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Supporting Information

Spin State Dependent Peroxidase Activity of Heme bound Amyloid β Peptides Relevant to Alzheimer's Disease

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Methods

General Methods

All reagents are of the highest grade, commercially available and are used without further purification. The two Aβ1-40 peptides that are used in this study are Wild type (WT) and site directed Tyr10Phe (Y10F) mutant. All peptides are purchased from Shanghai Yaxian Chemical Co., Ltd, China with >95% purity. Hemin, phosphate buffer, Serotonin and H_2O_2 are purchased from Sigma. All the experiments are done at pH 7.6 using 100 mM phosphate buffer. Freshly prepared hemin solution is used for all the experiments and is prepared in 1 M NaOH solution. For absorption, EPR and resonance Raman spectroscopy strength of peptide stock solutions are kept at 1 mM. For the preparation of low spin heme-Aβ, 1 equivalent of Aβ solution is incubated with 0.2 equivalent of heme solutions for ~1 h. H_2O_2 and serotonin (5-HT) was prepared in Millipore water with strength of H_2O_2 ranging from 5-20 mM and strength of 5-HT is taken to be 20 mM. Aliquots of H_2O_2 and serotonin are added to adjust the final concentration to the desired value (10 eq.).

Physical Methods

Peroxidase Activity

For low spin heme-Aβ, 3,3′,5,5′-Tetramethylbenzidine (TMB) is used as the substrate for peroxidase activity measurement. TMB stock solutions of 40-50 mM strength are used for the assay experiments and are prepared by dissolving TMB in glacial AcOH. The final concentration of low spin heme-Aβ, H_2O_2 and TMB in the cuvette are 0.5 μM, 200 μM and 40 μM respectively at pH 7.6, in 100 mM phosphate buffer. So, the experimental molar ratio of low spin heme-Aβ: H_2O_2: TMB = 1:400:80. Kinetic traces were obtained by monitoring the increase of the 650 nm absorption band with time. The control experiments are also done maintaining the aforementioned conditions i.e. free heme: H_2O_2: TMB/ Aβ: H_2O_2:

TMB = 1:400:80 and H_2O_2: TMB = 400:80. The final concentration of free heme, Aβ, H_2O_2 and TMB in the cuvette for the control experiments are 0.5 µM, 0.5 µM, 200 µM and 40 µM respectively at pH 7.6, in 100 mM phosphate buffer.

Absorption spectra

Absorption spectra of the reaction of low spin heme-Aβ and H_2O_2 are obtained by a UV-Vis diode array spectrophotometer (Agilent 8453). Absorption spectra are recorded by adding ~120 µL of the low spin heme-Aβ complex solution in a cuvette of 1 mm path length and kinetics studies are done by adding 10 eq. H_2O_2 to low spin heme-Aβ.

Serotonin Oxidation

Absorption spectra and kinetics of serotonin oxidation are monitored in a UV-vis diode array spectrophotometer (Agilent 8453). The concentration of serotonin stock solution is taken to be 20 mM and aliquot of serotonin is added to adjust 10 eq. of serotonin in the reaction mixture of low spin heme-Aβ and H_2O_2 to carry out serotonin oxidation.

In case of the catalytic serotonin oxidation by heme-Aβ(WT) and H_2O_2, serotonin stock solutions of 40-50 mM strength are used for the experiments. The final concentration of low spin heme-Aβ, H_2O_2 and serotonin in the cuvette are 0.5 µM, 200 µM and 40 µM respectively at pH 7.6 in 100 mM phosphate buffer. So, the experimental molar ratio of low spin heme-Aβ: H_2O_2: serotonin = 1:400:80. Kinetic traces were obtained by monitoring the increase of the 317 nm absorption band with time The control experiments are also done maintaining the aforementioned conditions i.e. free heme: H_2O_2: serotonin/ Aβ: H_2O_2: serotonin = 1:400:80 and H_2O_2: serotonin = 400:80. The final concentration of free heme, Aβ, H_2O_2 and serotonin in the cuvette for the control experiments are 0.5 µM, 0.5 µM, 200 µM and 40 µM respectively at pH 7.6, in 100 mM phosphate buffer.

EPR

EPR spectra are obtained with a Bruker EMX EPR spectrometer. The EPR samples are of 0.18 mM in concentration and are prepared by adding 10 equivalents of H_2O_2 low spin heme-Aβ Tyr10Phe mutant solution at different times (3 min and 7 min) and the samples are frozen in liq. N_2. The data are acquired at 77K. EPR settings are as follows: Freq. ≈ 9.31GHz, Power ≈ 1.26 mW, Receiver gain = 2×10^3, Modulation Frequency = 100 KHz, Modulation Amplitude = 20 G, Time Constant = 327.68 msec, Conversion Time = 80 msec, Sweep time = 163.84 sec.

Resonance Raman

Resonance Raman data are recorded using a Trivista 555 spectrograph (Princeton Instruments) by using 413.1 nm excitation from a Kr^+ laser (Coherent, Sabre Innova SBRC-DBW-K). The optics (plano-convex lens, mirror etc.), used for the collection of rR data are purchased from Sigma-Koki Japan. The power on the samples is kept at ~4-5 mW. The resonance Raman samples are of 0.18 mM in concentration and are prepared by adding 10 eq. of H_2O_2 to wild type (WT) low spin heme-Aβ complex and to the Tyr10Phe mutant and are frozen in liq. N_2. To minimize the interference of the background noise from quartz, charcoal data is acquired and subtracted from the experimental data.

