Electronic Supplementary Information (ESI)

Metal specificity of N-terminal and G-domain Ni(II) and Zn(II) binding sites in *E.coli* HypB

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Table S1 Protonation constants of HypB(164-199) peptide in 40 mM SDS solution of 4 mM $HClO_4$ at I = 100 mM $NaClO_4$. [L] = 0.5 mM, T = 298 K. The standard deviations are reported in parentheses as uncertainties on the last significant figure.

species	*logβ*	*pKa*
HL	9.85(2)	9.85 K
H_2L	17.86(4)	8.01 C
H_3L	24.31(5)	6.45 C
H_4L	30.15(5)	5.84 H
H_5L	35.51(5)	5.36 E
H_6L	40.38(7)	4.87 D
H_7L	43.35(9)	2.97 D
H_8L	46.03(8)	2.68 D

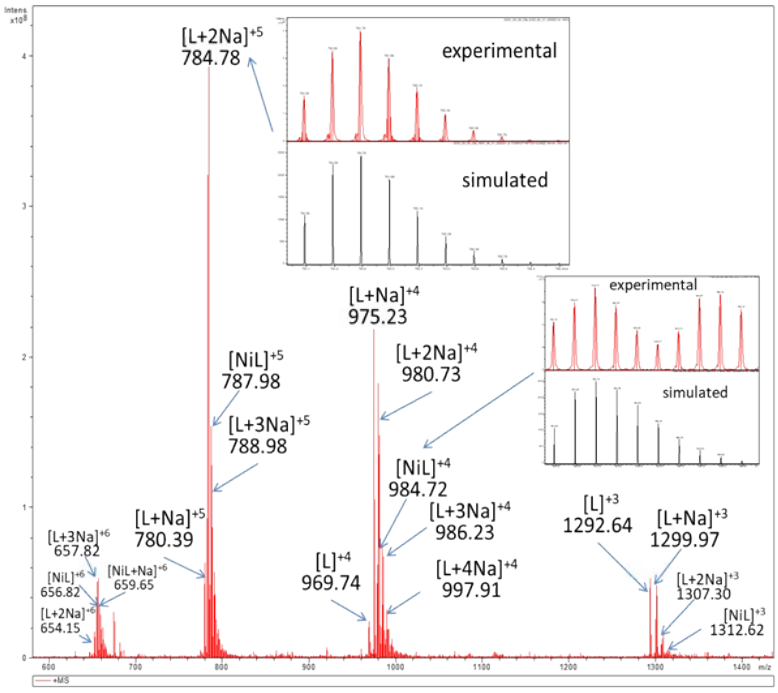

Figure S1. ESI-MS spectra of Ni(II)- HypB(164-199) system. Molar ratio for Ni(II) complexes 1:1. [L] = 0.5 mM. The complexes were prepared in a mixture of MeOH:H_2O (1:1) at pH 7. For chosen ligand and complex a comparison of experimental and simulated signals were performed on ESI-MS spectra.

