

Supporting Information for:

Investigation of the metal-binding site 2 of *Y. pestis* YfeA protein: solution studies of Fe(II), Mn(II), and Zn(II) complexes

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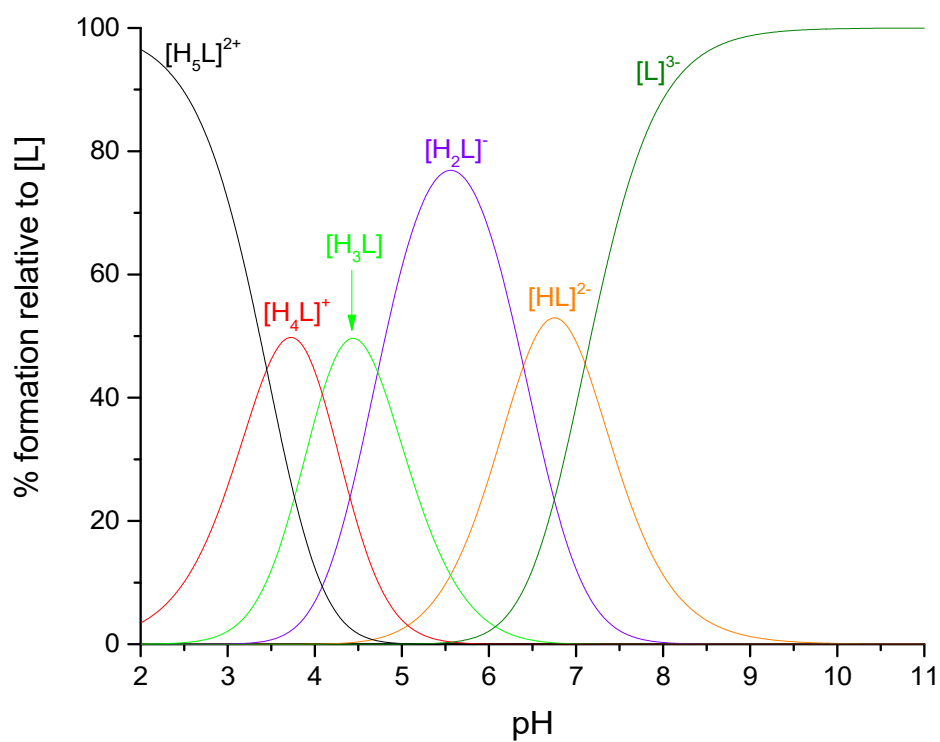
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Table S1. Hydrolysis constants for Mn(II), Zn(II) and Fe(II) ions for $I = 0.1\text{M}$ ionic strength, $T = 298\text{ K}$.^a

Species	$\log \beta$
Mn(II)	
Mn(OH)^+	-10.78
Mn(OH)_2	-22.39
Mn(OH)_3^-	-34.34
Mn(OH)_4^{2-}	-47.82
Zn(II)	
Zn(OH)^+	-9.12
Zn(OH)_2	-18.08
Zn(OH)_3^-	-27.97
Zn(OH)_4^{2-}	-39.50
Fe(II)	
Fe(OH)^+	-9.63
Fe(OH)_2	-20.73
Fe(OH)_3^-	-32.68

^a The hydrolysis constants for zero ionic strength⁴ were recalculated to 0.1 M ionic strength with an appropriate formula.⁵



Fig

re S1. Distribution diagram of the protonation equilibria of Ac-VEHDPHAHAET-NH₂.

$L_{[\text{tot}]} = 0.5\text{ mM}$.

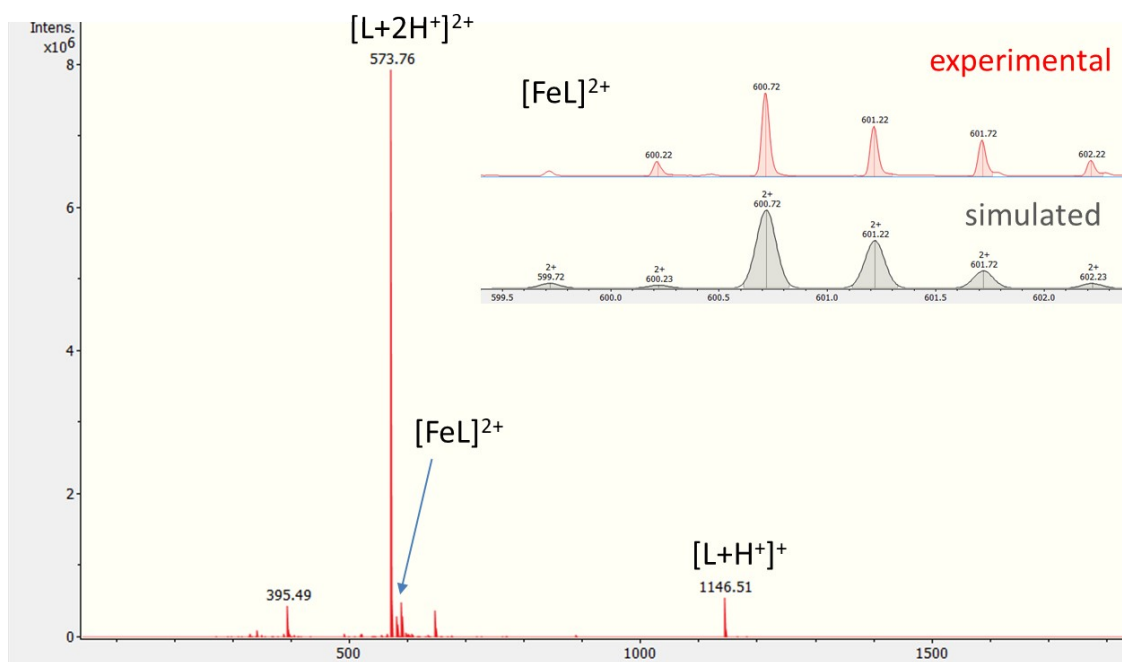


Figure S2. Mass spectrum acquired for Fe(II):L1 system. The comparison of the experimental and simulated isotopic pattern of the peak at $m/z = 599.72$ is shown on the right.

