Electronic Supplementary Material (ESI) for Dalton Transactions.

Exploring the Binding Preferences: Cu(II), Ni(II), and Zn(II) Complexes of Mycobacterial GroEL1 His-Rich and Glu/His-Rich Domains

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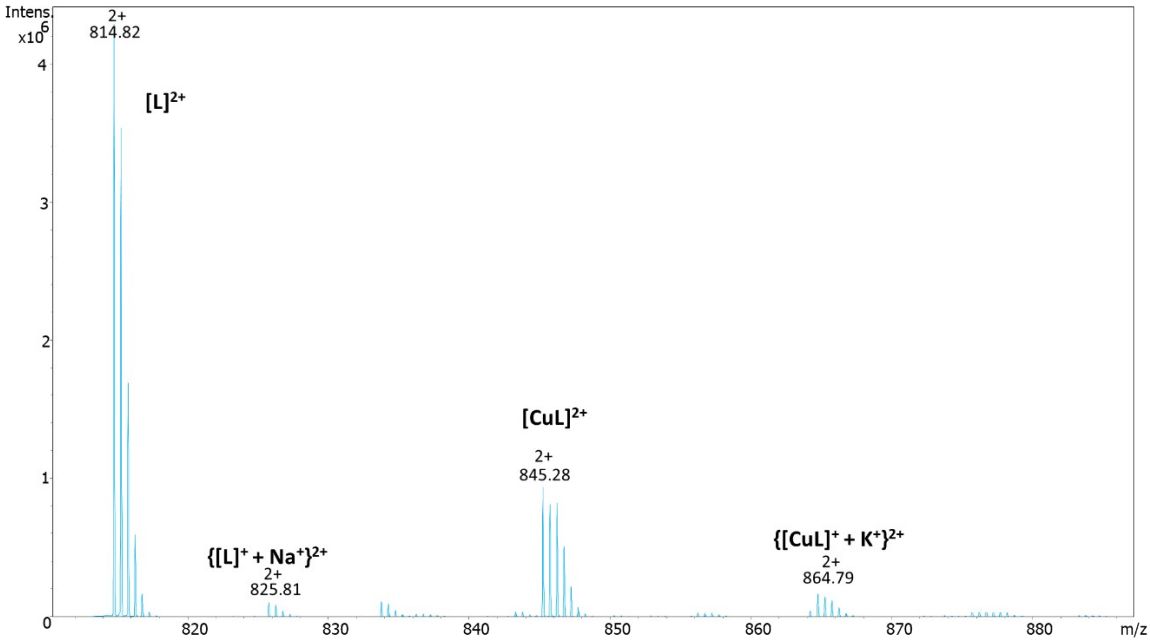


Figure S1. ESI-MS spectrum of a mixture of the XEN ligand and Cu(II) ions in the *m/z* range of 810–890. M:L = 1:1, pH = 7.4.

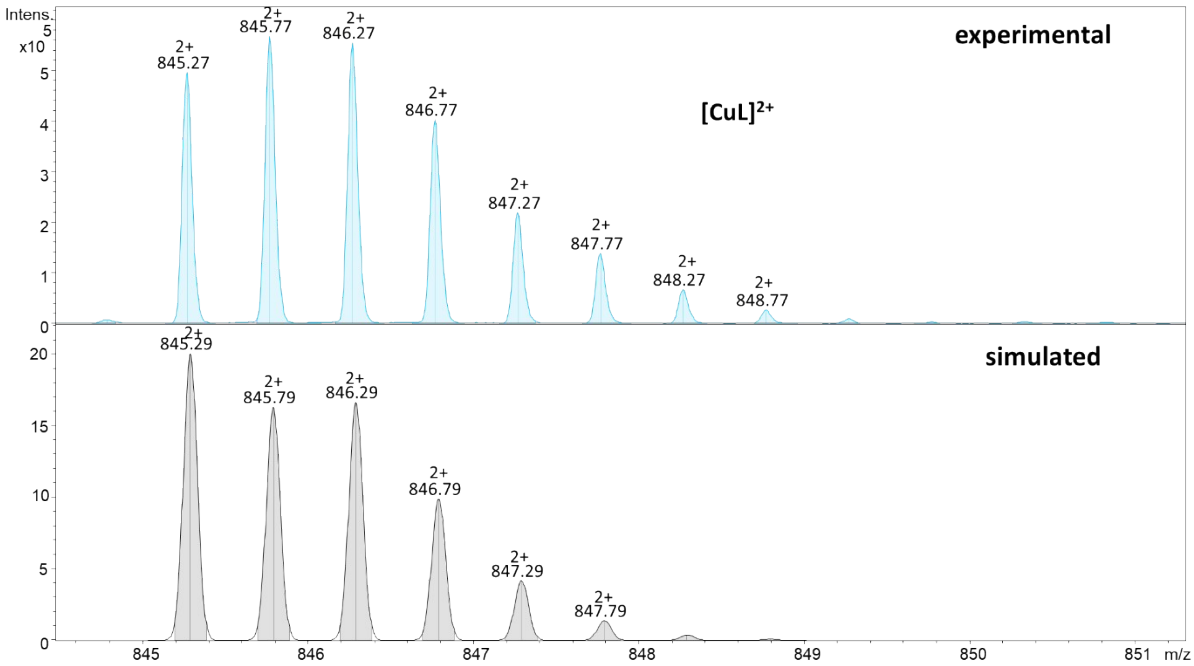


Figure S2. Comparison of the isotope distribution of the Cu(II) complex signal with XEN, $[CuL]^{2+}$, at *m/z* = 845.28 in the experimental and simulated spectra.

The first two forms of Cu(II)-XEN system, CuH_5L and CuH_4L (reaching maximum concentration at pH 4.5 and 4.9, respectively), likely result from the deprotonation of the side chain of histidine and glutamic acid residues. The pK_a value of 4.68 is comparable to the pK_a values associated with deprotonation of the glutamic acid side chain in the free ligand (4.07, 4.70, 4.91, 5.73). At least one of these two chemical groups, the histidine residue, may be involved in Cu(II) binding. This is confirmed by the appearance of a d-d transition band at around 699 nm in the UV-Vis spectrum of the studied system at pH 4.05 (indicating Cu(II) binding to one nitrogen atom) and the EPR spectrum parameters (A = 169.3, g∥ = 2.30) (**Figure S3A**, **B**).[15,16] This suggests a coordination type of $\{N_{im}\}$ for CuH_5L and CuH_4L. With an increase in pH to 5.08, the d-d band in the UV-Vis spectrum shifts to 640 nm, and the EPR parameters change to A = 175.8, g∥ = 2.27, indicating the involvement of a second imidazole nitrogen atom in Cu(II) binding (**Figure S3A**, **B**).[15,16] This is associated with the formation of two new complex forms, CuH_3L and CuH_2L (reaching maximum concentration at pH 5.3 and 6.0, respectively), likely corresponding to the deprotonation of a histidine residue and a glutamic acid residue, respectively, which can be assigned a coordination type of $\{2N_{im}\}$. The pK_a value of 4.96 for the CuH_3L form is lowered compared to the pK_a values associated with the deprotonation of the histidine side chain in the free ligand (6.71, 7.14, and 7.87), suggesting the binding character of another histidine residue. On the other hand, the pK_a value of 5.34 is comparable to several pK_a values associated with the deprotonation of the glutamic acid side chain in the free ligand (e.g., 4.91, 5.73). Further increase in pH to about 6.01 results in the shift of the d-d band in the UV-Vis spectrum to 622 nm and a change in the EPR spectrum parameters of the studied system (A = 179.7, g∥ = 2.25), indicating the existence of an equilibrium of forms at this pH that bind the Cu(II) ion through 2 or 3 nitrogen atoms.[15,17-19] This is related to the coexistence of the CuH_3L, CuH_2L, and CuHL forms at pH 6.01, with the CuHL form likely resulting from the deprotonation of the imidazole ring of the third histidine residue.

Three forms existing at basic pH: $CuH_{-1}L$, $CuH_{-2}L$, and $CuH_{-3}L$ (reaching maximum concentration at pH around 9.1, 10.2, and 11.0, respectively), correspond to the deprotonation of a non-binding metal ion lysine residue and three amidic bonds. In fact, $CuH_{-1}L$ comes from a deprotonation of two groups: lysine side chain and most probably, first amide bond. $CuH_{-2}L$, and $CuH_{-3}L$ correspond to the deprotonation of second and third amide bond. The amide nitrogen atoms may replace imidazole nitrogen atoms in the Cu(II) coordination sphere and form a complex with a square-planar geometry. This assumption is confirmed by the appearance of a positive Cotton band in the CD spectrum of the studied system at pH 9.02, which occurs in the formation of Cu(II) complexes with amidic nitrogen (**Figure S3C**).[21-24] The intensity of this band increases with an increase in pH, accompanied by a slight shift, indicating the involvement of additional amidic nitrogen atoms in Cu(II) binding. The parameters of the d-d band in the UV-Vis spectrum and the EPR spectrum of the studied system at pH around 10.0 and 11.0 (pH 10.13: λ_{max} = 573 nm, A = 192.2, g∥ = 2.20; pH 11.00: λ_{max} = 563 nm, A = 194.5, g∥ = 2.20) confirm the involvement of a fourth nitrogen atom in Cu(II) binding (**Figure S3A**, **B**).[16,21-24] Therefore, the complex

forms $CuH_{-1}L$, $CuH_{-2}L$, and $CuH_{-3}L$ can be respectively assigned coordination types of $\{3N_{im}, 1N^{-}\}$, $\{2N_{im}, 2N^{-}\}$, and $\{1N_{im}, 3N^{-}\}$.

