Supplementary Information

for the

Branched Peptide with Three Histidines for the Promotion of Cu^{II} Binding in a Wide pH Range – Complementary Potentiometric, Spectroscopic and Electrochemical Studies

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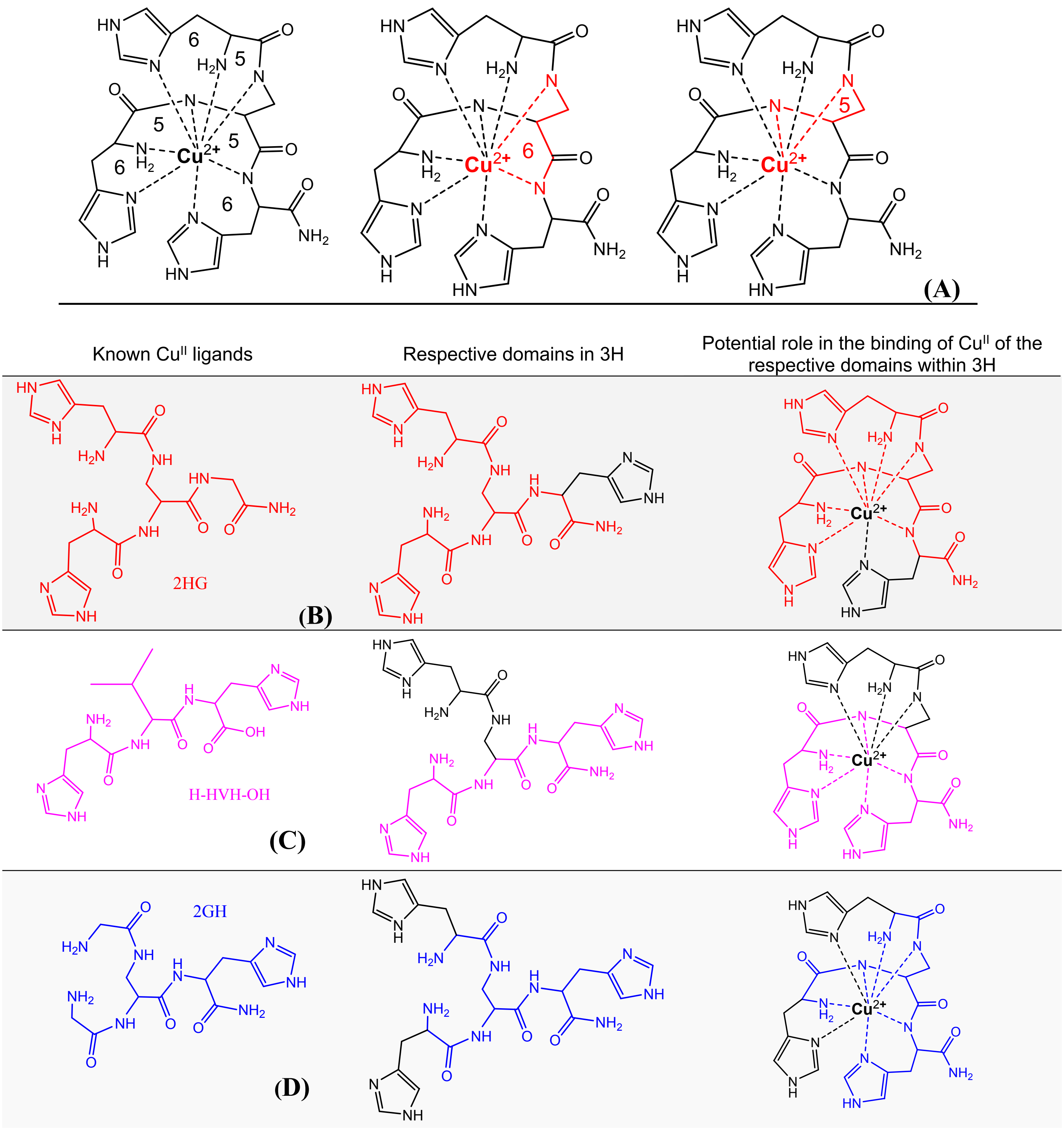

