Supplementary Information (SI)

Two sites, two stories: sequence-driven divergence in Cu(II) and Zn(II) binding to CusF

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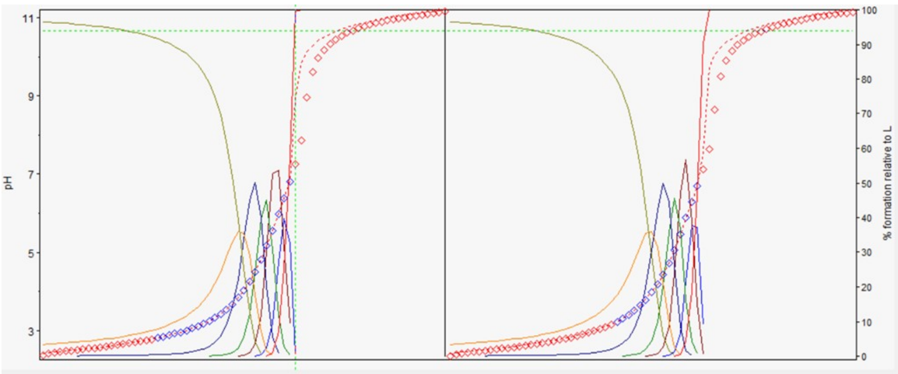
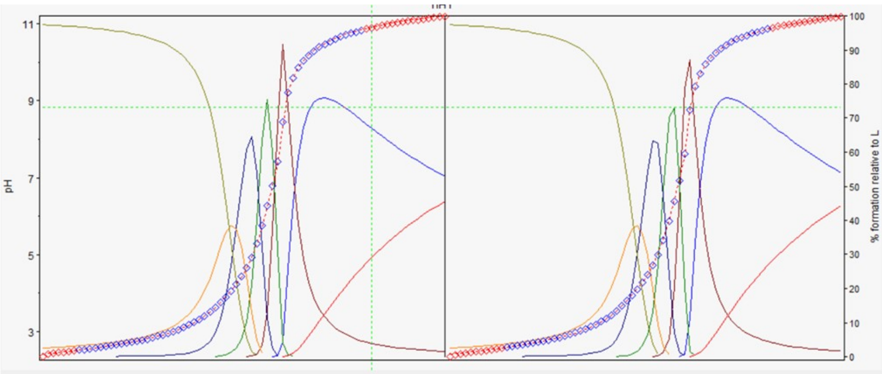
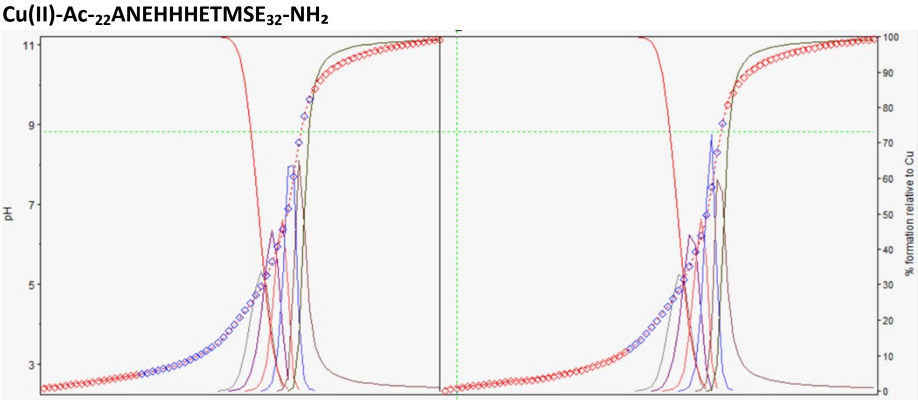
Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$