Peak fit

The curve fitting of absorption and resonance Raman data are done using the program *PeakFit v4.12*. Minimum numbers of bands were used to adequately fit key features in both spectra.

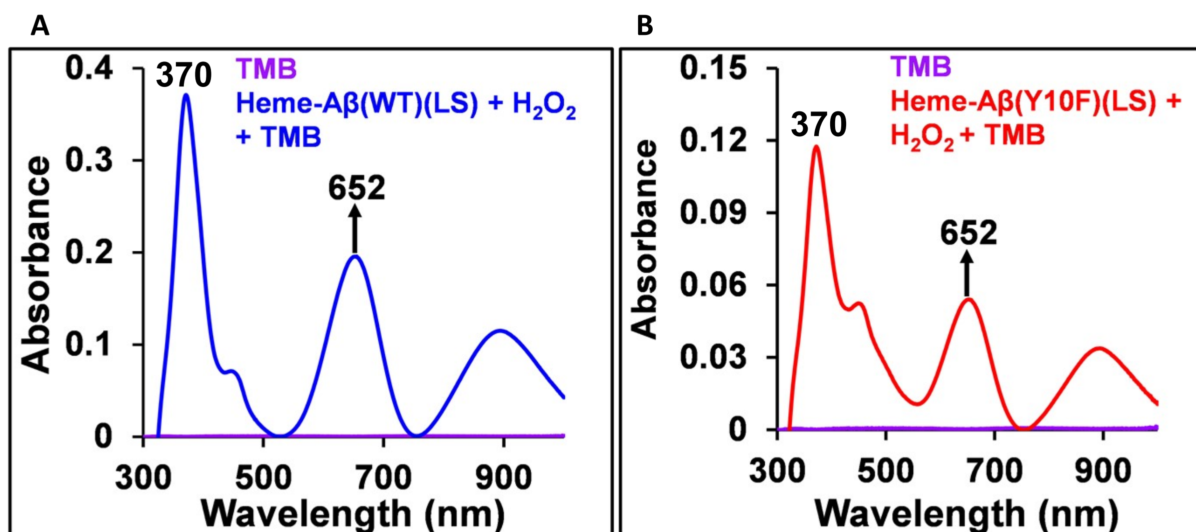


Figure S1. A) Absorption spectra of TMB (purple) and oxidized TMB for wild type low-spin heme-Aβ (WT, LS) (blue) B) Absorption spectra of TMB (purple) and oxidized TMB for low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) (red) in 100 mM phosphate buffer at pH 7.6.

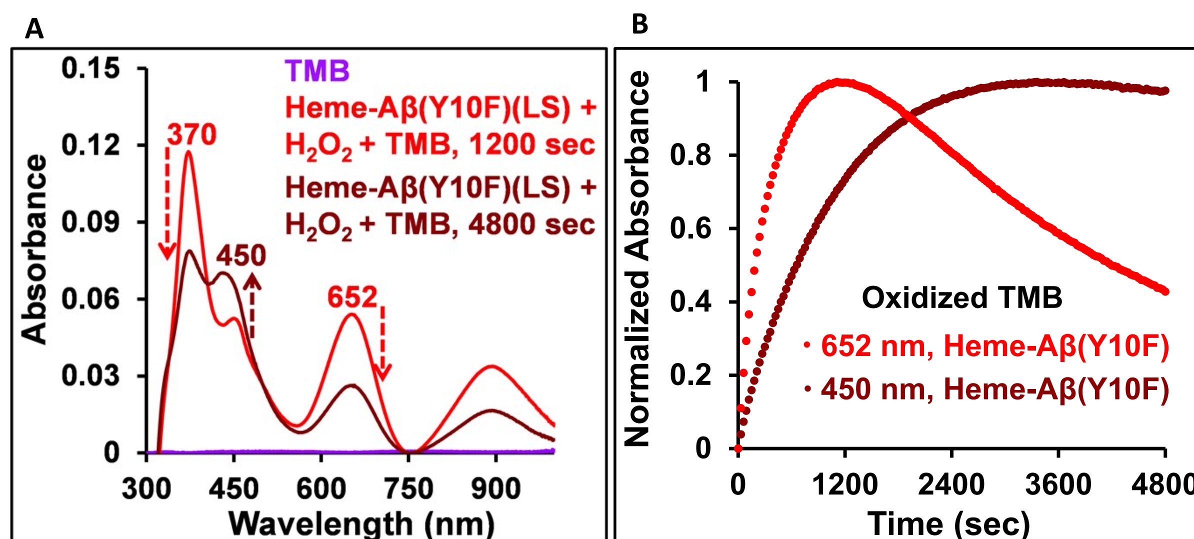


Figure S2. A) Absorption spectra of TMB (purple), oxidized TMB for low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and H_2O_2 at 1200 sec (red) and oxidized TMB for low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and H_2O_2 at 4800 sec (brown); B) Comparison of the rate of TMB oxidation for low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and H_2O_2 at 652 nm (red) and 450 nm (brown). The reaction is done in 100 mM phosphate buffer at pH 7.6.

