# Does hemopressin bind metal ions *in vivo*?

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# SUPPLEMENTARY INFORMATION

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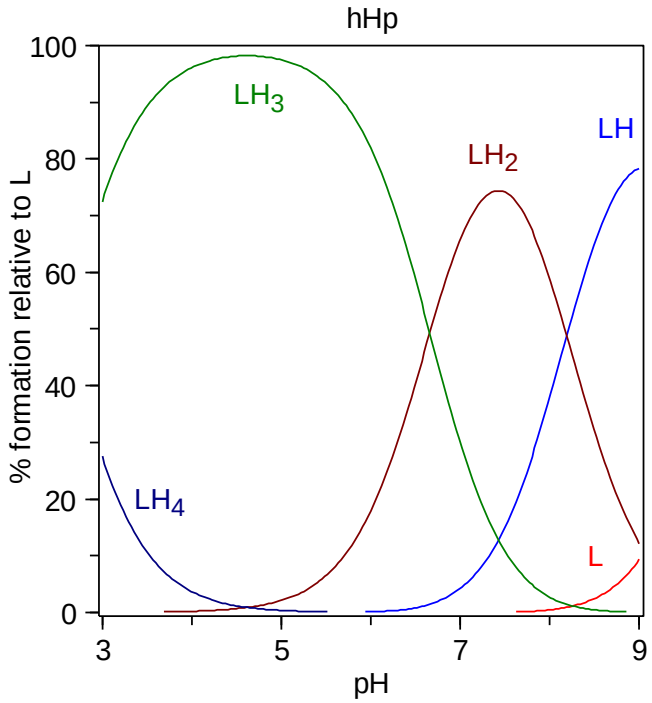



**Figure S1.** Representative distribution diagram for the protonation equilibria of hHp; C°L = $1 \cdot 10^{-3}$ mol $dm^{-3}$.

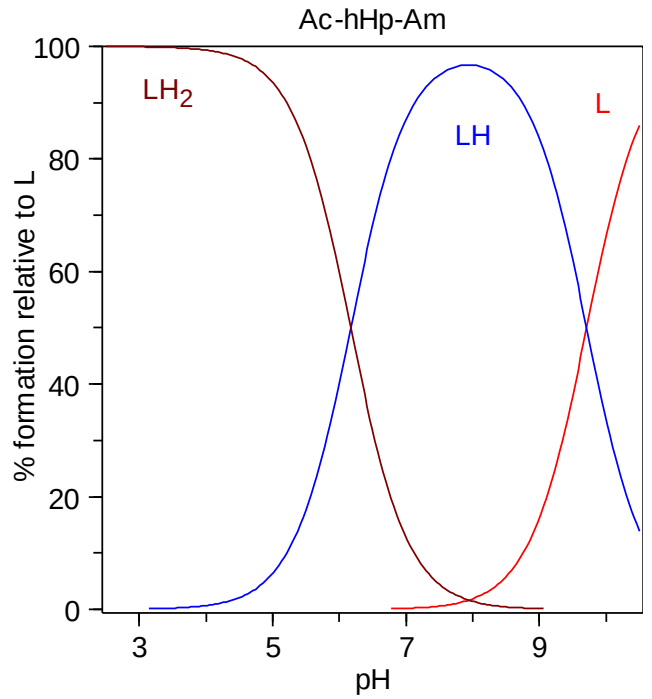



**Figure S2.** Representative distribution diagram for the protonation equilibria of Ac-hHp-Am; C°L = $1 \cdot 10^{-3}$ mol $dm^{-3}$.

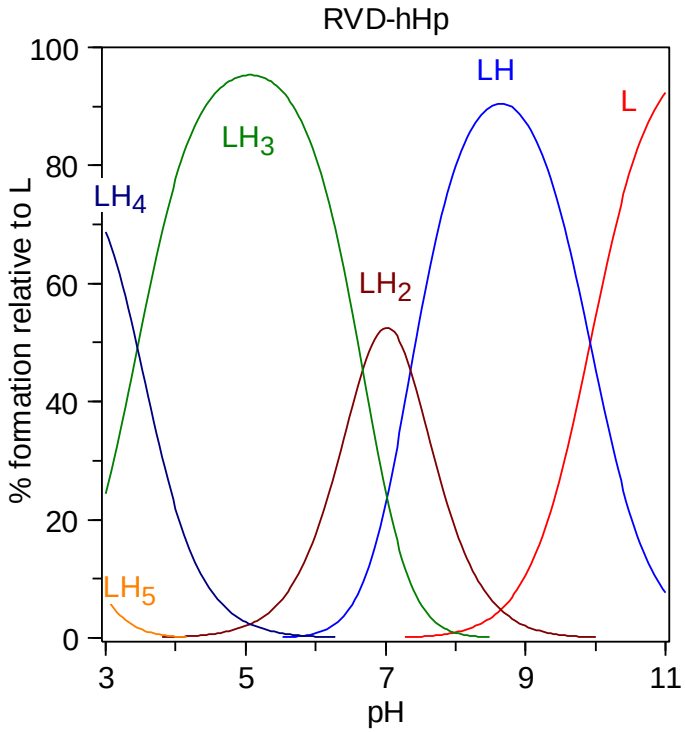



**Figure S3.** Representative distribution diagram for the protonation equilibria of RVD-hHp; C°L = $1 \cdot 10^{-3}$ mol $dm^{-3}$.

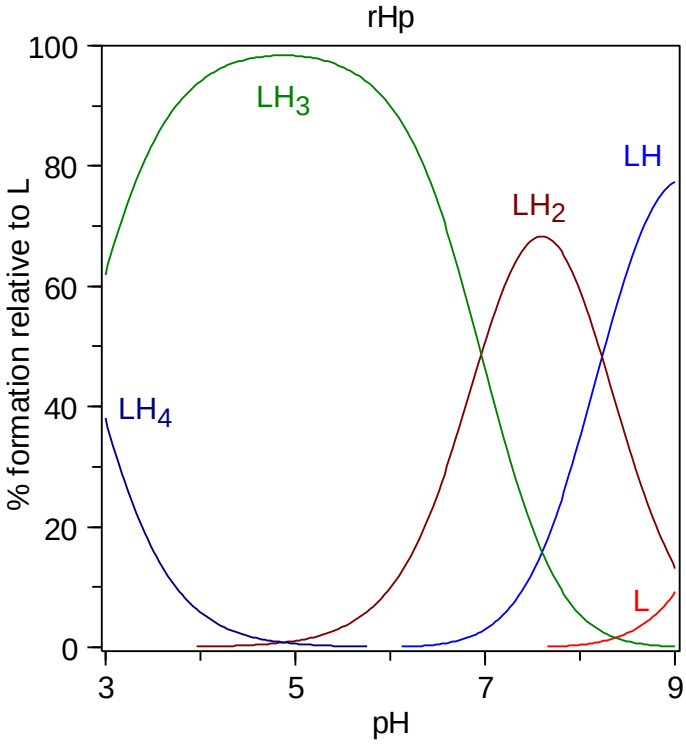
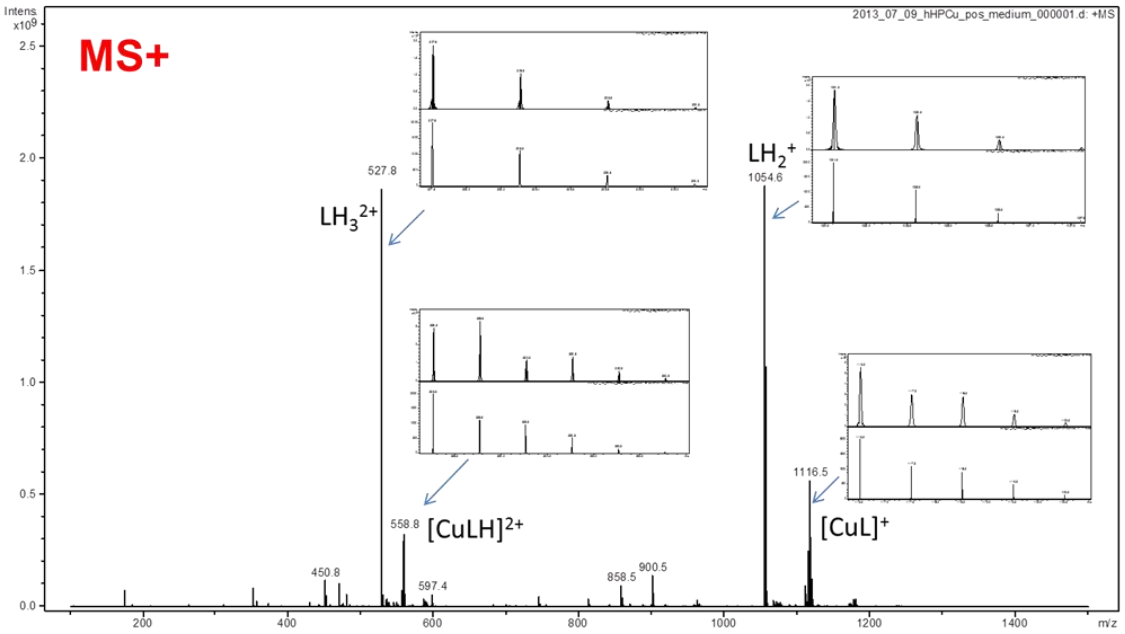



**Figure S4.** Representative distribution diagram for the protonation equilibria of rHp; C°L = $1 \cdot 10^{-3}$ mol $dm^{-3}$.

