

Supplementary information for

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A comparative study on the possible zinc binding sites of the human ZnT3 zinc transporter protein

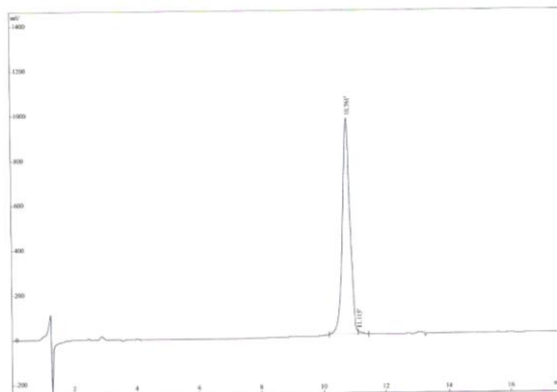
Table S1: EPR data of the complexes formed in the copper(II)-L¹ system

Species	g_{xx}	g_{yy}	g_{zz}	A_{xx}/G	A_{yy}/G	A_{zz}/G
Cu ²⁺	2.082	2.082	2.425	8.3	8.3	111.5
CuHL ¹	2.062	2.062	2.330	10.0	10.0	140.0
CuL ¹	2.058	2.058	2.274	23.8	23.8	165.2
CuH ₁ L ¹	2.051	2.051	2.240	32.0	32.0	180.0
CuH ₂ L ¹	2.036	2.044	2.209	15.4	33.2	184.5
CuH ₃ L ¹	2.031	2.046	2.188	20.1	35.2	192.8
Cu ₂ H ₂ L ¹				55.6		
Cu ₂ H ₃ L ¹				70.0		
Cu ₂ H ₄ L ¹				63.5		
Cu ₂ H ₅ L ¹ + Cu ₂ H ₆ L ¹				47.3		



HPLC Certificate

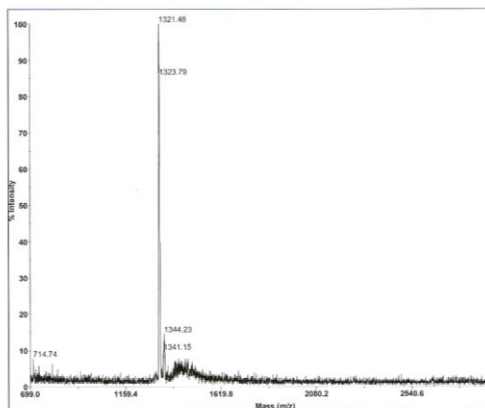
Lot No. P220410-04-01
PLC Column Agela (250×4.6mm I.D.) C18
Detection wavelength 220 nm
Gradient 3-23%B in 20min
Buffer A 0.05%TFA +2%CH₃CN
Buffer B 0.05%TFA +90%CH₃CN



Peak Results			
Rank	Time	Conc.	Area
1	10.761	99.46	16241834
2	11.115	0.54	88183
Total		100	16330017



Mass Spectrometry Certificate



Lot No.: P220410-04-01
Method: MALDI-TOF
Main Peak: 1321.48
MW [M+ H⁺]: 1321.48
MW: 1320.48
Theoretical MW: 1320.45
Match: Approved
Z=1

Figure S1. HPLC chromatogram and Maldi-TOF spectrum of L¹

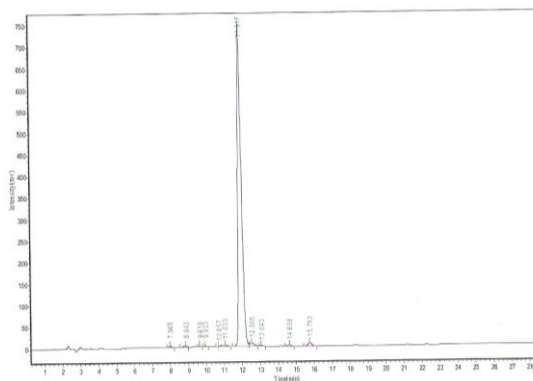


HPLC Certificate

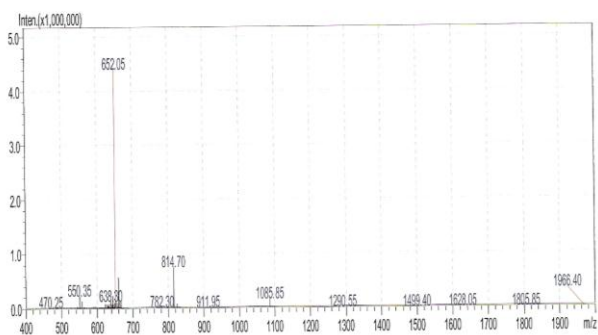
Lot No : P250310-04-01
Column : 4,6×250mm,Sinohrom ODS-BP-5
Solvent A : 0.1% trifluoroacetic in 100% acetonitrile
Solvent B : 0.1% trifluoroacetic in 100% water
Gradient :

