A comparative study on nickel binding to Hpn-like polipeptides from two *Helicobacter pylori* strains.

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Supplementary materials

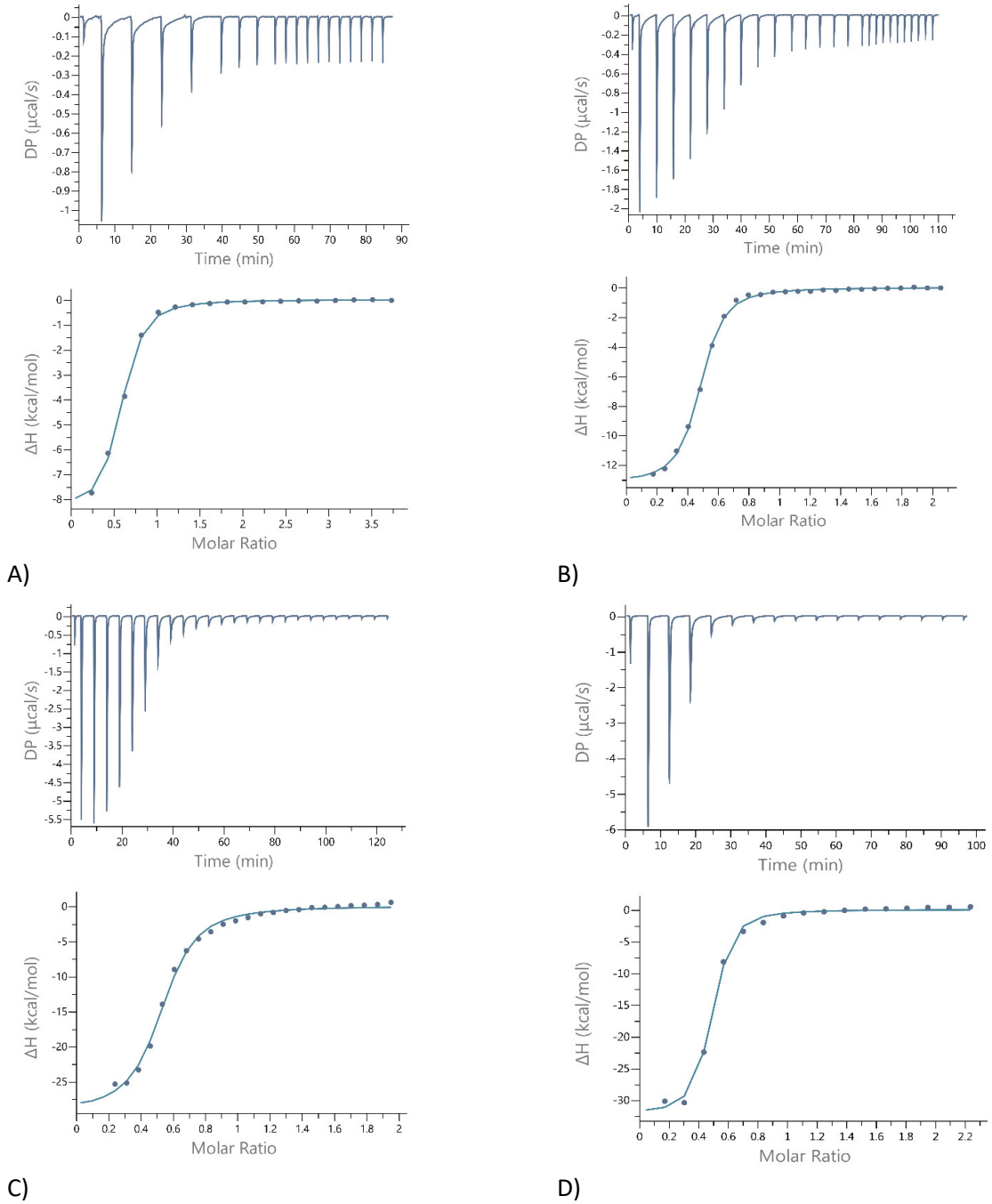


Figure S1. The best-fit ITC data for the 37 ˚C titration of 2mM Ni(II) into (**A**) Hpnl1, (B) Hpnl2, (**C**) Hpnl3, (**D**) Hpnl3a peptides in 20mM TRIS-HCl buffer + 500mM NaCl, pH 7.40. The peptides concentration was within the range of 100-200 µM.

