Zn(II) coordination influences the secondary structure, but not antimicrobial activity of the N-terminal histatin 3 hydrolysis product

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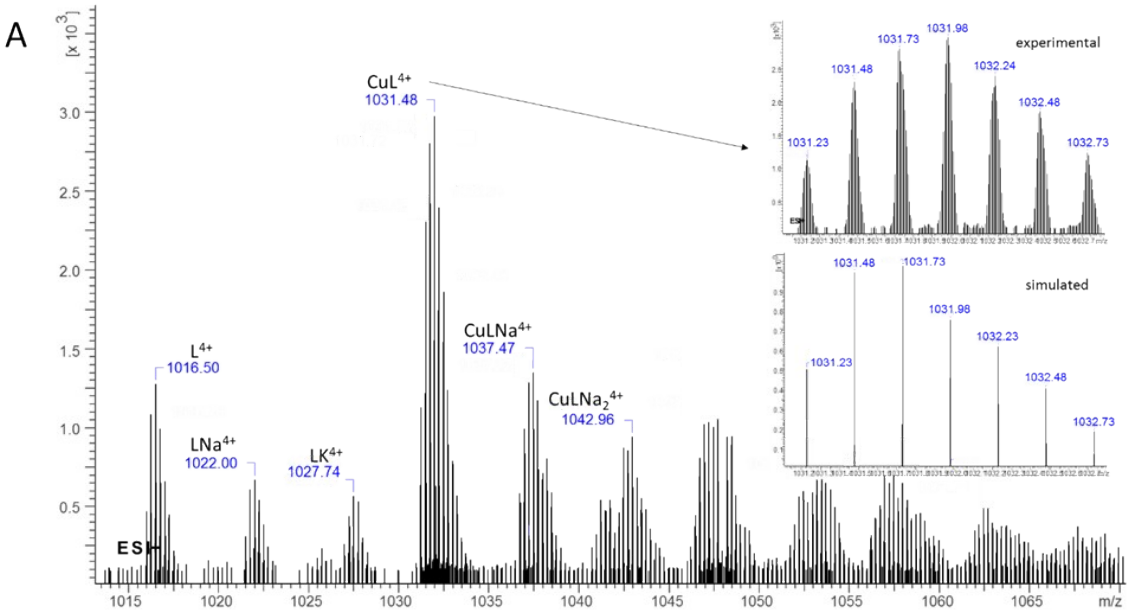

