Supporting Information

Formation of Compound I in Heme bound Aβ-peptides relevant to Alzheimer's Disease

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Methods

General Methods

All reagents were of the highest grade commercially available and were used without further purification. Aβ peptides of chain lengths 1-40 and 1-16 have been used for this study. The site directed mutants of Aβ(1-16) used were Arg5Asn and Tyr10Phe. All peptides were purchased from Ontores, China with >95% purity. Hemin, the buffers, Serotonin and m-CPBA were purchased from Sigma. All the experiments were done at pH 8 and Aβ(1-40), Aβ(1-16) and all the mutants were made in 50 mM phosphate buffer, while hemin solution was made in 1 M NaOH solution. Peptide stock solutions were 0.5 mM for absorption, EPR and resonance Raman studies. Heme-Aβ complexes were prepared by incubating 1 equivalent of Aβ solution with 0.8 equivalent of heme solutions for ~1 h. Serotonin (5-HT) was prepared in Millipore water and was of 5 mM strength. Meta-chloro perbenzoic acid (m-CPBA) was prepared by dissolving it in 20%(v/v) Acetonitrile-water mixture.

Physical Methods

Absorption spectra

Absorption spectra were recorded by adding ~100 μL of the heme-Aβ complex solution in a cuvette of 1 mm path length. Absorption spectra were obtained by a UV-Vis diode array spectrophotometer (Agilent 8453).

Stopped Flow

Stopped-flow analysis of the reaction of heme-Aβ complexes and it's mutants with m-CPBA were performed on SFM 4000 stopped-flow

absorption spectrophotometer (light source Xe lamp). The reactions were performed by mixing 0.3 mM of heme-Aβ complexes (wild type and mutants) with 2 mM m-CPBA in 1:10 concentration ratio.

The formation and decay time of Compound I was calculated from the kinetic trace followed at 675 nm. The time where kinetic trace at 675 nm shows maximum was considered to be the absorption spectrum of Compound I (e.g. 27 msec for heme-Aβ(1-16)). In order to observe the absorption bands of Compound I clearly, the initial spectrum of heme-Aβ (* a factor) was subtracted from that spectra to remove contribution of some unreacted heme-Aβ. The difference spectrum is commonly used to clearly observe the formation of new bands of low intensities.

Fitting of Kinetics Data

$y = y_0 + A_0 \exp(-kt)$

The above monophasic equation was used to fit the kinetics data and thus obtain the formation and decay rate constants. A positive pre-exponential factor 'A_0' was used to fit the decay kinetics while a negative value of the same was used to fit the formation kinetics. Here 'k' is the rate constant and 'y_0' is a constant.

Serotonin Oxidation

Absorption spectra and kinetics of serotonin oxidation was monitored in a UV-vis diode array spectrophotometer (Agilent 8453). The concentration of serotonin solution was 5 mM. Strength of heme- Aβ solution and m-CPBA were 0.3 mM and 500 mM respectively. Kinetics experiment was performed by adding 5 µl heme- Aβ solution and 8.5 µl m-CPBA to 70 µl serotonin taken in 1.2 ml 50 mM pH 8 phosphate buffer. The concentration ratio of Heme-Aβ, m-CPBA and serotonin was 1: 2360: 176.

Peroxidase Activity

3,3′,5,5′-Tetramethylbenzidine (TMB) was used as the substrate for peroxidase activity measurement. A 0.5 mg portion of TMB was dissolved in 50uL of glacial AcOH. Kinetics experiment was performed by adding 25 μl heme- Aβ solution (0.1 mM) and 15 μl m-CPBA (1 M) to 15 μL TMB (83 mM), taken in 1.2 ml 50 mM pH 8 phosphate buffer. The concentration ratio of Heme-Aβ, m-CPBA and TMB was 1: 6000: 498. Kinetic traces were obtained by monitoring the increase of the 652 nm absorption band with time.

EPR

EPR spectra were obtained with a JEOL FA200 spectrometer. The EPR samples were 0.35 mM in concentration and were prepared by adding 5 equivalents of m-CPBA to heme-Aβ (Tyr mutant) solution frozen in a low temperature n-Pentane bath (kept at -20^0C) and were run at 11K and 13K in liquid He setup(JANIS cryostat). The 77 K data of these samples were acquired in a liquid nitrogen finger dewar. EPR settings were as follows: Freq. ≈ 9.13GHz, Power ≈ 1mW, Mod. Width = 16 gauss, Amplitude = 50.00, Time Constant = 0.03 sec, Sweep time = 30 sec.