## Cu(II) – PVNFKLLSH (hHP)

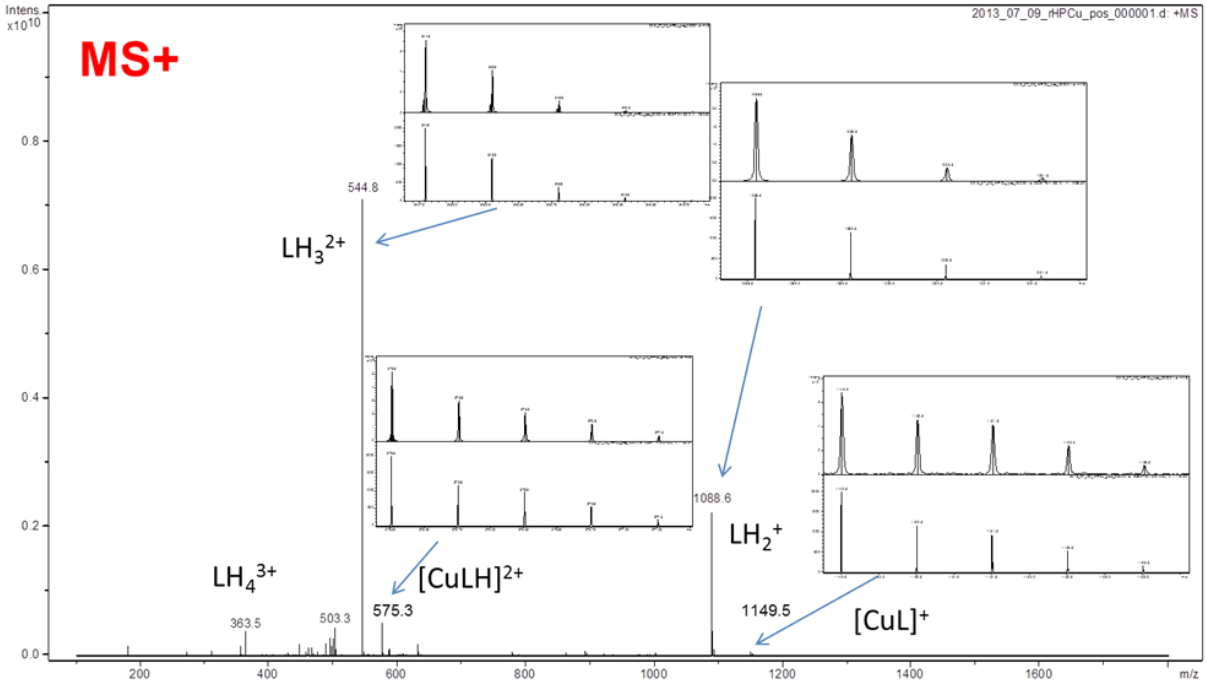
$C_{50}H_{79}N_{13}O_{12}$ = LH - MW = 1054.25 Da



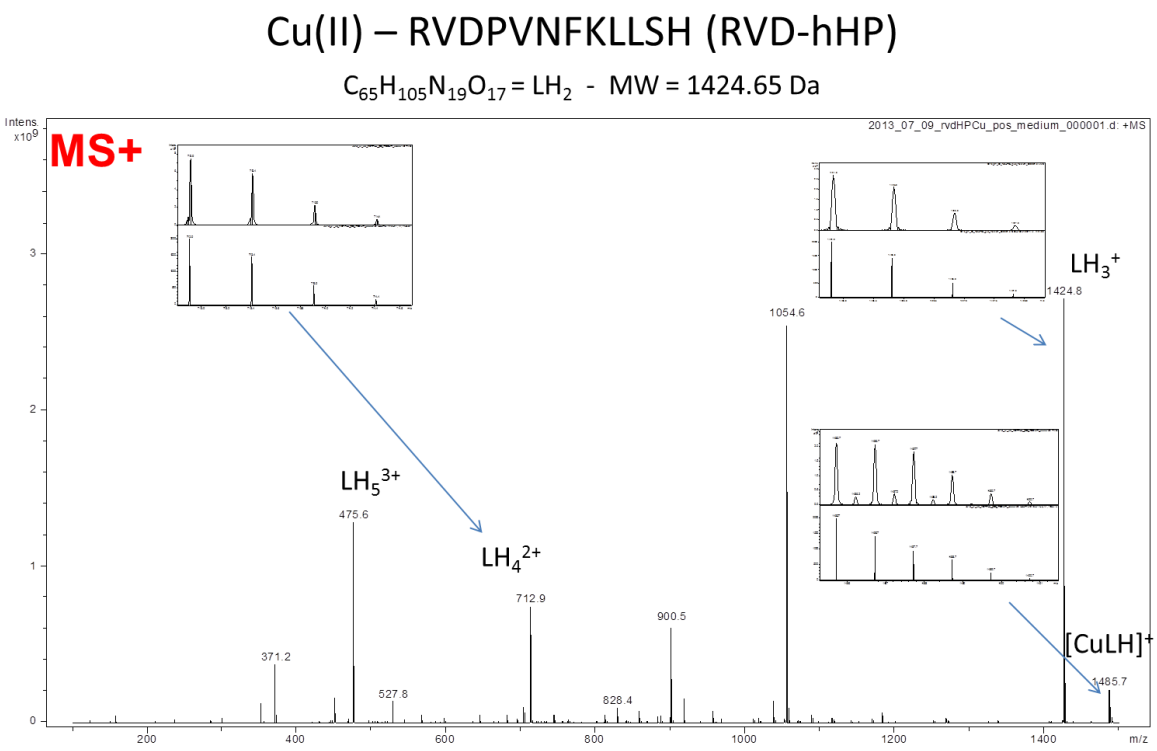

a)

## Cu(II) – PVNFKFLSH (rHp)

$C_{53}H_{77}N_{13}O_{12}$ = LH - MW = 1088.25 Da

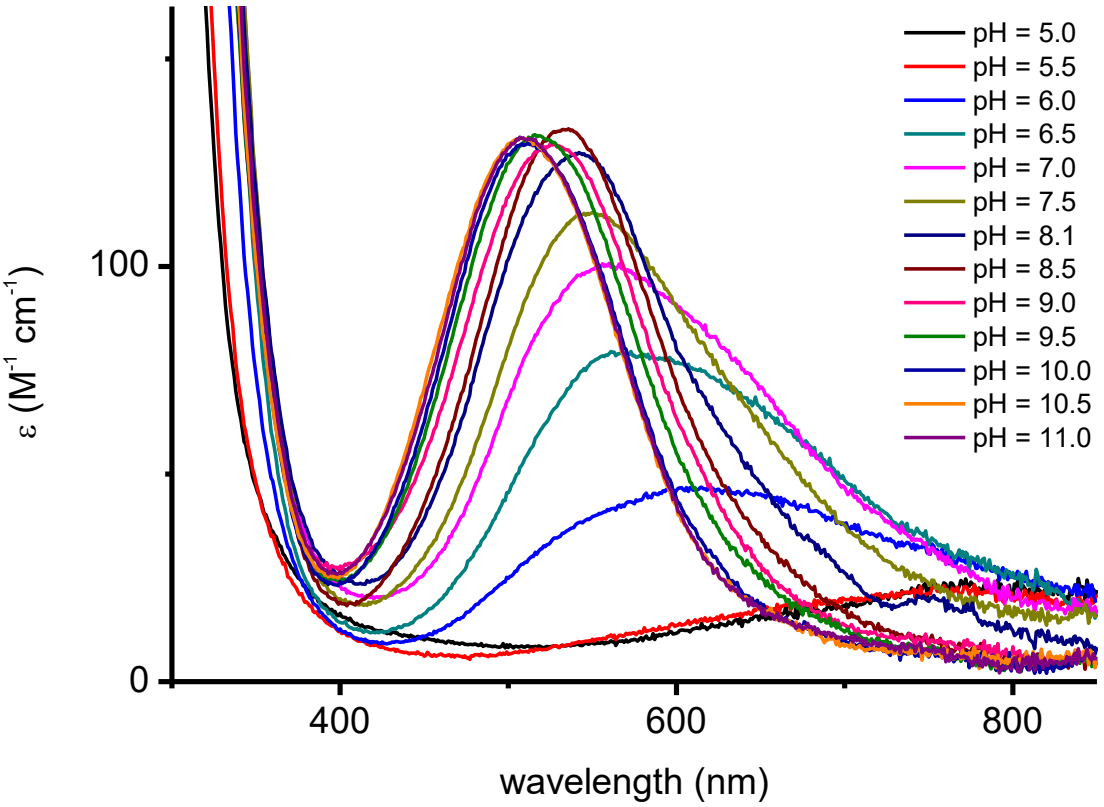



b)




c)

