Does hemopressin bind metal ions \textit{in vivo}?

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SUPPLEMENTARY INFORMATION
Figure S1. Representative distribution diagram for the protonation equilibria of hHp; C°L = 1·10^−3 mol dm^−3.

Figure S2. Representative distribution diagram for the protonation equilibria of Ac-hHp-Am; C°L = 1·10^−3 mol dm^−3.
Figure S3. Representative distribution diagram for the protonation equilibria of RVD-hHp; \( C^\circ L = 1 \cdot 10^{-3} \text{ mol dm}^{-3} \).

Figure S4. Representative distribution diagram for the protonation equilibria of rHp; \( C^\circ L = 1 \cdot 10^{-3} \text{ mol dm}^{-3} \).
Cu(II) – PVNFKLSSH (hHP)

C₅₀H₇₉N₁₃O₁₂ = LH - MW = 1054.25 Da

Cu(II) – PVNFKFLSH (rHp)

C₅₃H₇₇N₃₃O₁₂ = LH - MW = 1088.25 Da
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.
a) pH = 5.0
   pH = 5.5
   pH = 6.0
   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

b) pH = 4.0
   pH = 5.0
   pH = 5.5
   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
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.; \(C^+_{Cu} = 0.5 \times 10^{-3}\) M; M/L ratio = 0.9.
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.; $C_{Cu}^*$ = 0.5·10^{-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).
Figure S9. Vis spectra, at different pH values, for the systems: a) Ni(II)/hHp; b) Ni(II)/rHp; c) Cu(II)/RVD-hHp.; $C_{Cu}^c = 0.5 \cdot 10^{-3}$ M; M/L ratio = 0.9.
Figure S10. CD spectra, at different pH values, for the systems: a) Ni(II)/hHp; b) Ni(II)/rHp; c) Ni(II)/RVD-hHp; $C_{\text{Ni}}^*=0.5\cdot10^{-3}$ 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 rHpc $c = 0.85$ 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.