Resonance Raman

RR data were obtained using a Trivista 555 spectrograph (Princeton Instruments) using 415 nm excitation from a diode laser (MDL-E-415-50mW). The optics (plano-convex lens, mirror etc.), used for the collection of rR data were purchased from Sigma-Koki Japan. The power on the samples was ~5 mW. The rR samples were 0.35 mM in concentration and were prepared by adding 5 equivalents of m-CPBA to Tyr10Phe mutant, frozen in a low temperature n-Pentane bath (kept at -20^0C). For the preparation of compound I of the native peptide with m-CPBA , SFM-400 Rapid Freeze Quench (RFQ) technique was used and

samples were frozen in a low temperature n-Pentane bath (kept at – 60^{0}C).

HPLC

Oxidation products of 5-HT by heme–Ab complexes were separated by reversed-phase HPLC (RP-HPLC) using a Waters 1525 Separation Module coupled to a diode-array detector (Waters 2487). A Symmetry^R C18 reversed-phase column (250 mm 9 4.6 mm, 5 lm particle size) (Phenomenex) was used to separate 5-HT from its oxidation products using the linear gradient method. The mobile phase consisted of eluant A (1 % acetic acid and 1 mM ammonium acetate in water) and eluant B (100 % acetonitrile). The gradients applied were 0–10 min, 98–85 % eluant A; 10–12 min, 85–50 % eluant A; 12–14 min, 50 % eluant A. The flow rate was 0.8 mL/min. For the HPLC assay, 5 mM 5-HT was incubated with 335 mM H2O2/ m-CPBA and 0.3 mM heme–Ab in 100 mM Hepes, pH 8 with a conc. ratio of Heme-Aβ, m-CPBA and serotonin to be 1: 2360: 176. At specific time intervals, the oxidation products were analyzed by injecting 25 μL of the reaction mixture into the column. Serotonin and its oxidation products were analyzed with a UV-online detector set at 260 nm.

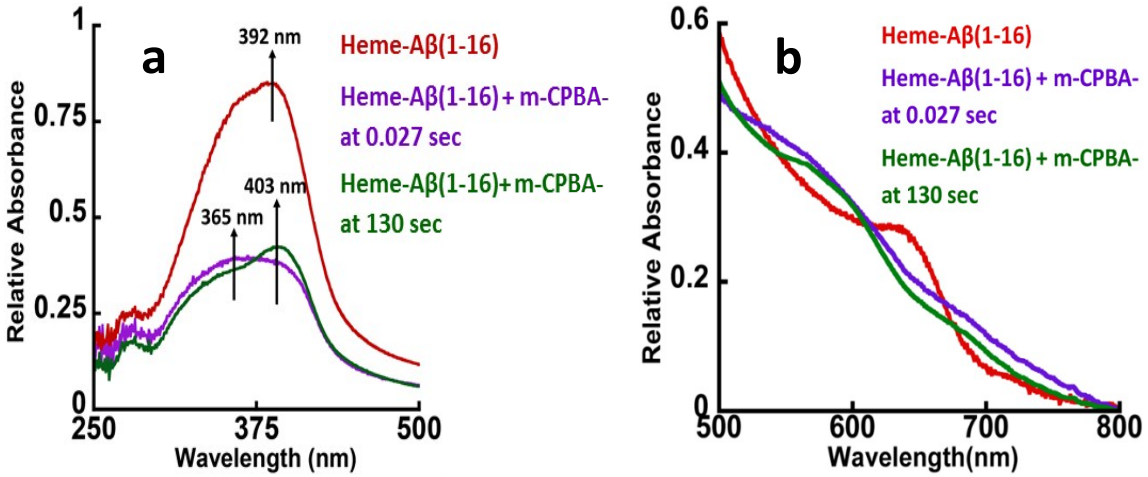

Figure S1. Absorption spectrum of heme-Aβ(1-16), red ; for the reaction of heme-Aβ(1-16) with m-CPBA, in 50 mM PO_4^{3-} buffer at pH 8 at 0.027 sec, purple ; and at 130 sec, green a) in the Soret region b) in the Q-band region.