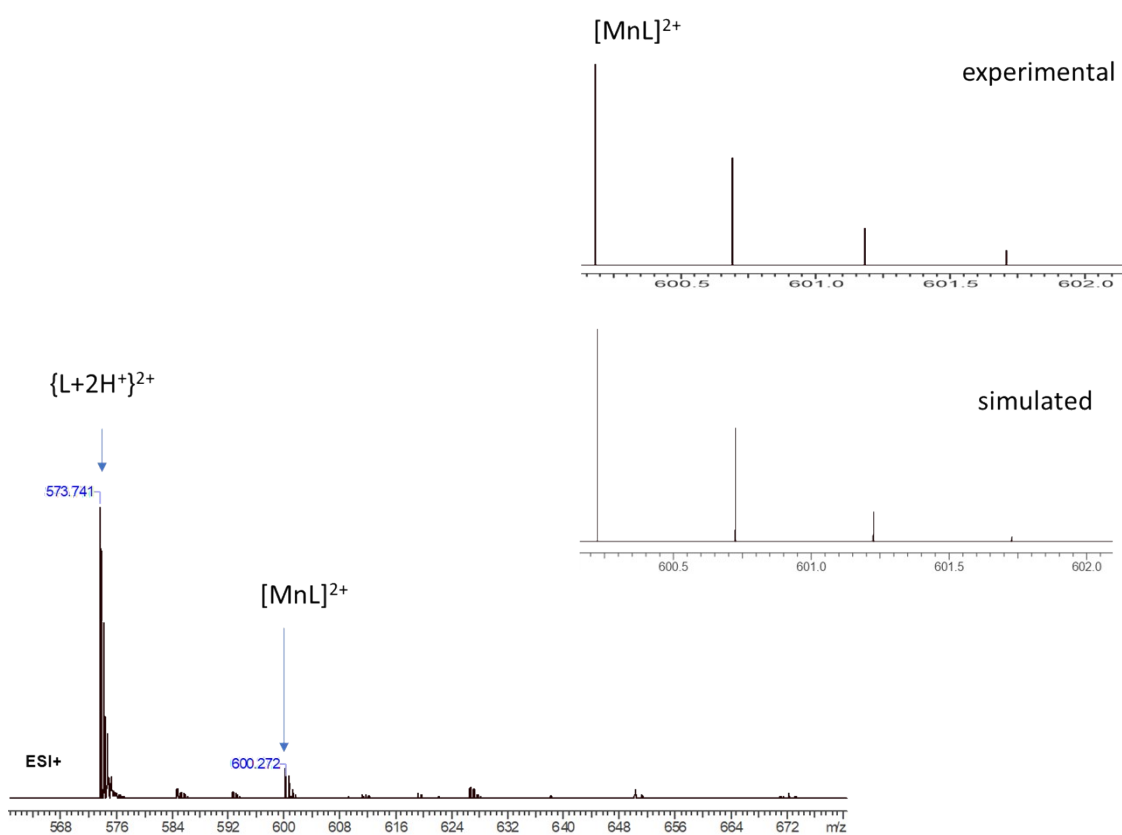


Figure S3. Mass spectrum acquired for Mn(II):L1 system. The comparison of the experimental and simulated isotopic pattern of the peak at $m/z = 600.27$ is shown on the right.

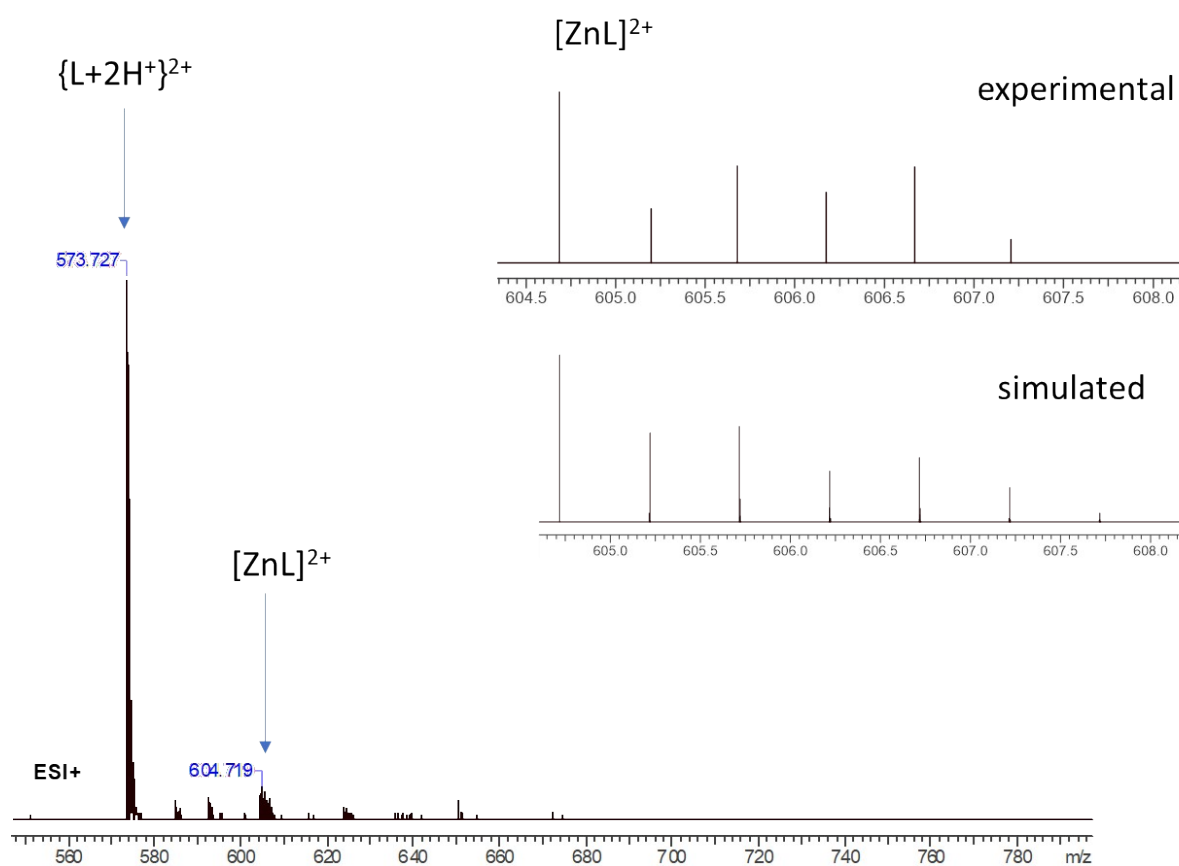


Figure S4. Mass spectrum acquired for Zn(II):L1 system. The comparison of the experimental and simulated isotopic pattern of the peak at $m/z = 604.72$ is shown on the right.

Table S2. Comparison of the m/z of the major signals observed in ESI-MS spectra for M(II):L1 systems, M:L=1:1.

System	Ion	m/z	
		experimental	simulated
Fe(II):L1	$[FeL]^{2+}$	599.72	599.72
	$\{[L]+2H\}^{2+}$	573.76	573.76
	$\{[L]+H^+\}^+$	1146.52	1146.52
Mn(II):L1	$[MnL]^{2+}$	600.21	600.22
	$\{[L]+2H\}^{2+}$	573.76	573.76
	$\{[L]+H^+\}^+$	1146.52	1146.52
Zn(II):L1	$[ZnL]^{2+}$	604.71	604.72
	$\{[L]+2H\}^{2+}$	573.76	573.76
	$\{[L]+H^+\}^+$	1146.51	1146.52