**Figure S5.** Mass spectra for the systems: a) Cu(II)/hHp; b) Cu(II)/rHp; c) Cu(II)/RVD-hHp. $C°_M = 0.5 \cdot 10^{-3}$ M; M/L molar ratio = 0.9; neutral pH. Experimental (up) and simulated (down) isotopic patterns are reported in the insets.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pH = 5.5
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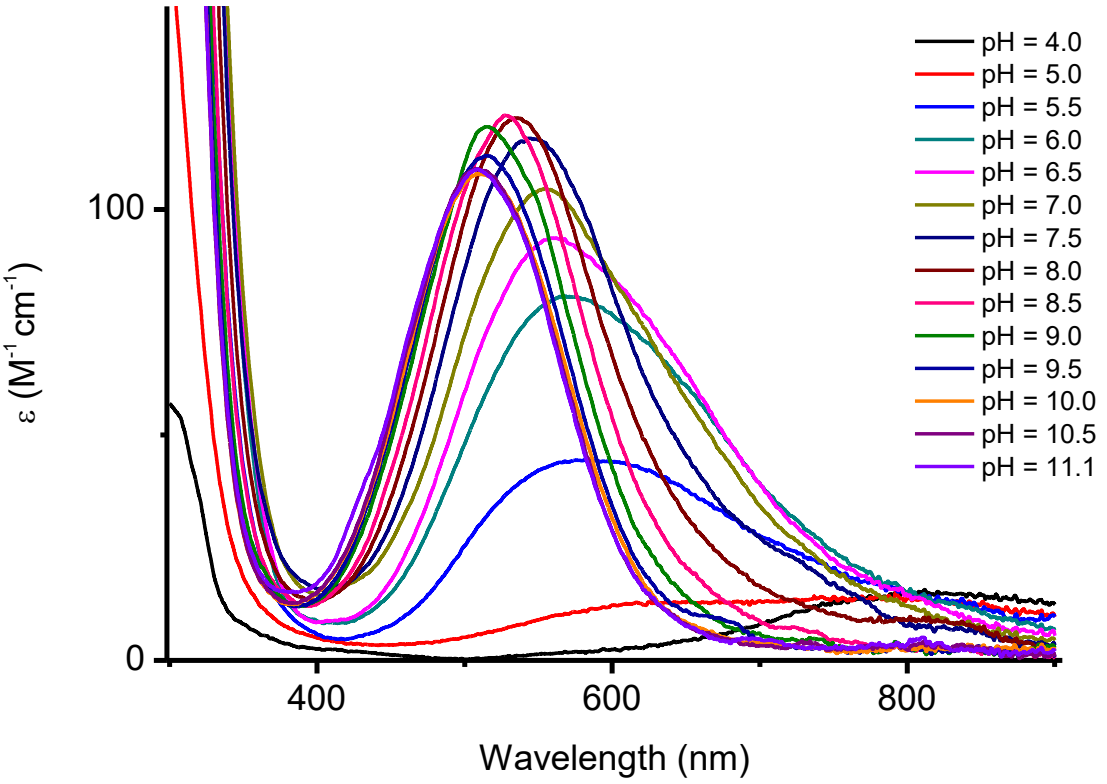
pH = 6.5
pH = 7.0
pH = 7.5
pH = 8.1
pH = 8.5
pH = 9.0
pH = 9.5
pH = 10.0
pH = 10.5
pH = 11.0
100
0
400
600
800
ε (M-1 cm-1)
wavelength (nm)

a)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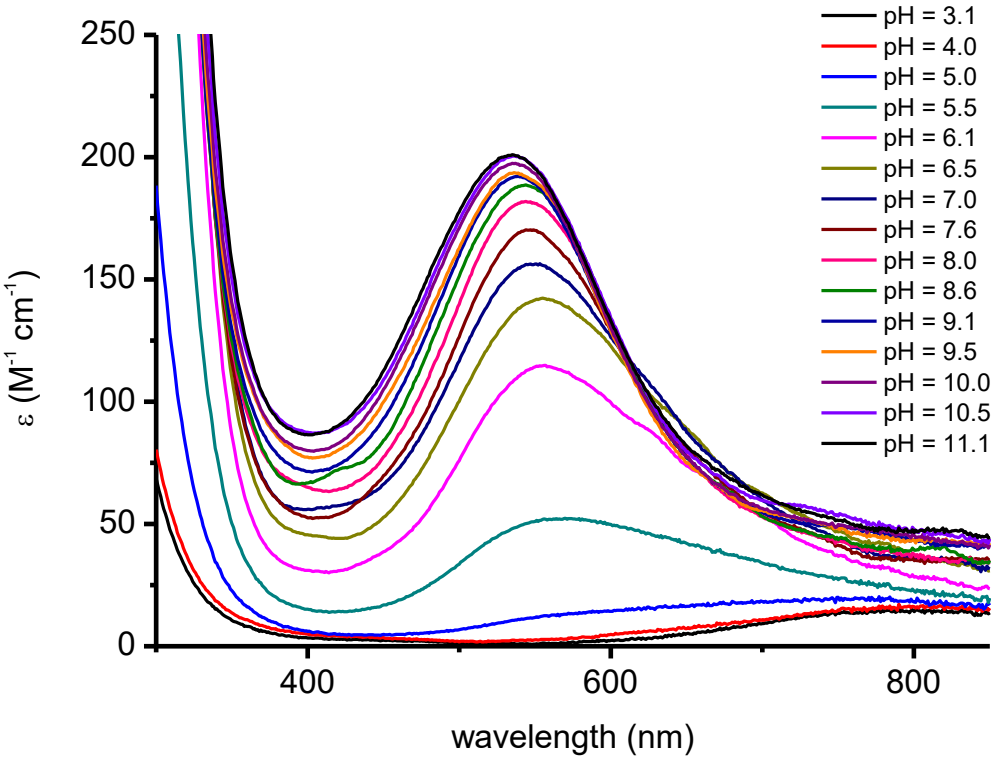
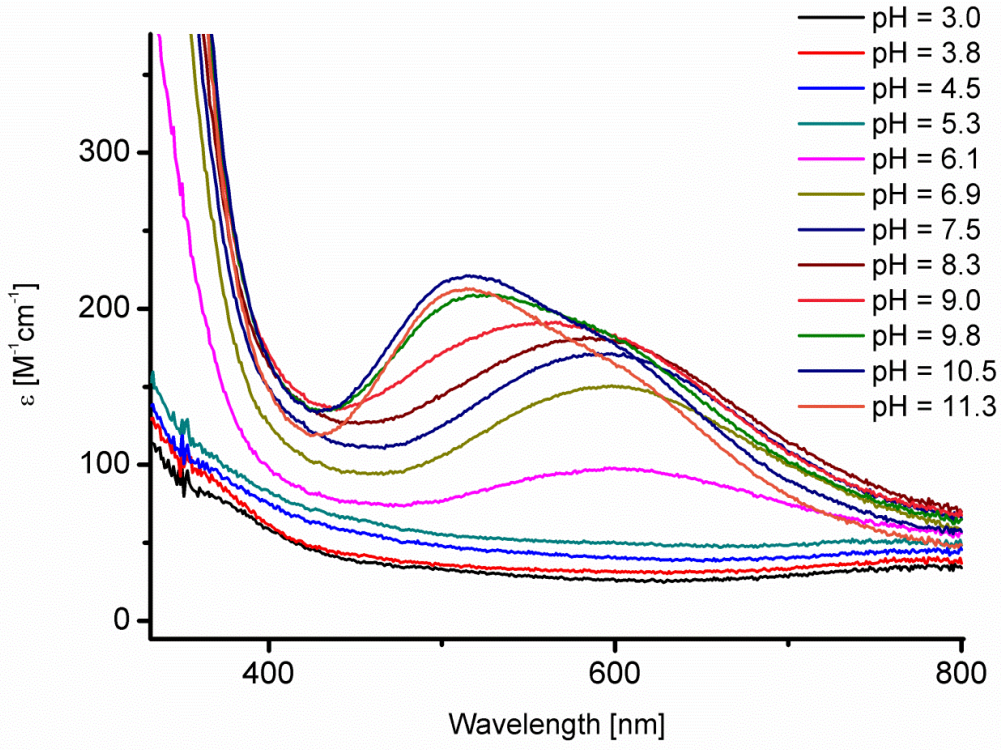
pH = 6.0
pH = 6.5
pH = 7.0
pH = 7.5
pH = 8.0
pH = 8.5
pH = 9.0
pH = 9.5
pH = 10.0
pH = 10.5
pH = 11.1
100
0
400
600
800
ε (M-1 cm-1)
Wavelength (nm)

b)

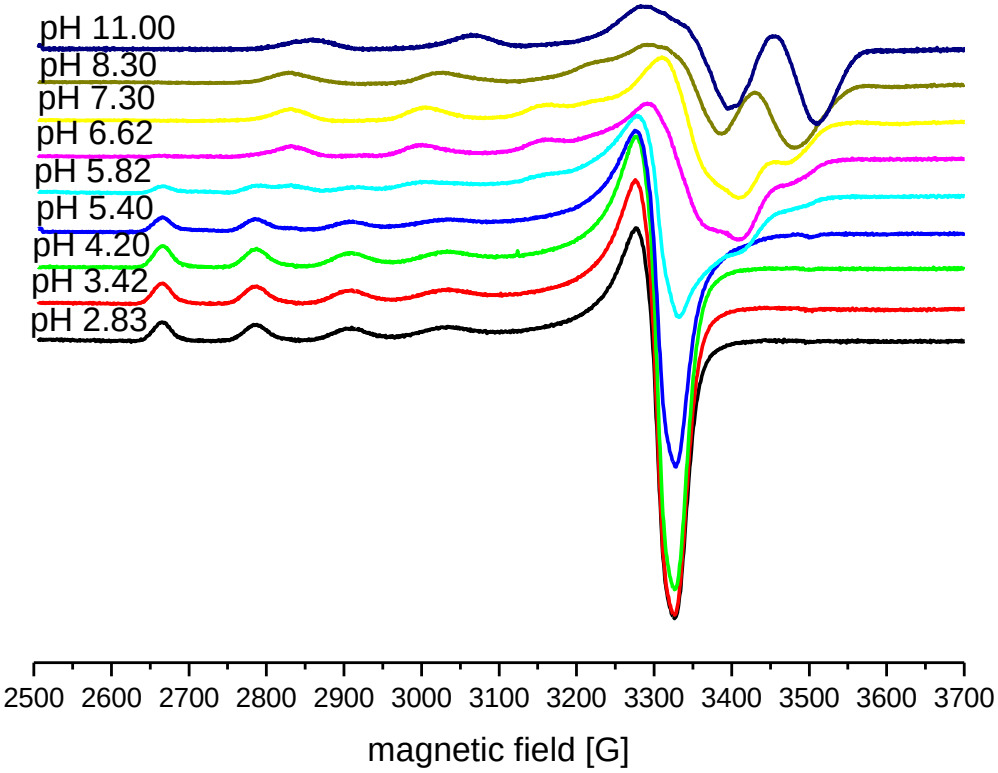



c)