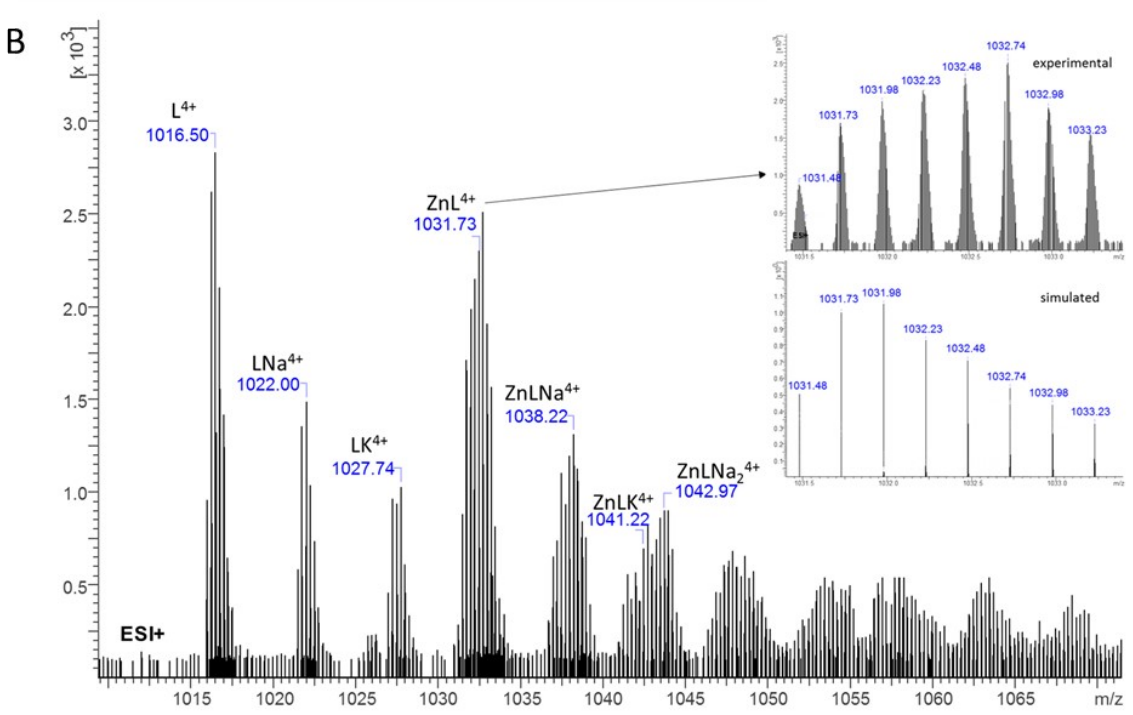
B
[x 10^3]
3.0
2.5
2.0
1.5
1.0
0.5
L4+
1016.50
LNa4+
1022.00
LK4+
1027.74
ZnL4+
1031.73
ZnLNa4+
1038.22
ZnLK4+
1041.22
ZnLNa2 4+
1042.97
ESI+
1015
1020
1025
1030
1035
1040
1045
1050
1055
1060
1065
m/z
experimental
1031.48
1031.73
1031.98
1032.23
1032.48
1032.74
1032.98
1033.23
simulated
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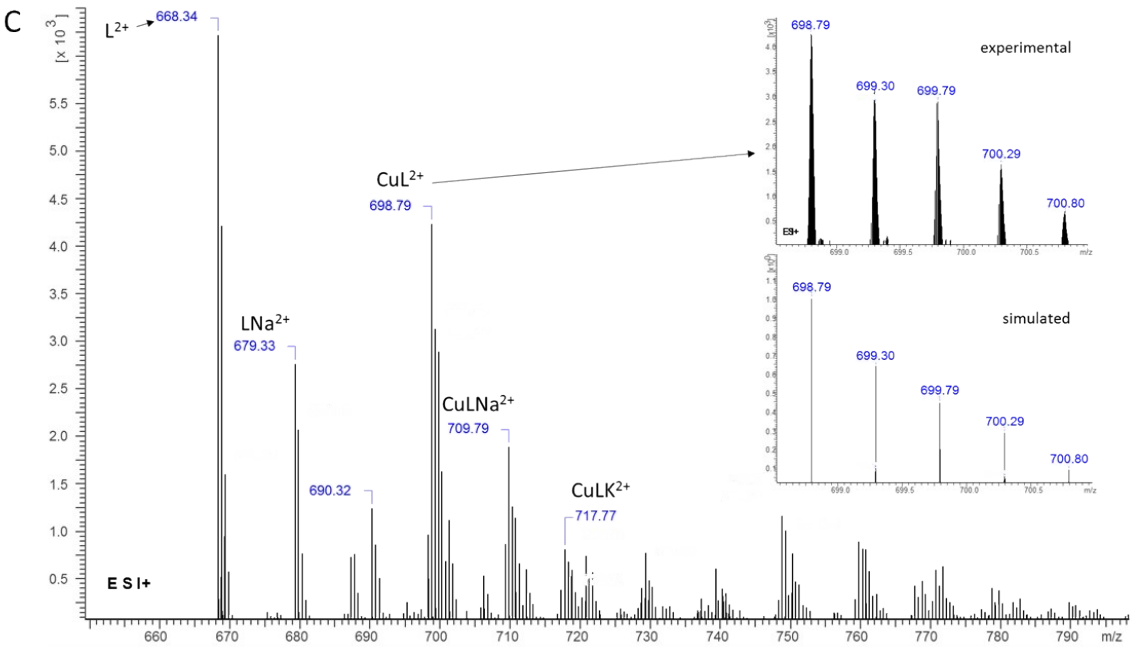
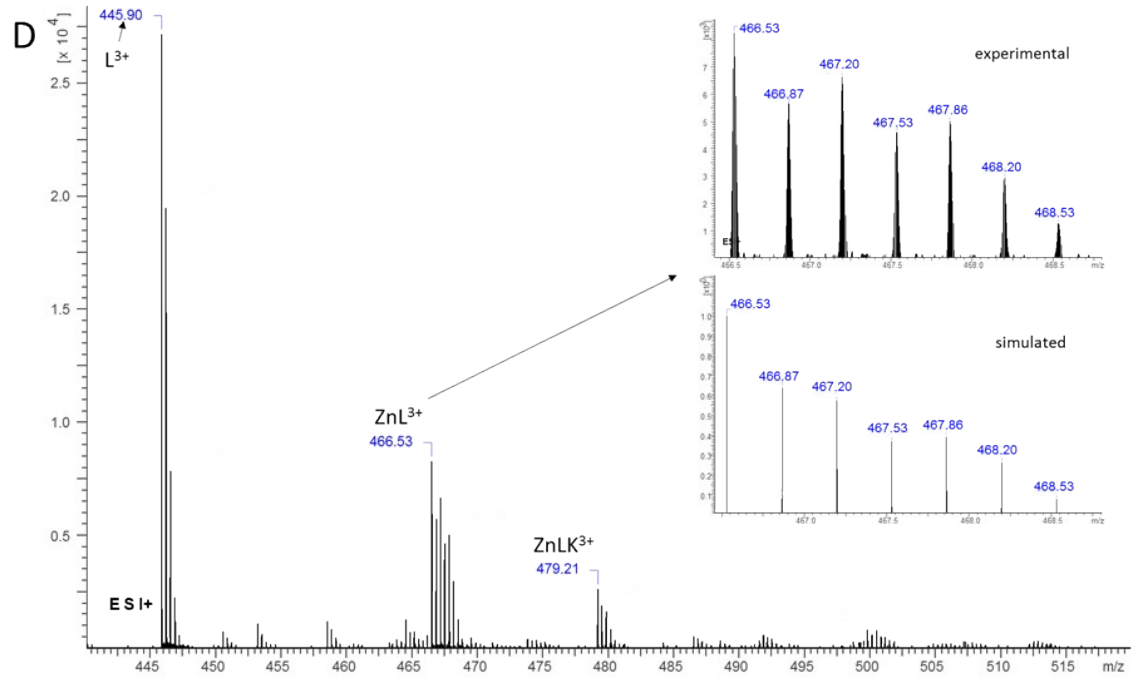
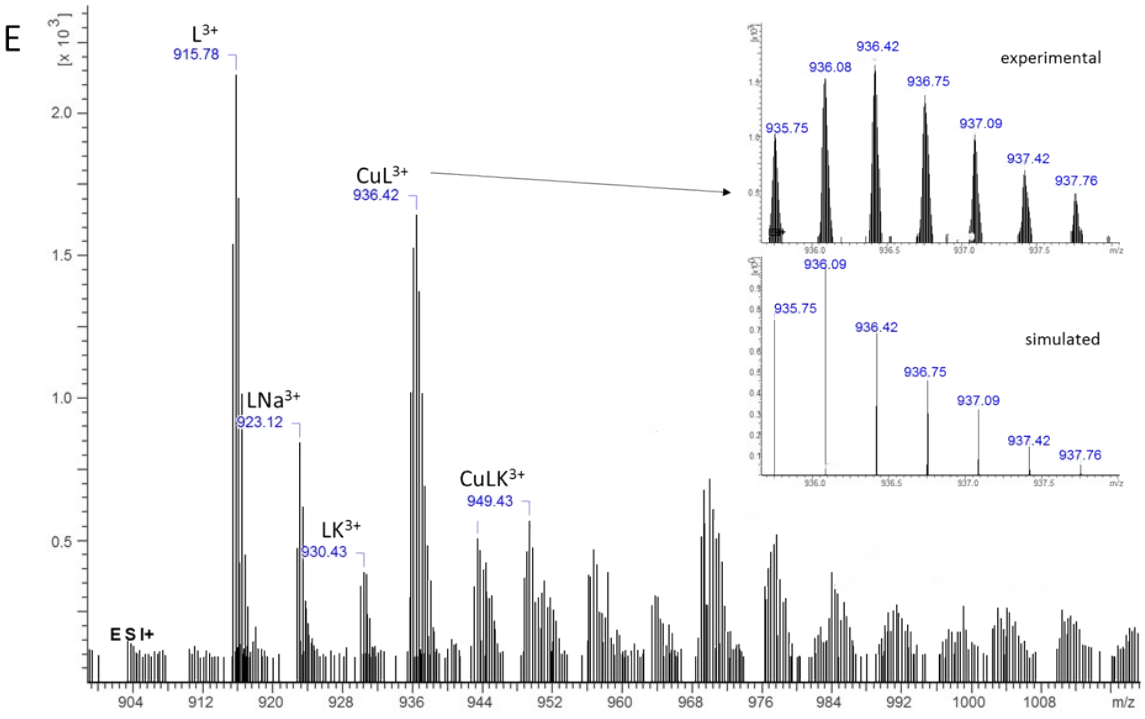
1031.73
1031.98
1032.23
1032.48
1032.74
1032.98
1033.23


C
[x 10^3]
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
L2+
668.34
LNa2+
679.33
690.32
CuL2+
698.79
CuLNa2+
709.79
CuLK2+
717.77
E S I+
660
670
680
690
700
710
720
730
740
750
760
770
780
790
m/z
experimental
698.79
699.30
699.79
700.29
700.80
ESI+
simulated
698.79
699.30
699.79
700.29
700.80


D
445.90
L3+
ZnL3+
466.53
ZnLK3+
479.21
ESI+
experimental
466.53
466.87
467.20
467.53
467.86
468.20
468.53
simulated
466.53
466.87
467.20
467.53
467.86
468.20
468.53
m/z


E
L3+
915.78
CuL3+
936.42
LNa3+
923.12
LK3+
930.43
CuLK3+
949.43
ESI+
experimental
936.42
936.08
936.75
935.75
937.09
937.42
937.76
simulated
936.09
935.75
936.42
936.75
937.09
937.42
937.76
m/z