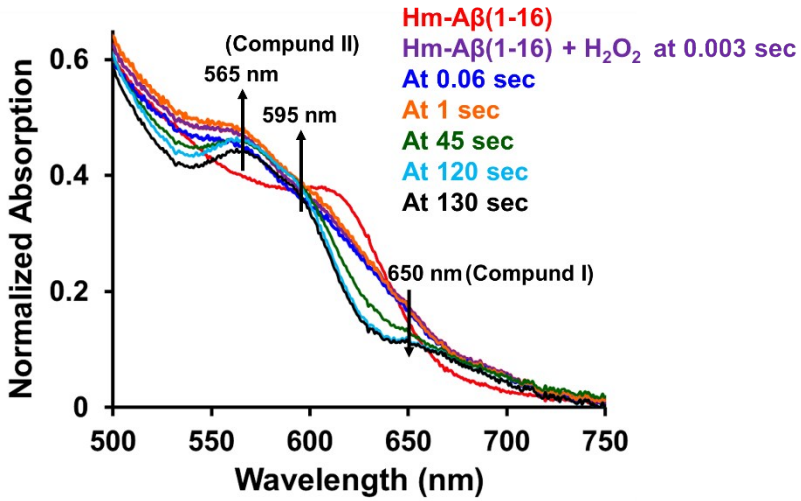

Figure S2. Absorption spectrum of heme-Aβ(1-16), red ; for the reaction of heme-Aβ(1-16) with H_2O_2, the difference spectrum at 0.003 sec, purple ; at 0.06 sec, blue; at 1 sec, orange; at 45 sec, green; at 120 sec, cyan and at 130 sec, black; in 50 mM PO_4^{3-} buffer at pH 8. The *arrows* indicate the direction of the spectral changes for compound I and compound II.

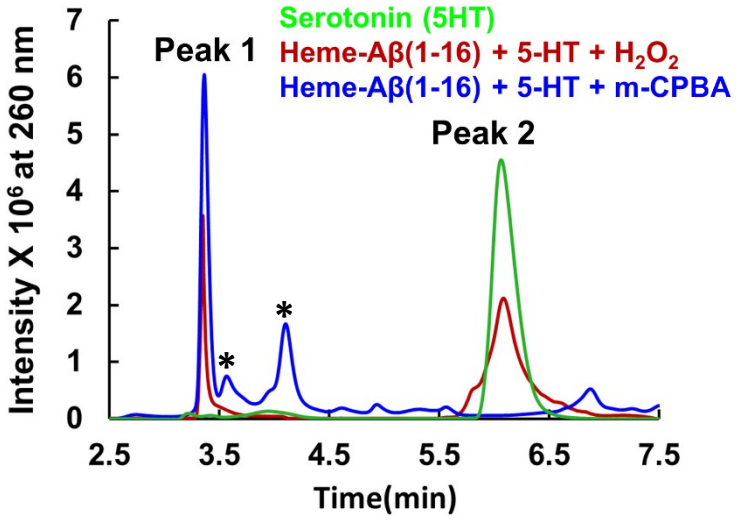

Figure S3. Separation of the 5-HT and its products by RP-HPLC after its incubation with heme–Ab(1–16) + H_2O_2 and .heme–Ab(1–16) + m-CPBA; 5-HT without reaction mixture (peak 2), green, separated reaction mixture injected 3.25 min after incubation resulting in major product (peak 1) for heme–Aβ(1–16) + H_2O_2, red and heme–Aβ(1–16) + m-CPBA, blue in 50 mM PO_4^{3-} buffer at pH 8. These peaks (*) are minor products which are likely the oxidized products of serotonin, 3,5-dihydroxy-3-ethyl amino-2-oxindole (3,5-DHEO) and 5,6-dihydroxytryptamine (5,6-DHT).[1]

