Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information

Rapid autoxidation of ferrous heme-Aβ complexes relevant to Alzheimer's Disease

Madhuparna Roy, Abhishek Dey* and Somdatta Ghosh Dey *

School of Chemical Sciences, Indian Association for the Cultivation of Science, 2A & 2B, Raja S. C. Mullick Road, Jadavpur, Kolkata 700032, India.

Experimental Details:

Materials:

All reagents were of the highest grade commercially available and were used without further purification. All peptides ((Aβ(1-16,WT), Aβ(1-40,WT) and Aβ(1-16,Tyr10Phe)) were purchased from Shanghai Yaxian Chemical Co. Ltd., China with >95% purity. Hemin, xylenol orange, buffers and D_2O (99%) were purchased from Sigma. $^{18}O_2$ isotope (99%) was purchased from ICON.

Instrumentation:

All the absorption spectra and kinetic traces were obtained using BioLogic SFM 4000 stopped-flow instrument (Xe lamp as light source) except for the xylenol orange assay for PROS calculation, for which Agilent Cary 3500 Compact UV-Vis Spectrophotometer was used. 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.

Sample Preparation and Spectral Characterization:

The peptide stock solutions were prepared in 1 mM concentration in 100 mM phosphate buffers at pH 6.5/7/7.5 or pD 7. Hemin stock solution was prepared in 1M NaOH in 5 mM concentration. The heme-Aβ complexes were formed by adding 0.8 equivalent of heme to 1 equivalent of Aβ, incubating the mixture for ~ 1 hour at room temperature and then adjusting the pH. The solutions were then degassed and the heme-peptide complexes were reduced using ~2 equivalents of sodium dithionite ($Na_2S_2O_4$) under anaerobic conditions in a glove box (mBRAUN, Germany).

For partially reduced oxygen species (PROS) calculation, the following xylenol orange assay was performed. A total of 4.9 mg of Mohr's salt and 3.9 mg of xylenol orange were dissolved in 5 mL of 250 mM H_2SO_4 and stirred for 10 min. A 200 μL portion of this solution was taken in 1.8 mL of nanopure water, and a calibration curve for the quantitative estimation of H_2O_2 was obtained for 0.05, 0.1, 0.5, 1, 2.5, 5, and 10 μM concentrations of H_2O_2 by recording their absorbance at

560 nm (Fig. S1A). The calibration curve was expressed as absorbance at a fixed wavelength of 560 nm versus concentration of H_2O_2 in micromolar units for a 2 mL volume. For the detection of PROS of the heme–Aβ(1–16, WT) and heme–Aβ(1–16, Tyr10Phe) complexes, a blank was obtained in the UV–Vis spectrophotometer with 1.8 mL of nanopure water in a cuvette. A total of 200 μL of the xylenol orange solution was added to the above cuvette, and the absorbance was measured. The heme–Aβ(1–16) complexes were reduced using a minimal amount of dithionite under anaerobic conditions, followed by their reoxidation by O_2 (monitored by absorption). A total of 200 μL of a reoxidized solution was added to the cuvette containing the control, and the absorbance of this solution was recorded (Fig. S1B). The value of the absorbance of the above solution (after the control was deducted) at 560 nm when plotted on the calibration curve yielded the corresponding H_2O_2 concentration. The amount of H_2O_2 produced is expressed as a percentage, where ~100% PROS implicates a two-electron-reduction pathway and 50% PROS implicates a one-electron-reduction pathway of O_2.

For monitoring the kinetics of the reaction between reduced heme-Aβ complexes and oxygen using the stopped-flow instrument, the cuvette holder was enclosed in a glove bag which was made oxygen free through repeated cycles of evacuation and refilling using N_2 (UHP) gas. The samples were transferred from the glove box to the glove bag (kept under high N_2 pressure) in septum-sealed anaerobic vials. The experiments were conducted using 0.2 mM heme-Aβ and ~0.22 mM O_2 (i.e., oxygen that remains dissolved in buffer) and the two were mixed in 1:3 volume ratio. The kinetic traces for the decay at 584 nm and the formation at 632 nm were fitted using the following biphasic equations respectively:

$$y = A_1*\exp(-k_1t) + A_2*\exp(-k_2t) + c$$

$$y = A_1*[1-\exp(-k_1t)] + A_2*[1-\exp(-k_2t)] + c$$

Here A_1 and A_2 are the pre-exponential factors and $A_1 + A_2 = 1$.