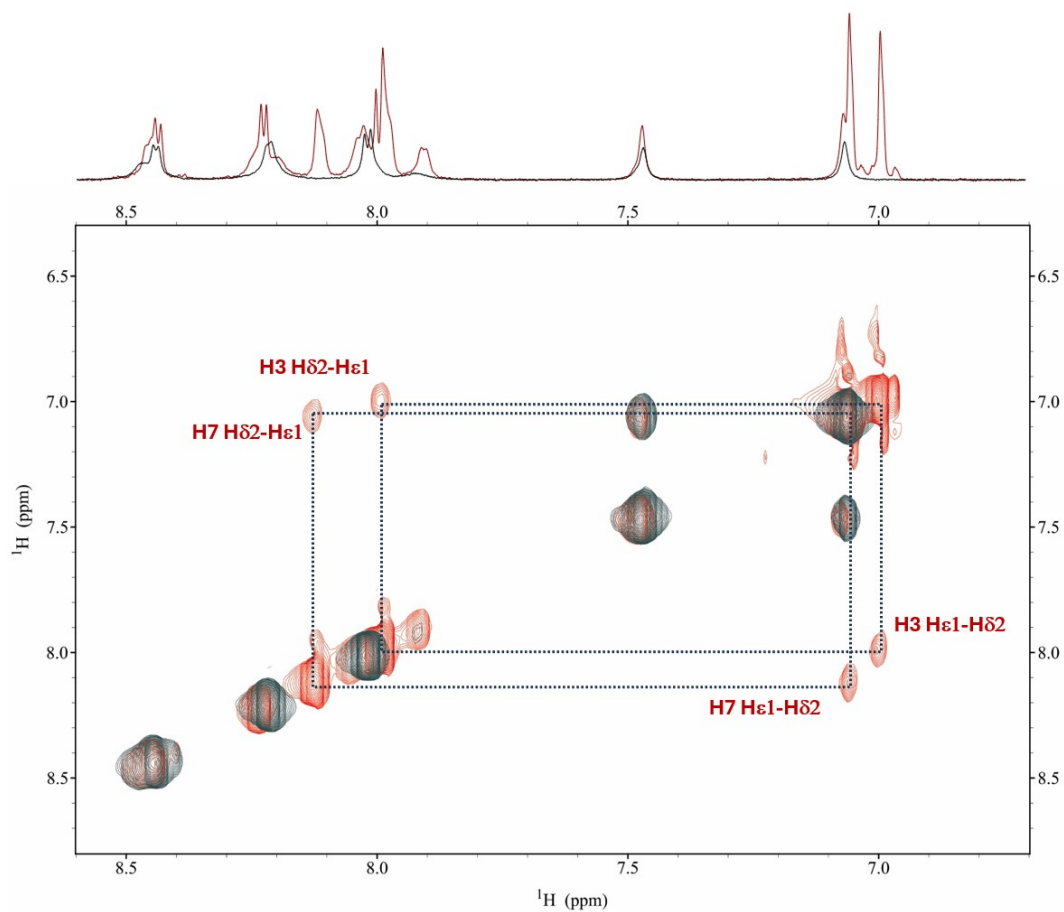


Figure S5. Comparison of the selected aromatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the $\text{Fe}(\text{II})$:**L1** complex (black) at a 0.3:1 molar ratio, at pH 7. Signals exhibiting significant chemical shift perturbations upon Fe^{2+} coordination are highlighted in red.

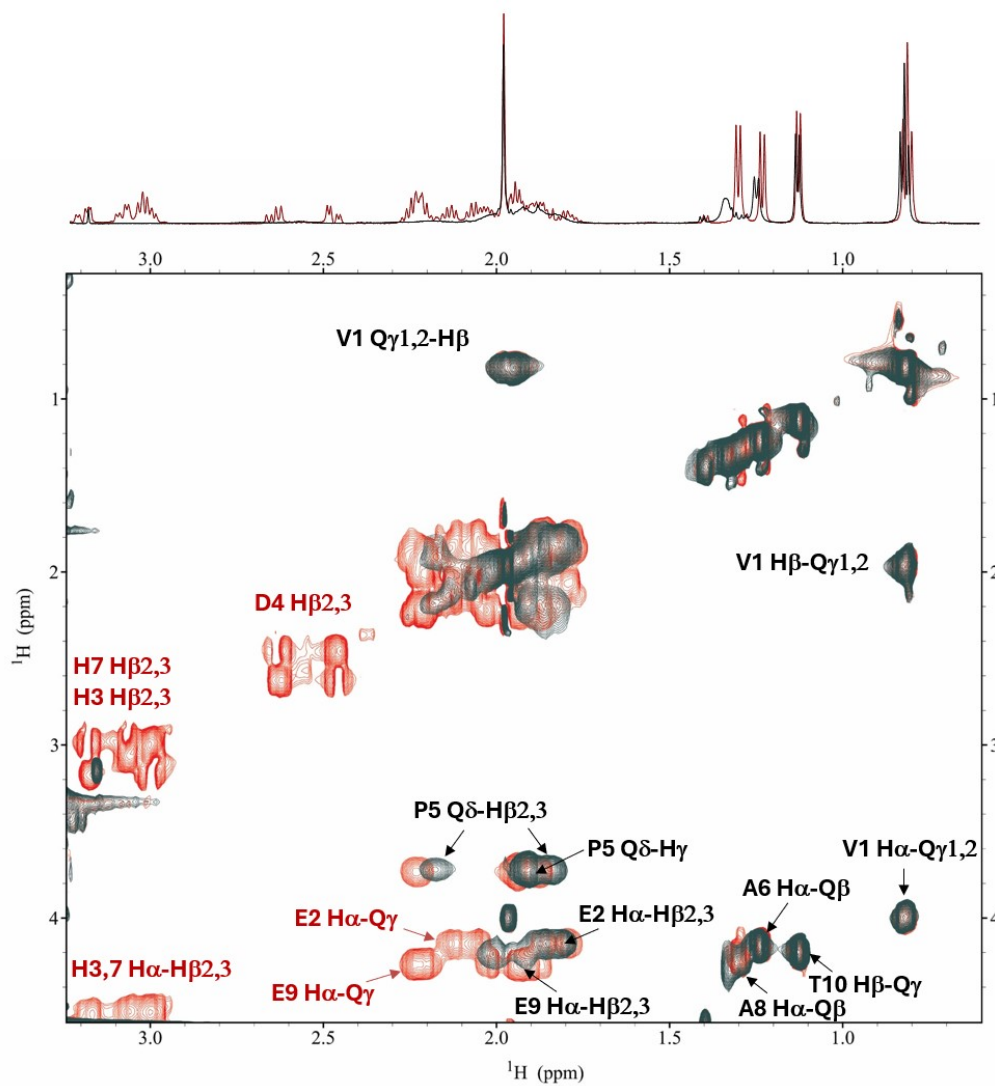


Figure S6. Comparison of the selected aliphatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Fe(II):L1 complex (black) at a 0.3:1 molar ratio, at pH 7. Signals exhibiting significant chemical shift perturbations upon Fe^{2+} coordination are highlighted in red.