Figure S2. (A) Schematic structures showing possible interactions (marked with dashed lines) between 3H and Cu^{2+} ions and the available 5 and 6 member chelate rings. (B)-(D) Schematic presentation of ligands known from literature (column I), respective domains in 3H (column II) and their possible role in metal binding within 3H (column III). The listed structures include all possibilities that have been considered during the study and do not necessarily represent the viable structures. Dashed lines do not indicate simultaneous coordination of all marked N donor groups, rather represent competing groups for metal binding. Color codes are meant to emphasize analogies of these competing groups with already known and characterized ligand fragments of 3H.

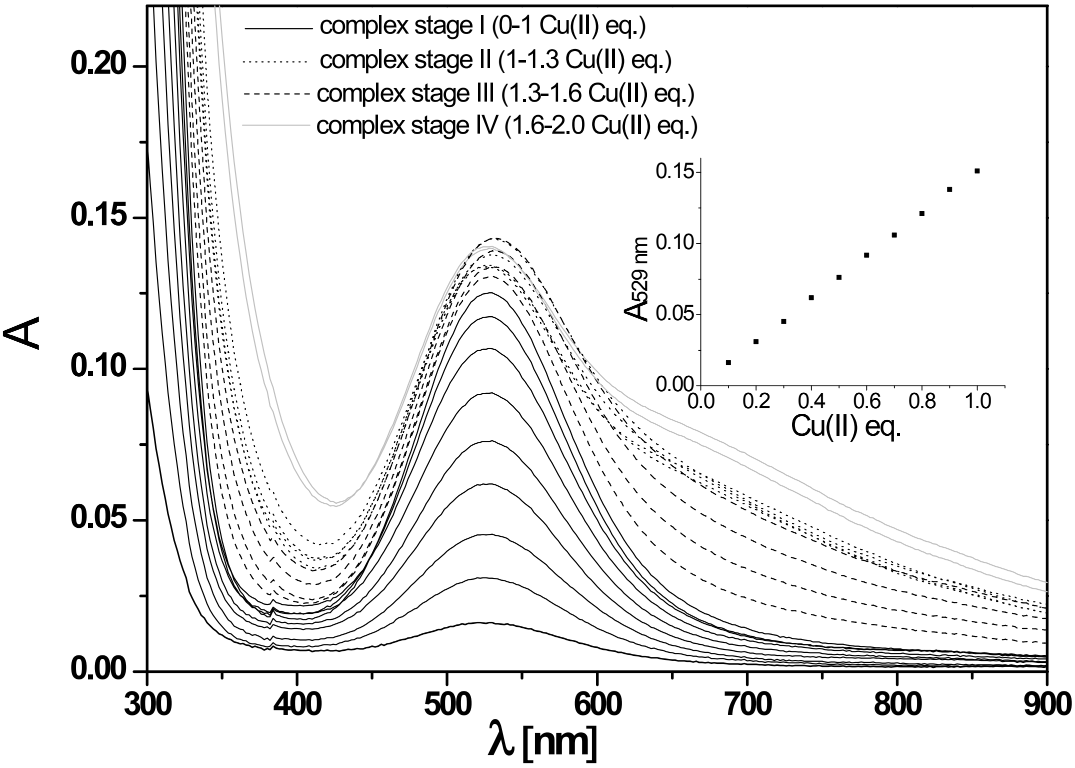

Figure S2. The UV-vis spectra showing the titration of 3H with Cu^{2+} at physiological pH (7.4) from 0.0 : 1.0 up to 2.0: 1.0 ratio (Cu^{II} : L).

Comment to Fig S2:

The results illustrate that form 0.0 : 1.0 up to 1.0 : 1.0 (Cu^{2+} : L) stoichiometry linear increase in the absorption band at 529 nm is observed. These data are in accordance with the ESI-MS spectra where only equimolar complexes in case of 0.4 : 1.0 and 0.7 : 1.0 (Cu^{II} : L) stoichiometry were detected.