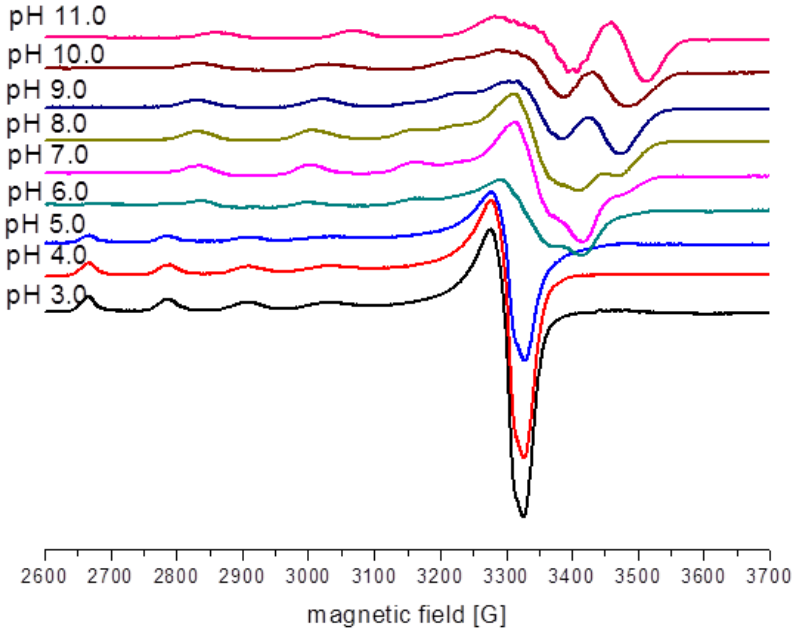



d)

**Figure S6.** Vis spectra, at different pH values, for the systems: a) Cu(II)/hHp; b) Cu(II)/rHp; c) Cu(II)/RVD-hHp; d) Cu(II)/Ac-hHp-Am.; $C°_{Cu} = 0.5 \cdot 10^{-3}$ M; M/L ratio = 0.9.


pH 11.00
pH 8.30
pH 7.30
pH 6.62
pH 5.82
pH 5.40
pH 4.20
pH 3.42
pH 2.83
2500 2600 2700 2800 2900 3000 3100 3200 3300 3400 3500 3600 3700
magnetic field [G]

**a)**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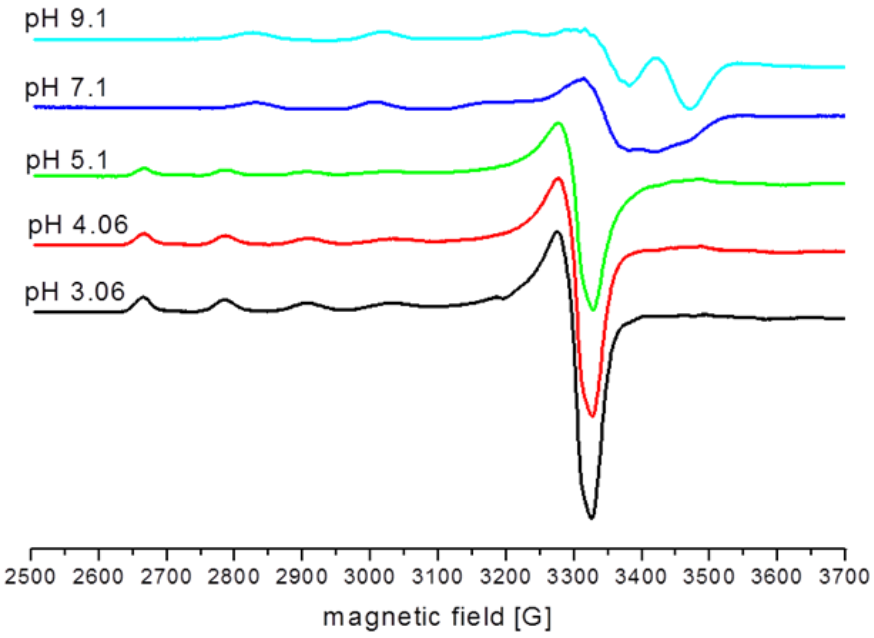
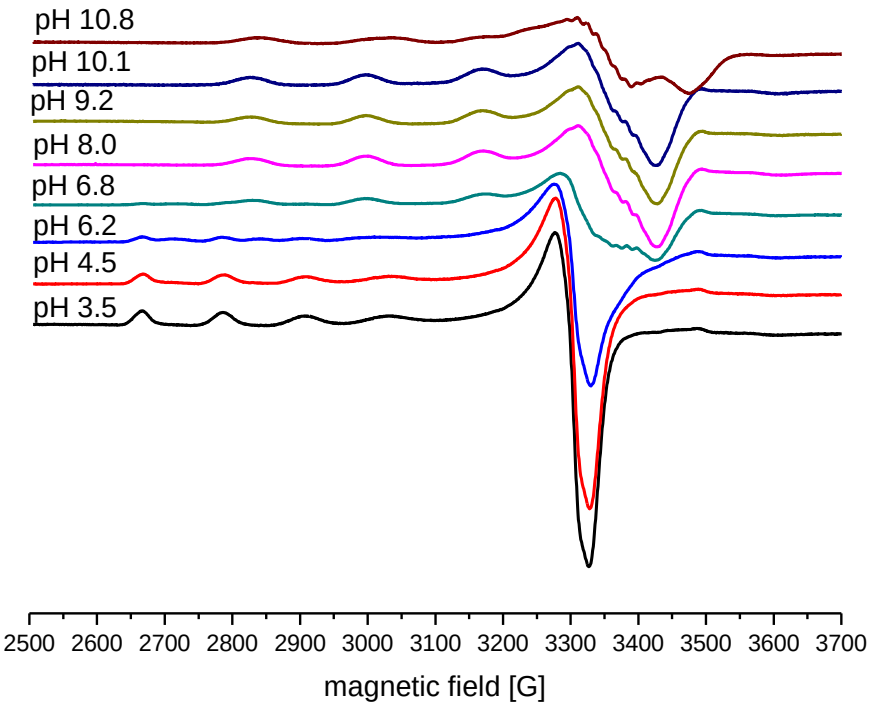
pH 10.0
pH 9.0
pH 8.0
pH 7.0
pH 6.0
pH 5.0
pH 4.0
pH 3.0
2600 2700 2800 2900 3000 3100 3200 3300 3400 3500 3600 3700
magnetic field [G]

**b)**




**c)**




**d)**

**Figure S7.** EPR spectra, at different pH values, for the systems: a) Cu(II)/hHp; b) Cu(II)/rHp; c) Cu(II)/RVD-hHp; d) Cu(II)/Ac-hHp-Am.; $C°_{Cu} = 0.5 \cdot 10^{-3}$ M; M/L ratio = 0.9.

