

Metal ions coordination and therapeutic potential of peptides derived from milk fermented with kombucha cultures.

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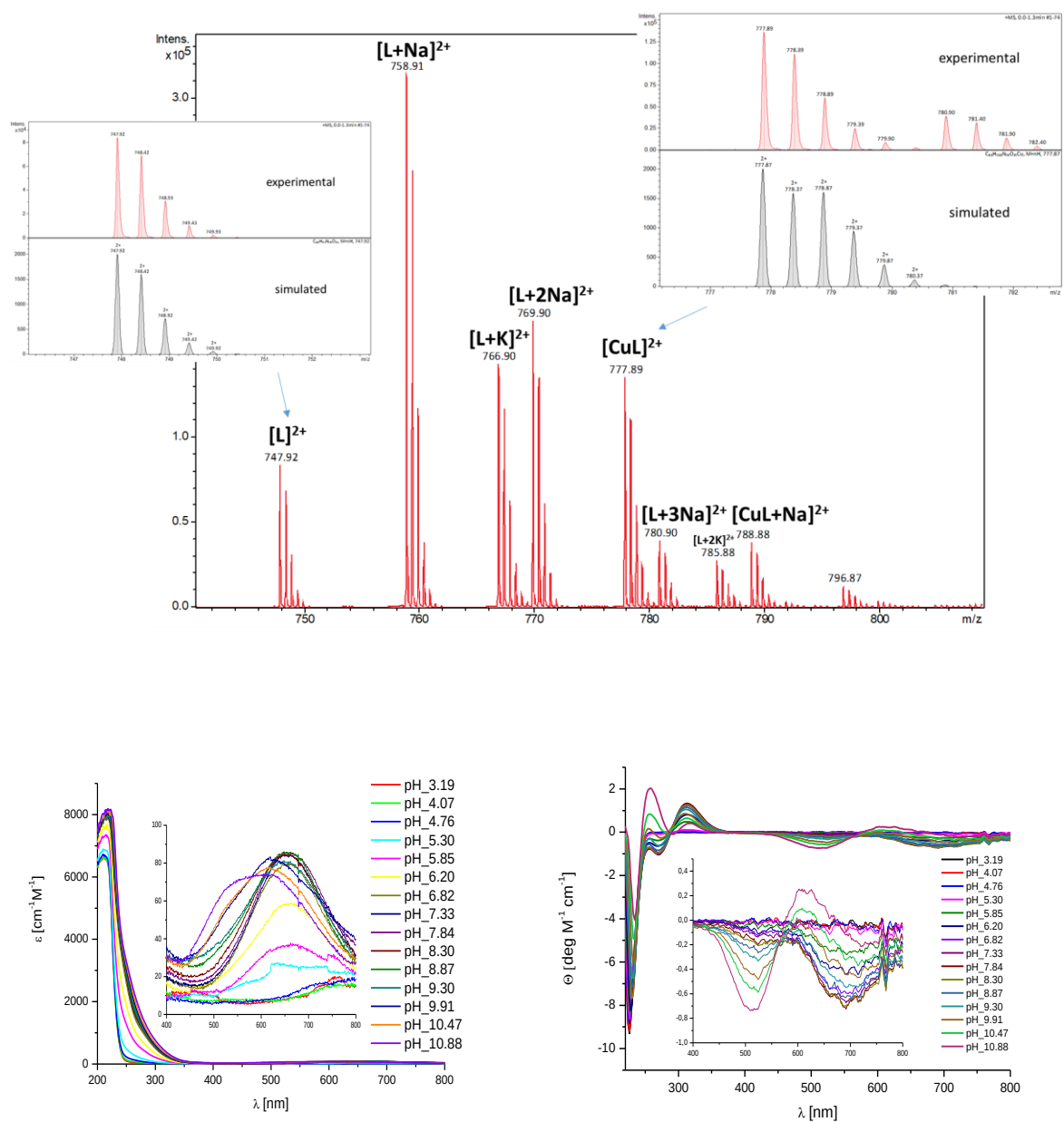


Figure S1. The upper panel show MS spectra of Cu(II)- AVPQEVLNENLLR (experimental and simulated) and the lower panel displays UV-Vis (left) and CD-spectroscopy (right) results of the Cu(II)-Pep1 titration over the pH range 3-11.