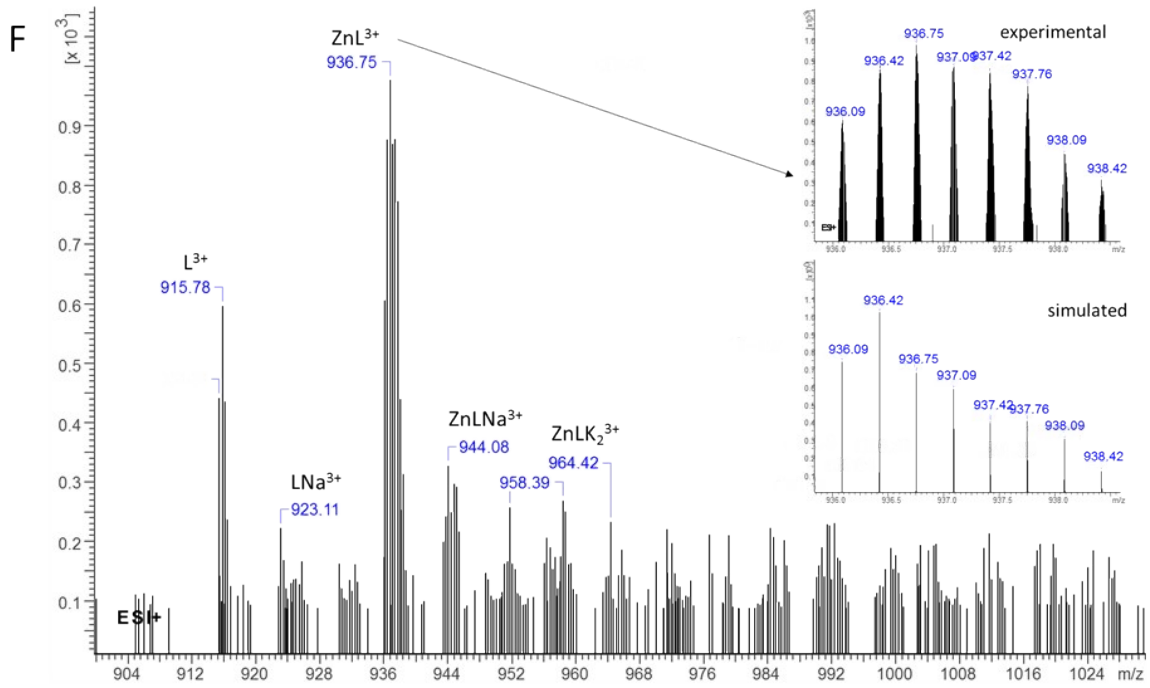
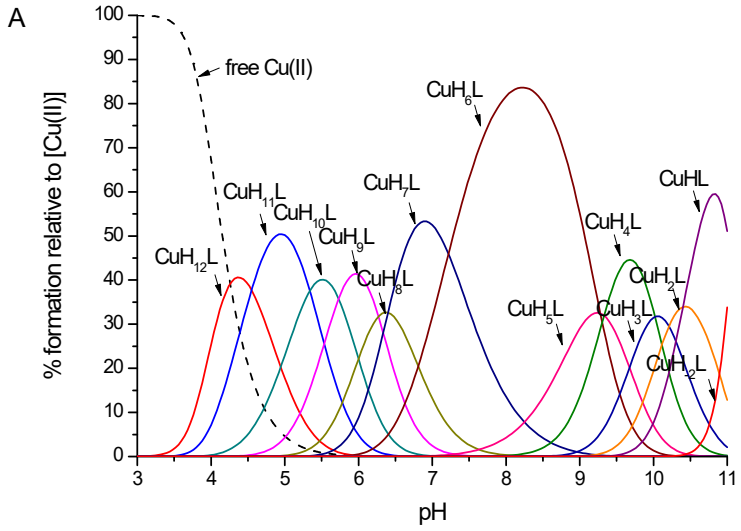


Fig. S1. ESI-MS spectra of: A) Cu(II)-histatin 3; B) Zn(II)-histatin 3; C) Cu(II)-histatin 3-4; D) Zn(II)-histatin 3-4; E) Cu(II)-histatin 4; F) Zn(II)-histatin 4; M:L molar ratio = 1:1, pH = 7.4 for Cu(II) complexes and pH = 8.0 for Zn(II) complexes.



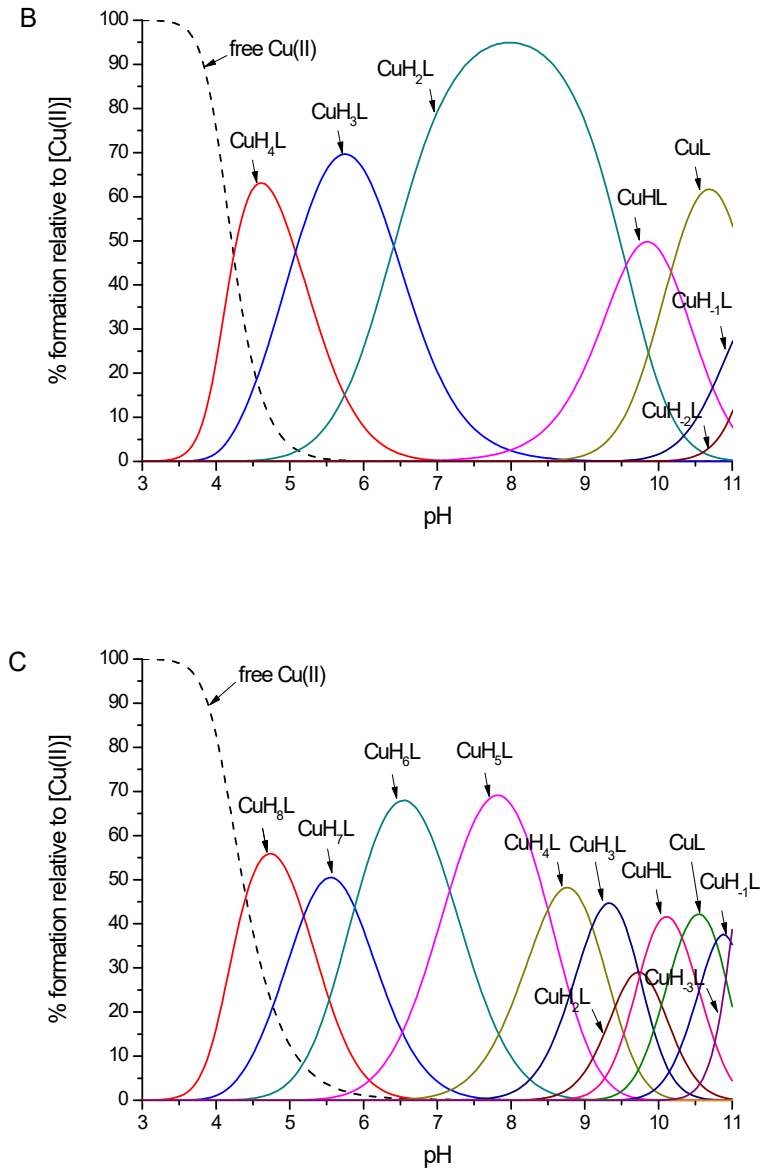
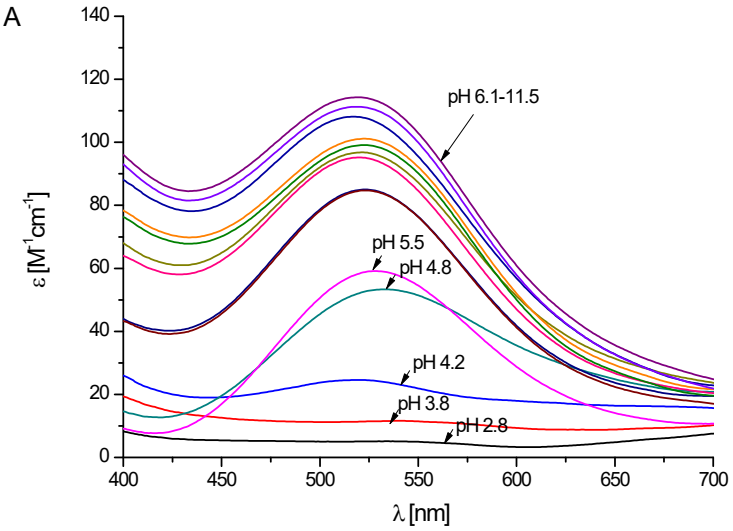
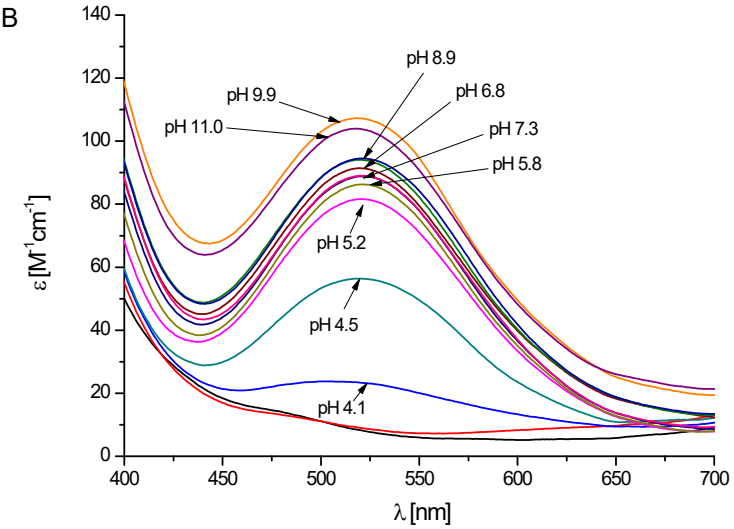