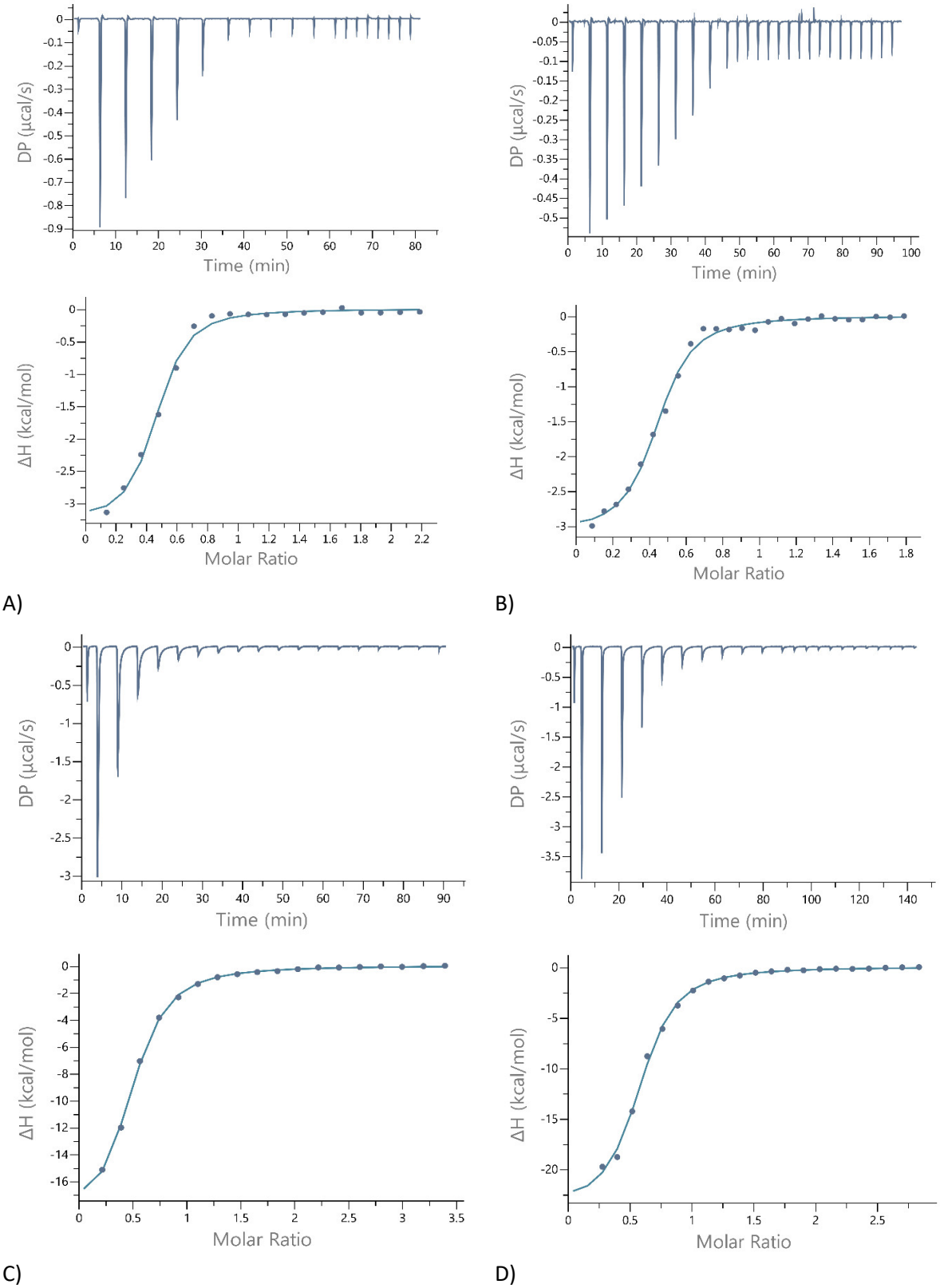


Figure S2. The best-fit ITC data for the 37 ˚C titration of 1-2mM Ni(II) into (**A**) Hpnl1, (**B**) Hpnl2, (**C**) Hpnl3, (**D**) Hpnl3a peptides in 20mM HEPES buffer + 100mM NaCl, pH 7.40. The peptides concentration was within the range of 109-128 µM.

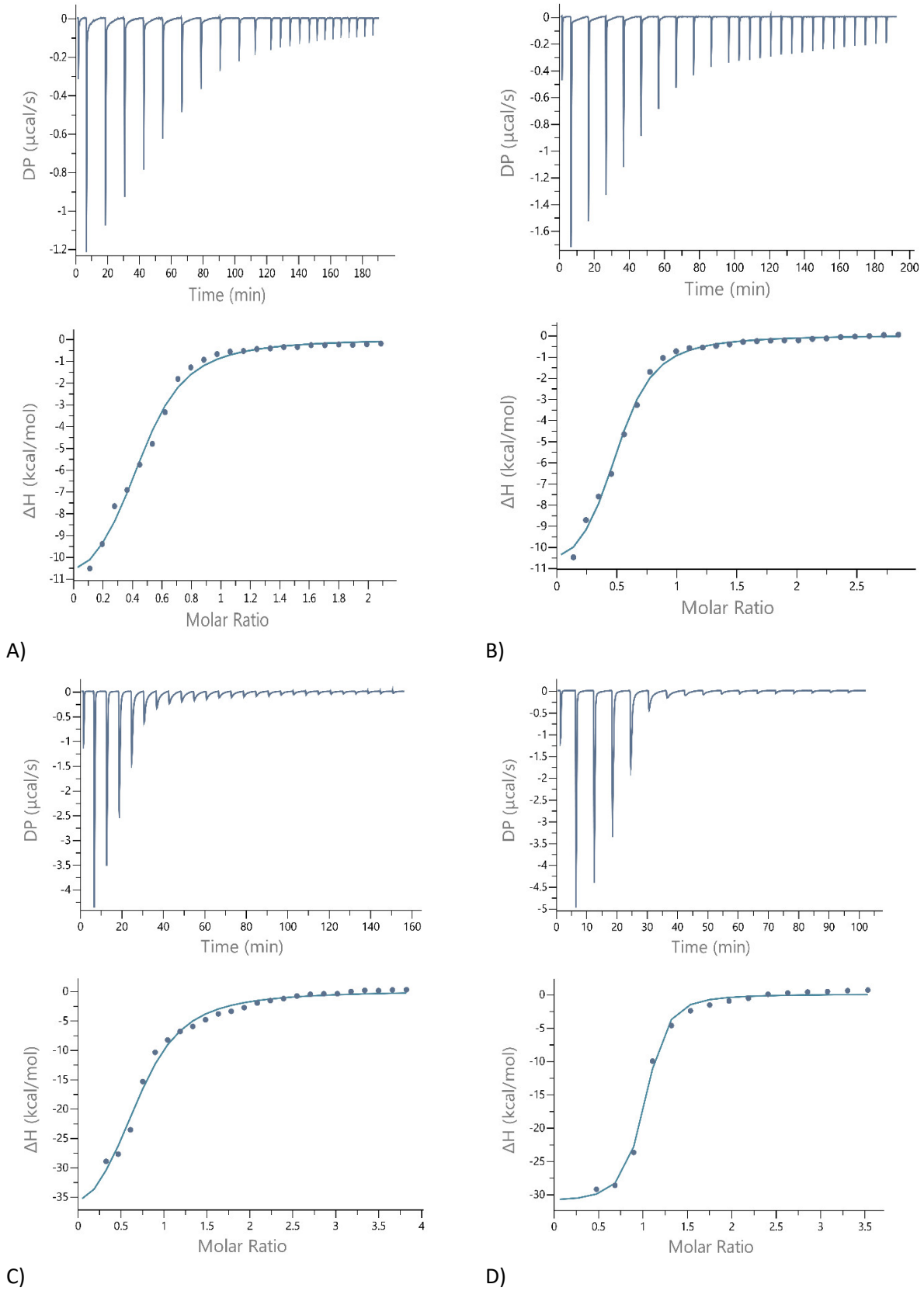


Figure S3. The best-fit ITC data for the 25 ˚C titration of 2mM Ni(II) into (**A**) Hpnl1, (**B**) Hpnl2, (**C**) Hpnl3, (**D**) Hpnl3a peptides in 20mM TRIS-HCl buffer + 500mM NaCl, pH 7.40. The peptides concentration was within the range of 106-171 µM.

