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Supporting information

Copper Induced Spin State Change of Heme-Aβ associated with Alzheimer's disease

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Experimental Details

Materials and methods

All the reagents were of highest grade commercially available and were used without any further purification. Aβ(1-40) peptide (sequence: Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val) used in this study was purchased from Ontores, China with >95 % purity (abbreviated as Aβ henceforth). Hemin, Copper sulfate and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) Hepes buffer were purchased from Sigma-Aldrich.

Absorption spectra were recorded by an UV-vis diode array spectrophotometer (Agilent 8453). Resonance Raman (rR) spectroscopy data were obtained using a Trivista 555 spectrograph (Princeton Instruments) and 413.1 nm excitation from a Kr^+ laser (Coherent, Sabre Innova SBRC-DBW-K). Raman shifts were calibrated with naphthalene and indene. The wavenumber accuracy was ± 1 cm^{-1} for well-defined peaks. The laser power was varied between 10-15 mW. The high frequency region of rR spectra for all the samples were recorded with an Olympus 1X73 microscope (Olympus, Tokyo, Japan) using a lens with 10× magnification (UPlanFLN, Olympus). The rR data were acquired using a drop of the liquid sample supported on a wire loop, held over the microscope lens. EPR samples were run at 77 K in a liquid nitrogen finger dewar and EPR spectra were obtained by Jeol (JES FA200) spectrophotometer with the following parameters for the high-field region: modulation width, 10 Gauss; amplitude, 250; time constant, 30 ms; power, 1 mW; frequency, 9.12 GHz.

In order to eliminate the effect of aggregation or oligomerization Aβ was monomerised using the following protocol.[1] The synthetic Aβ peptide was dissolved in 10 % (w/v) NH_4OH at 0.4 mg/mL. The peptide was incubated for 10 min at room temperature followed by sonication (5 min) and then dispensed in 0.5 mL microfuge tubes. The NH_4OH was removed by lyophilisation to yield a salt free fluffy white peptide. This lyophilized peptide was used to prepare Aβ stock solution of 0.5 mM by dissolving in 100 mM pH 8 Hepes buffer. Incubation of 10 minutes in 100 mM pH 8 hepes buffer is sufficient to dissolve and solubilize this monomerized peptide. This stock solution was incubated with requisite amount of heme to a final concentration of 0.1 mM heme for 30 minutes. The concentration of Aβ solution was determined by the absorbance at 275 nm using

the molar extinction coefficient for tyrosine ($\varepsilon_{275\ nm}$ = 1400 M^{-1} cm^{-1}).[2] A 5 mM hemin solution was prepared in 0.2 M NaOH solution and the concentration of the heme solution was determined using the $\varepsilon_{385\ nm}$ = 58.44 mM^{-1} cm^{-1}.[3] A copper sulfate solution of 10 mM strength was made in nanopure (MilliQ) water. The concentrated heme(III)-Aβ complexes were prepared by incubating the required amounts of heme and Aβ, following which the pH was adjusted to 7.5 with 0.5 M H_2SO_4. The final concentration of the concentrated 1:1 heme(III)-Aβ was 0.1 mM, and that of 1:5 heme(III)-Aβ was 0.1 mM with respect to heme. UV-vis, rR and EPR data were collected for the same samples (the absorption spectra of the concentrated samples were recorded using a cuvette of 1 mm path length). Cu(II) binding to both 1:1 heme(III)-Aβ as well as 1:5 heme(III)-Aβ was monitored by addition of 1 equivalent of $CuSO_4$ with respect to Aβ (concentration of Aβ ~ 0.5 mM). Cu(II) titration studies on both 1:1 and 1:5 heme(III)-Aβ complexes were done with increasing equivalents of Cu(II) from 0.1 mM to 0.5 mM with respect to the total Aβ concentration (0.5 mM with respect to Aβ). Around ~ 30 min were allowed after addition of each equivalent of Cu(II) for binding to Aβ during Cu(II) titration study.

