Supplementary Information for

Mass spectral studies reveal the structure of Aβ1-16-Cu^{2+} resembling ATCUN motif.

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Experimental Details

Materials-

Aβ1-16 peptide was purchased from USV chemicals, India. $CuSO_4 \cdot 5H_2O$ and $NiSO_4 \cdot 6H_2O$ were purchased from SRL, Mumbai, India. Ethanol was purchased from Molychem, India. Water used for all experiments was purified by Sartorius arium 611 water purification system with resistivity 18.2 MΩ x cm.

Peptide sample preparation-

Metal bound peptide samples were prepared by incubating respective metal ion with Aβ1-16 peptide overnight at $4^{O}C$.

Spectrophotometric analysis-

All spectrophotometric measurements were carried out on JASCO V-630 series.

Mass Spectrometry-

All mass spectral measurements were carried out on an AB Sciex 4800 MALDI-TOF/TOF mass spectrometer (AB Sciex, Framingham, MA) linked to 4000 series explorer software (v.3.5.3). Samples were mixed with CHCA (5mg of α cyano hydrocinnamic acid / ml of 50% ACN water + 1% TCA) matrix in 1:1 ratio and spotted on a MALDI target plate. Spots were then allowed to dry and loaded on MALDI. Mass spectra were recorded within a mass range from 800 to 4000 Da, using a Nd:YAG 355 nm laser. The acceleration voltage used was 20 kV and extraction voltage was 18 kV. The instrument was calibrated using 6 peptide standard mix that was purchased from AB Sciex. MS spectra were obtained in reflector mode using 900 laser shots with 4000 laser intensity. Further MS/MS spectra were acquired with a total accumulation of 1500 laser shots and collision energy of 2 kV.

Experimental Methods-

DEPC Assay

Two sets of 40 μM Aβ1-16 peptide solutions in ammonium acetate buffer (pH 7.0) were incubated with various concentrations of Cu^{2+} (prepared from $CuSO_4 \cdot 5H_2O$). One set was then treated with a 1.2 M excess ethanolic solution of DEPC for 30 min at 30 ºC, while the other set was treated just with ethanol. The reaction was terminated using excess Tris-HCl buffer (pH 8.3). The optical density of the DEPC-containing tubes was measured at around 240 nm using respective control tubes containing ethanol.

DEPC assay using mass spectrometry

Ten molar excess of DEPC was added to 100μM Aβ1-16 peptide in the presence and absence of 100μM Cu^{2+}in ammonium acetate buffer (pH-7) at 30^{o}C for 15 min and reaction was terminated by 50mM Tris-HCl (pH-8.3). The products were analyzed

using mass spectrometer. For nickel displacement assay, twenty molar excess of DEPC was added to 100μM Aβ1-16-Cu^{2+} complex in the presence of 100μM Ni^{2+} in ammonium acetate buffer (pH-7) at 30^{o}C for 30 min and reaction was terminated by 50mM Tris-HCl (pH-8.3). The product obtained was then analyzed using mass spectrometer.