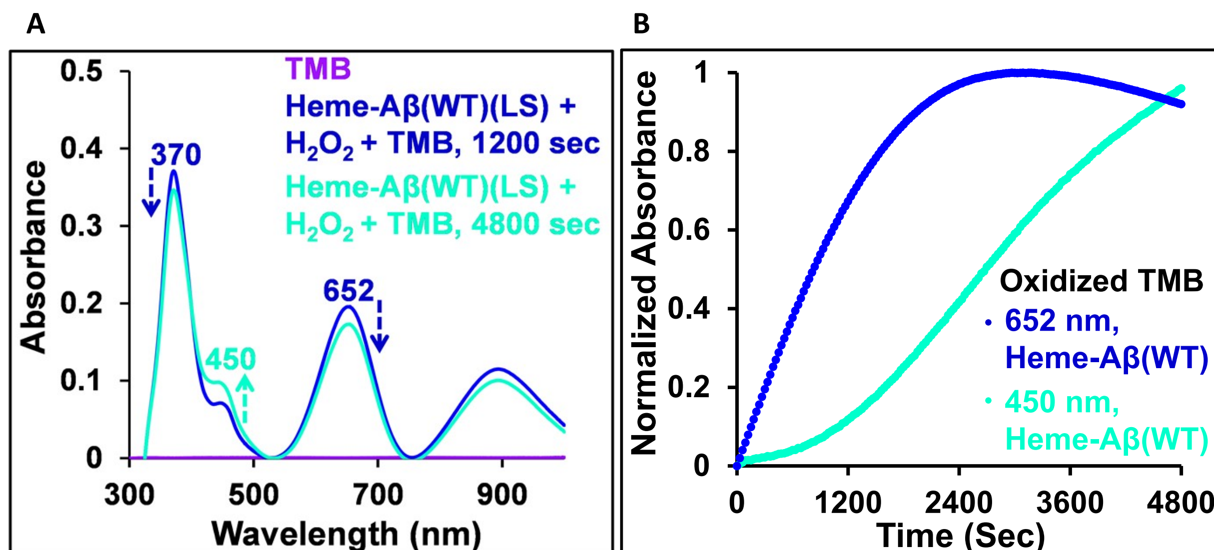
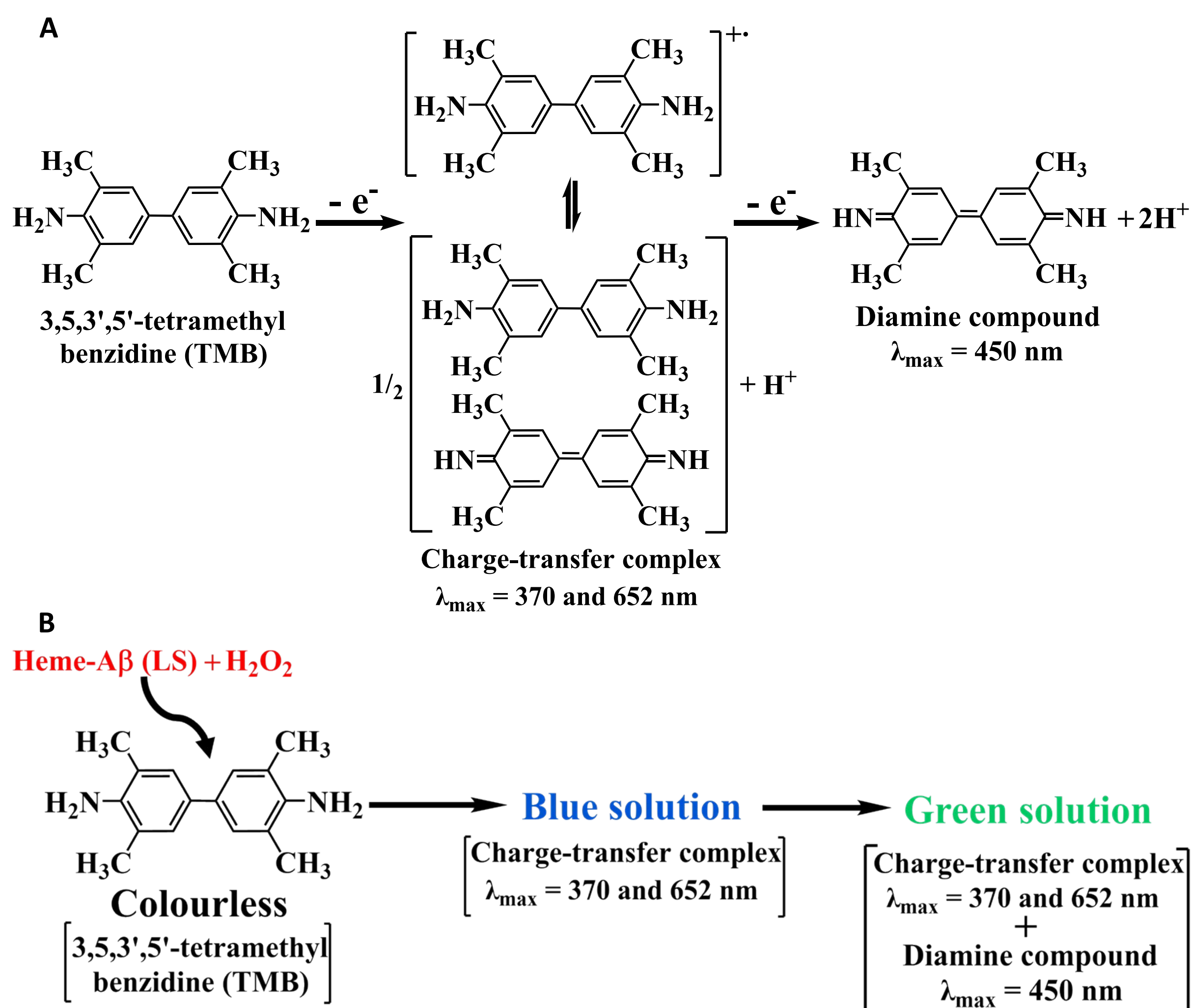


