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Electronic Supplementary Information for

Copper(II) complex of N-truncated amyloid-β peptide bearing a His-2 motif as a potential receptor for phosphate anions

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1. Experimental

Reagents and materials

Fmoc amino acids were purchased from Novabiochem. The TentaGel S RAM resin was obtained from RAPP Polymere. Diethyl ether was purchased from Chempur. Acetonitrile and trifluoroacetic acid (TFA) were received from Avantor and Merck, respectively. Potassium nitrate, nitric acid, hydrochloric acid, potassium hydroxide, sodium hydroxide, sodium chloride, sodium acetate, copper(II) nitrate hydrate, copper(II) chloride, adenosine 5′-monophosphate sodium salt (AMP), adenosine 5′-triphosphate disodium salt (ATP), hexaammineruthenium(III) chloride and adenine were received from Sigma-Aldrich. Dimethylformamide, monosodium phosphate and were purchased from Carl Roth. Sodium sulfate anhydrous were supplied by Fluka. All solutions used in electrochemical measurements were prepared with deionized water (resistivity 18 MΩcm^{-1}) passed through an arium® mini Lab Water System (Sartorius). The glassware utilized in the experiments was rinsed with 6 M HNO_3 followed by deionized water before use, to avoid metal ions contamination.

Peptide synthesis

The $A\beta_{5-9}$ peptide (Arg-His-Asp-Ser-Gly-NH_2) was synthesized on a Prelude™ peptide synthesizer (Protein Technologies, Inc. Tucson, AZ), according to the Fmoc strategy.[1] The peptide crude was purified by HPLC on a Breeze system (Waters) equipped with a Vydac semi-preparative column (5mm particle size, 10×250 mm). The mobile phase was a linear gradient of solution B (0.1% (v/v) TFA/ 90% (v/v) acetonitrile/ 9,9% (v/v) water) in solution A (0.1% (v/v) TFA/99,9% (v/v) water). The purity of the lyophilized peptide was verified by a Q-Tof Premier mass spectrometer (Waters), exhibiting correct molecular masses (see Figure S1).

UV–Vis spectroscopy

UV-Vis spectra were recorded at 25°C on a Cary 60 spectrophotometer (Agilent), over the spectral range 300–900 nm, using the 1 cm-path-length quartz cuvettes. UV-Vis measurements were performed to establish copper stock concentration, using absorption coefficient ε_{780}=12 M^{-1}cm^{-1}, to determine the peptide stock concentration by titration of peptide solution by the copper stock solution of known concentration, and to monitor the formation of peptide binary and ternary complexes.

[1] W. Chan and P. D. White, Fmoc Solid Phase Peptide Synthesis: A Practical Approach, Oxford University Press, Oxford 2000.

Circular Dichroism spectroscopy

Circular dichroism spectra were recorded at 25°C on a JASCO J-810 spectropolarimeter. Measurements were done in the 850−240 nm range with a 1 cm path quartz cuvette. $CuCl_2$ solution was added to the samples to reach 1:0.9 peptide-to-metal ratio. Titrations were performed in water by adding small amounts of concentrated NaH_2PO_4/Na_2HPO_4 or Na_2SO_4 or CH_3COONa solution. pH stability was checked for each titration point and adjusted with small amounts of concentrated NaOH/HCl solutions. Dilutions were included into the calculations**.**

