REDOX ACTIVE SUPRAMOLECULAR PROTEIN TEMPLATES FOR NANOPARTICLE SYNTHESIS AND ORGANIZATION

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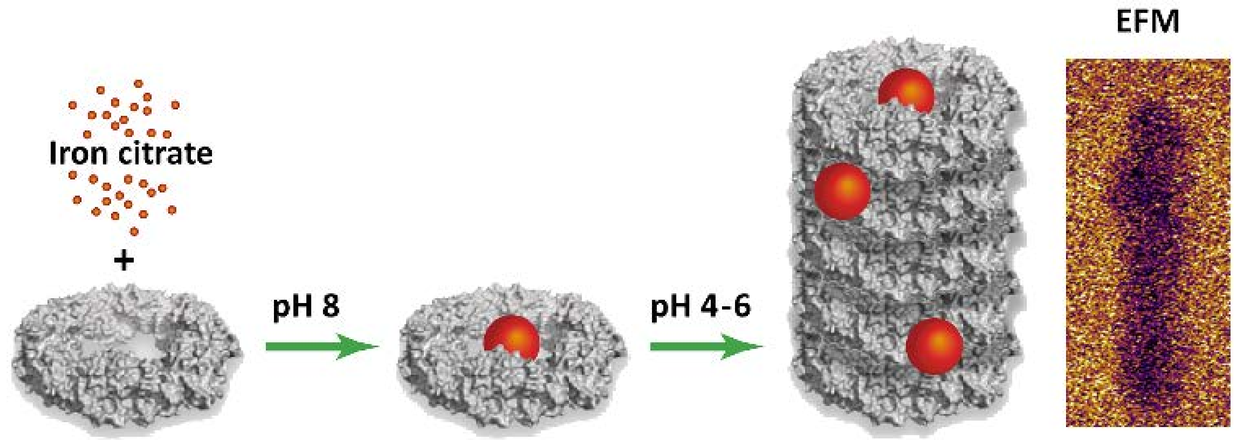
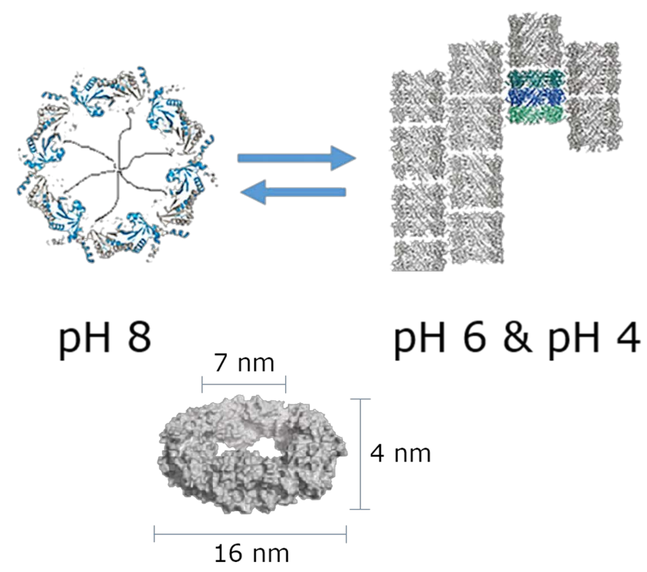
BACKGROUND

Hierarchical nanoparticle assemblies are a subject of wide interest for their emergent functions. The natural ability of proteins to form organized assemblies and their structural diversity makes them ideal candidates to build such assemblies. In this work, a toroidal shaped, redox active protein peroxiredoxin (Prx) was used to synthesize and organize iron oxyhydroxide nanoparticle assemblies. We report the synthesis, organization and characterization of Prx-nanoparticle assemblies by chromatography and microscopy.