Full Scale Mass Spectra-

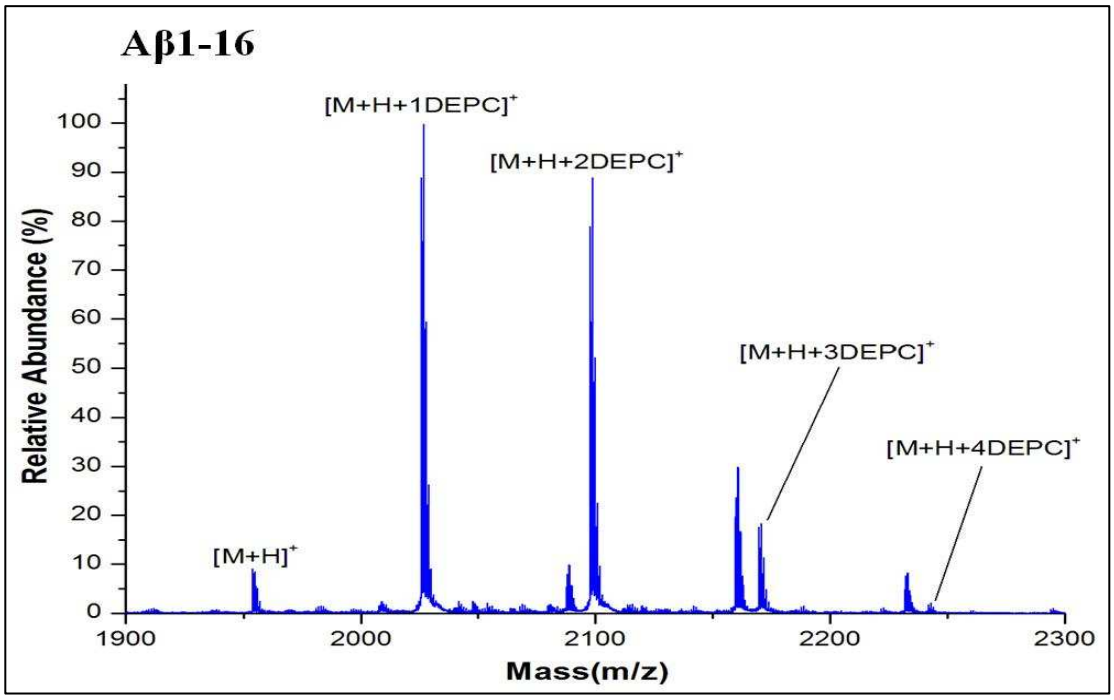

Figure S1- Mass spectrum of Aβ1-16 peptide.

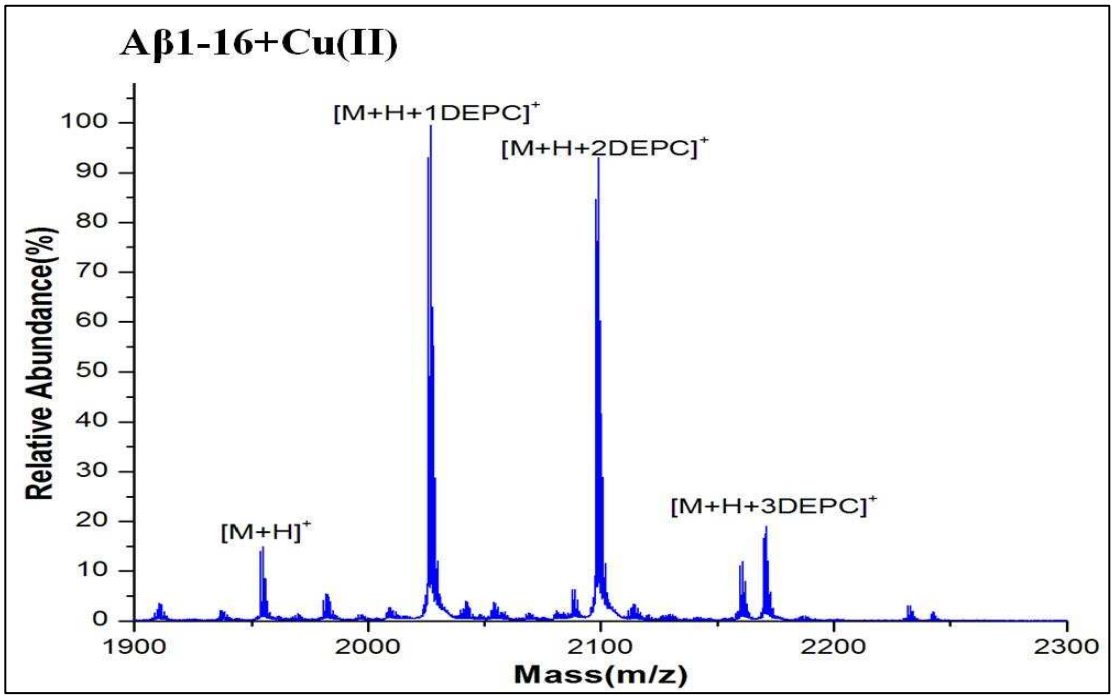

Figure S2- Mass spectrum of Aβ1-16-Cu(II) complex.