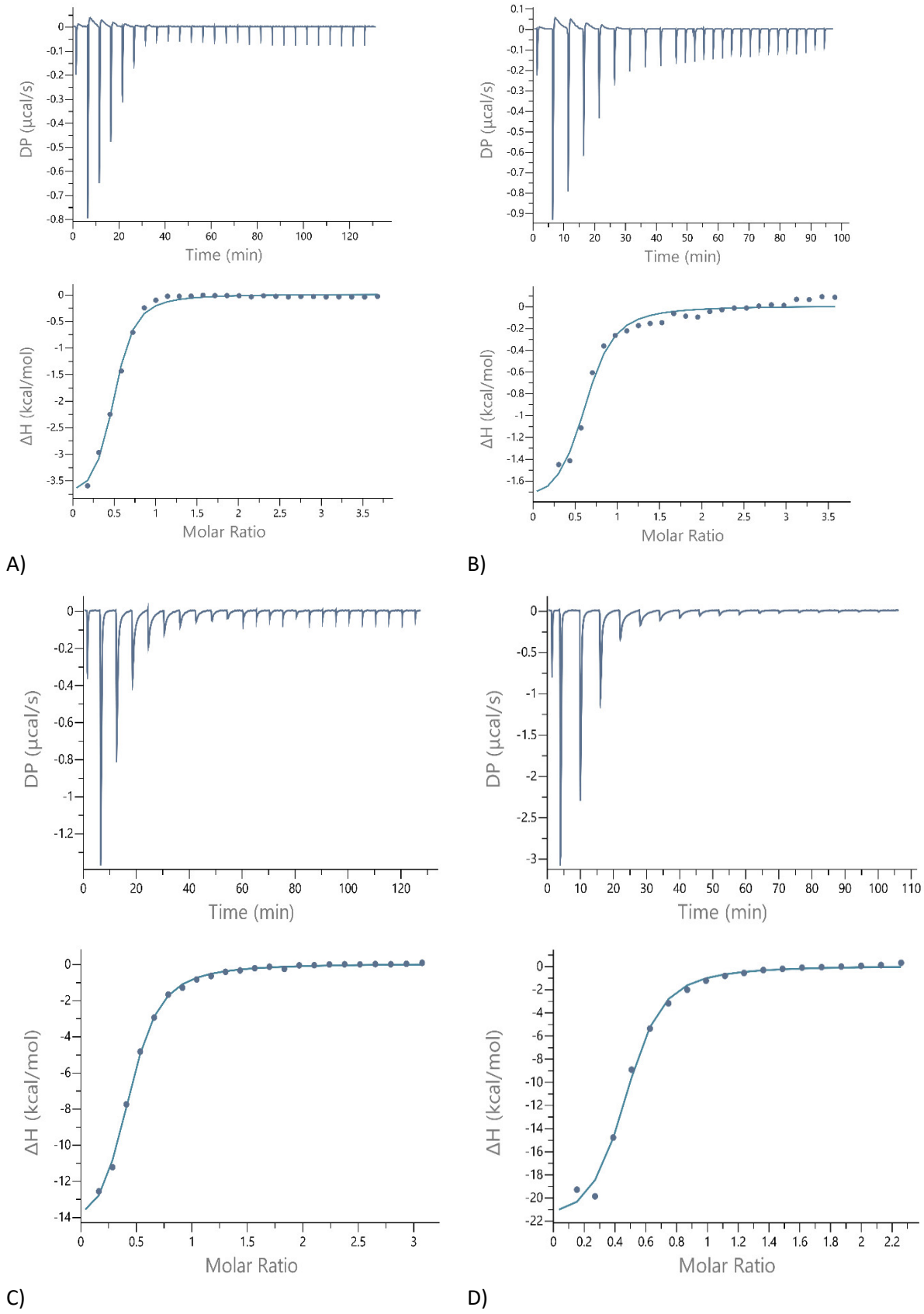


Figure S4. The best-fit ITC data for the 25 ˚C titration of 2mM Ni(II) into (**A**) Hpnl1, (**B**) Hpnl2, (**C**) Hpnl3, (**D**) Hpnl3a peptides in 20mM HEPES buffer + 100mM NaCl, pH 7.40. The peptides concentration was within the range of 109-122 µM.

Table S1. Potentiometric data for Ni (II) complexes with Hpnl1 (MAHHEQQQQQQA-NH_2) and Hpnl2 (MAHHEQQHQA-NH_2). Titrations were carried out over the pH range 2–11 at T = 25 ˚C in an aqueous solution with 4 mM HCl and 0.1 M KCl. The peptide concentration was 0.001 M and the Ni(II)-to-peptide ratios were 1:1, and 1:2.

Species	Hpnl1 MAHHEQQQQQQA-NH_2				Hpnl2 MAHHEQQHQA-NH_2			
	Ni:L 1:1		Ni:L 1:2		Ni:L 1:1		Ni:L 1:2	
	logβ	logK	logβ	logK	logβ	logK	logβ	logK
$NiLH_2$	17.61 (7)		18.31 (8)					
NiLH					12.15 (5)		12.23 (5)	
NiL	5.76 (7)		6.58 (6)					
$NiLH_{-1}$					-0.24 (3)		-0.81 (4)	
$NiLH_{-2}$	-6.64 (9)		-6.61 (8)		-7.46 (6)	7.22	-8.05 (9)	7.24
$NiLH_{-3}$			-13.72 (9)	7.11				

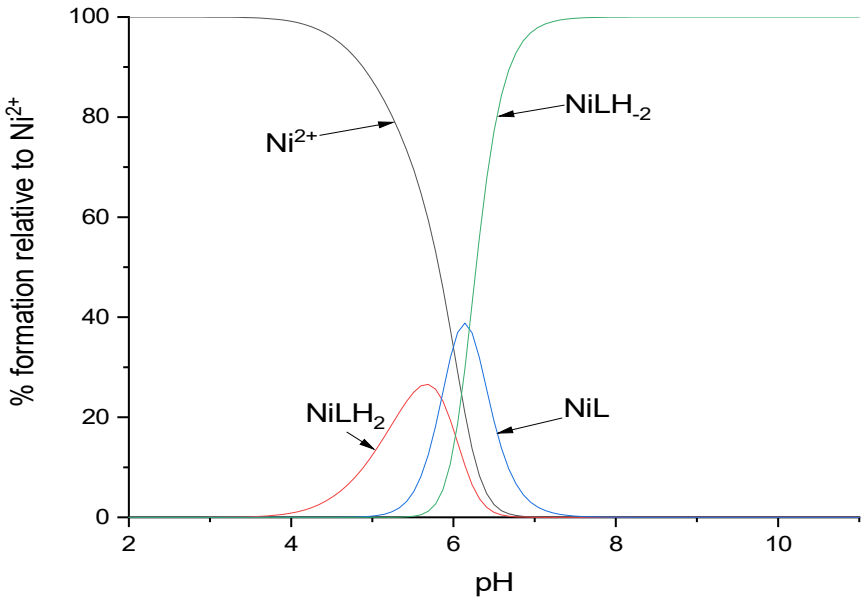


Figure S5. Species distribution profiles for Ni(II) complexes of MAHHEQQQQQQA-NH_2. Metal to ligand ratio = 1:1.

