Supplemental Material

Biologically Tunable Reactivity of Energetic Nanomaterials

Using Protein Cages

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MATERIALS AND METHODS

Assembly of single layer ferritin-nAl: Aluminum nanoparticles (80 nm, 80% active Al content), passivated with an amorphous aluminum oxide, were obtained from Novacentrix, Inc. For assembly, 100 µL of cationized ferritin from horse spleen (Sigma, 48 mg/mL protein) was added to 2 mg of nAl powder dispersed and sonicated in 500 µL of deionized water. These components were incubated for 1 hour to promote functionalization of nAl with protein cages and then purified to remove excess unbound cages by centrifugation at 4000 rpm for 10 minutes. The isolated nAl-ferritin pellet was redissolved in 500 µL of deionized water. To obtain AP-loaded cationized apoferritin-nano-Al hybrids, the iron oxide core of cationized ferritin was reductively dissolved by dissolution with 0.5% mercaptoacetic acid in 0.1 M acetate buffer pH 4.5 and repeated dialysis using 10 kDa MWCO dialysis tubing (Fisher brand)[22]. The empty cationized apoferritin cage was then subsequently filled via successive additions of 0.1M ammonium perchlorate (Sigma) in water or a rhodamine perchlorate analogue (Exciton) and multiple dialysis steps to obtain maximal loading.

LbL assembly of ferritin-nAl: nAl particles were first coated with a single layer of cationized ferritin described above. A second layer of 100 µL native ferritin from horse spleen (Sigma, 56 mg/mL) was added to the single layer of cationized ferritin on nAl and incubated for 15 minutes, purified by centrifugation at 4000 rpm for 10 minutes, and resuspended in 500 µL of deionized water to yield two-layers of protein cages surrounding nAl. This process was repeated to build additional protein layers

on nAl by using cationized ferritin for the third layer and native ferritin for the fourth to achieve 4 protein layers of ferritin on nAl.

Characterization of ferritin-nAl hybrids: Ferritin binding was determined by using a Q-Sense E4 QCM-D system with flow modules. QCM sensors were obtained with a coating of 100 nm aluminum oxide (α-Al_2O_3) film (Q-sense, QSX-309). These were cleaned by UZ/ozone treatment (Novascan PSD Pro Series Digital UV/Ozone system) for 10 minutes, immersed in a 2% SDS solution for 30 minutes, rinsed thoroughly with deionized water, dried under N_2, and exposed again to UZ/ozone for 10 minutes. After cleaning, sensors were mounted in QCM flow modules. Cationized ferritin (Sigma) and native ferritin (Sigma) at concentrations of 96 µg/mL and 56 µg/mL in deionized water were flowed across QCM sensors at 0.17 mL/min and monitored vs. time at the third overtone frequency for LbL assembly. XPS measurements were performed using an M-PROBE Surface Science XPS spectrometer utilizing charge neutralization. Samples were prepared by dropcasting 10 µL of an aqueous suspension of nano-Al-ferritin onto a polished silicon wafer (Wafer world) and air dried. Spectra were collected from 0-1000 eV at 1eV steps at a spot size of 800 um and averaged over 15 scans for standard resolution. For High resolution XPS scans, we used 0.01 eV steps and averaged over 200 scans over expanded 20 eV ranges. TEM images were obtained on a Phillips CM200 transmission electron microscope operating at 200 kV. Samples were prepared by pipetting 10 µL of nano-Al-ferritin onto a 3 mm 200 mesh copper TEM grid coated with ultrathin carbon film (Ted Pella) and air dried. Energy dispersive X-ray spectroscopy was performed using an integrated EDAX detector from 0-20 keV at an angle of 15°. Zeta potential measurements and dynamic light scattering analysis were obtained on a Malvern Instruments nano series Zetasizer with 500 µL of cationized ferritin and native ferritin and on LbL assembled structures. AP-loaded cationized apoferritin was characterized by size exclusion chromatography using a Bio-Rad 10 DG disposable 10 mL column. Both free rhodamine perchlorate and free cationized ferritin were added to the column and eluted using water/0.1% TFA. The eluted fractions were collected and analyzed in a 750 µL polished quartz

cuvette by absorbance at 280 nm on a Varian Cary 500 Scan UV-Vis-NIR spectrophotometer; while fluorescence was analyzed using a Varian Cary Eclipse fluorometer with an excitation at 480 nm.

