Electronic Supplementary Information
for

Dual mode of voltammetric studies on Cu(II) complexes of His2 peptides: phosphate and peptide sequence recognition

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1. Material and methods

Peptides Synthesis

All peptides were synthesized in the solid phase using Prelude automatic synthesizer (Protein Technologies) according to Fmoc protocol.[1] Synthesis was accomplished employing Fmoc amino acids (Novabiochem) on a TentaGel® S RAM resin (Rapp Polymere). Peptides were isolated by adding ice-cold diethyl ether (Chempur) and centrifuging. Crude peptides were purified by HPLC on a Breeze system (Waters) equipped with a semi-preparative column monitored at 220 nm. The mobile phase was a linear gradient of solution B [0.1% (v/v) TFA (Merck) in 90% (v/v) acetonitrile (Avantor)] in solution A [0.1% (v/v) TFA in water]. Identification and purity verification of lyophilized peptides were performed by mass spectrometry using Premier Q-Tof Premier mass spectrometer (Waters), exhibiting correct molecular masses.

UV-vis Spectroscopy

UV-vis spectra were recorded at room temperature (~25°C) on a Cary 60 spectrophotometer (Agilent) over a spectral range of 300-900 nm using a 1 cm-path quartz cuvette (Helma). UV-vis measurements were performed to determine $CuCl_2$ (Sigma-Aldrich), $Cu(NO_3)_2$ (Sigma-Aldrich), and $A\beta_{5-9}$ analogs stocks concentration and to perform the pH-metric titrations of Cu(II) complexes.

Stock solutions preparation:

All solutions were prepared in deionized water (the resistivity was 18 MΩ·cm) passed through an Arium mini Lab Water System (Sartorius).

The concentration of Cu(II) stock solutions (~50 mM) was estimated based on ε_{780} =12 $M^{-1}\cdot cm^{-1}$ of copper aqua complexes.[2] The concentration of peptide stock solutions was determined by $CuCl_2$ titration of the peptide in 20 mM HEPES (Sigma Aldrich) pH 7.4, monitoring absorbance at 600 nm (the typical d-d band of the complex), and assuming the 1:1 stoichiometry of the main Cu(II):peptide complex (Fig. S1).

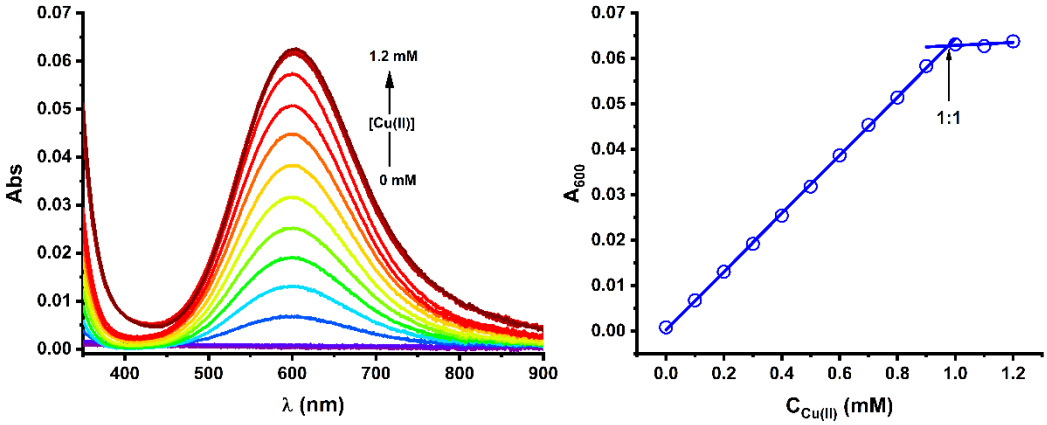


Fig. S1. UV-vis spectra registered during the titration of RHRSG-NH_2 (RHR) with Cu(II) carried out to determine the peptide concentration. The concentration of other peptides was determined similarly.