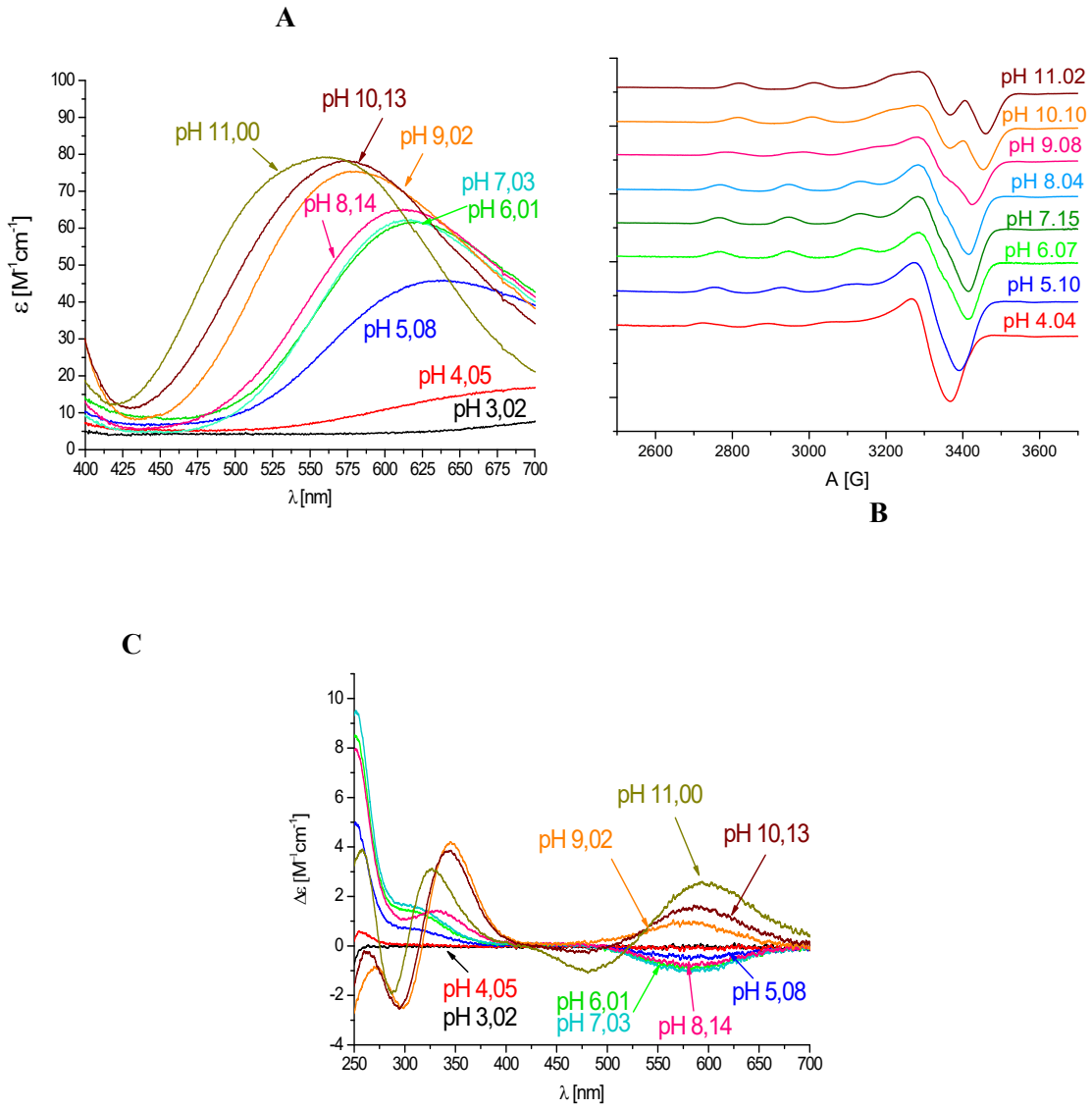


Figure S3. UV-Vis (A), EPR (B), and CD (D) spectra of Cu(II) complexes with the XEN ligand in the pH range of 2-11. Conditions: T = 298 K, metal-to-ligand molar ratio = 1:1; [Cu(II)] = 2.5 x 10^{-4} M.

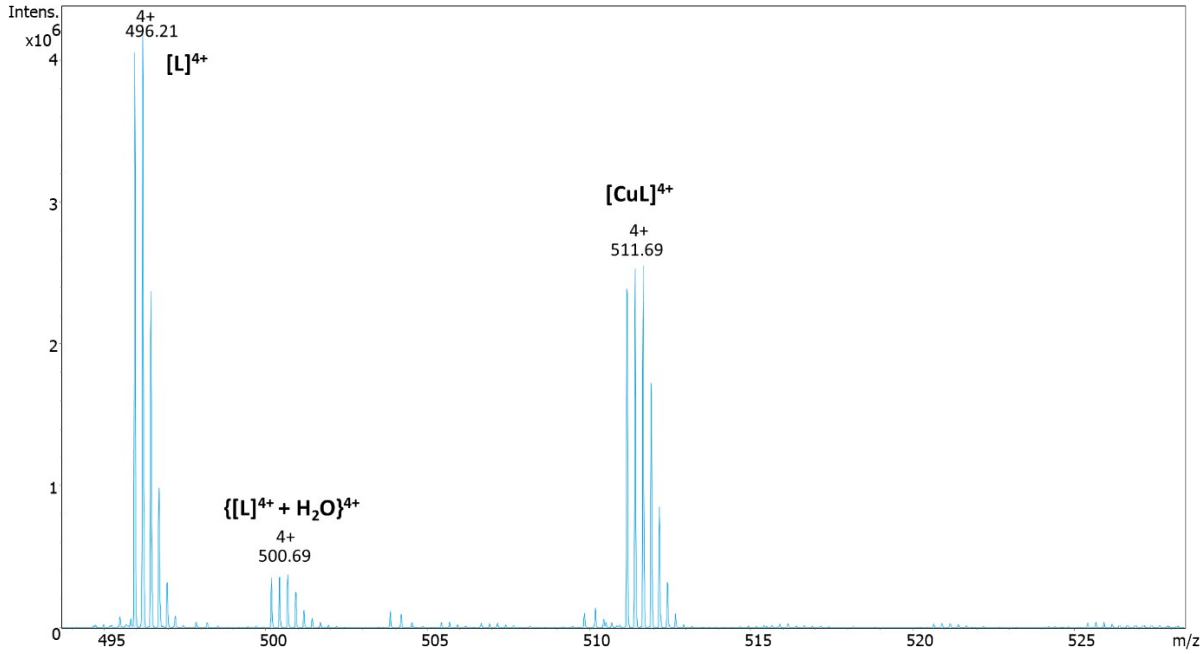


Figure S4. ESI-MS spectrum of the mixture of ABS ligand and Cu(II) ions in the *m/z* range of 490-530. M:L = 1:1, pH = 7.4.

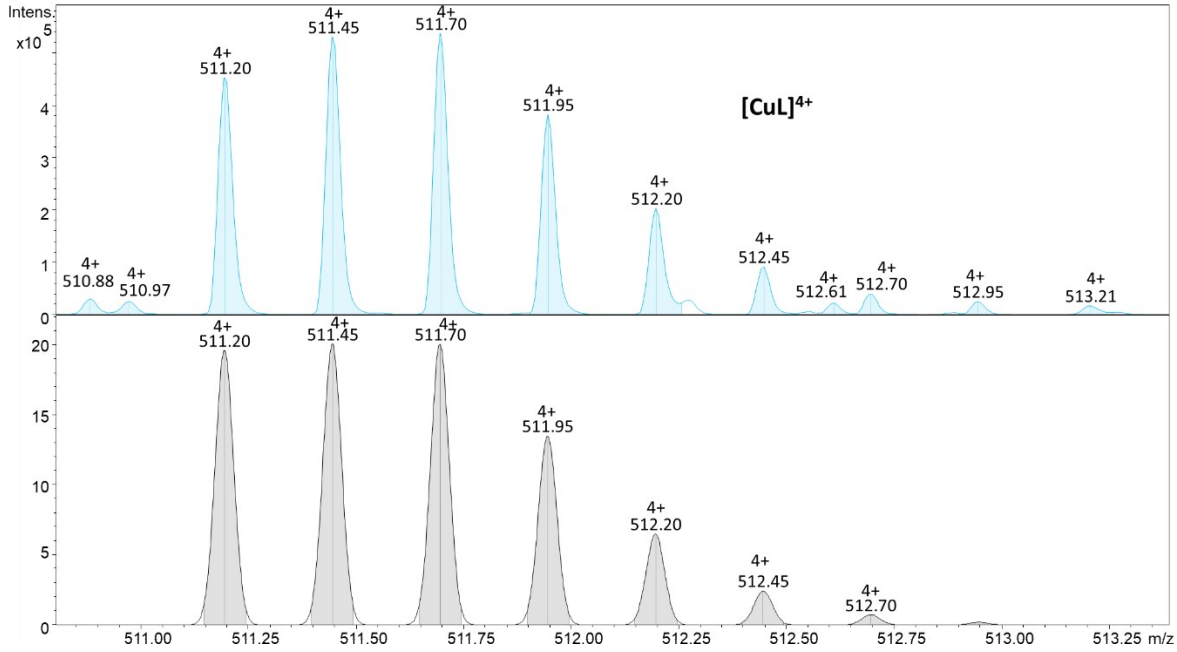


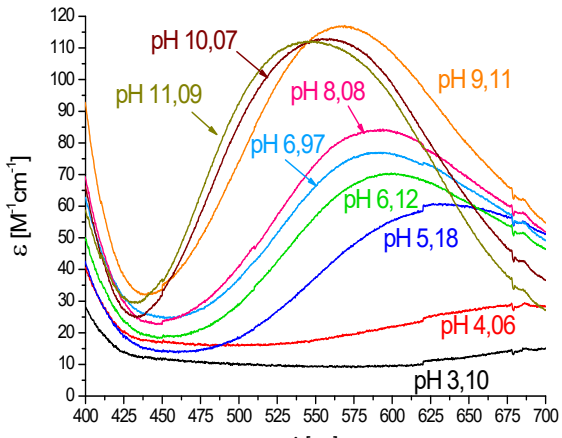
Figure S5. Comparison of the isotope distribution of the Cu(II) complex signal with ABS, $[CuL]^{4+}$, at *m/z* = 511.69 in the experimental and simulated spectra.