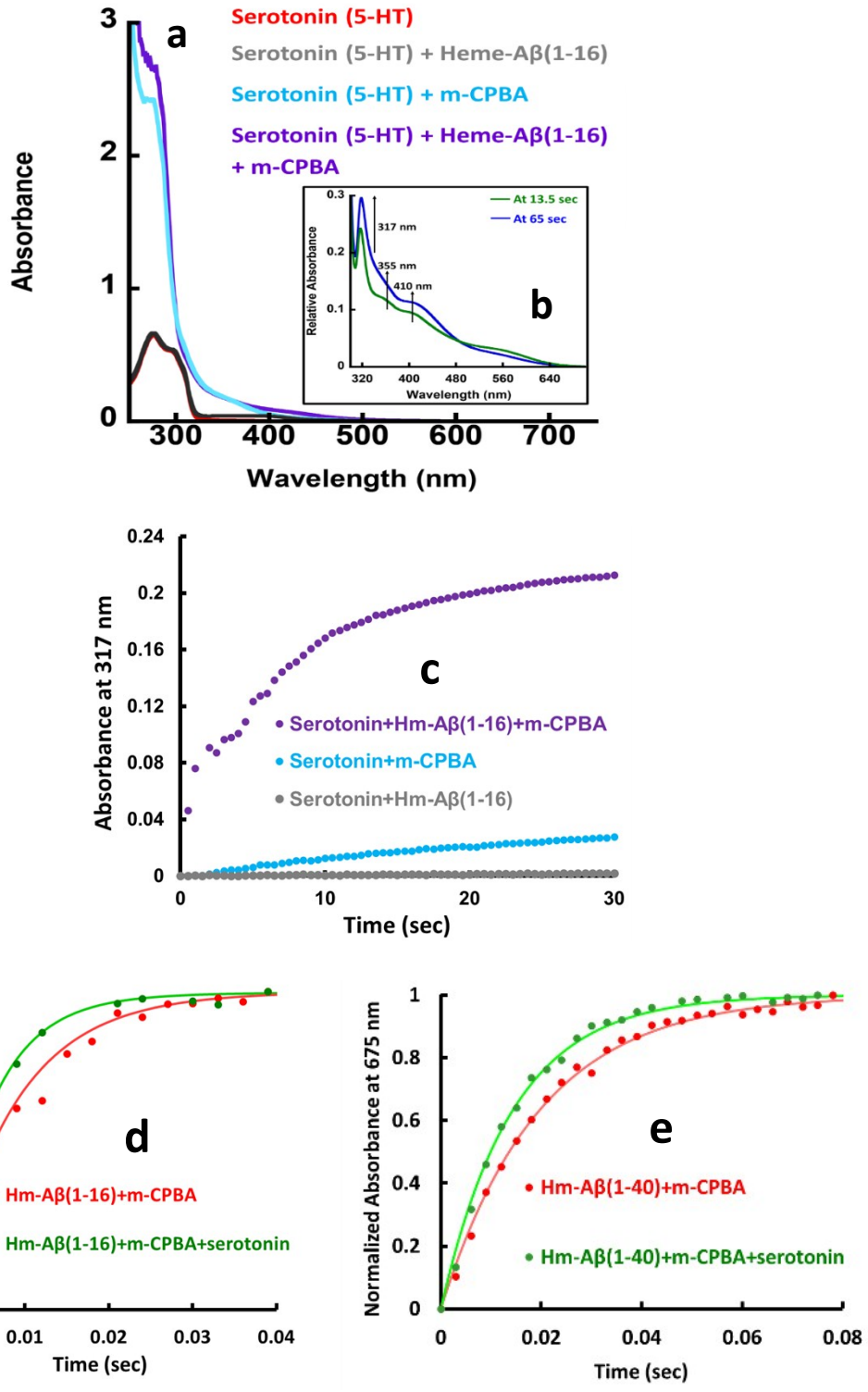

Figure S4. a) Absorption spectrum of serotonin (5-HT), red ; reaction of serotonin with heme-Aβ(1-16), grey ; serotonin with m-CPBA, cyan ; serotonin with heme-Aβ(1-16) and m-CPBA, violet ; b) Difference

Spectra of oxidized compared with unoxidized serotonin (5-HT) at 13.5 sec, green and 65 sec, blue respectively. c) Kinetic trace for the reaction of serotonin with heme-Aβ(1-16) and m-CPBA, purple ; serotonin with m-CPBA, cyan ; serotonin with heme-Aβ(1-16), grey d) Comparison of formation of compound I during reaction of heme-Aβ(1-16) with m-CPBA, in absence of substrate, red; and in presence of substrate, green.

e) Comparison of formation of compound I during reaction of heme-Aβ(1-40) with m-CPBA, in absence of substrate, red; and in presence of substrate, green.

