

# Supporting Information - Evidence of redox-active iron formation following aggregation of ferrihydrite and the Alzheimer's disease peptide $\beta$ -amyloid

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### **Anoxic Sampling**

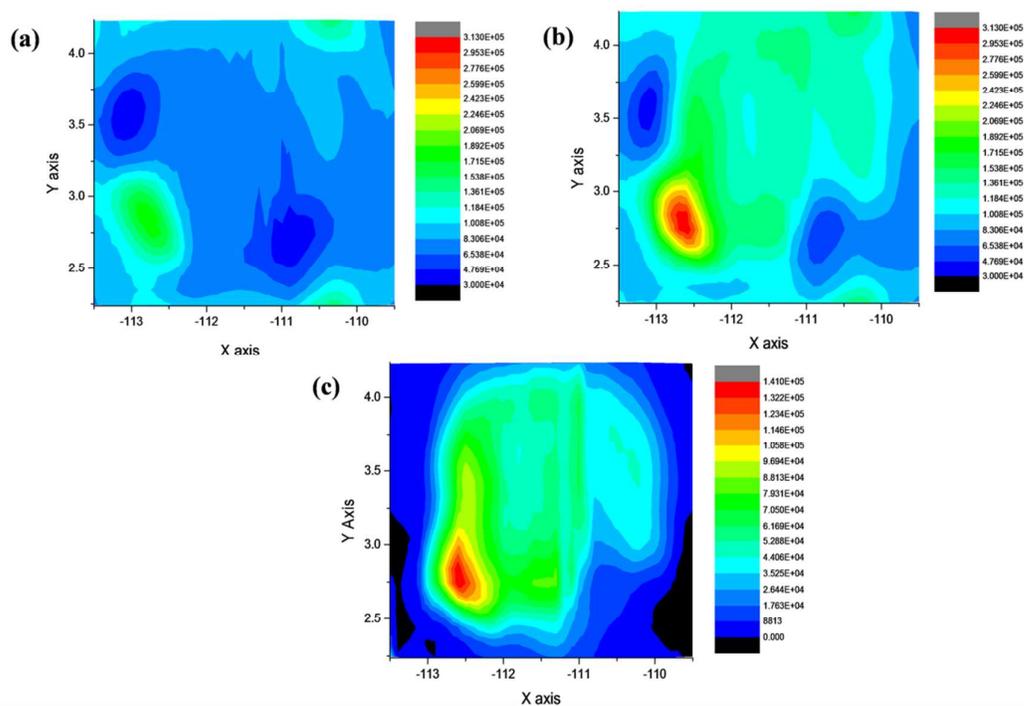
To prevent alterations to the oxidation state of the iron material used, anoxic conditions were created and maintained throughout the sampling and examination process. Sample manipulation was conducted under anoxic conditions within a nitrogen filled glove chamber. Nitrogen filled, O-ring sealed, plastic containers were used to transfer sample probes to the XAS/XMCD beamline for examination. Beamline chambers were backfilled with nitrogen preventing iron oxidation once probes had been inserted. Sample materials were exposed to oxygen for a maximum of one second when being lowered into the beamline chamber. Such methodology has previously been utilized by Coker *et al.* (2009)<sup>1</sup>.

### **Two-dimensional x-ray absorption iron mapping**

To visualize the iron content of the sample area under XAS/XMCD examination, two dimensional maps revealing areas of iron accumulation were created. This was achieved by performing sample area scans just below (706 eV) and on the iron  $L_3$  peak energies (710.5 eV) at a spatial resolution of 100  $\mu\text{m}$ . Off peak x-ray absorption values were then subtracted from the equivalent peak iron x-ray absorption values, resulting in the removal of background absorption signals and highlighting areas of iron accumulation (Figure S1). Once identified these areas of iron accumulation were examined in greater detail by performing x-ray absorption scans over the entire iron  $L$ -edge (700-740 eV).

