Electronic Supplementary Material (ESI) for Metallomics.

Suplementary Information

Pneumococcal histidine triads – involved not only in Zn(II), but also Ni(II) binding?

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        10         20         30         40         50
MKINKKYLVG SAAALILSVC SYELGLYQAR TVKENNRVSY IDGKQATQKT
        60         70         80         90        100
ENLTPDEVSK REGINAEQIV IKITDQGYVT SHGDHYHYYN GKVPYDAIFS
       110        120        130        140        150
EELLMKDPNY KLKDEDIVNE VKGGYVIKVD GKYYVYLKDA AHADNVRTKE
       160        170        180        190        200
EINRQKQEHS QHREGGTPRN DGAVALARSQ GRYTTDDGYI FNASDIIEDT
       210        220        230        240        250
GDAYIVPHGD HYHYIPKNEL SASELAAAEA FLSGRGNLSN SRTYRRQNSD
       260        270        280        290        300
NTSRTNWVPS VSNPGTTNTN TSNNSNTNSQ ASQSNDIDSL LKQLYALPLS
       310        320        330        340        350
QRHVESDGLV FDPAQITSRT ARGVAVPHGD HYHFIPYSQM SELEERIARI
       360        370        380        390        400
IPLRYRSNHW VPDSRPEQPS PQPTPEPSPG PQPAPNLKID SNSSLVSQLV
       410        420        430        440        450
RKVGEGYVFE EKGISRYVFA KDLPSETVKN LESKLSKQES VSHTLTAKKE
       460        470        480        490        500
NVAPRDQEFY DKAYNLLTEA HKALFENKGR NSDFQALDKL LERLNDESTN
       510        520        530        540        550
KEKLVDDLLA FLAPITHPER LGKPNSQIEY TEDEVRIAQL ADKYTTSDGY
       560        570        580        590        600
IFDEHDIISD EGDAYVTPHM GHSHWIGKDS LSDKEKVAAQ AYTKEKGILP
       610        620        630        640        650
PSPDADVKAN PTGDSAAAIY NRVKGEKRIP LVRLPYMVEH TVEVKNGNLI
       660        670        680        690        700
IPHKDHYHNI KFAWFDDHTY KAPNGYTLED LFATIKYYVE HPDERPHSND
       710        720        730        740        750
GWGNASEHVL GKKDHSEDPN KNFKADEEPV EETPAEPEVP QVETEKVEAQ
       760        770        780        790        800
LKEAEVLLAK VTDSSLKANA TETLAGLRNN LTLQIMDNNS IMAEAEKLLA
       810
LLKGSNPSSV SKEKIN
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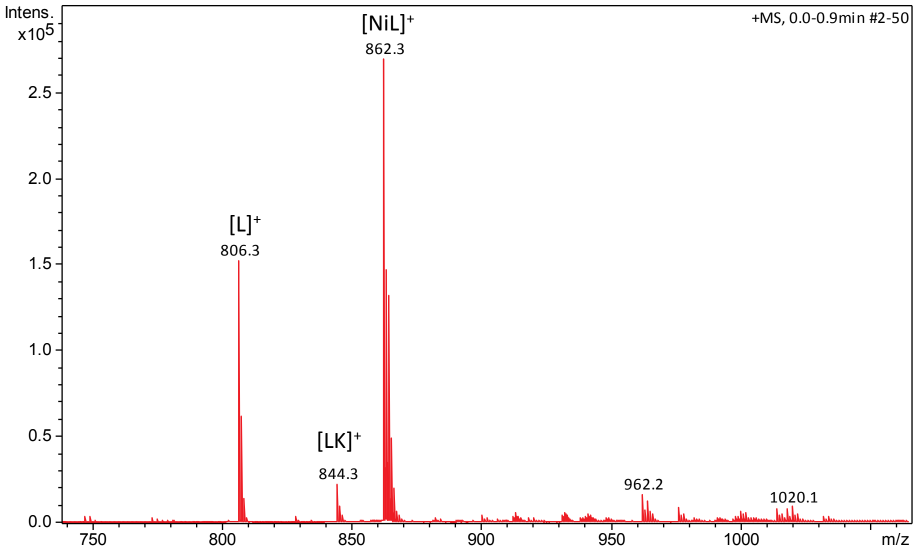
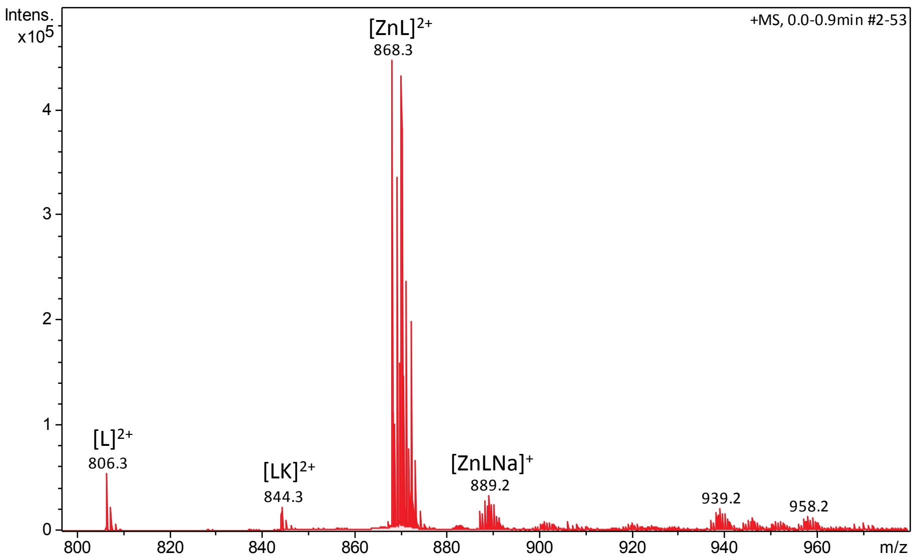
Figure S1. PhtA sequence from *S. pneumoniae*; Uniprot accession numberA0A0T8RGX3. The studied histidine triads are underlined in red.

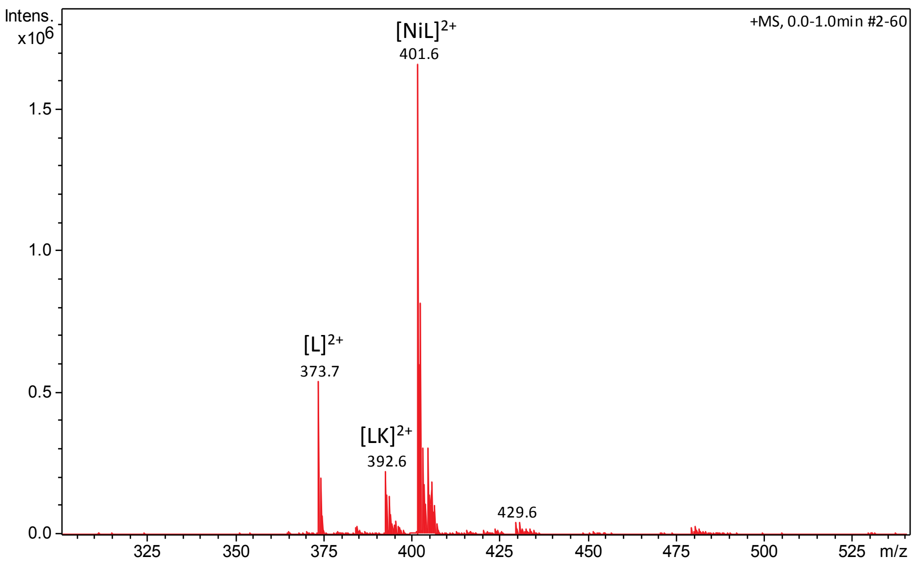
A)


Intens.
x10^5
+MS, 0.0-0.9min #2-50
[NiL]+
862.3
[L]+
806.3
[LK]+
844.3
962.2
1020.1
m/z