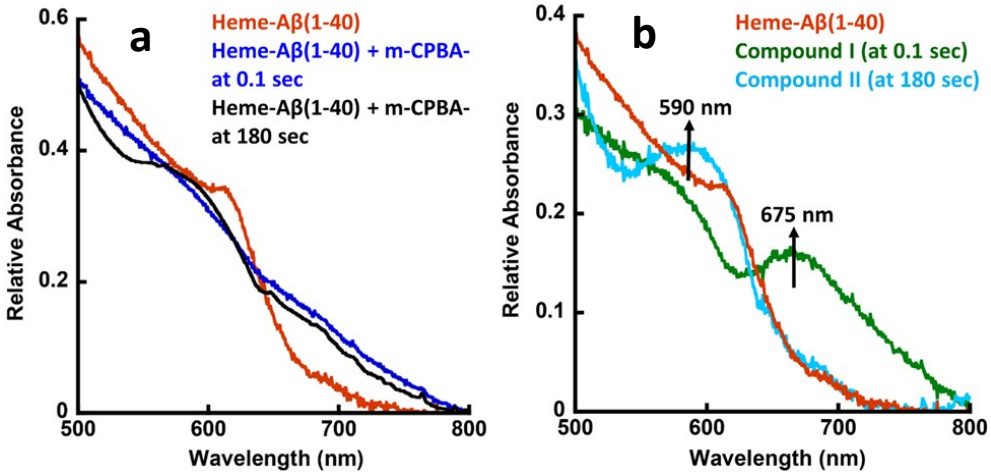

Figure S5. a) Absorption spectra of heme-Aβ(1-40), orange ; heme-Aβ(1-40) with m-CPBA, at 0.1 sec, blue ; and at 180 sec, black. b) Absorption spectra of heme-Aβ(1-40), orange ; difference spectrum of heme-Aβ(1-40) with m-CPBA, at 0.1 sec (Compound I), green and at 180 sec (Compound II), cyan. The *arrows* indicate the direction of the spectral changes. Data acquired in 50 mM PO_4^{3-} buffer at pH 8.

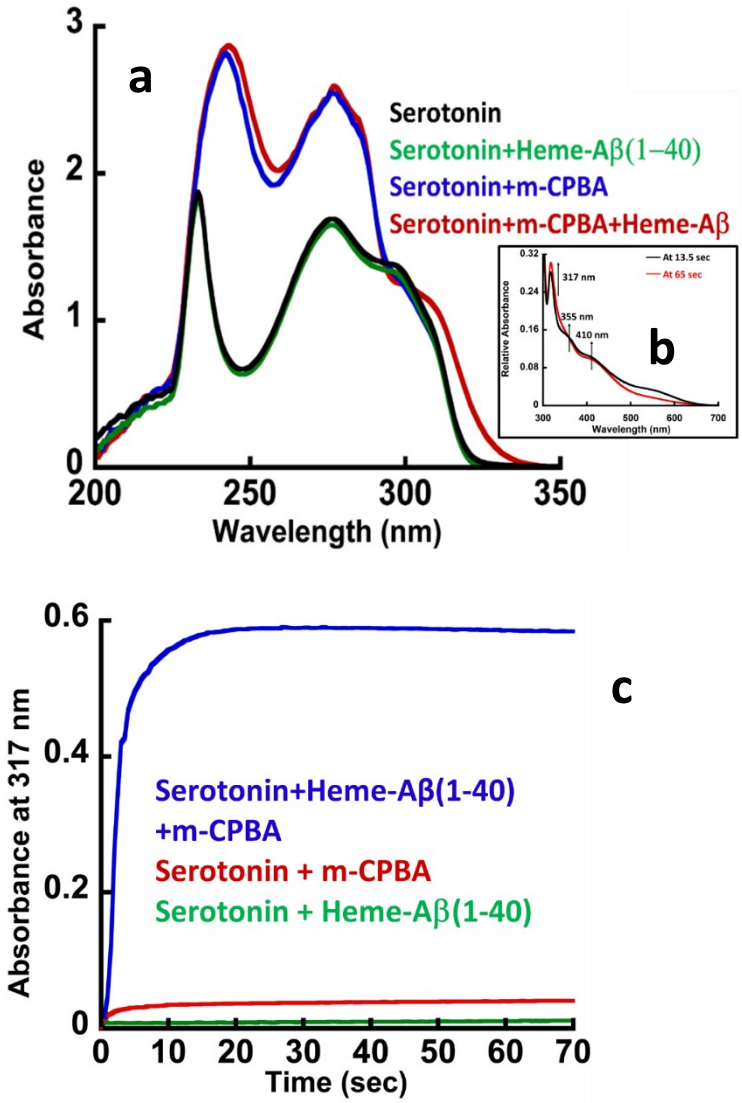

Figure S6. a) Absorption spectrum of serotonin (5-HT), black ; reaction of serotonin with heme-Aβ(1-40), greeen ; serotonin with m-CPBA, blue ; serotonin with heme-Aβ(1-16) and m-CPBA, red ; b) Difference Spectra of oxidized compared with unoxidized serotonin (5-HT) at 13.5 sec, black and 65 sec, red respectively. c) Kinetic trace for the reaction of serotonin with heme-Aβ(1-40) and m-CPBA, blue ; serotonin with m-CPBA, red ; serotonin with heme-Aβ(1-16), green.