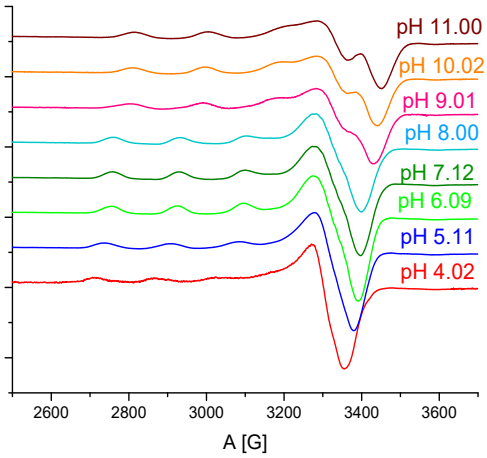
The first two forms of Cu(II)-ABS system, CuH_6L and CuH_5L (reaching maximum concentration at pH 4.4 and 4.8, respectively), likely result from the deprotonation of the side chain of the first and second histidine residues. Imidazole nitrogen atoms of both amino acid residues are involved in Cu(II) binding. This is confirmed by the appearance of a d-d transition band at around 669 nm in the UV-Vis spectrum of the studied system at pH 4.06 (corresponding to Cu(II) binding to two nitrogen atoms), where CuH_6L

and CuH_5L forms coexist. Additionally, this finding is supported by the EPR spectrum parameters of the studied system: A = 171.9, g∥ = 2.28, at pH 4.06 (**Figure S6A**, **B**).[15,16] The coordination type for CuH_6L and CuH_5L is thus $\{1N_{im}\}$ and $\{2N_{im}\}$, respectively. Further, an increase in pH to 5.18 does not induce significant changes in the EPR and UV-Vis spectra, indicating the non-binding character of the next two His residues. Their deprotonation leads to the formation of coexisting CuH_4L and CuH_3L forms at pH 5.06, also with a coordination type of $\{2N_{im}\}$. They reach their maximum concentration at pH 5.5 and 6.0, respectively, and the pK_a values of 4.85 and 5.62 for these forms could correspond to the pK_a values of 6.21 and 6.50, respectively, derived from the deprotonation of His residues in the free ligand. Upon raising the pH to 6.12, there is a shift of the d-d transition band in the UV-Vis spectrum (λ_{max} = 600 nm) and a change in the EPR spectrum parameters of the studied system (A = 168.0, g∥ = 2.27), indicating an equilibrium of forms in which two or three nitrogen atoms create the coordination sphere of the Cu(II) ion (**Figure S6A**, **B**).[15,17-19] This is related to the formation of another form, CuH_2L (reaching maximum concentration at pH 6.7), resulting from the deprotonation of the fifth histidine residue. The pK_a value of 6.20 for this form likely corresponds to a pK_a value of 8.11 for the same amino acid residue in the free ligand, and it is lowered, suggesting the binding character of the discussed chemical group and a coordination type of $\{3N_{im}\}$ for CuH_2L.

The subsequent forms of the complex at alkaline pH, $CuH_{-1}L$, $CuH_{-2}L$, and $CuH_{-3}L$, are formed by the deprotonation of the non-binding metal lysine residue and three amidic bonds, where nitrogen atoms successively replace 2 out of 3 histidine residues in the coordination sphere of the copper(II) ion, forcing the formation of a square-planar complex geometry. This assumption is verified by the appearance of a positive band in the CD spectrum of the studied system at pH 9.11, which occurs in the interaction of Cu(II) with the amidic nitrogen atom (**Figure S6C**).[21-24] With the increase in pH, the intensity of this band increases, accompanied by a slight shift, indicating the gradual involvement of additional amide nitrogens in the coordination sphere of the copper(II) ion. The parameters of the d-d transition band in the UV-Vis spectrum and the EPR spectrum of the studied system at pH around 10.0 and 11.0 (pH 10.13: λ_{max} = 573 nm, A = 192.2, g∥ = 2.20; pH 11.00: λ_{max} = 563 nm, A = 194.5, g∥ = 2.20) confirm the involvement of a fourth nitrogen atom in binding the copper(II) ion (**Figure S6A**, **B**).[16,21-24] Therefore, the complex forms $CuH_{-1}L$, $CuH_{-2}L$, and $CuH_{-3}L$ can be respectively assigned coordination types of $\{3N_{im}, 1N^-\}$, $\{2N_{im}, 2N^-\}$, and $\{1N_{im}, 3N^-\}$.

B





C

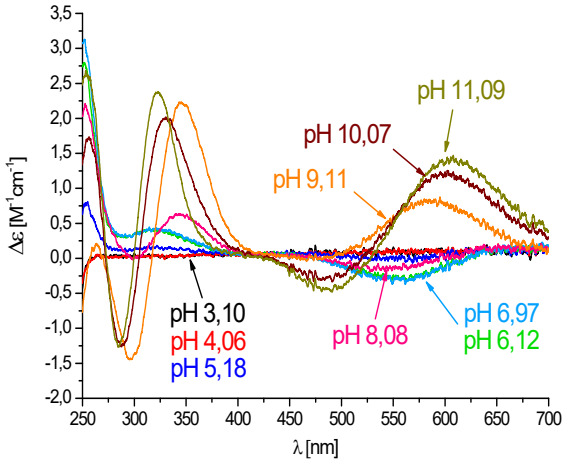


Figure S6. UV-Vis (A), EPR (B), and CD (D) spectra of Cu(II) complexes with the ABS ligand in the pH range of 2-11. Conditions: T = 298 K, metal-to-ligand molar ratio = 1:1; [Cu(II)] = 2.5 x 10^{-4} M.

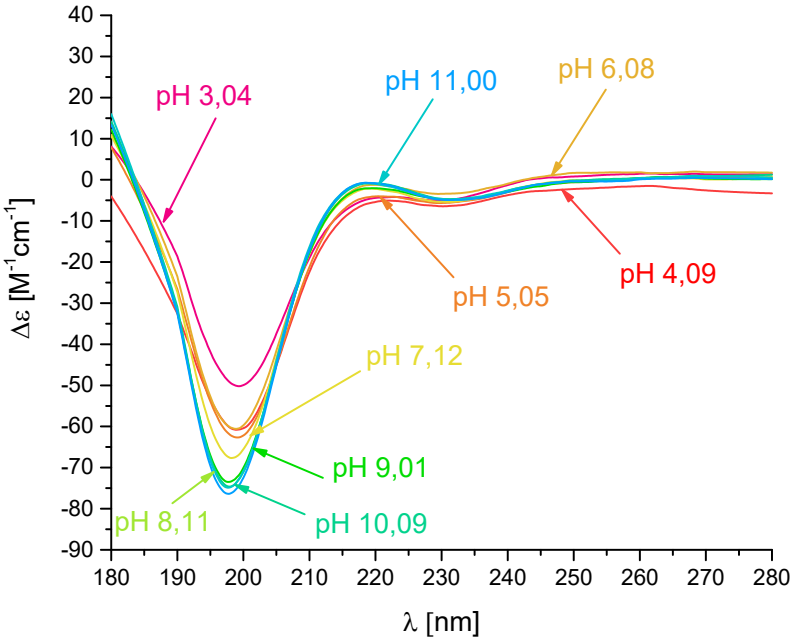


Figure S7. CD spectrum in the range of 180-280 nm for the free ABS peptide.

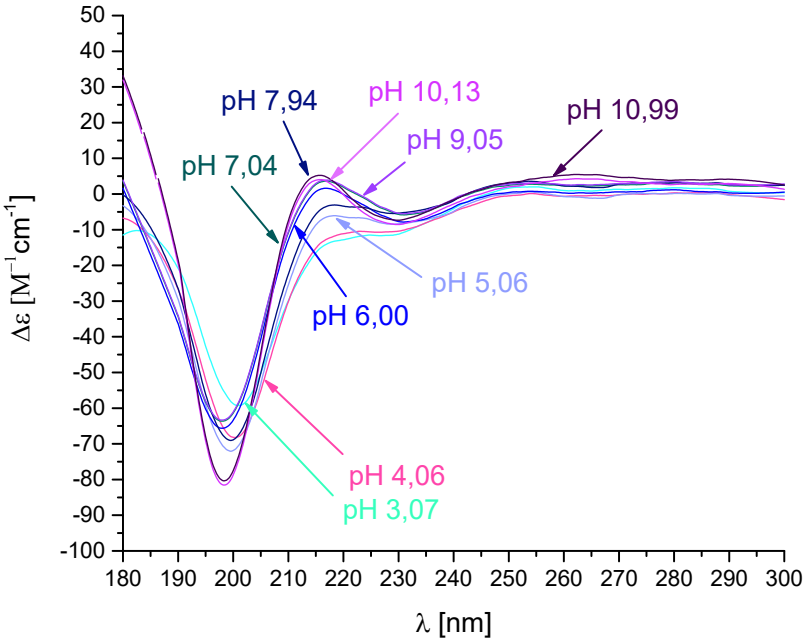


Figure S8. CD spectrum in the range of 180-300 nm for the Cu(II)-XEN system.

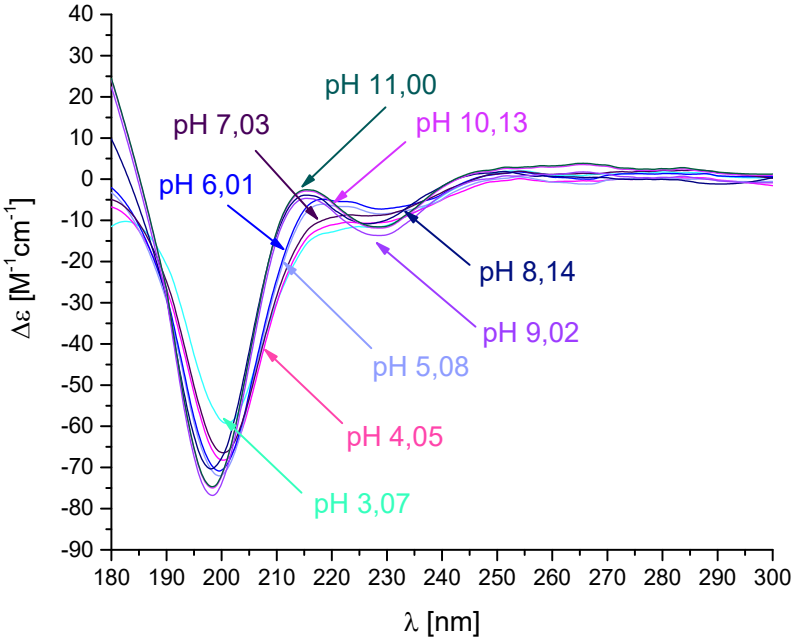


Figure S9. CD spectrum in the range of 180-300 nm for the Cu(II)-ABS system.