Initial spectra were obtained at 12 ms and the duration of the experiments were 2.5 min.

For trapping and characterizing the Fe-O_2 intermediate formed during the afore mentioned reaction using resonance Raman spectroscopy, ~0.6 mM reduced heme-Aβ (prepared in glove box) and oxygen saturated (1-1.2 mM concentration) 100mM pH 7 PO_4^{3-} buffer were used and the two were

mixed in 1: 2 volume ratio within septum sealed anaerobic EPR tubes placed in a 0°C bath (water and ethanol mixture) and then frozen at different time intervals in liquid nitrogen at 77K.

The heme-Aβ complexes in their oxidized and reduced forms were characterized using both absorption and resonance Raman spectroscopies. For the former, the stopped flow instrument, with its cuvette holder placed in a glove bag kept under anaerobic conditions, was employed. Mixing 0.2 mM of heme(III)-Aβ/heme(II)-Aβ solutions with degassed buffer in 1:3 volume ratio ensured the final concentration remained the same as during the kinetics experiments. After a short run time of 3s the desired spectra were obtained (Fig. S2). In case of rR spectroscopy, ~0.6 mM heme(III)-Aβ/heme(II)-Aβ solution was mixed with degassed buffer in 1:2 volume ratio in septum sealed anaerobic EPR tubes, to keep the sample concentrations uniform during all rR experiments allowing comparison of spectra. Finally, the samples were frozen in liquid nitrogen at 77 K (Fig. S5).

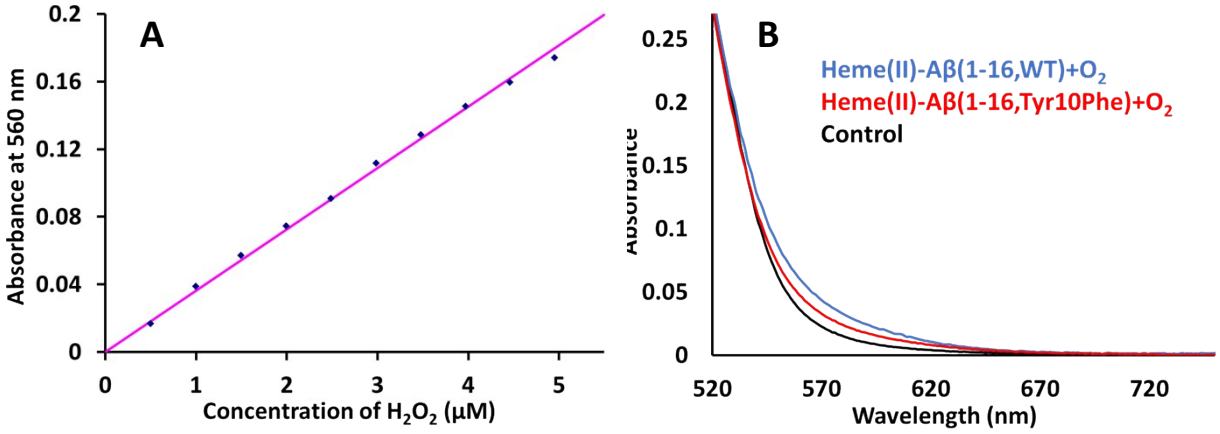
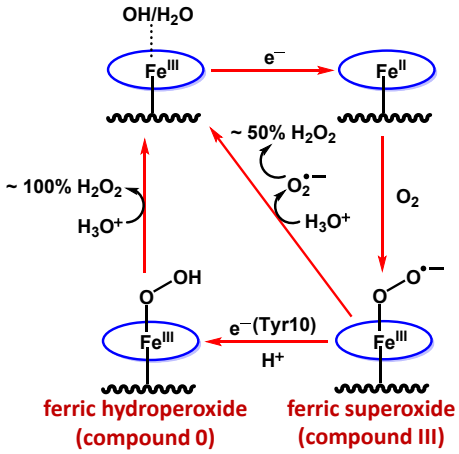


Fig. S1 (A) Calibration curve for H_2O_2 detection by the xylenol orange assay protocol, (details in the Sample Preparation and Spectral Characterization section). Absorbance at 560 nm vs. different concentrations of H_2O_2 (μM) have been plotted to prepare the calibration curve. (B) Xylenol orange incubated sample, heme(II)-Aβ(1-16,WT)+O_2, blue ; heme(II)-Aβ(1-16,Tyr10Phe)+O_2, red; and control i.e., only xylenol orange, black. Measuring the absorbance at 560 nm for the sample solutions and subtracting from them the absorbance at the same wavelength for the control and plotting that value on the calibration curve gives the H_2O_2 concentration in the sample solution. Ratio of this H_2O_2 concentration with that of the sample itself (i.e., of the heme-Aβ complex) when expressed as percentage helps to quantify the H_2O_2 generated.