The Cu(II) binding kinetics were followed by monitoring the absorption spectra between 250 nm and 900 nm. To determine the initial rate of the decay of bis-His low spin species upon 1 equivalent Cu(II) binding to 1:1 heme(III), absorption spectra were monitored for a final concentration of 100 µM 1:1 heme(III)-Aβ, incubated with 100 µM $CuSO_4$ in a final volume of 100 µL of 100 mM Hepes buffer at pH 7.5 at 298 K. While the rate of bis-His low spin heme species decay upon binding of 1 equivalent Cu(II) binding (equimolar ratio of Cu(II) and Aβ) to 1: 5 heme(III)-Aβ, absorption spectra were monitored for a final concentration of 100 µM 1:5 heme(III)-Aβ (0.5 mM Aβ) incubated with 500 µM $CuSO_4$ in a final volume of 100 µL of 100 mM Hepes buffer at pH 7.5 at 298 K. The biphasic rate for the 1:1 heme(III)-Cu(II)-Aβ and 1:5 heme(III)-Cu(II)-Aβ were calculated using the following equation:

$$y = A_1 e^{-K_1 x} + A_2 e^{-K_2 x}$$

where K_1 is the initial rate. The rate constants were calculated by considering that in 1:1 heme(III)-Aβ has around 30 % of bis-His low spin species, while 1:5 heme(III)-Aβ has almost 80 % bis-His

low spin species. The xylenol orange assay was used for the detection of PROS as described in previous literature.[4, 5]

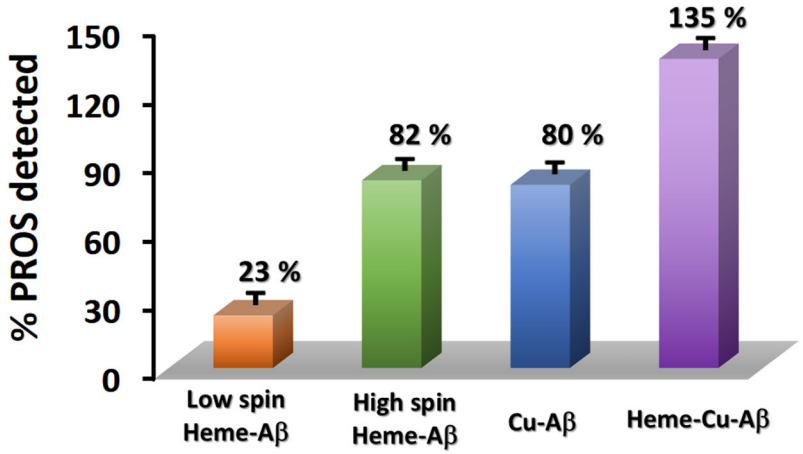

Figure S1. Pictorial representation of amount of PROS detected for bis-His bound low spin heme-Aβ, mono-His bound high spin heme-Aβ, Cu-Aβ and heme-Cu-Aβ in their respective reduced states.

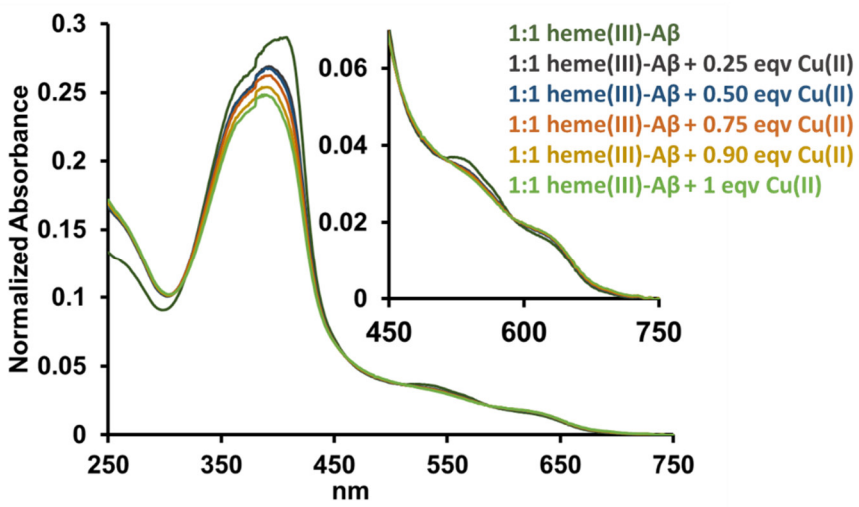

Figure S2. Absorption spectra of titration of Cu(II) with increasing equivalence from 0.25 to 1 eqv with 0.1 mM 1:1 heme(III)-Aβ in 100 mM Hepes at pH 7.5 at 298 K.