UV-vis titration with NaOH:

Small amounts of a concentrated solution of NaOH (Sigma-Aldrich) were added gradually to a reaction mixture in a cuvette (0.45 mM $CuCl_2$/0.50 mM peptide). The pH stability was checked using a SevenCompact pH meter (Mettler-Toledo) with an InLab Micro Pro combination pH electrode (Mettler-Toledo). The UV-vis spectra of reaction mixtures were collected at selected pH values.

pK values were calculated based on the pH dependence of absorbance at the given wavelength (A_λ) provided in Fig.2 according to the Hill equation below:

$$A_\lambda = \frac{p1 + p2 \times 10^{n\times(pH-pK)}}{1 + 10^{n\times(pH-pK)}} \qquad \text{eqn (S1)}$$

where p1 and p2 are the fitted absorbance values for 0% and 100% of the formed complex, respectively, and n is the Hill factor.

Electrochemical Experiments

Voltammetric measurements were performed using CHI 1030 potentiostat (CH Instruments) in a three-electrode arrangement: Ag/AgCl as a reference electrode (MINERAL), glassy carbon (GCE, 3 mm diameter, BASi) as a working electrode, and platinum wire (MINERAL) as an auxiliary electrode. Prior to each measurement, GCE was polished to a mirror-like surface with alumina suspensions (1.0 μm, 0.3 μm) on a polishing cloth (Buehler), followed by 1 min ultrasonication in deionized water. All electrochemical experiments were carried out in 100 mM KNO_3 (Sigma-Aldrich) at room temperature (~25°C) under an argon atmosphere.

The concentration of the His2 peptides in samples tested using voltammetric techniques was

0.50 mM, whereas $Cu(NO_3)_2$ 0.45 mM (1.0:0.9 molar ratio) to avoid $Cu(OH)_2$ precipitation and its adsorption on GCE surface during experiments. Stock solutions of NaCl (Sigma-Aldrich), Na_2SO_4 (Fluka), CH_3COONa (Sigma-Aldrich), and NaH_2PO_4 (Sigma-Aldrich) were prepared daily in deionized water, adjusting the pH to 7.4 with NaOH. In selectivity tests, a stock of mentioned salts was added to the Cu(II)/peptide solution to obtain 10-fold excess of anions over the initial peptide concentration. The strict pH control was maintained by adding, if required, small aliquots of concentrated KOH or HNO_3 solutions.

The applied techniques were Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV). In the line with our previous studies,[3,4] during CV measurements, scan rate (v) was 100 mV/s, whereas the following parameters were used in DPV: a pulse amplitude of 0.05 V, a pulse width of 0.1 s, a sample width of 0.005 s, and a pulse period of 1s.

Chemometric analysis

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to evaluate the potential of the studied systems for the discrimination of peptides with a different type of residue in the proximity of His2 in their sequences. Before chemometric modeling, differential pulse voltammetric curves recorded by scanning the potential between 0.5 V and 1.4 V were arranged in the data matrices (samples x currents). The Savitzky-Golay first derivative and mean centering were used as data preprocessing steps. Ward method was applied for combining clusters in HCA. The chemometric analysis was performed in Solo version 9.0 (Eigenvector Research Inc.), while figures were generated using Origin (OriginLab Corporation).