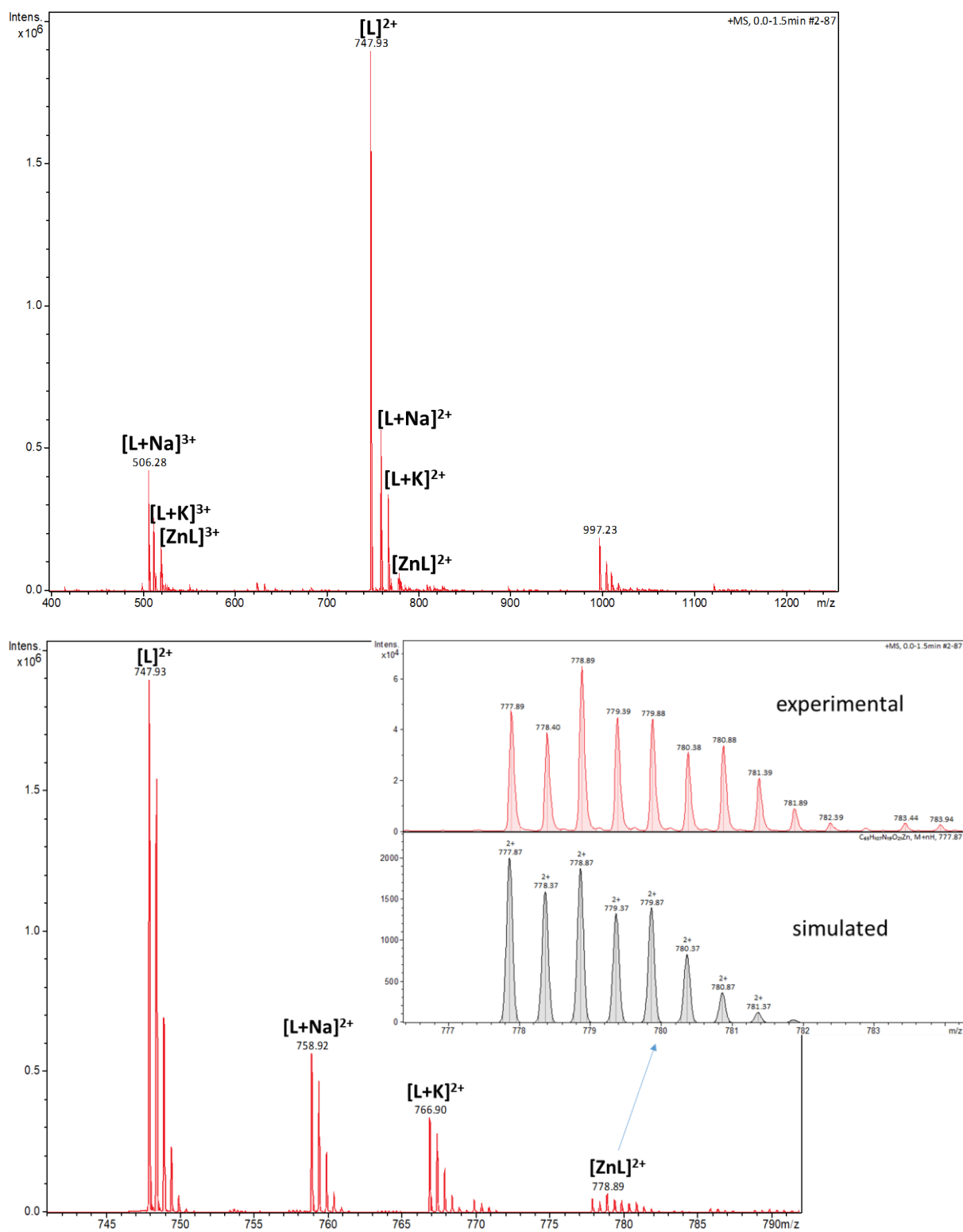


Figure S2. MS spectra of Zn (II)- AVPQEVLENLLR. The upper panel shows the whole range and the lower panel displays chosen MS region (experimental and simulated)