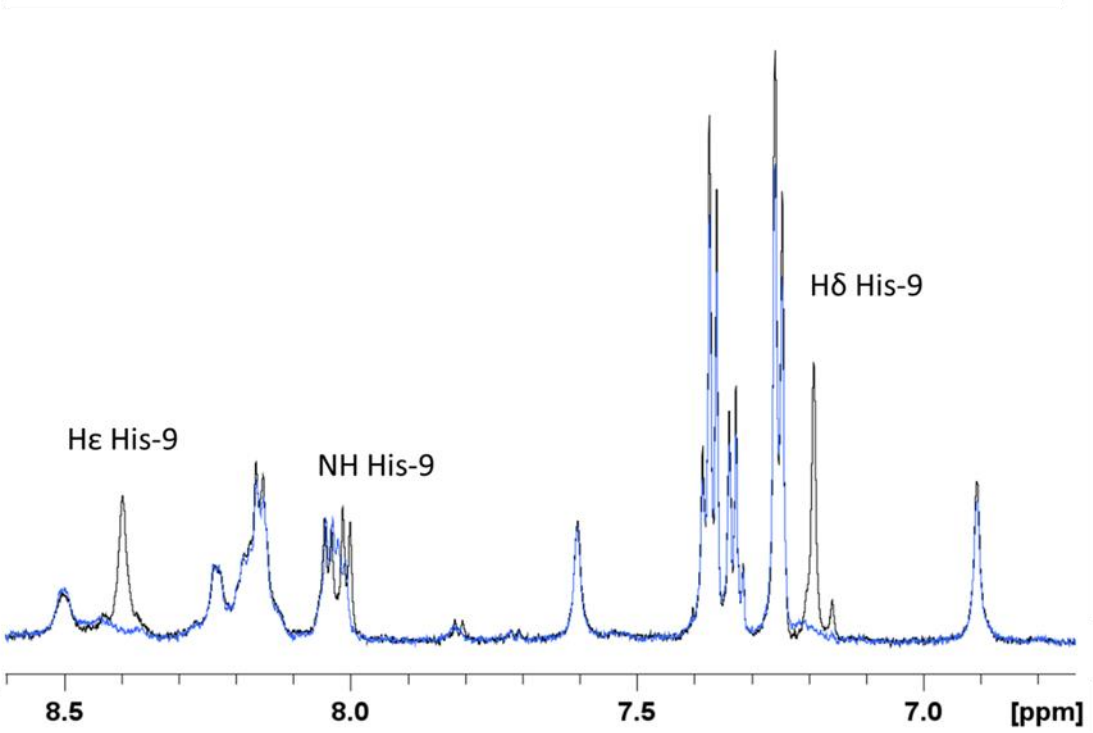
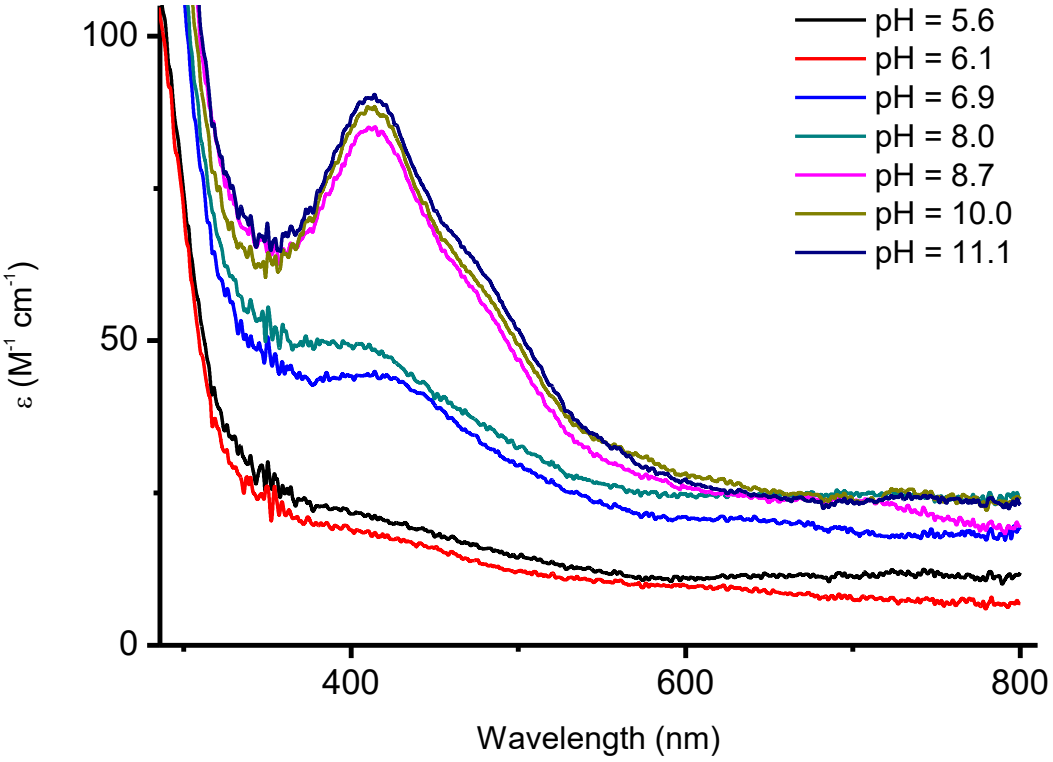



**Figure S8.** $^{1}$H NMR spectra of hHP 0.8 mM at pH 6.2, T=298 K in absence (black) and in presence of 0.1 Cu(II) eq. (blue).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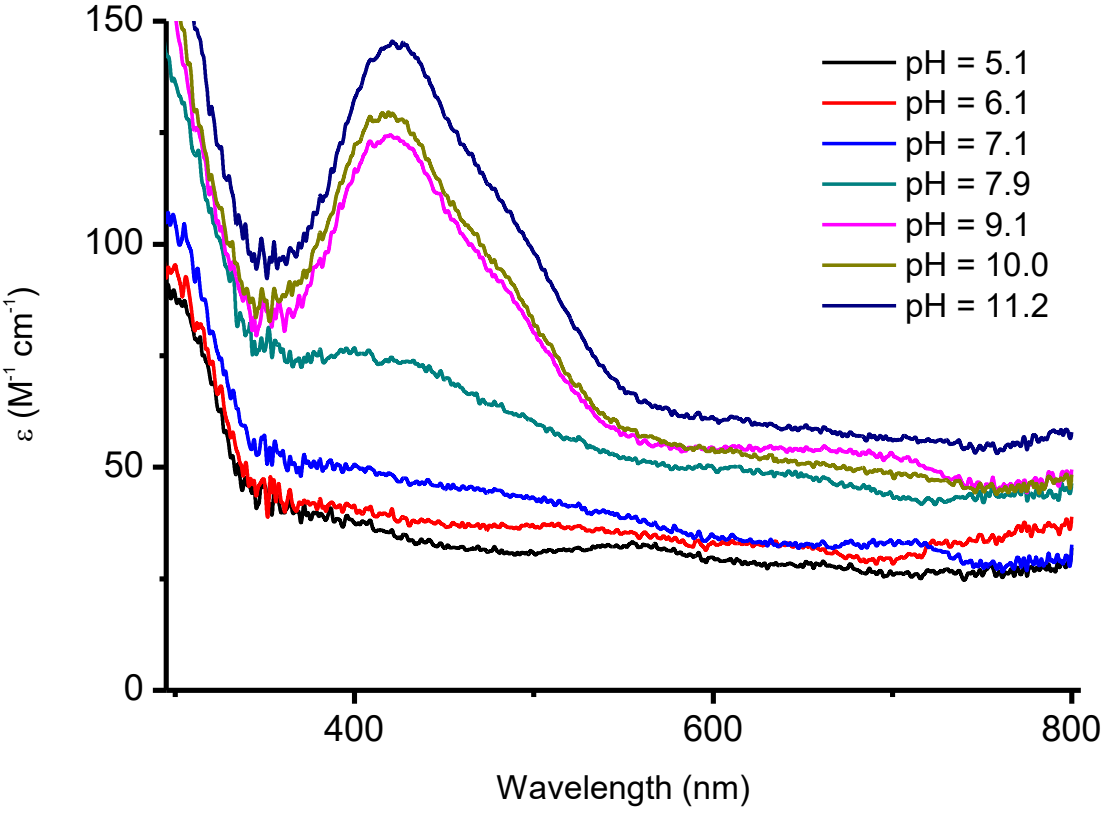
pH = 6.9
pH = 8.0
pH = 8.7
pH = 10.0
pH = 11.1
ε (M-1 cm-1)
100
50
0
400
600
800
Wavelength (nm)