2. Spectroscopic characterization of Cu(II)-His2 complexes

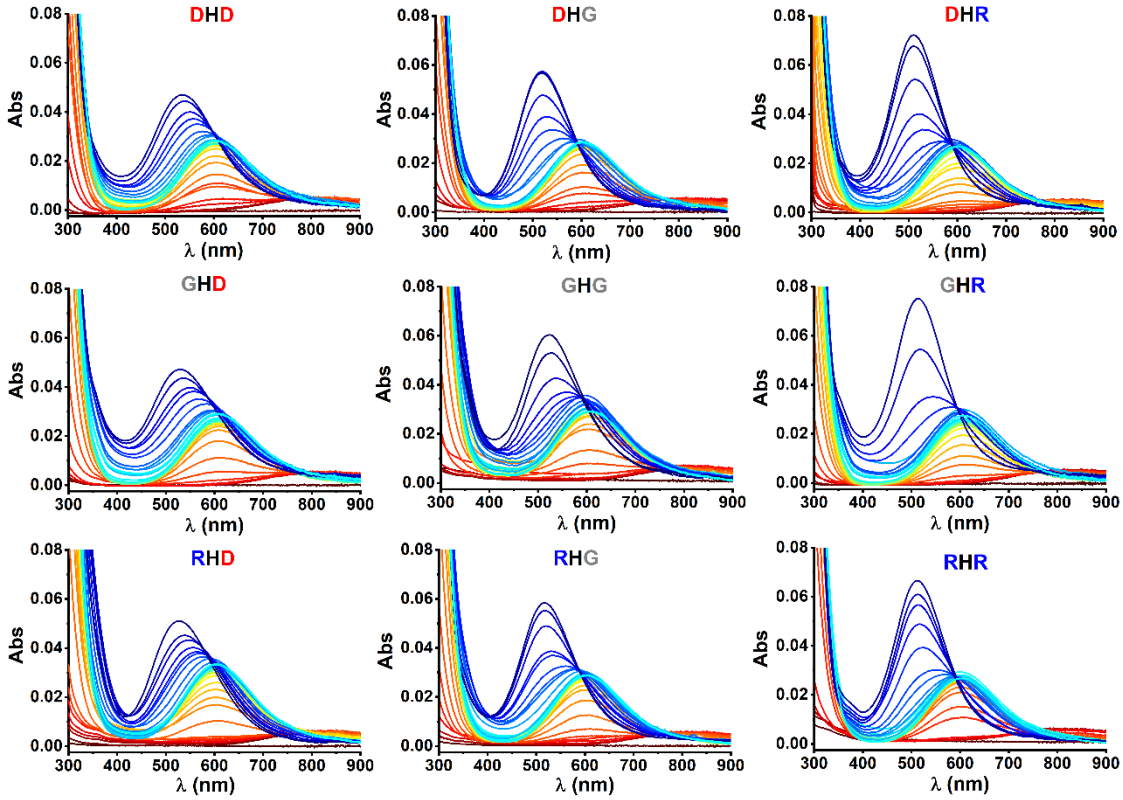


Fig. S2. UV−vis spectra registered during the titrations of 0.5 mM peptide/0.45 mM Cu(II) with NaOH within pH range 2.5−11.7 coded with colors from red (acidic pH) to blue (basic pH).