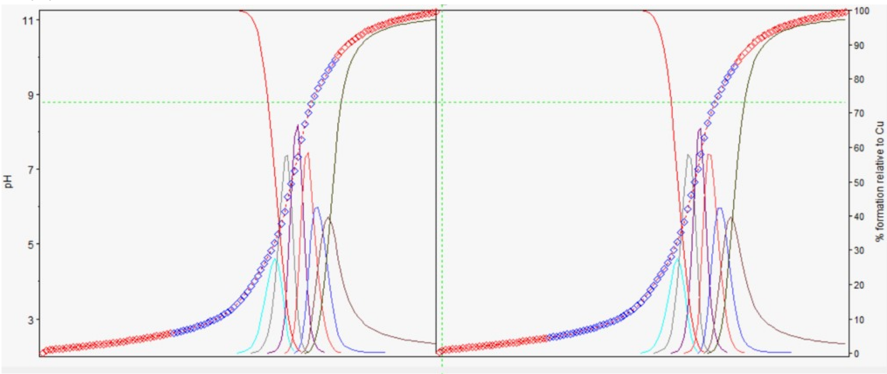
Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$



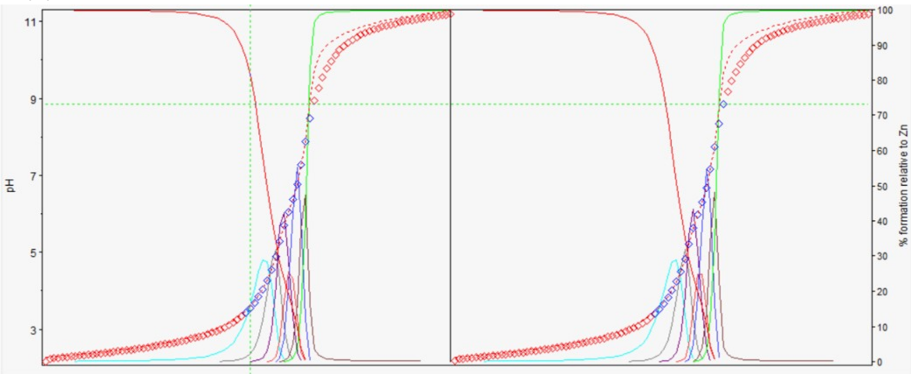
Cu(II)-Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$



Cu(II)-Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$



Zn(II)-Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$



Zn(II)-Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$

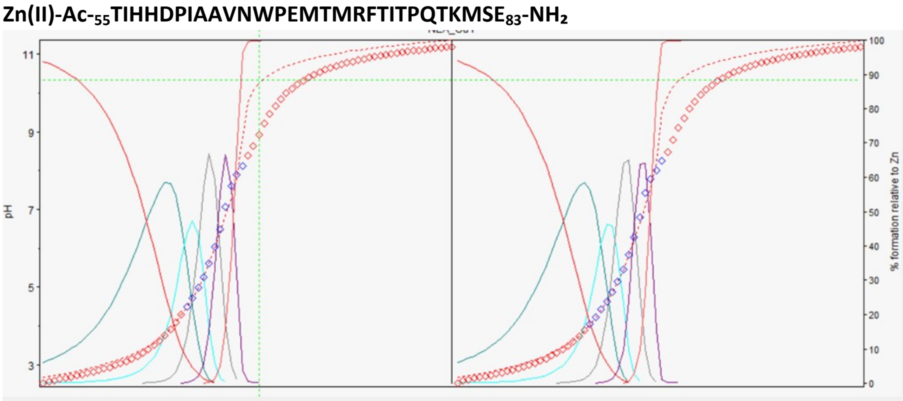


Figure S1 Experimental and fitted potentiometric titration curves together with species distribution diagrams for the studied systems: Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$; Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$; Cu(II)-Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$; Cu(II)-Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$; Zn(II)-Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$; Zn(II)-Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$. Experimental points are shown as open diamonds, and calculated curves are shown as dashed lines.

Table S1 Equilibrium constants for Zn(II) and Cu(II) hydrolysis. [1] C.F. Baes and R.E. Mesmer, The Hydrolysis of Cations. Wiley, New York, 1976, p. 246.[2] G. Arena, R. Cali, E. Rizzarelli, S. Sammartano, Thermodynamic study on the formation of the cupric ion hydrolytic species, Thermochim. Acta, 16 (3) (1976), pp. 315-321.