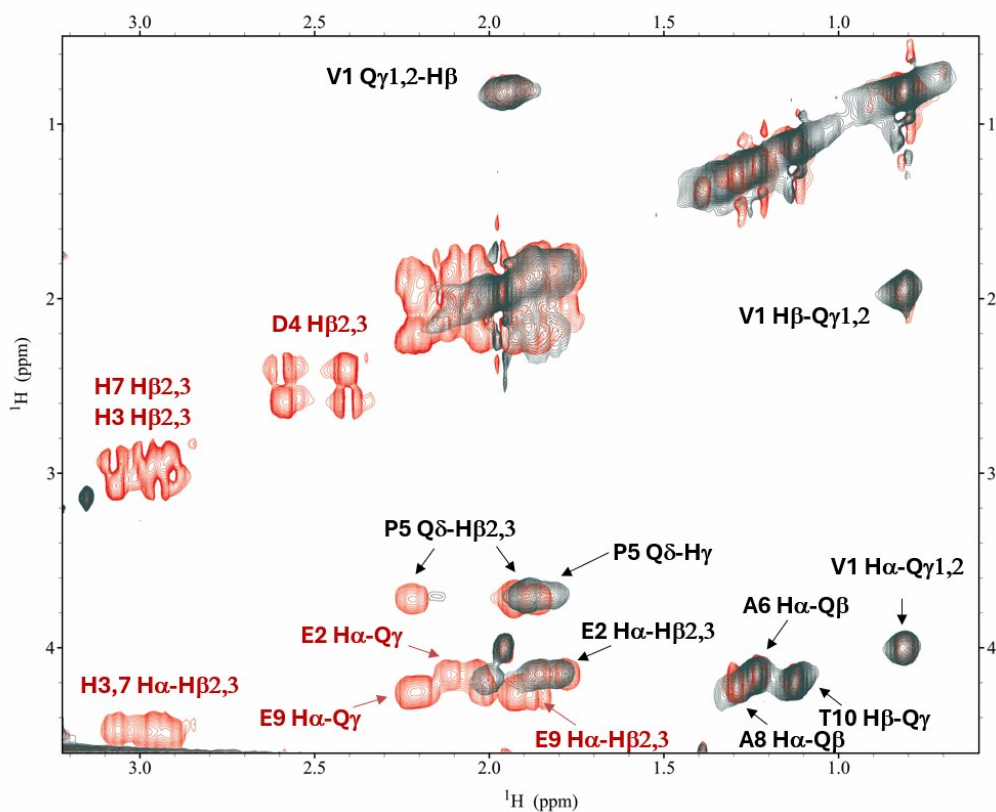


Figure S7. Comparison of the selected aliphatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Fe(II):L1 complex (black) at a 0.3:1 molar ratio, at pH 8.9. Signals exhibiting significant chemical shift perturbations upon Fe^{2+} coordination are highlighted in red.

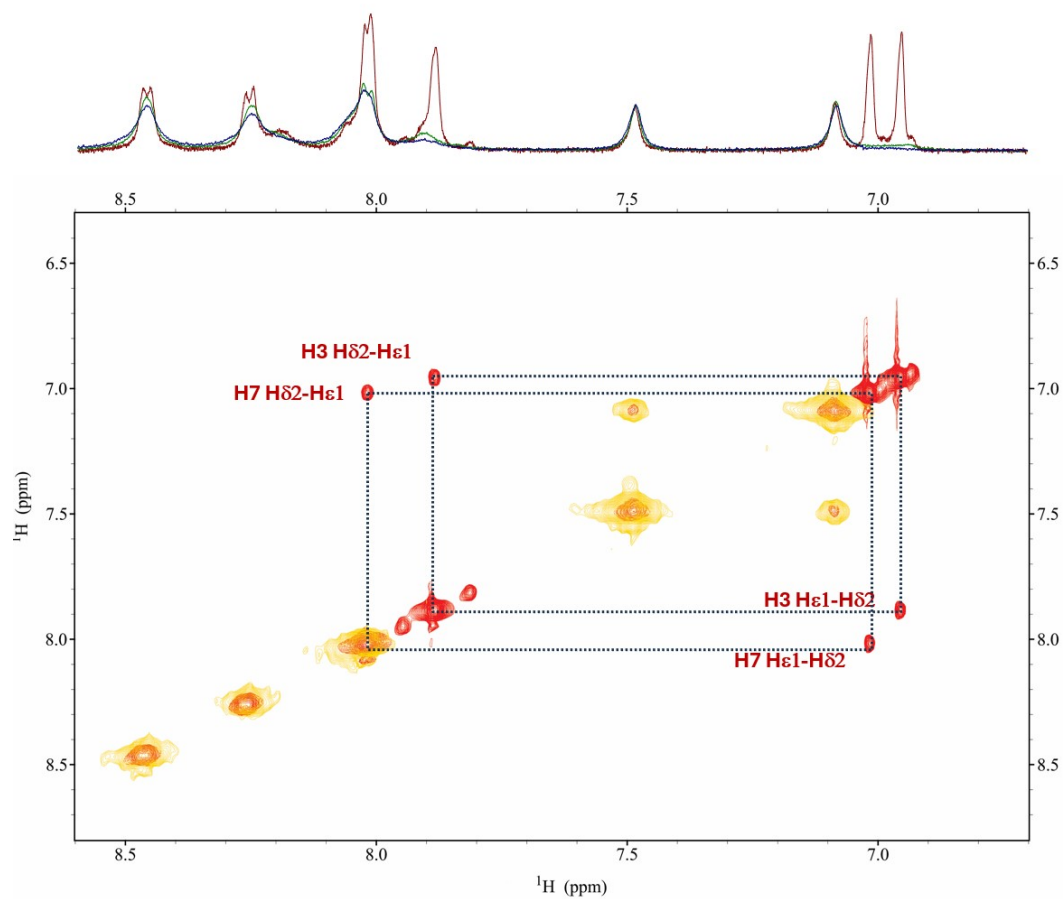


Figure S8. Comparison of the selected aromatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Mn(II):L1 complex (gold) at a 0.005:1 molar ratio, at pH 7. Signals exhibiting significant chemical shift perturbations upon Fe^{2+} coordination are highlighted in red.