a)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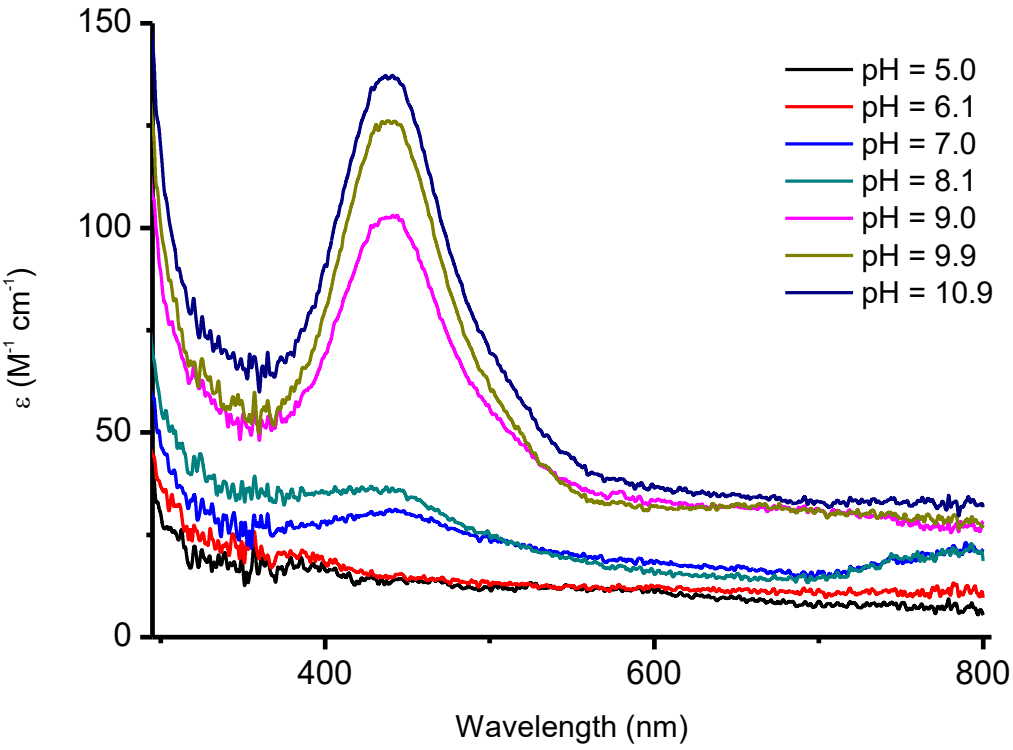
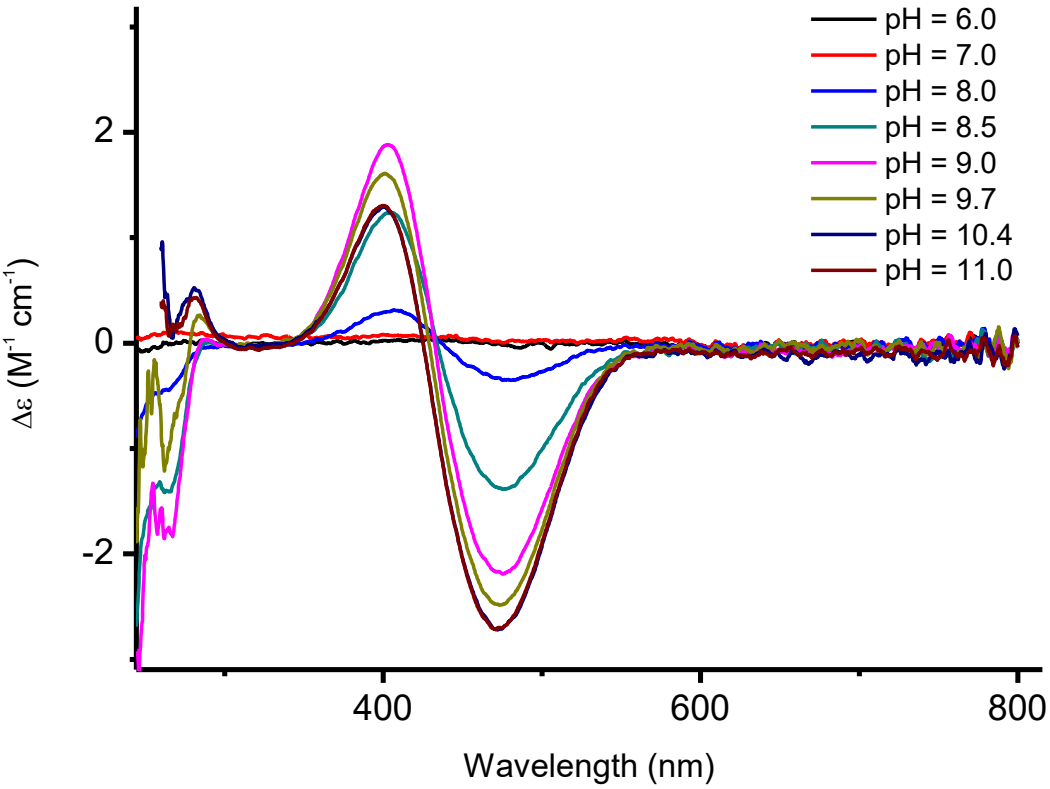
pH = 6.1
pH = 7.1
pH = 7.9
pH = 9.1
pH = 10.0
pH = 11.2
ε (M-1 cm-1)
150
100
50
0
400
600
800
Wavelength (nm)

b)




**c)**

**Figure S9.** Vis spectra, at different pH values, for the systems: a) Ni(II)/hHp; b) Ni(II)/rHp; c) Cu(II)/ RVD-hHp.; $C°_{Cu}$ = $0.5 \cdot 10^{-3}$ M; M/L ratio = 0.9.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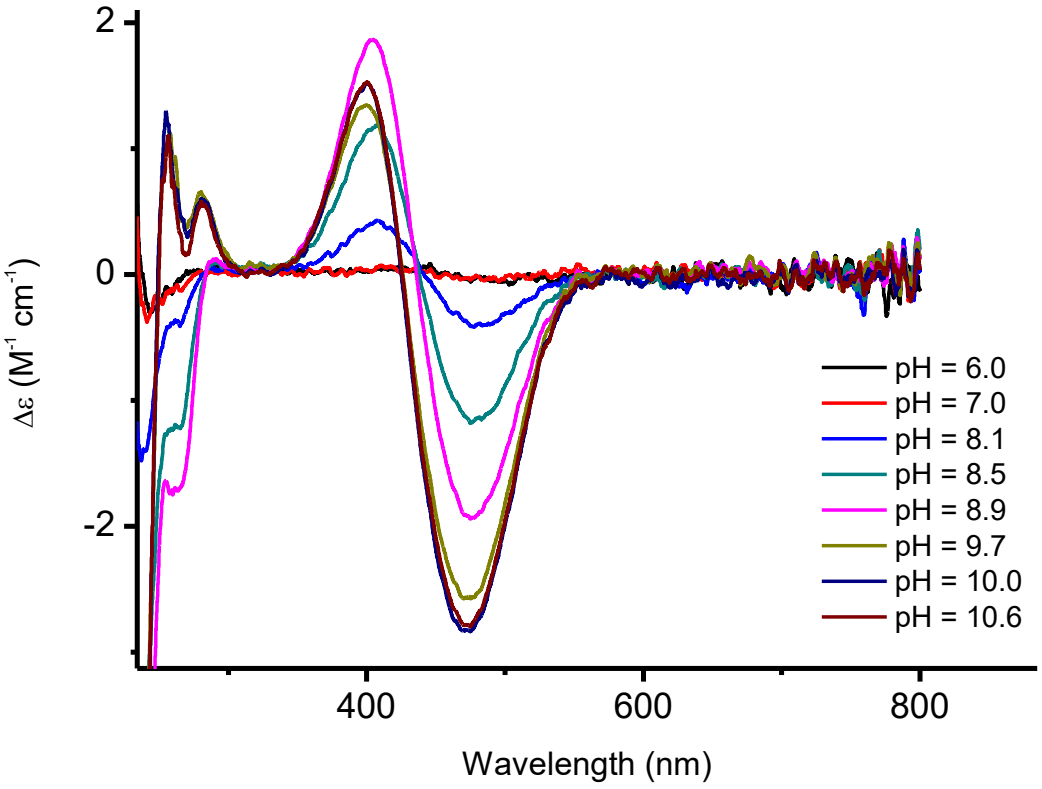
pH = 8.0
pH = 8.5
pH = 9.0
pH = 9.7
pH = 10.4
pH = 11.0
2
0
-2
Δε (M-1 cm-1)
400
600
800
Wavelength (nm)

a)


2
0
-2
Δε (M-1 cm-1)
pH = 6.0
pH = 7.0
pH = 8.1
pH = 8.5
pH = 8.9
pH = 9.7
pH = 10.0
pH = 10.6
400
600
800
Wavelength (nm)

b)

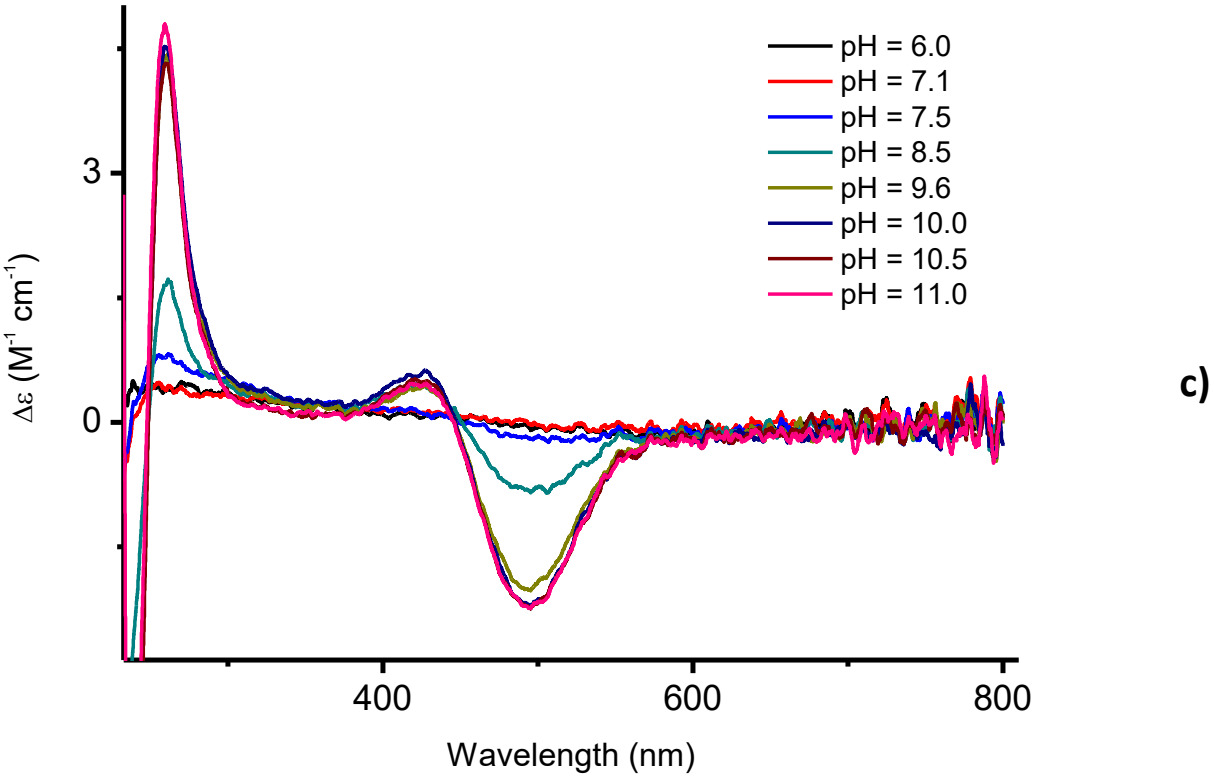



**Figure S10.** CD spectra, at different pH values, for the systems: a) Ni(II)/hHp; b) Ni(II)/rHp; c) Ni(II)/RVD-hHp.; $C°_{Cu}$ = $0.5 \cdot 10^{-3}$ M; M/L ratio = 0.9.

