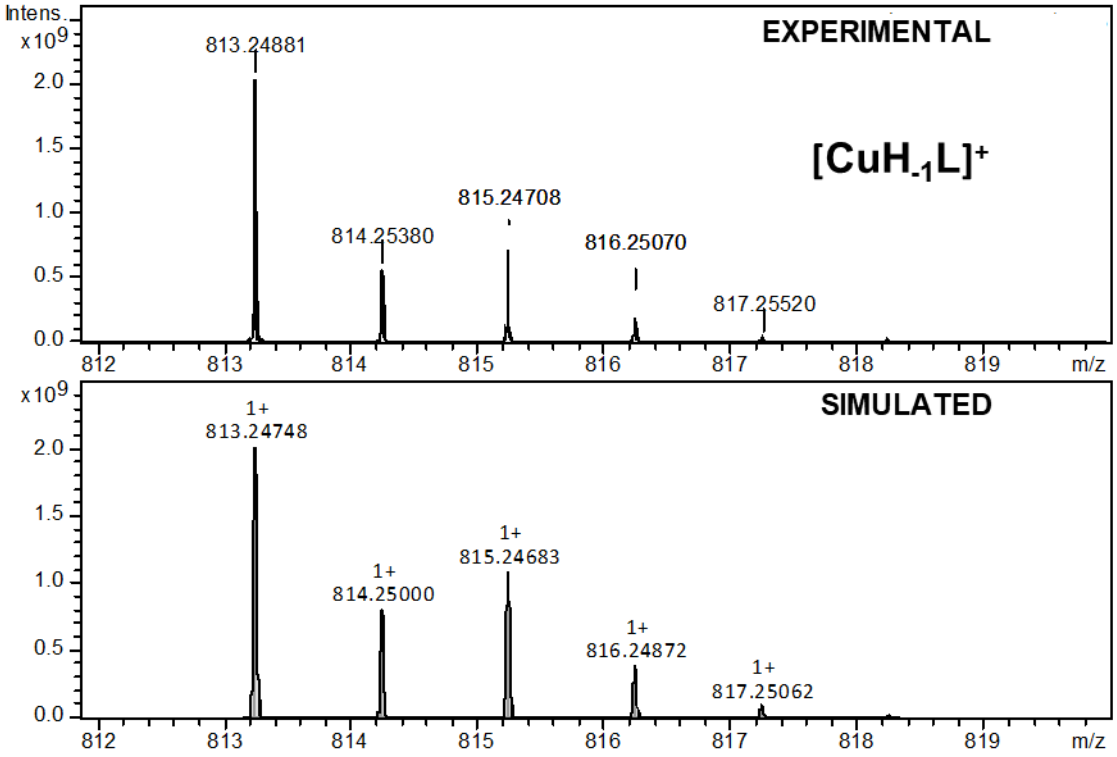
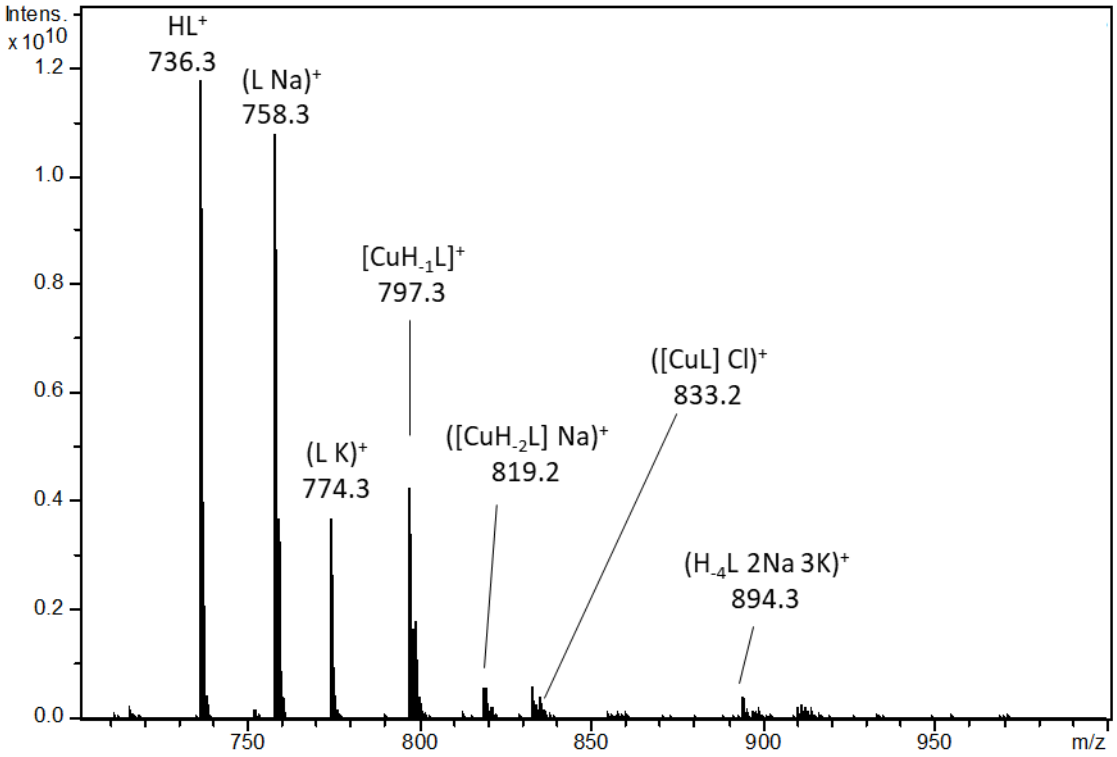


Figure S1. A) ESI-MS spectrum for Cu(II)/**L1** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

A)



B)

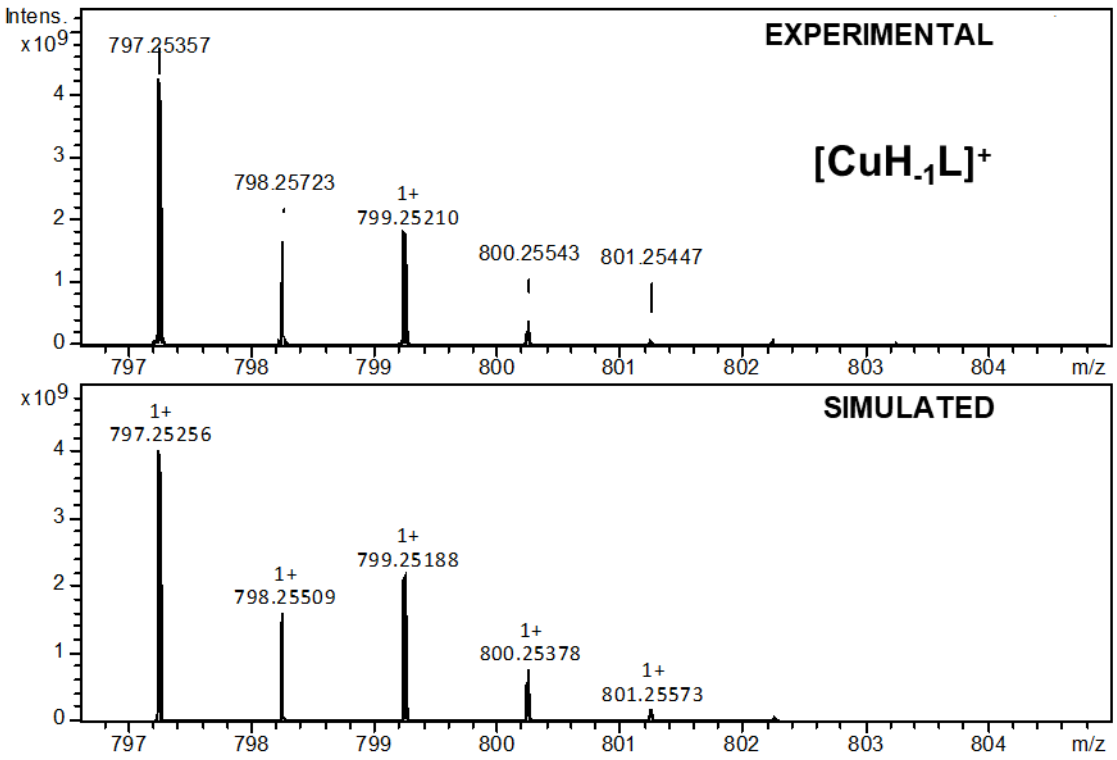
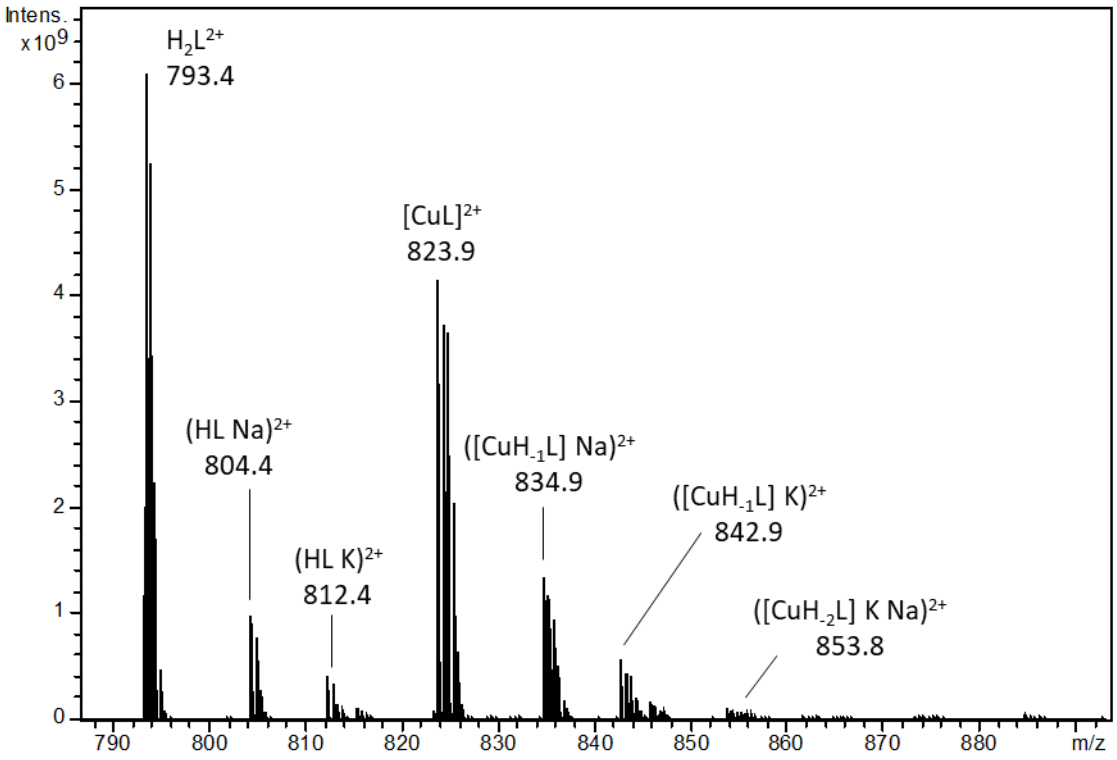