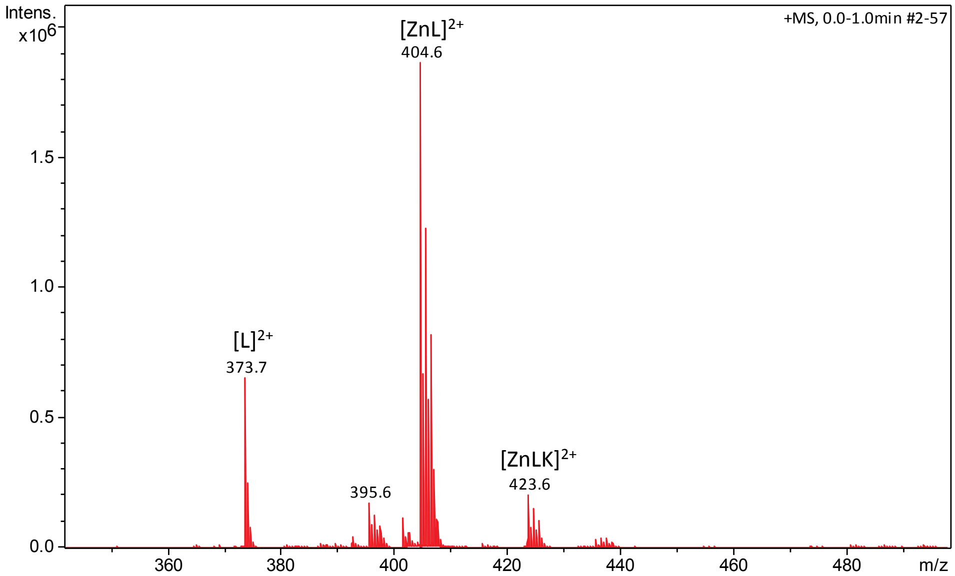
B)


Intens.
x10^5
+MS, 0.0-0.9min #2-53
[ZnL]2+
868.3
[L]2+
806.3
[LK]2+
844.3
[ZnLNa]+
889.2
939.2
958.2
m/z

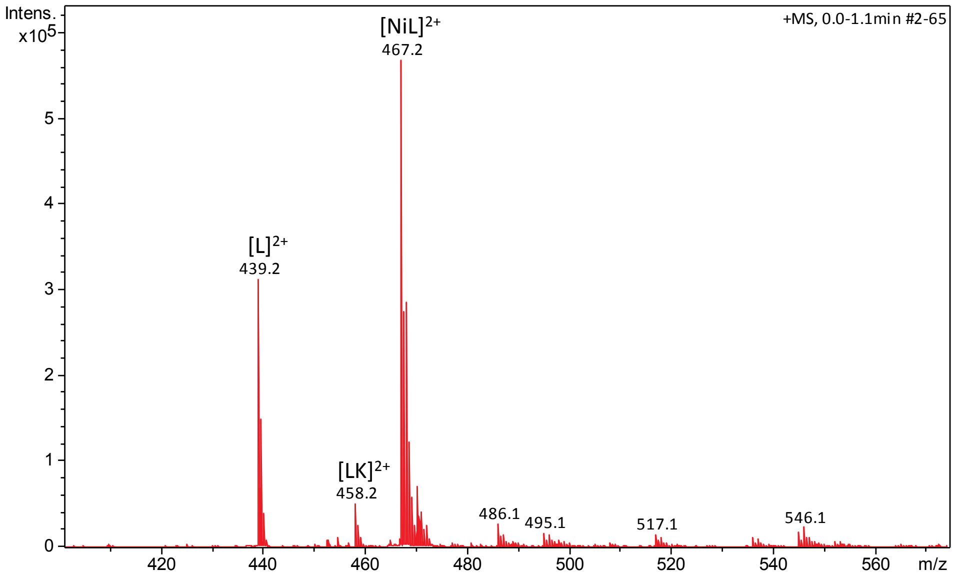
C)


Intens.
x10^6
+MS, 0.0-1.0min #2-60
[NiL]2+
401.6
[L]2+
373.7
[LK]2+
392.6
429.6
m/z

D)


Intens.
x10^6
+MS, 0.0-1.0min #2-57
[ZnL]2+
404.6
[L]2+
373.7
395.6
[ZnLK]2+
423.6
1.5
1.0
0.5
0.0
360
380
400
420
440
460
480
m/z

E)


Intens.
x10^5
+MS, 0.0-1.1min #2-65
[NiL]2+
467.2
[L]2+
439.2
[LK]2+
458.2
486.1
495.1
517.1
546.1
5
4
3
2
1
0
420
440
460
480
500
520
540
560
m/z

F)

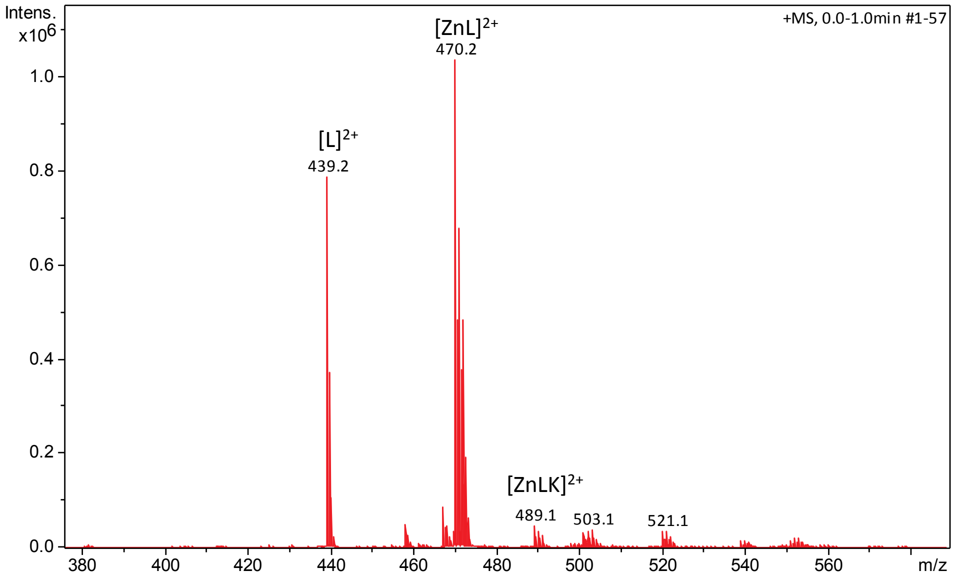
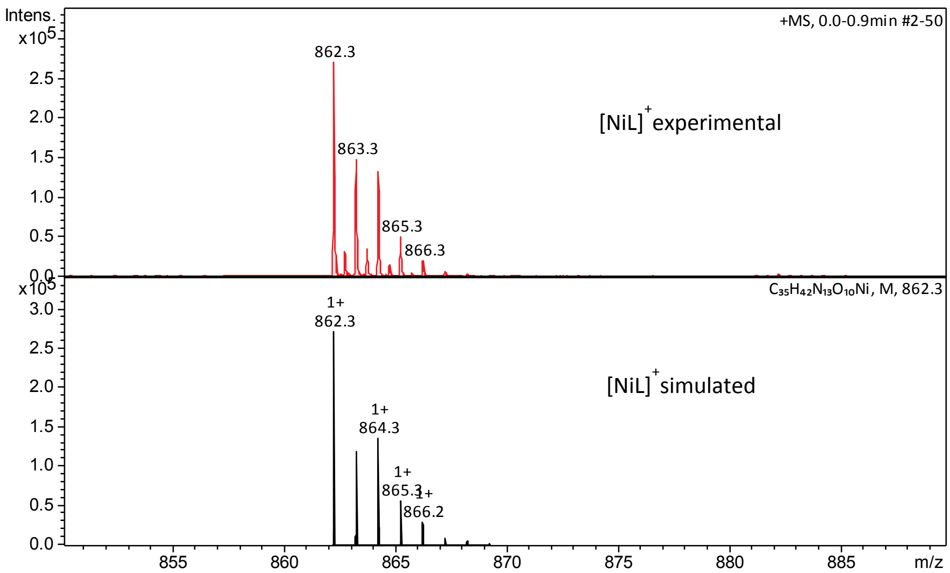
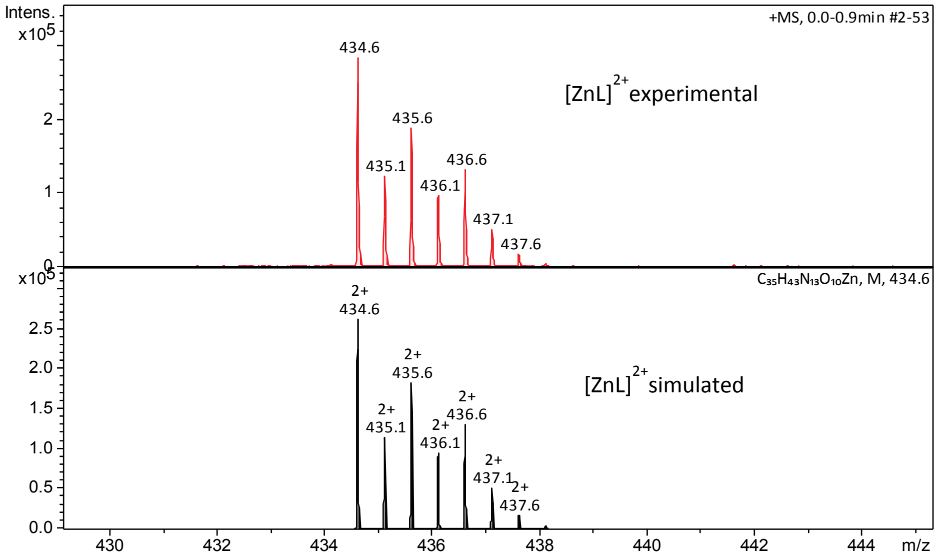