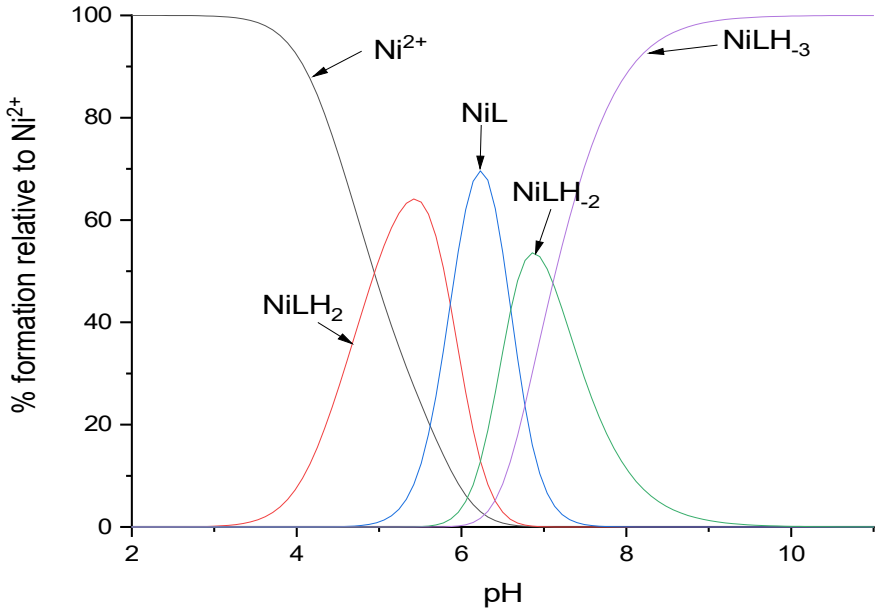


Figure S6. Species distribution profiles for Ni(II) complexes of MAHHEQQQQQQA-NH_2. Metal to ligand ratio = 1:2.

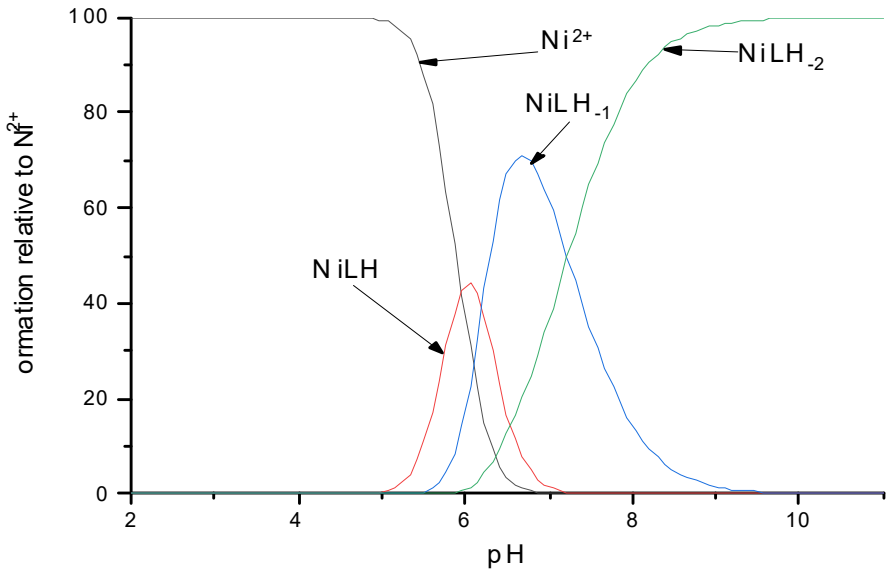


Figure S7. Species distribution profiles for Ni(II) complexes of MAHHEQQHQA-NH_2. Metal to ligand ratio = 1:1.

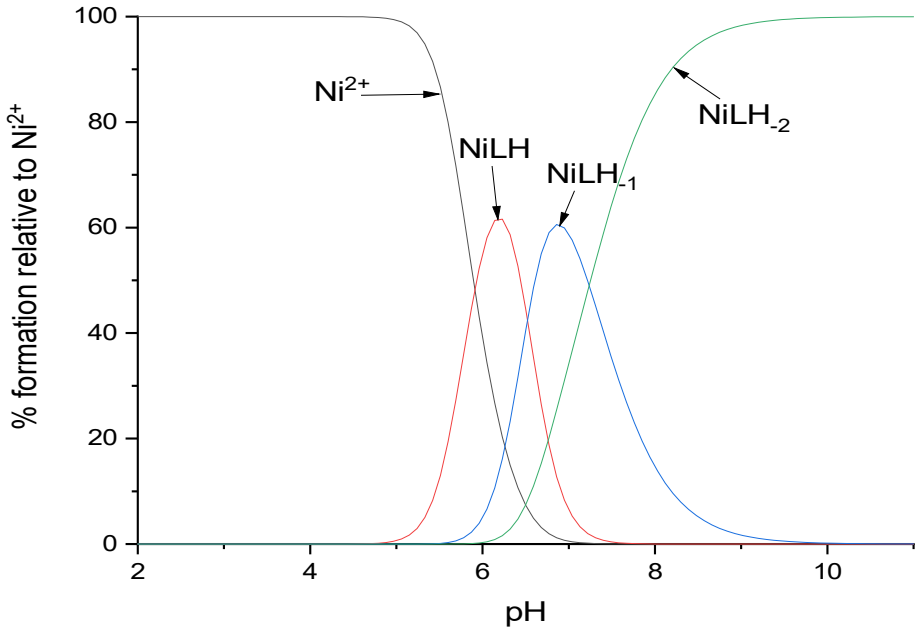


Figure S8. Species distribution profiles for Ni(II) complexes of MAHHEQQHQA-NH_2. Metal to ligand ratio = 1:2.

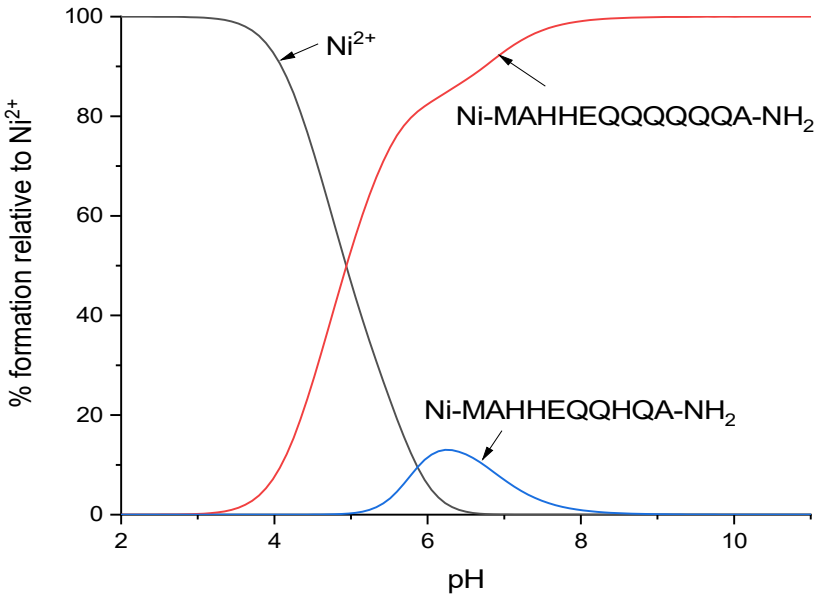
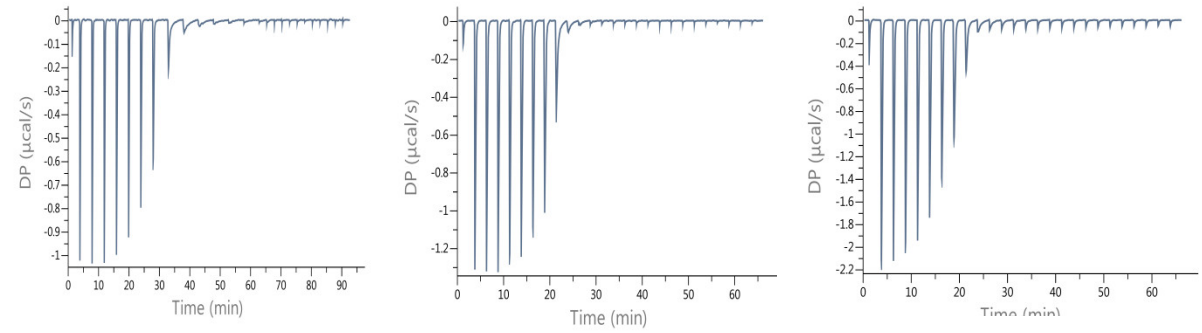