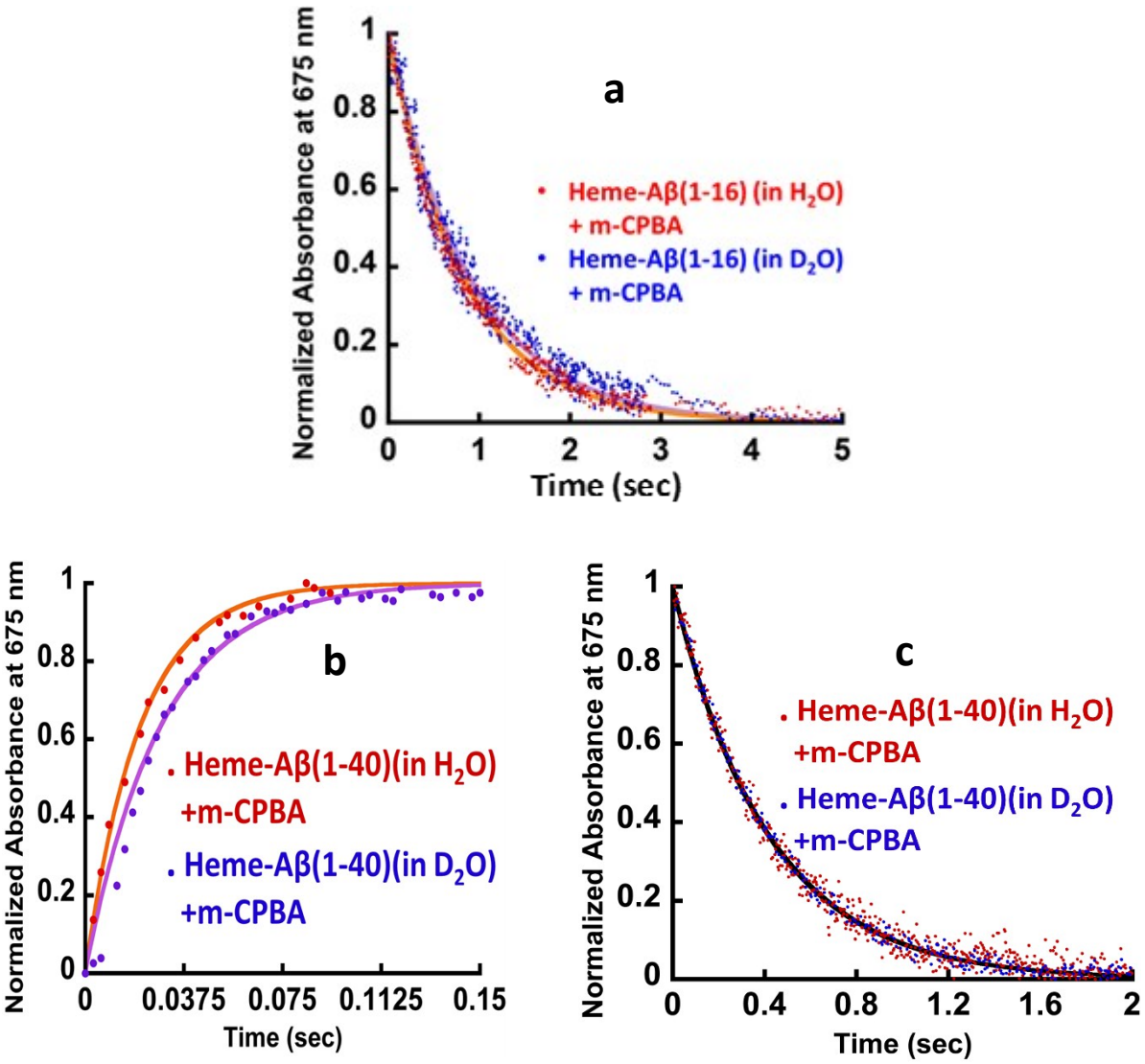

Figure S7. Kinetic trace for a) decay of Compound I of heme-Aβ(1-16) in H_2O (pH 8), red; and in D_2O (pD 8), blue ;.b) formation of Compound I of heme-Aβ(1-40) in H_2O (pH 8), red; and in D_2O (pD 8), blue; and c) decay of Compound I of heme-Aβ(1-40) in H_2O (pH 8), red; and in D_2O (pD 8), blue ; followed at 675 nm

