Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

Analogues of Oxy-heme Aβ: Reactive Intermediates relevant to Alzheimer's Disease

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Experimental Details:

Materials:

All the studies were made using dry and degassed DMF (Dimethyl Formamide) as solvent. DMF was purchased from Sigma. The peptide was purchased from GL Biochem (Shanghai) Ltd. with >95% purity. Picket fence porphyrin: Bromo [meso-tetra (α,α,α,α,-o-pivalamidophenyl)porphyrin]iron-(III) (Fe(α4-T_{piv}PP)Br)) and Fe-E_4 (α4-tetra-2-(4-carboxymethyl-1,2,3-triazolyl)-phenylporphyrinato iron (III) bromide ([α4-FeE_4]Br)) were synthesized according to previous reports.[1-3] $^{18}O_2$ isotope (99%) was purchased from ICON.

Instrumentation:

All the absorption spectra were obtained by a UV-vis diode array spectrophotometer (Agilent 8453). EPR spectra were obtained by a Jeol (JES FA200) spectrophotometer. Resonance Raman data were collected using a Trivista 555 triple spectrograph (Princeton Instrument) and 413.1 nm excitation from Kr^+ laser (Coherent, Sabre Innova SBRC-DBW-K). Gratings used in the three stages were 900, 900 and 1800 grooves/mm. The optics (plano-convex lens, mirror etc), used for the collection of Raman data were purchased from Sigma-Koki Japan. Power on the sample was ~10 to 15 mW. Raman shifts were calibrated with naphthalene and indene. Data acquisitions were done for 300 seconds. The wave number accuracy was ± 1 cm^{-1} for well defined peaks.

Sample Preparation and Spectral Characterization:

The peptide stock solution was prepared in 1 mM concentration. Porphyrin stock solution was prepared in 10 mM concentration. The peptide and porphyrin solution were mixed in 1:1 ratio and kept ~ 1 hour for pfp and ~ 30 min for Fe-E_4 at room temperature for the formation of porphyrin-Aβ complex. Absorption spectra were taken by adding 12.5 μL of the porphyrin-Aβ complex solution in 1 mL DMF. For the formation of oxy complex, the porphyrin-Aβ complexes were prepared in dry and degassed DMF in glove box (mBRAUN, Germany) under anaerobic conditions. Na_2S was used as reducing agent and was prepared in dry and degassed MeOH. Na_2S concentration was 25 mM. The reduced porphyrin-Aβ complex was cooled in a -80 ^{0}C bath prepared with dry ice & methanol. The solution of reduced complexes was oxygenated for ~ 2 minutes and was kept ~ 5 minutes at this temperature to react. For isotope study $^{18}O_2$ was used instead of

$^{16}O_2$. Absorption spectra of the reduced porphyrin-Aβ complex and oxy-adducts were measured in septum-sealed anaerobic cuvettes maintaining the temperature at -80˚ C. Reactive intermediates with relatively small life times do not allow their trapping and characterization at room temperature. At low temperatures, where the thermal energy is not enough for the reaction to further progress, the lifetimes of these intermediates increase significantly. Hence the Fe-O_2 intermediate was stabilized at -80 ^{o}C. Several analogues of key reaction intermediates of metallo-enzyme active sites have been trapped and characterized at low temperatures.[3-8] None of these intermediates, involved in the reaction, could be observed at room temperature.

EPR data for all the oxidized, reduced and oxy complexes were recorded at 77 K. rR data for the reduced and oxy complex were collected on the same EPR samples in septum-sealed anaerobic EPR tubes. rR spectra of the oxy and reduced complexes were obtained at 77 K where as the oxidized samples were obtained at room temperature.

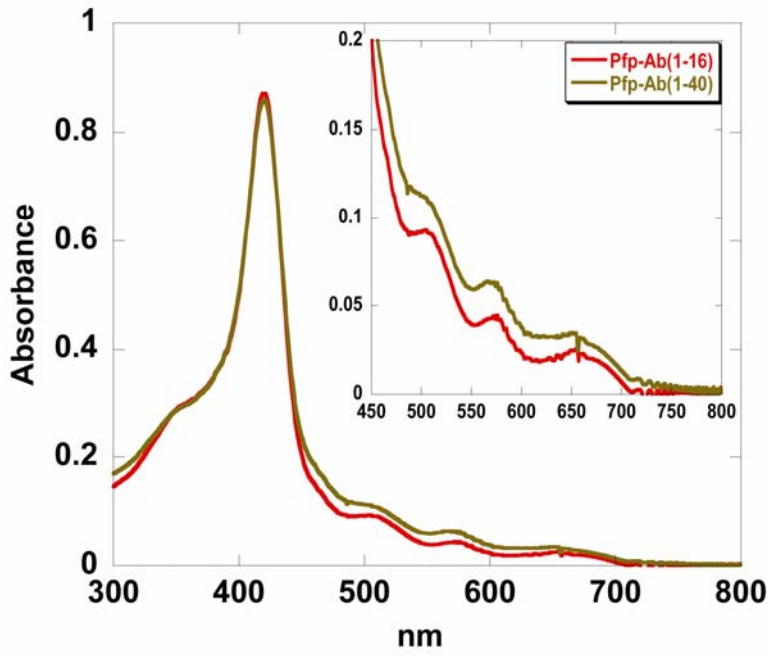

Figure S1. Absorption spectra of pfp-Aβ(1-16) (red) and pfp-Aβ(1-40) (dark yellow).