Figure S2. A) ESI-MS spectrum for Cu(II)/**L2** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

A)



B)

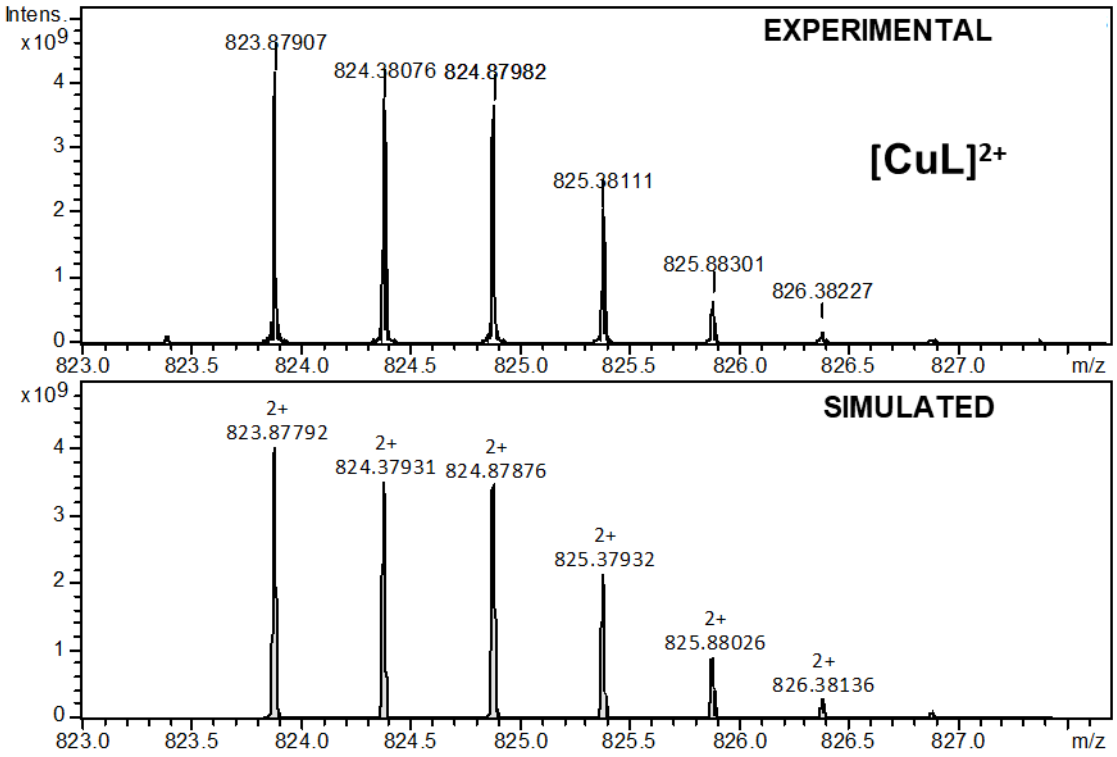
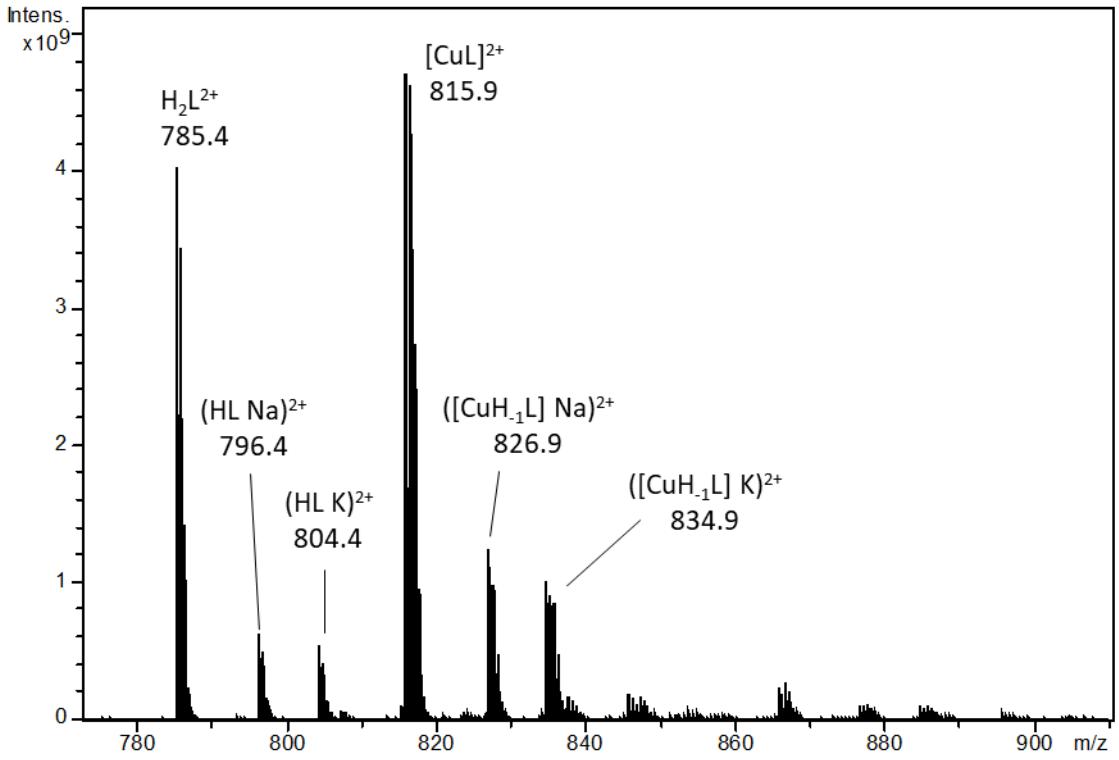


Figure S3. A) ESI-MS spectrum for Cu(II)/**L3** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

A)



B)

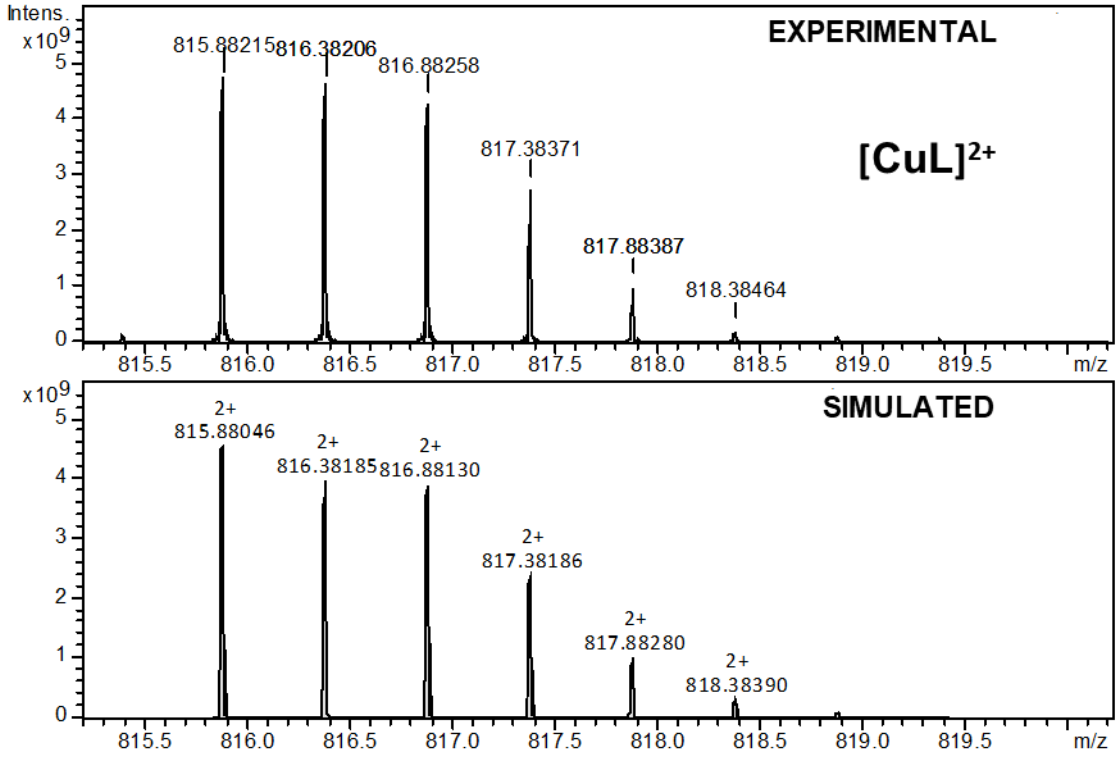


Figure S4. A) ESI-MS spectrum for Cu(II)/**L4** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