Energetic characterization of nAl-ferritin: Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) measurements were performed in a TA Instruments SDT Q 600. Samples (5 to 10 mg) were placed into a tared alumina crucible with an empty alumina crucible serving as the reference. All data was collected in dynamic mode under flowing argon (100 mL/min) from room temperature up to 1000°C at a rate of 5°C/min. Control samples were prepared by mixing 30 wt % AP and nAl or iron oxide (Fe_2O_3) nano-powder with nAl at the appropriate stoichiometric ratios. Combustion experiments were performed by placing approximately 10 mg of the respective bio-thermite powder onto a flat steel gauze substrate in a vented fragmentation chamber under an air atmosphere. The powders were initiated by a butane flame from directly below. A NAC Image Technology Memrecam® GX8 digital high speed video camera, collecting full frame, full color images at 5,000 frames per second, was used to record the combustion event.

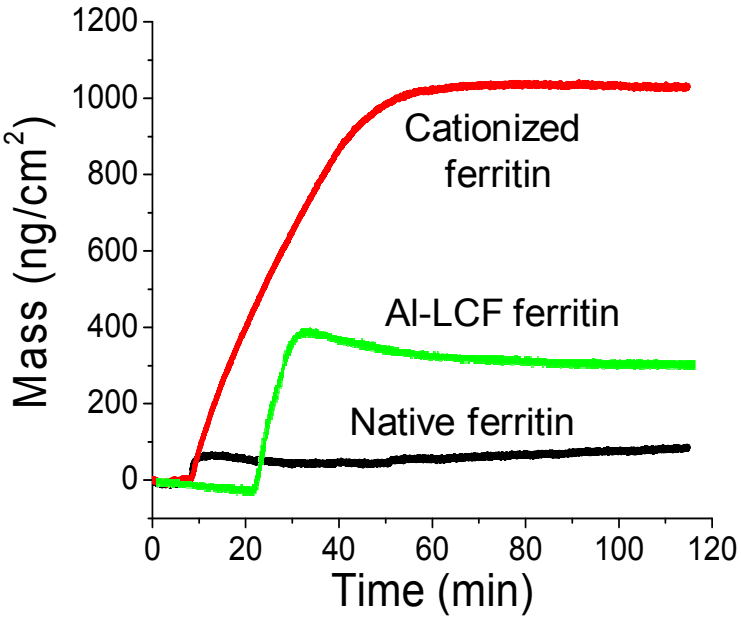

Figure S1. QCM binding plot of chemically and peptide modified ferritin on an aluminum oxide coated quartz QCM sensor (QSX-309, Q-sense) using an E4 Q-Sense QCM-D system.