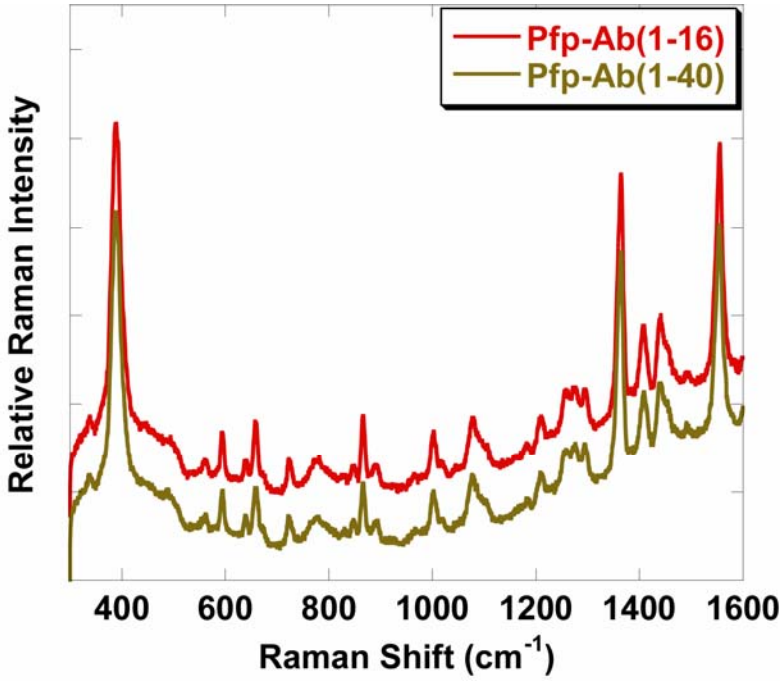

Figure S2. Resonance Raman spectra of pfp-Aβ(1-16) (red) and pfp-Aβ(1-40) (dark yellow).

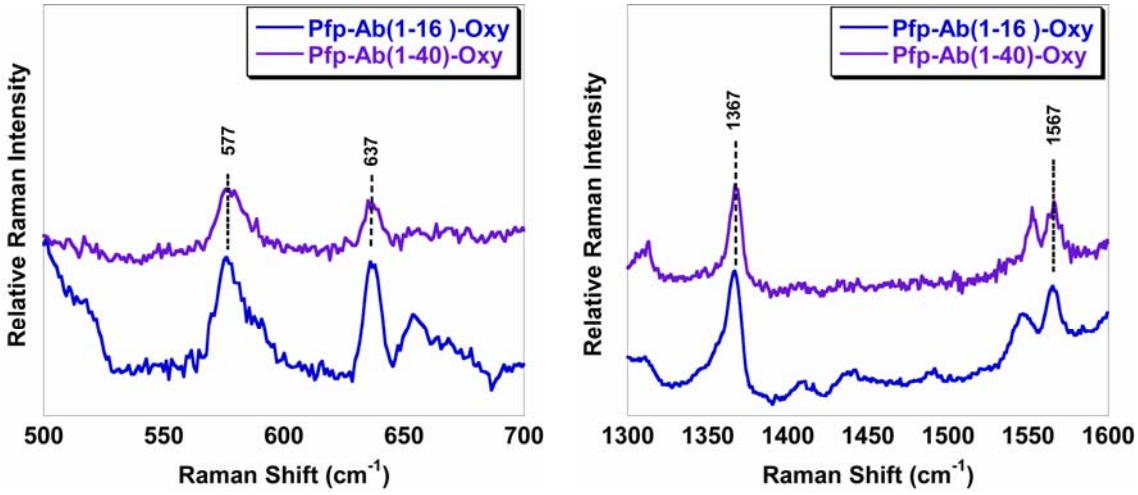

Figure S3. Resonance Raman spectra of oxy intermediate of pfp-Aβ(1-16) (blue) and pfp-Aβ(1-40) (violet) in DMF.

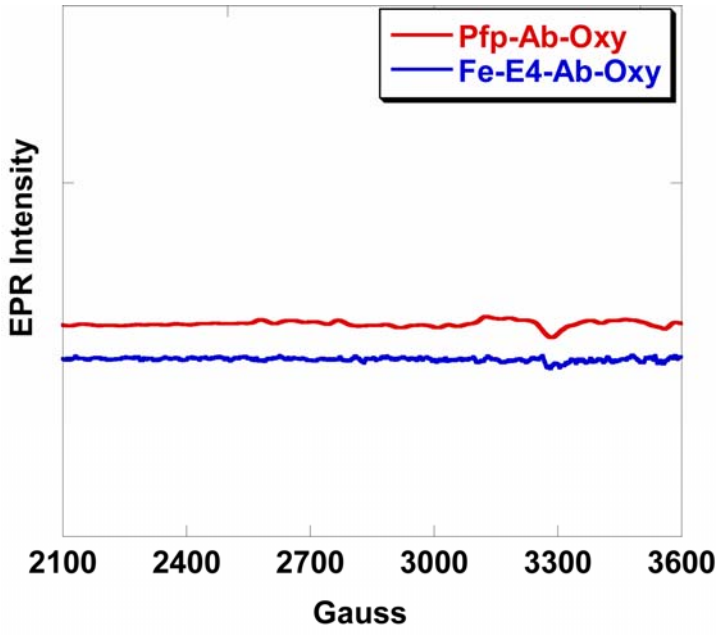

Figure S4. High field low spin region EPR spectra of the oxy complex of pfp-Aβ (red) and Fe-E_4-Aβ (blue).

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