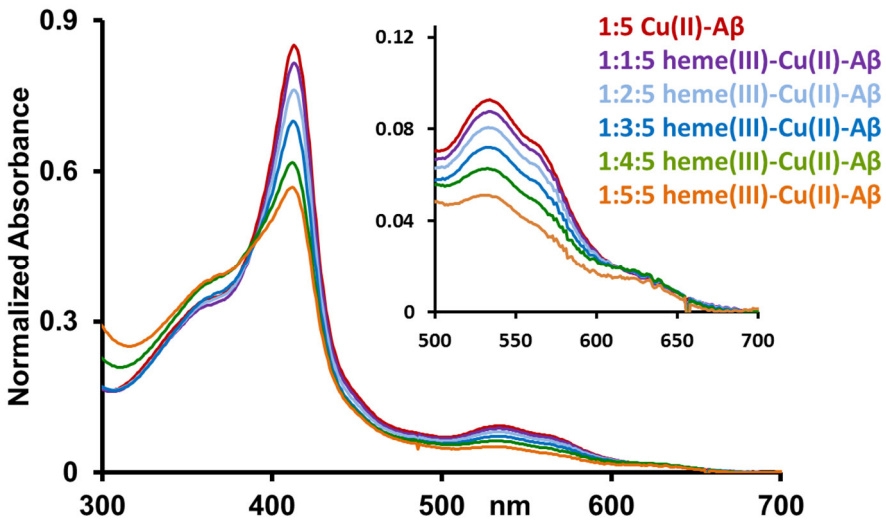

Figure S3. Absorption spectra of titration of Cu(II) with increasing equivalence from 1 to 5 eqv with respect to heme in 0.1 mM 1:1 heme(III)-Aβ (concentration of Aβ ~ 0.5 mM) in 100 mM Hepes at pH 7.5 at 298 K.

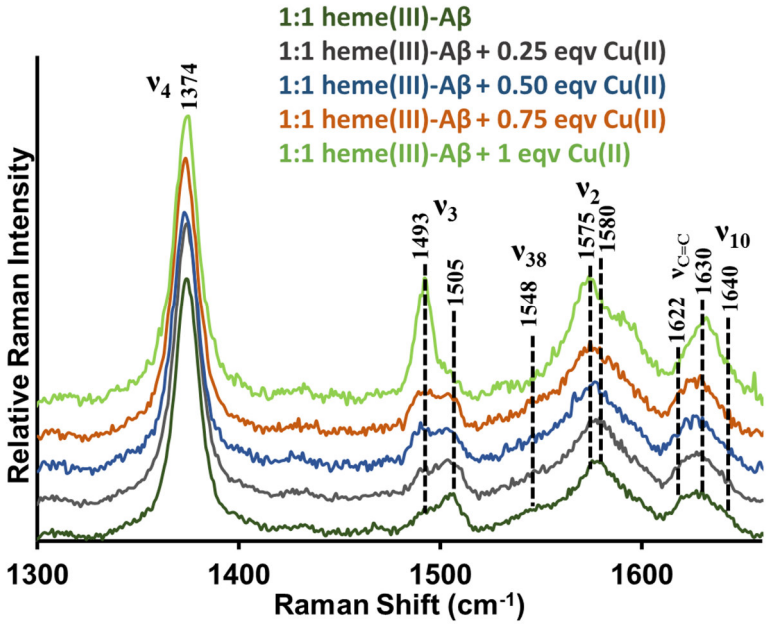

Figure S4. High Frequency rR spectra of titration of Cu(II) with increasing equivalence from 0.25 to 1 eqv with respect to heme in 0.1 mM 1:1 heme(III)-Aβ (concentration of Aβ ~ 0.1 mM) in 100 mM Hepes at pH 7.5 at 298 K.