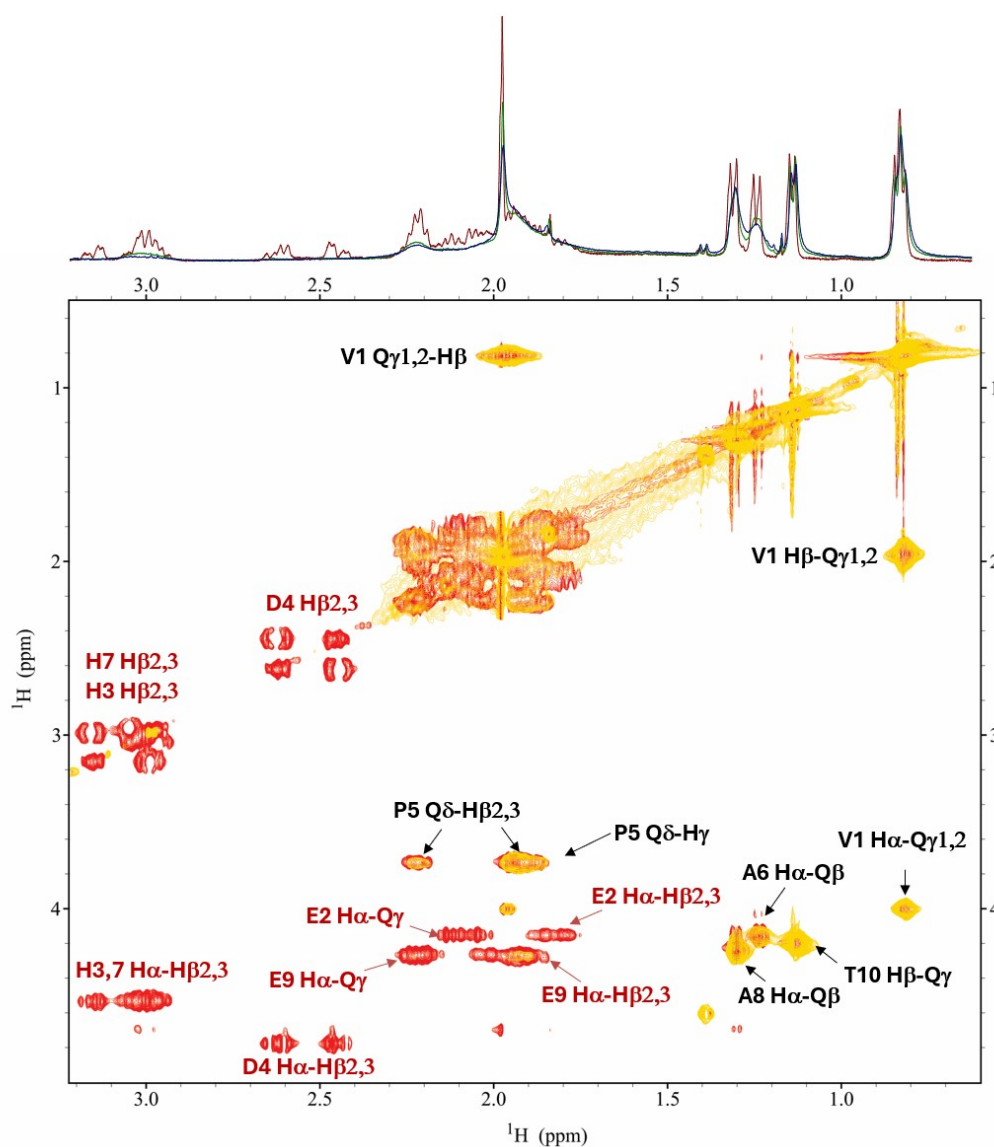


Figure S9. Comparison of the selected aliphatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Mn(II):L1 complex (gold) at a 0.0,05:1 molar ratio, at pH 7. Signals exhibiting significant chemical shift perturbations upon Mn^{2+} coordination are highlighted in red.

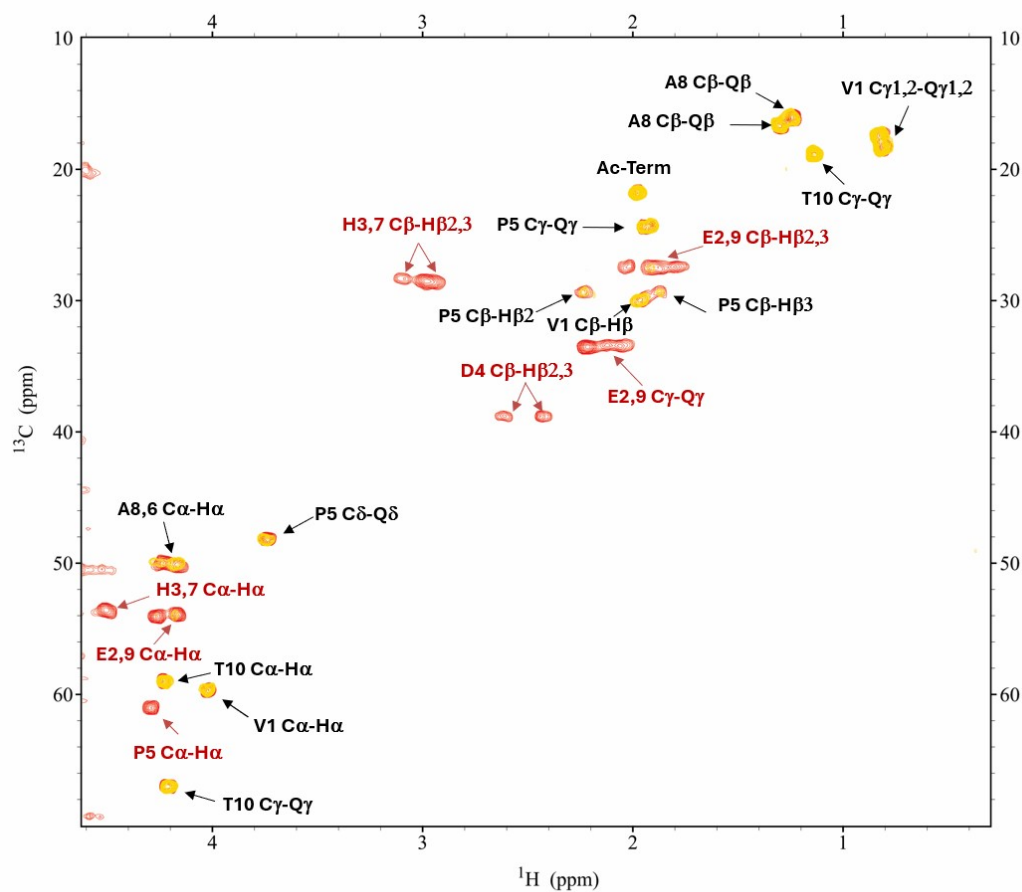


Figure S10. Comparison of the selected aromatic region of the ^1H - ^{13}C HSQC spectra for the free peptide **L1** (red) and the Mn(II):**L1** complex (gold) at a 0.005:1 molar ratio, at pH 9. Signals exhibiting significant chemical shift perturbations upon Mn²⁺ coordination are highlighted in red.

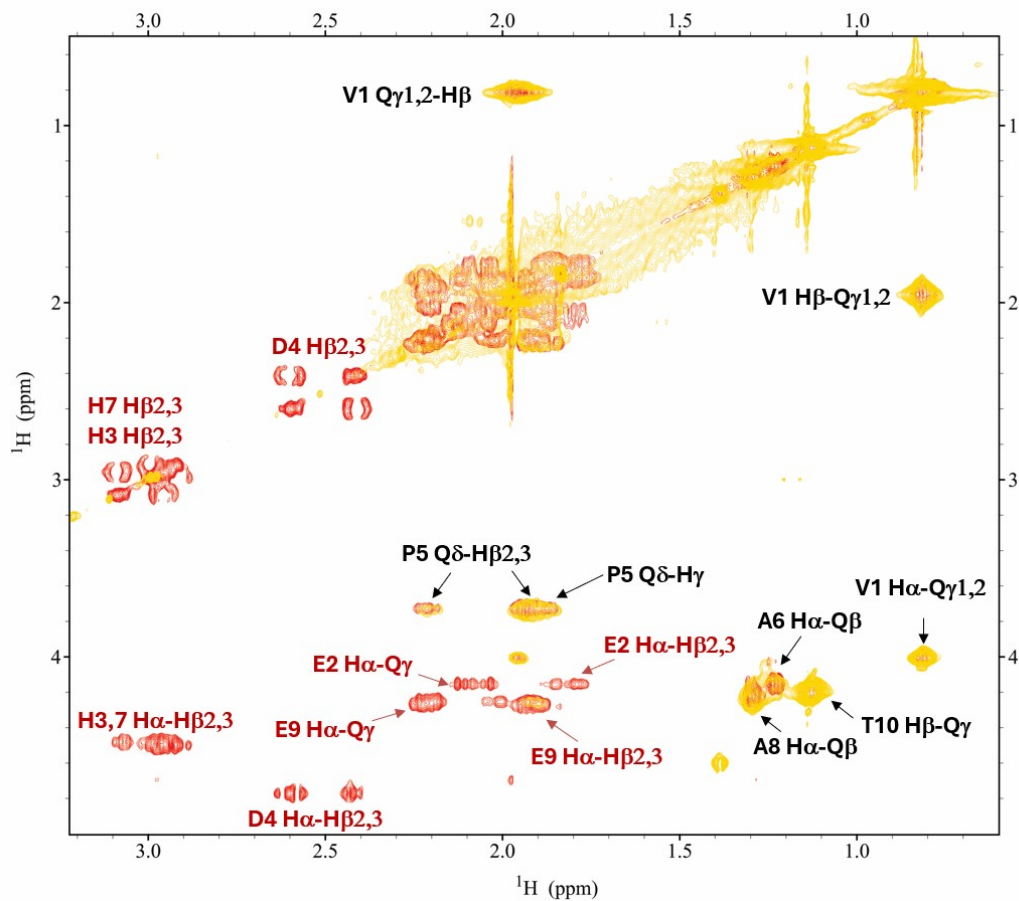


Figure S11. Comparison of the selected aliphatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Mn(II):L1 complex (gold) at a 0.0,05:1 molar ratio, at pH 9. Signals exhibiting significant chemical shift perturbations upon Mn^{2+} coordination are highlighted in red.