Fig. S2. Distribution diagrams for the formation of: A) Cu(II) complex with histatin 3; B) Cu(II) complex with histatin 3-4; C) Cu(II) complex with histatin 4. Conditions: T = 298 K, *I* = 0.1 M $NaClO_4$, [Cu(II)] = $0.4x10^{-3}$ M; M:L molar ratio = 0.8:1.

A


pH 6.1-11.5
pH 5.5
pH 4.8
pH 4.2
pH 3.8
pH 2.8
ε [M-1cm-1]
λ [nm]

B


pH 8.9
pH 9.9
pH 6.8
pH 11.0
pH 7.3
pH 5.8
pH 5.2
pH 4.5
pH 4.1
ε [M-1cm-1]
λ [nm]

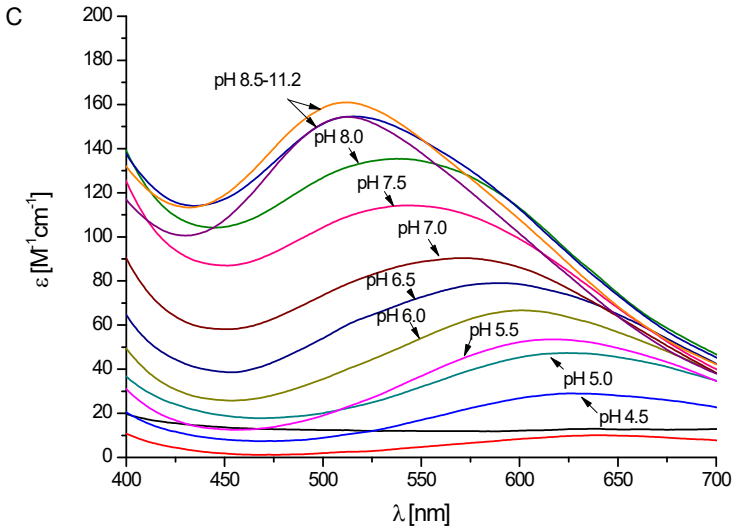
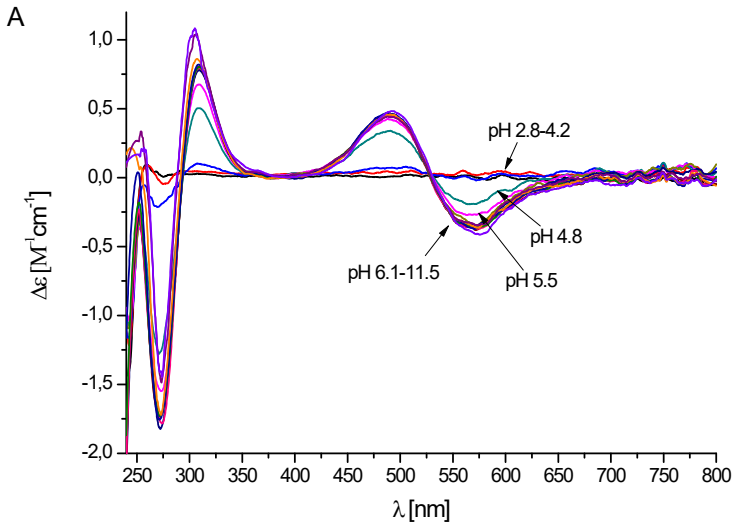


Fig. S3. UV-Vis spectra of Cu(II) complexes with: A) histatin 3; B) histatin 3-4; C) histatin 4; in pH range 2-11. Conditions: T = 298 K, *I* = 0.1 M $NaClO_4$, [Cu(II)] = $0.4.10^{-3}$ M; M:L molar ratio = 0.8:1.