In case of Cu^{II} : L ratio from 1.0 : 1:0 to 2.0 : 1.0 three different stages of complex formation were observed. Analysis with ESI-MS was not preformed due to poor solubility of complexes in stages II, III and IV (slight precipitation was observed). In case of equivalency of Cu^{II} higher than 2.0 : 1.0 strong precipitation was observed.

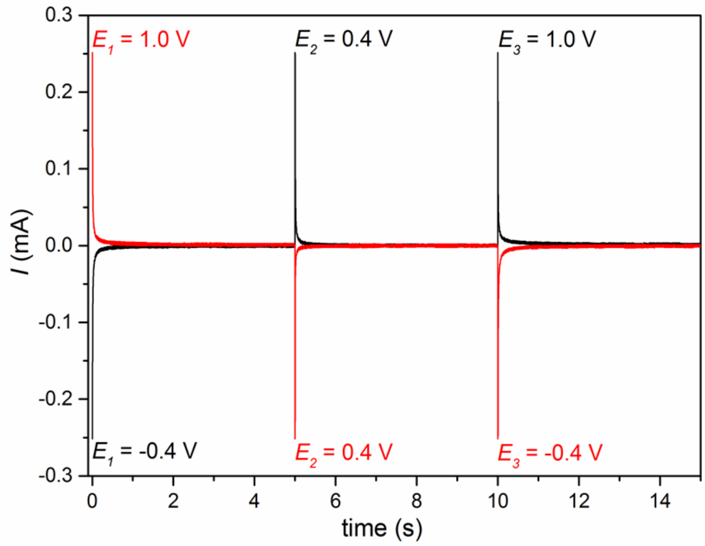

Figure S3. Chronoamperometry (CA) plot showing a forward and reverse sequence of three potentials (1.0, 0.4 and -0.4 V vs. Ag/AgCl) set consecutively to the GC working electrode for 5 seconds. Data indicate that the observed oxidation and reduction (assigned as the $Cu^{III/II}$ and $Cu^{II/I}$ redox transitions) involve equal number of electrons. Conditions are identical to those of CV scans at pH 6.98.

Table S1. Formal potentials derived from SWV for the $Cu^{III/II}$ redox transition of the Cu-3H system at different pH values.

pH	$E_{net}(Cu^{III/II})$ *vs.* (V)[a]	$\lvert I_{for}/I_{red}\rvert$ (μA)
7.04	0.858(2)	0.93
7.24	0.848(2)	0.94
7.37	0.843(2)	0.90
7.51	0.839(3)	0.96
7.64	0.837(3)	0.94
7.77	0.836(2)	0.91
7.96	0.832(3)	0.95
8.14	0.829(3)	0.91
8.42	0.820(3)	0.94
8.79	0.810(3)	0.92

[a] error in last digit is indicated in parenthesis

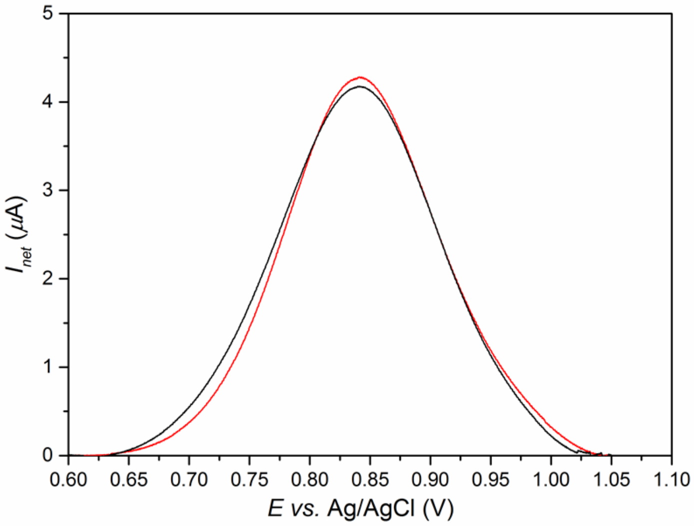

Figure S4. Background-corrected SWVs showing the $Cu^{III/II}$ response for Cu-3H at pH = 7.51(7.55) obtained from two independent measurements on two different samples using identical experimental settings. The average error in the current peak potential value is ~4 mV.