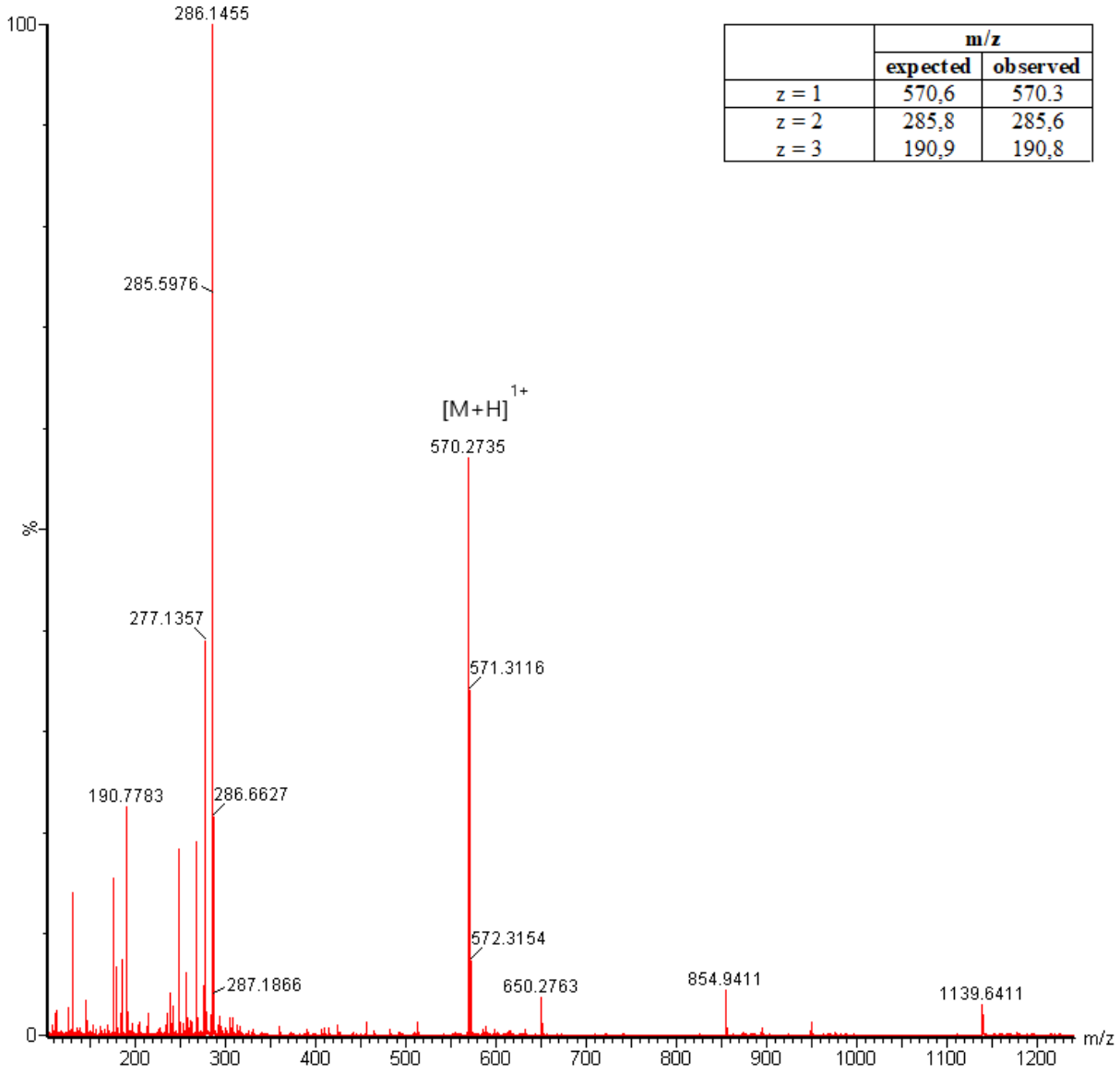
Voltammetry

The electrochemical experiments were done in a three-electrode arrangement with a glassy carbon electrode (GCE, BASi, 3 mm diameter) as the working electrode, an Ag/AgCl electrode as the reference (separated from the working solution by an electrolytic bridge filled with 96 mM KNO_3/4 mM HNO_3) and a platinum wire as the counter electrode. The potential of the reference electrode was calibrated using ruthenium electrode (the formal potential of this process was E_f = −173 mV). The GC electrode was sequentially mechanically polished with 1.0 and 0.3 μm alumina powder on a Buehler polishing cloth. In order to remove the remaining powder, the electrode was sonicated for 1 min in deionized water.

All electrochemical measurements were carried out in 96 mM KNO_3 solution containing 4 mM HNO_3 in pH 7.4. The pH was adjusted with submicroliter volumes of concentrated KOH/HNO_3 solutions. The pH was closely controlled before, during and at the end of each voltammetric measurement using SevenCompact pH-meter (Mettler-Toledo) with InLab Micro Pro micro combination pH electrode (Mettler-Toledo). The concentration of peptide was 0.5 mM, whereas $Cu(NO_3)_2$ solution was added to the samples to obtain 1:0.9 peptide-to-metal ratio (thus preventing the interference from metal cations not bound to the peptide). In the next step, NaCl, Na_2SO_4, CH_3COONa, NaH_2PO_4/Na_2HPO_4, AMP, ATP or adenine were added to the Cu(II)-peptide complex. Oxygen was removed from the sample solution by passing argon for 5 min before all measurements and argon blanket was maintained over the solution during the experiments carried out at 25°C.

