Supplementary Material (ESI) for Journal of Materials Chemistry

Supplemental Information

Biomimetic Sythesis of photoactive α-Fe_2O_3 templated by the hyperthermophilic ferritin from *Pyrococus furiosus*

Pf_Fn Purification from E.coli: Starter cultures of approximately 10 mL of sterile Luria-Bertani (LB) broth (10 g/L peptone from casein, 5 g/L yeast extract, 10 g/L NaCl, 30 µg/mL kanamycin) were inoculated with E. coli carrying the pET30a plasmid[1] with the Pf_Fn gene and allowed to grow 6-8 hours at 37ºC or until the starter culture became opaque.[2] 500 µL of starter culture was added to 1-liter of sterile LB broth. The liter cultures were allowed to grow for 10-12 hours at 37ºC. The cells were pelleted from solution by centrifugation (20 min at 3700 x g), resuspended in 40 mL of lysis buffer (50 mM phosphate, 100 mM sodium chloride (NaCl), pH 7.0, 20 mg/mL DNase, 30 mg/mL RNase, 15 mg/mL lysozyme) per 10 g of cell mass, and allowed to incubate for 45 min on ice at room temperature. The slurry was French pressed (American Power Press) and sonicated (3 X 5 min) and separated by centrifugation (45 min at 12,000 x g). The supernatant was heated to 90ºC for 10 min, cooled on ice, and then was re-centrifuged to remove excess cellular debris (20 min at 12,000 x g). The resulting supernatant was allowed to dialyze against 25 mM acetate, 50 mM NaCl buffer at pH 4.5 overnight, and then was allowed to dialyze against 50 mM 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES), 100 mM NaCl buffer at pH 7.0 overnight. The dialysis product was centrifuged (20 min at 12,000 x g) to clear the solution and the supernatant was loaded onto a calibrated Superose 6 size exclusion column (Amersham Pharmacia) equilibrated with the same buffer on a BioRad QuadTec system (BioRad, Richmond, CA). The flow rate was 0.5 mL min-1 and elution of the protein was monitored at 260, 280, and 410 nm. Protein purity was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, subunit molecular weight = 20309 Da) using 12% gels stained with Coomassie Brilliant Blue R-250. The mass of apo-Pf_Fn was confirmed using a Waters Q-TOF Premier mass spectrometer with an electrospray ionization source. Protein concentration was determined by spectrophotometry using an extinction coefficient of 29870 $M^{-1}cm^{-1}$.

Transmission electron microscopy: TEM data were collected on a Leo 912, with Ω filter, operating at 80 keV. Samples were concentrated using microcon ultrafilters (Microcon YM-100) with 100 kDa Mw cutoff and transferred to carbon coated copper grids. Samples were imaged both stained with uranyl acetate and unstained. Electron diffraction data were collected on these samples, and *d*-spacing was calculated and compared to powder diffraction files for α-Fe_2O_3 after calibration of the instrument with a Au standard.

Dynamic Light Scattering: Dynamic light scattering (DLS) measurements were made on a Brookhaven Instruments ZetaPals (phase analysis light scattering) particle size analyzer. DLS was measured at 90° using a 661 nm diode laser, and the

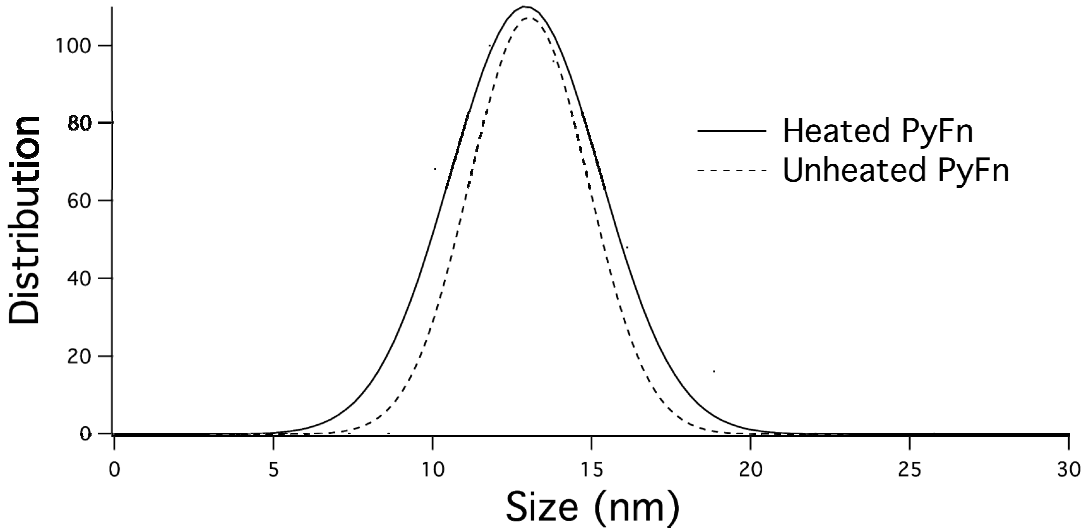
correlation functions were fit using a non-negatively constrained least-squares analysis.

Size exclusion chromatography*:* SEC was performed on a Biologic Duo-Flow fast protein liquid chromatography system equipped with a quad-tech UV-Vis detector and using a Superose 6 (Pharmacia) size exclusion chromatography column.

Photocurrent measurements*:* Photocurrents were measured using indium tin oxide coated glass slides as a working electrode (Aldrich, 70-100 Ω/cm^2). Another glass slide containing 1cm wide strips of vapor deposited ITO (100 Ω/cm^2) and Pt was used. Various concentrations of the Pf_Fn/hematite composite were placed in 1mL of a 20% ethanol solution and injected between the two glass electrodes. The photocurrents were then measured on a CV-50W Voltammetric Analyzer (Bioanalytical Systems Inc, West Lafayette, IN) while being illuminated by a 420nm LED light (17W, 700mA, ADVANCE, Rosemont, IL).

References:

1. Tatur, J; Hagedoorn, PL; Overeijnder, ML; Hagen, WR. *Extremophiles*, **2006**, *10*, 139.
2. Parker, M.J.; Ramsay, B.; Allen, M.A.; Klem, M.T.; Young, M.; Douglas, T. *Chem. Mater.* **2008**, *4*, 1541.



S1. Dynamic Light scattering data for heated Pf_Fn (solid) and unheated Pf_Fn (dashed). Both samples share similar hydrodynamic radii, 12.9 ± 0.5 nm vs 13.0 ± 0.2 nm for unheated and heated, respectively.

hkl	Measured d-spacing	Calculated d-spacing
104	2.80	2.70
024	1.97	1.90
116	1.66	1.69
300	1.42	1.45
134	1.14	1.14

Table 1. *d*-spacings for α-Fe_2O_3 (determined from **Fig. 1A** insert) and measured *d*-spacings (Å) for 97 ºC in Pf_Fn.