	A	B
0.01min	17%	83%
25min	57%	43%
25.1min	100%	0%
30min		STOP

Flow rate : 1.0 mL/min
Wavelength : 220nm
Volume : 5ul



Mass Spectrometry Certificate



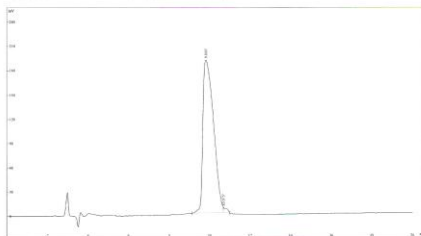
Lot No.: P250310-04-01
Method: ESI
Main Peak: 652.05
MW [M+ 5H⁺]: 3260.25
MW: 3255.25
Theoretical MW: 3254.59
Match: Approved
Z=5

Figure S2. HPLC chromatogram and ESI-MS spectrum of L²



HPLC Certificate

Lot No. P311008-01-01
PLC Column Merck (250×4.6mm I.D.) C18
Detection wavelength 210 nm
Gradient 0-30%B in 30min
Buffer A 0.05%TFA +2%CH₃CN
Buffer B 0.05%TFA +90%CH₃CN

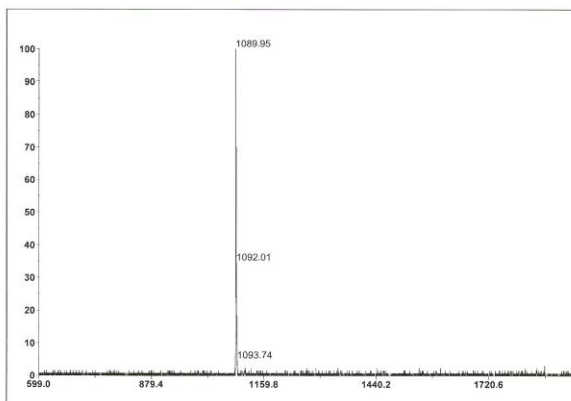


Peak Results

Rank	Time	Conc.	Area
1	9.808	98.86	6918346
2	10.675	1.146	80219
Total		100	6998565



Mass Spectrometry Certificate



Lot No.: P311008-01-01
Method: MALDI
Main Peak: 1089.95
MW [M+ H⁺]: 1089.95
MW: 1088.95
Theoretical MW: 1089.20
Match: Approved
Z=1

Figure S3. HPLC chromatogram and MALDI-TOF spectrum of L³

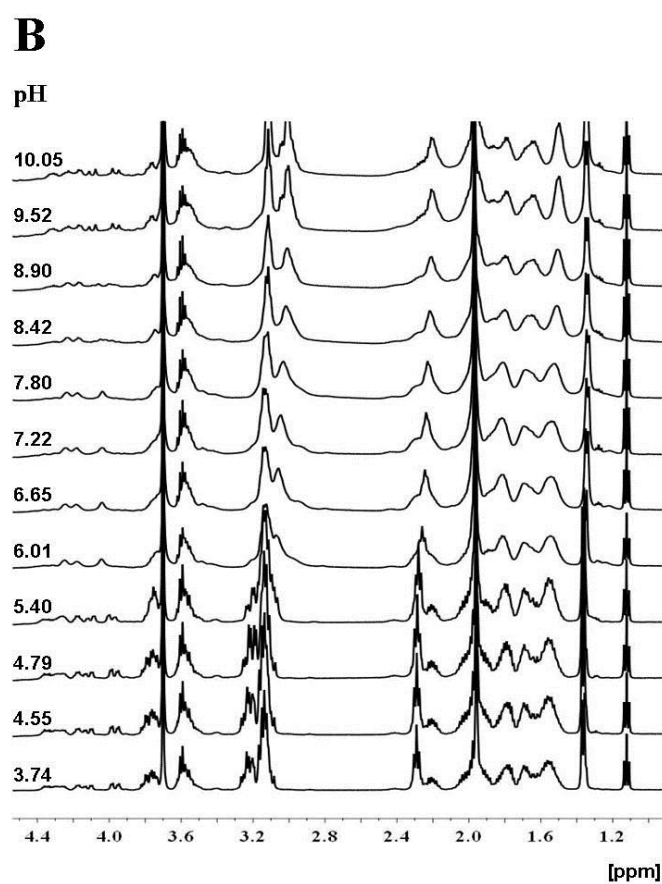
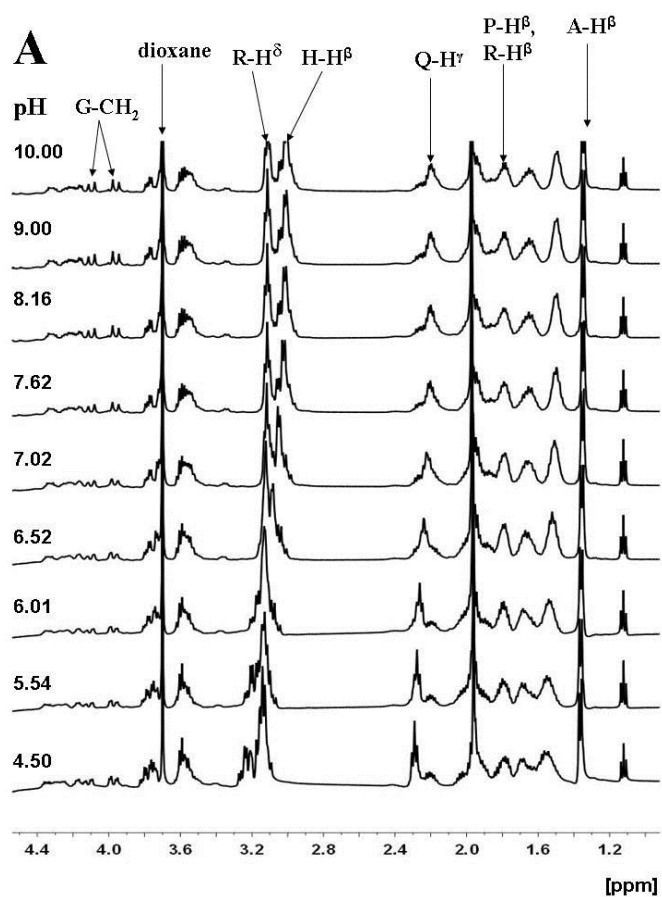