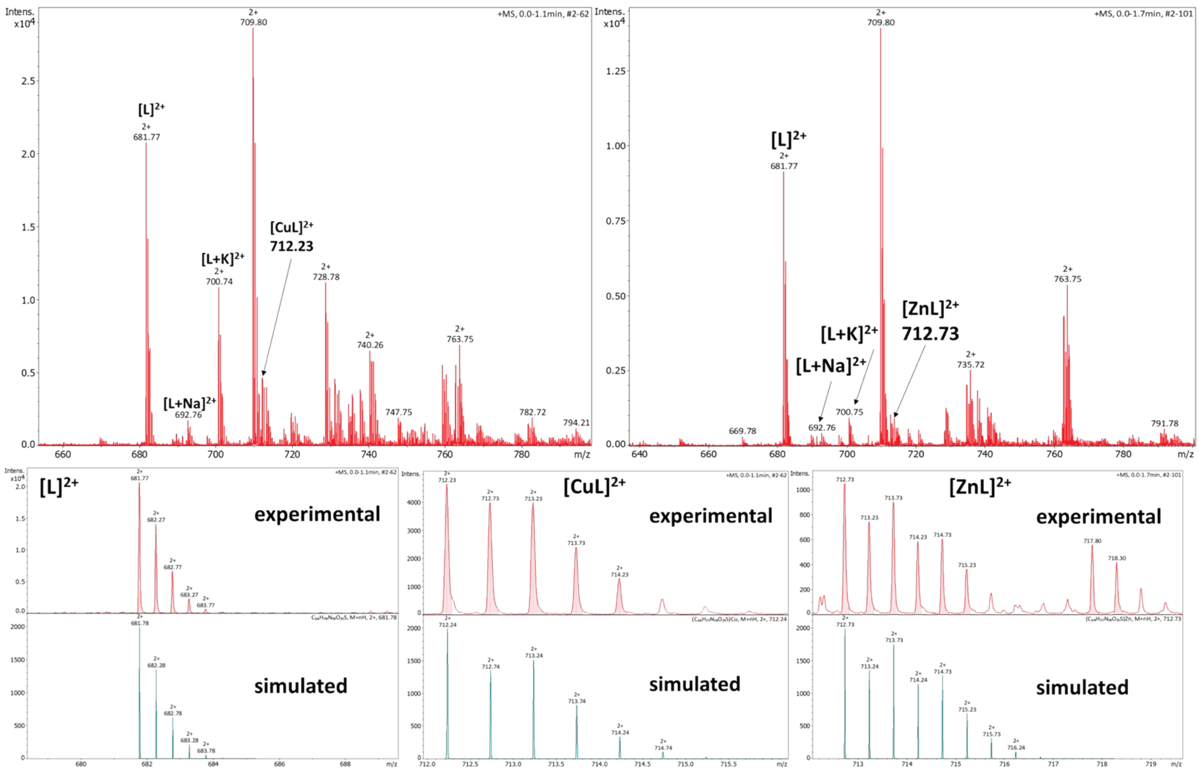
Equilibrium reactions	**logK at infinite dilution and T = 298 K**
Zn(II)[1]	
$Zn^{2+} + H_2O \rightleftharpoons ZnOH^+ + H^+$	−8.96
$Zn^{2+} + 2\ H_2O \rightleftharpoons Zn(OH)_2 + 2\ H^+$	−16.9
$Zn^{2+} + 3\ H_2O \rightleftharpoons Zn(OH)_3^- + 3\ H^+$	−28.4
$Zn^{2+} + 4\ H_2O \rightleftharpoons Zn(OH)_4^{2-} + 4\ H^+$	−41.2
Cu(II)[2]	
$Cu^{2+} + H_2O \rightleftharpoons CuOH^+ + H^+$	-26.8

$Cu^{2+} + 2\ H_2O \rightleftharpoons Cu(OH)_2 + 2\ H^+$	-39.9

Table S2 Stoichiometry, molecular formula and average m/z value for the species present in ESI-MS spectra of Cu(II) and Zn(II) complexes with the A) Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$ and B) Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$, M:L molar ratio = 0.9:1 in water:methanol 50:50 solution