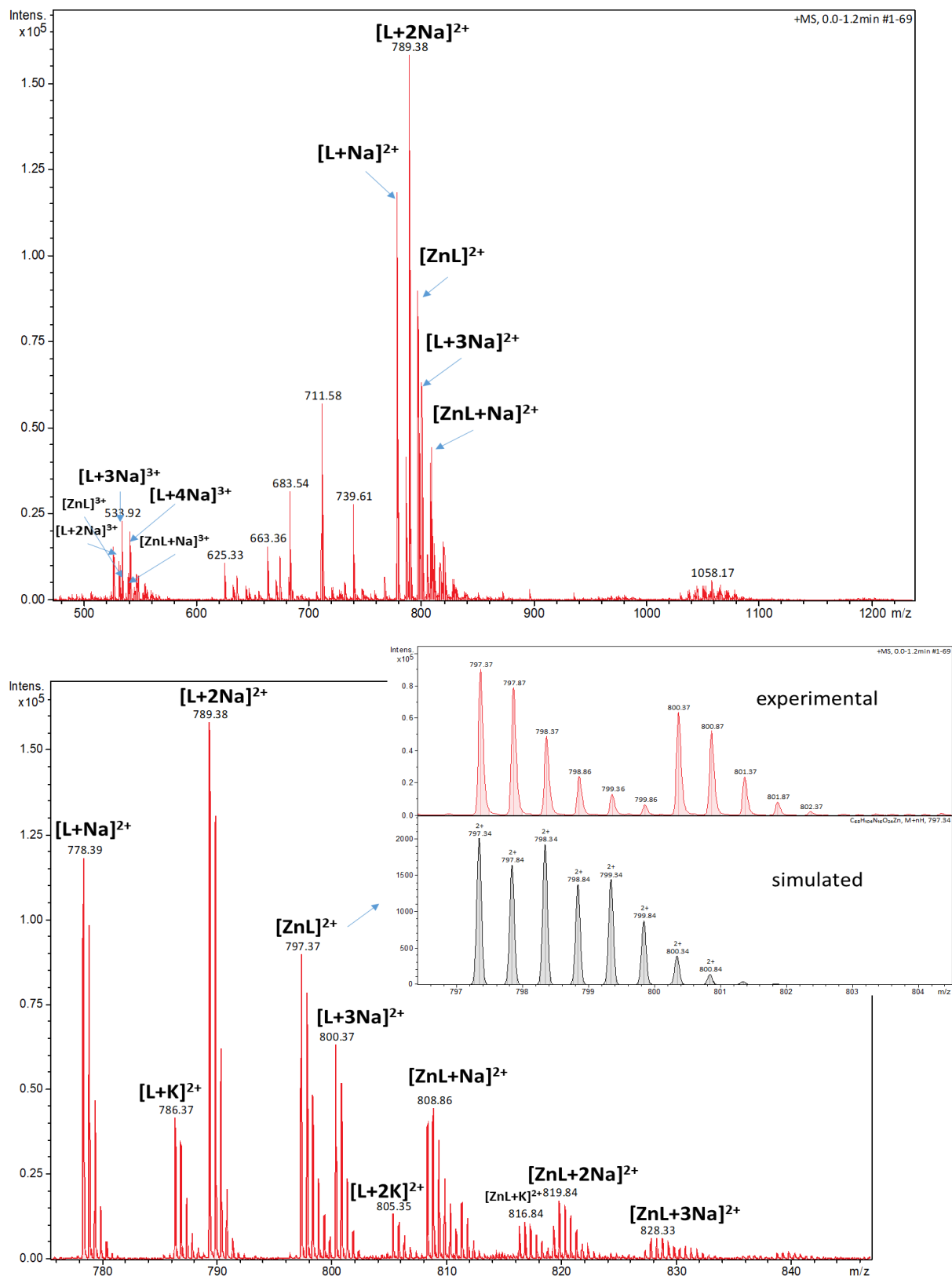


Figure S4. MS spectra of Zn (II)- YLQGSNLVVPLTDD. The upper panel shows the whole range and the lower panel displays chosen MS region (experimental and simulated)