Figure S3. A) Absorption spectra of TMB (purple) and oxidized TMB for wild type low-spin heme-Aβ(WT, LS) and H_2O_2 at 1200 sec (blue) and oxidized TMB for wild type low-spin heme-Aβ(WT, LS) and H_2O_2 at 4800 sec (cyan); B) Comparison of the rate of TMB oxidation for low-spin heme-Aβ WT and H_2O_2 at 652 nm (blue) and 450 nm (cyan). The reaction is done in 100 mM phosphate buffer at pH 7.6.



Scheme S1. A) Schematic representation of oxidation of TMB oxidation in a general peroxidase mechanism;[1,2] B) Schematic representation of oxidation of TMB oxidation with low-spin heme-Aβ and H_2O_2. The initial blue solution produced in the reaction indicates that formation of the charge-transfer complex (one electron oxidation of TMB) and the final green solution corresponds to the mixture of blue the charge-transfer complex (one electron oxidation product of TMB) and the yellow diamine compound (two electron oxidation product of TMB) as evident from absorption spectroscopy.

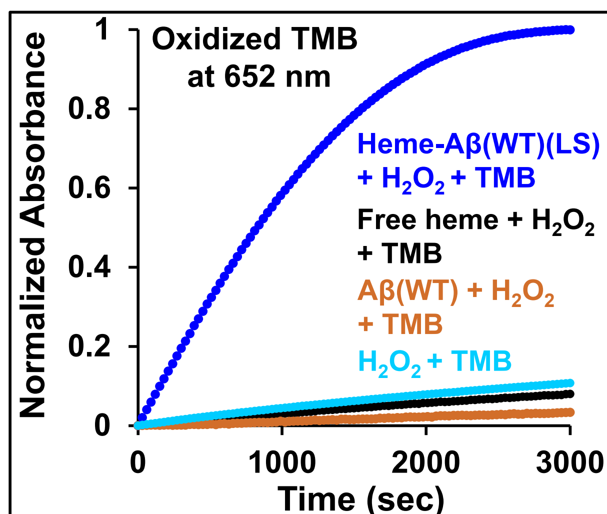


Figure S4. Comparison of the rate of peroxidase activity (TMB oxidation) of wild type low-spin heme-Aβ(WT, LS) + H_2O_2 + TMB (blue), free heme + H_2O_2 + TMB (black), only Aβ(WT) + H_2O_2 + TMB (brown) and H_2O_2 + TMB (cyan); monitored at 652 nm in 100 mM phosphate buffer at pH 7.6.

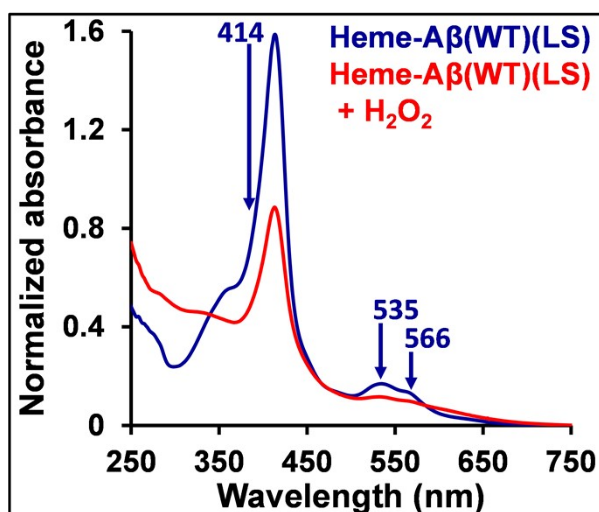


Figure S5. Absorption spectrum of low spin heme-Aβ (blue), for the reaction of low-spin heme-Aβ(WT, LS) with H_2O_2 (red). The reaction is done in 100 mM phosphate buffer at pH 7.6.

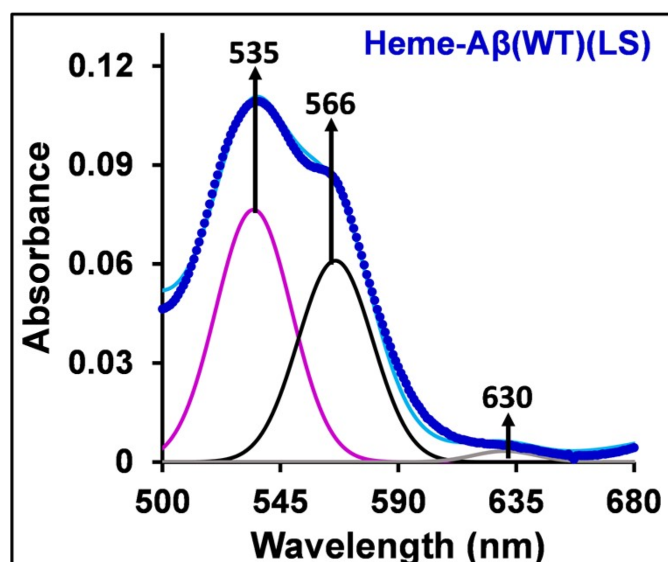


Figure S6. Experimental absorption spectrum (Q-band) of low-spin heme-Aβ (WT, LS) (blue dotted line) and simulated spectrum (cyan solid line). The simulated line shows three bands, one doublet at 535 and 566 nm for low-spin heme-Aβ and another band at 630 nm, which represent a minor water bound high spin heme-Aβ component.