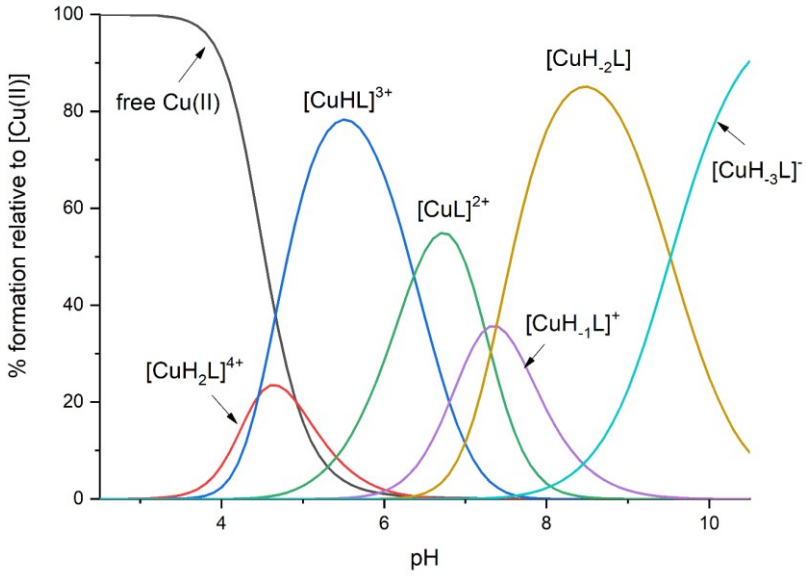


Figure S5. Species distribution diagram relative to Cu(II)/**L2** complexes; M:L molar ratio = 0.9:1; $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

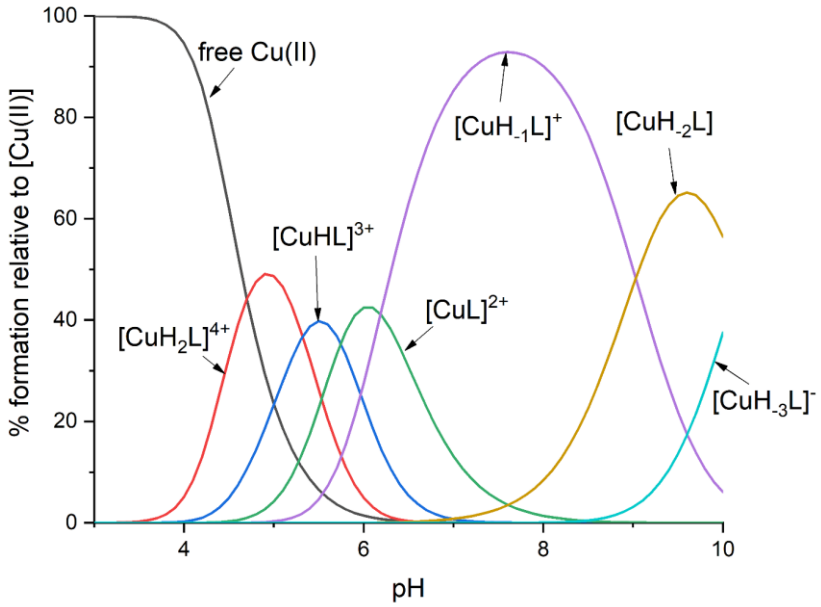


Figure S6. Species distribution diagram relative to Cu(II)/**L3** complexes; M:L molar ratio = 0.9:1; $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

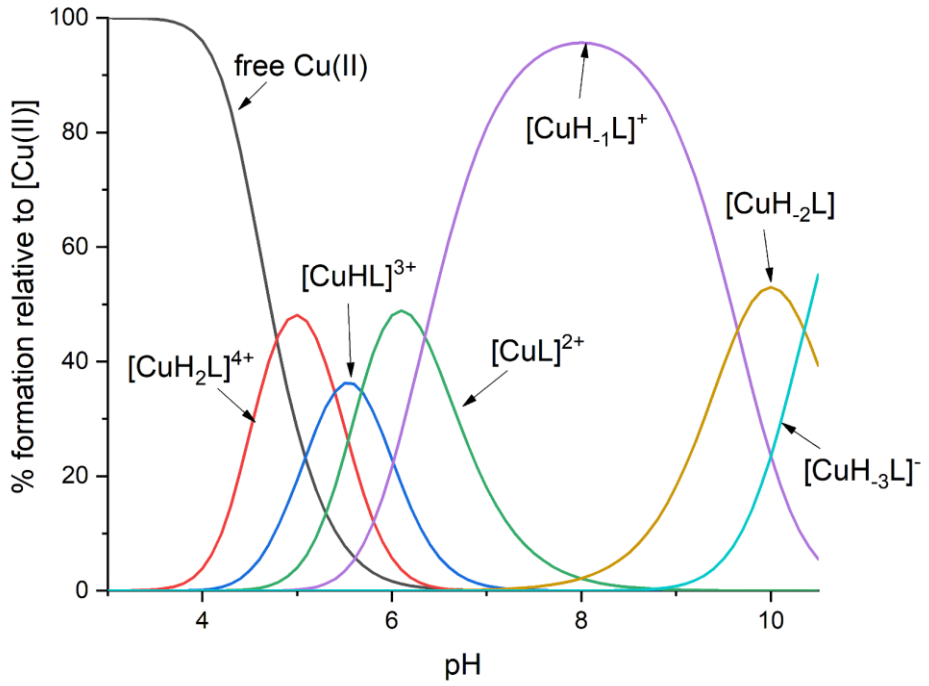


Figure S7. Species distribution diagram relative to Cu(II)/**L4** complexes; M:L molar ratio = 0.9:1; $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

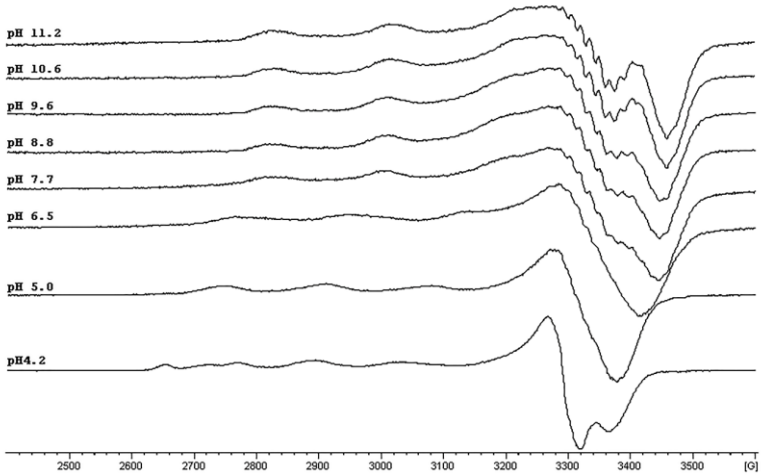


Figure S8. EPR spectra for Cu(II) complexes with **L1**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.9 \cdot 10^{-3}$ mol dm^{-3}.

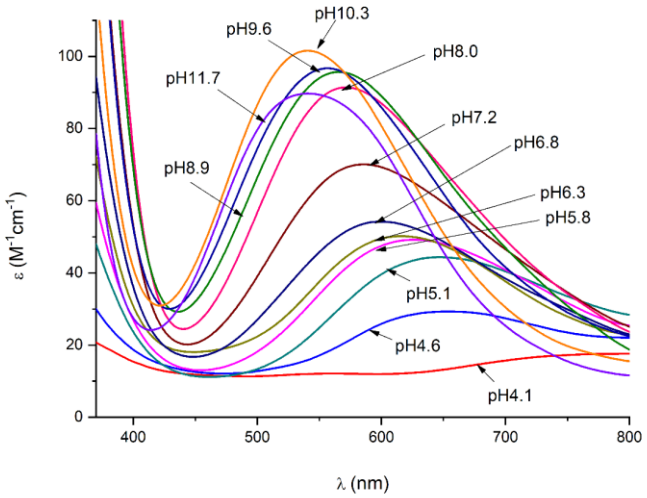


Figure S9. Vis absorption spectra for Cu(II) complexes with **L2**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}, optical path 1 cm.

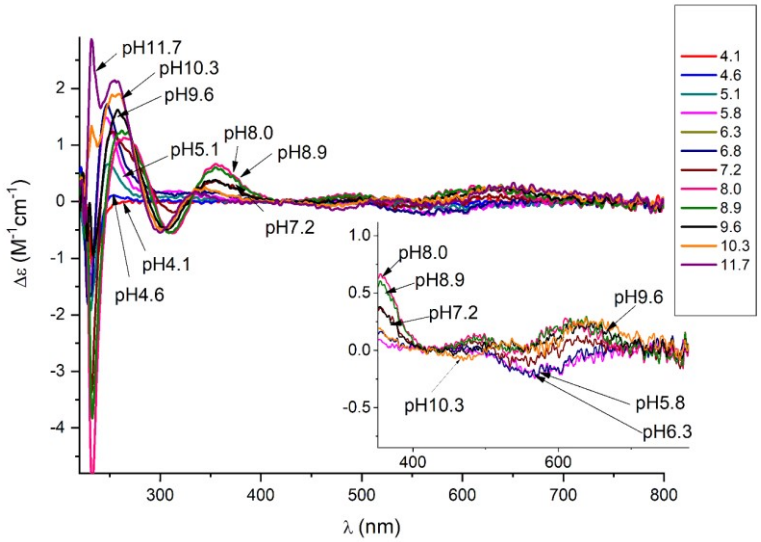


Figure S10. CD spectra for Cu(II) complexes with **L2**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}, optical path 1 cm.