Figure S4 : The aliphatic part of the ¹H NMR spectra of **L**¹ as a function of pH in the absence (**A**) and presence (**B**) of zinc(II) (T = 298 K, [**L**¹] = 0.0046 M, [Zn²⁺] = 0.00425 M).

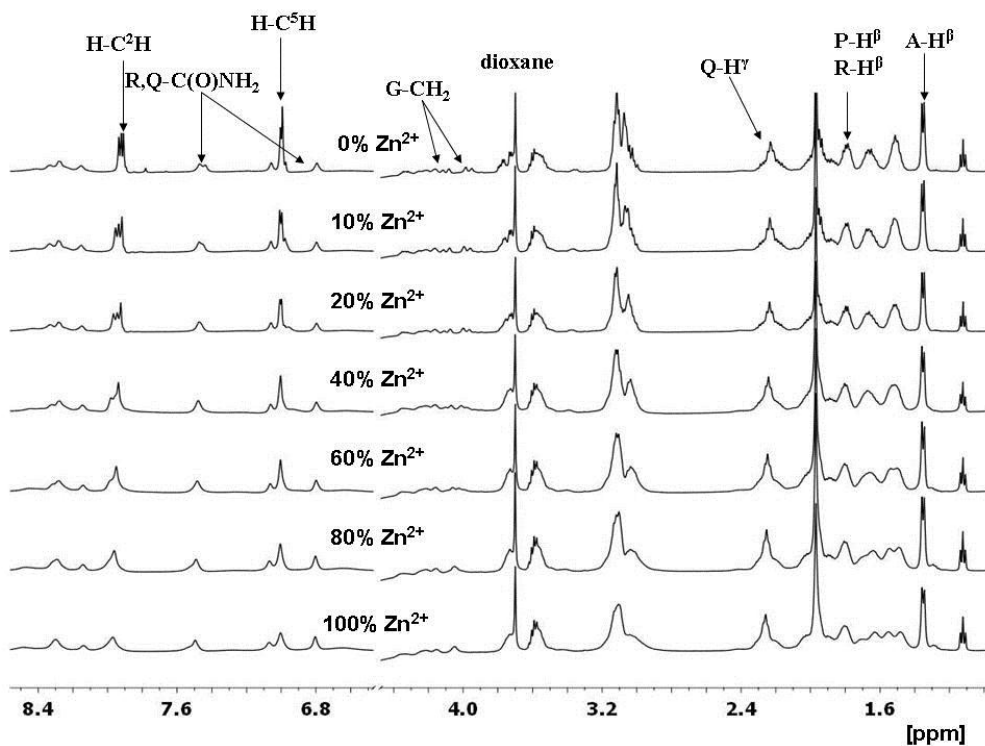


Figure S5: The ¹H NMR spectra of L¹ at pH = 7.0 as a function of zinc(II) concentration (T= 298 K).