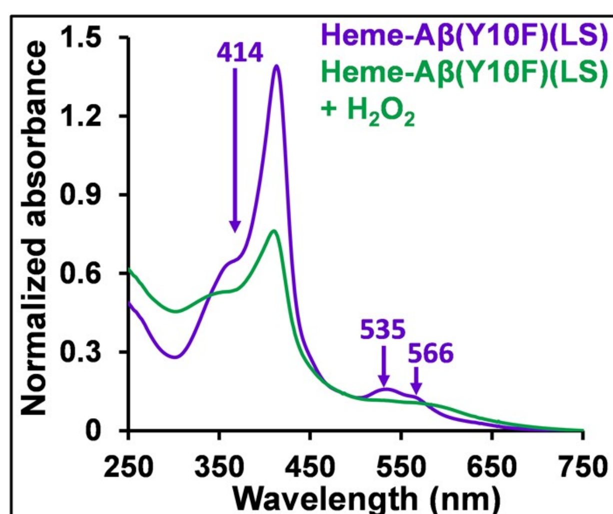


Figure S7. Absorption spectrum of low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) (purple), for the reaction of low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) with H_2O_2 (green). The reaction is done in 100 mM phosphate buffer at pH 7.6.

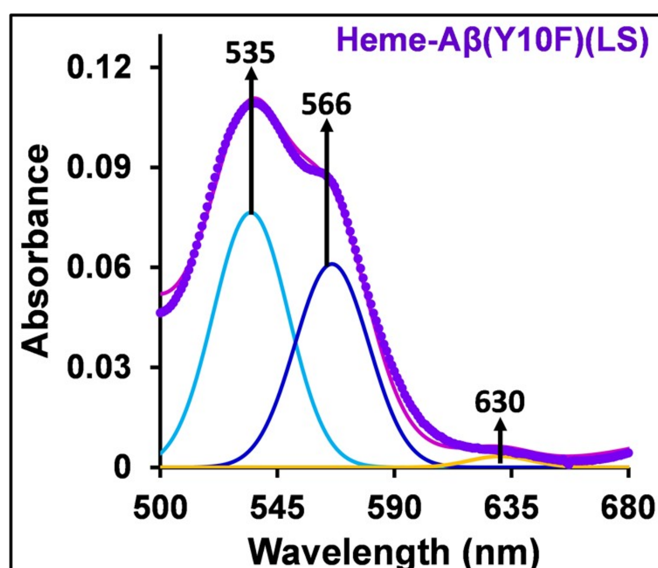


Figure S8. Experimental absorption spectrum (Q-band) of low-spin heme-Aβ (Y10F, LS) (purple dotted line) and simulated spectrum (pink solid line). The simulated line shows three bands, one doublet at 535 and 566 nm for low-spin heme-Aβ and another band at 630 nm, which represent a minor water bound high spin heme-Aβ component.

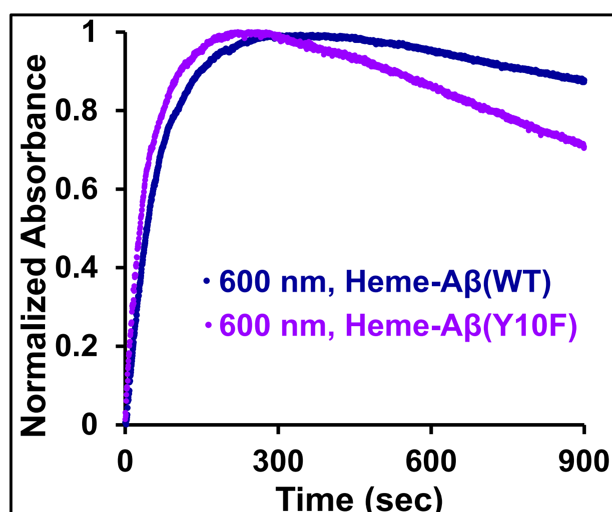


Figure S9. Overlay of reaction kinetics for the 600 nm band formed in the reaction of low-spin heme-Aβ (WT, LS) with H_2O_2 (blue) and low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) with H_2O_2 (purple). The reaction is done in 100 mM phosphate buffer at pH 7.6.