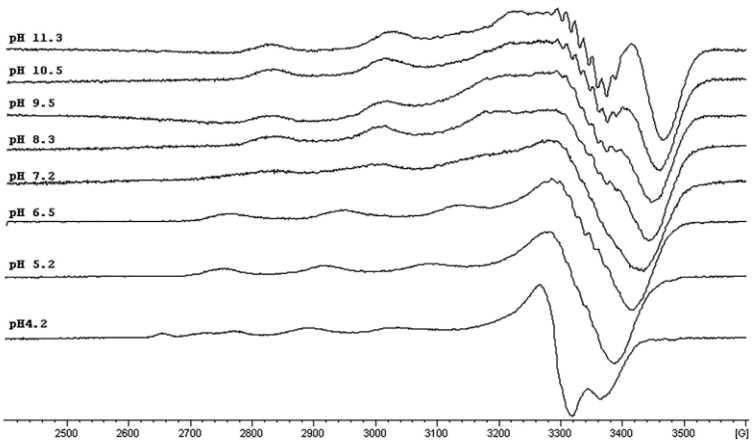


Figure S11. EPR spectra for Cu(II) complexes with **L2**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.9 \cdot 10^{-3}$ mol dm^{-3}.

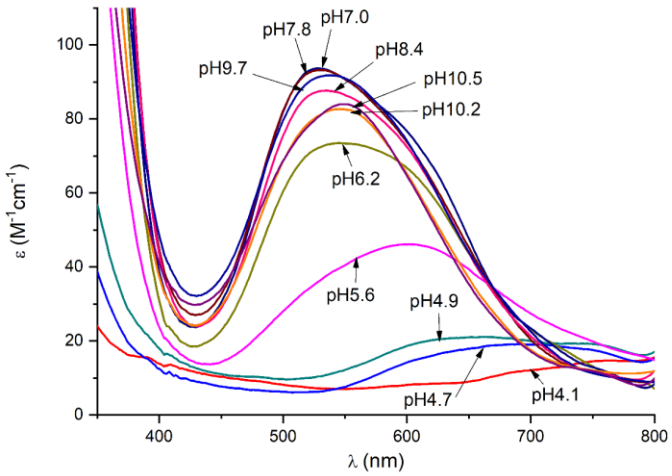


Figure S12. Vis absorption spectra for Cu(II) complexes with **L3**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}, optical path 1 cm.

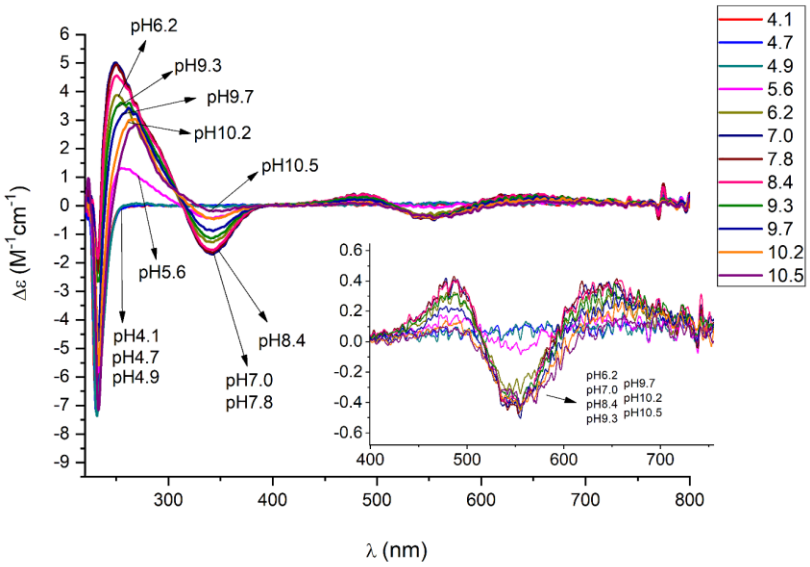


Figure S13. CD spectra for Cu(II) complexes with **L3**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}, optical path 1 cm.

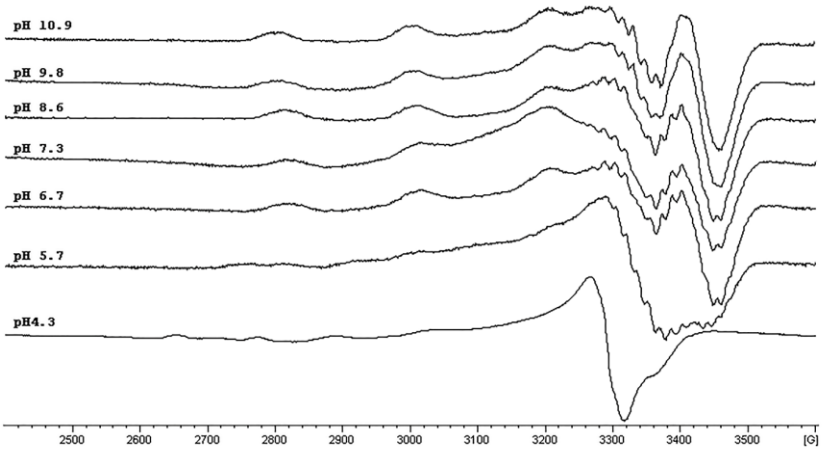


Figure S14. EPR spectra for Cu(II) complexes with **L3**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.9 \cdot 10^{-3}$ mol dm^{-3}.

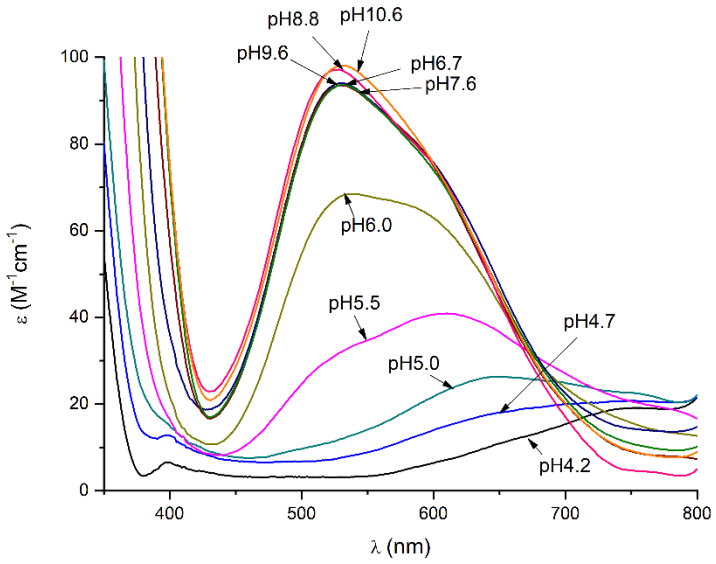


Figure S15. Vis absorption spectra for Cu(II) complexes with **L4**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}, optical path 1 cm.

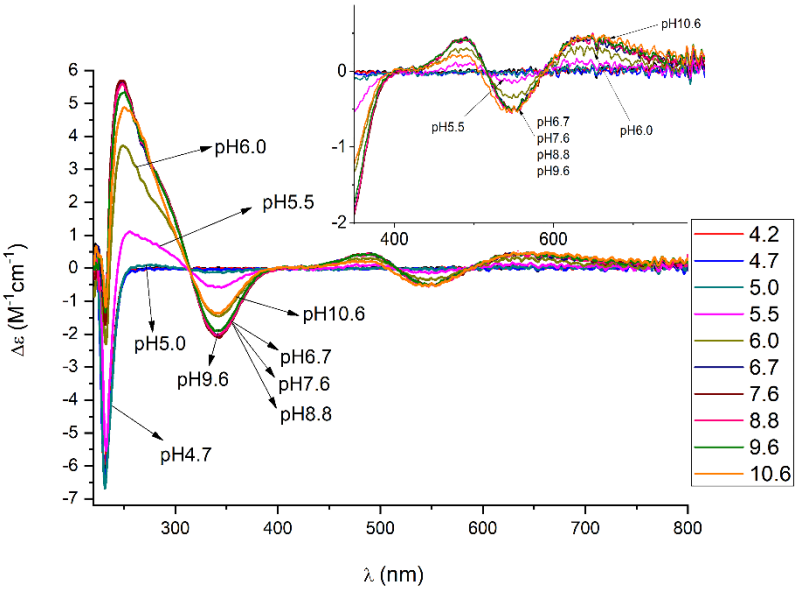


Figure S16. CD spectra for Cu(II) complexes with **L4**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}, optical path 1 cm.