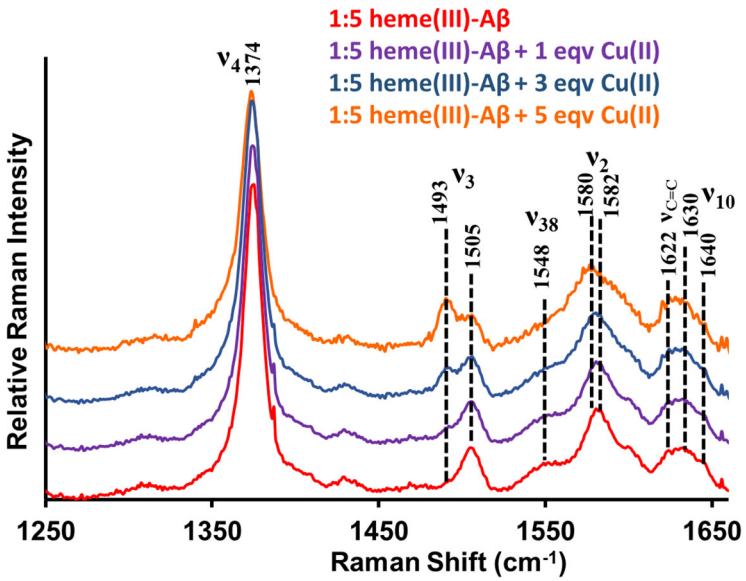

Figure S5. High Frequency rR spectra of titration of Cu(II) with increasing equivalence from 1 eqv to 5 eqv with respect to heme in 0.1 mM 1:5 heme(III)-Aβ (concentration of Aβ ~ 0.5 mM) in 100 mM Hepes at pH 7.5 at 298 K.

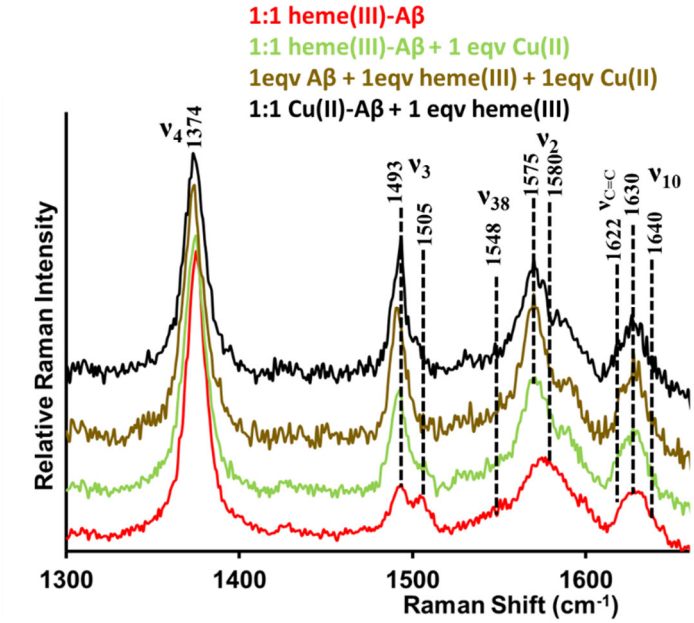

Figure S6. High frequency rR spectra of different incubation of heme(III) and Cu(II) with Aβ, pre-incubated 0.05 mM 1:1 heme(III)-Aβ, red; 0.05mM Cu(II) incubated with 0.05 mM 1:1 heme(III)-Aβ forming 0.05mM 1:1:1 heme(III)-Cu(II)-Aβ, light green; 0.05mM heme(III), 0.05 mM Cu(II) and 0.05 mM Aβ all incubated at once and 0.05 mM heme(III) incubated to pre-incubated 0.05 mM 1:1 Cu(II)-Aβ, black in 100 mM Hepes pH 7.5 buffer at 298 K.

Supporting References

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