Biomimetic FePt nanoparticle synthesis within *Pyrococcus furiosus* ferritins and their layer-by-layer formation **

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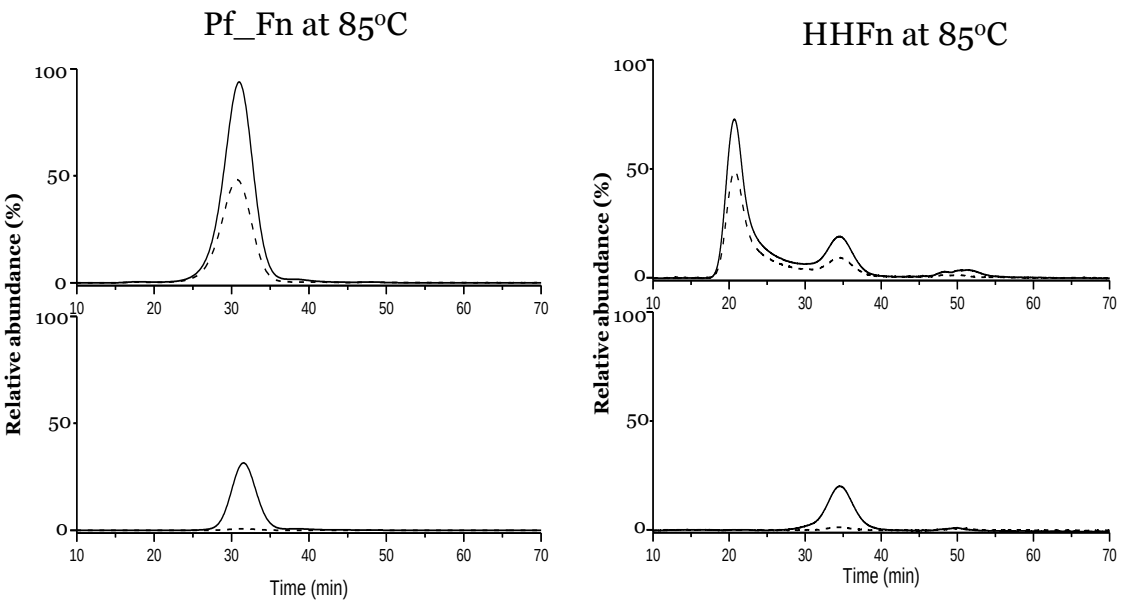
Experimental Section

Biomimetic FePt nanoparticle synthesis : Pf_Fn was diluted to 0.5 mg/mL in a buffer containing 50 mM Tris at pH 8 and pre-heated at 85^{o}C for 5 min. The temperature in the reaction vessel was maintained at 85^{o}C by flowing water through a jacketed flask. Deaerated 6.25 mM solutions of $(NH_4)_2Fe(SO_4)_2$ and K_2PtCl_4 were added with a desired flow for 15 min using a syringe pump until the loading of 500 each metal atoms/cage was achieved. To reduce Fe^{2+} and Pt^{2+} to FePt, 25 mM $NaBH_4$ was added simultaneously to the reaction mixture at the same rate. Reaction was maintained for further 30 min to be annealed at 85^{o}C. After cooling down, reaction was loaded onto a 10 x 300 mm Superose 6 (GE healthcare) size exclusion column and eluted with buffer containing 50 mM phosphate, 100 mM NaCl (pH6.5) at a rate of 0.5 ml/min. To obtain large quantities of FePt mineralized Pf_Fn, we scaled up reaction to 100 ml (total 50 mg protein/reaction and 12 mg FePt/reaction) reaction and still did not see any noticeable amount of aggregation or large cluster on the SEC separation. We obtained 50 mg FePt particles or more by combining 5 reactions together and used for VSM.

Chemical modification of FePt mineralized Pf_Fn: Pf_Fn were incubated with 5 molar equivalents of maleimide-PEG_2-biotin (MPB) or fluorescein-5-maleimide (F5M) at room temperature with vigorous shaking for 3 hrs. Reactions were loaded onto a 10 x 300 mm superose 6 size exclusion column and eluted with buffer containing 50 mM phosphate, 100 mM NaCl, pH6.5 at a rate of 0.5 ml/min.