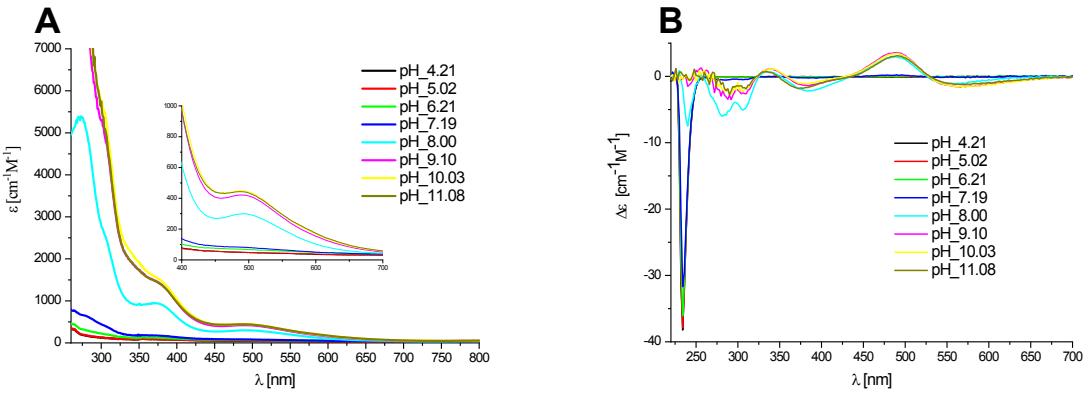

Figure S2. The absorption spectra (A) UV-Vis and (B) CD for the Ni(II)- HypB(164-199) system in 40 mM SDS solution of 4mM $HClO_4$ at I = 100 mM $NaClO_4$. [L] = 0.5 mM, molar ratio M:L – 1:1.d-d bands in the UV-Vis spectra are enlarged. Optical path lengths of 1 cm.

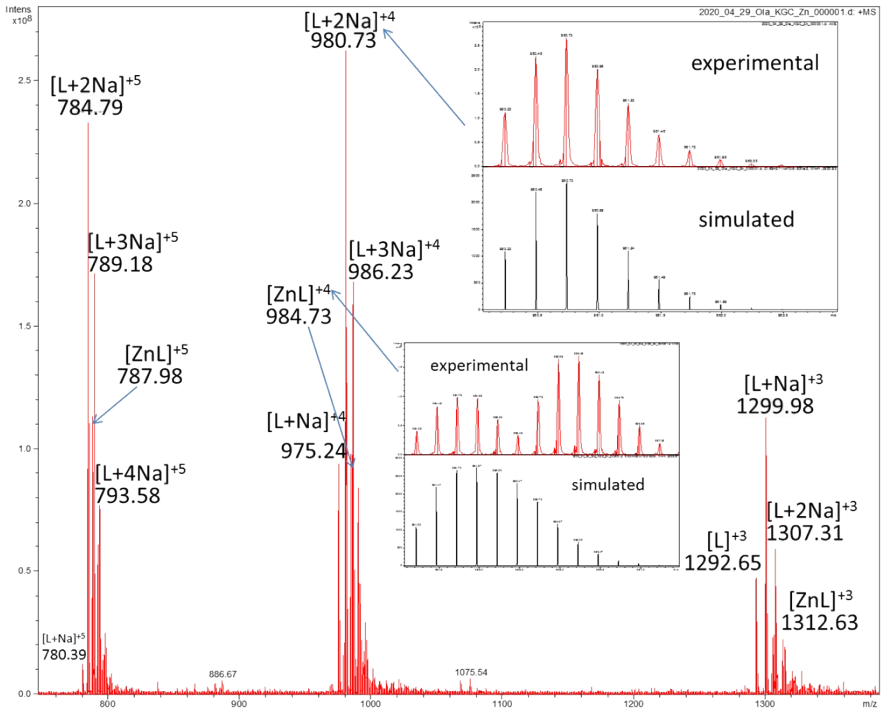

Figure S3. ESI-MS spectra of Zn(II)- HypB(164-199) system. Molar ratio for Zn(II) complexes 1:1. [L] = 0.5 mM. The complexes were prepared in a mixture of MeOH:H_2O (1:1) at pH 7. For chosen ligand and complex a comparison of experimental and simulated signals were performed on ESI-MS spectra.

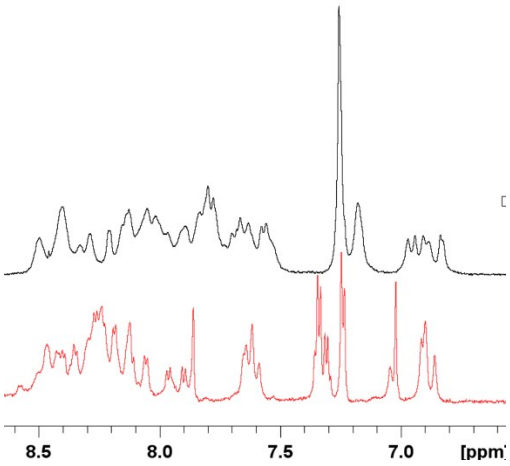

Figure S4. Comparison between amide regions of 1D ^{1}H NMR spectra of HypB(93-128) fragment in aqueous solutions in presence (black lines) and in absence of 40 mM SDS (red lines). C_M = 0.5 mM, pH = 8.0, T = 298 K.