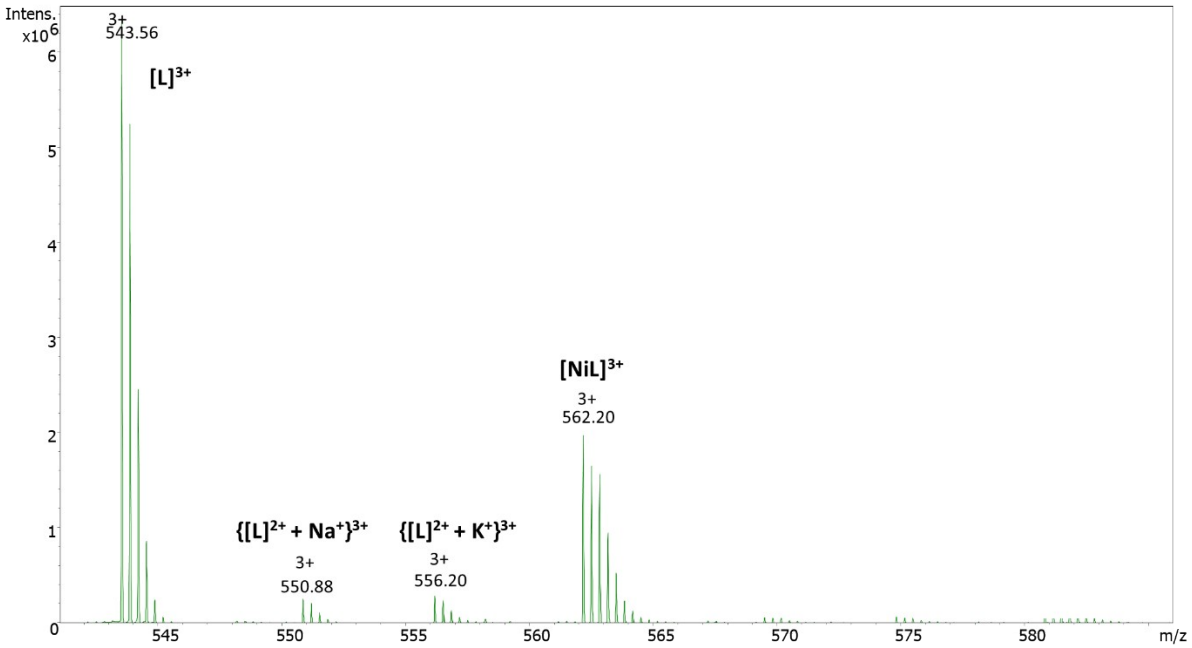


Figure S10. ESI-MS spectrum of the mixture of XEN ligand and Ni(II) ions in the *m/z* range of 540-585. M:L = 1:1, pH = 7.4.

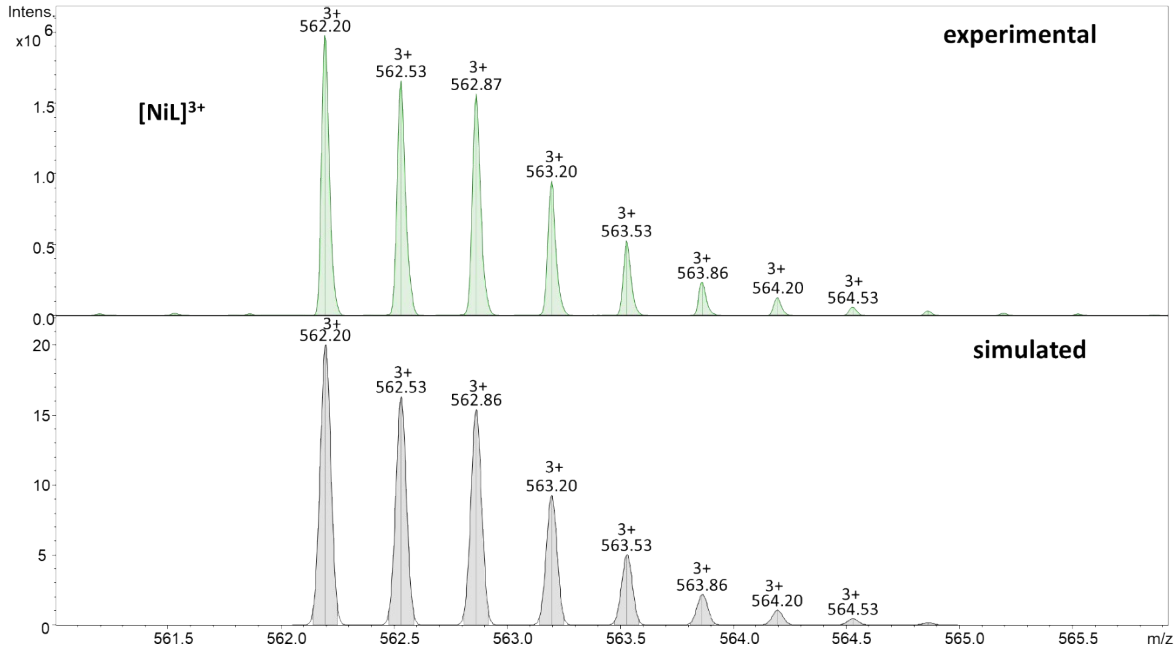


Figure S11. Comparison of the isotope distribution of the Ni(II) complex signal with XEN, $[NiL]^{3+}$, at *m/z* = 562.20 in the experimental and simulated spectra.

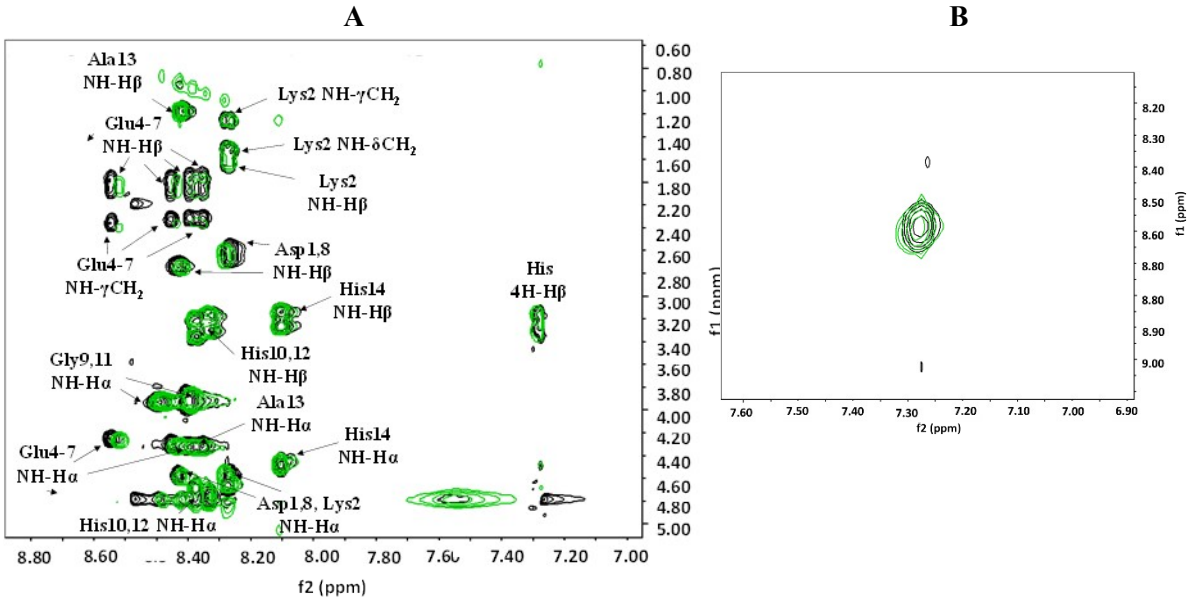


Figure S12. ^{1}H-^{1}H TOCSY NMR spectra of the XEN ligand (black) and its Ni(II) complex (green) at pH 5.2 in: (A) fingerprint region, (B) aromatic region; M : L = 0.4 : 1, T = 298 K.

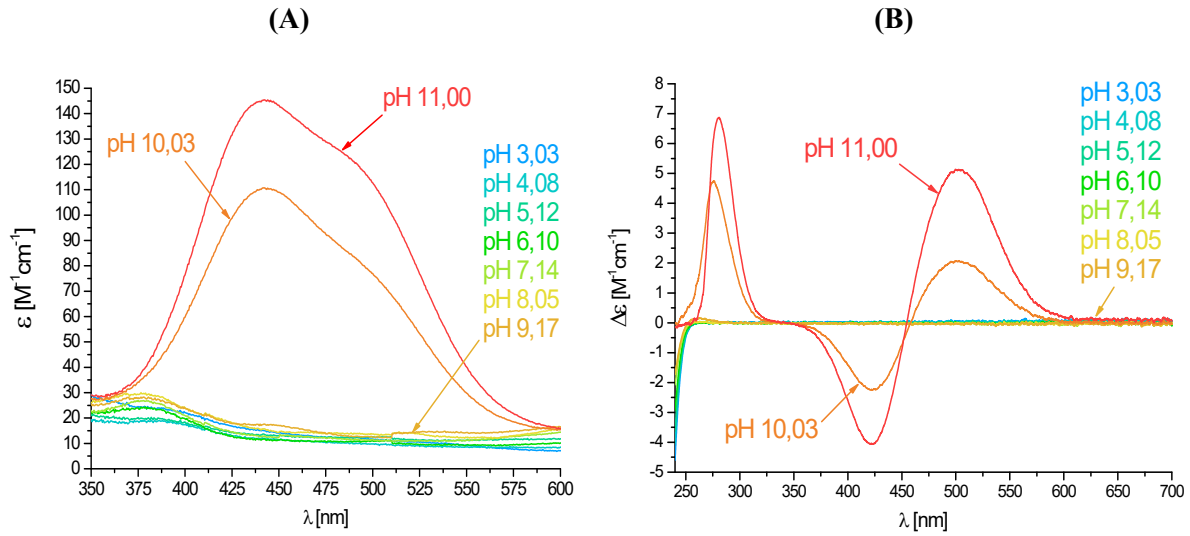


Figure S13. UV-Vis (A) and CD (B) spectra of Ni(II) complexes with the XEN ligand in the pH range 2-11. Conditions: T = 298 K, metal-to-ligand molar ratio = 1:1; [Ni(II)] = 2.5 x 10^{-4} M.