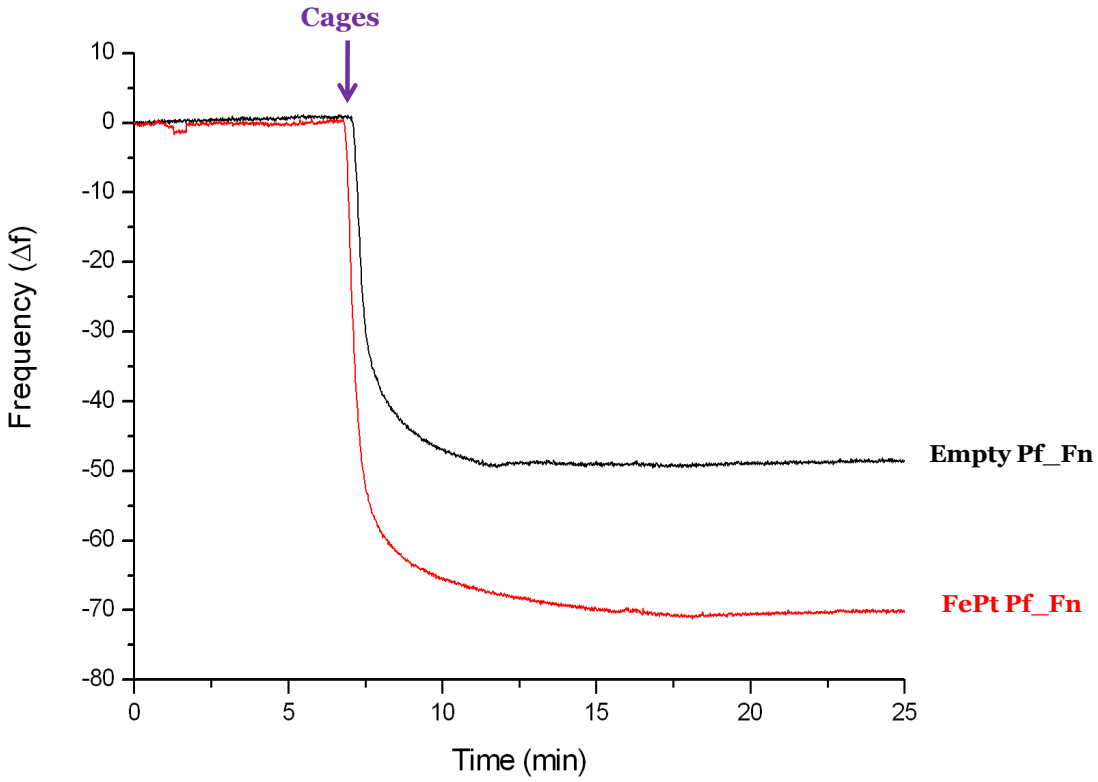
Quartz crystal microbalance (QCM) measurement: The experiments were performed using Q-Sense E4 and standard gold QCM sensors (Q-Sense). The system was operated in flow mode with a pump and temperature was maintained at 25.0 ± 0.1^{o}C. Each sample solution was introduced to the measurement chamber with a pump and continuously measured for 3 min prior to subsequent introductions. Chemically modified FePt mineralized Pf_Fn and streptavidin were introduced at concentrations of approximately 100 μg/ml and 50 μg/ml in phosphate buffer (50 mM MES, 100 mM NaCl, pH6.5), respectively. Resonance frequencies were measured at seven harmonics (5, 15, 25, 35, 45, 55 and 65 MHz) simultaneously and normalized frequency of the third overtone was presented for clarity.

Supporting Figure 1.