Cyclic (CV) and differential pulse (DPV) voltamperometry experiments were performed using the CHI 1030 potentiostat (CH Instrument, Austin, USA). During CV measurements, the scan rate (v) was 0.1 V/s, whereas the following parameters were used in DPV: pulse amplitude 50 mV and pulse width 100 ms.

2. Figures



	m/z	
	expected	observed
z = 1	570,6	570.3
z = 2	285,8	285,6
z = 3	190,9	190,8

Figure S1. ESI-MS (+) spectrum of the purified $A\beta_{5\text{-}9}$. The comparison of expected and observed m/z signals for different z values are given in the inset.

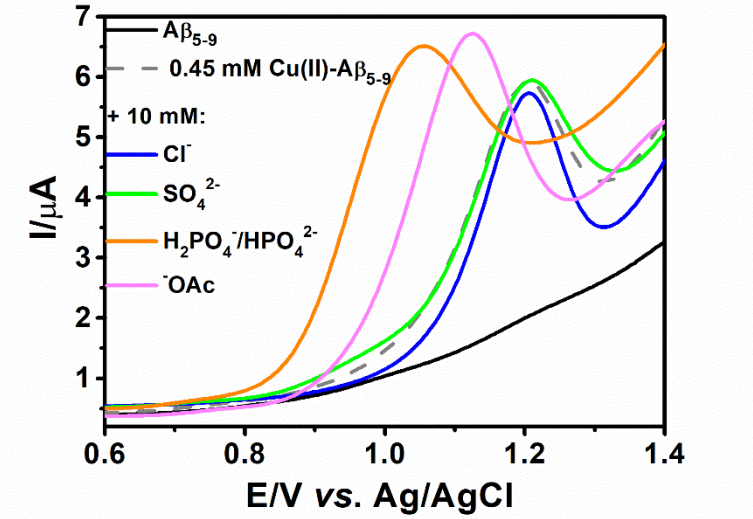


Figure S2. DPV obtained for Cu(II) complexes with $A\beta_{5-9}$ (0.9:1.0 molar ratio) at pH 7.4 recorded in 96 mM KNO_3/4 mM HNO_3 after addition 10 mM of selected anions.

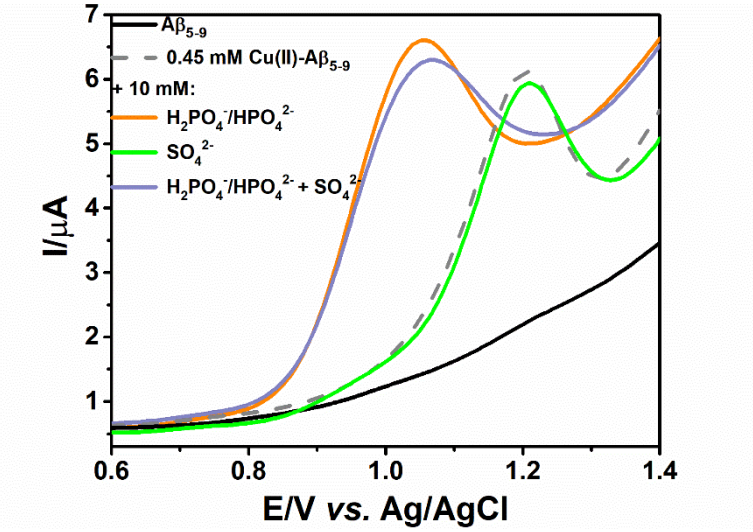


Figure S3. DPV obtained for Cu(II) complexes with $A\beta_{5-9}$ (0.9:1.0 molar ratio) at pH 7.4 recorded in 96 mM KNO_3/4 mM HNO_3 after adding 10 mM of phosphate and sulfate anions (separately and in the mixture (1:1 molar ratio)).