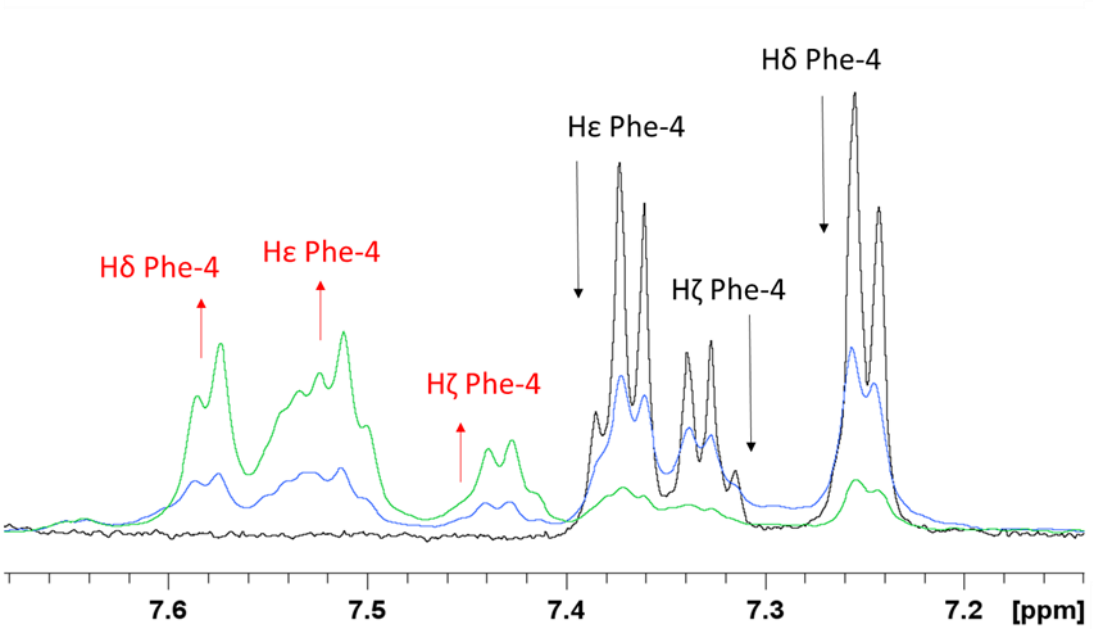



**Figure S11.** $^{1}$H NMR spectra of hHP 0.5 mM at pH 7.8, T=298 K, in absence (black) and in presence of 0.9 Ni(II) eq. at different time intervals, t=0 hrs (blue), t=72 hrs (green).

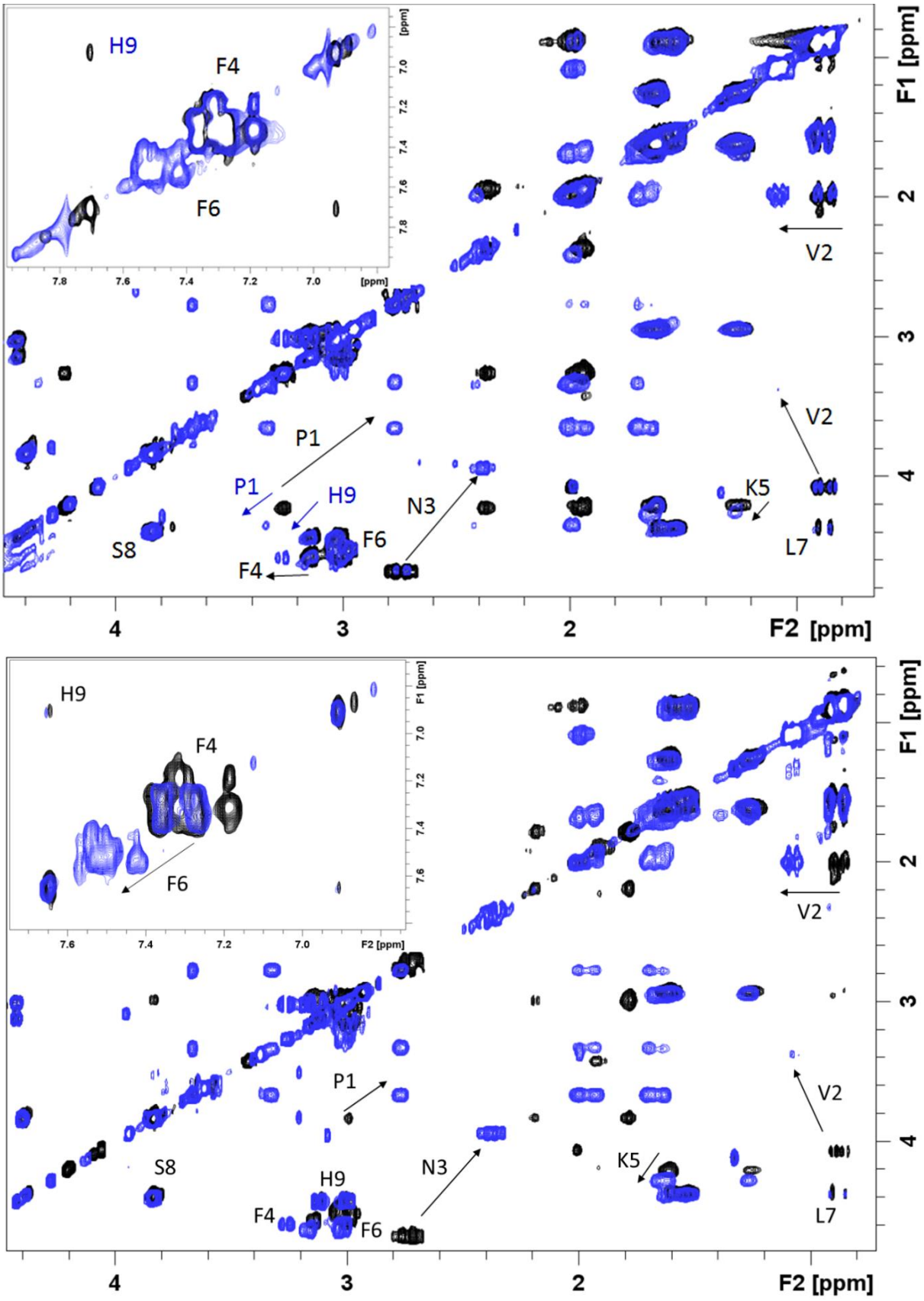



**Figure S12.** Superimposition of $^{1}$H-$^{1}$H TOCSY experiments of rHp c = 0.85 mM in absence (black contours) and in presence of 0.9 Ni(II) eq. (blue contours) at pH 7.8 (up), and at pH 8.9 (down), T=298 K. The insets show selected aromatic regions of the spectra.