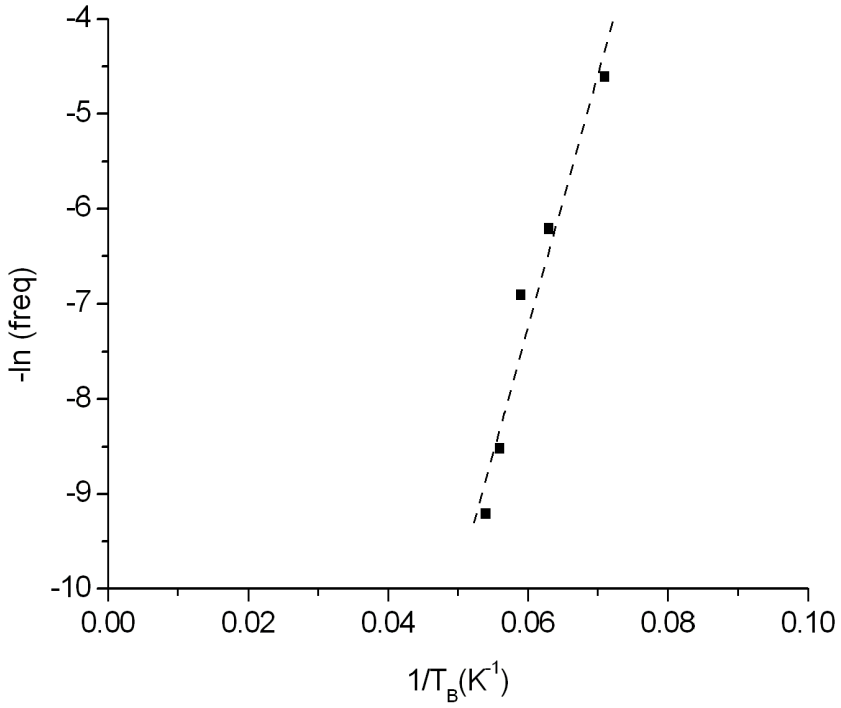
Supporting Figure 1. Size exclusion elution profiles (280 nm (solid lines) and 350 nm (dashed lines) of the FePt-mineralized (top) and empty (bottom) Pf_Fn (left) and HHFn (right). The samples were loaded onto a 10 x 300 mm superose 6 (GE healthcare) size exclusion column which is pre-equilibrated with 50 mM phosphate, 100 mM NaCl (pH 6.5) and eluted with same buffer at a rate of 0.5 ml/min.

Supporting Figure 2.



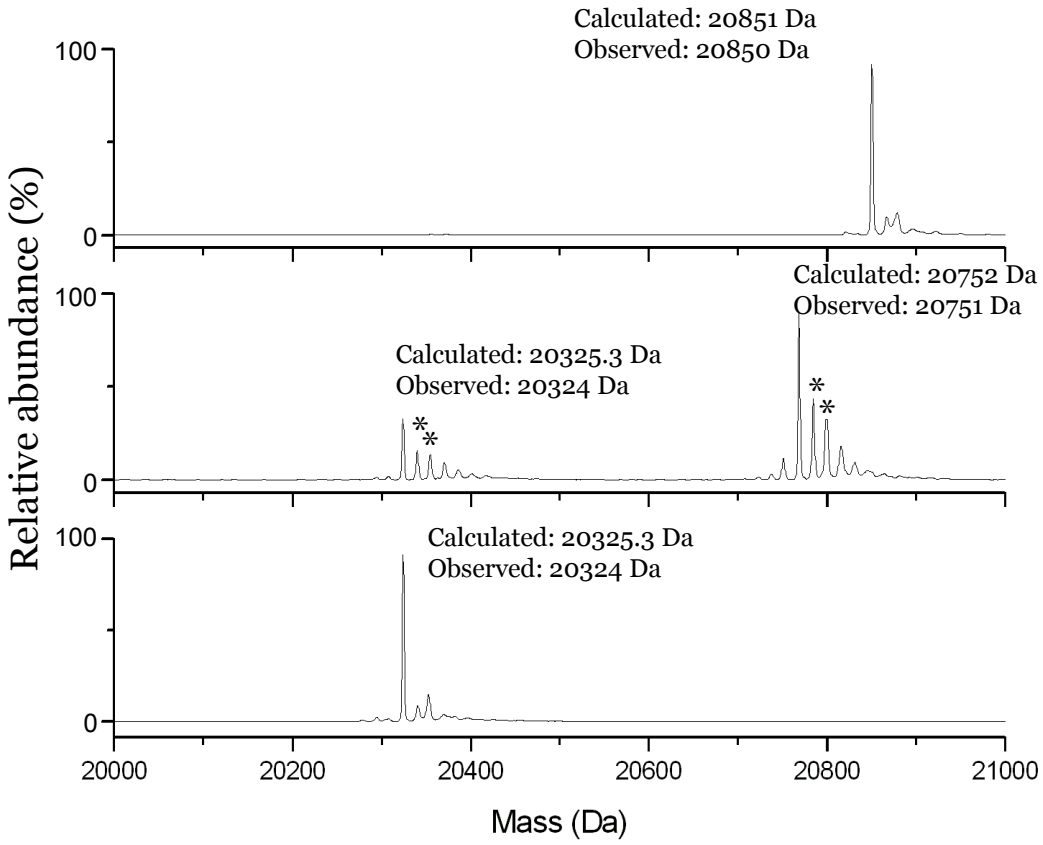
Supporting Figure 2. QCM resonance frequency change (-Δ*F*) profiles of Pf_Fn with (red line) or without (black line) FePt core on the gold QCM sensors.