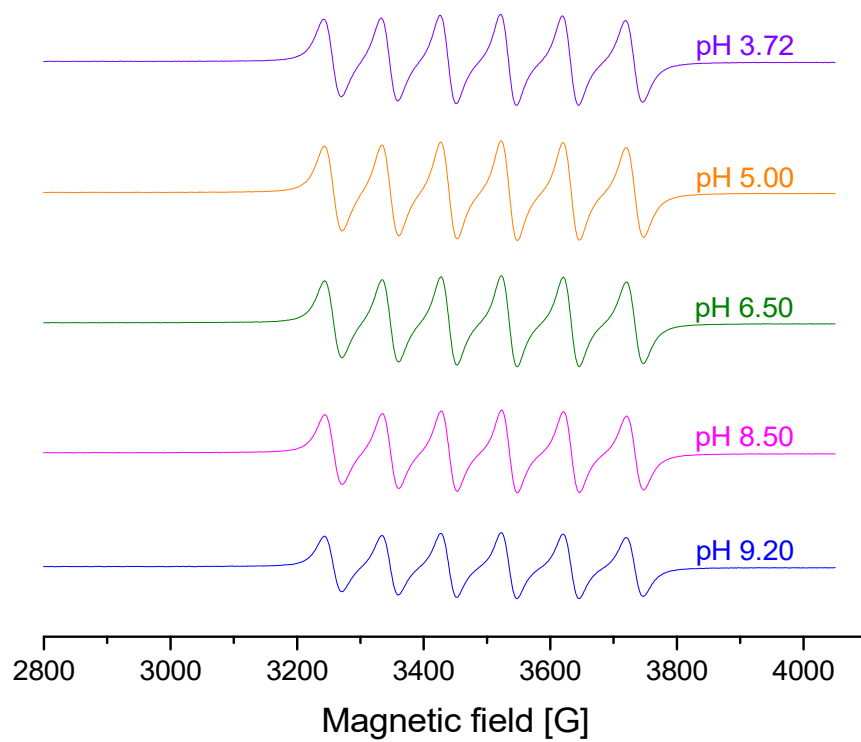


Figure S12. Spectra acquired for Mn(II):L1 system at various pH values at room temperature. $L_{\text{[tot]}} = 1 \text{ mM}$, Mn(II):L = 1:1.1.

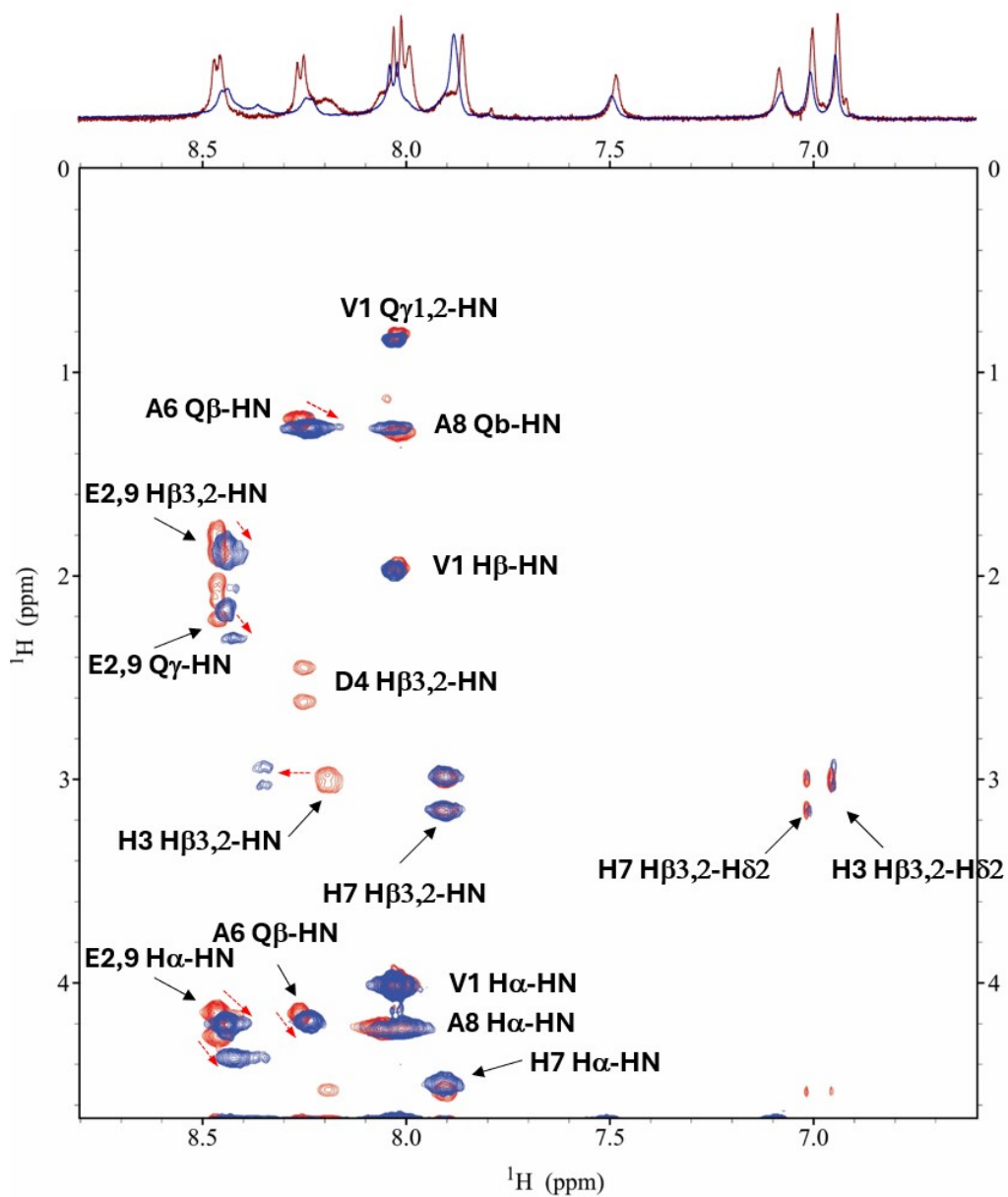


Figure S13. Comparison of the selected aromatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Zn(II):L1 complex (blue) at a 1:1 molar ratio, at pH 7. Signals showing significant chemical shift changes upon Zn^{2+} coordination are indicated by red arrows.