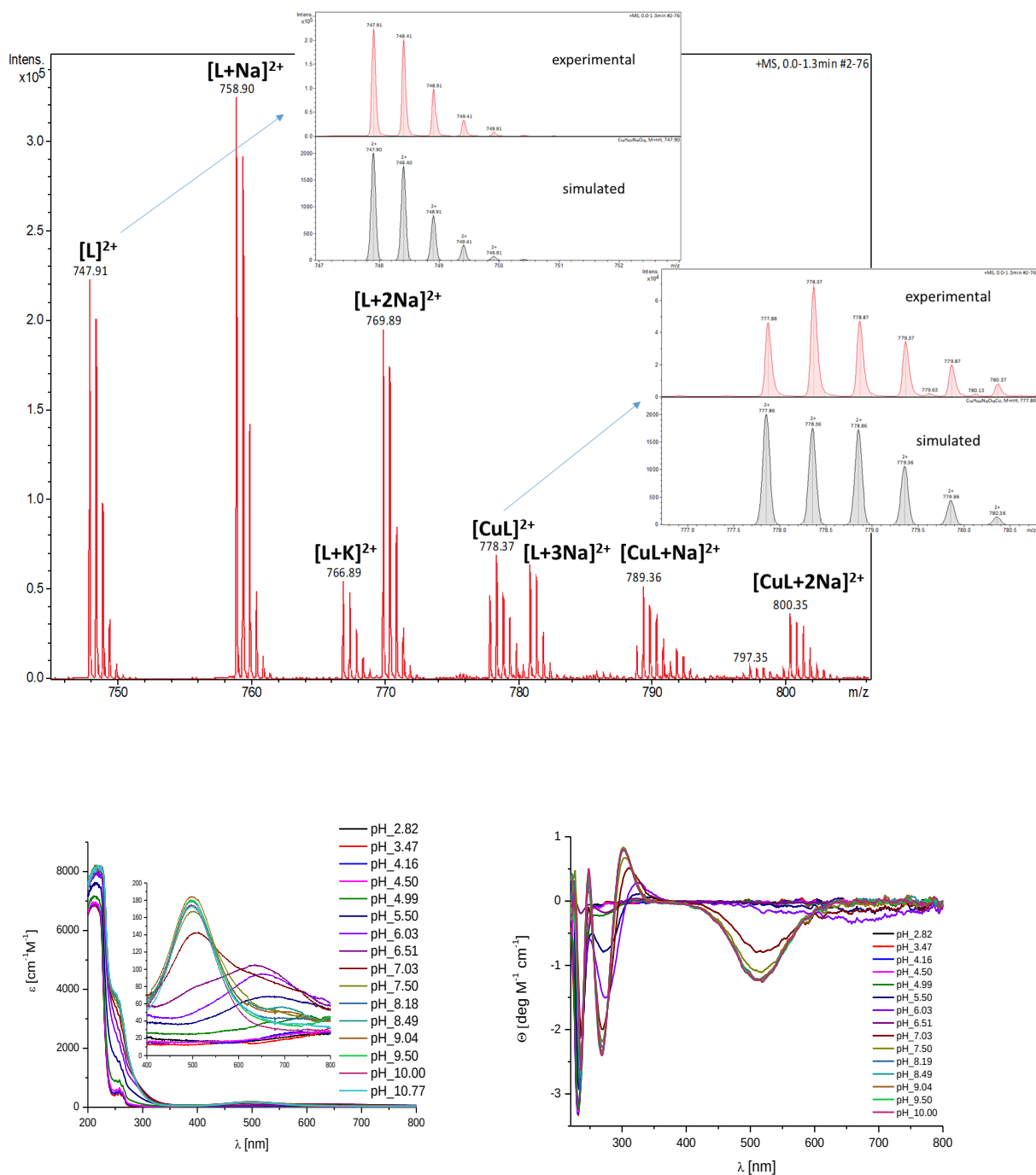


Figure S5. The upper panel shows MS spectra of Cu(II)- KFKGFVEPFPAVE (experimental and simulated) and the lower panel displays UV-Vis (left) and CD-spectroscopy (right) results of the Cu(II)-Pep3 titration over the pH range 3-11.

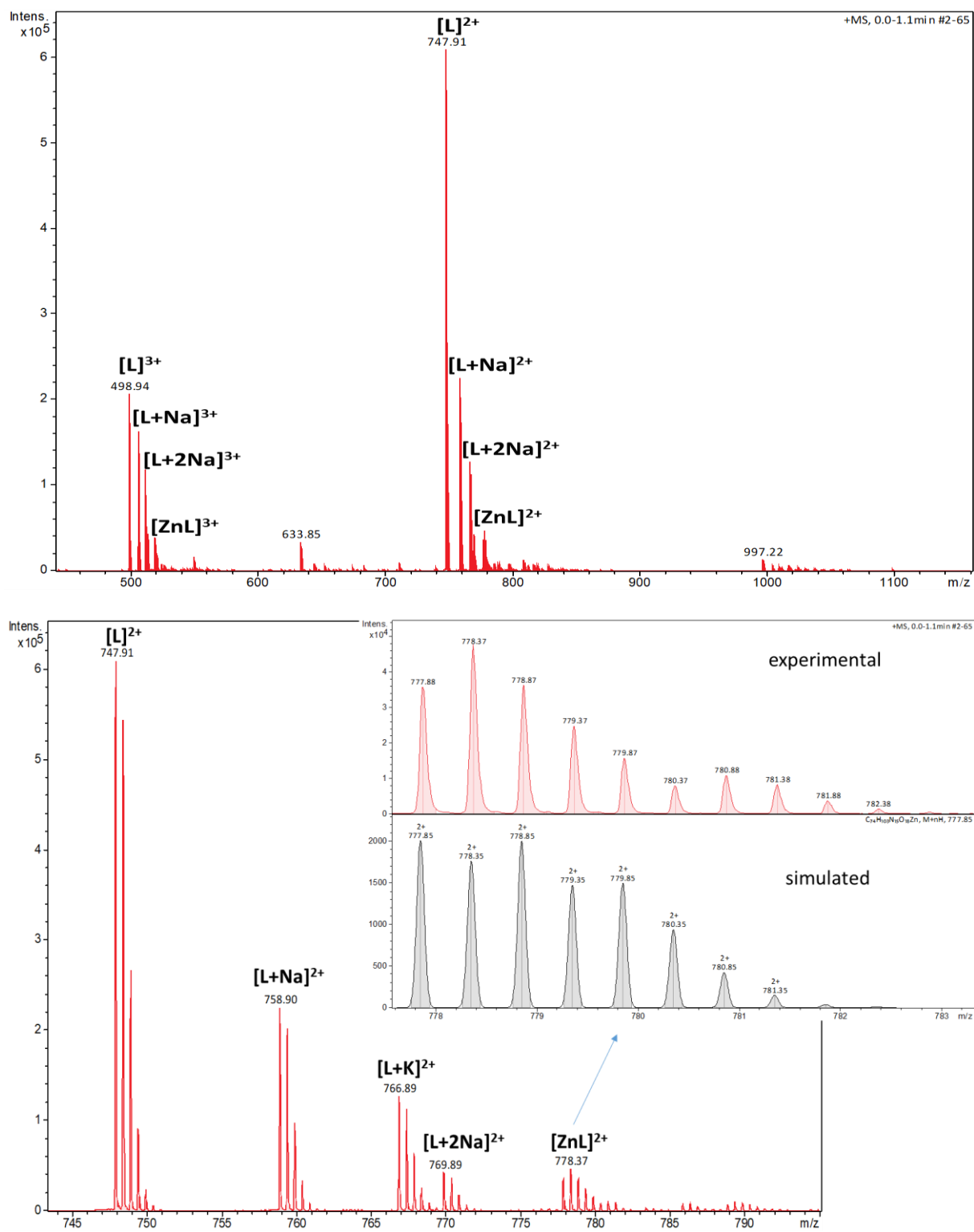


Figure S6. MS spectra of Zn (II)- KFKGFVEFPFAVE. The upper panel shows the whole range and the lower panel displays chosen MS region (experimental and simulated)