Scheme S1 Schematic representation of probable pathways of PROS formation by heme-Aβ complexes resulting in almost 100% or nearly 50% PROS formation. From the amount of PROS detected, heme-Aβ(1-16,WT) is expected to follow the 2e⁻ pathway while heme-Aβ(1-16,Tyr10Phe) is expected to follow the 1 e⁻ pathway. The plausible oxygen bound intermediates are mentioned in red.

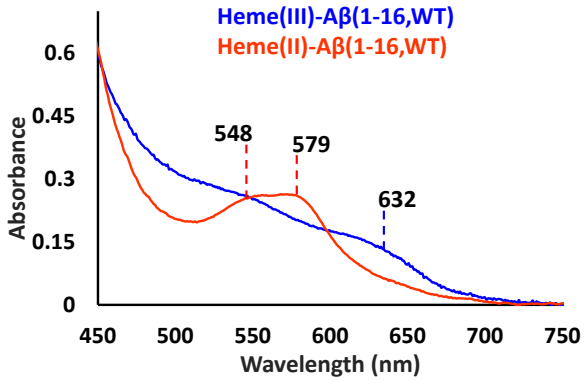


Fig. S2 Absorption spectra of heme(III)-Aβ(1-16,WT), blue; and heme(II)-Aβ(1-16,WT), red; in 100mM pH 7 PO_4^{3-}buffer.

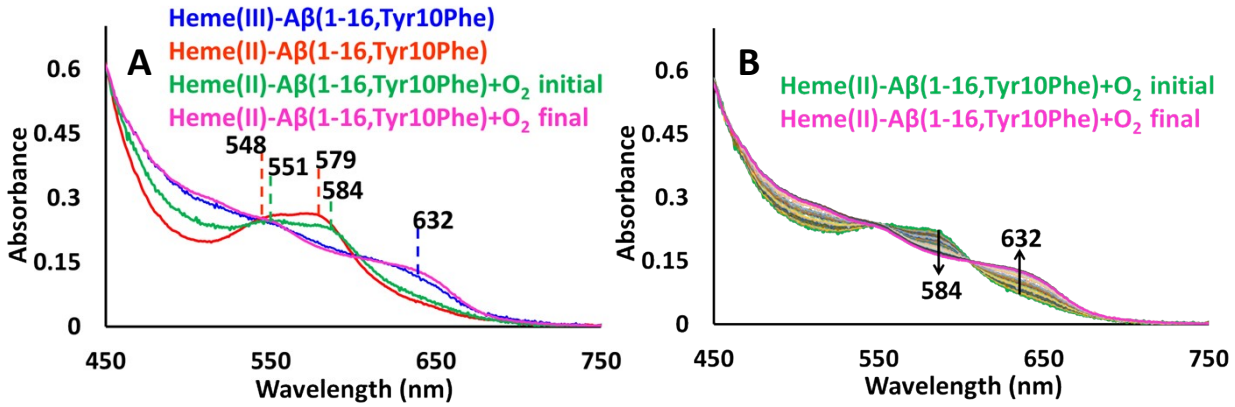


Fig. S3 (A) Absorption spectrum of high spin oxidized heme-Aβ(1-16,Tyr10Phe), blue; reduced heme-Aβ(1-16,Tyr10Phe), red; for the reaction of reduced heme-Aβ(1-16,Tyr10Phe) with O_2 in 100mM pH 7 PO_4^{3-}buffer, initial, green and final, pink. (B) Progression of the reaction between heme(II)-Aβ(1-16,Tyr10Phe) and O_2 with the arrows showing the direction of spectral changes.