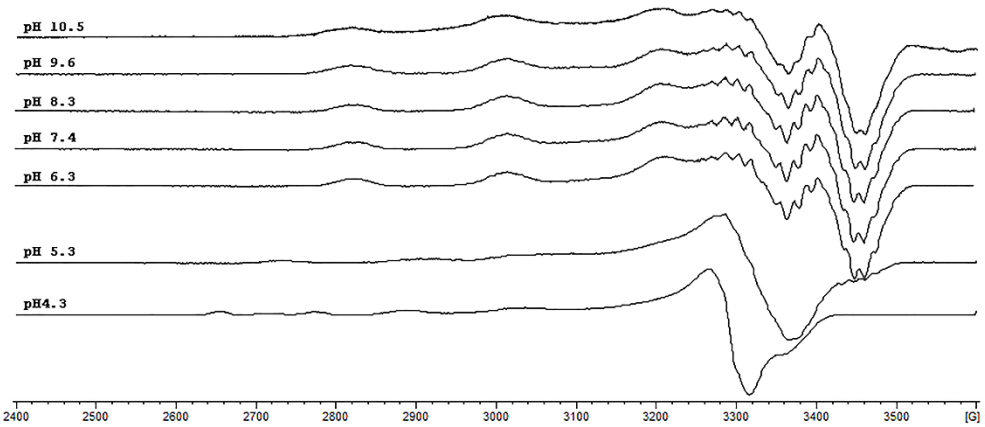
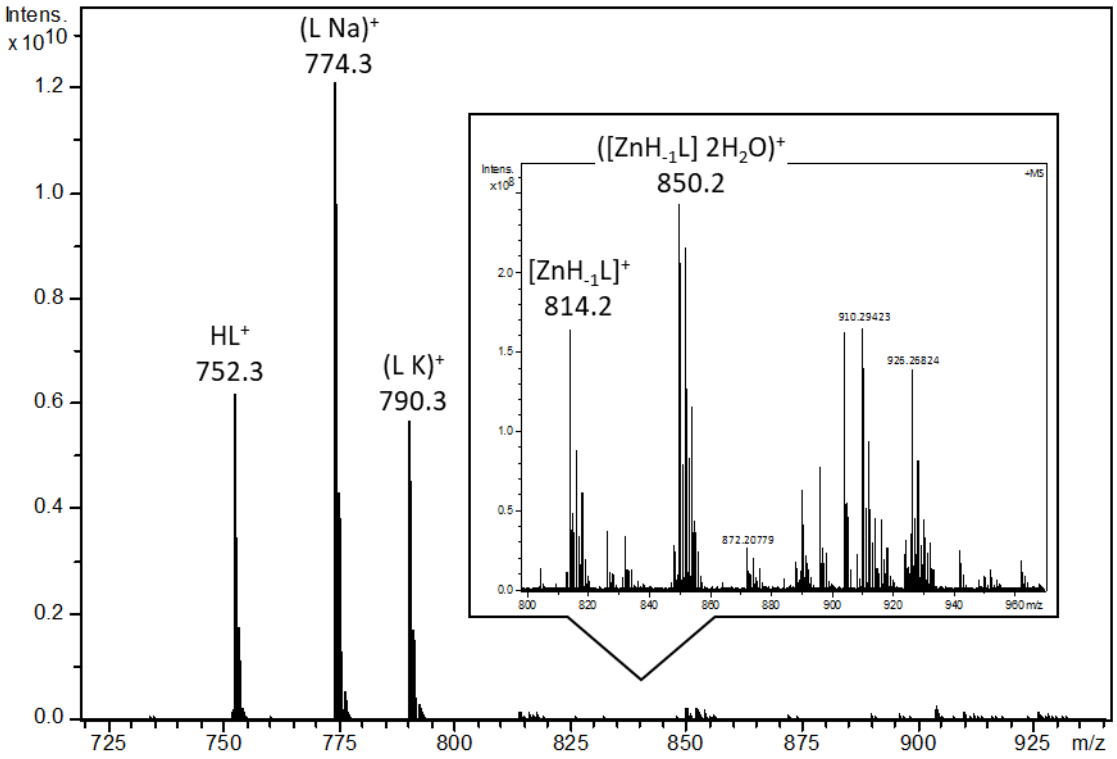


Figure S17. EPR spectra for Cu(II) complexes with **L4**, M:L ratio = 0.9:1, $C_{Cu(II)} = 0.9 \cdot 10^{-3}$ mol dm^{-3}.

A)



B)

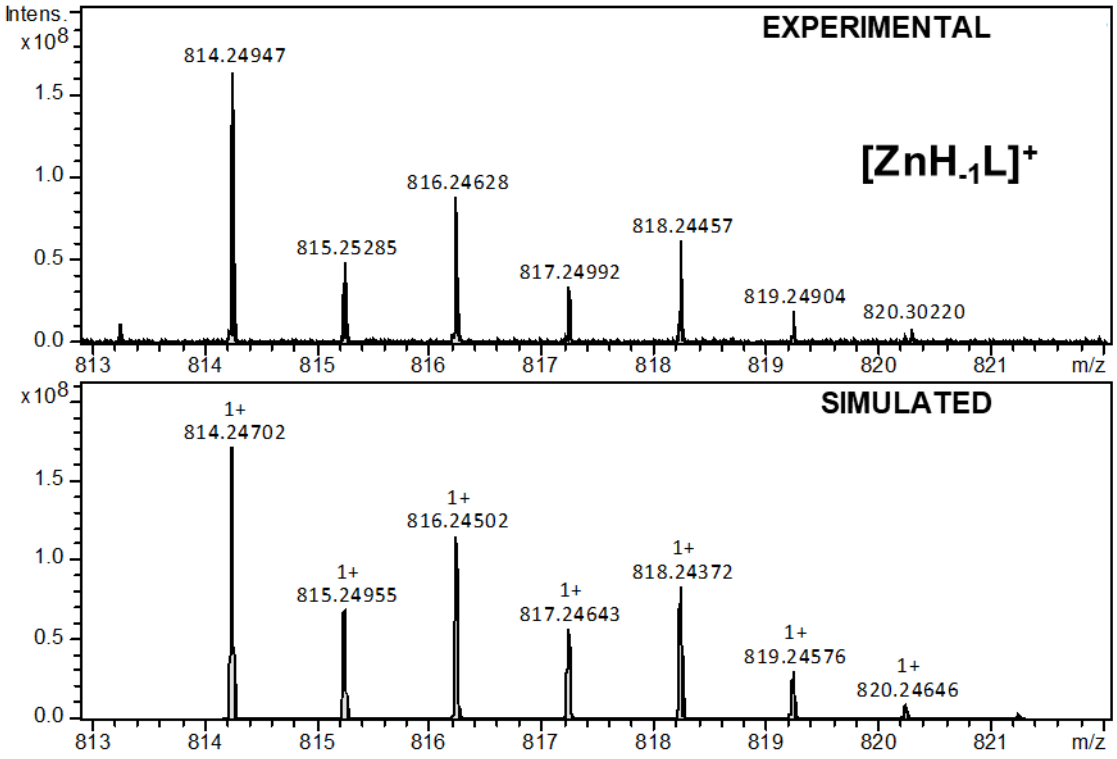
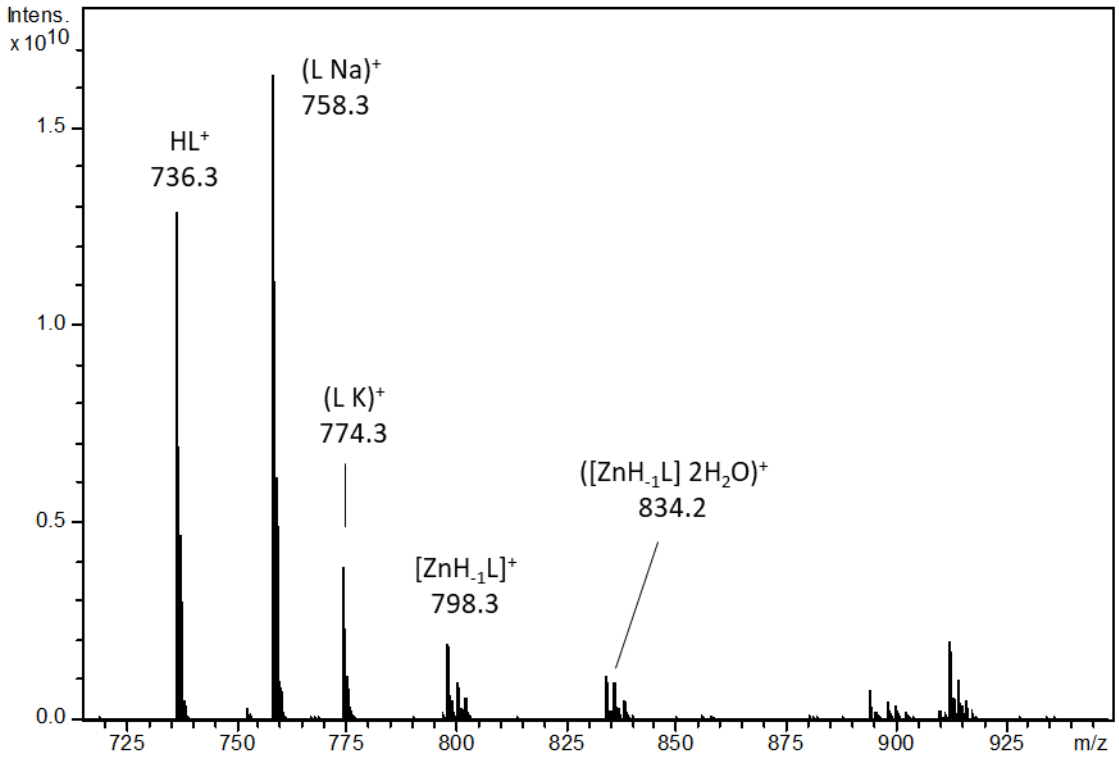


Figure S18. A) ESI-MS spectrum for Zn(II)/**L1** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

A)



B)