Figure S2. ESI-MS spectra of: A) Ni^{2+}-Ac-HGDHYH-NH_2; B) Zn^{2+}-Ac-HGDHYH-NH_2; C) Ni^{2+}-Ac-HMGHSH-NH_2; D) Zn^{2+}-Ac-HMGHSH-NH_2; E)Ni^{2+}-Ac-HKDHYH-NH_2; F) Zn^{2+}-Ac-HKDHYH-NH_2; M^{2+}:L molar ratio=1:1, pH 6.

A)

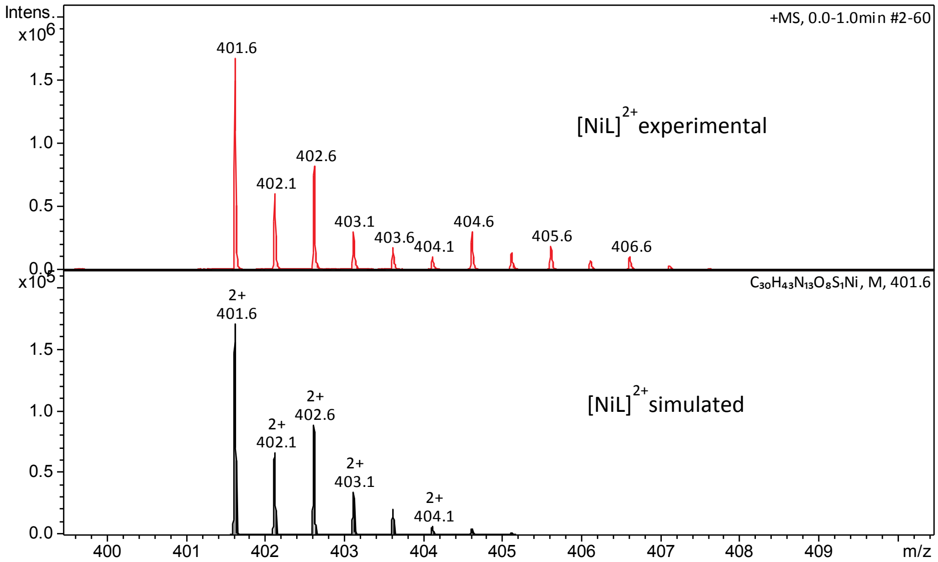


B)



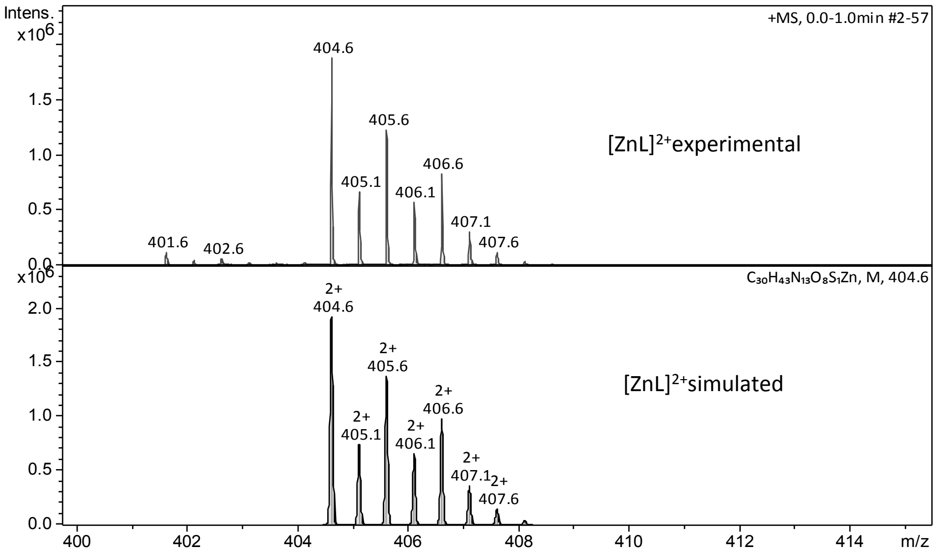
Intens.
x10^5
+MS, 0.0-0.9min #2-53
[ZnL]2+ experimental
434.6
435.1
435.6
436.1
436.6
437.1
437.6
C35H43N13O10Zn, M, 434.6
[ZnL]2+ simulated
m/z

C)



Intens.
x10^6
+MS, 0.0-1.0min #2-60
[NiL]2+ experimental
401.6
402.1
402.6
403.1
403.6
404.1
404.6
405.6
406.6
x10^5
C30H43N13O8S1Ni, M, 401.6
[NiL]2+ simulated
m/z

D)



Intens.
x10^6
+MS, 0.0-1.0min #2-57
[ZnL]2+experimental
401.6
402.6
404.6
405.1
405.6
406.1
406.6
407.1
407.6
C30H43N13O8S1Zn, M, 404.6
[ZnL]2+simulated
m/z