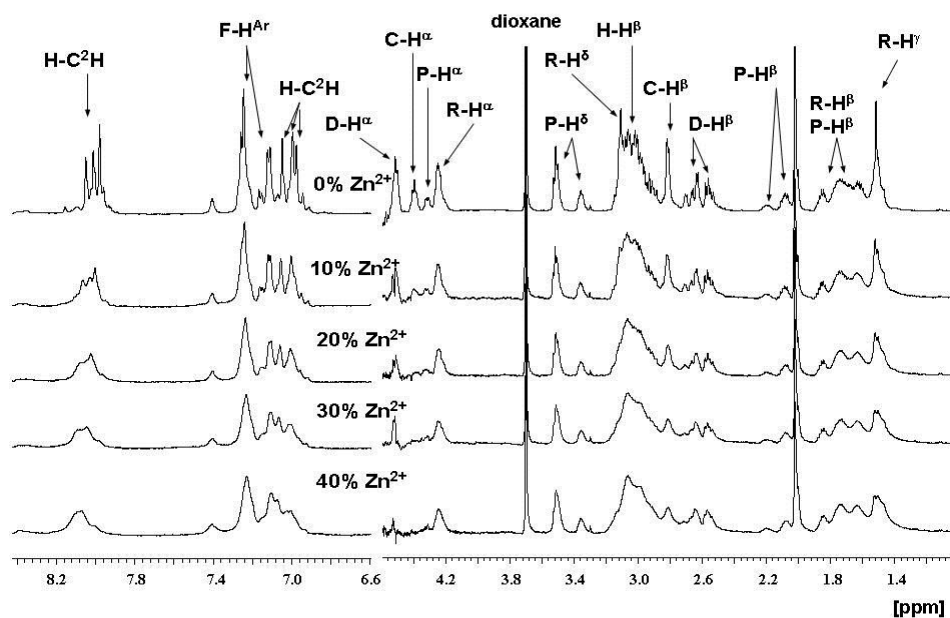


Figure S6: ¹H NMR spectra of L³ as a function of zinc(II) concentration at pH = 7.0 (T=298 K)

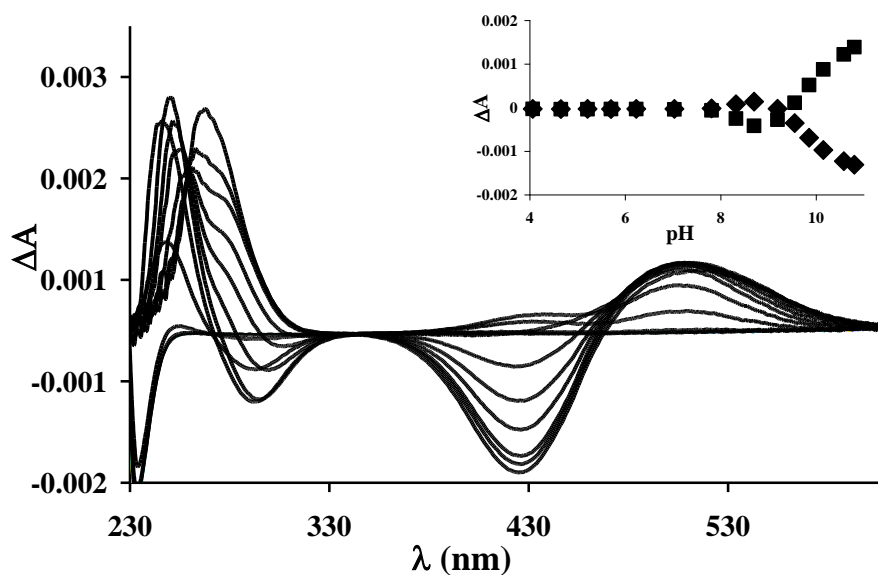


Figure S7: pH-dependent CD spectra of the nickel(II)- L^1 system ($[L^1]= 0.0007$ M, $[Ni^{2+}]= 0.00035$ M, $T = 298$ K, $I= 0.1$ M NaCl). The insert shows the pH dependence of the CD intensities at 280 (■) and 423 nm (▲).

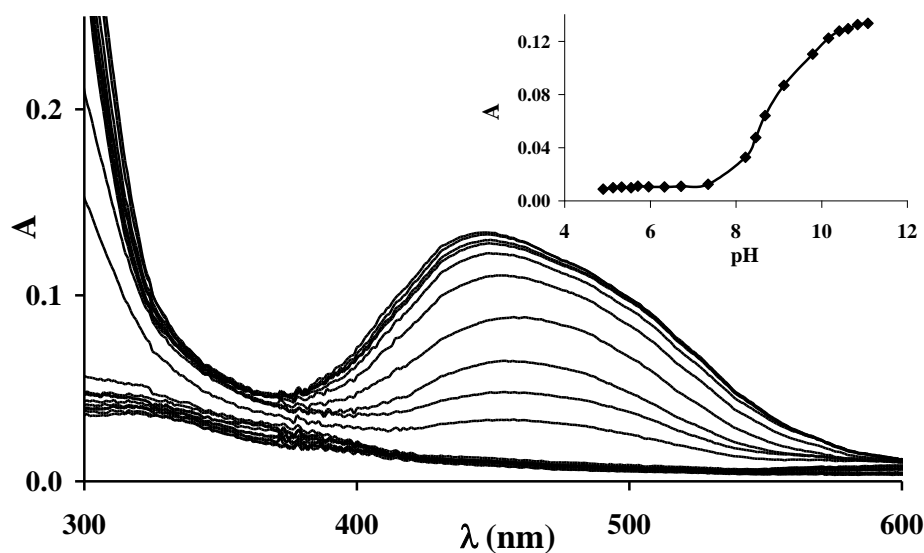


Figure S8: pH-dependent UV-Vis spectra of Ni(II)- L^1 system, and the absorption measured at 450 nm as a function of pH ($[L^1]= 0.001$ M, $[Ni^{2+}]= 0.0005$ M, $T = 298$ K, $I= 0.1$ M NaCl),