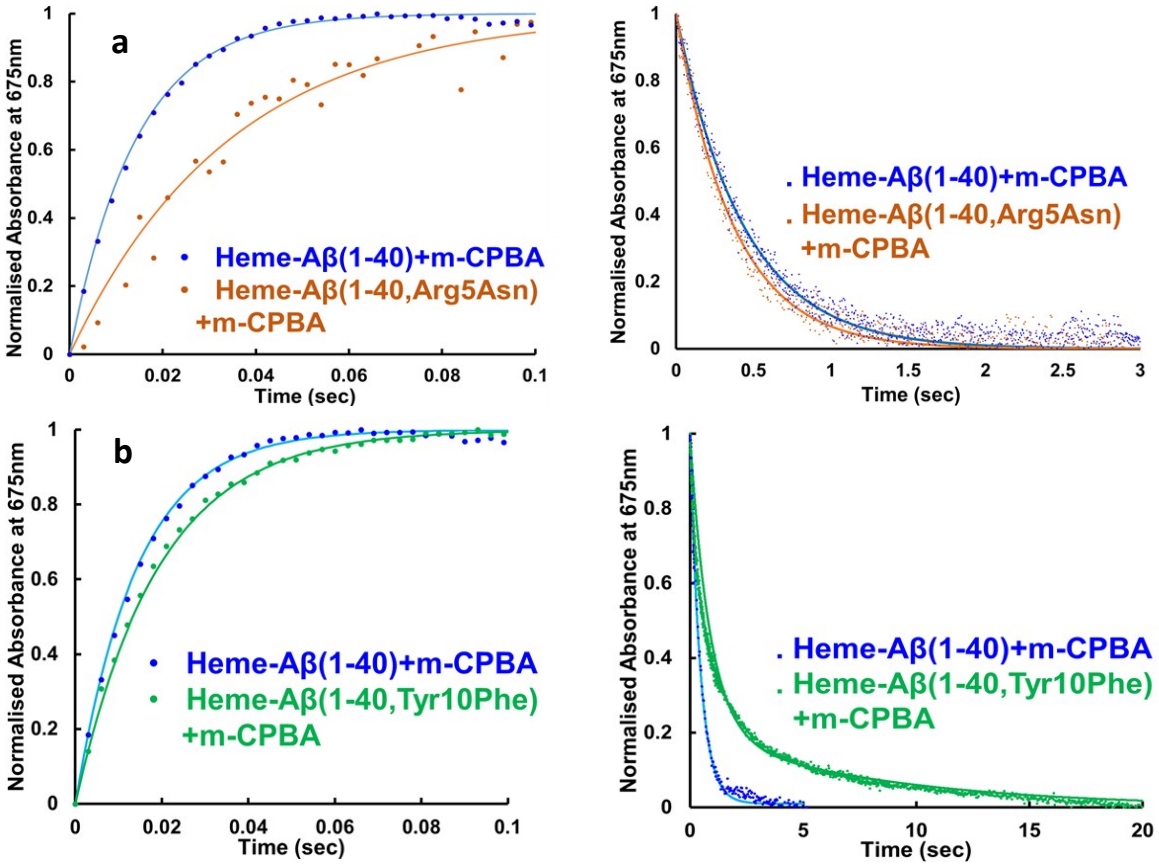

Figure S8. a) Formation and decay of Compound I during reaction between heme-Aβ(1-40) and m-CPBA, blue; and between heme-Aβ(1-40, Arg5Asn) and m-CPBA, orange. b) Formation and decay of Compound I during reaction between heme-Aβ(1-40) and m-CPBA, blue; and between heme-Aβ(1-40, Tyr10Phe) and m-CPBA, green followed at 675 nm, in 50 mM PO_4^{3-}buffer at pH 8.

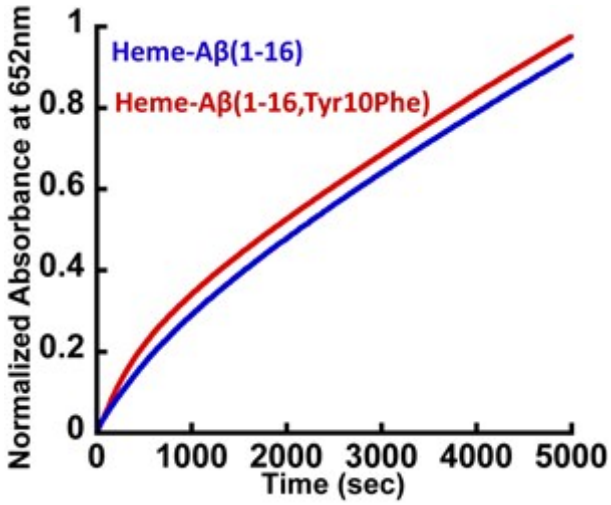

Figure S9. Kinetic trace of peroxidase activity of Heme-Aβ(1-16), blue and Heme-Aβ(1-16,Tyr10Phe), red; followed at 652 nm for the oxidation of TMB at pH 8 in 50 mM PO_4^{3-} buffer.