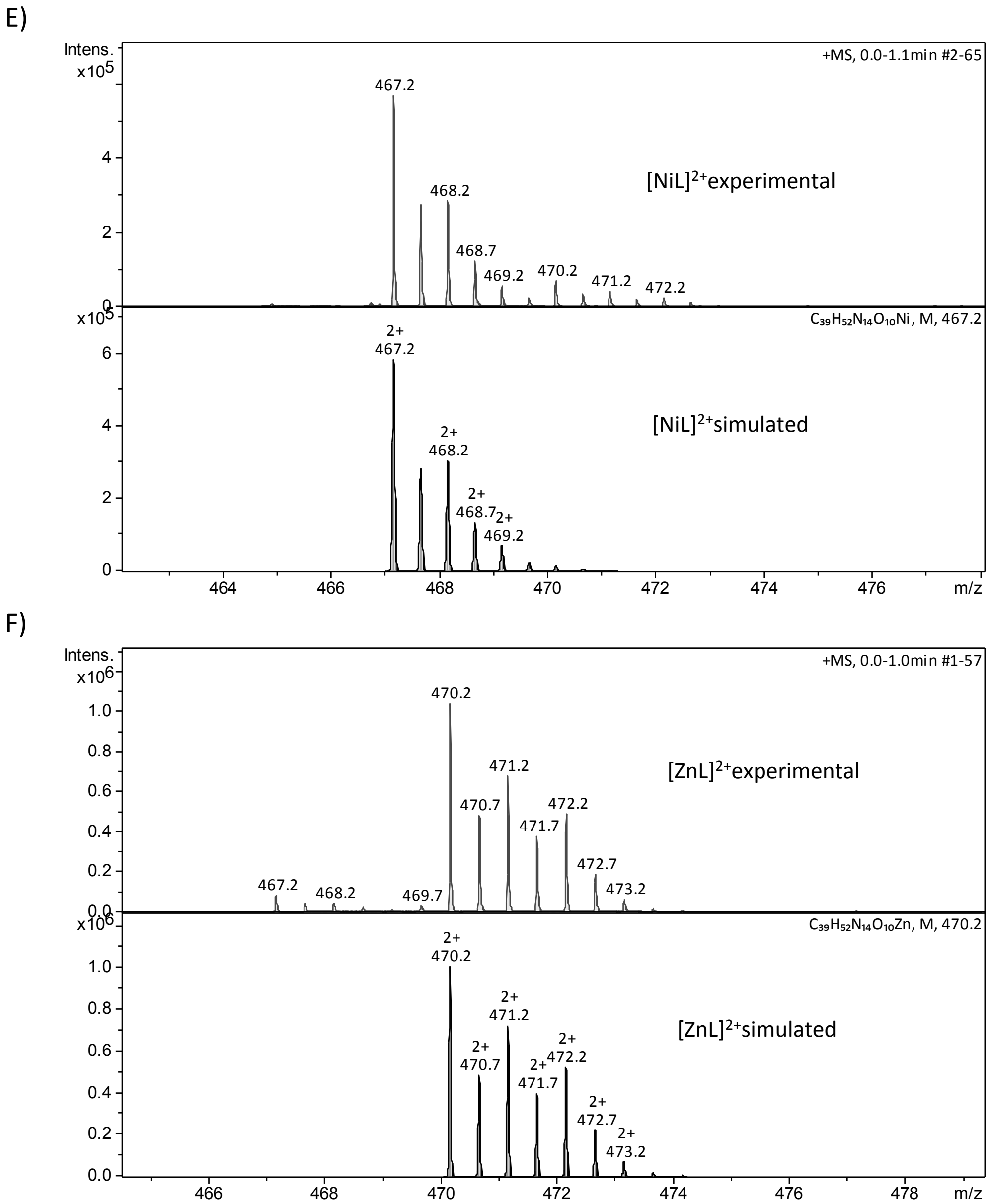
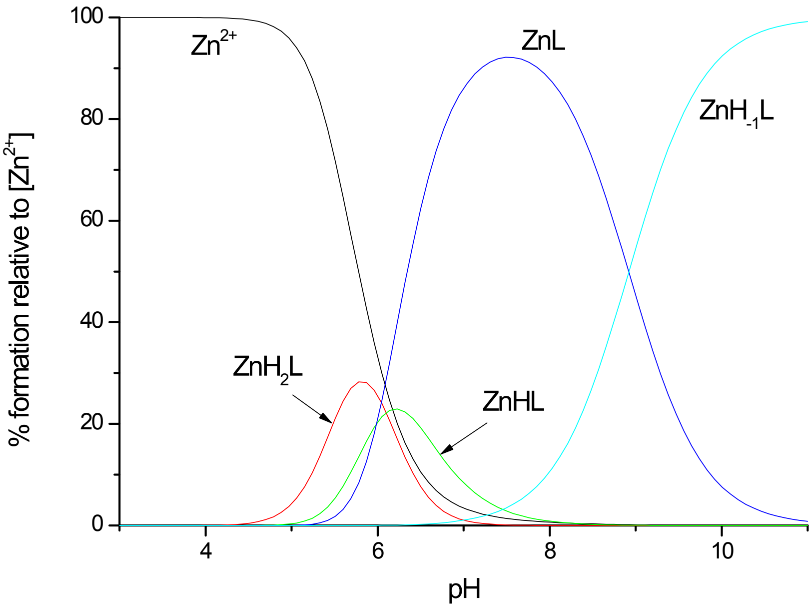


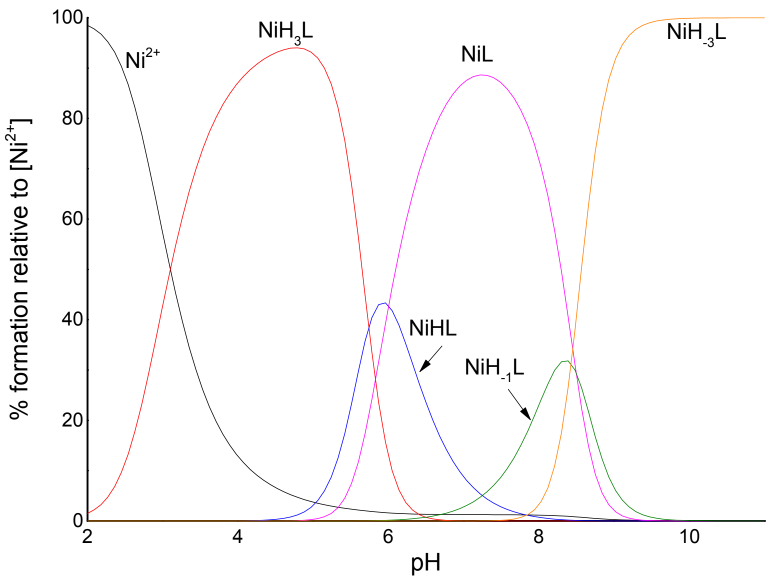
Figure S3. Isotopic distribution of: A) Ni^{2+}-Ac-HGDHYH-NH_2; B) Zn^{2+}-Ac-HGDHYH-NH_2; C) Ni^{2+}-Ac-HMGHSH-NH_2; D) Zn^{2+}-Ac-HMGHSH-NH_2; E) Ni^{2+}-Ac-HKDHYH-NH_2; F) Zn^{2+}-Ac-HKDHYH-NH_2; M^{2+}:L molar ratio=1:1, pH 6.

A)



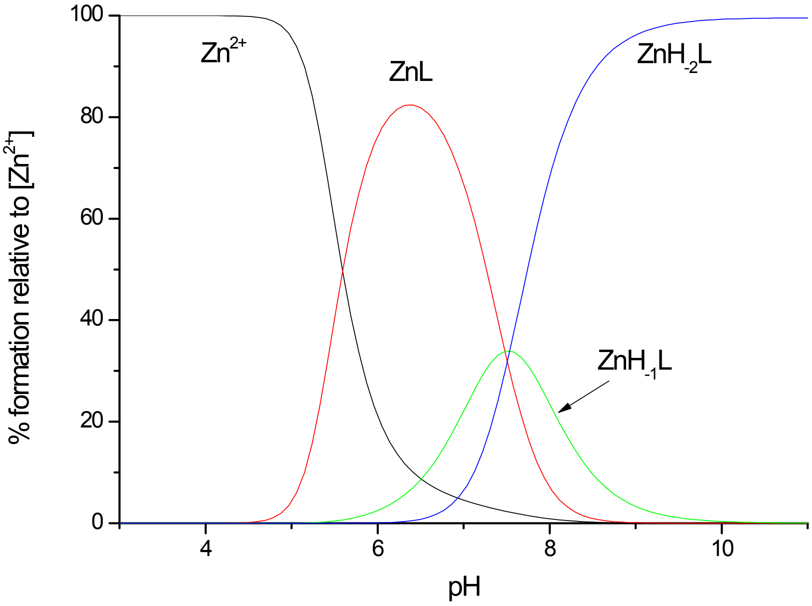
100
80
60
40
20
0
4
6
8
10
pH
% formation relative to [Zn2+]
Zn2+
ZnL
ZnH-1L
ZnH2L
ZnHL

B)



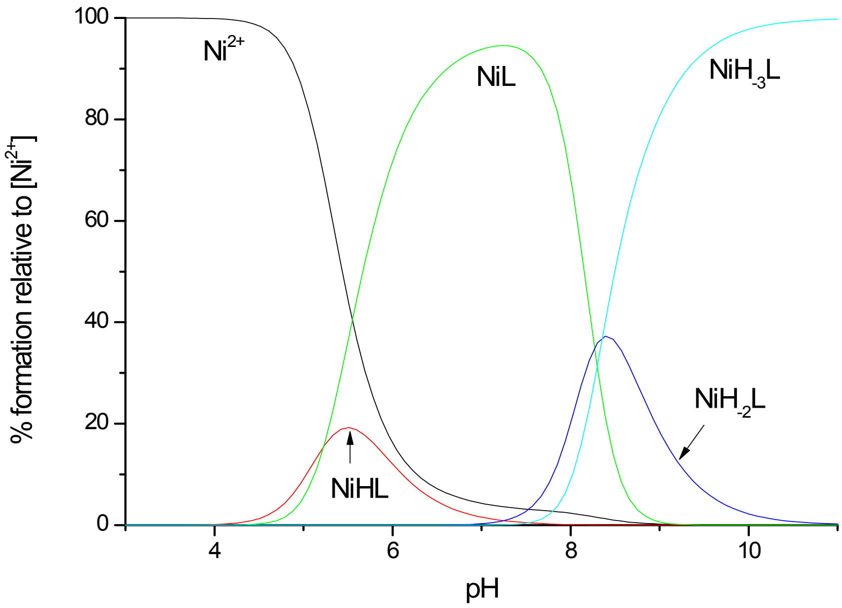
100
80
60
40
20
0
2
4
6
8
10
pH
% formation relative to [Ni2+]
Ni2+
NiH3L
NiL
NiH-3L
NiHL
NiH-1L