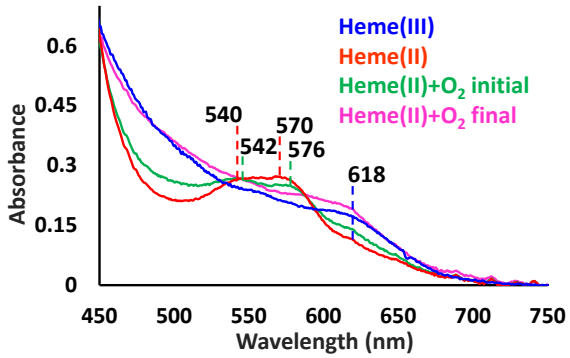


Fig. S4 (A) Absorption spectrum of oxidized heme, blue; reduced heme, red; for the reaction of reduced heme with O_2 in 100mM pH 7 PO_4^{3-}buffer, initial, green and final, pink. The nature of spectral changes is similar to that for the heme-Aβ complexes but the absolute values are different.

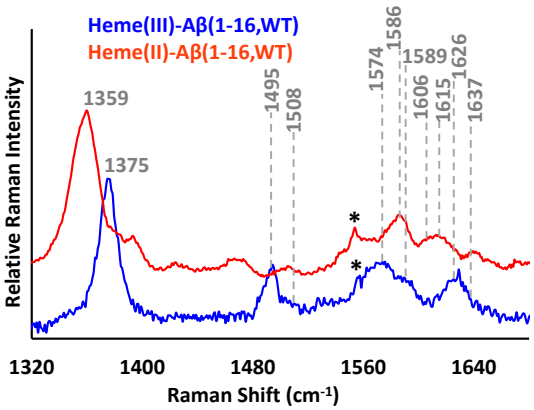


Fig. S5 The high frequency resonance Raman spectra of heme(III)-Aβ(1-16,WT), blue; and heme(II)-Aβ(1-16,WT), red. The peaks marked with * imply plasma lines. All samples are prepared using 100mM PO_4^{3-} buffer at pH 7 and data are obtained using an excitation wavelength of 413.1 nm at 77 K.

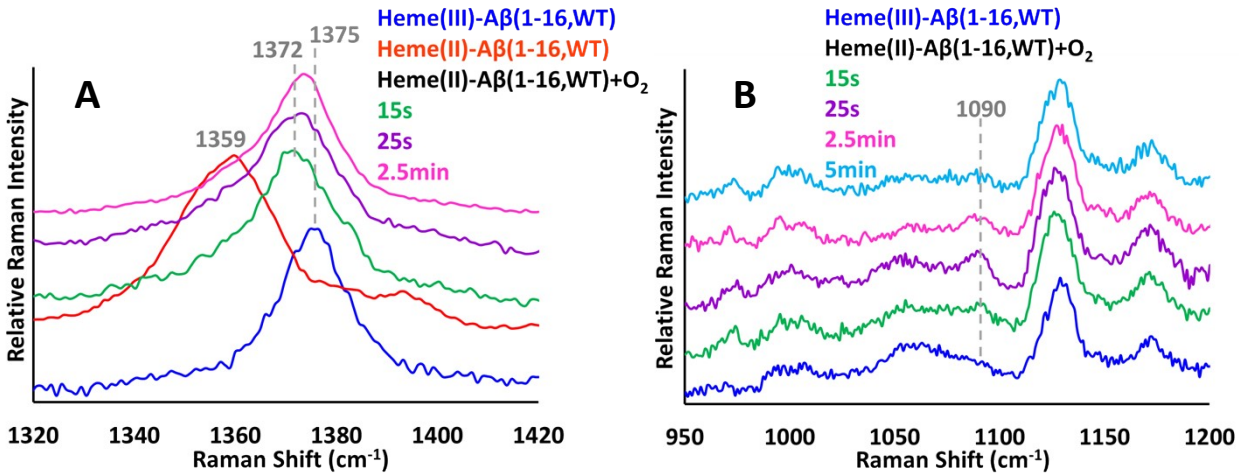


Fig. S6 (A) The ν_4 (oxidation state) marker band region and (B) the low frequency region of the resonance Raman spectra of heme(III)-Aβ(1-16,WT), blue; heme(II)-Aβ(1-16,WT), red; heme(II)-Aβ(1-16,WT) reacted with O_2 at 0°C and frozen at reaction times 15s, green; 25s, purple; 2.5 min, pink; and 5 min, cyan. All samples are prepared using 100mM PO_4^{3-} buffer at pH 7 and data are obtained using an excitation wavelength of 413.1 nm at 77 K.