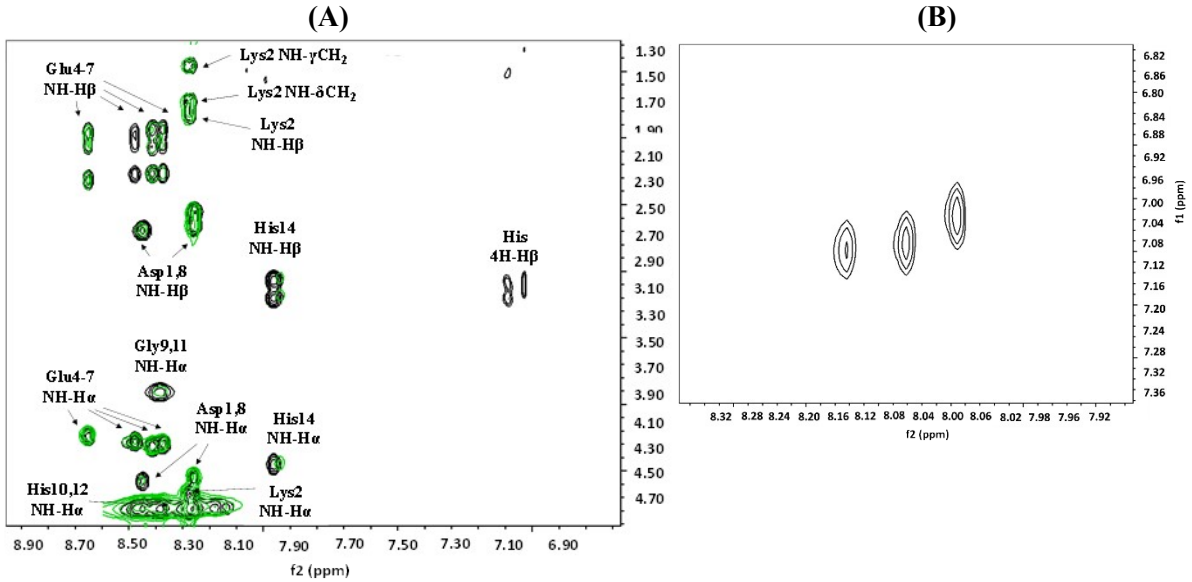


Figure S14. ^{1}H-^{1}H TOCSY NMR spectra of the XEN ligand (black) and its Ni(II) complex (green) at pH 7.4 in: (A) fingerprint region, (B) aromatic region; M : L = 0.4 : 1, T = 298 K.

The two complex forms of Ni(II)-XEN system, named (i) $NiH_{-1}L$, and (ii) $NiH_{-3}L$ (with maximum concentration at pH around 9.7 and 11.0, respectively), correspond to the deprotonation of (i) one lysine residue and first amide bond, (ii) second and third amide bonds, with nitrogen atoms potentially replacing the imidazole nitrogen atoms in the coordination sphere of the Ni(II) ion, forming a complex with a square planar geometry. This assumption is confirmed by the appearance of a positive-negative band (at 420 and 500 nm) and a charge transfer band of $N^{-}\rightarrow$Ni(II) type at λ_{max} = 280 nm in the CD spectrum of the studied system at pH 10.03, which occurs when Ni(II) complexes are formed with amide nitrogen atoms (**Figure S13B**).[31-32] The intensity of this band increases with increasing pH, indicating the involvement of additional amide nitrogen atoms in binding the Ni(II) ion. Therefore, the $NiH_{-1}L$,

and $NiH_{-3}L$ complex forms can probably be assigned coordination types $\{3N_{im}, 1N^{-}\}$ and $\{1N_{im}, 3N^{-}\}$, respectively.

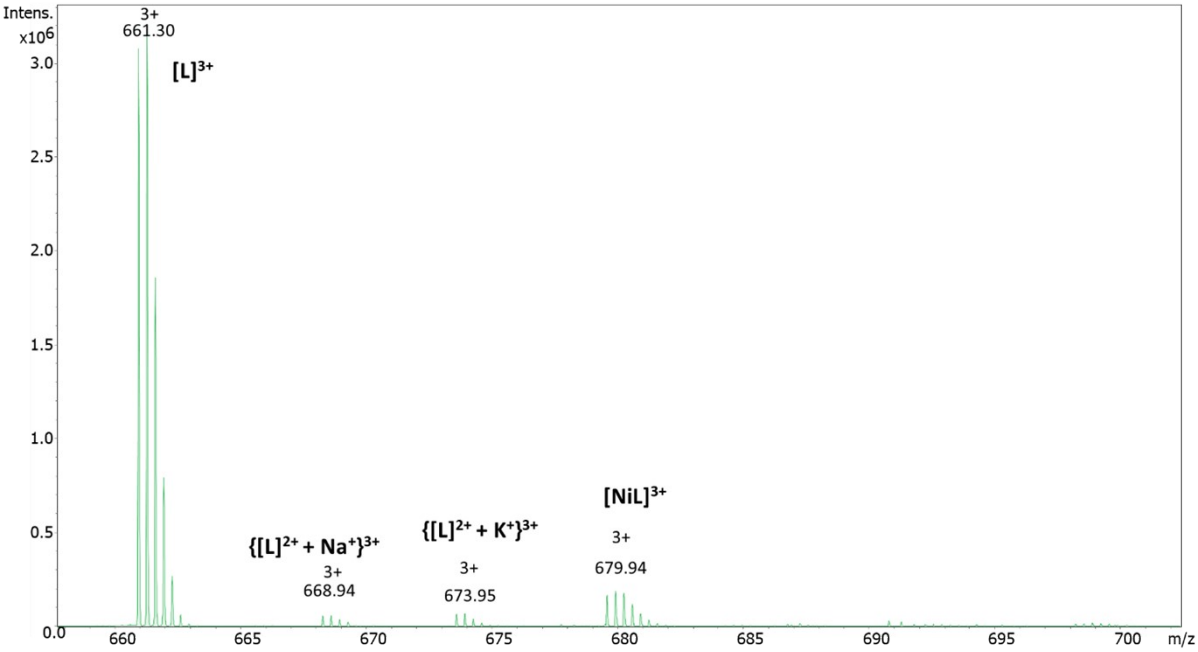


Figure S15. ESI-MS spectrum of the mixture of ABS ligand and Ni(II) ions in the *m/z* range of 655-705. M:L = 1:1, pH = 7.4.

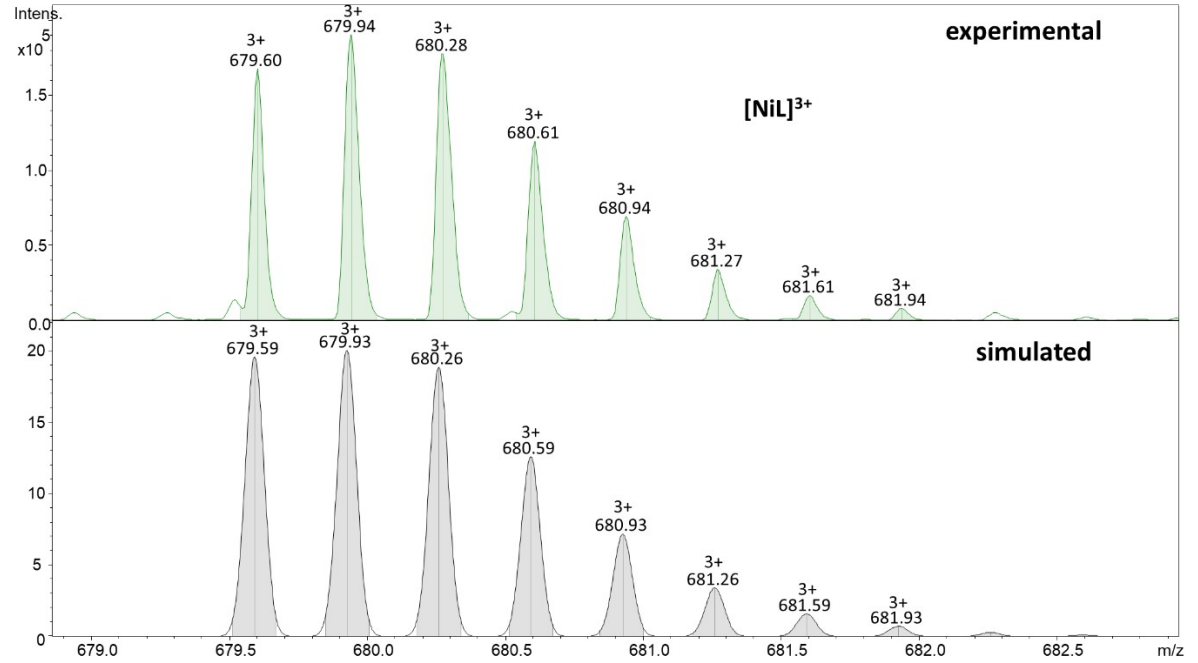


Figure S16. Comparison of the isotope distribution of the Ni(II) complex signal with ABS, $[NiL]^{3+}$, at *m/z* = 679.94 in the experimental and simulated spectra.