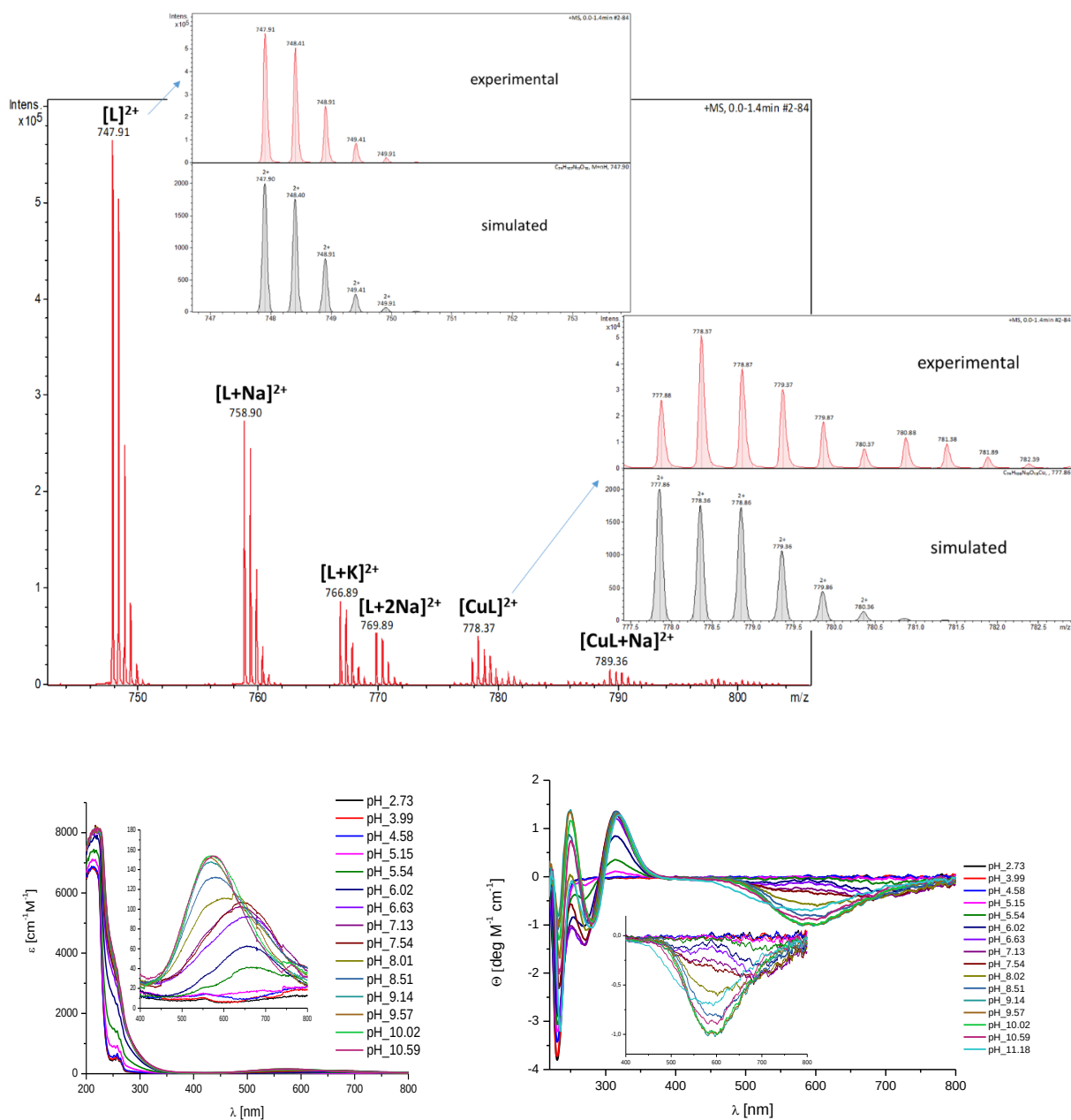


Figure S7. The upper panel show MS spectra of Cu(II)- FVAPEPFVFGKEK (experimental and simulated) and the lower panel displays UV-Vis (left) and CD-spectroscopy (right) results of the Cu(II)-Pep4 titration over the pH range 3-11.