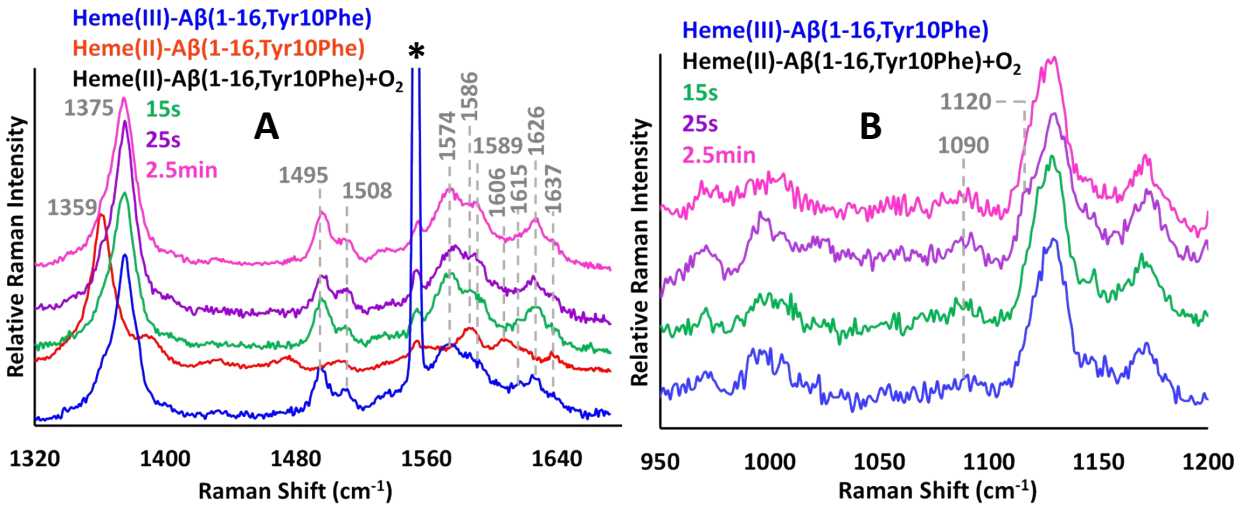


Fig. S7 (A) The high frequency region and (B) the low frequency region of the resonance Raman spectra of heme(III)-Aβ(1-16,Tyr10Phe), blue; heme(II)-Aβ(1-16,Tyr10Phe), red; heme(II)-Aβ(1-16,Tyr10Phe) reacted with O_2 at 0°C and frozen at reaction times 15s, green; 25s, purple; and 2.5 min, pink. The peak marked with * implies plasma line. All samples are prepared using 100mM PO_4^{3-} buffer at pH 7 and data are obtained using an excitation wavelength of 413.1 nm at 77 K.

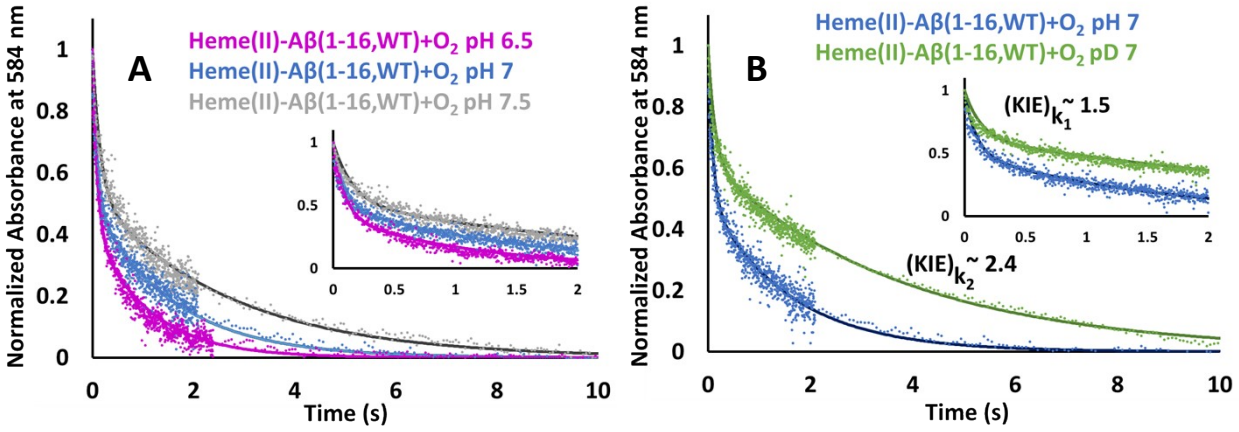


Fig. S8 Comparison of the decay of the Aβ bound heme(III)-$O_2^{\bullet-}$ intermediate formed during the reaction of heme(II)-Aβ(1-16,WT) with O_2 in 100 mM PO_4^{3-} buffer at (A) pH 6.5, purple; pH 7, blue; pH 7.5, gray and also at (B) pH 7, blue and pD 7, green. All kinetics were followed at 584 nm.