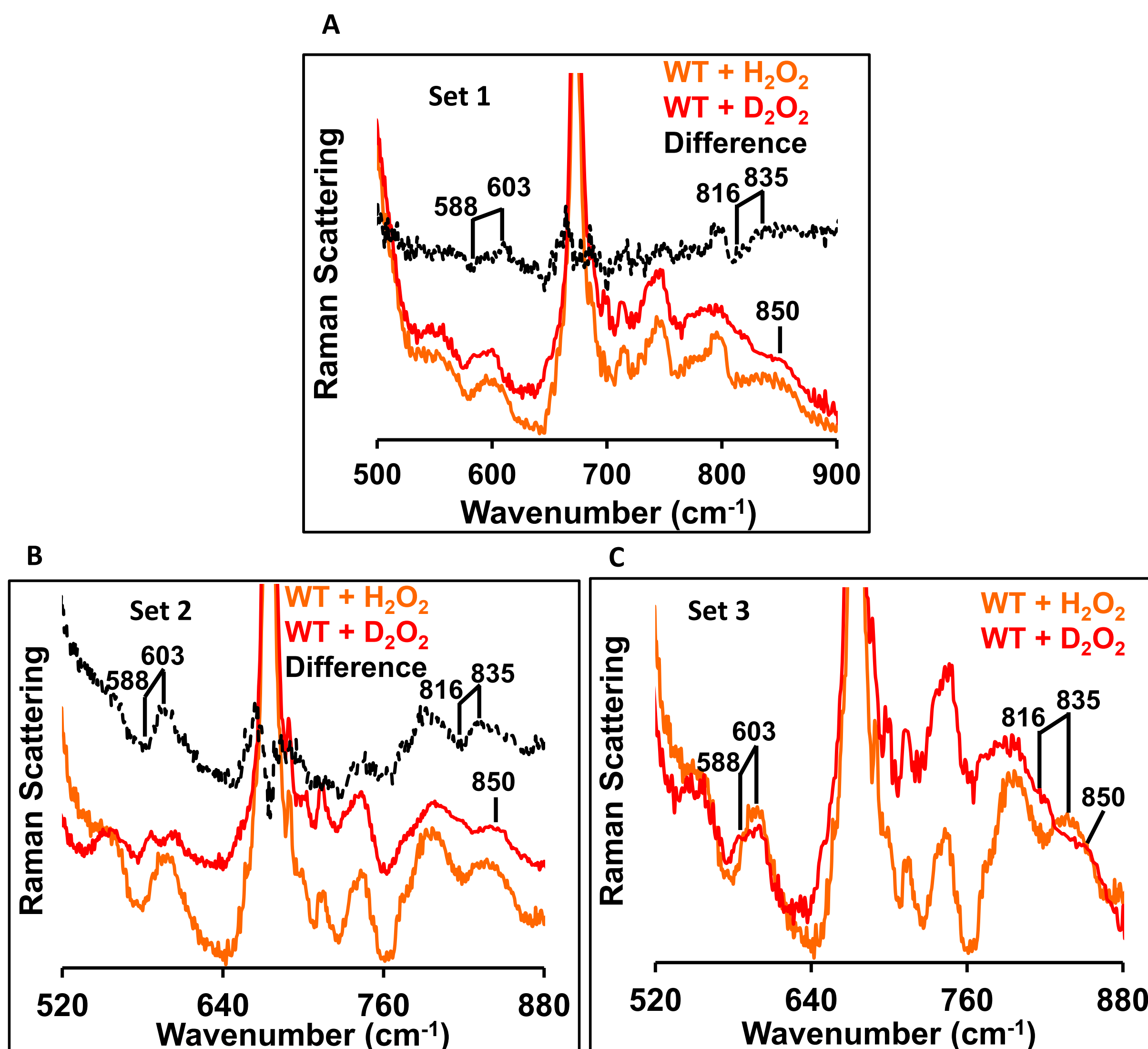


Figure S10. Different sets of reproducible data of low frequency region of the resonance Raman spectra for the reaction of low-spin heme-Aβ (WT, LS) and H_2O_2 (orange) and low-spin heme-Aβ (WT, LS) and D_2O_2 (red) A) set 1; B) set 2; C) set 3. The reaction is done in 100 mM phosphate buffer at pH 7.6 and data are obtained with an excitation wavelength of 413.1 nm (5 mW) at 77 K.