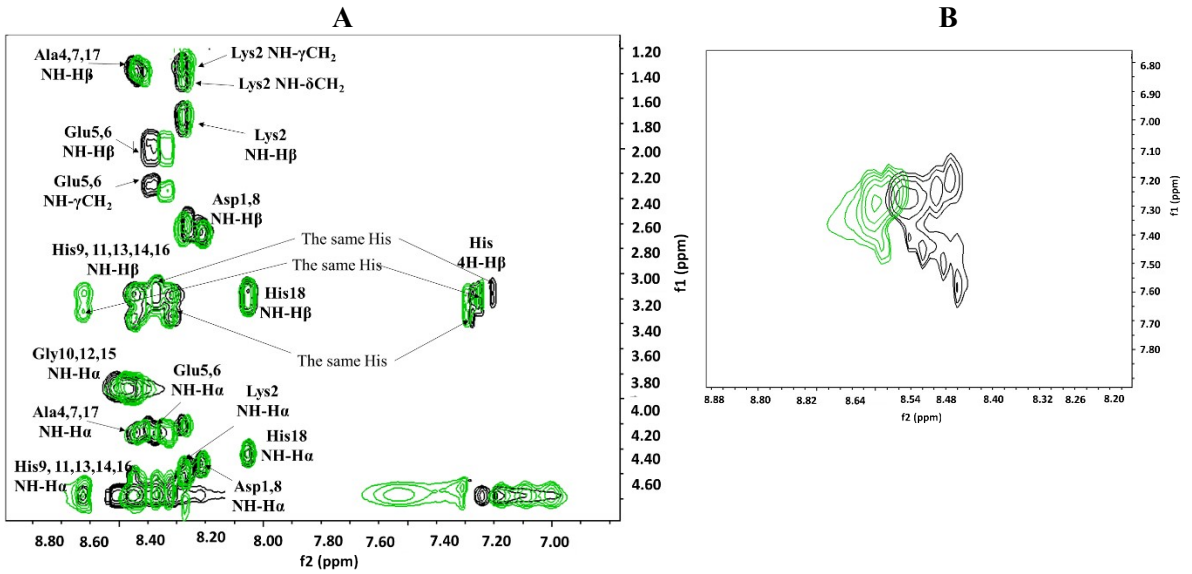


Figure S17. ^{1}H-^{1}H TOCSY NMR spectra of the ABS ligand (black) and its Ni(II) complex (green) at pH 5.2 in: (A) fingerprint region, (B) aromatic region; M : L = 0.4 : 1, T = 298 K.

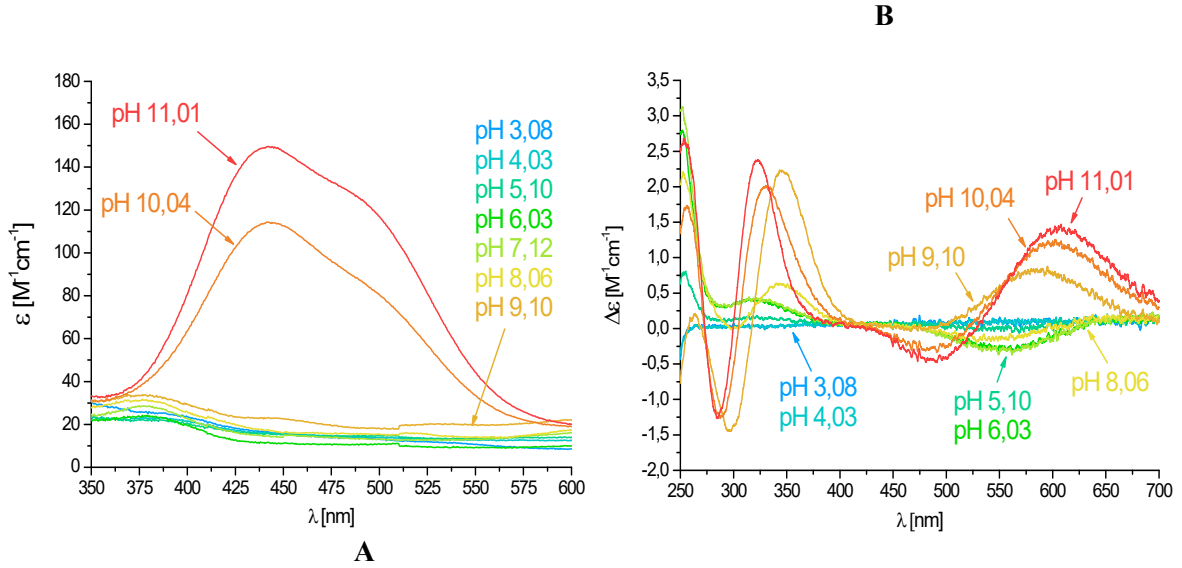


Figure S18. UV-Vis (A) and CD (B) spectra of Ni(II) complexes with the ABS ligand in the pH range 2-11. Conditions: T = 298 K, metal-to-ligand molar ratio = 1:1; [Ni(II)] = 2.5 x 10-4 M.

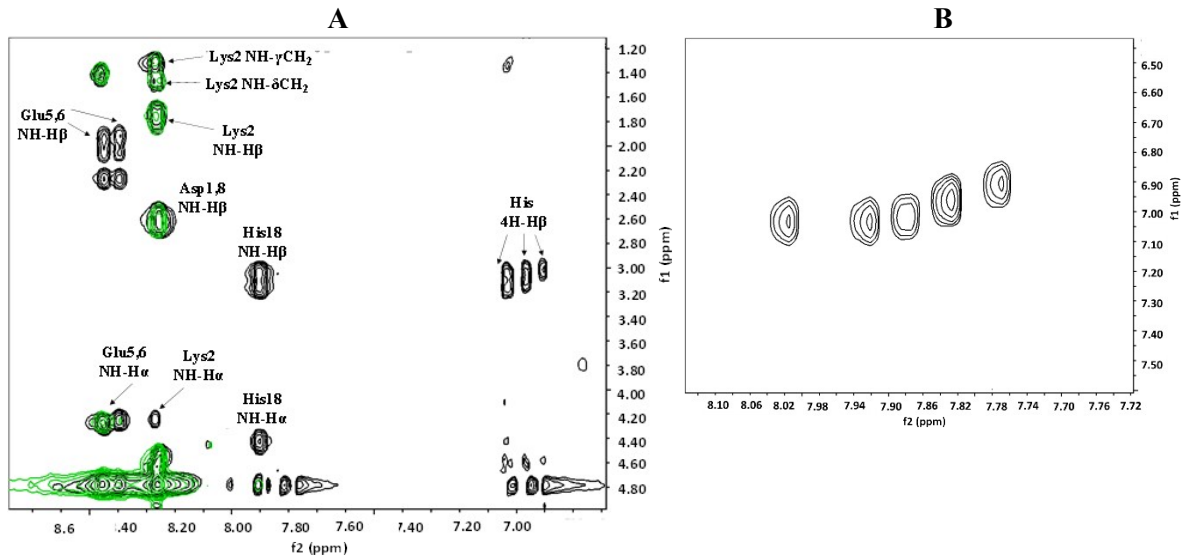


Figure S19. ^{1}H-^{1}H TOCSY NMR spectra of the ABS ligand (black) and its Ni(II) complex (green) at pH 7.5 in: (A) fingerprint region, (B) aromatic region; M : L = 0.4 : 1, T = 298 K.

The two complex forms of Ni(II)-ABS, named $NiH_{-1}L$ and $NiH_{-3}L$ (with maximum concentration at pH around 9.9 and 11.0, respectively), corresponding to the deprotonation of one lysine residue and three amide bonds, with nitrogen atoms potentially replacing the imidazole nitrogen atoms in the coordination sphere of the Ni(II) ion, forming a complex with a square planar geometry. This is indicated by the appearance of a positive-negative band at 420 and 500 nm and a charge transfer band of $N^{-}\rightarrow$Ni(II) type in the CD spectrum of the studied system at pH 9.10, characteristic of Ni(II) complexes with an amide nitrogen atom (**Figure S18B**).[31-32] The intensity of this band increases with increasing pH, indicating the involvement of additional amide nitrogen atoms in binding the Ni(II) ion. Therefore, the NiH–1L and $NiH_{-3}L$ complex forms can probably be assigned coordination types $\{3N_{im}, 1N^{-}\}$ and $\{1N_{im}, 3N^{-}\}$, respectively.

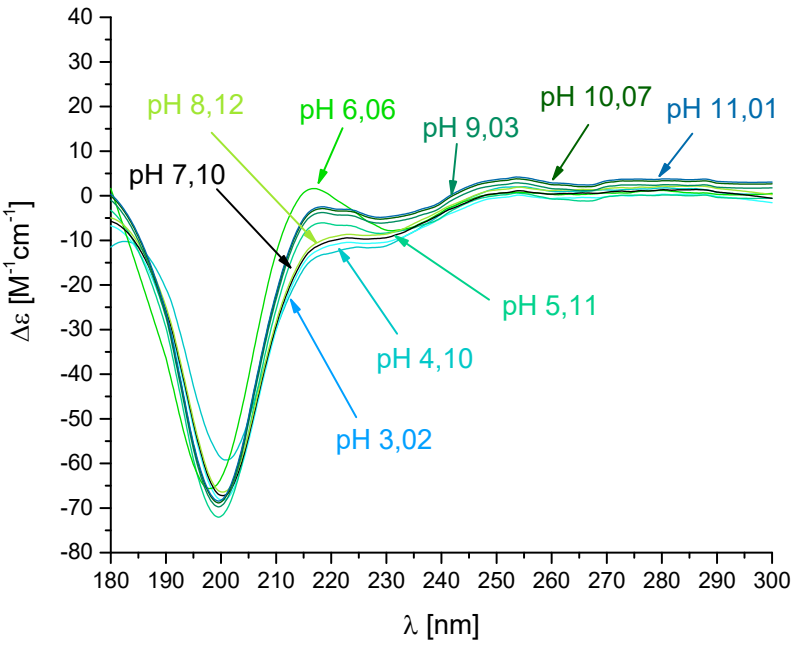


Figure S20. CD spectrum in the range of 180-300 nm for the Ni(II)-XEN system.

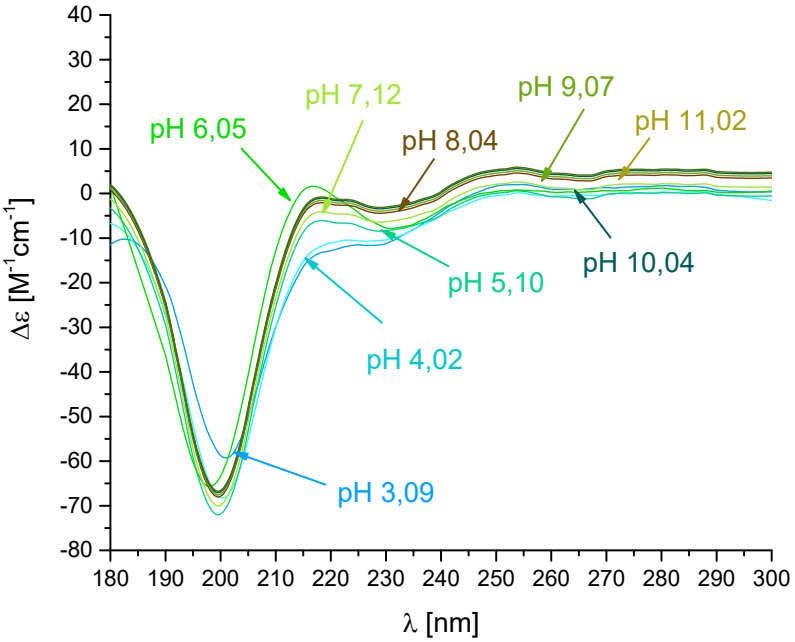


Figure S21. CD spectrum in the range of 180-300 nm for the Ni(II)-ABS system.