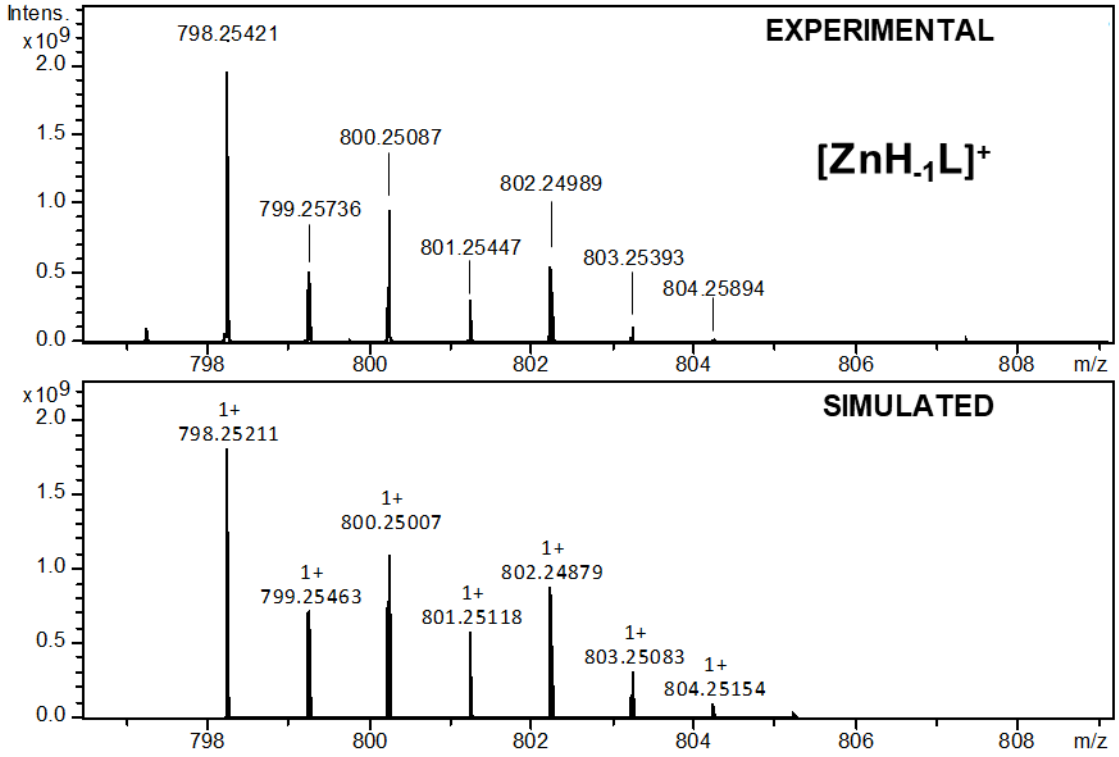
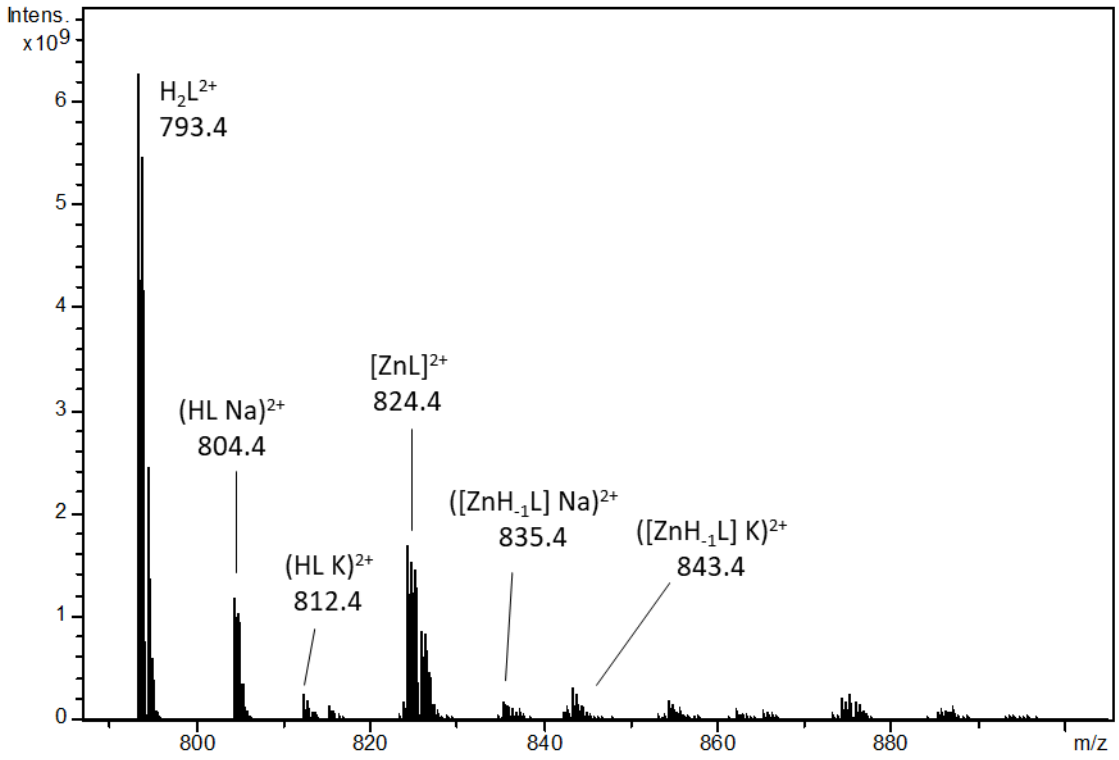


Figure S19. A) ESI-MS spectrum for Zn(II)/**L2** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

A)



B)

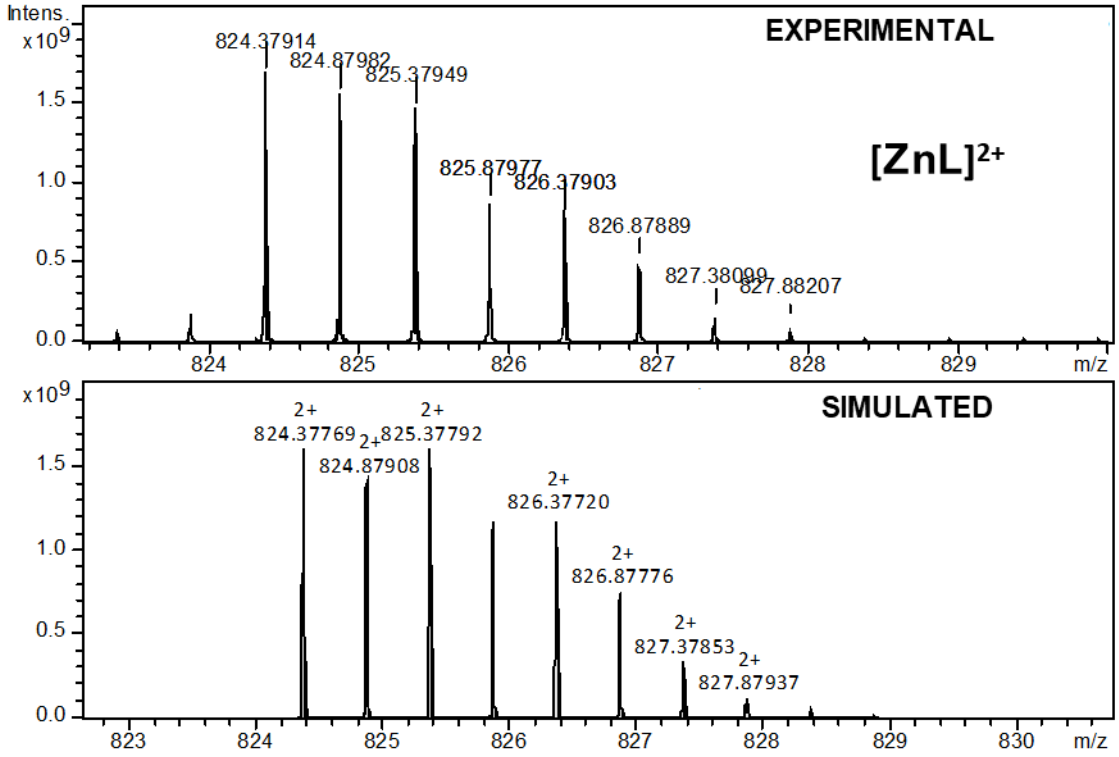
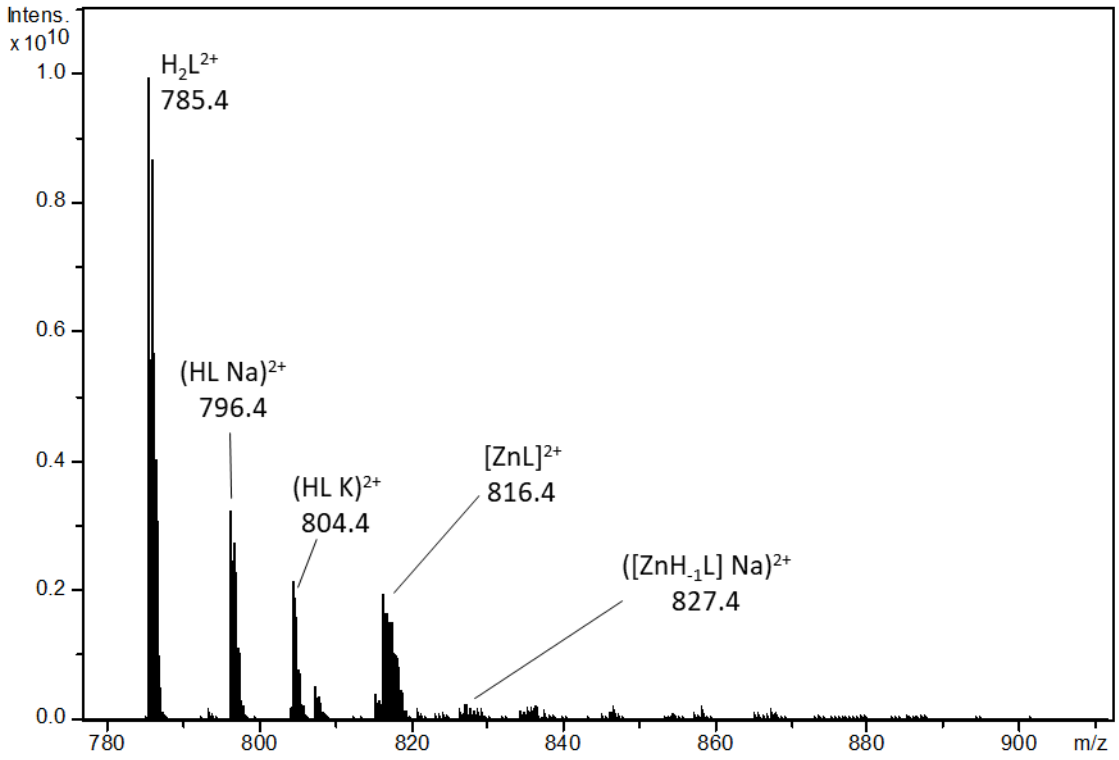


Figure S20. A) ESI-MS spectrum for Zn(II)/**L3** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

A)



B)