Ion signal	*m/z*	*Chemical formula*
	A) Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$	
$[L]^+$	1362.54	C54H80N19O21S
$[L+Na]^+$	1384.50	C54H79N19O21SNa
$[L+K]^+$	1400.48	C54H79N19O21SK
$[CuL]^+$	1423.45	C54H78N19O21SCu
$[L]^{2+}$	681.77	C54H81N19O21S
$[L+Na]^{2+}$	692.76	C54H80N19O21SNa
$[L+K]^{2+}$	700.74	C54H80N19O21SK
$[CuL]^{2+}$	712.23	C54H79N19O21SCu
$[ZnL]^{2+}$	712.73	C54H79N19O21SZn
	B) Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$	
$[L]^{2+}$	1713.32	C151H237N41O44S3
$[CuL]^{2+}$	1741.35	C151H230N41O44S3Cu
$[L]^{3+}$	1142.55	C151H238N41O44S3
$[CuL]^{3+}$	1161.24	C151H231N41O44S3Cu
$[L]^{4+}$	856.92	C151H239N41O44S3
$[CuL]^{4+}$	871.18	C151H232N41O44S3Cu
$[ZnL]^{2+}$	1741.35	C151H229N41O44S3Zn
$[ZnL]^{3+}$	1161.24	C151H230N41O44S3Zn
$[ZnL]^{4+}$	871.18	C151H231N41O44S3Zn

A)