Figure S9. Competition plot for MAHHEQQQQQQA-NH_2, MAHHEQQHQA-NH_2, and Ni(II) showing the relative amount of each complex at different pH values for the hypothetical situation in which equimolar amounts of the two species are mixed. [Ni(II)]=0.0005 M. Ni(II) to ligand ratio of 0.5:1:1.



A B C

Figure S10. ITC traces of 1mM Ni(II) to 0,1 mM EDTA titration in (**A**) HEPES, (**B**) MOPS, and (**C**) TRIS buffer, pH 7.4. The average ΔH_{ITC} for at least 2 best fit titration was -7.94±0.2 kcal/mol; -9.73±0.21 kcal/mol; and -17.4±0.16 kcal/mol for HEPES, MOPS, and TRIS buffer, respectively.

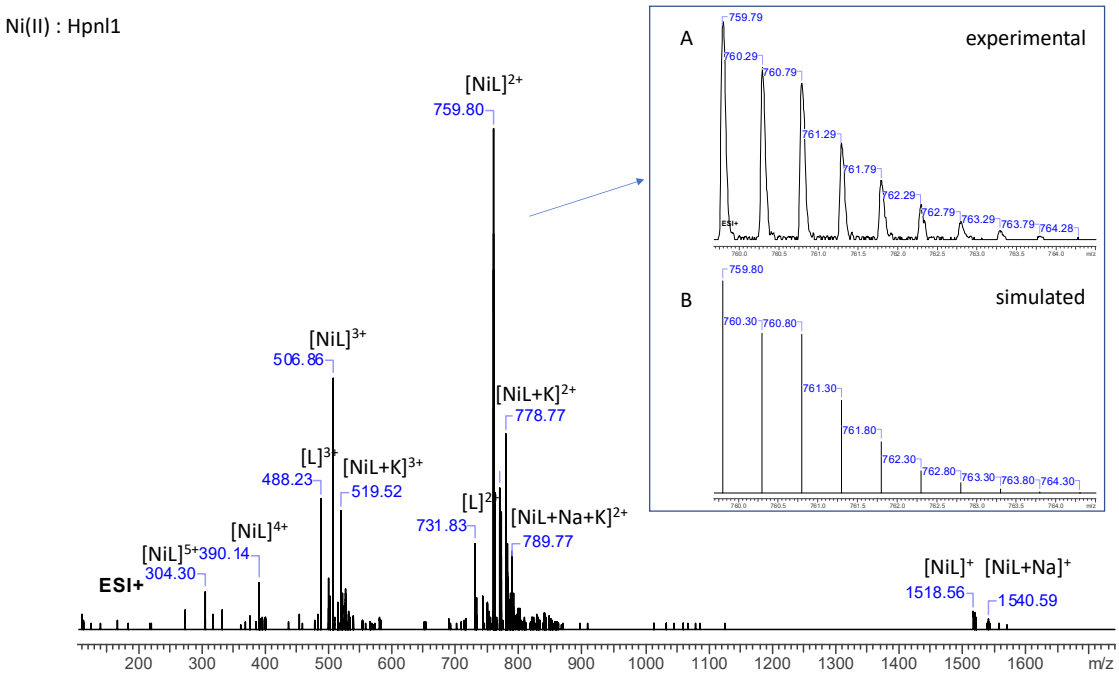


Figure S11. ESI positive mass spectrum (ESI-MS) for the Ni(II):Hpnl1 system with experimental (**A**) and simulated (**B**) spectra for chosen signal, $[NiL]^{2+}$. Metal:ligand in a 1:1 stoichiometry, where $[ligand]_{tot}$= 0.1 mM. Measurements were prepared in water/methanol (50/50, v/v) mixture at pH 7.40.