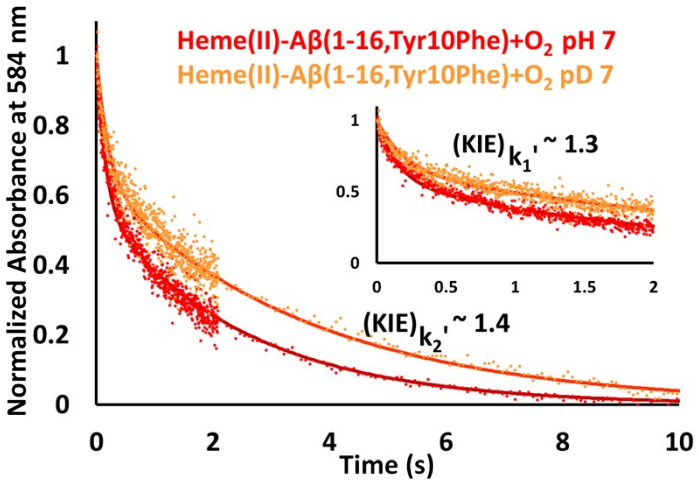


Fig. S9 Comparison of the decay of the Aβ bound heme(III)-$O_2^{\bullet-}$ intermediate formed during the reaction of heme(II)-Aβ(1-16,Tyr10Phe) with O_2 in 100 mM PO_4^{3-} buffer at pH 7, red and pD 7, orange. All kinetics were followed at 584 nm.

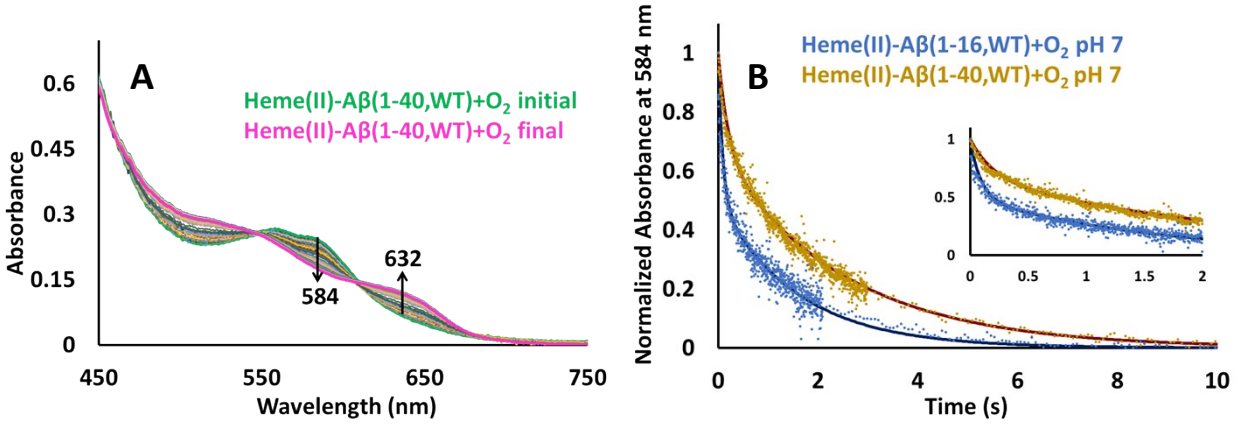


Fig. S10 (A) Progression of the reaction between heme(II)-Aβ(1-40,WT) and O_2 in 100 mM PO_4^{3-} buffer at pH 7 monitored using absorption spectroscopy with the arrows showing the direction of spectral changes. (B) Comparison of the decay of the Aβ bound heme(III)-$O_2^{\bullet-}$ intermediate formed during the reaction of heme(II)-Aβ(1-16,WT) with O_2, blue; and heme(II)-Aβ(1-40,WT) with O_2, brown, in 100 mM PO_4^{3-} buffer at pH 7. All kinetics were followed at 584 nm.