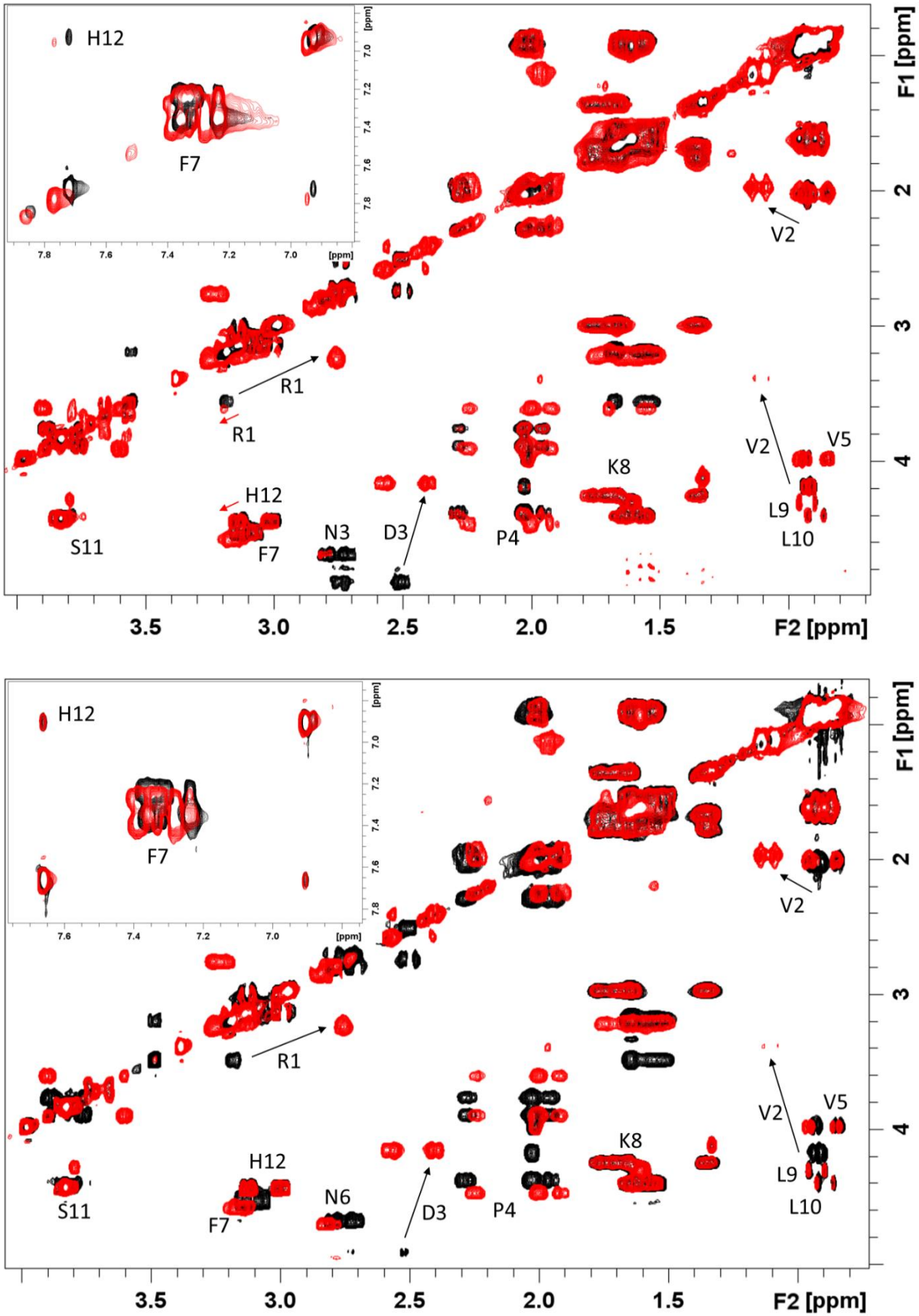



**Figure S13.** Superimposition of $^{1}$H-$^{1}$H TOCSY experiments of RVD-hHp c = 0.85 mM in absence (black contours) and in presence of 0.9 Ni(II) eq. (red contours) at pH 7.8 (up), and at pH 8.9 (down), T=298 K. The insets show selected aromatic regions of the spectra.

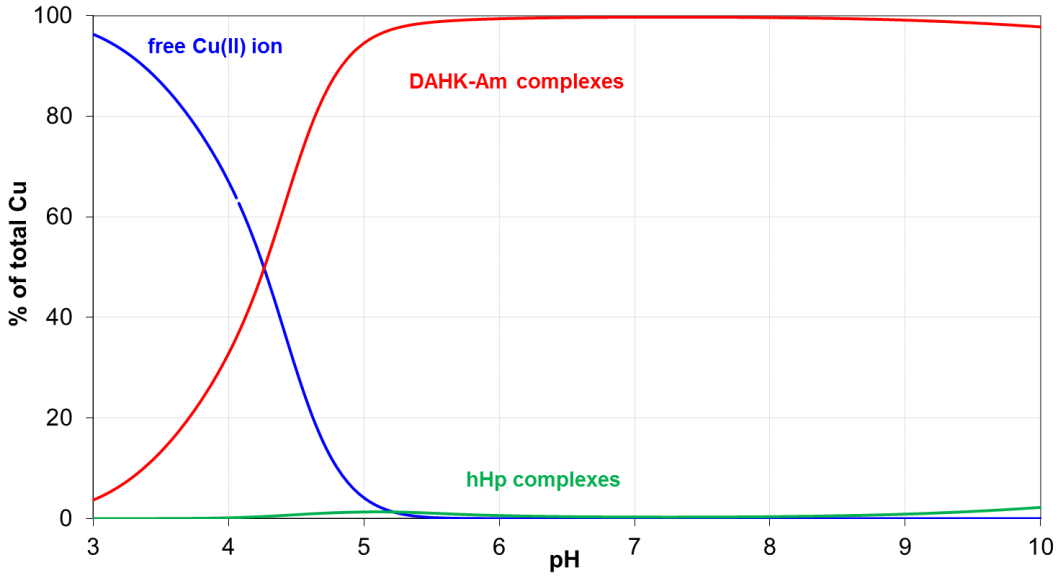



**Figure S14.** Competition between hHp and the peptide DAHK-Am, that mimics the N-terminal copper and nickel (ATCUN) binding site of human albumin, to bind Cu(II). The total concentration of both the metal and each ligand is 0.001 M. Protonation and complex formation constant of DAHK-Am are taken from: M. Sokolowska, A. Krezel, M. Dyba, Z. Szewczuk and W. Bal, Eur. J. Biochem. 2002, 269, 1323-1331.

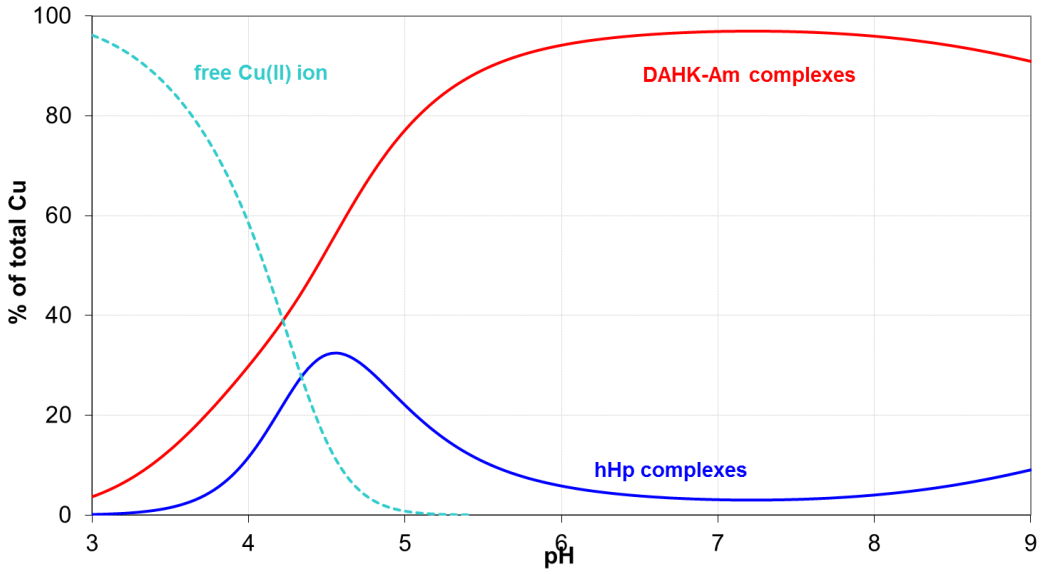



**Figure S15.** Competition between hHp and the peptide DAHK-Am, that mimics the N-terminal (ATCUN) binding site of human albumin. The total concentration of both copper and DAHK-Am is 1 mM, while total concentration of hHp is 100 mM. Protonation and complex formation constant of DAHK-Am are taken from: M. Sokolowska, A. Krezel, M. Dyba, Z. Szewczuk and W. Bal, Eur. J. Biochem. 2002, 269, 1323-1331.

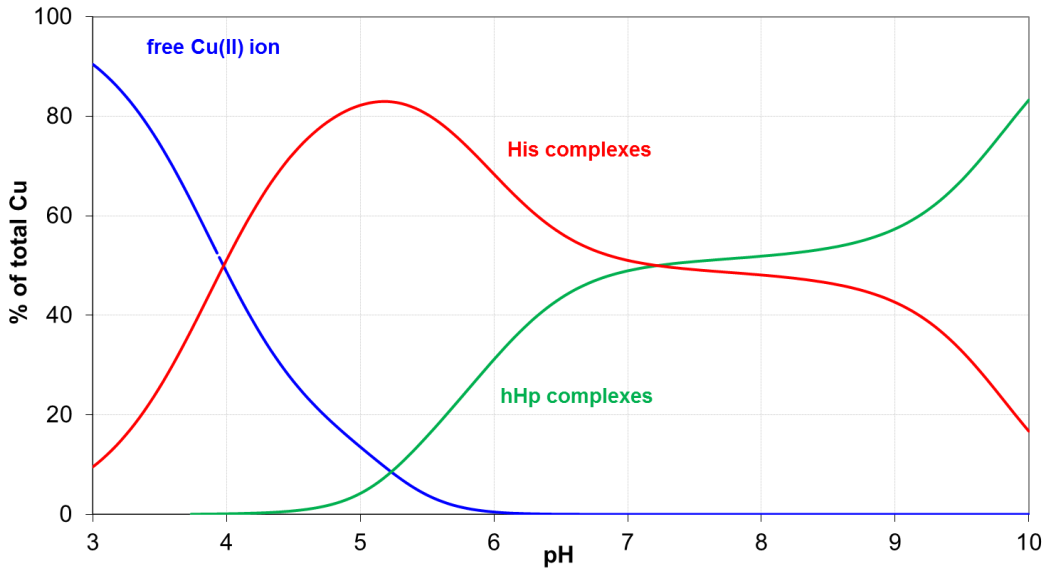



**Figure S16.** Competition between hHp and histidine, to bind Cu(II). The total concentration of both the metal and each ligand is 1 mM. Protonation and complex formation constant of His are taken from: G. Borghesani, F. Pulidori, M. Remelli, R. Purrello and E. Rizzarelli, *J. Chem. Soc.-Dalton Trans.* 1990, 2095-2100.