Supporting Figure 3.



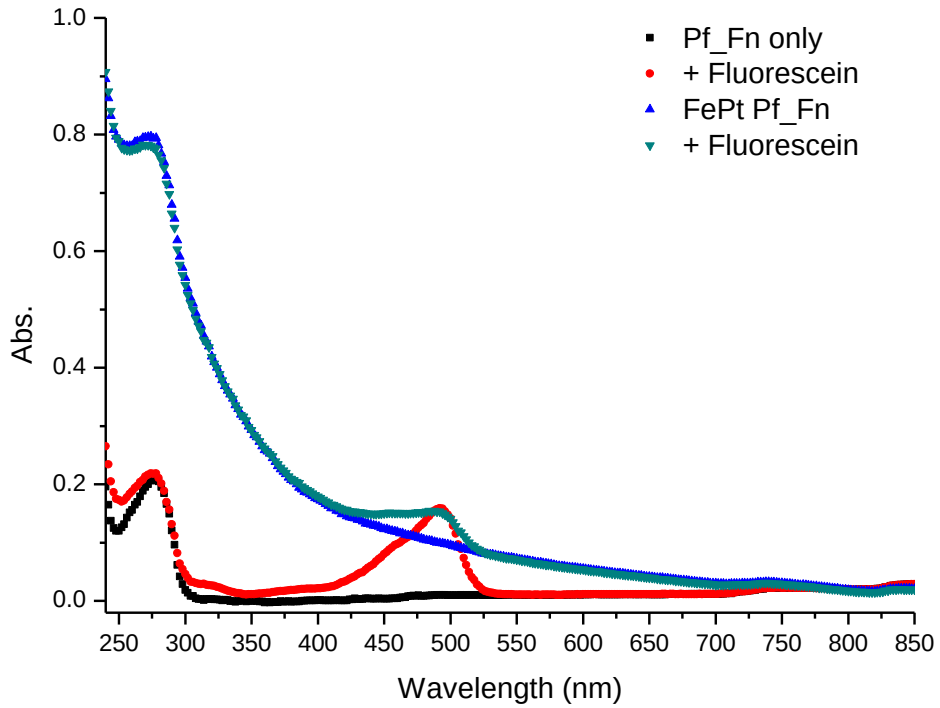
Supporting Figure 3. Néel-Arrhenius fits of the alternating current magnetic susceptibility (ACMS) data.

Supporting Figure 4.



Supporting Figure 4. Mass spectrometric analyses. Molecular mass spectra of dissociated subunits of untreated (bottom), F5M treated (middle) and MPB treated (top) Pf_Fn. Calculated and observed molecular masses of dissociated subunits are indicated. *Peaks resulted from methionine oxidation.

Supporting Figure 5.



Supporting Figure 5. UV/Vis spectra of untreated (black square) and F5M labelled (red circle) empty Pf_Fn and untreated (blue triangle) and F5M labelled (green reverse triangle) FePt mineralized Pf_Fn.