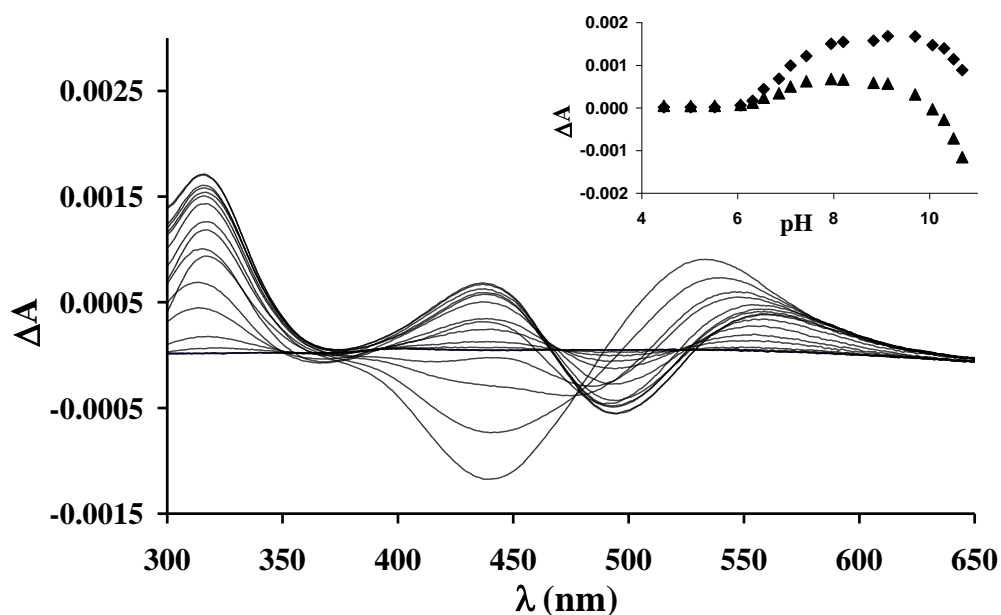


Figure S9: pH-dependent CD spectra of the nickel(II)- L^3 system ($[L^3] = 0.0007$ M, $[Ni^{2+}] = 0.0007$ M, $T = 298$ K, $I = 0.1$ M NaCl). The insert shows the pH dependence of the CD intensities at 312 (◆) and 435 nm (▲).

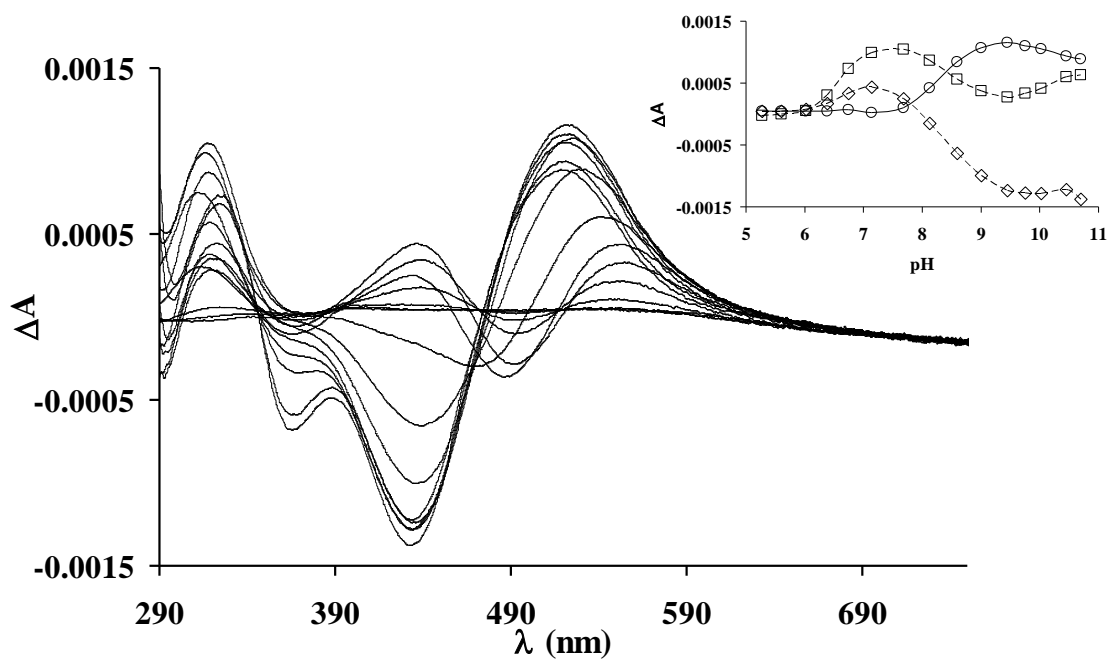


Figure S10: pH-dependent CD spectra of the nickel(II)- L^3 system ($[L^3] = 0.00064$ M, $[Ni^{2+}] = 0.00122$ M, $T = 298$ K, $I = 0.1$ M NaCl). The insert shows the pH dependence of the CD intensities at 316 (□), 433 (◇) and 520 (○) nm.