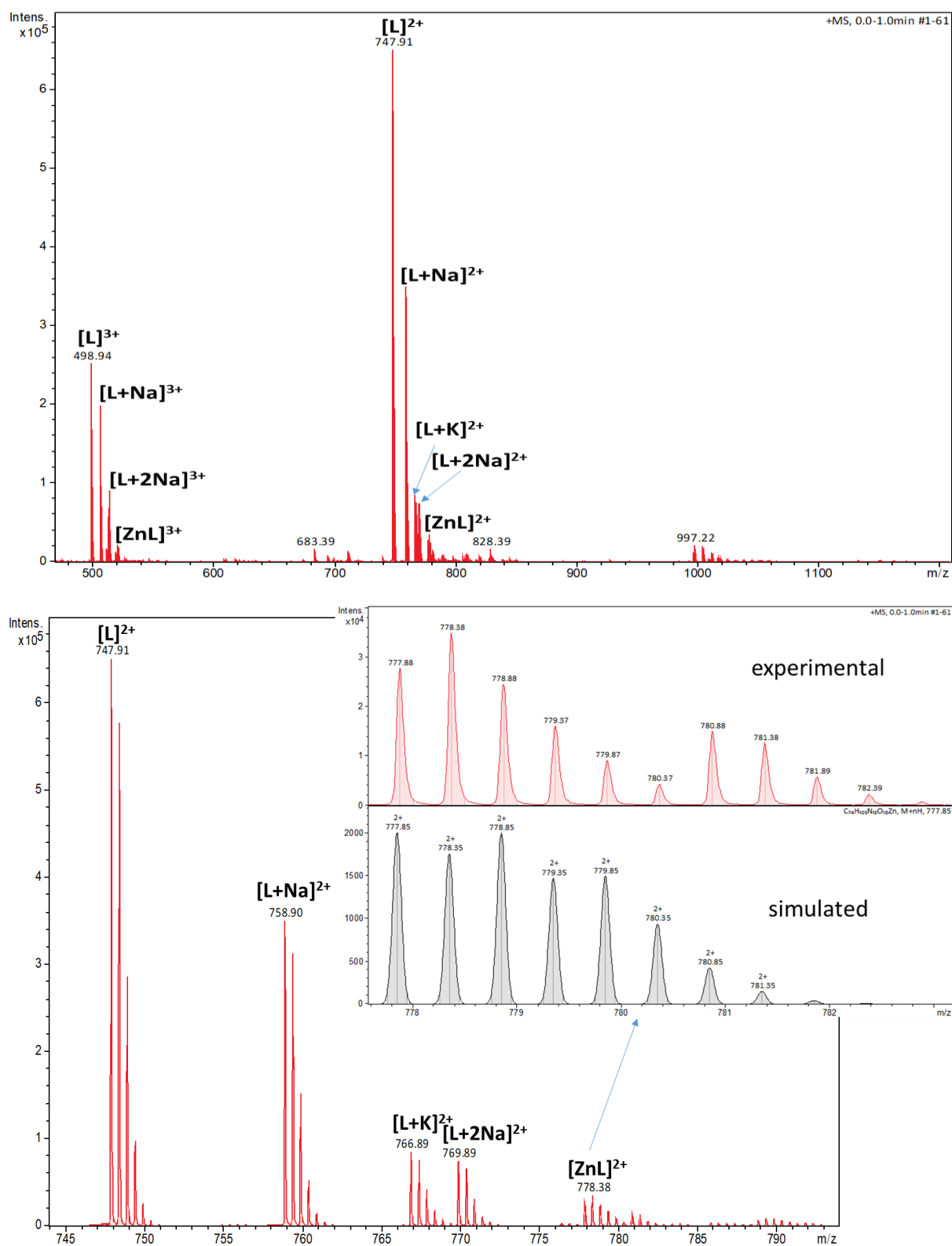


Figure S8. MS spectra of Zn (II)- FVAPEPFVFGKEK . The upper panel shows the whole range and the lower panel displays chosen MS region (experimental and simulated)