C)



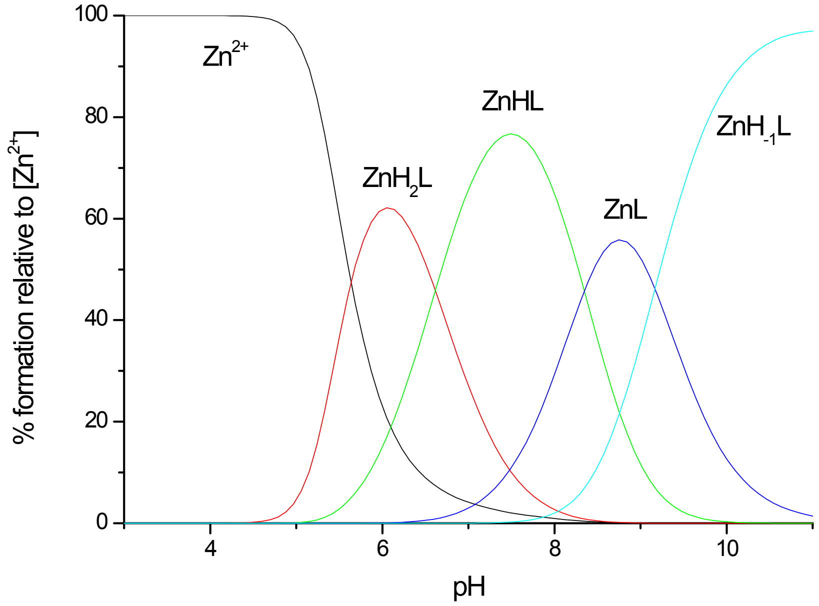
100
80
60
40
20
0
% formation relative to [Zn2+]
4
6
8
10
pH
Zn2+
ZnL
ZnH-2L
ZnH-1L

D)



100
80
60
40
20
0
% formation relative to [Ni2+]
4
6
8
10
pH
Ni2+
NiL
NiH-3L
NiH-2L
NiHL

E)



F)

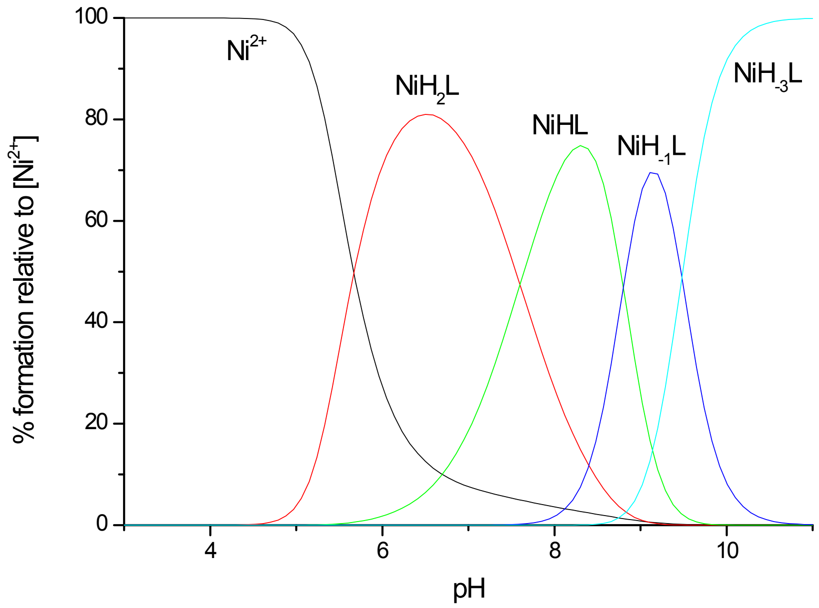
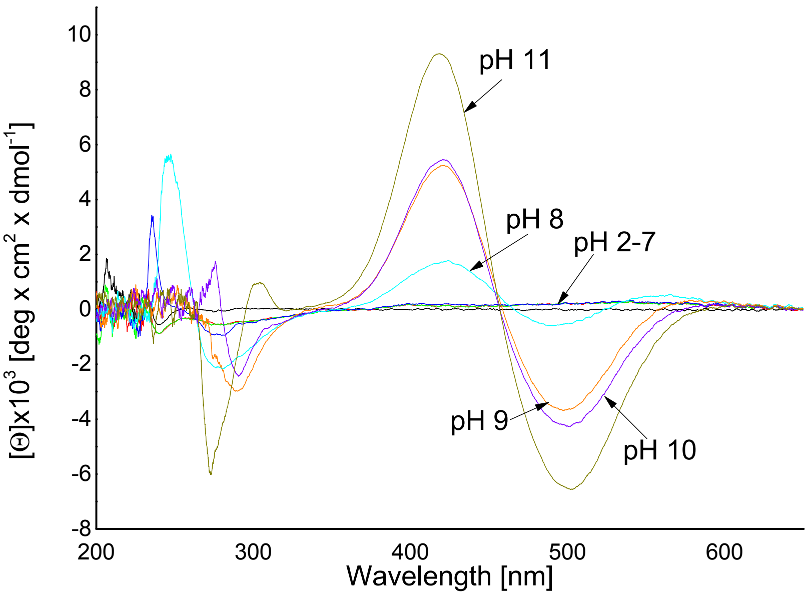
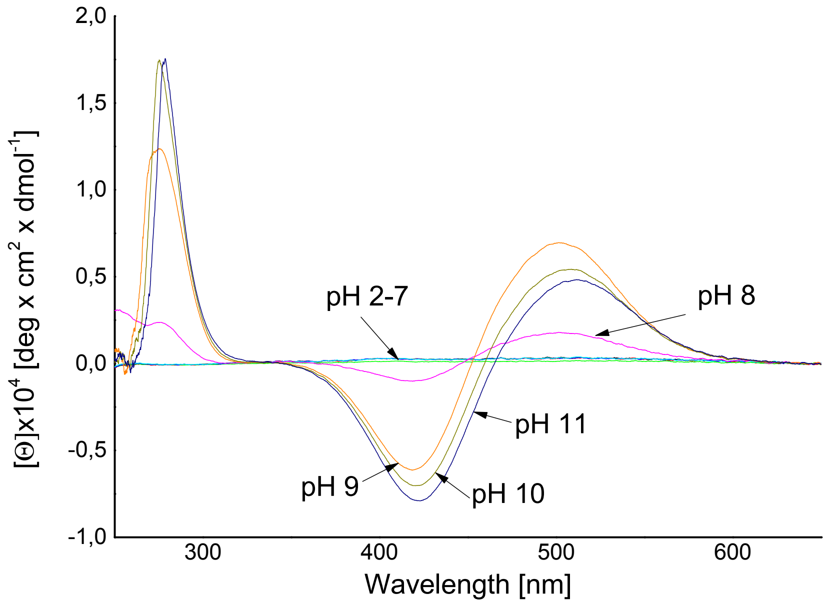


Figure S4. Distribution diagrams for the formation of: A) Zn^{2+} complex with Ac-HGDHYH-NH_2; B) Ni^{2+} complex with Ac-HGDHYH-NH_2C) Zn^{2+} complex with Ac-HMGHSH-NH_2; D) Ni^{2+} complex with Ac-HMGHSH-NH_2; E) Zn^{2+} complex with Ac-HKDHYH-NH_2; F) Ni^{2+} complex with Ac-HKDHYH-NH_2; T=298K, I=0,1M, $[M^{2+}]=0.5{\cdot}10^{-3}$M; M^{2+}:L molar ratio=1:1. K_d values at pH 7.4 for appropriate complexes were equal to: A) Zn^{2+} complex with Ac-HGDHYH-NH_2 = 7.80*10^-10; B) Ni^{2+} complex with Ac-HGDHYH-NH_2 = 1.28*10^13; C) Zn^{2+} complex with Ac-HMGHSH-NH_2 = 3.73*10^-8; D) Ni^{2+} complex with Ac-HMGHSH-NH_2 = 4.23*10^-7; E) Zn^{2+} complex with Ac-HKDHYH-NH_2 = 6.37*10-9; F) Ni^{2+} complex with Ac-HKDHYH-NH_2 = 4.36*10-6. Appropriate metal and ligand concentrations were taken from the Hyss programme.