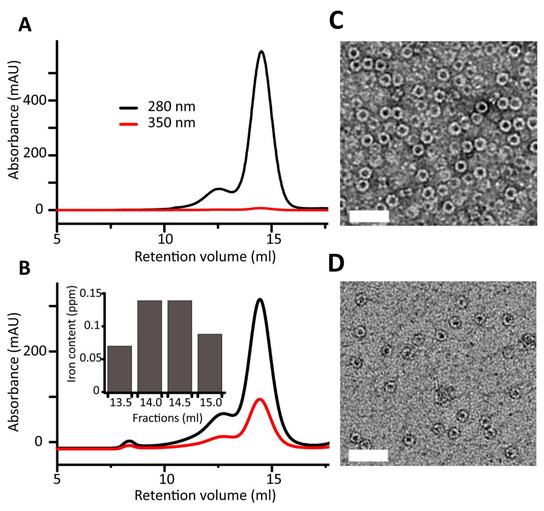
PEROXIREDOXIN

Peroxiredoxin (Prx) is a class of peroxidases that is capable of forming an array of Supramolecular assemblies. In solution, at pH 8.0 they exist as a toroidal dodecameric ring and assembles into higher order structures from pH 7.2 and below. Histidine functionalized pore, as a centre for metal ion mineralization.



- Salt bridges between key residues glutamic acid (E), lysine(L) and histidines (H) present on the surface play an important role.
- Change in pH and ionic strength can alter electrostatic interactions forcing rings to form higher order structures.

SEQUESTRATION OF IRON INTO Prx PORE



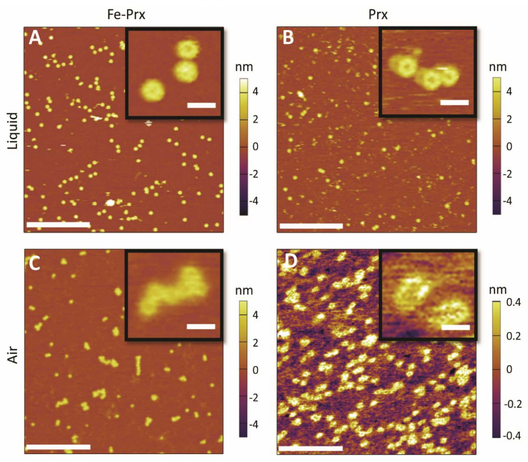
- To sequester iron into Prx, we used a citrate based strategy
- Absorbance was monitored at 280 nm for the protein and at 350 nm for the mineral respectively.
- Size exclusion chromatography (SEC) and Inductively coupled plasma-mass spectrometry (ICP-MS) confirmed the presence of iron in the protein.

SEC profiles of A) Prx B) Fe-Prx C) & D) corresponding TEM images.

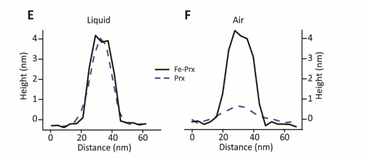
$$Fe^{2+} + Citrate \rightleftharpoons Fe^{2+}\text{-}Citrate \rightleftharpoons Fe^{3+} + Citrate \rightleftharpoons Fe^{3+}\text{-}Citrate$$

Mechanism of Fe binding to Prx

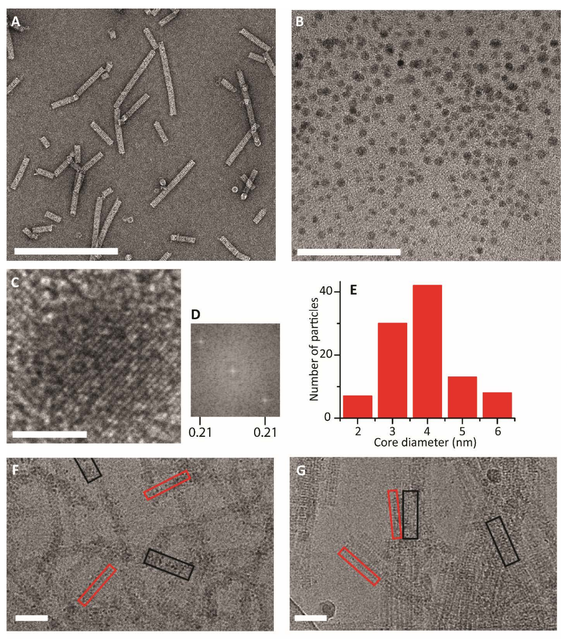
Fe-Prx RINGS



- AFM images reveal intact protein rings with clearly resolved central cavity in Fe-Prx and Prx rings.
- Fe-Prx did not show any reduction in height when imaged in air.
- Possibly due to the mechanical reinforcement caused by the nanoparticle mineralization.