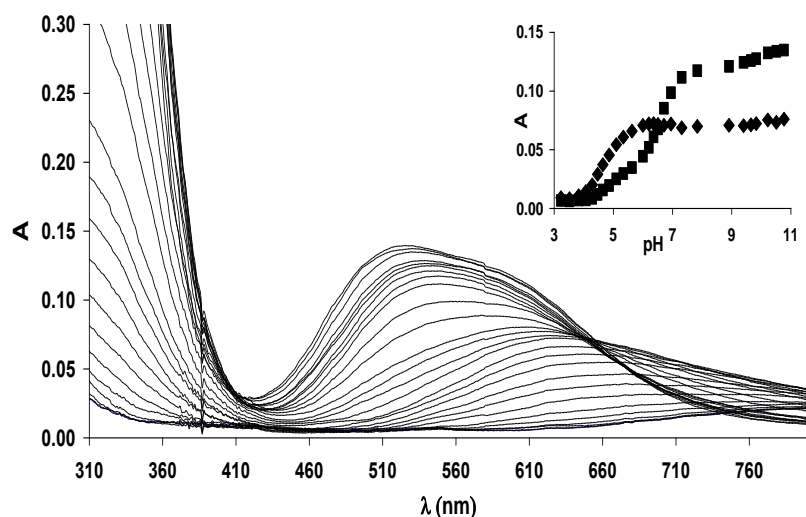


Figure S11: UV-Vis spectra of the copper(II)- L^1 system as a function of pH ($[L^1]=0.0013$ M, $[Cu^{2+}]=0.0013$ M, $T=298$ K, $I=0.1$ M NaCl). The insert shows the pH-dependence of absorbances at 550 (■) and 650 nm (◆).

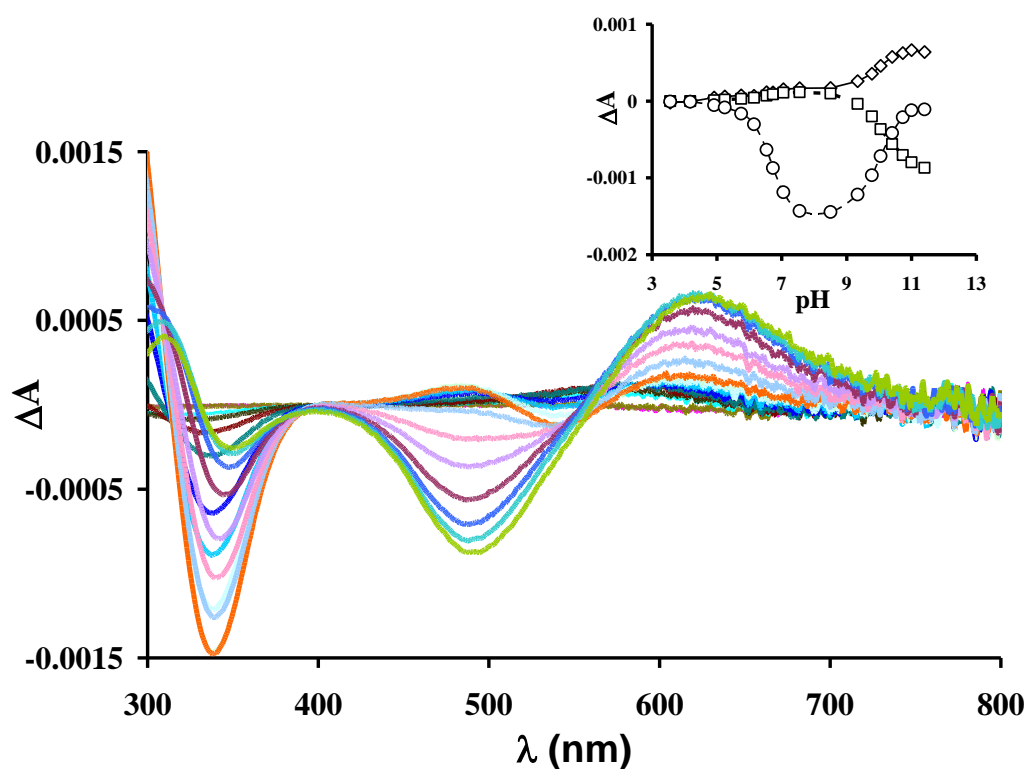


Figure S12: CD spectra of the copper(II)- L^1 system as a function of pH ($[L^1]=[Cu^{2+}]=8\times 10^{-4}$ M, $I=0.1$ M NaCl, $T=298$ K, the insert shows the absorbances at $\lambda=335$ (○), 490(□) and 620(◇) nm).