A)


pH 11
pH 8
pH 2-7
pH 9
pH 10
10
8
6
4
2
0
-2
-4
-6
-8
200
300
400
500
600
Wavelength [nm]
[Θ]x10^3 [deg x cm^2 x dmol^-1]

B)


pH 2-7
pH 8
pH 11
pH 9
pH 10
2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
300
400
500
600
Wavelength [nm]
[Θ]x10^4 [deg x cm^2 x dmol^-1]

C)

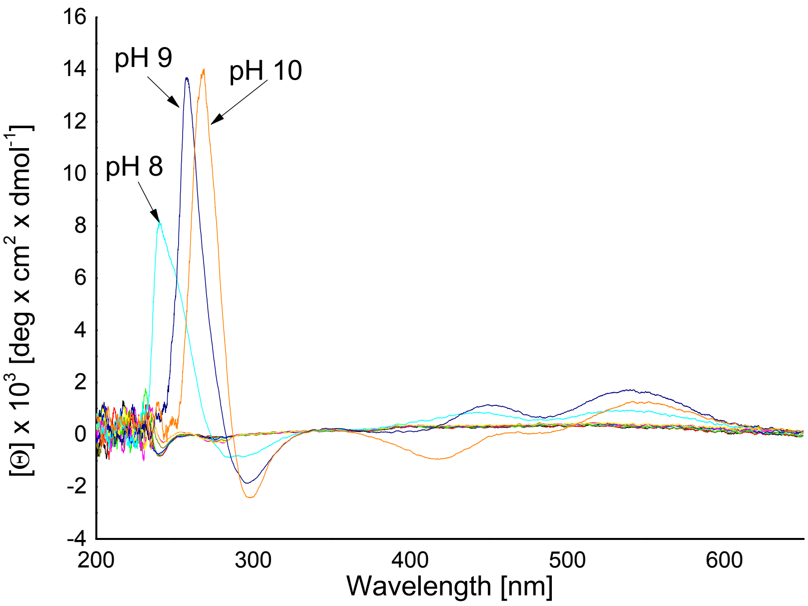
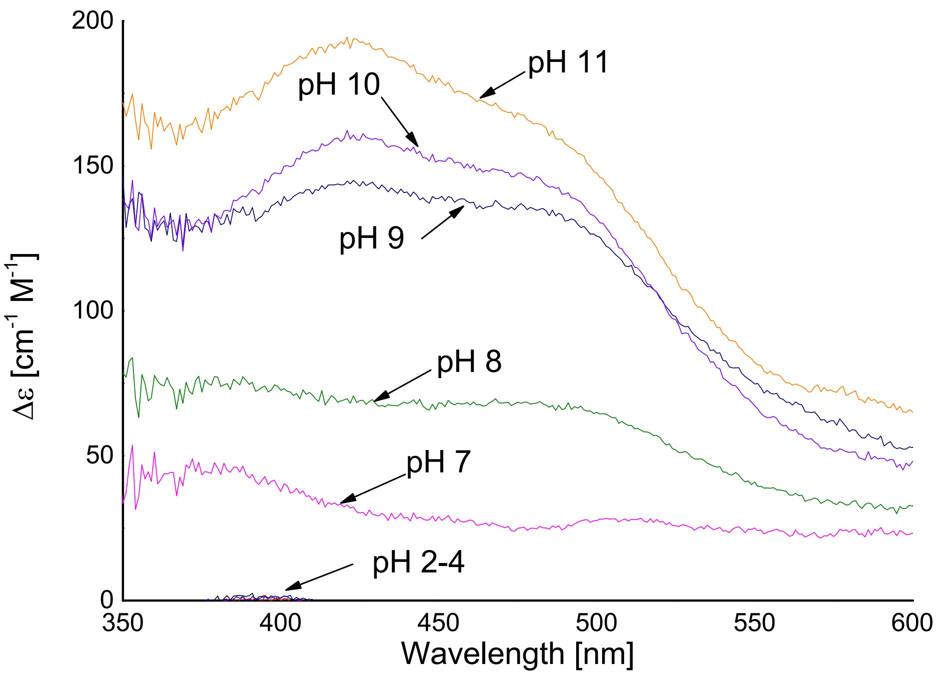
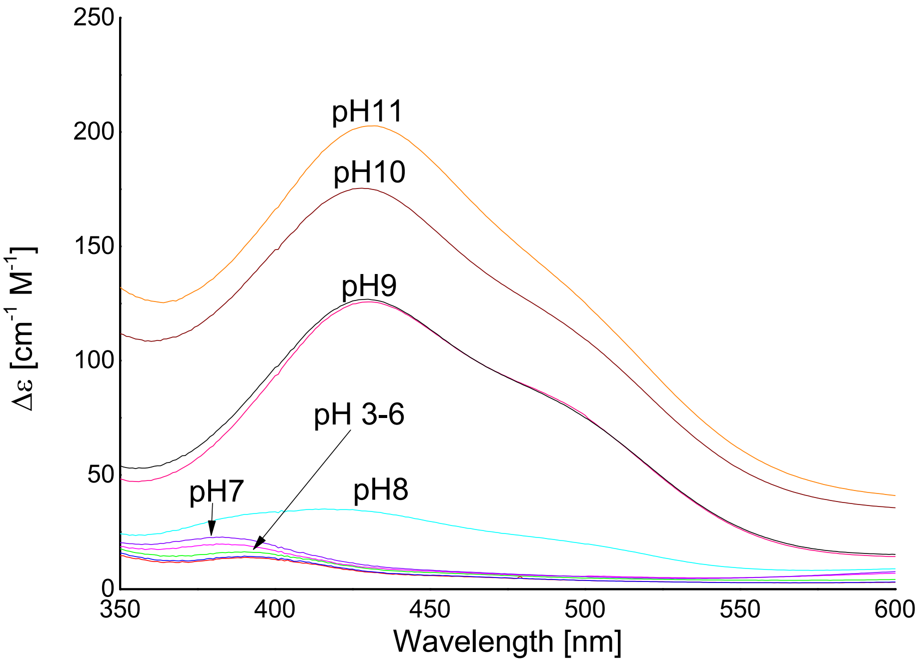


Figure S5. CD spectra of Ni^{2+} complexes with: A) Ac-HGDHYH-NH_2; B) Ac-HMGHSH-NH_2; C) Ac-HKDHYH-NH_2; in pH range 3-11.