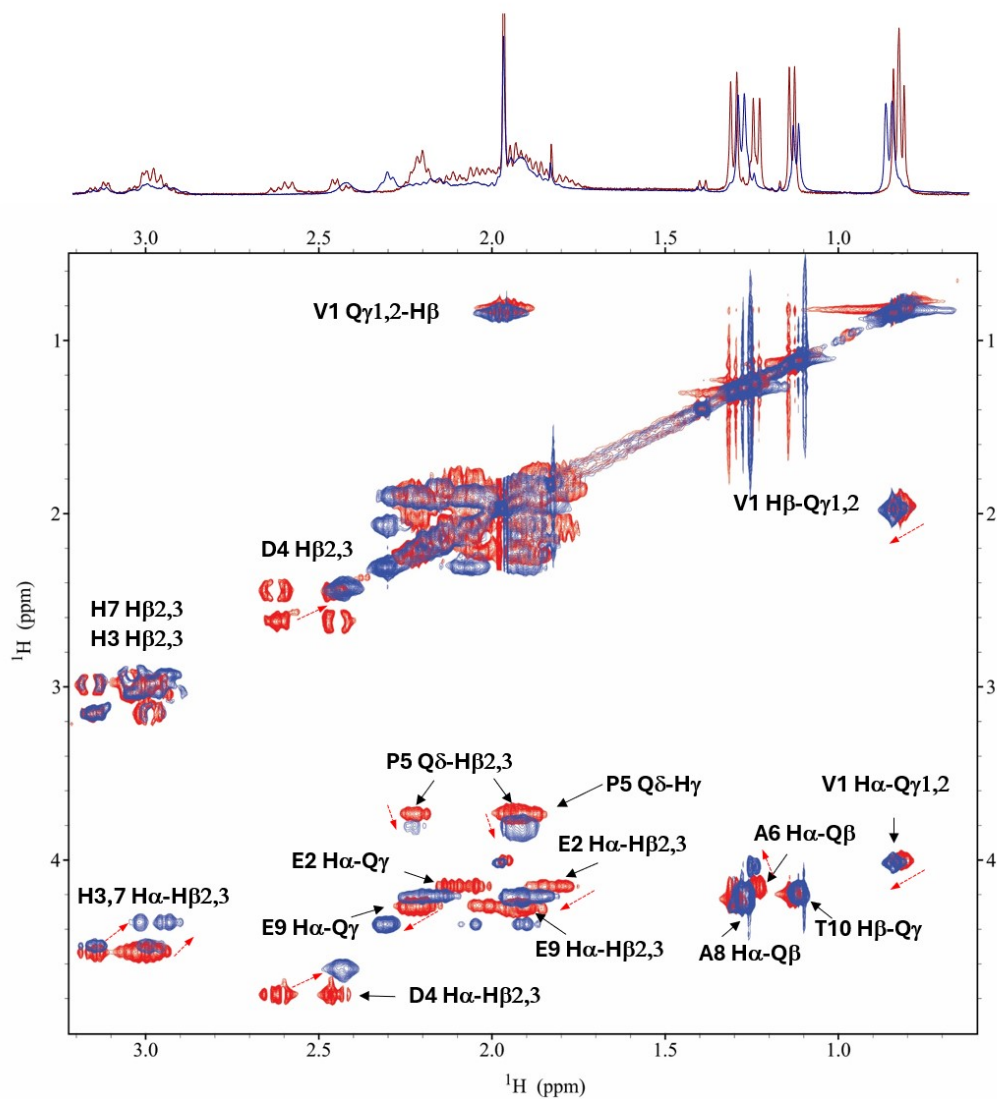


Figure S14. Comparison of the selected aliphatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Zn(II):L1 complex (blue) at a 1:1 molar ratio, at pH 7. Signals showing significant chemical shift changes upon Zn^{2+} coordination are indicated by red arrows.

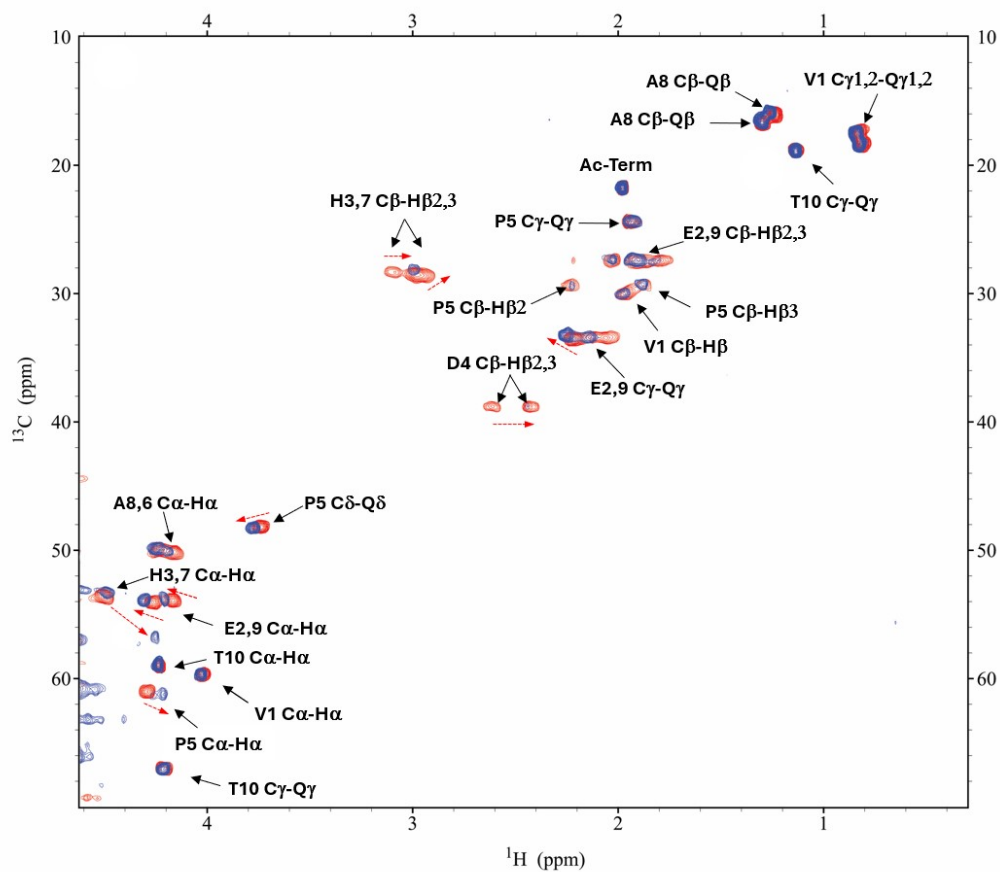


Figure S15. Comparison of the aliphatic region of the ^1H - ^{13}C HSQC spectra for the free peptide **L1** (red) and the Zn(II):L1 complex (blue) at a 1:1 molar ratio, at pH 9.1. Signals exhibiting significant chemical shift changes upon Zn^{2+} coordination are highlighted in red arrows.