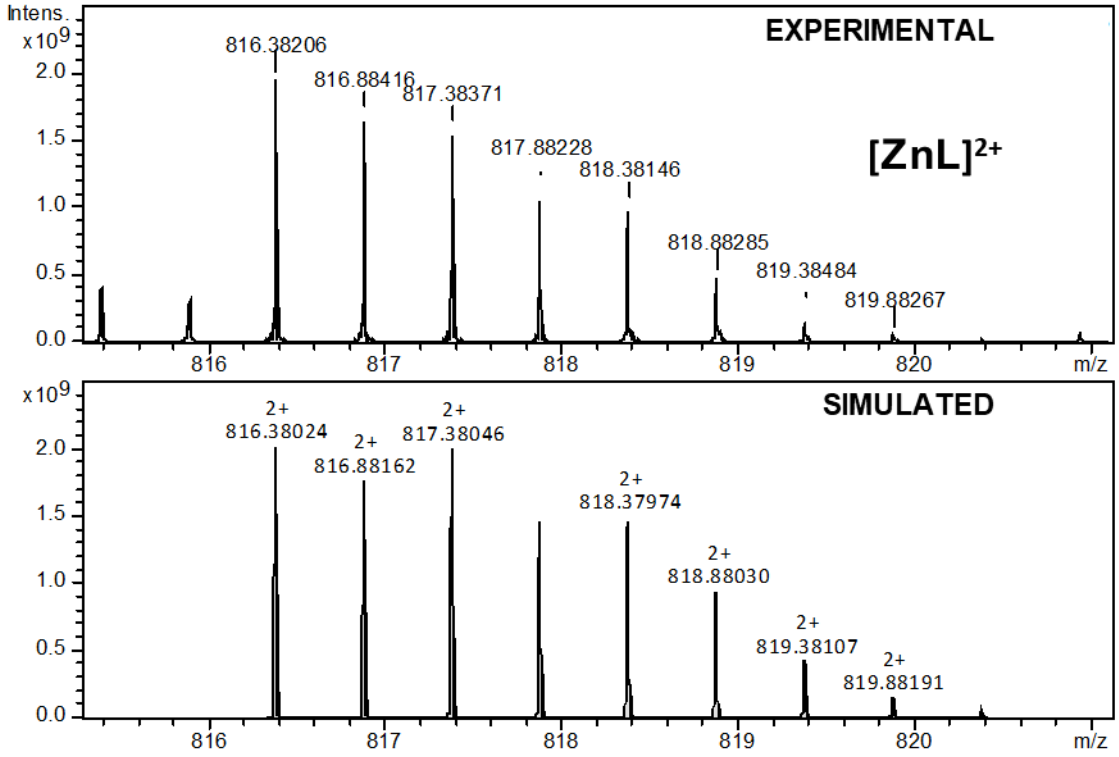


Figure S21. A) ESI-MS spectrum for Zn(II)/**L4** system at L:M molar ratio=1:0.9 in MeOH:H_2O (1:1) mixture solution at pH=7. $C_L = 0.5 \cdot 10^{-3}$ mol dm^{-3}. B) Comparison of experimental and simulated isotopic pattern of the chosen metal complex.

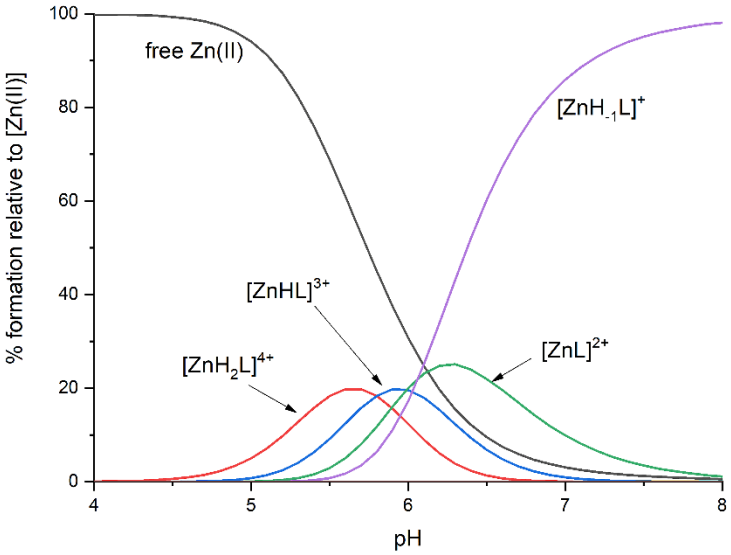


Figure S22. Species distribution diagram relative to Zn(II)/**L2** complexes; M:L molar ratio = 0.9:1; $C_{Zn(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

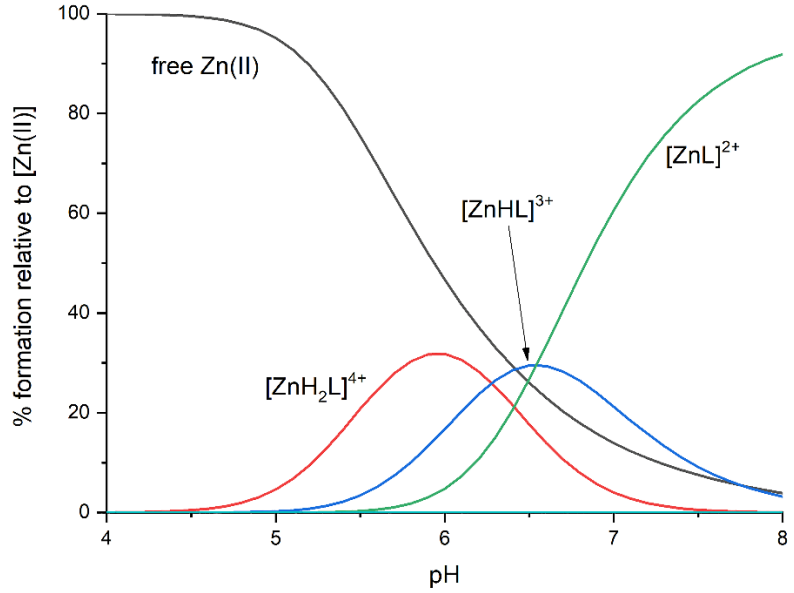


Figure S23. Species distribution diagram relative to Zn(II)/**L3** complexes; M:L molar ratio = 0.9:1; $C_{Zn(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

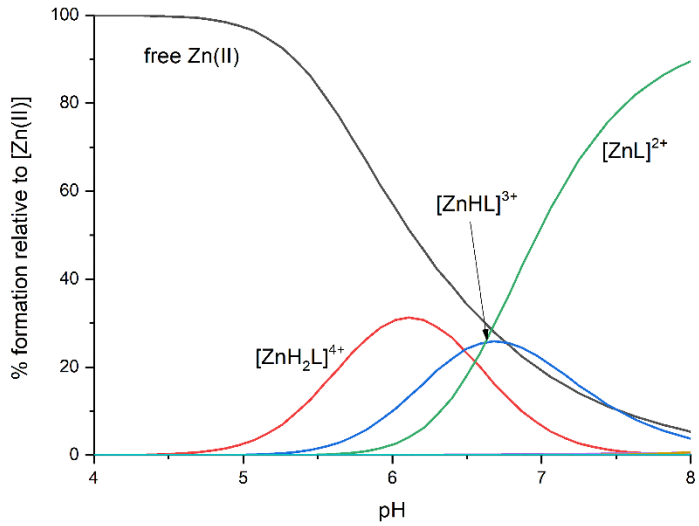


Figure S24. Species distribution diagram relative to Zn(II)/**L4** complexes; M:L molar ratio = 0.9:1; $C_{Zn(II)} = 0.45 \cdot 10^{-3}$ mol dm^{-3}.

A) **B)**

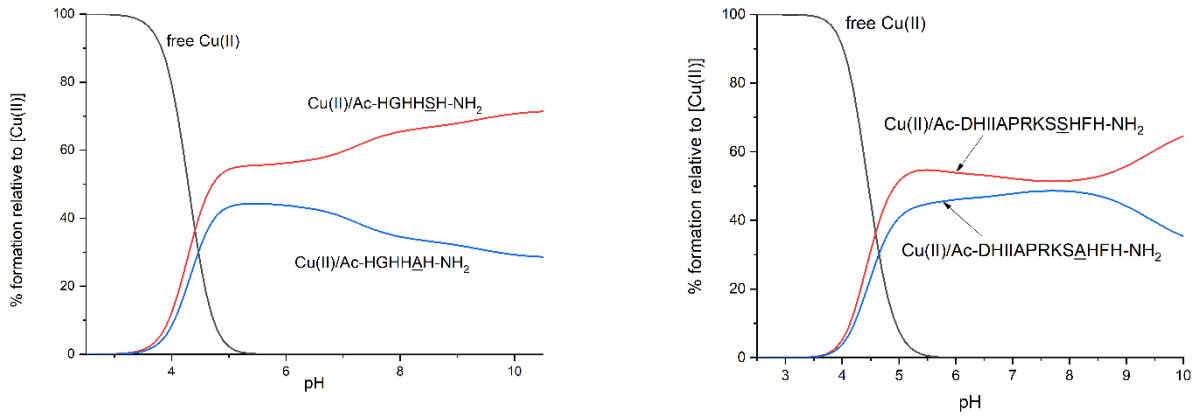


Figure S25. Competition plots for a solution containing equimolar concentration ($1 \cdot 10^{-3}$ mol dm^{-3}) of A) Cu(II), **L1** = Ac-HGHHSH-NH_2 and **L2** = Ac-HGHHAH-NH_2; B) Cu(II), **L3** = Ac-DHIIAPRKSSHFH-NH_2 and **L4** = Ac-DHIIAPRKSAHFH-NH_2.