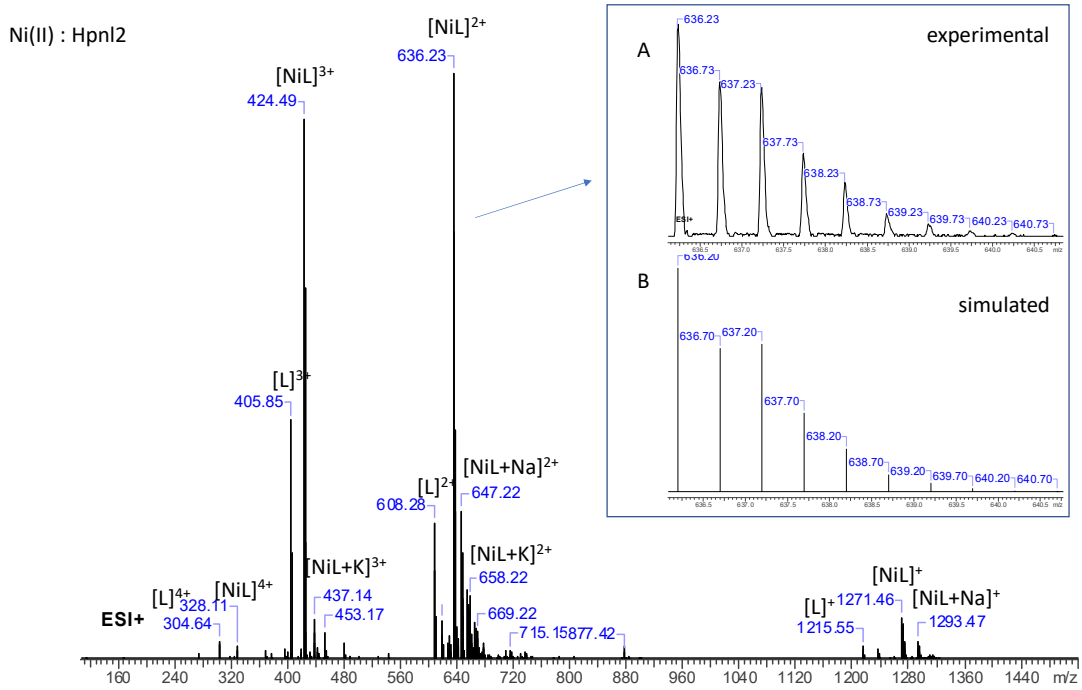


Figure S12. ESI positive mass spectrum (ESI-MS) for the Ni(II):Hpnl2 system with experimental (**A**) and simulated (**B**) spectra for chosen signal, $[NiL]^{2+}$. Metal:ligand in a 1:1 stoichiometry, where $[ligand]_{tot}$= 0.1 mM. Measurements were prepared in water/methanol (50/50, v/v) mixture at pH 7.40.

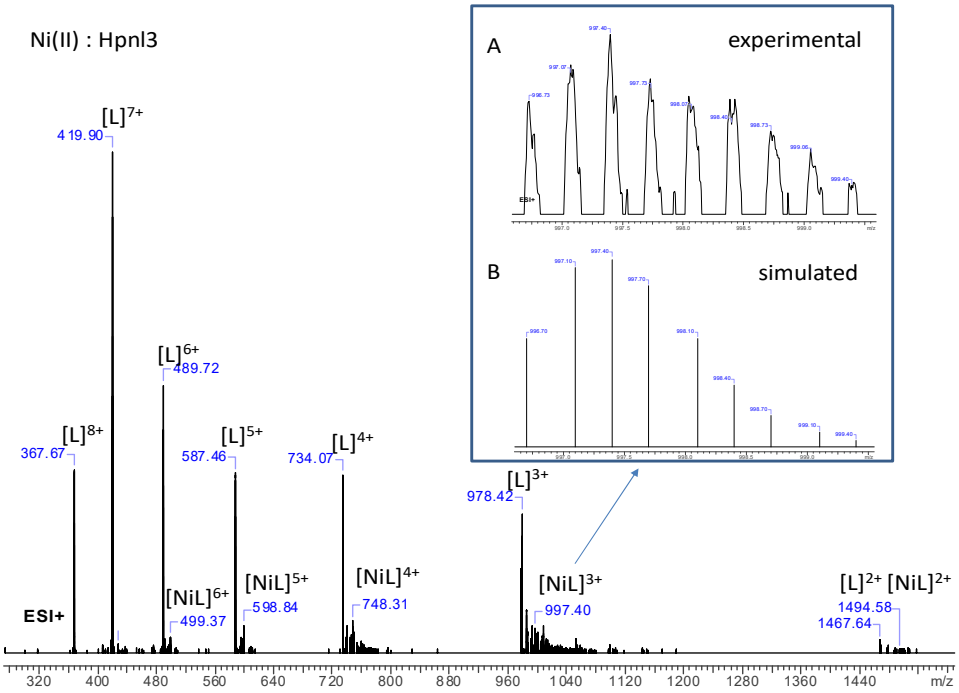


Figure S13. ESI positive mass spectrum (ESI-MS) for the Ni(II):Hpnl3 system with experimental (**A**) and simulated (**B**) spectra for chosen signal, $[NiL]^{3+}$. Metal:ligand in a 1:1 stoichiometry, where $[ligand]_{tot}$ = 0.1 mM. Measurements were prepared in water/methanol (50/50, v/v) mixture at pH 7.40.

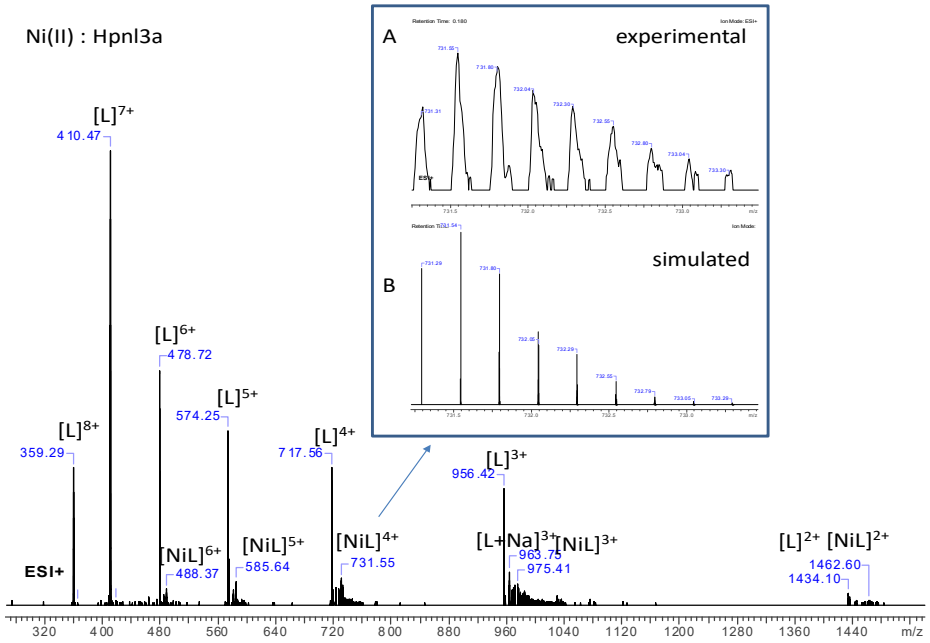
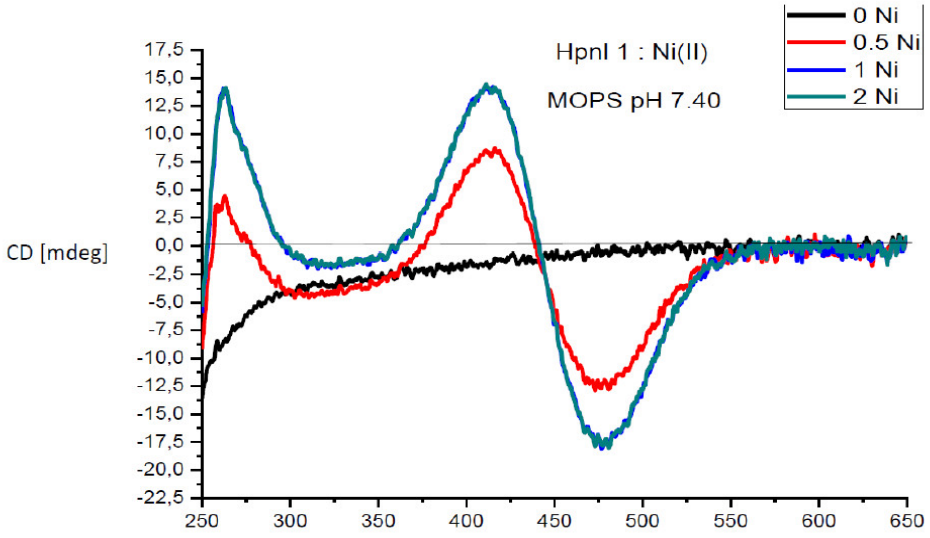
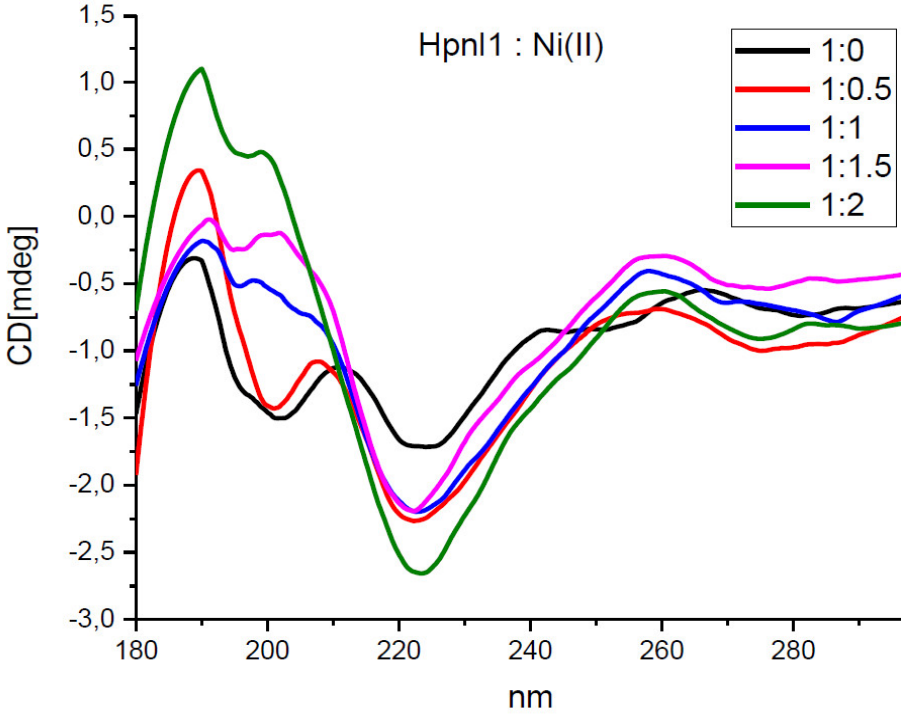