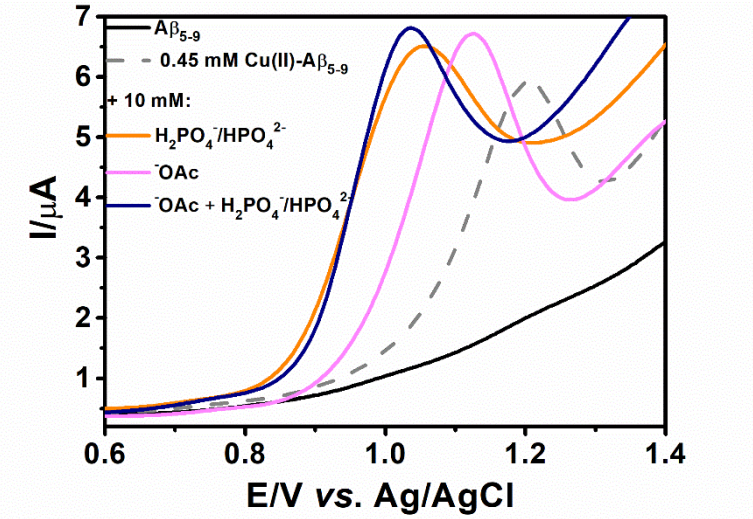


Figure S4. DPV obtained for Cu(II) complexes with $A\beta_{5-9}$ (0.9:1.0 molar ratio) at pH 7.4 recorded in 96 mM KNO_3/4 mM HNO_3 after adding 10 mM of phosphate and acetate anions (separately and in the mixture (1:1 molar ratio)).

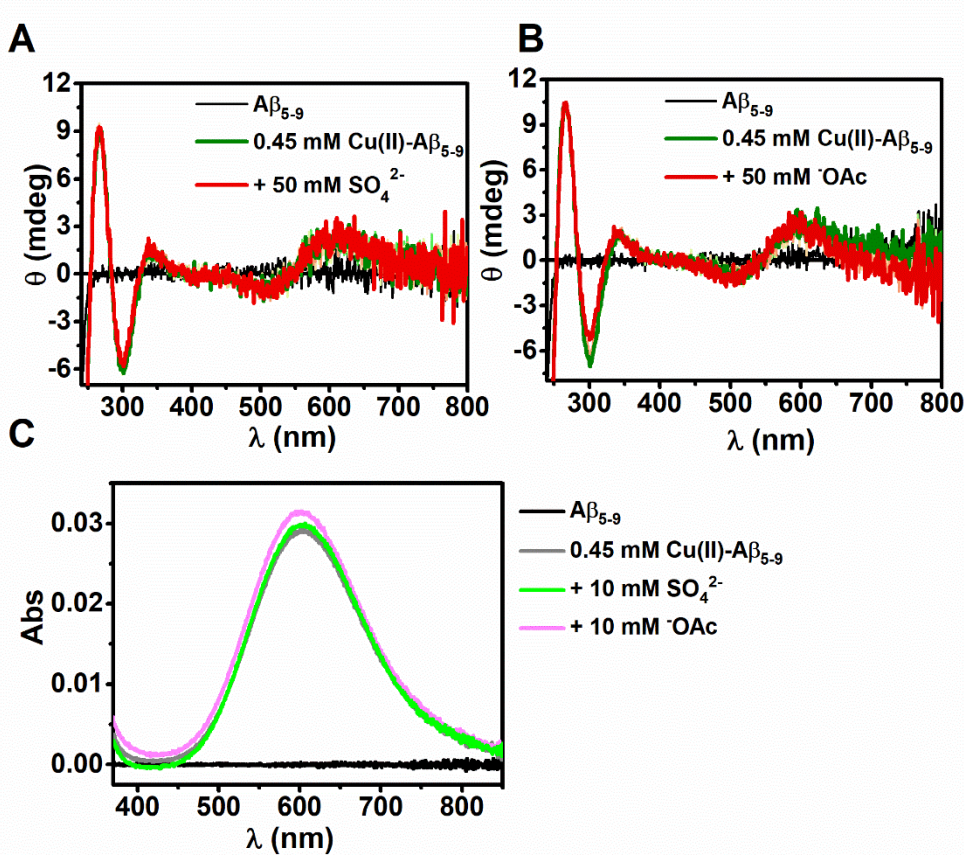


Figure S5. CD spectra of 0.5 mM $A\beta_{5-9}$ and 0.45 mM $CuCl_2$ titrated with Na_2SO_4 (A) and CH_3COONa (B), pH 7.4. (C) UV-Vis spectra obtained for Cu(II) complexes with $A\beta_{5-9}$ (0.9:1.0 molar ratio) after addition 10 mM of sulfate or acetate anions at pH 7.4 in 96 mM KNO_3/4 mM HNO_3. The black line represents apopeptide.