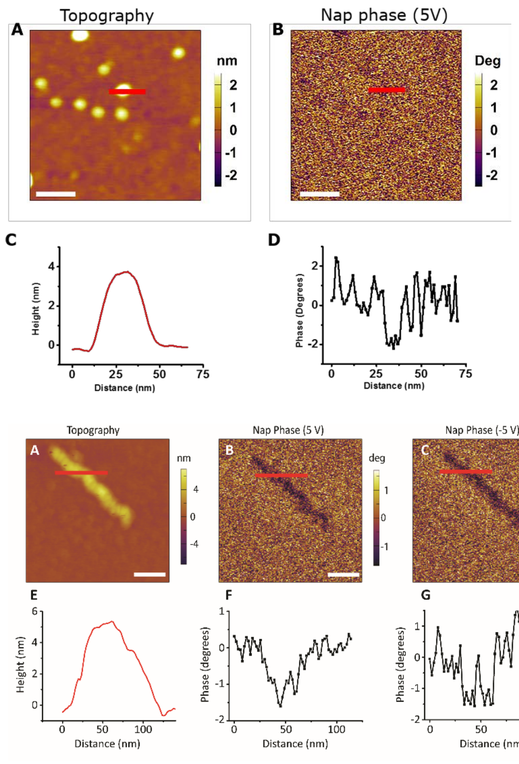


1D Fe-Prx ASSEMBLIES

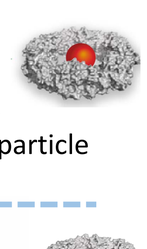


- Prx-Fe was dialyzed into citrate buffer to form higher order assemblies
- Unstained images revealed small nanoparticles of size 4.6±0.9 nm
- HR-TEM and its corresponding FFT revealed particles with a d-spacing of 0.21 nm corresponding to the ferrihydrite space.
- Cryo-TEM indicated at pH 6, particles appear to be associated with the outer surface of the protein (red) and some within the pore (black).
- Particles were released due to protonation of histidines at acidic pH.

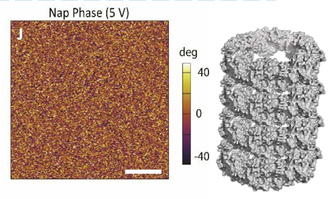
ELECTROSTATIC FORCE MICROSCOPY OF Fe-Prx & ASSEMBLIES



- Particle formation and assembly were monitored with EFM
- A clear EFM phase response was obtained for Prx-Fe rings indicating particle formation.

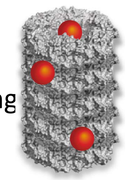


- No EFM phase response was obtained for control



Control!

- EFM phase response was obtained along the length of the stack indicating particle retention



ACKNOWLEGMENTS

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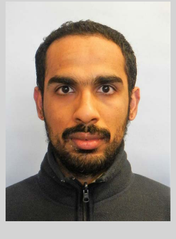
ABOUT THE AUTHOR



Sesha Manuguri is a final year PhD student at the school of chemical sciences, The University of Auckland. My research interests include self-assembly and functional atomic force microscopy. For further information please contact at sman708@aucklanduni.ac.nz

REFERENCES

1.Manuguri, S.; Webster, K.; Yewdall, N. A.; An, Y.; Venugopal, H.; Bhugra, V.; Turner, A.; Domigan, L. J.; Gerrard, J. A.; Williams, D. E.; Malmström, J. *Nano Letters* **2018**, *18*, 5138.
2 Yewdall, N. A.; Venugopal, H.; Desfosses, A.; Abrishami, V.; Yosaatmadja, Y.; Hampton, Mark B.; Gerrard, Juliet A.; Goldstone, David C.; Mitra, Alok K.; Radjainia, M. *Structure* **2016**, *24*, 1120.

CONCLUSIONS

Prx as a building block to synthesize and organize 1D-nanoparticle assemblies. EFM studies indicated the formation of organized nanoparticles assemblies. Such assemblies can be of an interest for electrical and magnetic applications