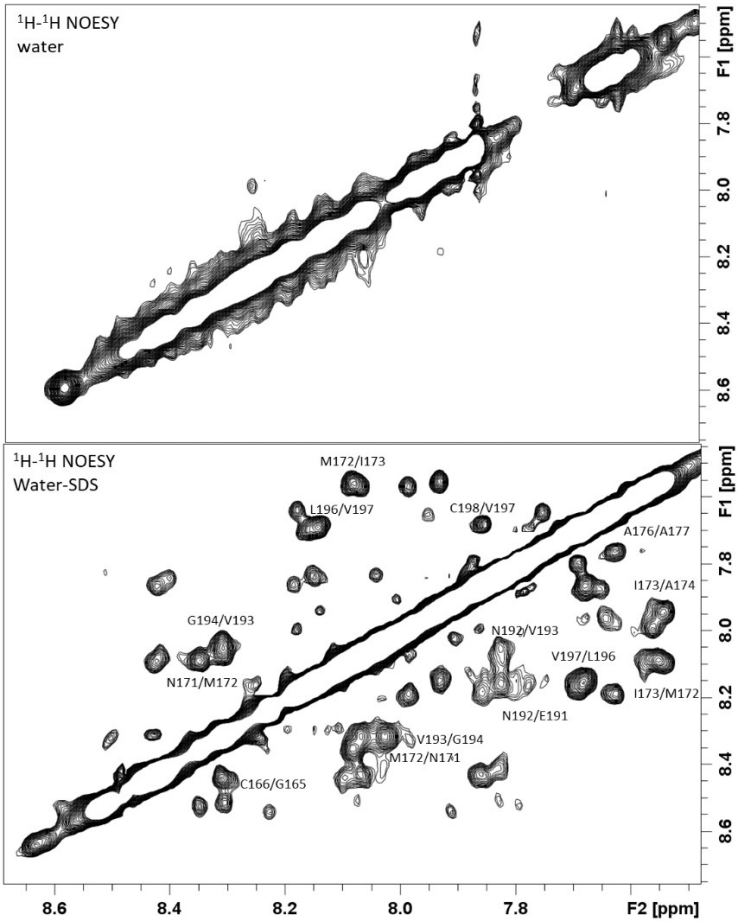

Figure S5. 2D NMR Spectra of ^{1}H-^{1}H NOESY spectra of HypB(164-199) at pH 7.8, T 298 K in absence (top) and in presence (bottom) of 40 mM SDS.

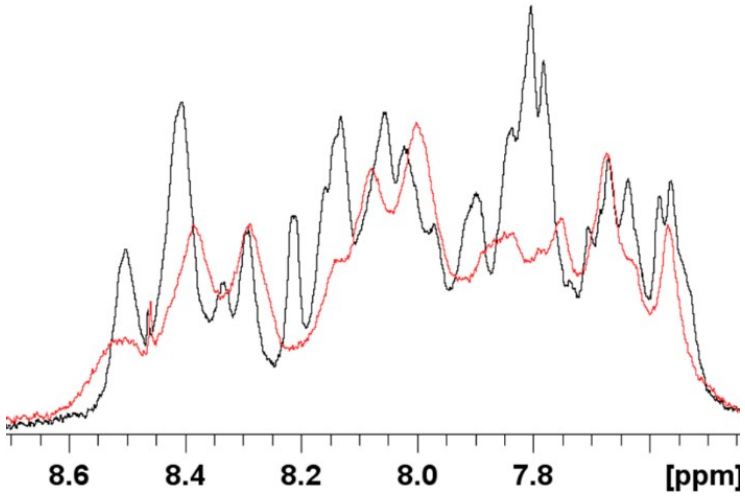

Figure S6. Comparison between amide regions of 1D ^{1}H NMR spectra of HypB(164-199) fragment in aqueous solutions containing 40 mM SDS, C_M = 0.5 mM, pH = 8.0, T = 298 K, in absence (black lines) and in presence of 1.0 Zn(II) eqs. (red lines).