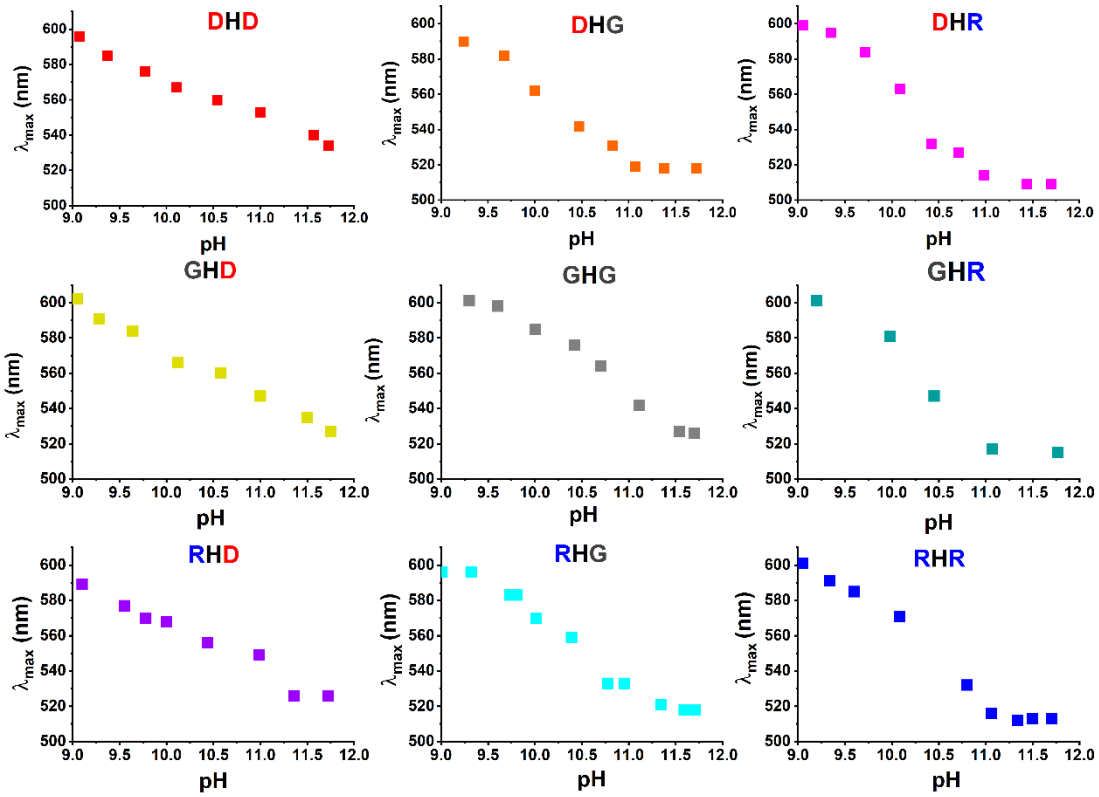


Fig. S3. The pH dependence of wavelengths at maximum absorbance associated with the formation of the $3N+OH^-$ complex of Cu(II) for all studied His2 peptides.

Table S1. Spectroscopic parameters of Cu(II) complexes of the His2 peptides at pH 11.7.

Complex	pH 11.7	
	λ_{max}	ε ($M^{-1}cm^{-1}$)
Cu(II)-RHR	513	148
Cu(II)-DHD	539	104
Cu(II)-DHG	519	126
Cu(II)-DHR	511	160
Cu(II)-RHG	518	130
Cu(II)-RHD *Cu(II)-Aβ$_{5-9}$*	529	116
Cu(II)-GHR	515	167
Cu(II)-GHD	531	105
Cu(II)-GHG	525	134

3. Voltammetric data

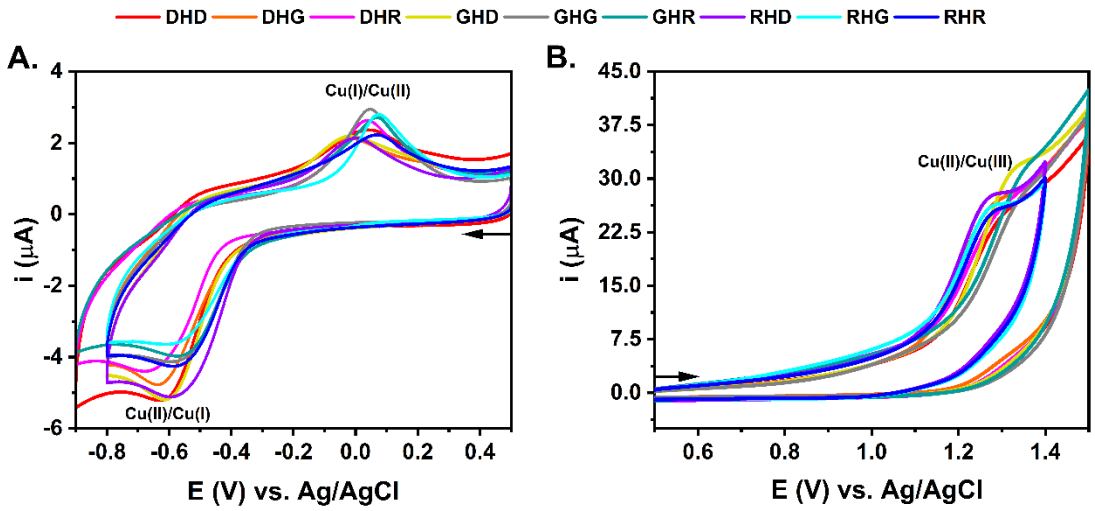


Fig. S4. CV curves obtained for Cu(II) complexes of the His2 peptides (0.9:1.0 ratio) scanned towards negative (A.) and positive (B.) potential recorded in 100 mM KNO_3, pH 7.4. Scan rate v = 100 mV/s.