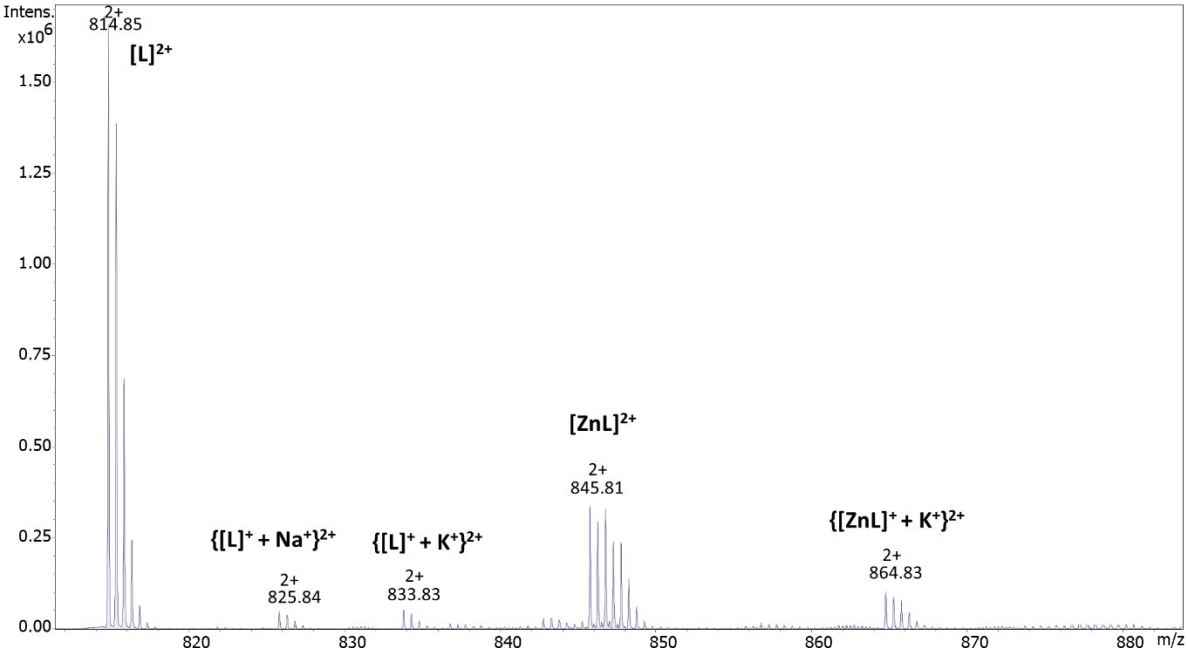


Figure S22. ESI-MS spectrum of the mixture of XEN ligand and Zn(II) ions in the *m/z* range of 810-880. M:L = 1:1, pH = 7.4.

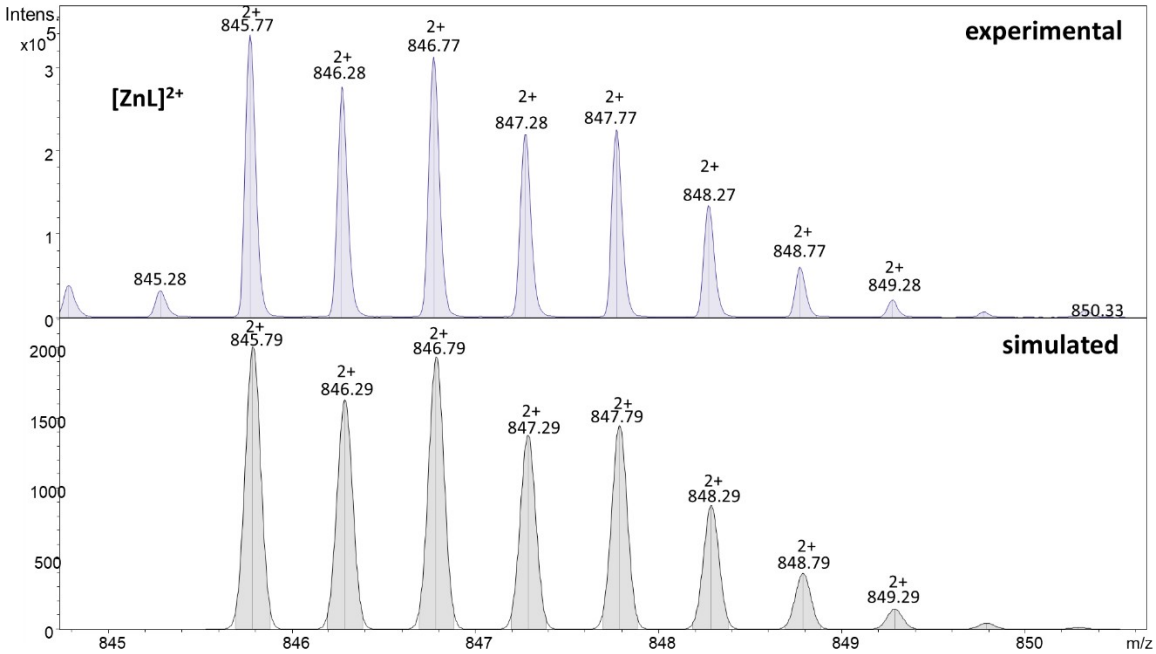


Figure S23. Comparison of the isotope distribution of the Zn(II) complex signal with XEN, $[ZnL]^{2+}$, at *m/z* = 845.81 in the experimental and simulated spectra.

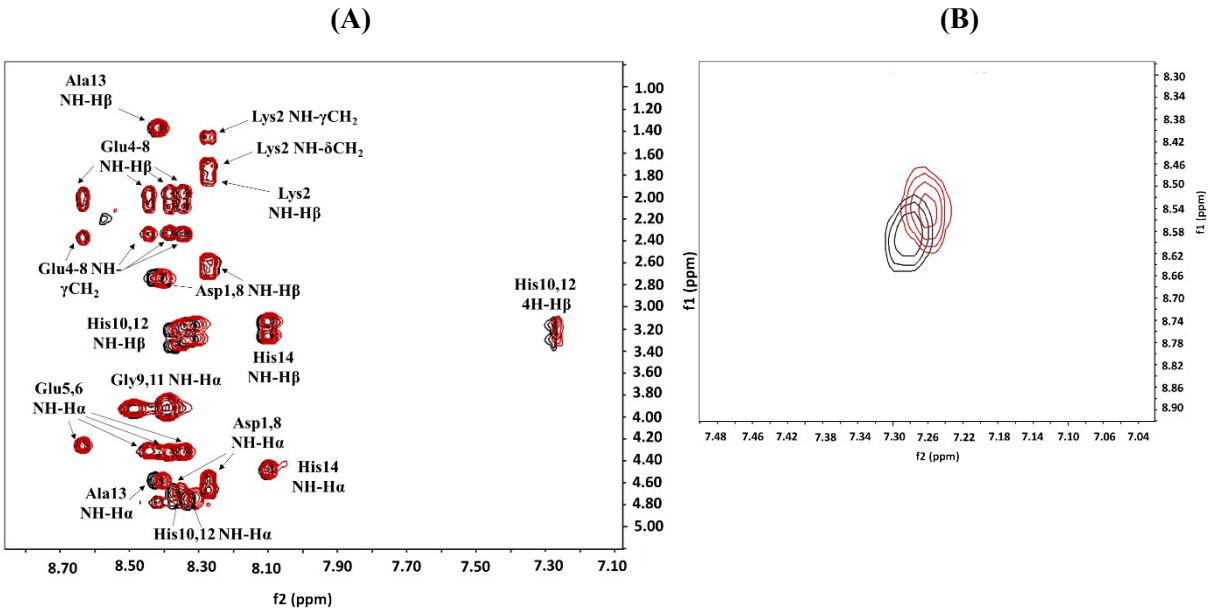


Figure S24. ^{1}H-^{1}H TOCSY NMR spectra of the XEN ligand (black) and its Zn(II) complex (red) at pH 5.2 in: (A) fingerprint region, (B) aromatic region; M : L = 0.8 : 1, T = 298 K.

ZnL and ZnH_2L forms of the Zn(II)-XEN system come from the deprotonation of water molecules, attached to Zn(II), and $ZnH_{-1}L$ is related to the deprotonation of Lys side chain deprotonation that is not involved in Zn(II) binding.

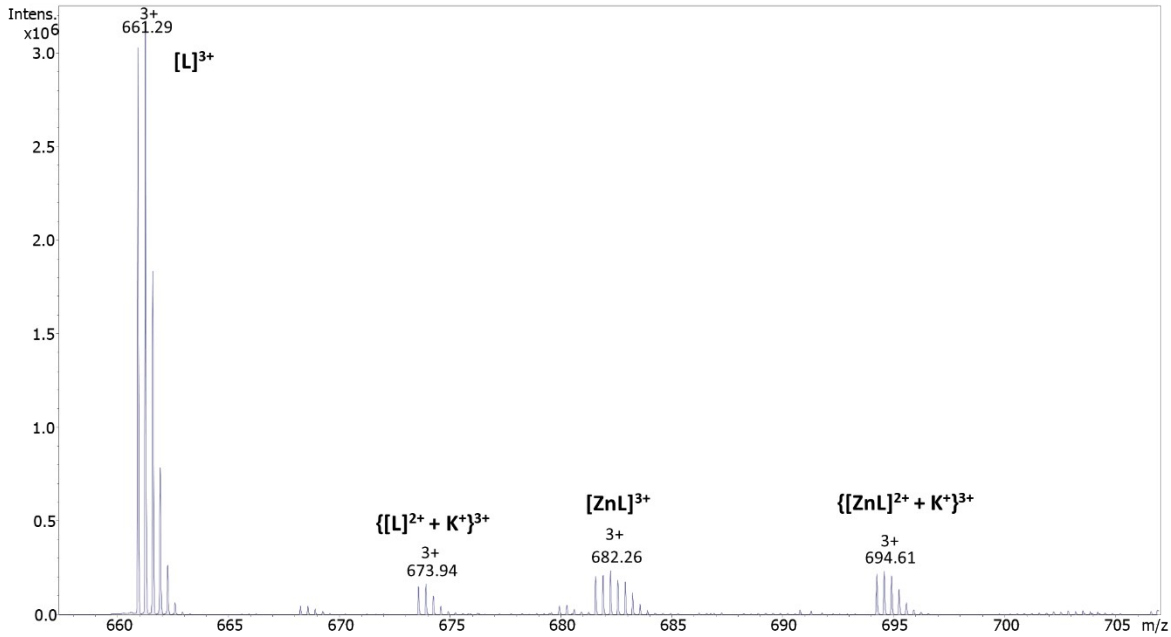


Figure S25. ESI-MS spectrum of the mixture of ABS ligand and Zn(II) ions in the *m/z* range of 655-705. M:L = 1:1, pH = 7.4.