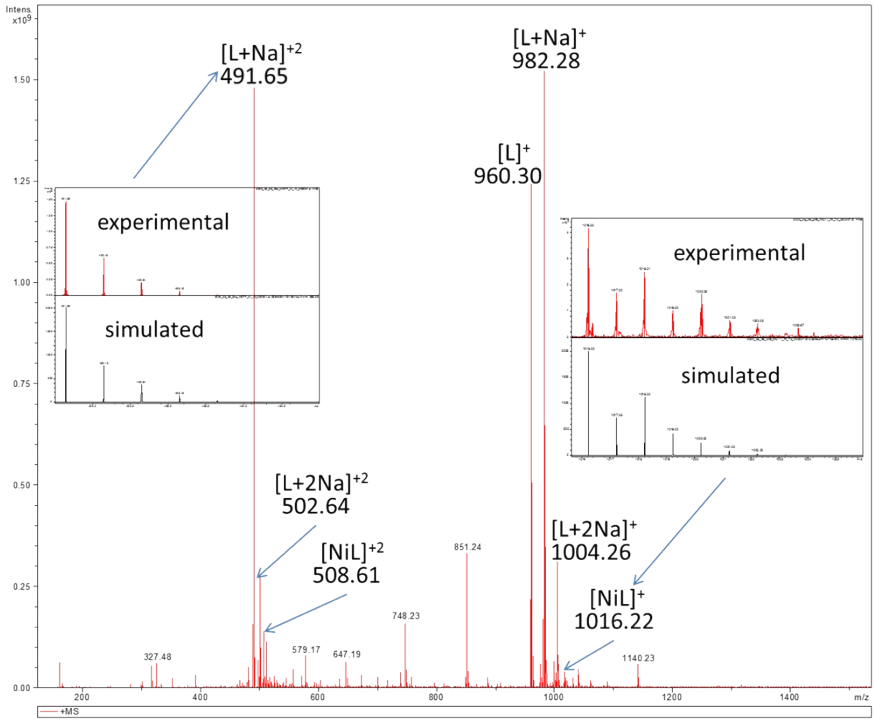

Figure S7. ESI-MS spectra of Ni(II)- HypB(1-10) system. Molar ratio for Ni(II) complexes 1:1. [L] = 0.5 mM. The complexes were prepared in a mixture of MeOH:H_2O (1:1) at pH 7. For chosen ligand and complex a comparison of experimental and simulated signals were performed on ESI-MS spectra.

Table S2 Protonation constants of HypB(1-10)peptide in A) H_2O and B) 40 mM SDS solution of 4 mM $HClO_4$ at I = 100 mM $NaClO_4$. [L] = 0.5 mM, T = 298 K. The standard deviations are reported in parentheses as uncertainties on the last significant figure.

species	***logβ***	***pKa***
A) NH_2-MCTTCGCGEG-NH_2 in H_2O		
HL	9.22(2)	9.22 N-terminus
H_2L	17.99(2)	8.77 C
H_3L	25.94(3)	7.95 C
H_4L	32.99(4)	7.05 C
H_5L	37.60(7)	4.61 E
B) NH_2-MCTTCGCGEG-NH_2 in SDS		
HL	9.04(2)	9.04 N-terminus
H_2L	17.25(2)	8.21 C
H_3L	24.71(4)	7.46 C
H_4L	30.02(6)	5.31 C
H_5L	33.36(8)	3.34 E