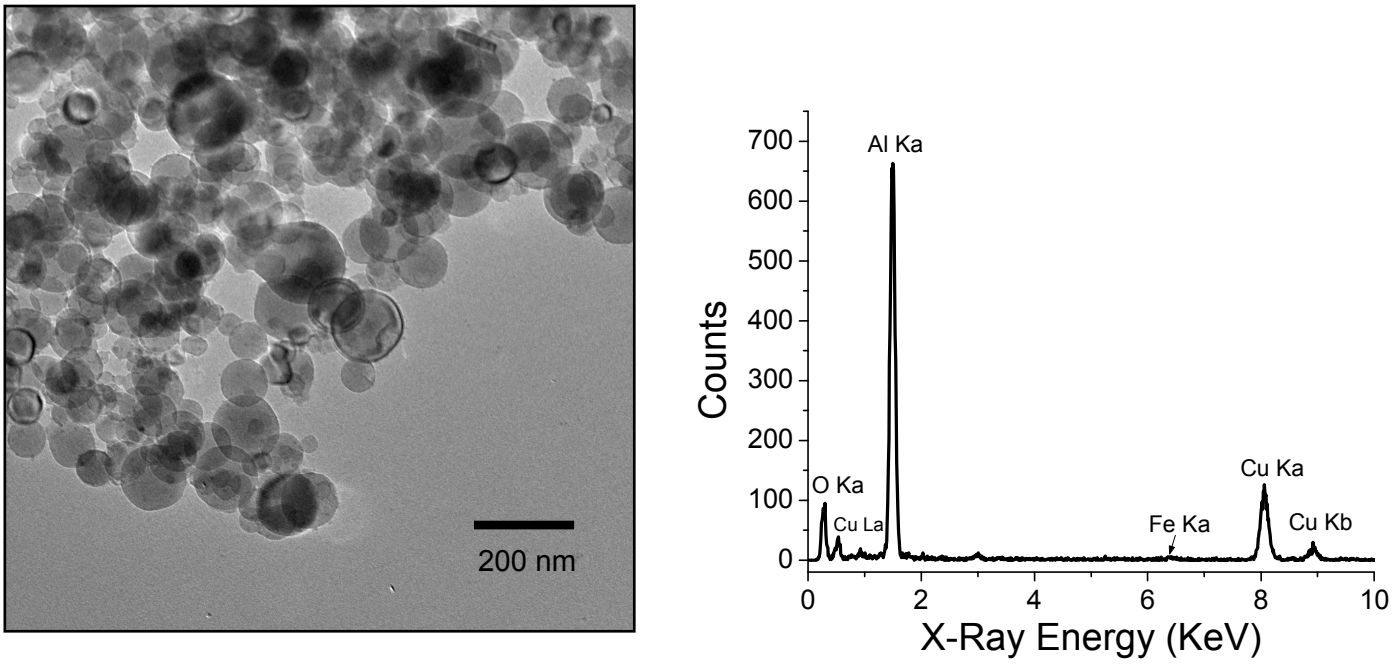

Figure S2. TEM image and EDS spectrum of nAl assembled with native ferritin (negatively charged) loaded with FeO(OH).

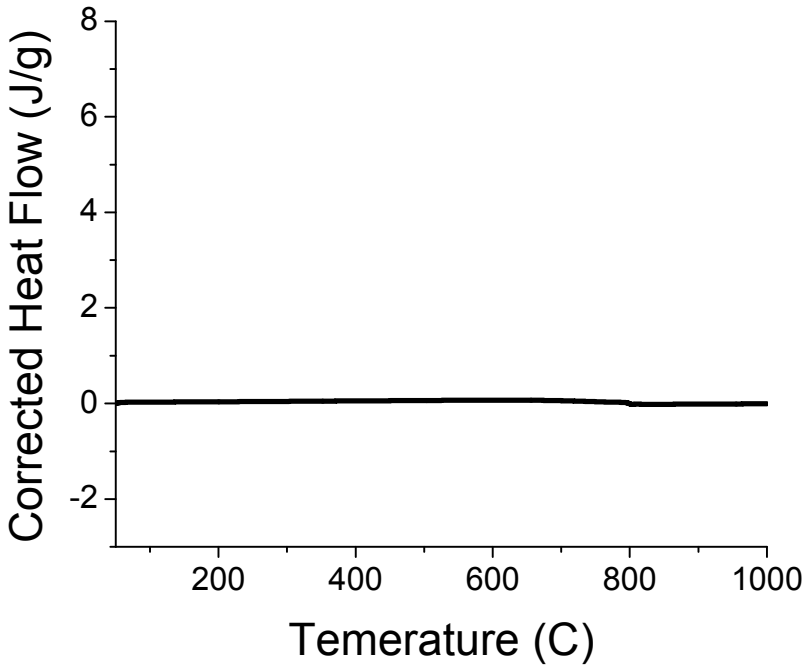

Figure S3. DTA profile of ferritin only powder loaded with FeO(OH) corrected for wt using TGA values.