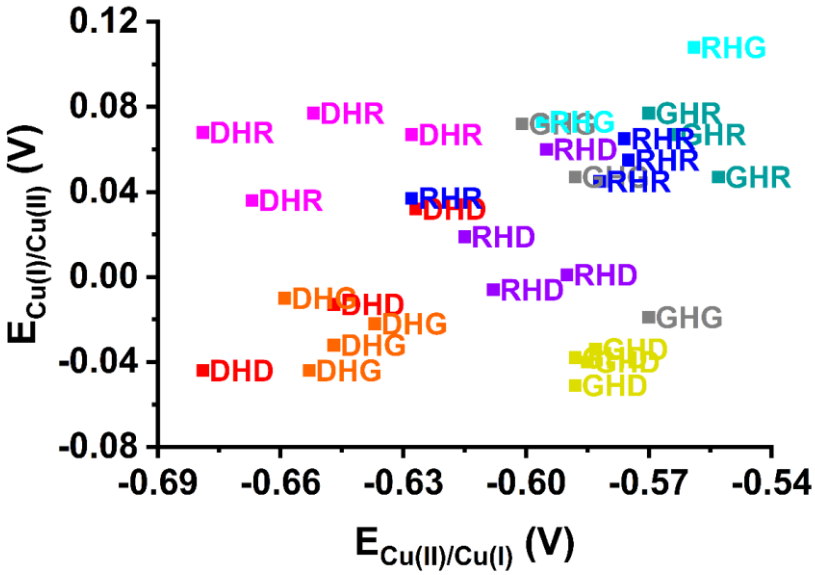


Fig. S5. Cu(II)/Cu(I) reduction and Cu(I)/Cu(II) oxidation potential for Cu(II) complexes of the His2 peptides from CV curves showed in Fig. S4A.