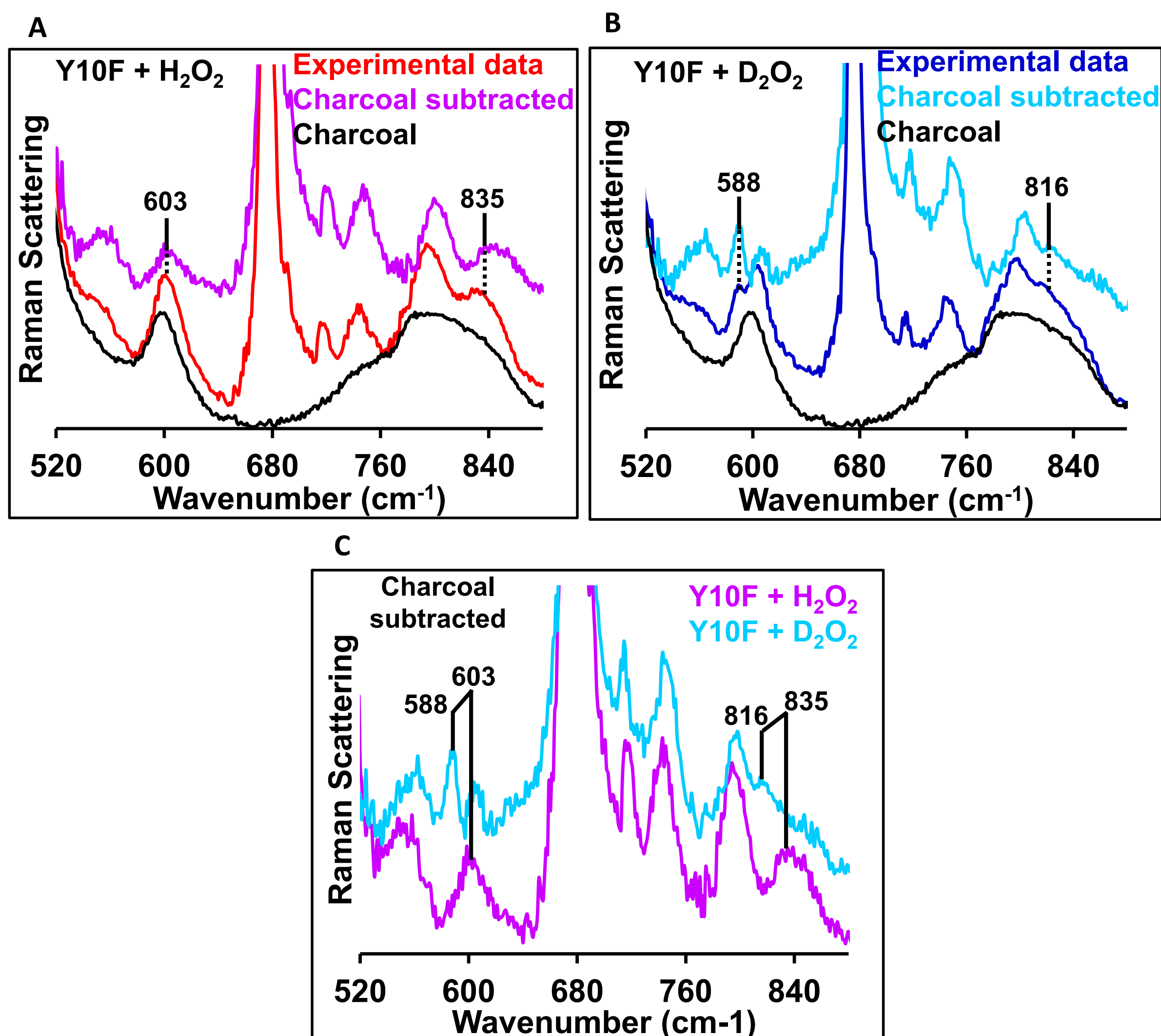


Figure S11. Low frequency region of the resonance Raman spectra of A) low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and H_2O_2: experimental spectrum (red), charcoal subtracted spectrum (pink) and spectrum of charcoal (black).

B) low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and D_2O_2: experimental spectrum (blue), charcoal subtracted spectrum (cyan) and spectrum of charcoal (black).

C) Overlay of charcoal subtracted data of low-spin heme-Aβ Tyr10Phe mutant (Y10F, LS) + H_2O_2 (pink) and low spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and D_2O_2 (cyan). Subtraction of charcoal spectrum from the experimental spectra is done to cancel background noise from the quartz tube.