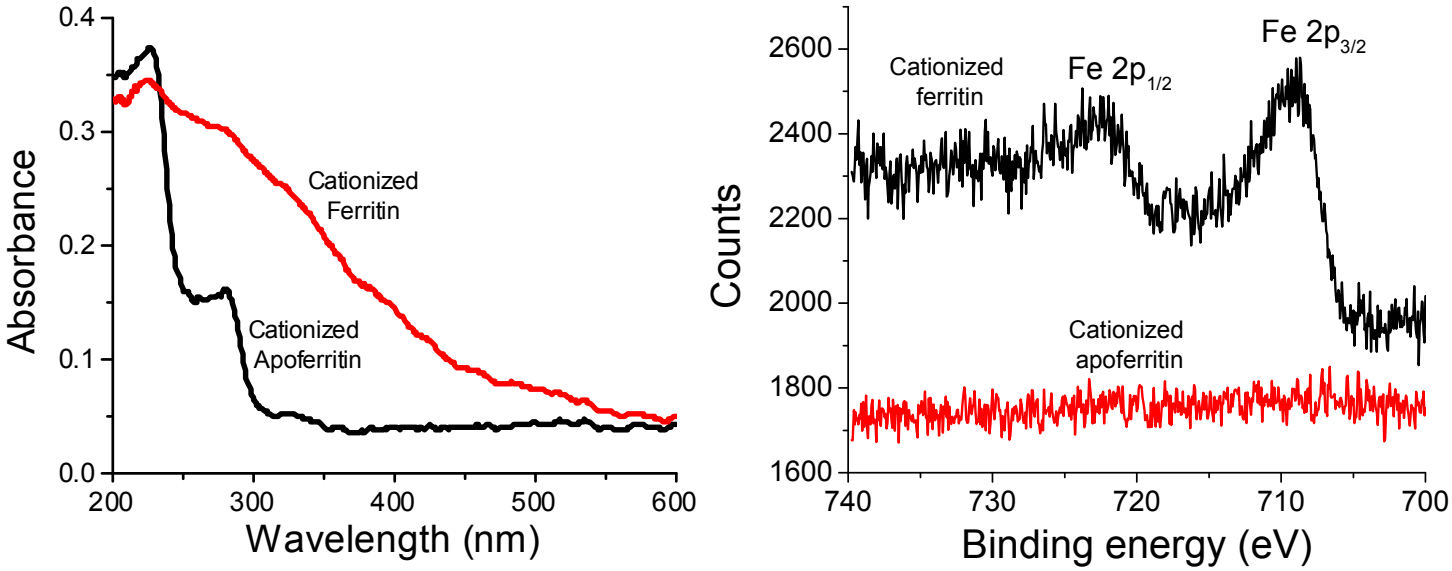

Figure S4. UV-Vis absorbance and high resolution XPS spectra of cationized ferritin and cationized apoferritin after FeO(OH) dissolution.