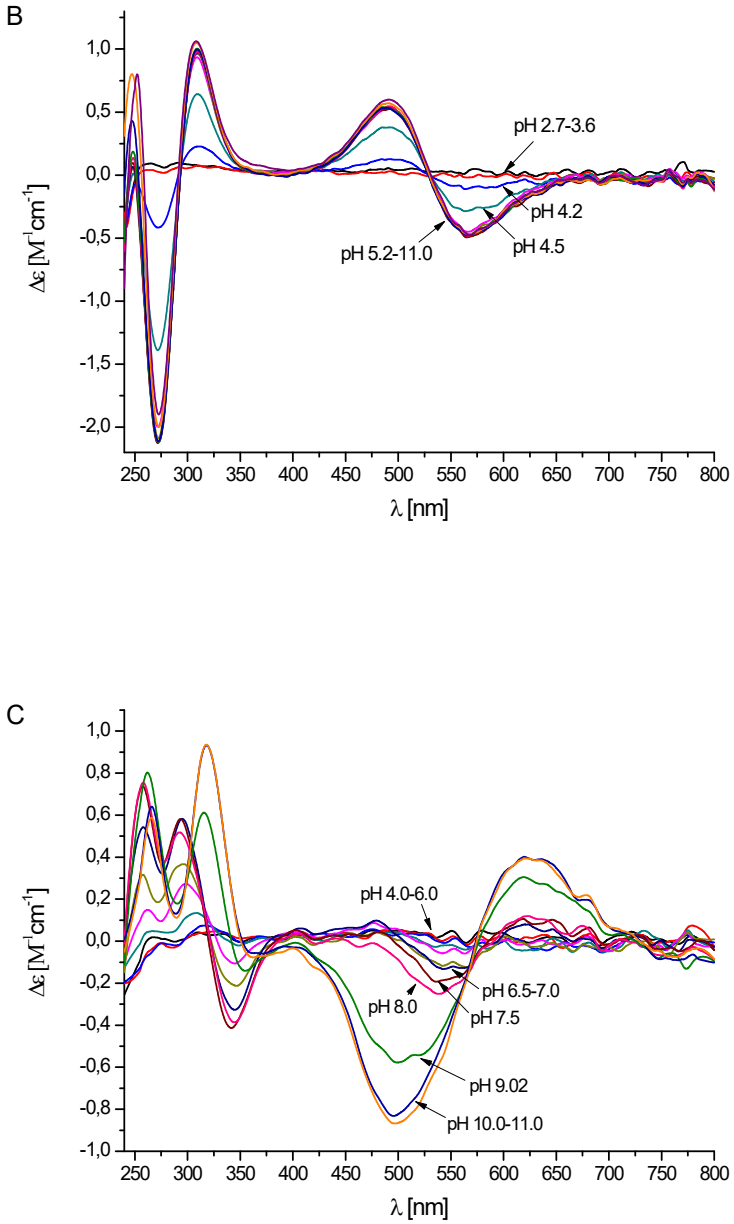


Fig. S4. CD spectra of Cu(II) complexes with: A) histatin 3; B) histatin 3-4; C) histatin 4; in pH range 2-11. Conditions: T = 298 K, *I* = 0.1 M $NaClO_4$, [Cu(II)] = $0.4.10^{-3}$ M; M:L molar ratio = 0.8:1.

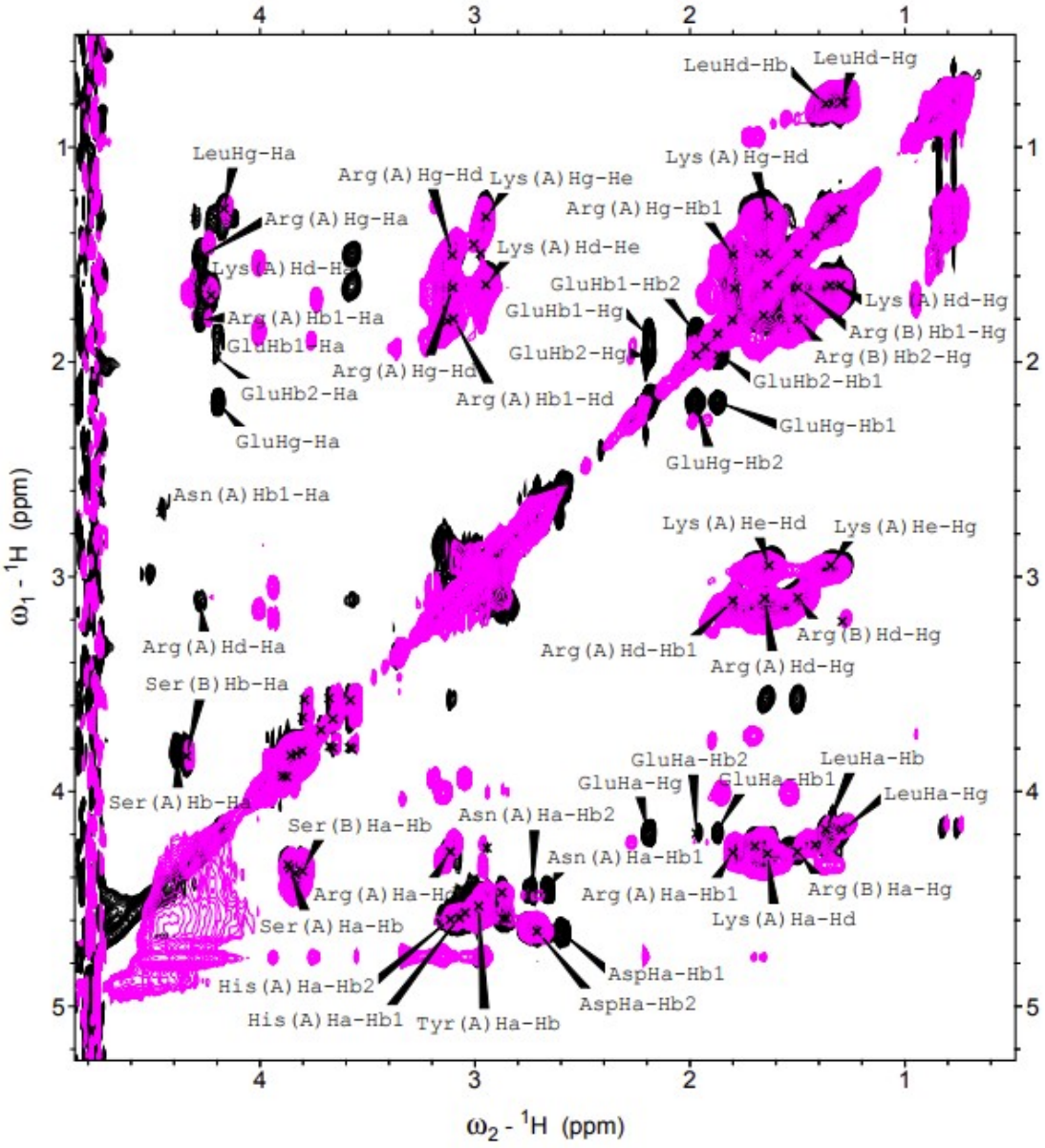
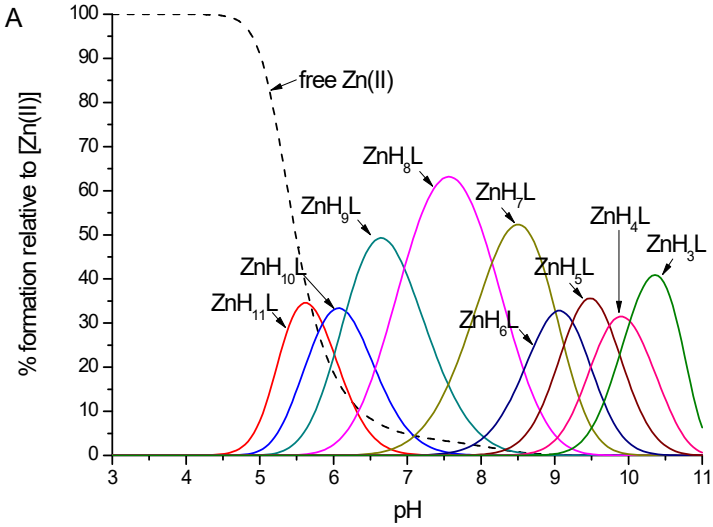
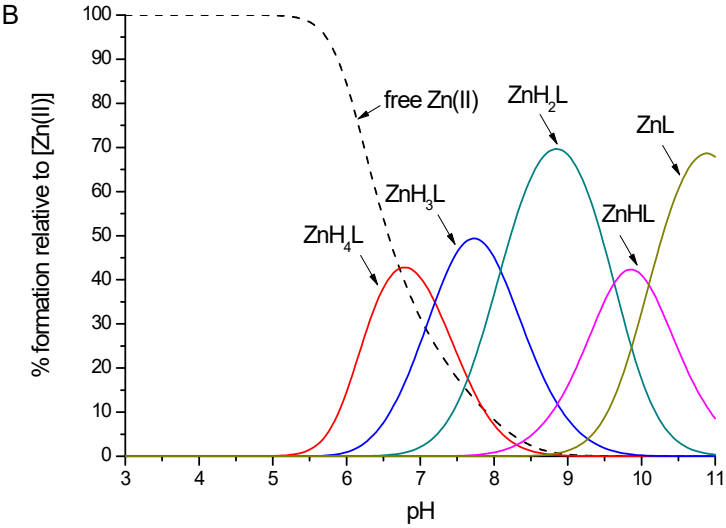