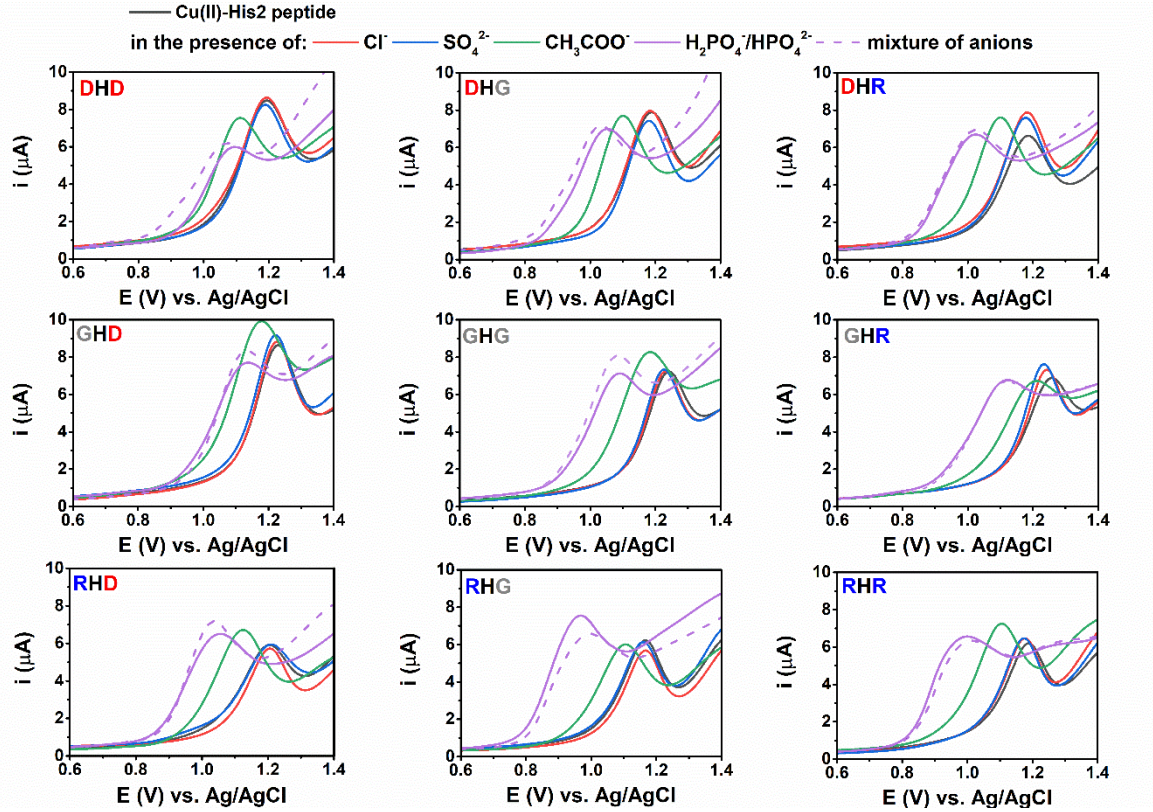


Fig. S6. DPV curves obtained for 0.45 mM Cu(II)-His2 peptide complexes recorded in 100 mM KNO_3, pH 7.4, in the presence of 10 mM of selected anions.