A)



B)



C)

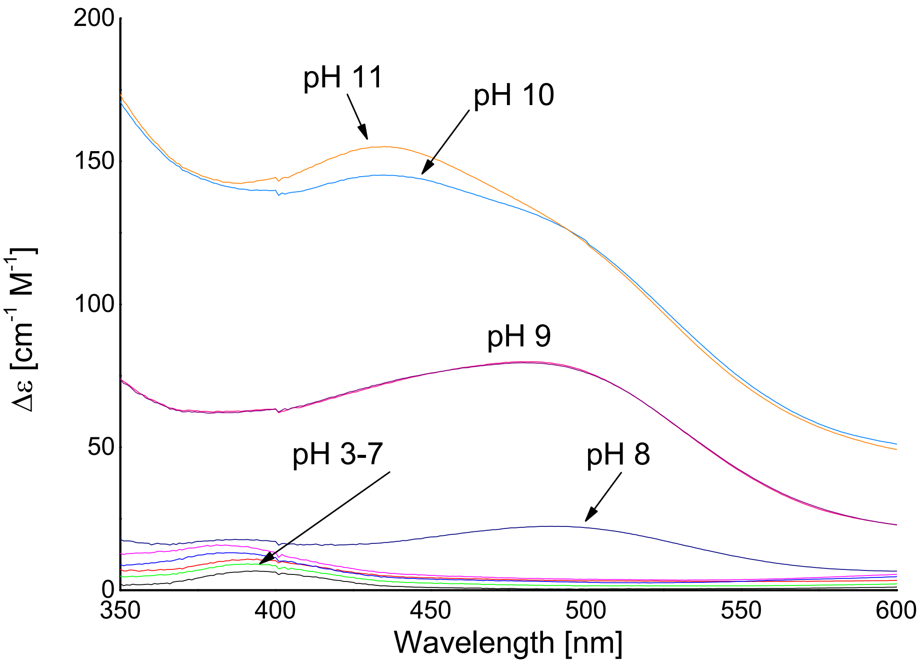
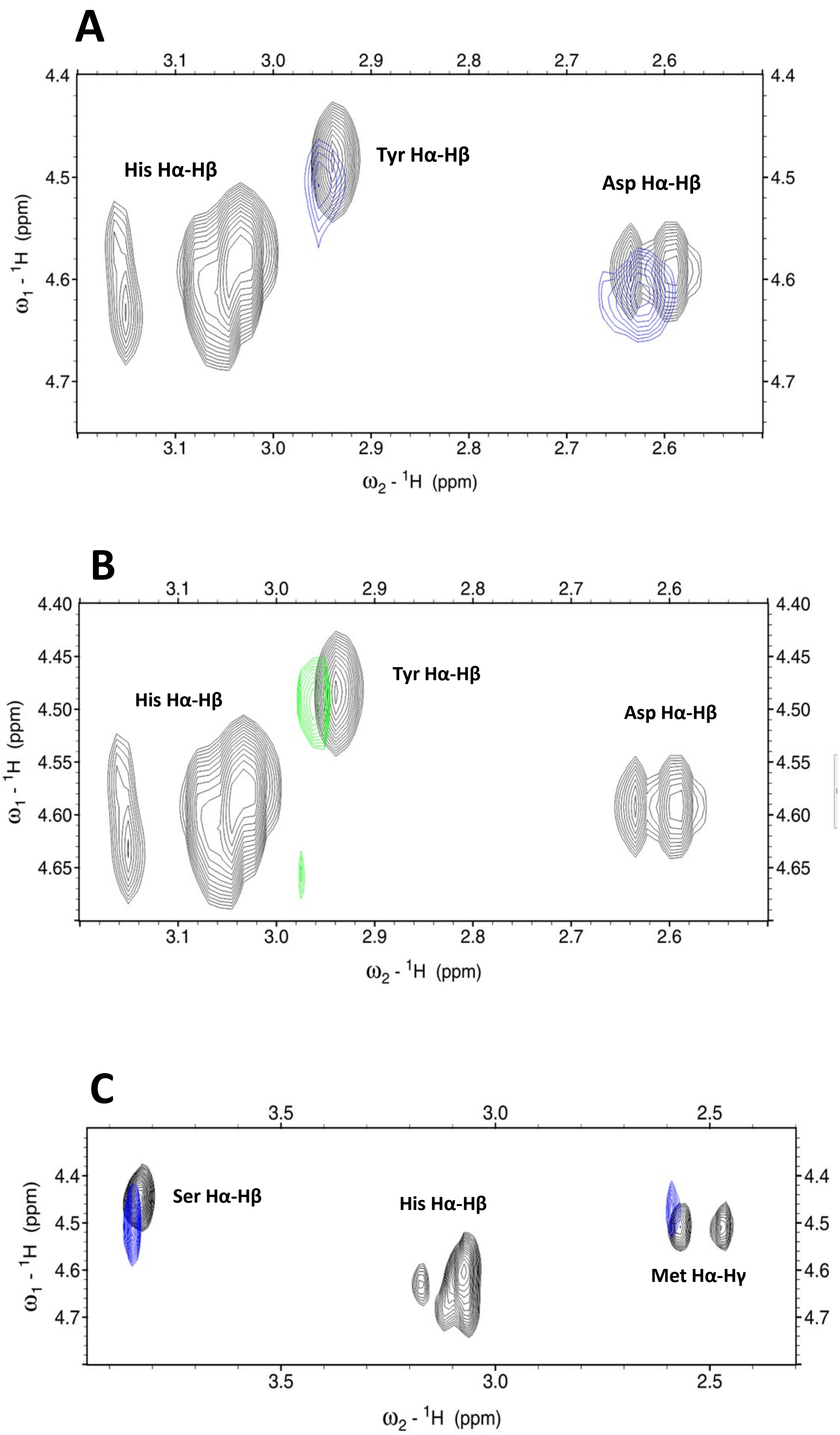


Figure S6. UV-Vis spectra of Ni^{2+} complexes with: A) Ac-HGDHYH-NH_2; B) Ac-HMGHSH-NH_2; C) Ac-HKDHYH-NH_2; in pH range 3-11.



A
His Hα-Hβ
Tyr Hα-Hβ
Asp Hα-Hβ
ω1 - 1H (ppm)
ω2 - 1H (ppm)
B
His Hα-Hβ
Tyr Hα-Hβ
Asp Hα-Hβ
ω1 - 1H (ppm)
ω2 - 1H (ppm)
C
Ser Hα-Hβ
His Hα-Hβ
Met Hα-Hγ
ω1 - 1H (ppm)
ω2 - 1H (ppm)