Figure S14. ESI positive mass spectrum (ESI-MS) for the Ni(II):Hpnl3a system with experimental (**A**) and simulated (**B**) spectra for chosen signal, $[NiL]^{3+}$. Metal:ligand in a 1:1 stoichiometry, where $[ligand]_{tot}$= 0.1 mM. Measurements were prepared in water/methanol (50/50, v/v) mixture at pH 7.40.



A)



B)

Figure S15. (**A**) The visible CD and (**B**) far-UV CD spectra of Ni(II) complexes of MAHHEQQQQQQA-NH2 (Hpnl1) peptide, pH 7,4.

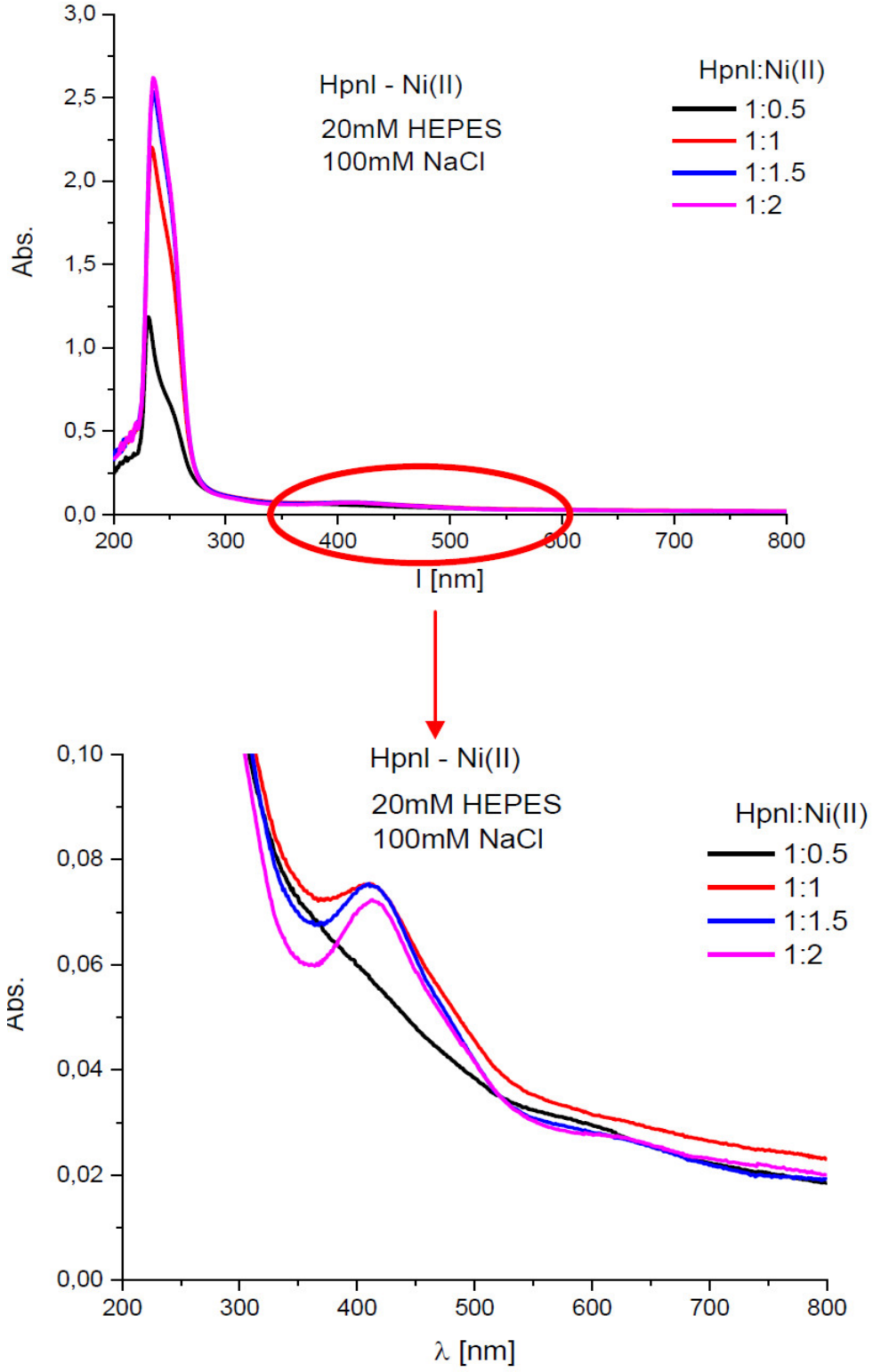
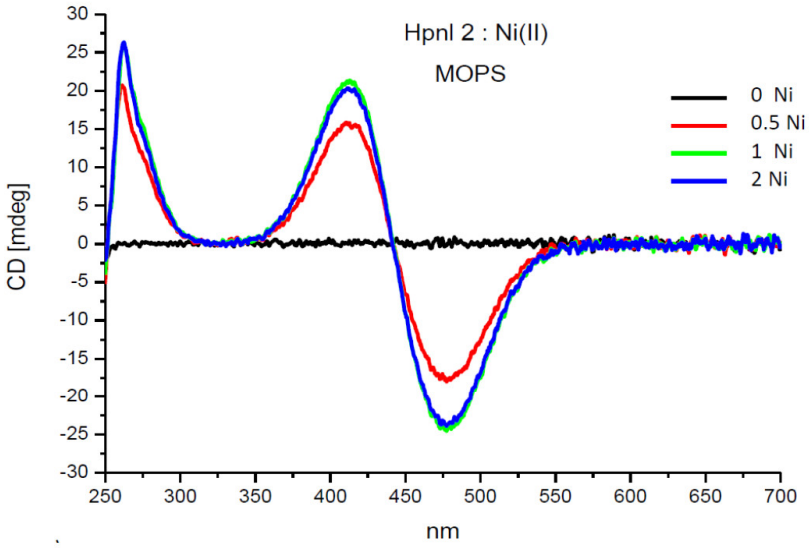
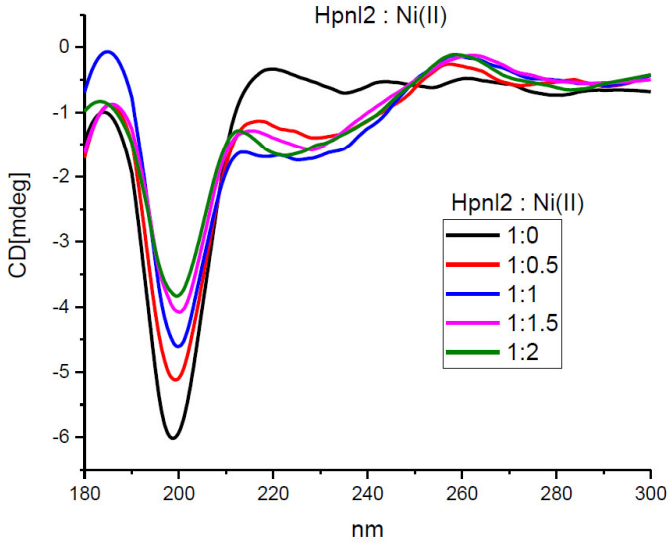