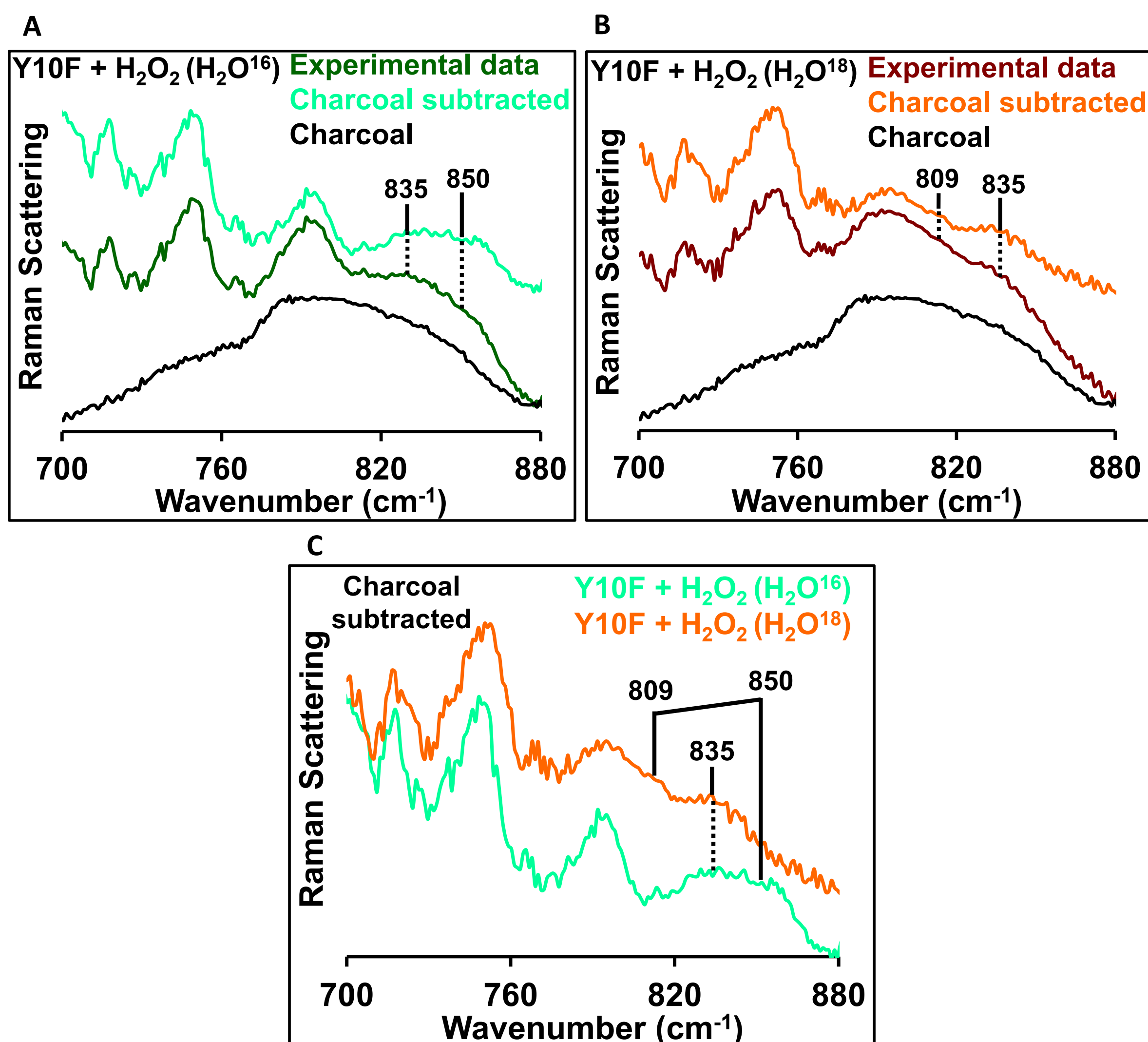


Figure S12. Low frequency region of the resonance Raman spectra of A) low spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and H_2O_2 (in H_2O^{16}): experimental spectrum (dark green), charcoal subtracted spectrum (light green) and spectrum of charcoal (black).

B) low spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and H_2O_2 (in H_2O^{18}): experimental spectrum (brown), charcoal subtracted spectrum (orange) and spectrum of charcoal (black).

C) Overlay of charcoal subtracted data of low spin heme-Aβ Tyr10Phe mutant (Y10F, LS) + H_2O_2 (in H_2O^{16}) (light green) and low spin heme-Aβ Tyr10Phe mutant (Y10F, LS) and H_2O_2 (in H_2O^{18}) (orange). Subtraction of charcoal

spectrum from the experimental spectra is done to cancel background noise from the quartz tube.

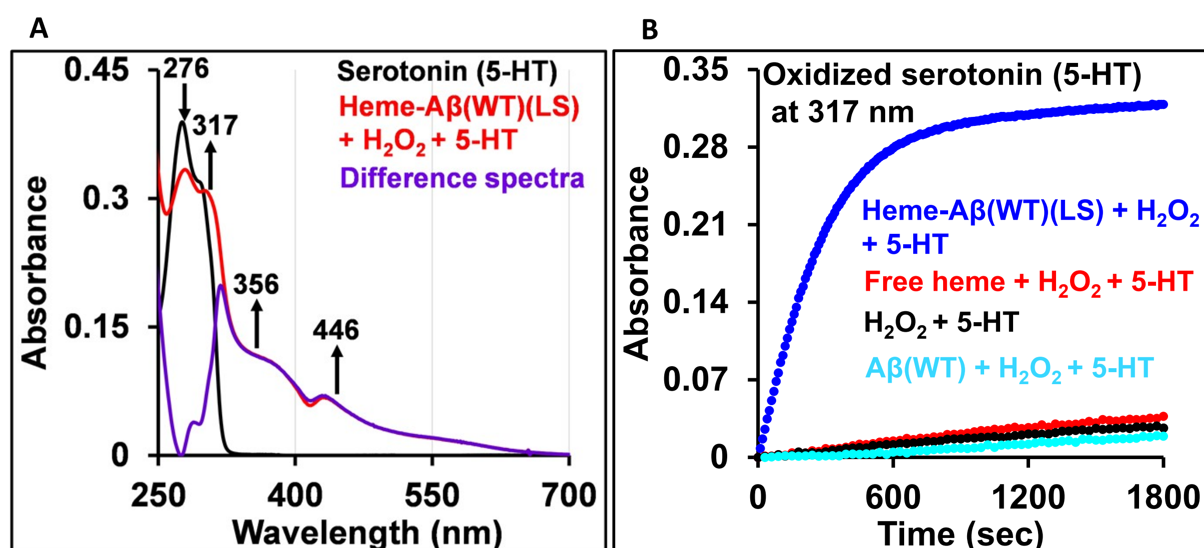


Figure S13. Catalytic serotonin (5-HT) oxidation by wild type low spin heme-Aβ(WT, LS) in presence of H_2O_2, A) Absorption spectra of serotonin (5-HT) (black), oxidized serotonin (5-HT) for wild type low-spin heme-Aβ(WT, LS) and H_2O_2 (red) and difference spectrum obtained by subtracting the spectrum of serotonin (5-HT) from the spectrum of the reaction mixture of wild type low spin heme-Aβ (WT, LS) + serotonin (5-HT) + H_2O_2 (1:400:80) (purple); B) Comparison of the rate of serotonin (5-HT) oxidation of wild type low-spin heme-Aβ(WT, LS) + H_2O_2 + serotonin (5-HT) (blue), free heme + H_2O_2 + serotonin (5-HT) (red), H_2O_2 + serotonin (5-HT) (black) and only Aβ(WT) + H_2O_2 + serotonin (5-HT) (cyan); monitored at 317 nm in 100 mM phosphate buffer at pH 7.6.

References

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2 L. A. Marquez and H. B. Dunford, *Biochemistry*, 1997, **36**, 9349–9355.