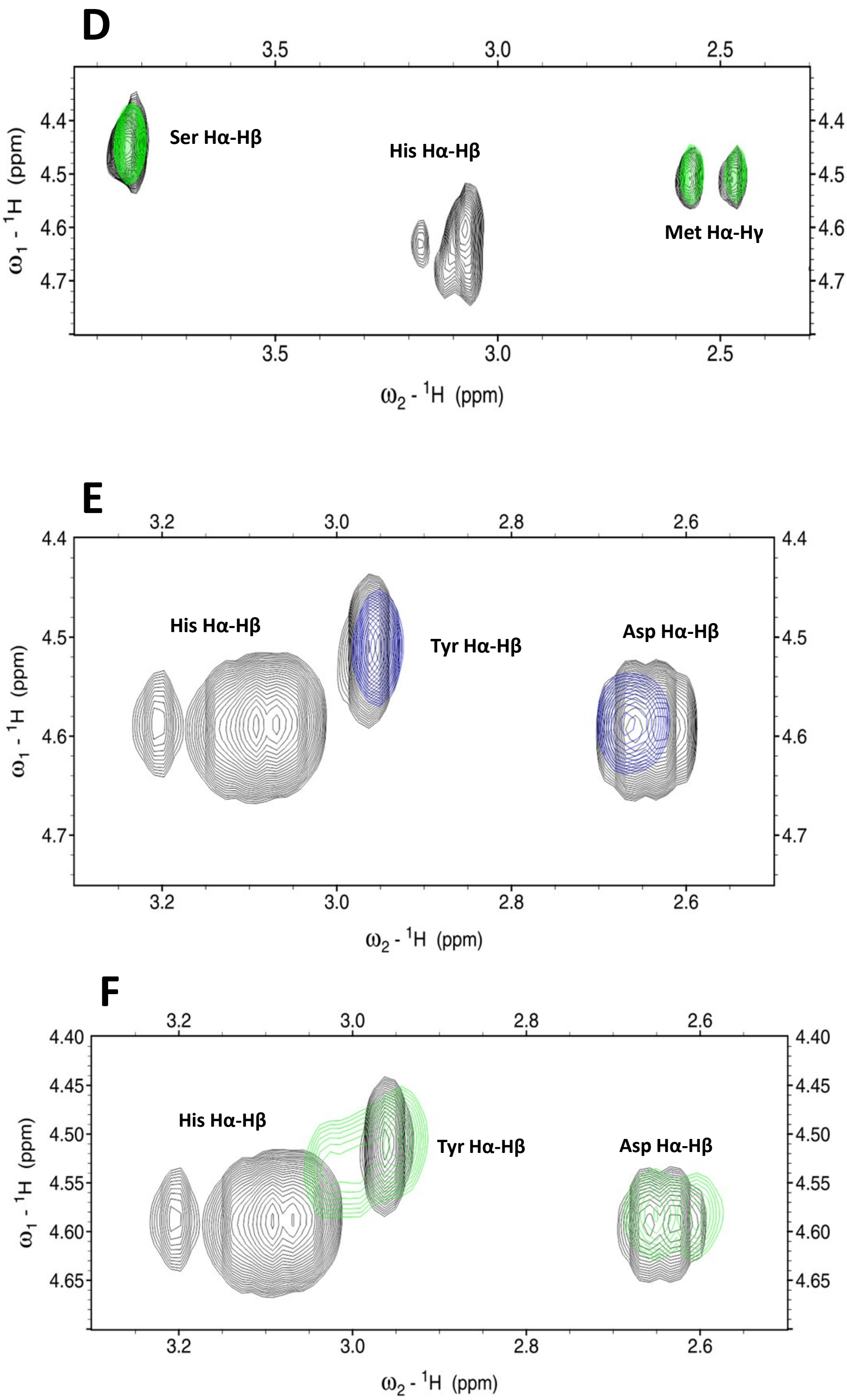


Figure S7. ^1H-^1H TOCSY NMR spectra fragment of ligand (black), Zn^{2+} complex (blue) and Ni^{2+} complex (green), with ligand Ac-HGDHYH-NH_2(A, B), Ac-HMGHSH-NH_2 (C, D) and Ac-HKDHYH-NH_2 (E, F); [ligand]=3mM, [Zn^{2+}]=3mM, [Ni^{2+}]=1mM, pH=7, T=298K.

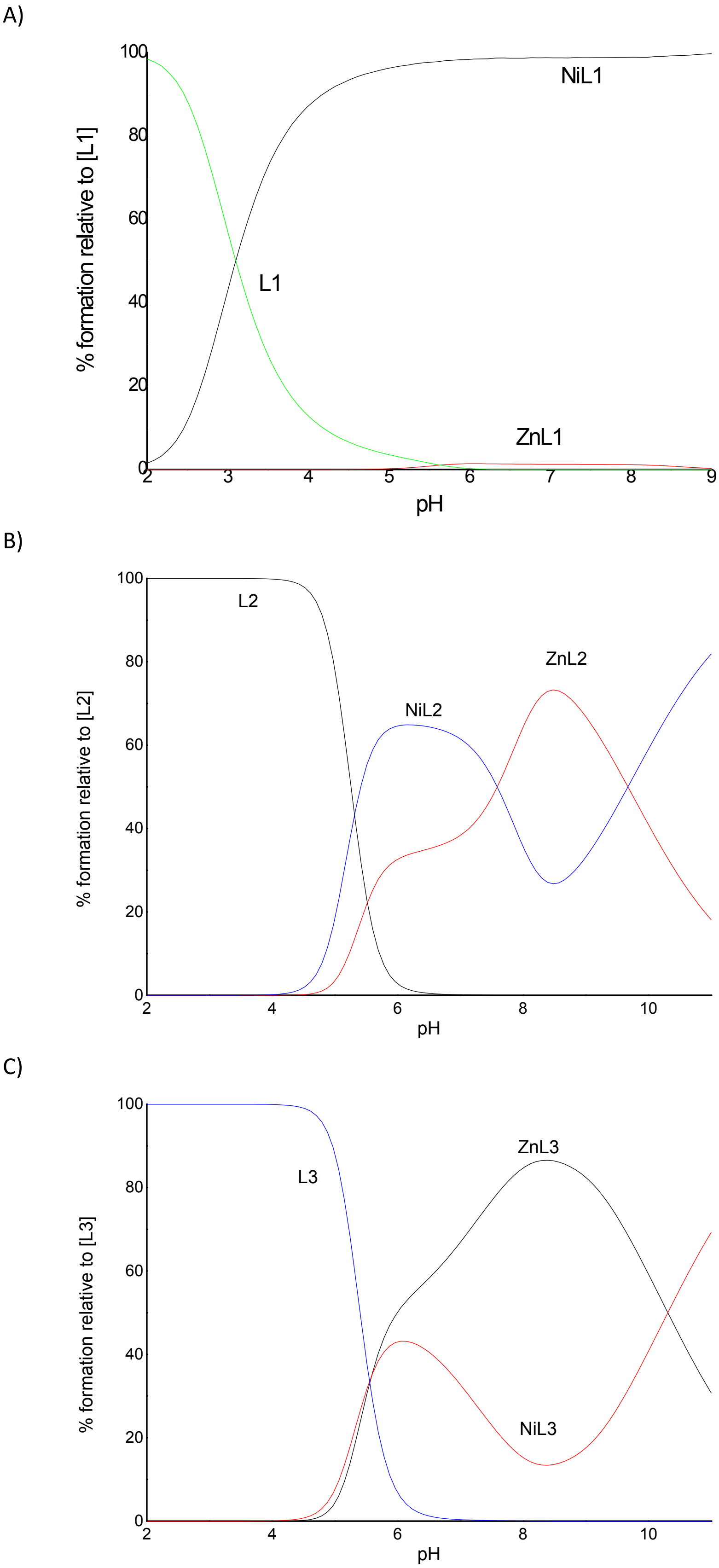


Figure S8. Competition plots between: A) Ac-HGDHYH-NH_2 (L1), Zn^{2+} and Ni^{2+};B) Ac-HMGHSH-NH_2 (L2), Zn^{2+} and Ni^{2+}; C) Ac-HKDHYH-NH_2 (L3), Zn^{2+} and Ni^{2+}. Plots describe complex formation at different pH values in a hypothetical situation, in which equimolar amounts of the reagents are mixed. Calculations are based on binding constants from Table 1. T=298K, I=0,1M, $[Zn^{2+}]=[Ni^{2+}]=[Ac\text{-}HGDHYH\text{-}NH_2]=[Ac\text{-}HMGHSH\text{-}NH_2]=[Ac\text{-}HKDHYH\text{-}NH_2]=0.001M$.

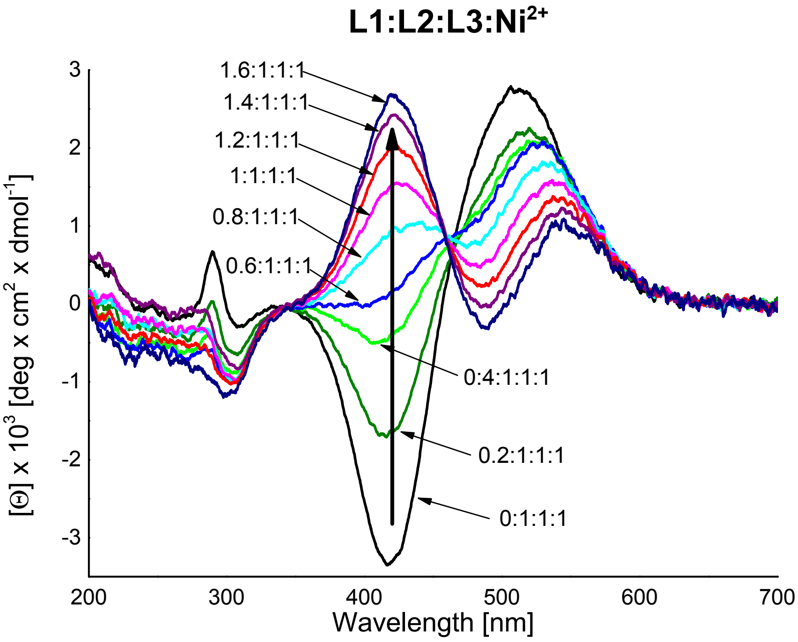


Figure S9. CD spectra of a Ni^{2+}, Ac-HMGHSH-NH_2 and Ac-HKDHYH-NH_2 solution titrated with Ac-HGDHYH-NH_2 at pH 10. $[Ni^{2+}]$ = [Ac-HMGHSH-NH_2] (L2) = [Ac-HKDHYH-NH_2] (L3) = 1 mM. 0.2 equivalents of Ac-HGDHYH-NH_2 were added in each step. A stepwise change of the typical Ni^{2+}-L2 CD spectrum to a typical Ni^{2+}-L1 spectrum is observed.