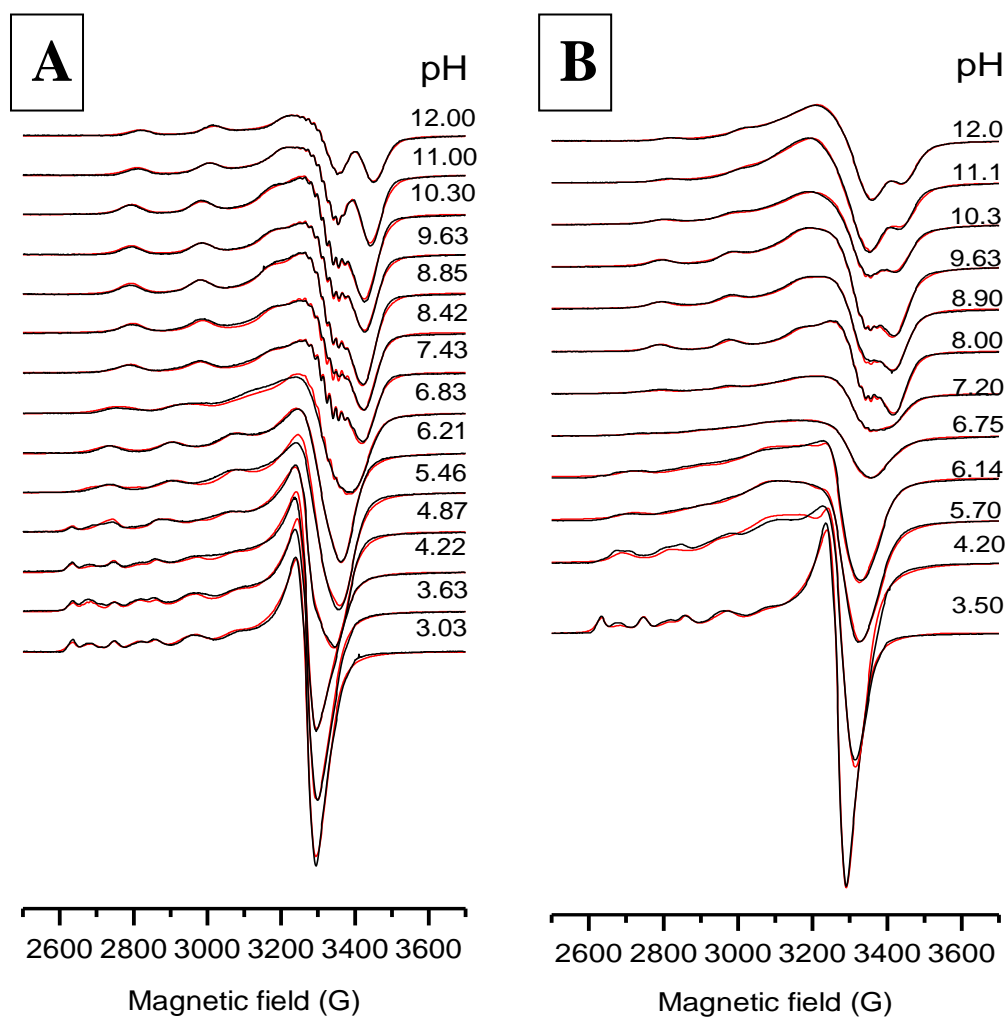


Figure S13: The measured (black) and calculated (red) anisotropic EPR spectra of the copper(II)-L1 system as a function of pH (A: $[Cu^{2+}] = 0.00130$ M, $[L^1] = 0.00131$ M; B: $[Cu^{2+}] = 0.00213$ M, $[L^1] = 0.00118$ M, T = 80 K).

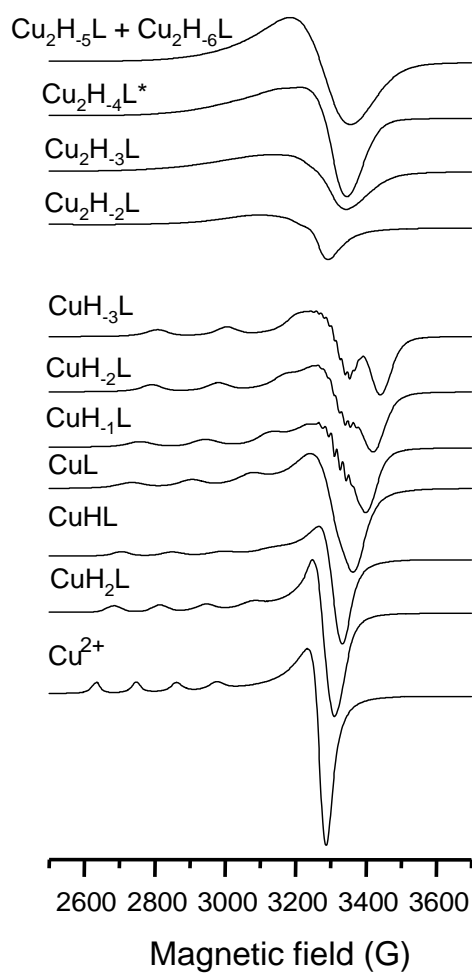


Figure S14: The calculated individual anisotropic EPR spectra of the specieses formed in the copper(II)- L^1 system