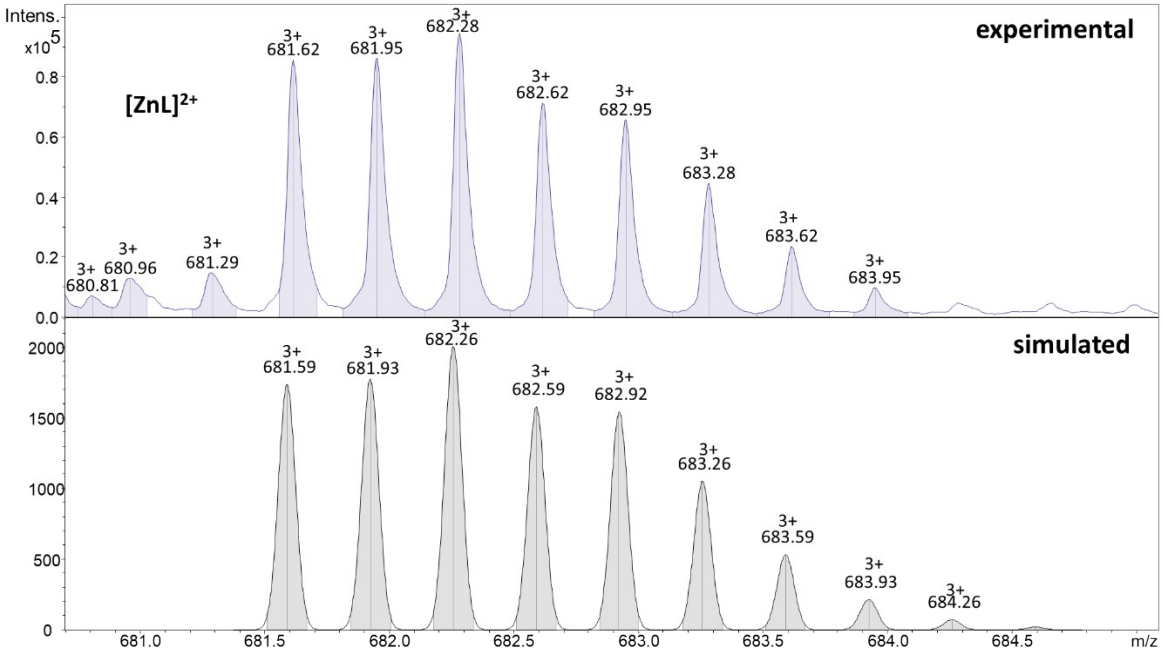


Figure S26. Comparison of the isotope distribution of the Zn(II) complex signal with ABS, $[ZnL]^{2+}$, at *m/z* = 682.26 in the experimental and simulated spectra.

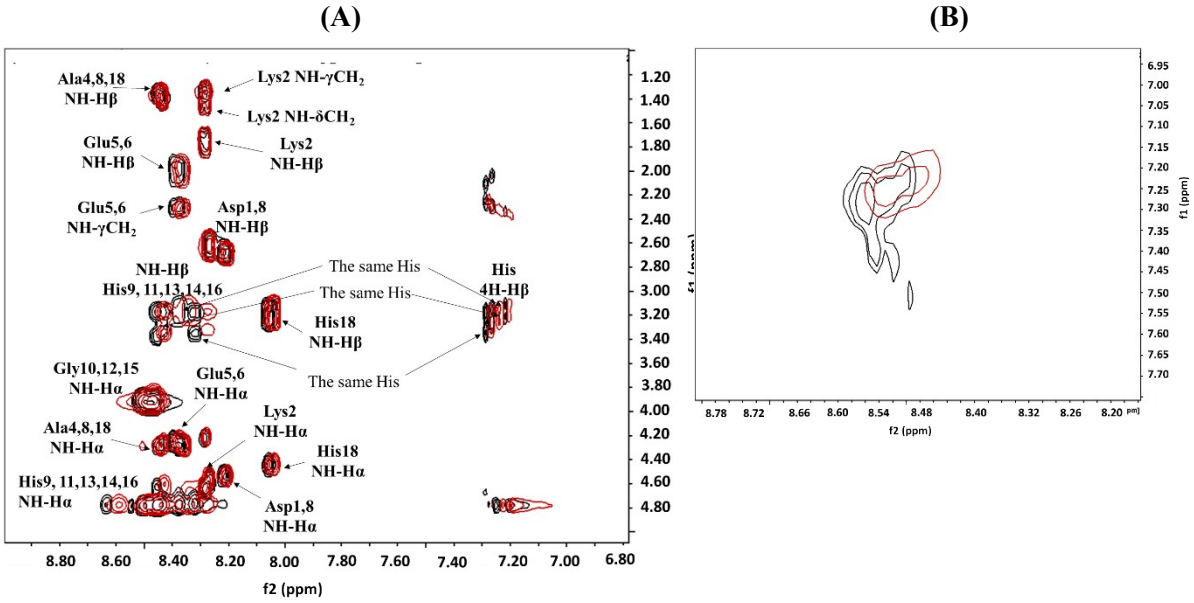


Figure S27. ^{1}H-^{1}H TOCSY NMR spectra of the ABS ligand (black) and its Zn(II) complex (red) at pH 5.2 in: (A) fingerprint region, (B) aromatic region; M : L = 0.8 : 1, T = 298 K.

Three complex forms of Zn(II)-ABS named, ZnL, $ZnH_{-1}L$, and $ZnH_{-2}L$, come from the deprotonations of water molecules attached to Zn(II) and one non-metal-binding Lys residue.

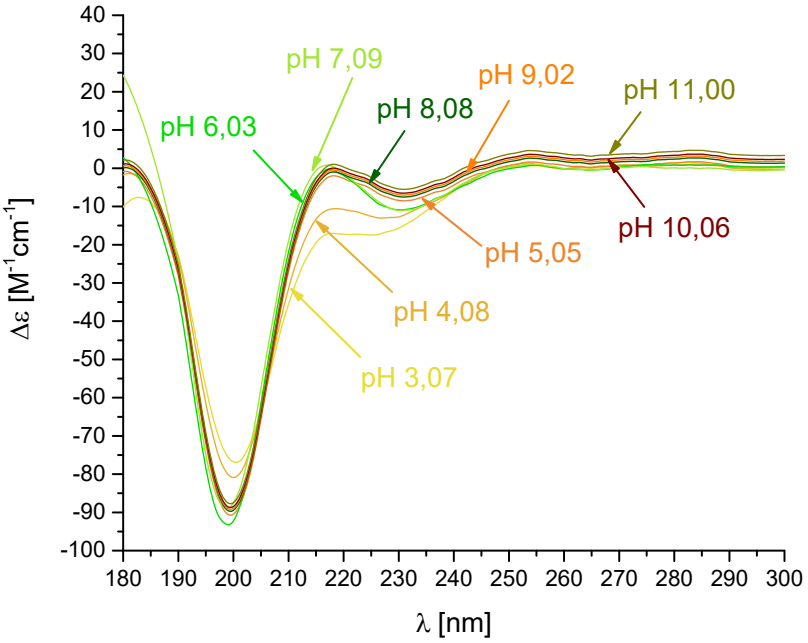


Figure S28. CD spectrum in the range of 180-300 nm for the Zn(II)-XEN system.

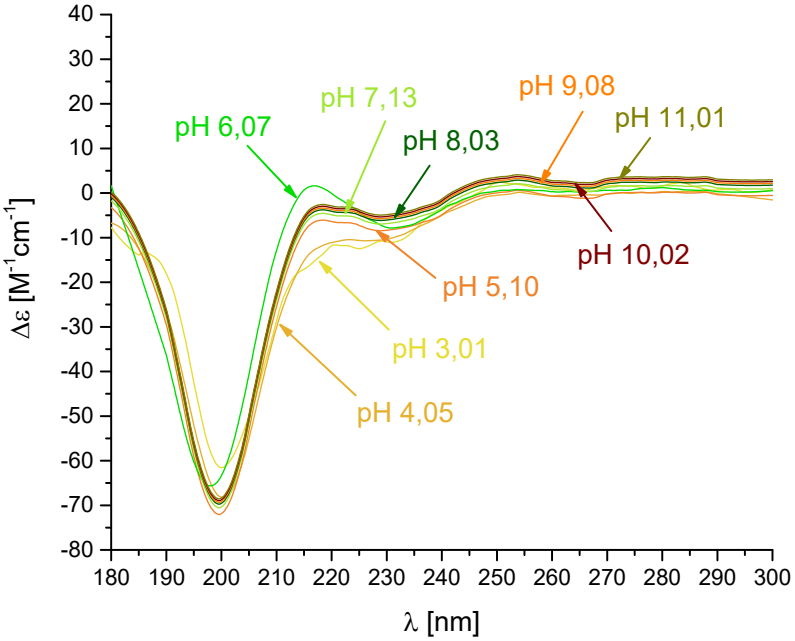


Figure S29. CD spectrum in the range of 180-300 nm for the Zn(II)-ABS system.

***All the references are listed in the References section of the Manuscript main body.**