A) **B)**

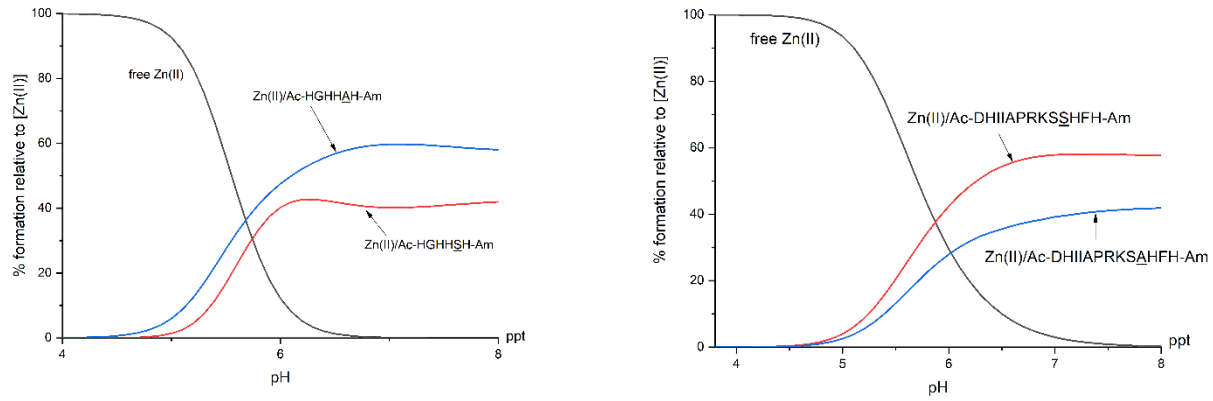


Figure S26. Competition plots for a solution containing equimolar concentration ($1 \cdot 10^{-3}$ mol dm^{-3}) of A) Zn(II), **L1** = Ac-HGHHSH-NH_2 and **L2** = Ac-HGHHAH-NH_2; B) Zn(II), **L3** = Ac-DHIIAPRKSSHFH-NH_2 and **L4** = Ac-DHIIAPRKSAHFH-NH_2.

A) **B)**

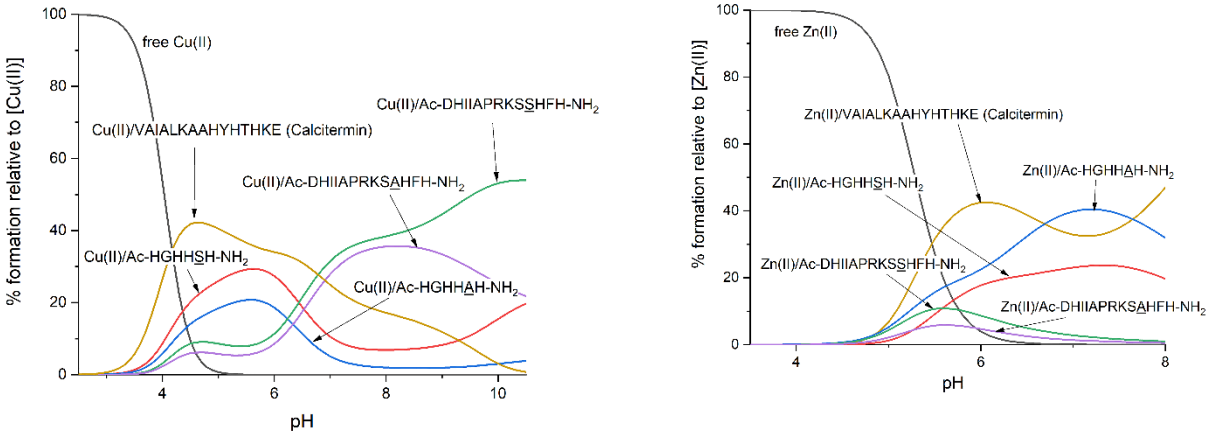


Figure S27. Competition plots for a solution containing equimolar concentration ($1 \cdot 10^{-3}$ mol dm^{-3}) of metal ion, **L1** = Ac-HGHHSH-NH_2, **L2** = Ac-HGHHAH-NH_2, **L3** = Ac-DHIIAPRKSSHFH-NH_2, **L4** = Ac-DHIIAPRKSAHFH-NH_2 and calcitermin VAIALKAAHYHTHKE. A) Cu(II), B) Zn(II).

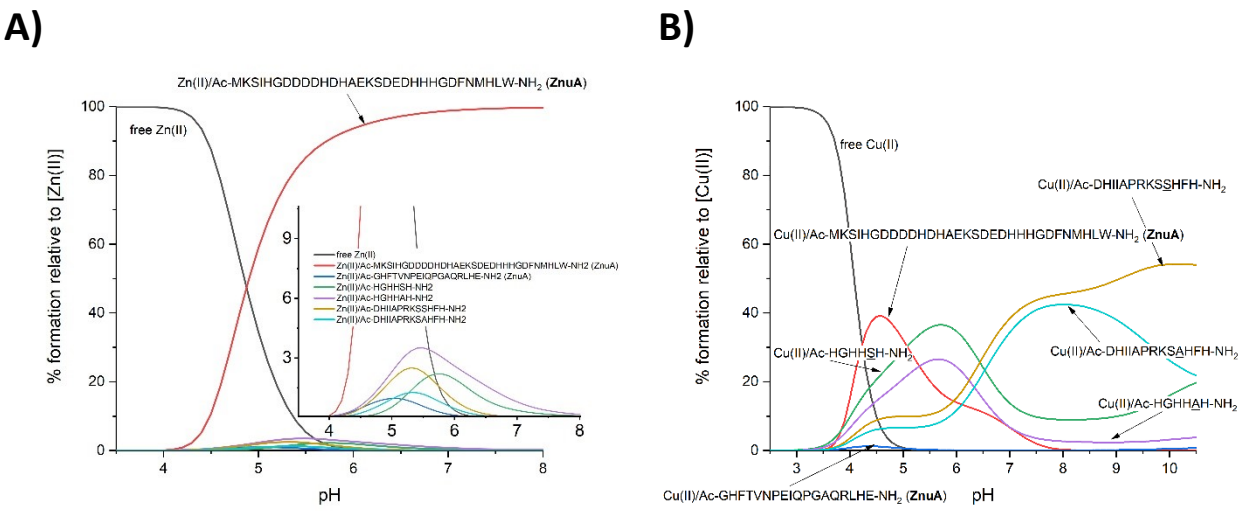


Figure S28. Competition plots for a solution containing equimolar concentration ($1 \cdot 10^{-3}$ mol dm^{-3}) of metal ion, **L1** = Ac-HGHHSH-NH_2, **L2** = Ac-HGHHAH-NH_2, **L3** = Ac-DHIIAPRKSSHFH-NH_2, **L4** = Ac-DHIIAPRKSAHFH-NH_2, *E. coli* ZnuA peptide Ac-MKSIHGDDDDHDHAEKSDEDHHHGDFNMHLW-NH_2 and *E. coli* ZnuA peptide Ac-GHFTVNPEIQPGAQRLHE-NH_2. A) Zn(II), B) Cu(II).

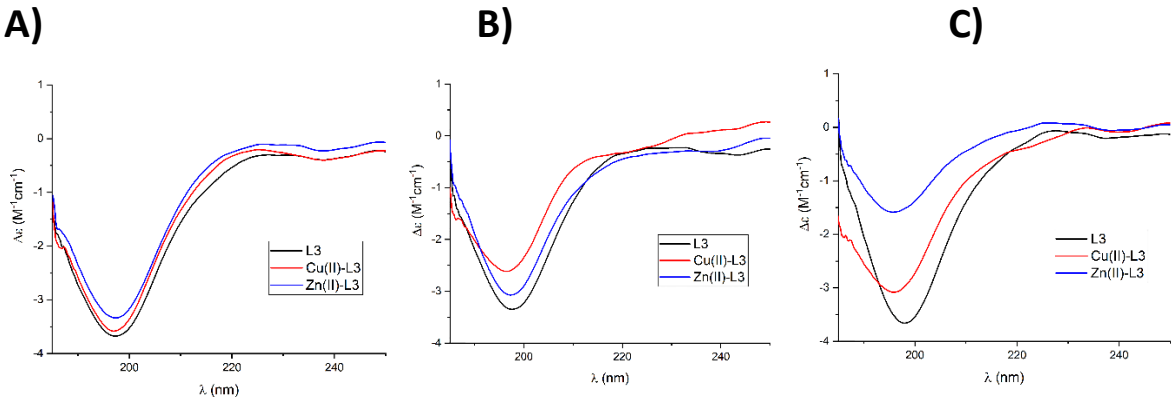


Figure S29. Comparison of near-UV CD spectra of **L3** apo-peptide and its Cu(II) and Zn(II) complexes. M:L molar ratio = 0.9:1, $C_L = 0.1 \cdot 10^{-3}$ mol dm^{-3}. Optical path 0.01 cm. A) pH = 4.5; B) pH = 7; C) pH = 9.

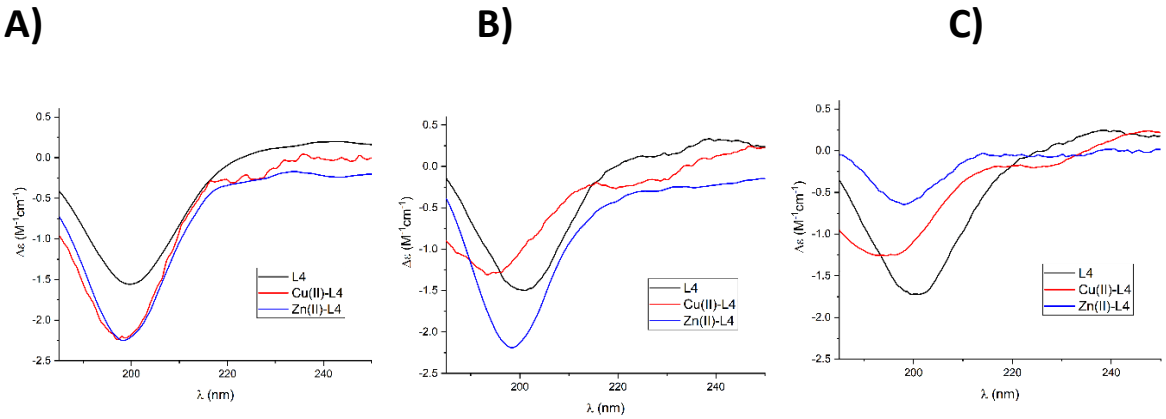


Figure S30. Comparison of near-UV CD spectra of **L4** apo-peptide and its Cu(II) and Zn(II) complexes. M:L molar ratio = 0.9:1, $C_L = 0.1 \cdot 10^{-3}$ mol dm^{-3}. Optical path 0.01 cm. A) pH = 4.5; B) pH = 7; C) pH = 9.