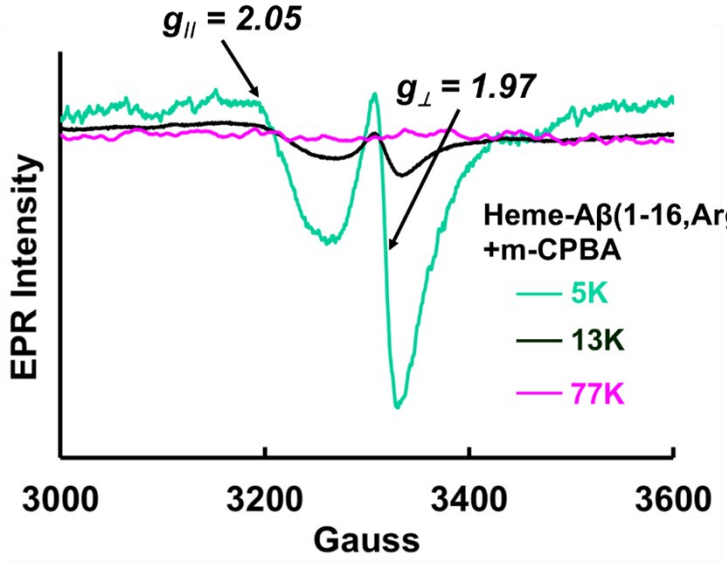

Figure S10. Temperature dependent EPR spectra of heme-Aβ(1-16, Arg5Asn) complex on reaction with m-CPBA (mixing time 5 sec), at 5 K, green; at 13 K, black and at 77 K, pink; in 50 mM PO_4^{3-} buffer at pH 8.

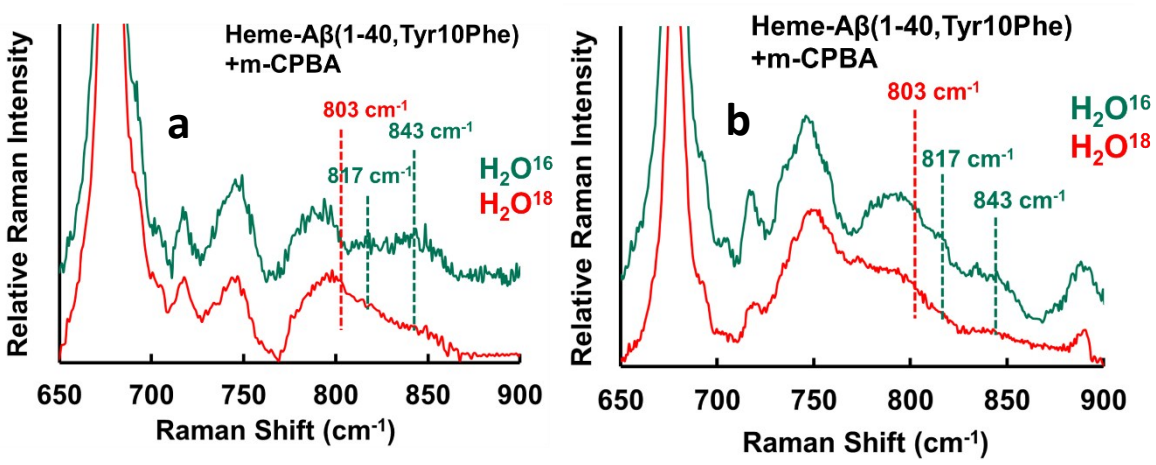

Figure S11. Low frequency region of resonance Raman spectra of heme−Aβ(1-40, Tyr10Phe) + m-CPBA in H_2O^{16},green; H_2O^{18} , red; a) data set 1, b) data set 2; data were obtained with an excitation wavelength of 413.1 nm (15 mW) at 77 K.

Table S1. Absorption bands of Compound I above 600 nm

	Heme Proteins / Porphyrins	Axial Ligand	Absorption Band of Compound I	References
1	HRP	Histidine	650	*Biochemistry* 1984, *23*, 4743
2	CPO	Cysteine	688	*Biochemistry* 1984, *23*, 6809
3	P450 (CYP119)	Cysteine	690	*Science* 2010, 330, 933
4	Catalase	Tyrosine	657	*Biochemistry* 2007, *46*, 11
5	TMP	Imidazole	667	*Inorg. Chem.* 1997, *36*, 6142

Table S2. Rate constants of formation and decay of Compound I in D_2O, in 50 mM PO_4^{3-} buffer at pD 8 and in H_2O, in 50 mM PO_4^{3-} buffer at pH 8.

Heme-Aβ(1-40) complex	Formation rate constant	Decay rate constant
D_2O	**35 ± 2**	**2.4 ± 0.1**
H_2O	**47 ± 1**	**2.4 ± 0.2**

Reference:

1. M. Z. Wrona, Z. Yang, M. McAdams, S. O'Connor-Coates and G. Dryhurst, *J. Neurochem.*, 1995, 64, 1390-400.