4. Chemometric modeling

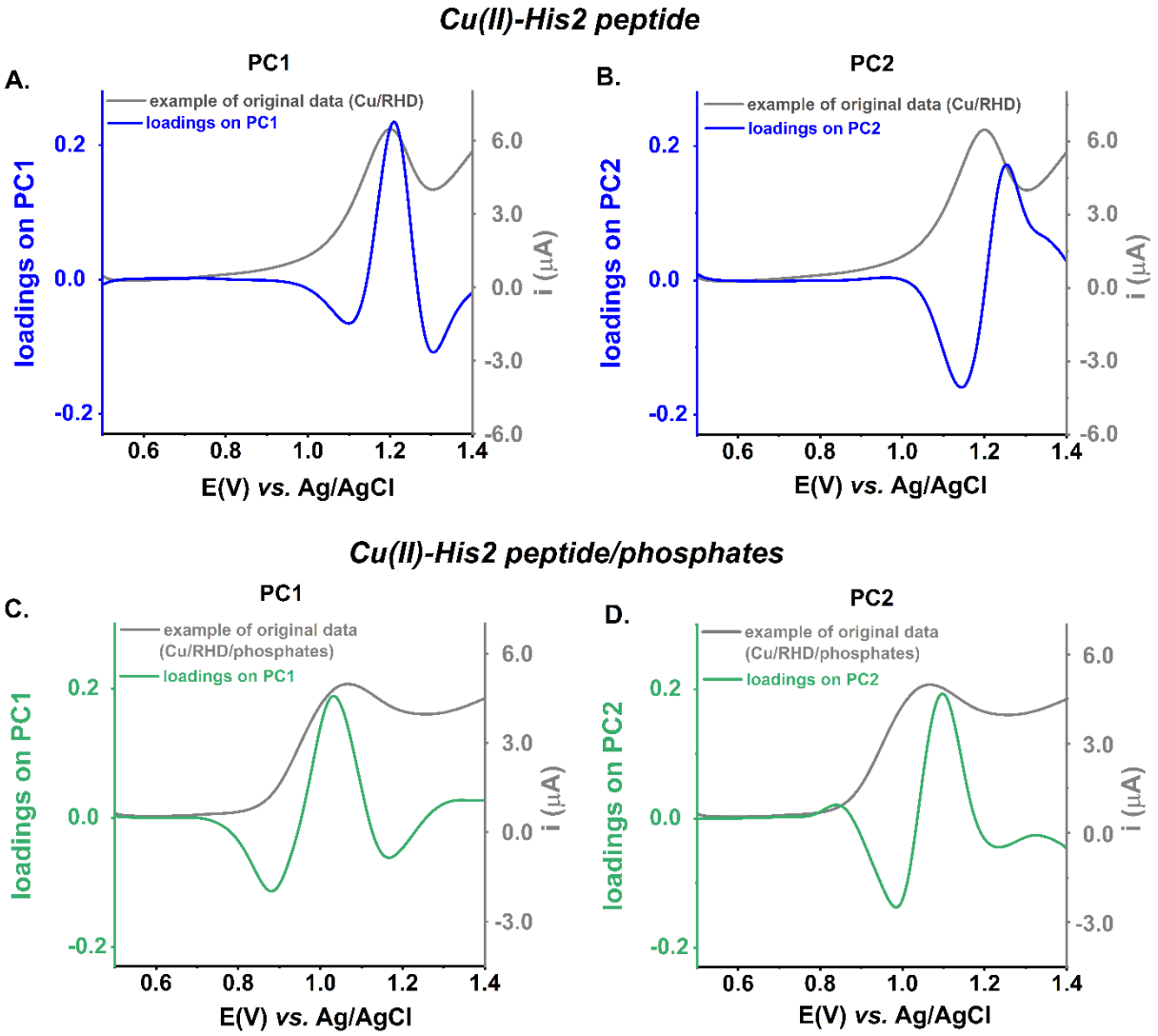


Fig. S7. Loadings plots on PC1 (A, C) and PC2 (B, D) showing the input of each variable to a respective principal component (PC). They were obtained based on voltammetric data recorded for the binary system Cu(II)-His2 peptide (A, B) and ternary system Cu(II)-His2 peptide/phosphates (C, D). The respective PCA score plots are provided in Fig. 6A, B. The loadings (blue or green solid lines) are overlapped by examples of original data (solid grey lines).

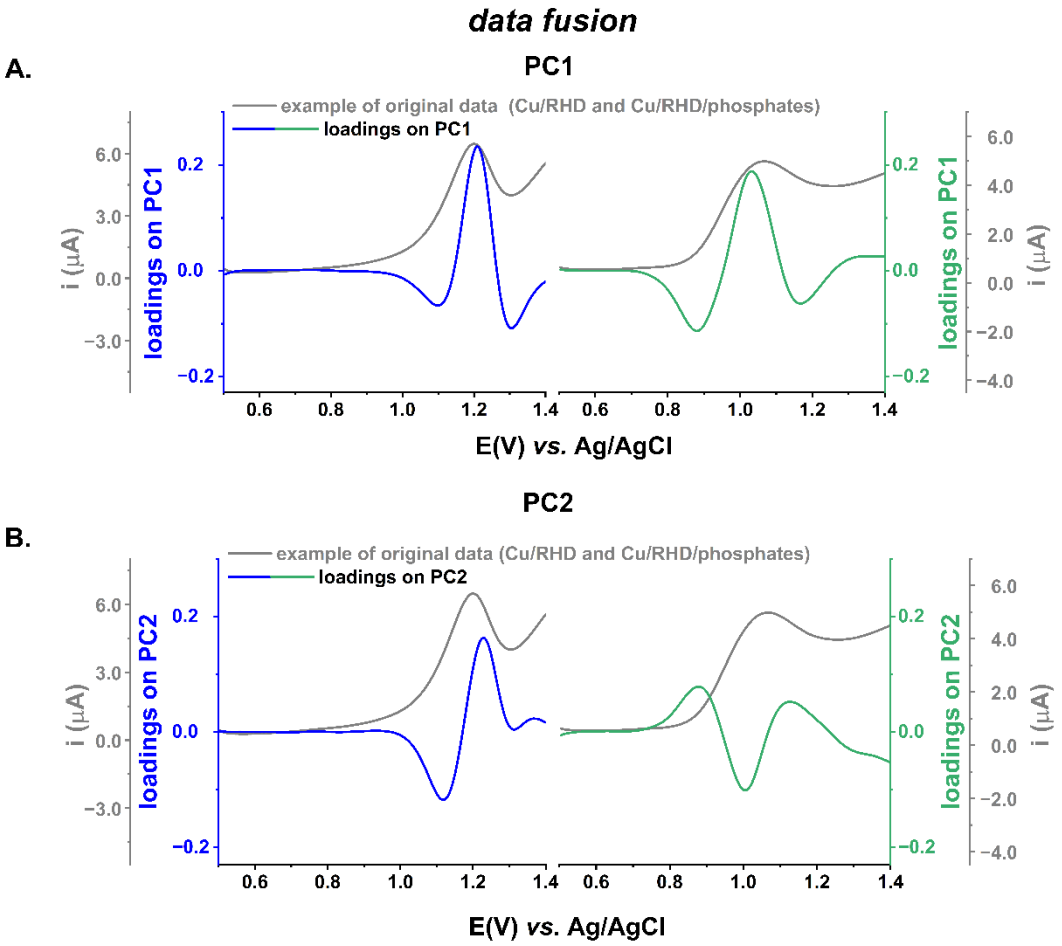
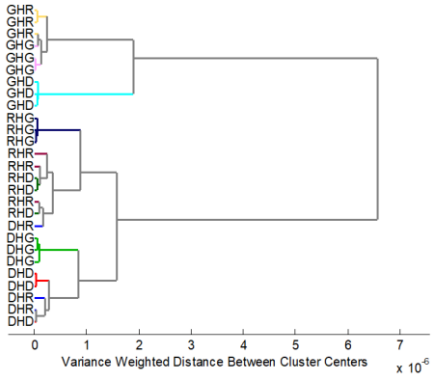
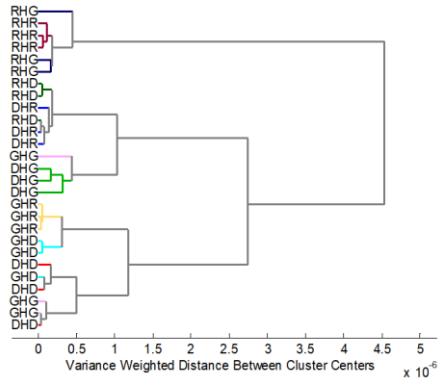