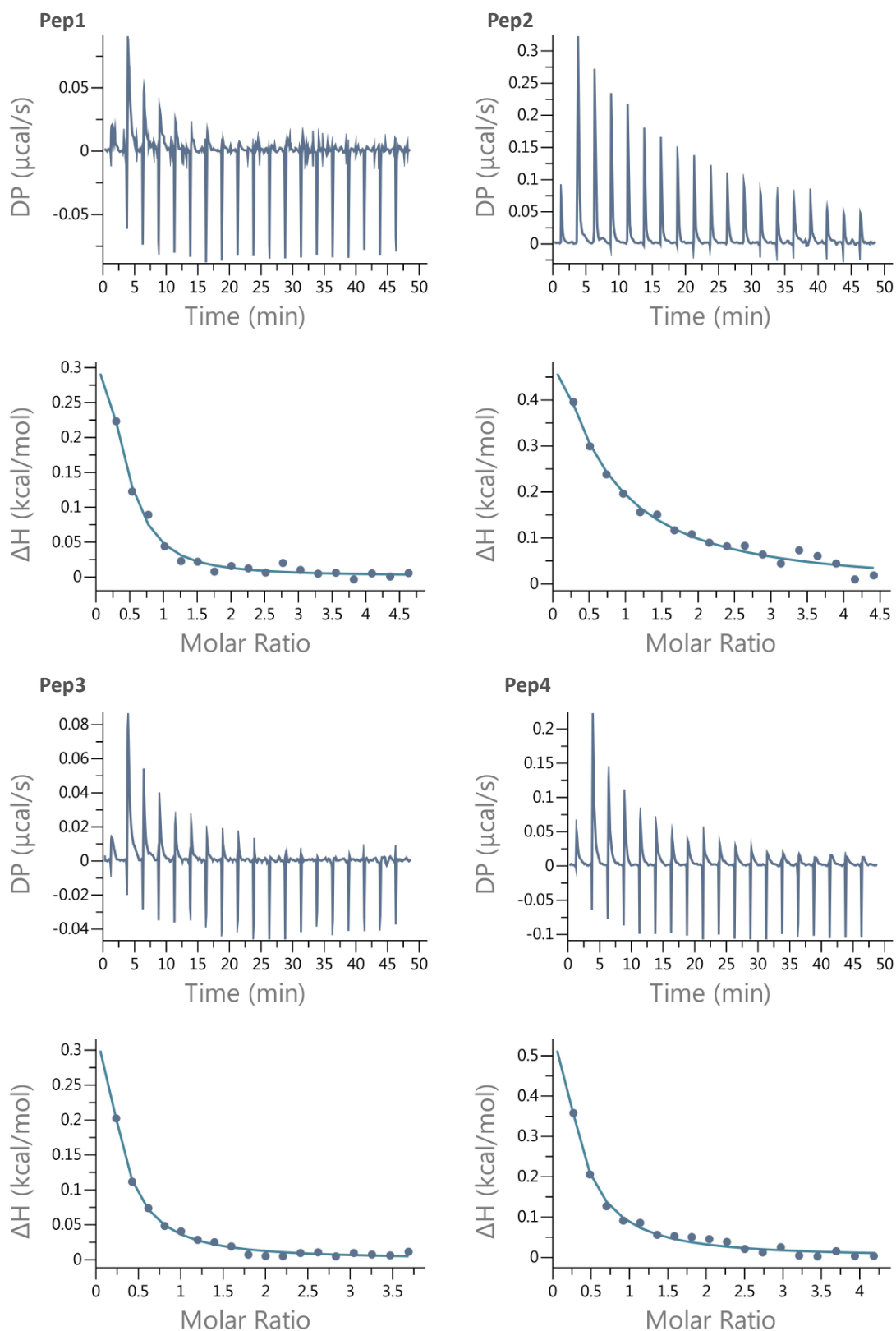
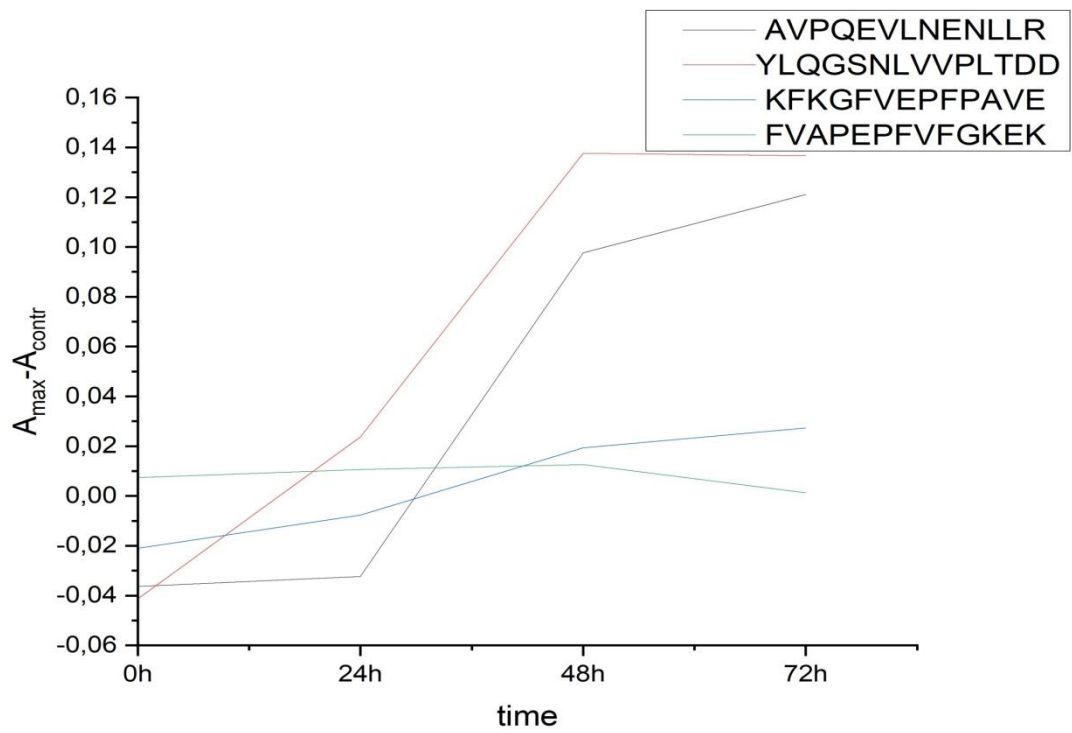
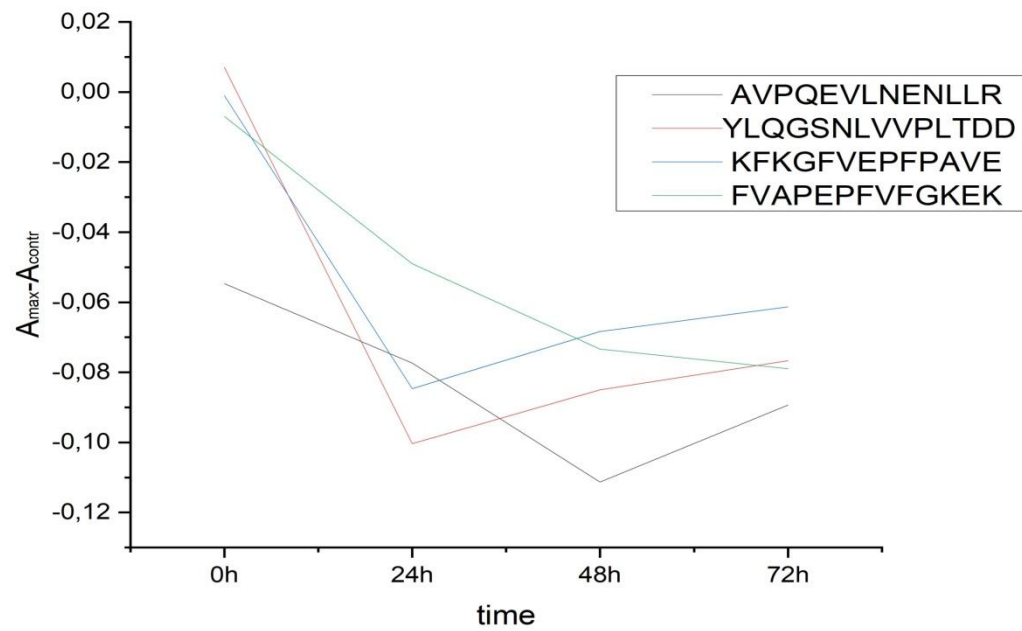