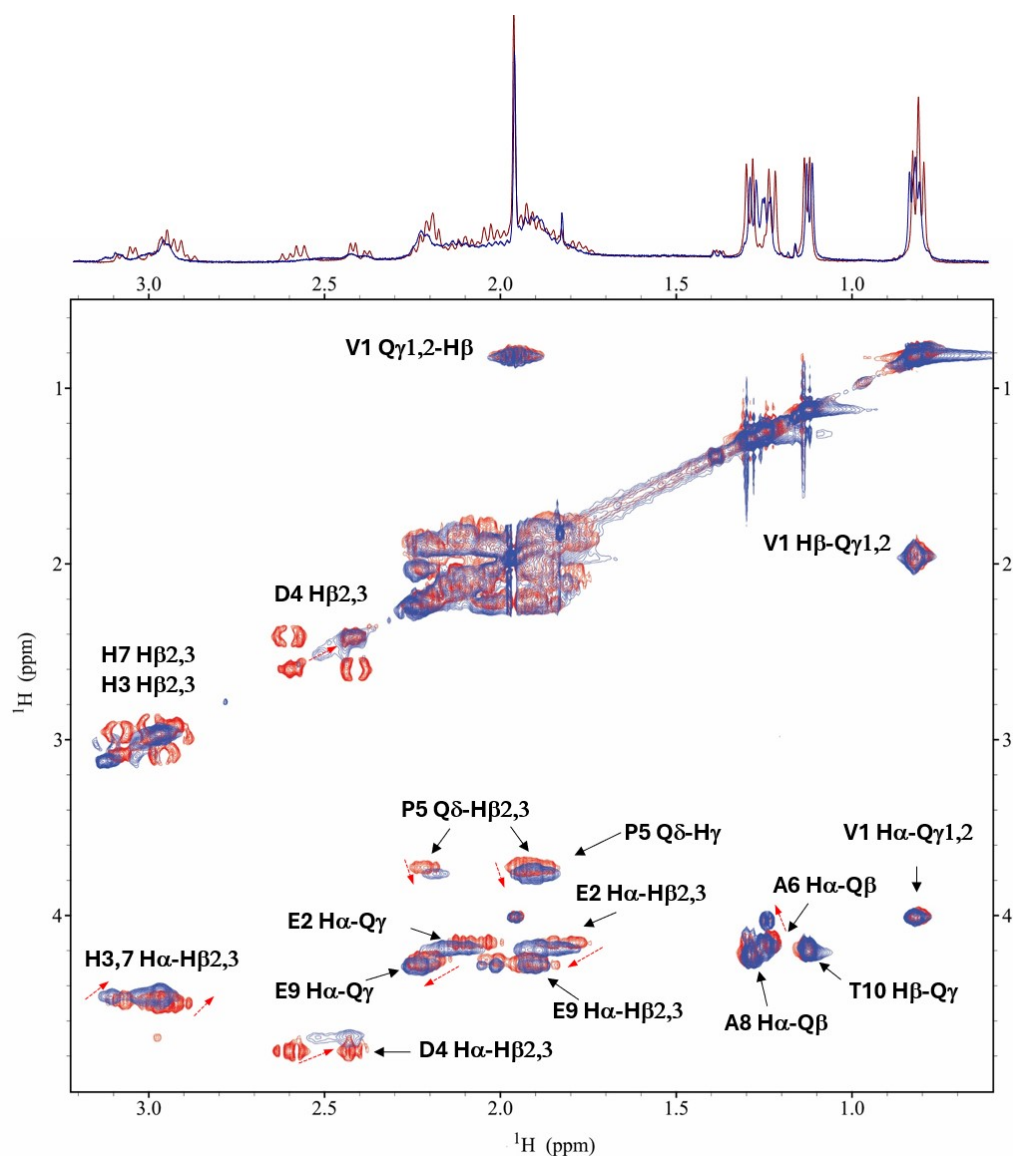


Figure S16. Comparison of the selected aliphatic region of the ^1H - ^1H TOCSY spectra for the free peptide **L1** (red) and the Zn(II):L1 complex (blue) at a 1:1 molar ratio, at pH 9.1. Signals showing significant chemical shift changes upon Zn^{2+} coordination are indicated by red arrows.

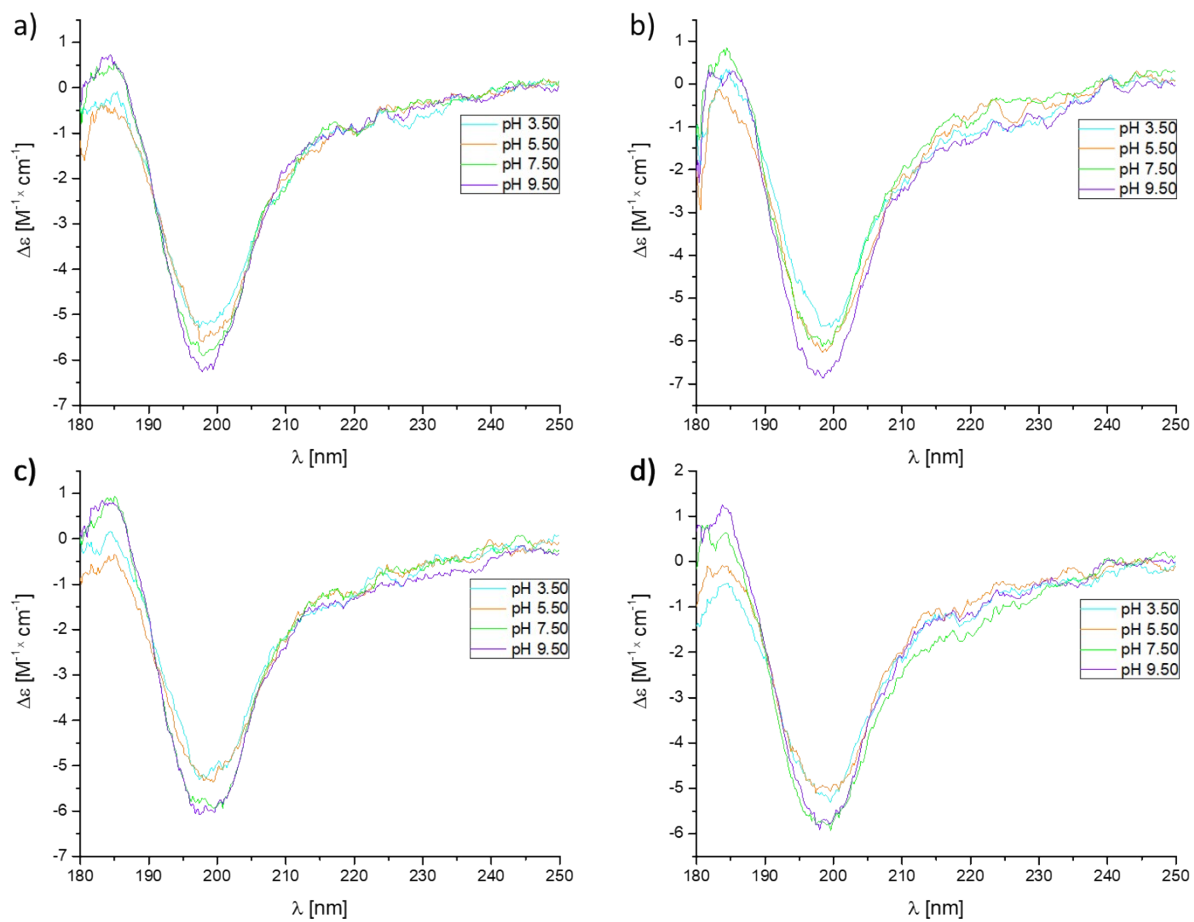


Figure S17. Far UV CD spectra of the systems: a) free L1, b) Fe(II):L1, c) Mn(II):L1, d) Zn(II):L1. $L_{[tot]} = 0.35$ mM, M:L= 1:1.1.