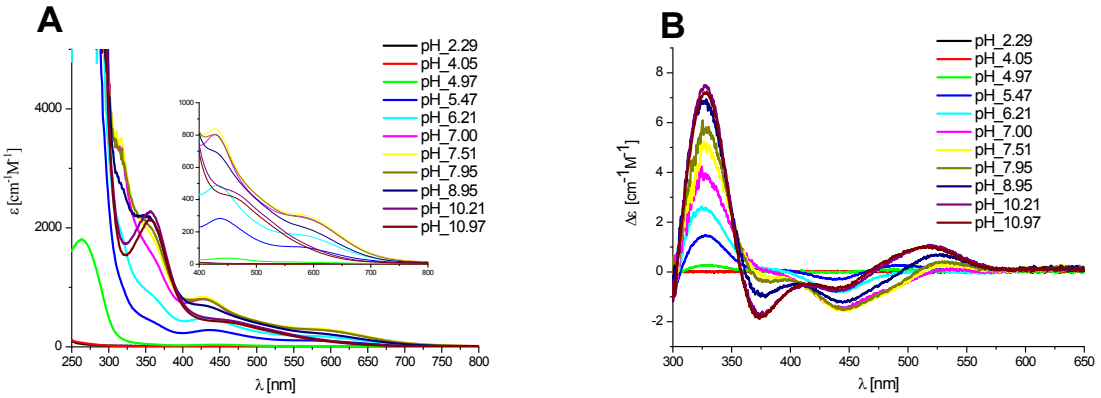

Figure S8. The absorption spectra (A) UV-Vis and (B) CD for the Ni(II)- HypB(1-10)system in H_2O solution. [L] = 0.5 mM, molar ratio M:L – 1:1.d-d bands in the UV-Vis spectra are enlarged. Optical path lengths of 1 cm.

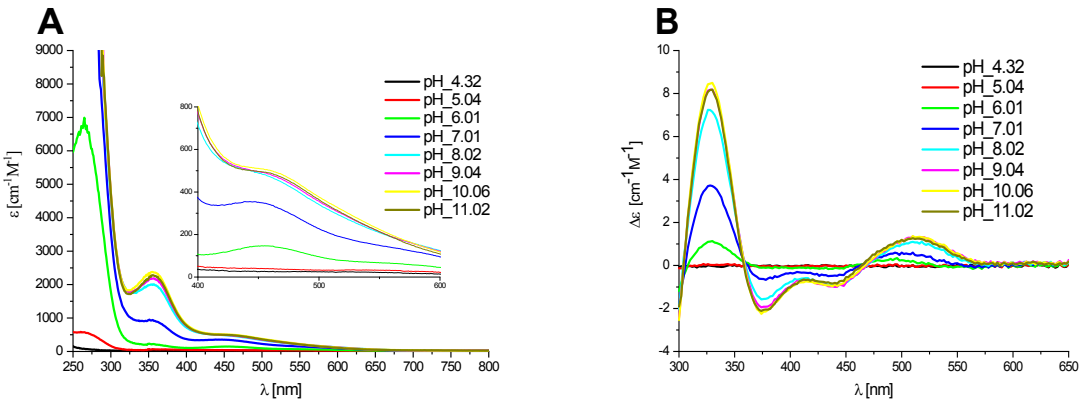

Figure S9. The absorption spectra (A) UV-Vis and (B) CD for the Ni(II)- HypB(1-10) system in 40 mM SDS solution. [L] = 0.5 mM, molar ratio M:L – 1:1.d-d bands in the UV-Vis spectra are enlarged. Optical path lengths of 1 cm.