Figure S16. UV-Vis spectra of Ni(II) complexes of MAHHEQQQQQQA-NH2 (Hpnl1) peptide, pH 7,4.



A)



B)

Figure S17. (**A**) The visible CD and (**B**) far-UV CD spectra of Ni(II) complexes of Hpnl 2 (MAHHEQQHQA-NH2) peptide, pH 7,4.

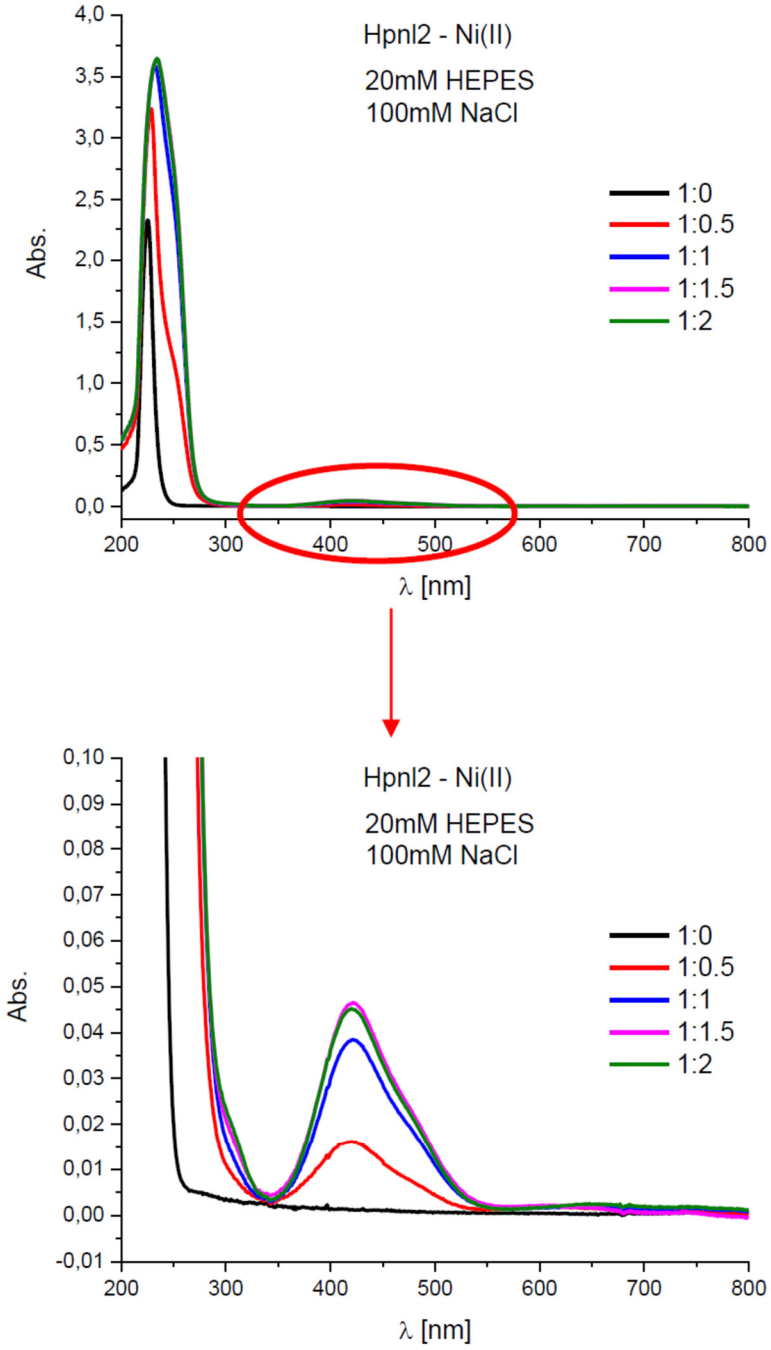


Figure S18. UV-Vis spectra of Ni(II) complexes of Hpnl 2 (MAHHEQQHQA-NH2) peptide, pH 7,4.