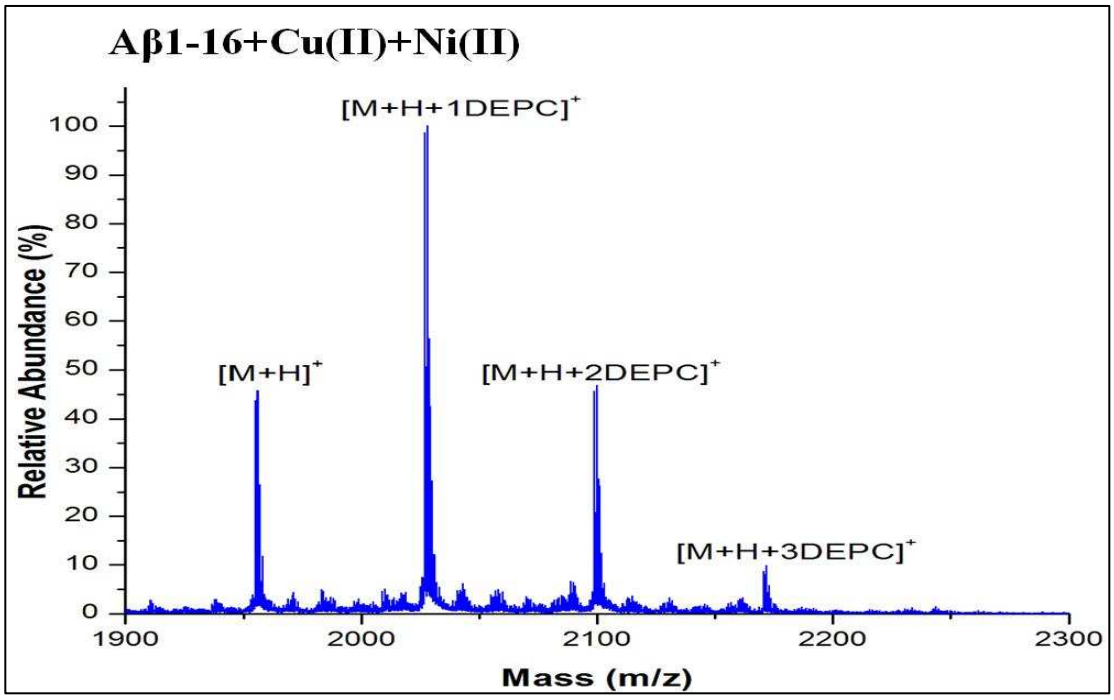

Figure S3- Mass spectrum of Aβ1-16-Cu(II)-Ni(II) complex.

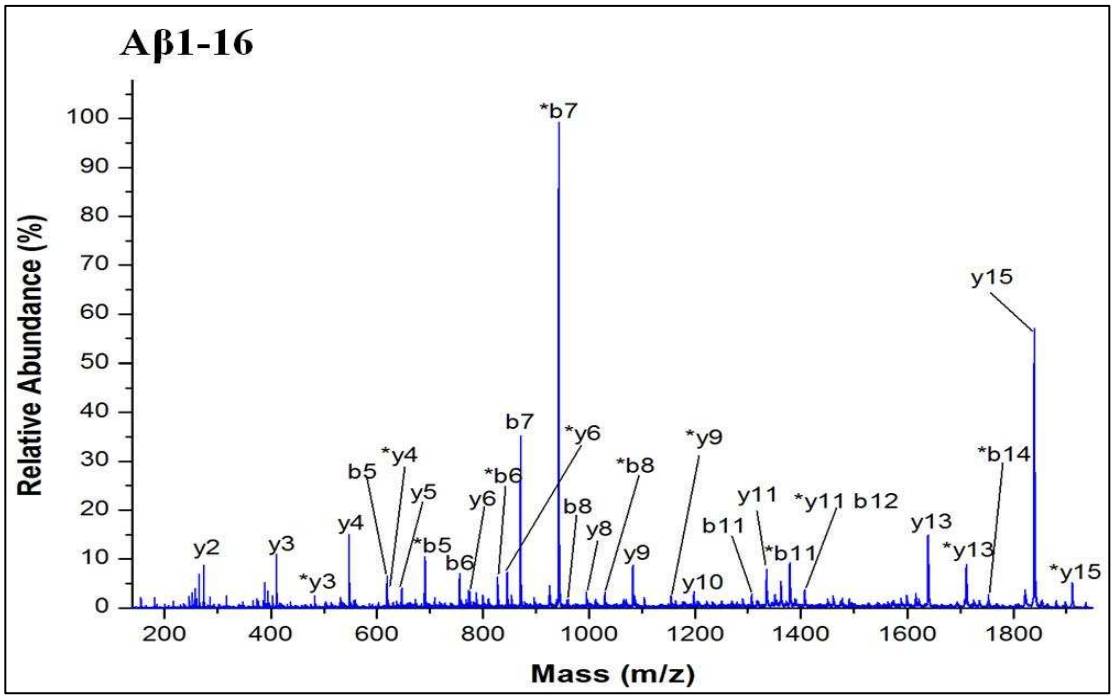

Figure S4- MS/MS of Aβ1-16 peptide.