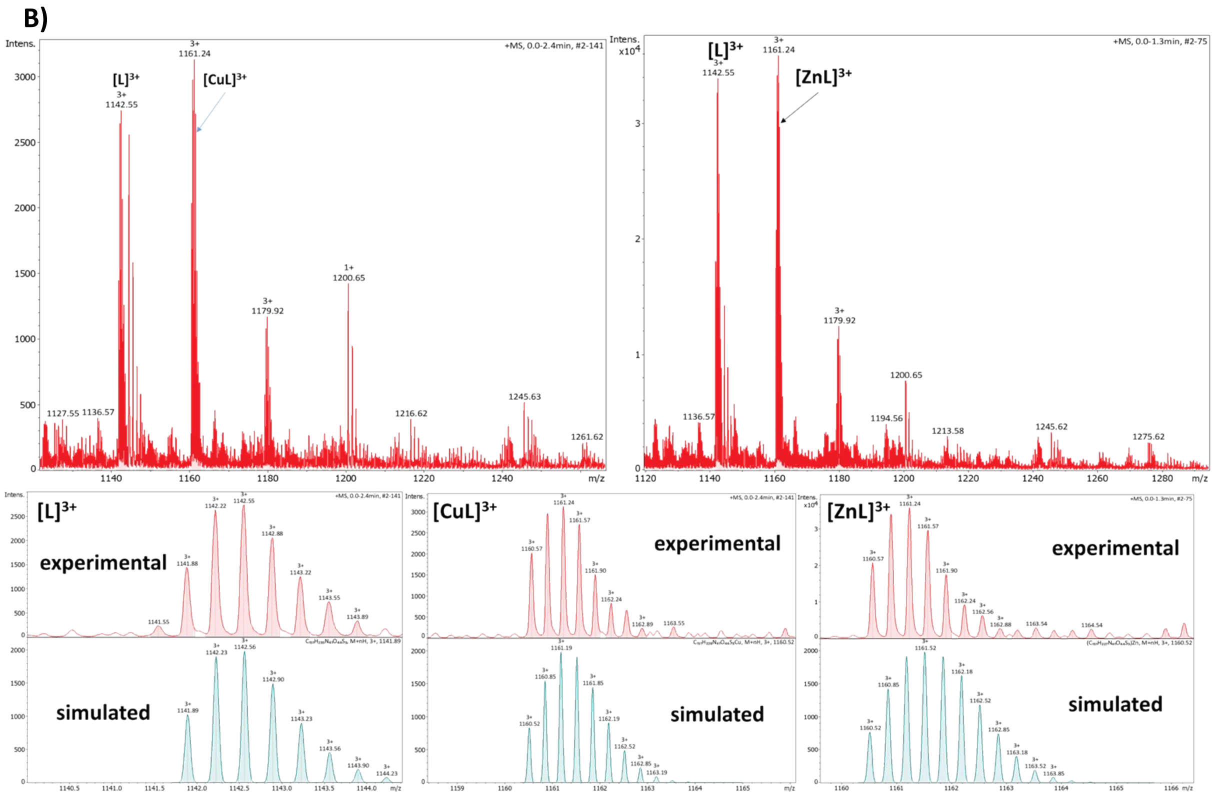


Figure S2 High-resolution ESI-MS spectra of Cu(II) and Zn(II) complexes with CusF-derived peptides. (A) Spectrum of the Cu(II)-ANE and Zn(II)-ANE (Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$) showing the 2+ charge state region. Below: comparison of experimental and simulated isotopic patterns for [L]$^{2+}$, [CuL]$^{2+}$, and [ZnL]$^{2+}$ species. (B) Spectrum of the Cu(II)-TIH and Zn(II)-TIH (Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$) showing the 3+ charge state region. Below: comparison of experimental and simulated isotopic patterns for [L]$^{3+}$, [CuL]$^{3+}$, and [ZnL]$^{3+}$ species.

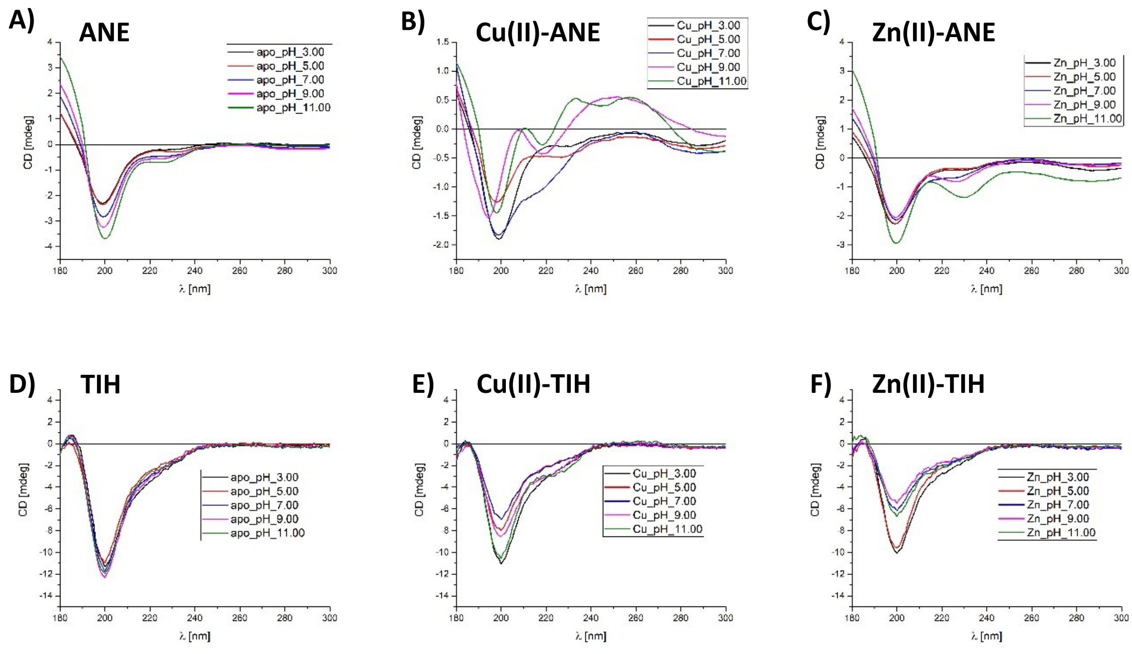


Figure S3 CD spectra for A) Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$, B) Cu(II)-Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$, C) Zn(II)-Ac-$_{22}$ANEHHHETMSE$_{32}$-NH$_2$,, D) Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$, E) Cu(II)-Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH$_2$, F)

Zn(II)-Ac-$_{55}$TIHHDPIAAVNWPEMTMRFTITPQTKMSE$_{83}$-NH_2 in aqueous solution of 4 mM $HClO_4$ with I = 0.1 M $NaClO_4$ at 25 °C. C_L *= 0.1 mM; M:L molar ratio = 0.9:1.*