Mass spectrometry of individual ferritin cores using size-selected gold clusters as mass standards in the electron microscope

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Ferritin is an iron storage protein which is believed to be capable of sequestering up to 4500 oxidised iron atoms within its hollow core [1]. The exact stoichiometry of the core is not well understood, with the closest approximation being ferrihydrite ($Fe_5HO_8 \bullet 4H_2O$). Here we demonstrate a novel method for measuring the number of iron atoms present in individual protein cores.

In this study we use scanning transmission electron microscopy (STEM) in the high angle annular dark field (HAADF) mode where the signal recorded is proportional to atomic number. This signal is detected by an annular scintillator detector below the sample, which can be adjusted to collect a different angular range of scattered electrons. In this experiment a 200kV Jeol 2100F STEM fitted with CEOS aberration corrector is used.

Size-selected gold clusters have previously been used as mass standards in quantitative intensity measurements [2], though not for a biologically relevant sample. The clusters are prepared in the gas phase using a cluster beam method [3]; atoms are plasma sputtered from a metal (in this case gold) target, allowed to condense in a flowing rare gas stream and then enter a lateral time-of-flight mass filter, which is used to select clusters of a particular size prior to deposition onto a copper electron microscopy grid covered with an amorphous carbon support film. The high mass resolution (5%) of the deposited clusters enables them to be used as precision mass standards [2].

Electron microscopy grids covered with an amorphous carbon support film were half-decorated with size-selected gold clusters. Equine spleen ferritin (Sigma-Aldrich, UK) was then deposited from solution onto the other half of the grid. This method minimizes the variability in imaging conditions between the target and reference samples. Images were taken in HAADF mode; at exactly the same magnification, collection angles and beam current.

Atomic resolution images of the ferritin protein core confirm a subunit structure in the core [4]. The mass spectrometry measurement gives a nuclearity of 5540 ± 1140 iron atoms per core, assuming a ferrihydrite composition and a negligible contribution from the protein shell. This compares with the accepted value for the maximum loading of 4500 iron atoms per core [1], which is dependent on the assumed stoichiometry.

In conclusion we have shown for the first time that size-selected clusters can be used as mass balances to characterise the metal loading in biological molecules, which in the case of ferritin, is essential for providing insight into the iron uptake process and the ultimate saturation level of the protein compared to that found in nature. To this end, a further study has been carried out whereby the ferritin protein shell is both systematically filled up with iron, and the iron core reduced to allow escape from the shell to model the physiological environment. These results will be presented where the mass of the resultant cores will be calibrated against size-selected clusters using the method described above.

References

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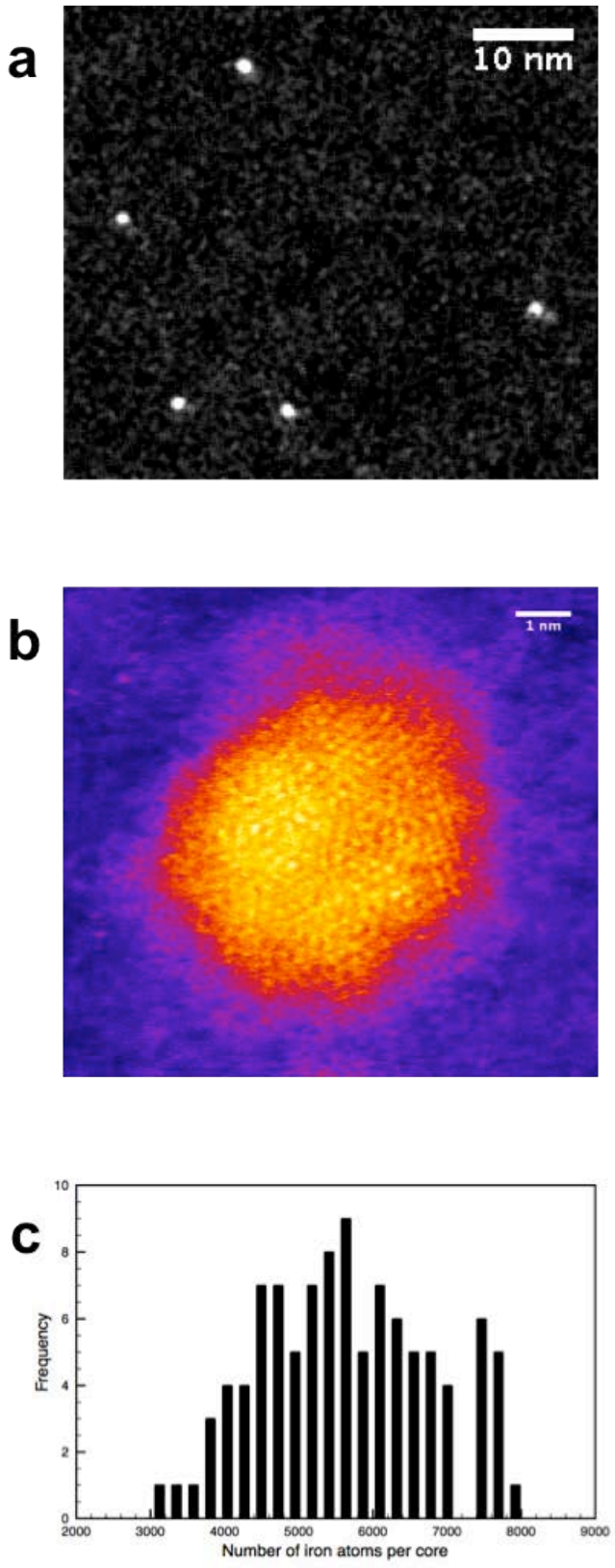

***Figure 1**. **a.** It can be seen that gold size-selected clusters (55 atoms) are mono-disperse in both diameter and intensity and are therefore excellent candidates for use as mass standards. **b.** An atomic resolution STEM image of a single ferritin core in HAADF mode (false colour applied), atomic columns within the iron oxide core can clearly be resolved. **c.** Distribution of the number of iron atoms per ferritin core.*