Fig. S8. Loadings plots on PC1 (A) and PC2 (B) showing the input of each variable to the respective principal component (PC). They were obtained based on data fusion upon the integration of voltammetric data recorded for the binary system Cu(II)-His2 peptide and ternary system Cu(II)-His2 peptide/phosphates. The respective PCA score plots are provided in Fig. 6C. The loadings (blue or green solid lines) are overlapped by examples of original data (solid grey lines).

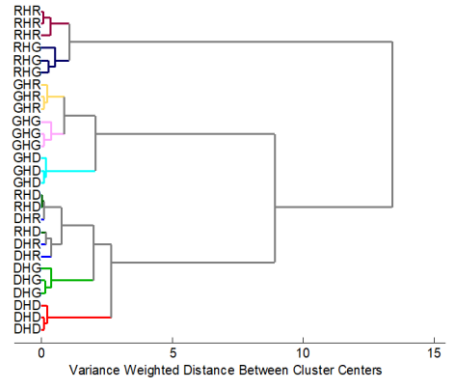
A.



B.



C.



D.

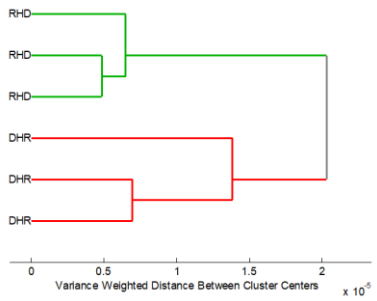


Fig. S9. Hierarchical Cluster Analysis (HCA) showing the differentiation of the His2 peptides depending on the origin of voltammetric data. A) binary system Cu(II)-His2 peptide, B) ternary system Cu(II)-His2 peptide/phosphates, C) data fusion (upon the integration of data from binary and ternary systems). The best differentiation was observed for data fusion with three main groups of His2 peptides: (i) Gly1 peptides, (ii) Asp peptides without Gly1, and (iii) Arg1 peptides without Asp (see corresponding data in Fig. 6). For data fusion, RHD and DHR peptides could be discriminated between by clustering based on current values (D).

5. References

1 W. C. Chan and P. D. White, in *Fmoc Solid Phase Peptide Synthesis : A Practical Approach*, eds. W. C. Chan and P. D. White, Oxford University Press, New York, 2000, pp. 41–76.

2 A. Santoro, G. Walke, B. Vileno, P. P. Kulkarni, L. Raibaut and P. Faller, *Chem. Commun.*, 2018, **54**, 11945–11948.

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