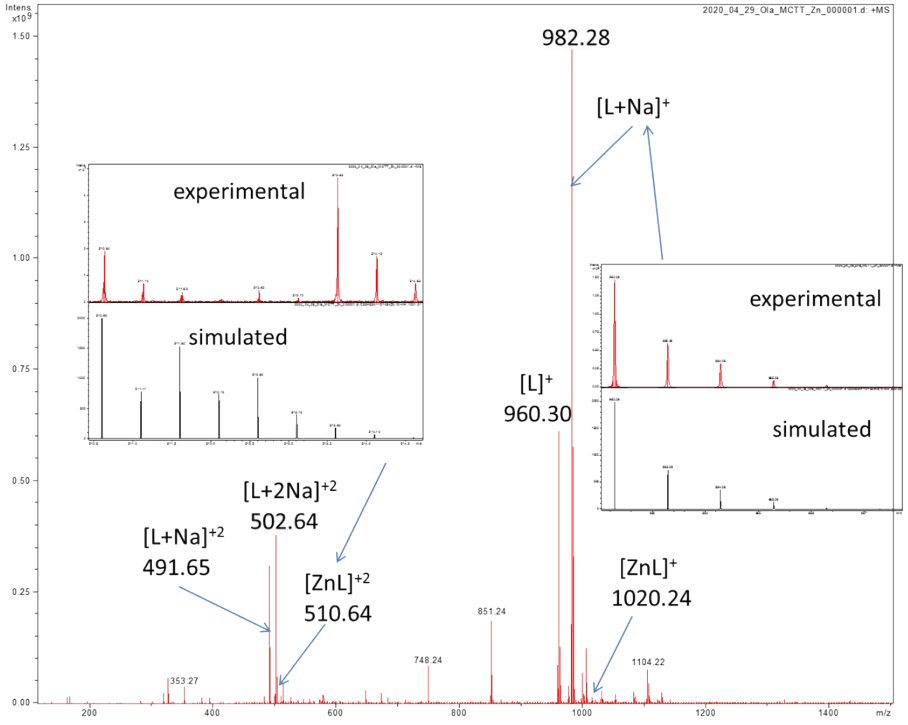

Figure S10. ESI-MS spectra of Zn(II)- HypB(1-10) system. Molar ratio for Ni(II) complexes 1:1. [L] = 0.5 mM. The complexes were prepared in a mixture of MeOH:H_2O (1:1) at pH 7. For chosen ligand and complex a comparison of experimental and simulated signals were performed on ESI-MS spectra.

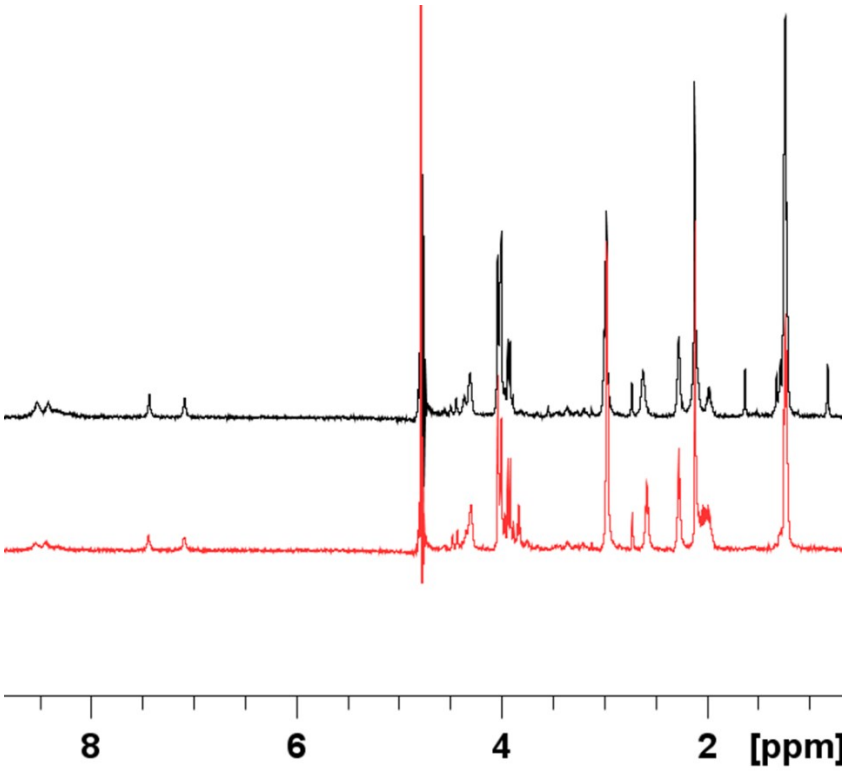

Figure S11. Comparison between amide regions of 1D ^{1}H NMR spectra of HypB(1-10) fragment in aqueous solutions in presence (black lines) and in absence of SDS micelles (red lines). C_M = 0.5 mM, pH = 8.0, T = 298 K.