Fig. S5. ^{1}H–^{1}H TOCSY NMR spectra of a fragment of the ligand (black) and the Cu(II) complex (pink) with the histatin 4, where (A) and (B) in the case of Ans, Arg, Lys, His, Ser and Tyr mean amino acid residues present in histatin 4, but without indicating a specific position in the peptide chain. Conditions: [histatin 4] = 1 mM, [Cu(II)] = 0.3 mM, pH = 7.4, T = 298 K.

A


% formation relative to [Zn(II)]
pH
free Zn(II)
ZnH8L
ZnH9L
ZnH7L
ZnH4L
ZnH3L
ZnH10L
ZnH5L
ZnH11L
ZnH6L

B


% formation relative to [Zn(II)]
pH
free Zn(II)
ZnH2L
ZnL
ZnH3L
ZnHL
ZnH4L

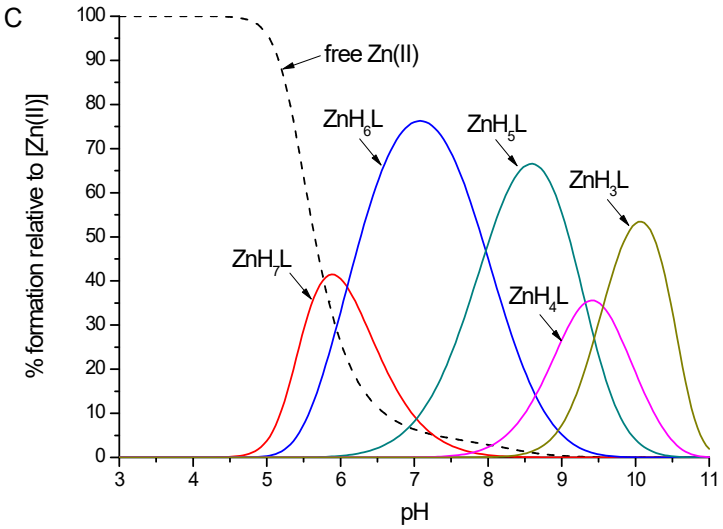
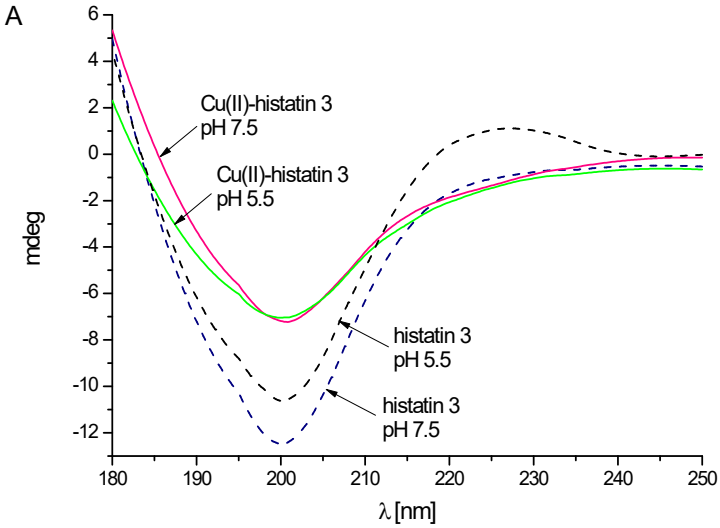
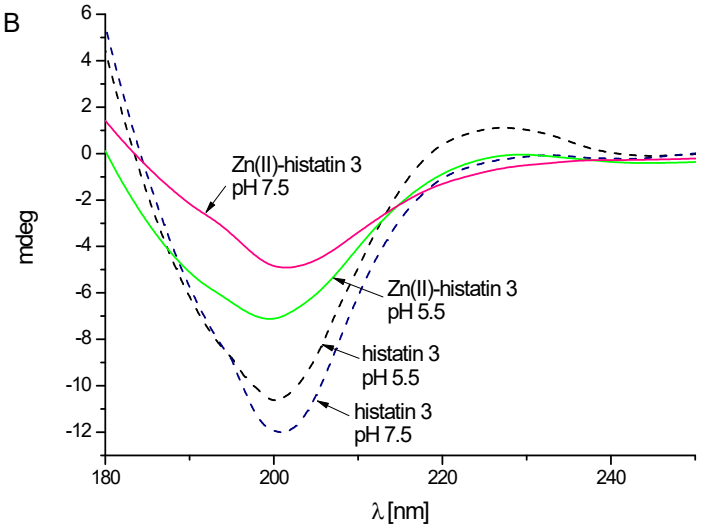


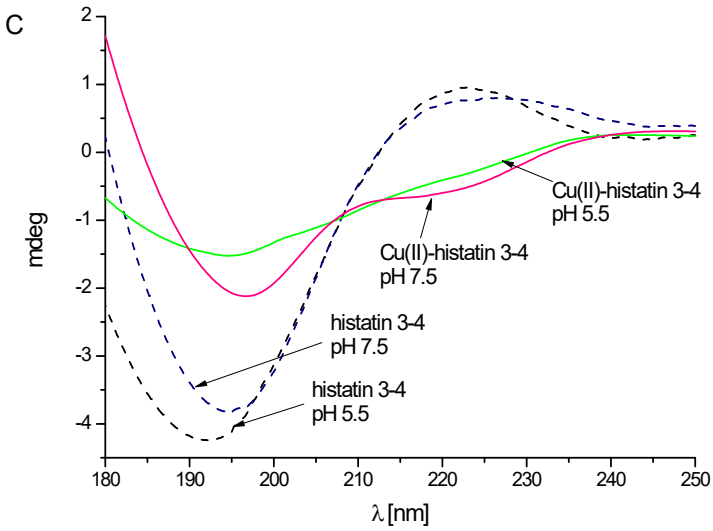
Fig. S6. Distribution diagrams for the formation of: A) Zn(II) complex with histatin 3; B) Zn(II) complex with histatin 3-4; C) Zn(II) complex with histatin 4. Conditions: T = 298 K, *I* = 0.1 M $NaClO_4$, [Zn(II)] = $0.4x10^{-3}$ M; M:L molar ratio = 0.8:1.



