The redox potentials of the ferroxidase iron-oxo center of *Pyrococcus furisosus* ferritin determined by EPR monitored equilibrium titration

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Ferritins are small alpha-helical proteins that polymerize into 24-mers in the shape of a hollow shell of 8 nm inner diameter. They have the catalytic capacity to oxidize Fe(II) ions and to form, inside their shell, a 'ferrihydrite' mineral core. There is a number of reasons why redox studies on ferritins are indicated: (i) the biological activities of ferritins are redox processes; (ii) the oxidative substrate of ferritins in anaerobes is unknown; (iii) the reductive redox partner of any ferritin is unknown; (iv) the prosthetic redox group of ferritins have only partially been characterized; and (v) nucleation site(s) and early intermediates in core formation have not been extensively characterized.

Recombinant ferritin from *P. furiosus* is expressed in *E. coli* as a 24-mer with *circa* one Fe per subunit. In EPR monitored, dye mediated, redox equilibrium titrations the protein exhibits a mixed valence (Fe^{3+}-μO-Fe^{2+}) S=1/2 signal at intermediate potentials that is a high-resolution homolog of the signal previously described for horse spleen apoferritin anaerobically incubated with Fe(III)/Fe(II) mixtured. *P. furiosus* apo-ferritin when incubated either anaerobically with Fe(III)/Fe(II) or aerobically with Fe(III) and then reduced to intermediate potentials exhibits the same mixed-valence signal, which is assigned to the ferroxidase active center. The subsequent midpoint potentials at neutral pH are both positive, suggesting that the iron storage/release system is wired into cellular metabolism via a much higher potential than previously suggested on the basis of direct coulometric titrations of iron loaded ferritin.