### **X-ray beam reduction**

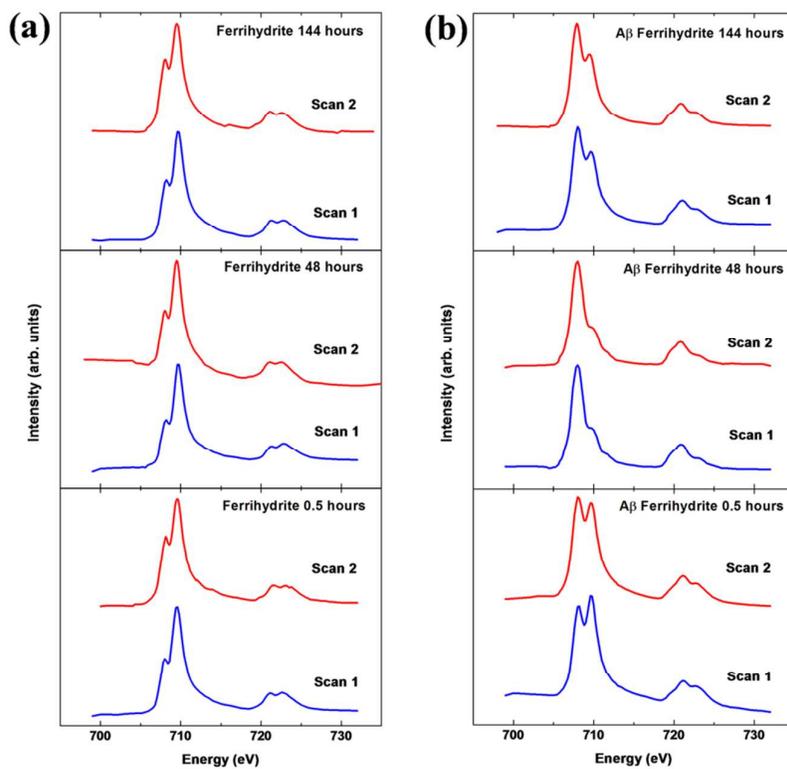
Generally, crystalline iron oxides are not susceptible to x-ray beam induced reduction effects at the soft x-ray energies used in this study. However, as we explored amorphous and weakly crystalline phases it was necessary to assess the influence of the x-ray beam on these phases. To do this we performed successive repeated iron  $L_{2,3}$  x-ray absorption scans on iron accumulation areas and monitored changes in the XAS profile as a function of beam exposure. An example of this process is shown for ferrihydrite solutions incubated in the presence and absence of  $\text{A}\beta$  in Figure S2. Where ferrihydrite was incubated in the absence of  $\text{A}\beta$  (Figure S2a) there was no substantial increase in the  $\text{Fe}^{2+}$  cation peak with successive XAS/XMCD scans for the sample series. This is probably because the ferrihydrite crystalline structure is maintained and therefore the iron mineral is less susceptible to x-ray beam reduction.



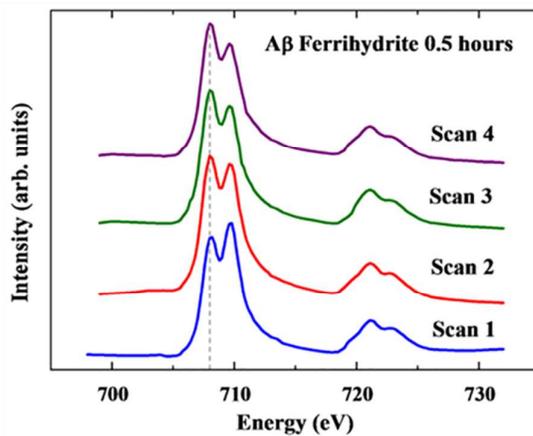
**Figure S1.** Typical x-ray absorption maps of a sample grid containing  $\text{A}\beta$  + ferrihydrite. (a) Off iron peak image (706 eV) and (b) on-peak iron image (710.5 eV). (c) Difference map

showing distribution of iron: for this example an accumulation of iron is seen in the lower left quadrant of the grid area. Iron x-ray absorbance values are shown to the right of each image.