Figure S9. ITC traces and binding isotherms of titration of the Pep1-Pep4 solutions with Zn(II) ions.



A



B

Figure S10. Growth of *Candida albicans* cells on a Sabouraud dextrose liquid medium (pH 5.85) containing peptides (0.5mg/ml each) without Cu (II) (A) and with addition of Cu(II) ions (B).

Table S1. Potentiometric data for proton and Zn(II) complexes with Pep1. Titrations were carried out over the pH range 2–11 at T = 298 K in an aqueous solution with 4 mM HClO₄ and 0.1 M NaClO₄. Standard deviations are shown in brackets.

species	log β	pKa	Proposed donors
HL	7.94(2)	7.94 (N-terminal NH ₃ ⁺)	
H ₂ L	12.78(3)	4.84 (E)	
H ₃ L	16.66(3)	3.88 (E)	
H ₄ L	19.17(5)	2.51 (C-terminal COOH)	
ZnH ₂ L	18.67(6)		
ZnHL	14.57(6)	4.10	E
ZnL	9.87(8)	4.70	NH ₂
			NH ₂ ,H ₂ O
ZnH ₂ L	-5.90(9)		NH ₂ ,2H ₂ O
ZnH ₃ L	-13.78(9)	7.88	NH ₂ ,3H ₂ O

Table S2. Potentiometric data for proton and Zn(II) complexes with Pep2. Titrations were carried out over the pH range 2–11 at T = 298 K in an aqueous solution with 4 mM HClO₄ and 0.1 M NaClO₄. Standard deviations are shown in brackets.

species	log β	pKa	proposed donors
HL	9.71(1)	9.71(Y)	
H ₂ L	16.92(2)	7.21(N-terminal NH ₃ ⁺)	
H ₃ L	21.87(3)	4.95(D)	
H ₄ L	25.79(3)	3.92(D)	
H ₅ L	28.33(5)	2.54(C-terminal COOH)	
ZnH ₃ L	26.91(2)		
ZnH ₂ L	22.73(4)	4.18	D
ZnHL	17.66(6)	5.07	NH ₂
ZnL	9.86(8)	7.80	NH ₂ ,H ₂ O
ZnH ₁ L	-		NH ₂ ,2H ₂ O
ZnH ₂ L	-5.92(6)		NH ₂ ,3H ₂ O
ZnH ₃ L	-15.54(7)	9.62	NH ₂ ,3H ₂ O

Table S3. Potentiometric data for proton and Zn(II) complexes with Pep3. Titrations were carried out over the pH range 2–11 at T = 298 K in an aqueous solution with 4 mM HClO₄ and 0.1 M NaClO₄. Standard deviations are shown in brackets.

species	log β	pKa	Proposed donors
HL	11.00(1)	11.00(K)	
H ₂ L	20.94(1)	9.94(K)	
H ₃ L	28.11(2)	7.17(N-terminal NH ₃ ⁺)	
H ₄ L	32.92(2)	4.81 (E)	
H ₅ L	36.93(2)	4.01 (E)	
H ₆ L	39.76(2)	2.83 (C-terminal COOH)	
ZnH ₃ L	33.45(7)		
ZnH ₂ L	28.36(9)	5.09	NH ₂
ZnHL	21.07(9)	7.29	NH ₂ ,H ₂ O
ZnL	-		NH ₂ ,2H ₂ O
ZnH ₁ L	-		NH ₂ ,3H ₂ O
ZnH ₂ L	-2.69(9)		NH ₂ ,4H ₂ O

Table S4. Potentiometric data for proton and Zn(II) complexes with Pep4. Titrations were carried out over the pH range 2–11 at T = 298 K in an aqueous solution with 4 mM HClO₄ and 0.1 M NaClO₄. Standard deviations are shown in brackets.

species	log β	pKa	Proposed donors
HL	10.61(1)	10.61 (K)	
H ₂ L	20.22(1)	9.61(K)	
H ₃ L	27.49(3)	7.27 (N-terminal NH ₃ ⁺)	
H ₄ L	32.35(4)	4.86 (E)	
H ₅ L	36.33(4)	3.98 (E)	
ZnH ₃ L	33.93(6)		
ZnH ₂ L	29.66(5)	4.27	NH ₂
ZnHL	24.63(8)	5.03	NH ₂ ,H ₂ O
ZnL	16.72(9)	7.91	NH ₂ ,2H ₂ O
ZnH ₁ L	-		NH ₂ ,3H ₂ O
ZnH ₂ L	1.49(8)		NH ₂ ,4H ₂ O