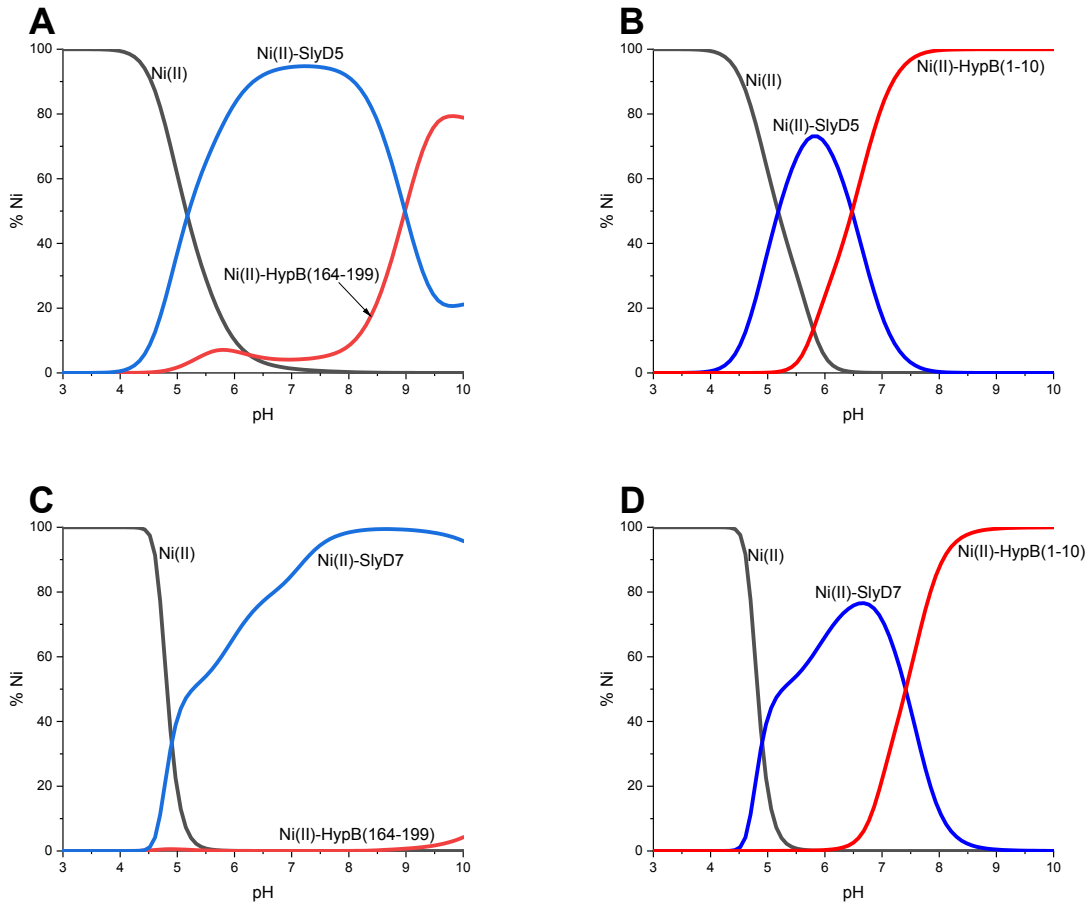

Figure S12. Competition plots for A) Ni(II)-HypB(164-199) and Ni(II)-SlyD5 (Ac-GHGHDHGHEHG-NH_2); B) Ni(II)-HypB(1-10) and Ni(II)-SlyD5 (Ac-GHGHDHGHEHG-NH_2); C) Ni(II)-HypB(164-199) and Ni(II)-SlyD7 (Ac-AHGHVHGAHDHHHD-NH_2) and D) Ni(II)-HypB(1-10) and Ni(II)-SlyD7 (Ac-AHGHVHGAHDH).