B


mdeg
λ [nm]
Zn(II)-histatin 3
pH 7.5
Zn(II)-histatin 3
pH 5.5
histatin 3
pH 5.5
histatin 3
pH 7.5

C


mdeg
λ [nm]
Cu(II)-histatin 3-4
pH 5.5
Cu(II)-histatin 3-4
pH 7.5
histatin 3-4
pH 7.5
histatin 3-4
pH 5.5

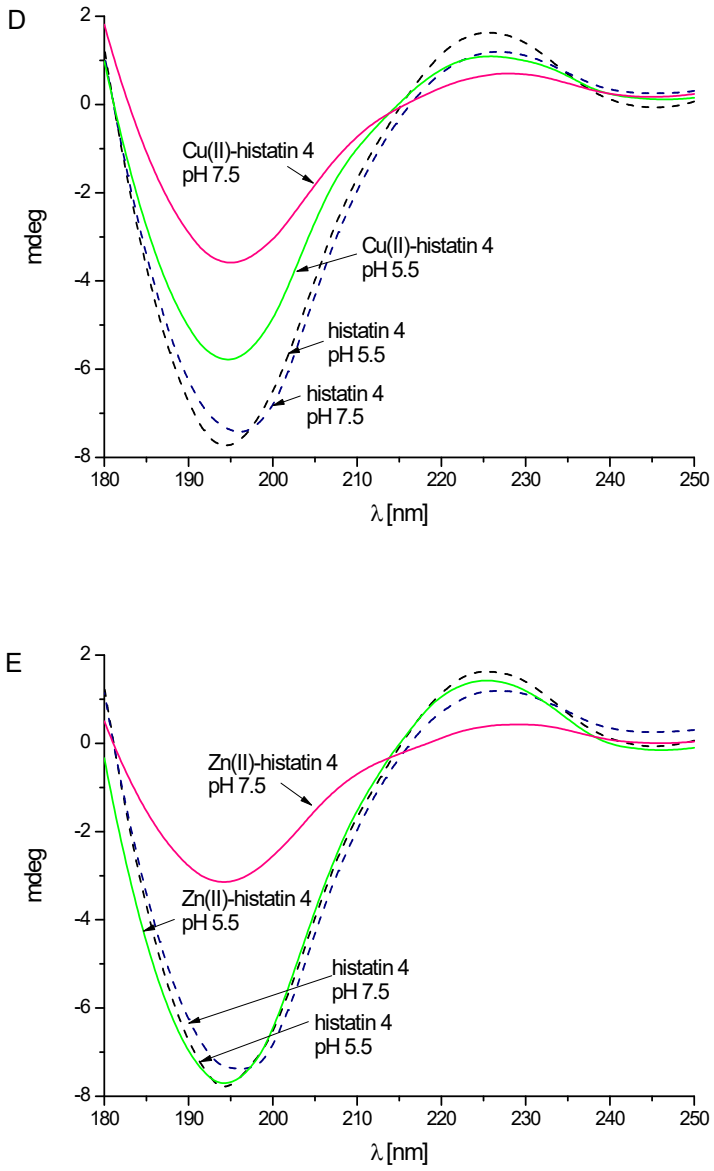


Fig. S7. Comparison of CD spectra of (A) histatin 3 and its Cu(II) complex, (B) histatin 3 and its Zn(II) complex, (C) histatin 3-4 and its Cu(II) complex, (D) histatin 4 and its Cu(II) complex and (E) histatin 4 and its Zn(II) complex at pH 5.5 and 7.5. Conditions: T = 298 K, I = 0.1 M $NaClO_4$, [Cu(II)] = [Zn(II)] = $0.4.10^{-3}$ M; M:L molar ratio = 0.8:1, optical path = 0.01 cm.

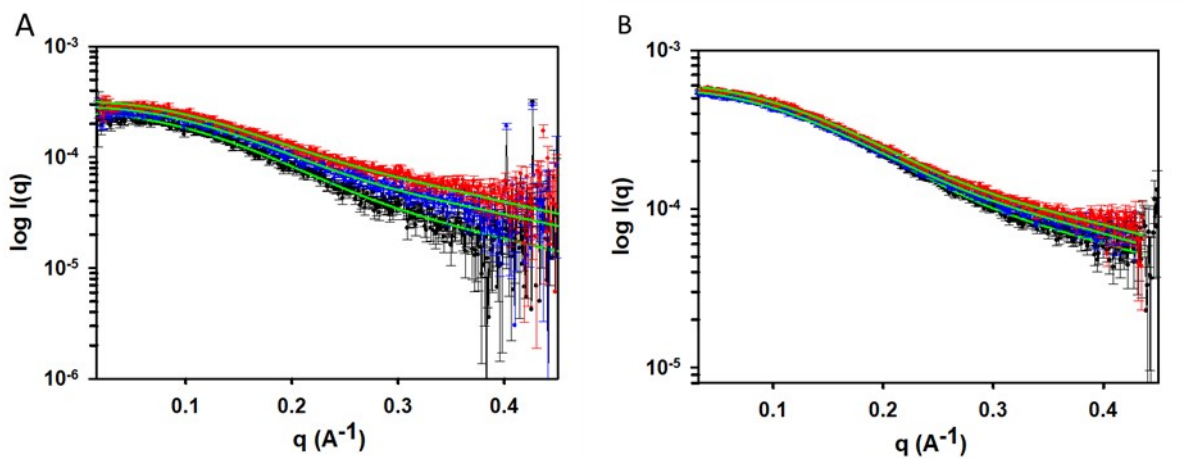


Fig. S8. The plots show the log of the scattering intensity (I, arbitrary units [a.u.]) as a function of momentum transfer ($q = 4\pi\sin(\theta)/\lambda$) for (A) histatin 3-4 and (B) histatin 5-8 obtained in the absence (black) and in the presence of Zn(II) at a Zn(II):peptide ratio of 1:1 (blue) and at 1.5:1 (red). The scattering curves were obtained setting the sample/detector distance at 630 mm. The line green line in the plots of panel B represents the GNOM fit.