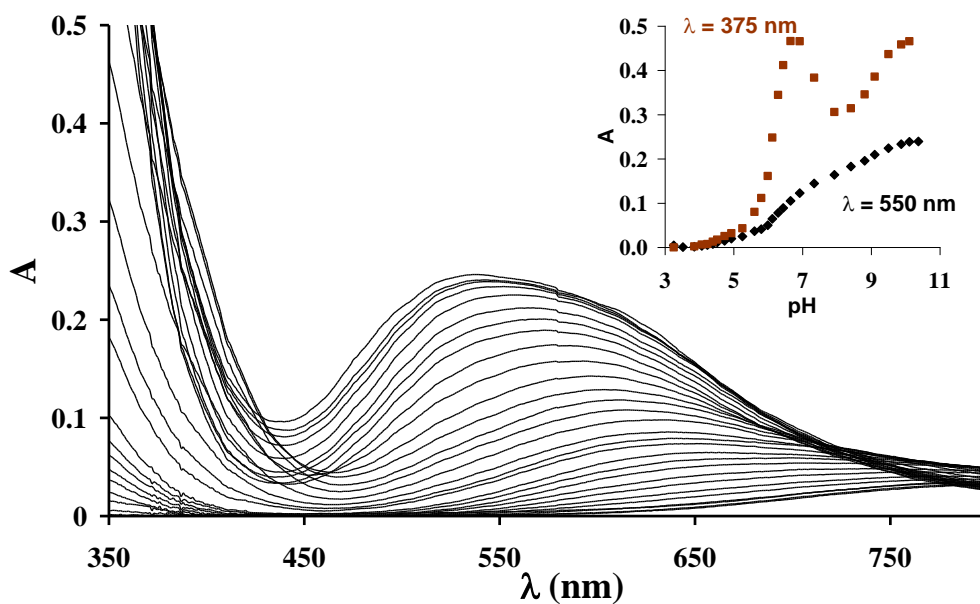


Figure S15: UV-Vis spectra of the copper(II)-L¹ system at 1:2 L¹/Cu²⁺ ratio as a function of pH ([Cu²⁺] = 0.00241 M, [L¹] = 0.00127 M, T = 298 K, the insert shows the pH-dependence of the absorbances at 375 and 550 nm).

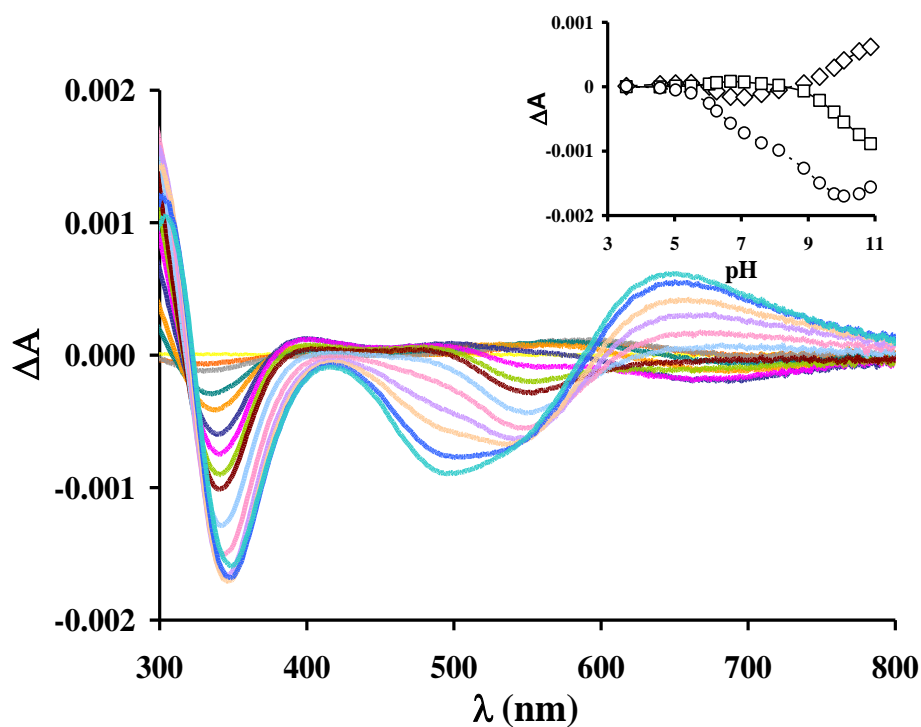


Figure S16: CD spectra of the copper(II)-L¹ system at 1:2 L¹/Cu²⁺ ratio as a function of pH pH ([L¹] = 0.000697 M, [Cu²⁺] = 0.00132 M, I = 0.1 M NaCl, T = 298 K, the insert shows the absorbances at λ = 345(○), 490(□) and 645(◇) nm).