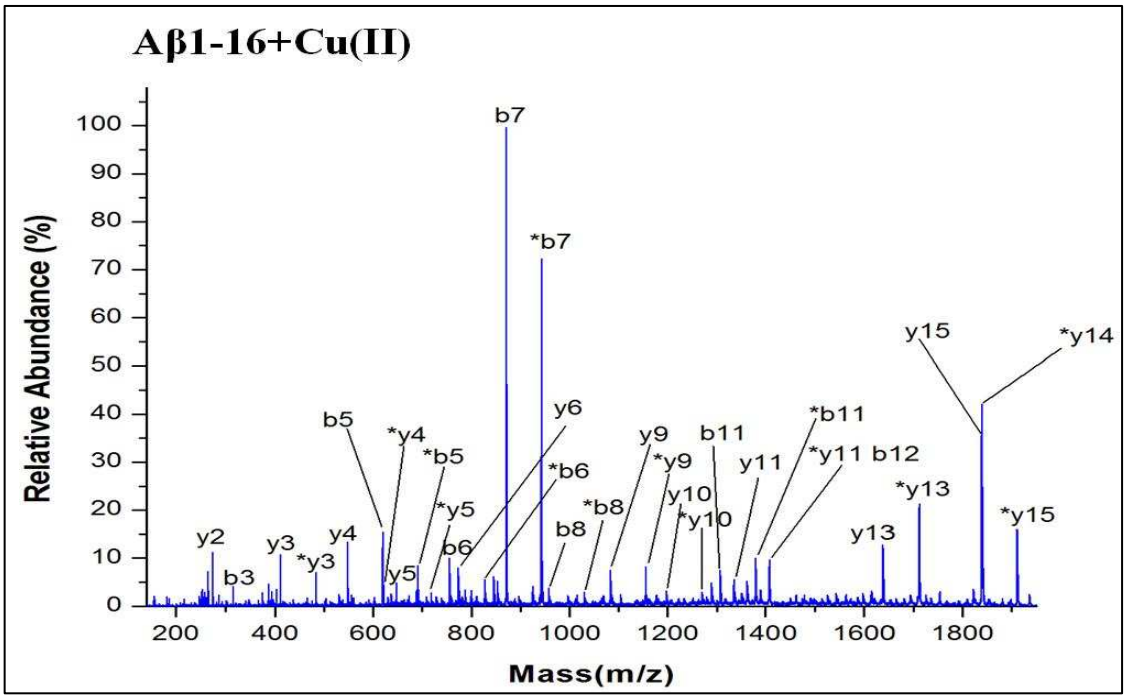

Figure S5- MS/MS of Aβ1-16-Cu(II) complex.

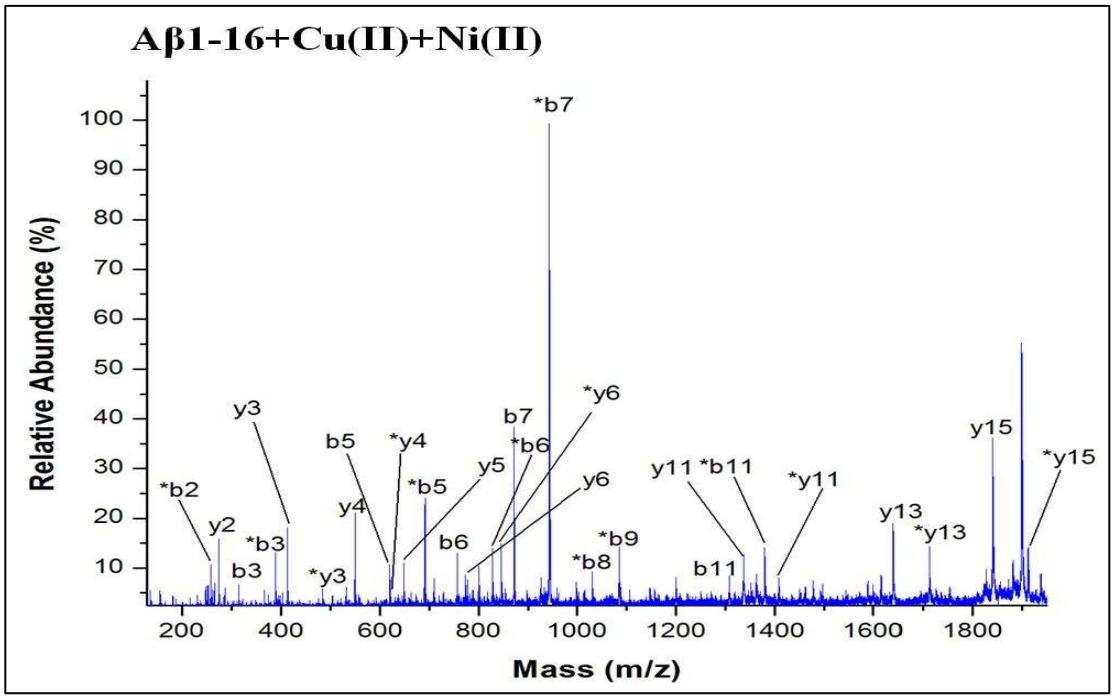

Figure S6- MS/MS of Aβ1-16-Cu(II)-Ni(II) complex.