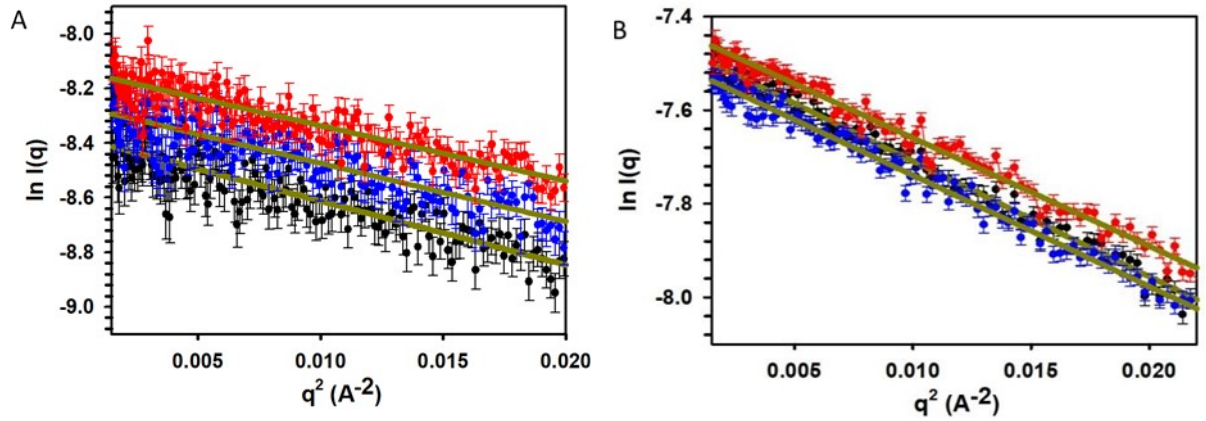


Fig. S9. Guinier plots of the SAXS curves corresponding to the low q region of (A) histatin 3-4 and (B) histatin 5-8 in the absence (black) and in the presence of Zn(II) at a Zn(II):peptide ratio of 1:1 (blue) and at 1.5:1 (red). The Green lines represent the fitting of the experimental data to the Gunier equation.

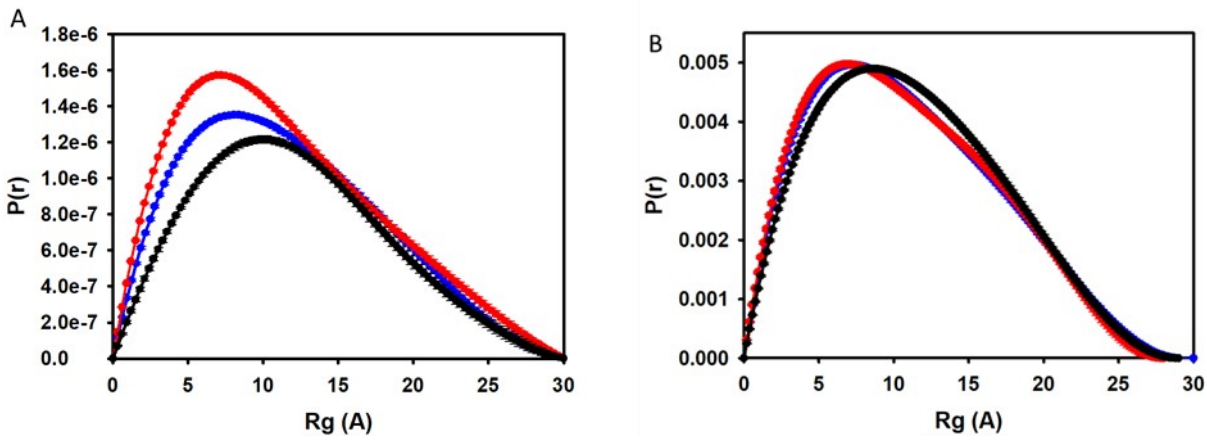


Fig. S10. Pair distance distribution functions of the scattering curve obtained for (A) histatin 3-4 and (B) histatin 5-8 in the absence (black) and in the presence of Zn(II) at a Zn(II):peptide ratio of 1:1 (blue) and 1.5:1 (red). The P(r) values were determined using scattering data collected with the 0.03-0.45 A-1 q range.

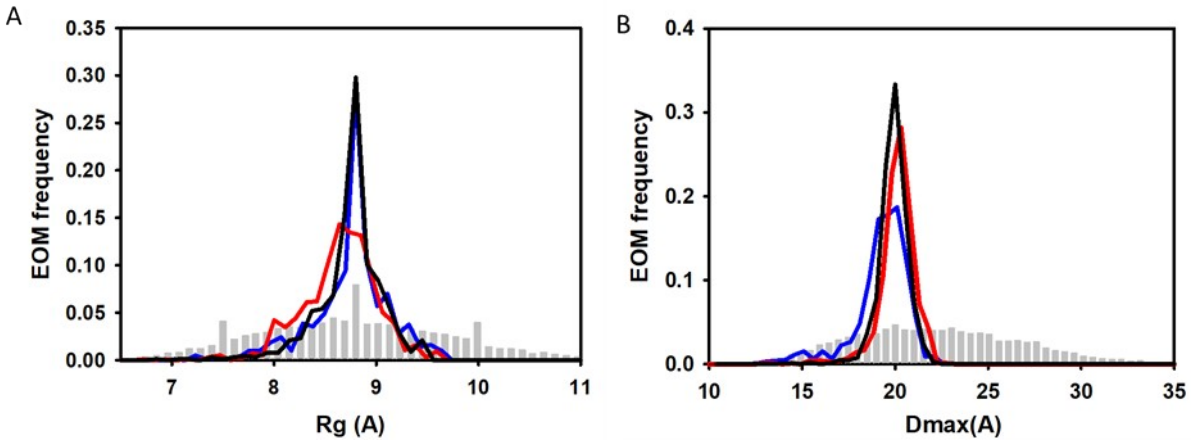


Fig. S11. SAXS analysis by Ensemble optimization method (EOM). Distribution of (A) Rg and (B) Dmax values for the generated pool (grey bars) and the histatin 5-8 conformational ensemble (lines) obtained in the absence (black) and in the presence of Zn(II) at a Zn(II):peptide ratio of 1:1 (blue) and 1.5:1 (red).

Table S1. Examples of MIC breakpoints values from EUCAST/2023/06/29 for bacteria.[1]

Enterococcus ssp. (for *E. faecalis*)	MIC breakpoints (µg/mL)		*Staphylococcus* spp. (for *S. aureus*)	MIC breakpoints (µg/mL)		*Candida* spp. (for *C.albicans*)	MIC breakpoints (µg/mL)	
	S≤	R>		S≤	R>		S≤	R>
Ampicillin	4	8	Azithromycin	2	2	Amphotericin B	1	1
Ampicillin-Sulbactam	4	8	Ceftobiprole	2	2	Fluconazole	2	4
-Amoxicillin	4	8	Ciprofloxacin	0.001	1	Micafungin	0.16	0.16
Amoxicillin-clavulanic acid	4	8	Daptomycin	1	1	Itraconazole	0.06	0.06
Imipenem	0.001	4	Maxifloxacin	0.25	0.25			
Ciprofloxacin	4	4	Amikacin	16	16			
Levofloxacin	4	4	Gentamicin	2	2			
Vancomycin	4	4	Tobramycin	2	2			
Linezolid	4	4	Teicoplanin	2	2			
Nitrofurantoin	64	64	Clindamycin	0.25	0.25			

References:

1. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. http://www.eucast.org.