In fact for this series only one sample, ferrihydrite incubated with A $\beta$  over a period of 30 minutes, showed evidence of an x-ray beam reduction effect (Figure S2b, bottom), where the Fe<sup>2+</sup> cation peak can be seen to increase with successive scans. In this case it appears that an unstable (possibly amorphous) phase that is prone to reduction by the x-ray beam, has been formed following 30 minutes of incubation with the amyloid. However, despite evidence of x-ray mediated iron reduction for amorphous/unstable aggregates, we clearly found that x-ray exposure was *not* capable of forming the pure iron (II) phases seen in the A $\beta$ /ferrihydrite series. This is demonstrated in Figure S3, where four successive scans were performed over the iron  $L_{2,3}$  absorption edge of an ferrihydrite aggregate known to be unstable to the x-ray beam. Despite an increase in the Fe<sup>2+</sup> cation peak throughout the first three scans, the reduction effect plateaus after the fourth scan, where an iron(II)/iron(III) intermediate and not a pure iron (II) phase is present. Most importantly, in all cases where amyloid induced iron reduction occurred, the samples were found to be in a heavily reduced state on first XAS examination (e.g. Figure S2b, 48 hour and 144 hour incubations), and the XAS profiles were stable to subsequent x-ray exposure. Therefore, even for samples forming unstable aggregates, it was easily possible to separate reduction caused by amyloid interaction from the effects caused by x-ray exposure.



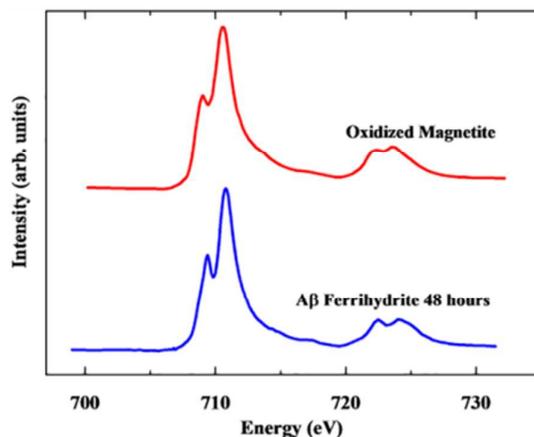
**Figure S2.** X-ray absorption spectra of the ferrihydrite iron *L*-edge in the absence (a) and presence (b) of A $\beta$ , for successive XAS measurement repetitions. Scan number and incubation times are shown above the XAS spectra.



**Figure S3.** X-ray absorption spectra of an unstable ferrihydrite aggregate following four successive XAS measurements. Fe<sup>2+</sup> cation peak is indicated by a grey dotted line at 708eV.

### Oxidation and re-examination of iron(II) phases

The pure iron (II) phase found in the A $\beta$ /ferrihydrite sample after 48 hours of incubation was left to oxidize in air (over a period of several months) before being re-examined using XAS/XMCD. Examination over the iron  $L_{2,3}$  absorption edge (Figure S4) showed iron to be in a pure iron (III) phase with no evidence of iron reduction. This iron was stable in the x-ray beam and did not show enhancement of Fe<sup>2+</sup> cation features after successive XAS scans. The Fe<sup>3+</sup> cation shoulder feature present at 708 eV appeared more pronounced than seen in oxidized magnetite standards (Figure S4) which could suggest the formation of a pure iron (III) mineral different from the oxidation product of magnetite, although further measurements are required to confirm this observation. These data combined with electron diffraction examination of equivalent oxidized A $\beta$ /ferrihydrite samples (Figure 5, main text) suggest that the pure iron (II) phase formed via A $\beta$ /ferrihydrite interaction, oxidizes to an iron mineral with similar lattice parameters to magnetite, but without the magnetic properties of magnetite.



**Figure S4.** X-ray absorption spectrum of ferrihydrite incubated with A $\beta$  for 48 hours with subsequent prolonged air exposure, compared to an oxidized magnetite standard.

(1) Coker, V. S.; Telling, N. D.; van der Laan, G.; Pattrick, R. A. D.; Pearce, C. I.; Arenholz, E.; Tuna, F.; Winpenny, R. E. P.; Lloyd, J. R. *Acs Nano* **2009**, *3*, 1922-1928.