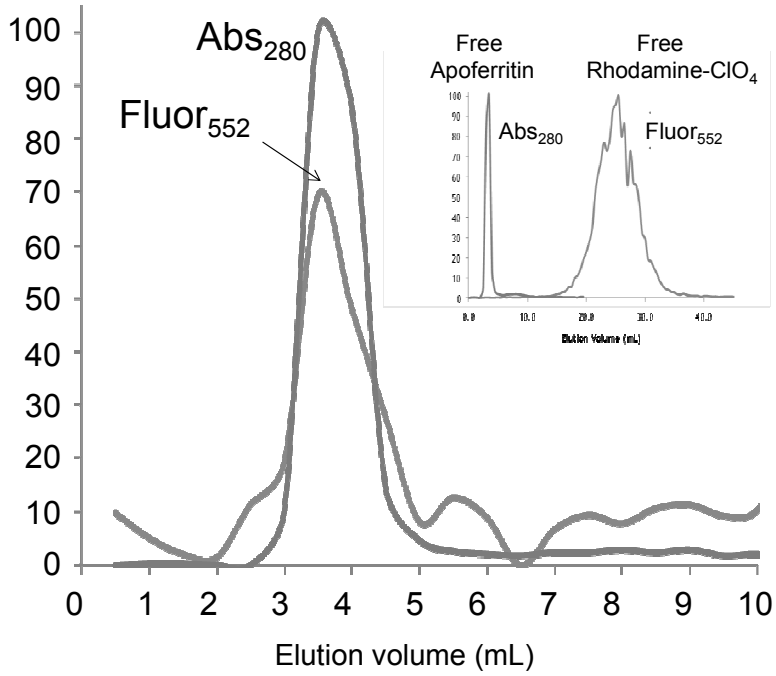

Figure S5. Size exclusion chromatography plot of cationized apoferritin loaded with rhodamine perchlorate. Absorbance at 280 nm was measured for ferritin while fluorescence at 552 nm was measured for rhodamine perchlorate (exc. 480 nm) and plotted vs. volume eluted. Inset shows elution plot of free cationized apoferritin at absorbance$_{280nm}$ and free rhodamine perchlorate at absorbance$_{552nm}$.

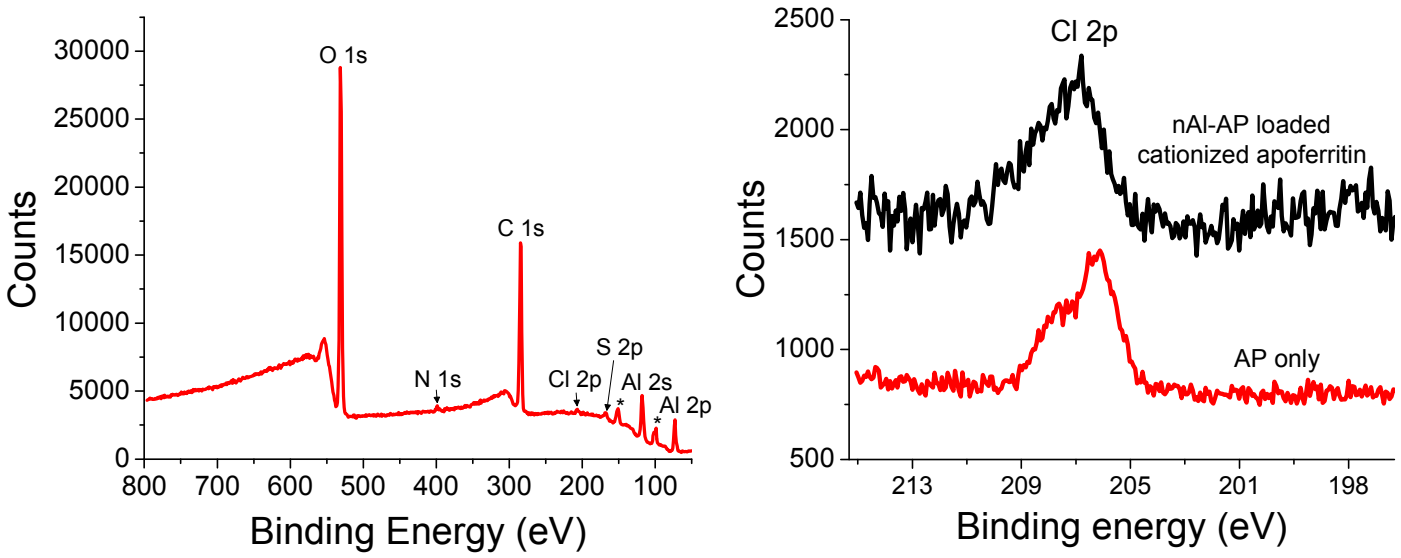

Figure S6. XPS spectra of 1 layer nAl-AP loaded cationized apoferritin showing survey scan and high resolution scan of Cl 2p region. Asterisk indicates Silicon peaks from substrate.

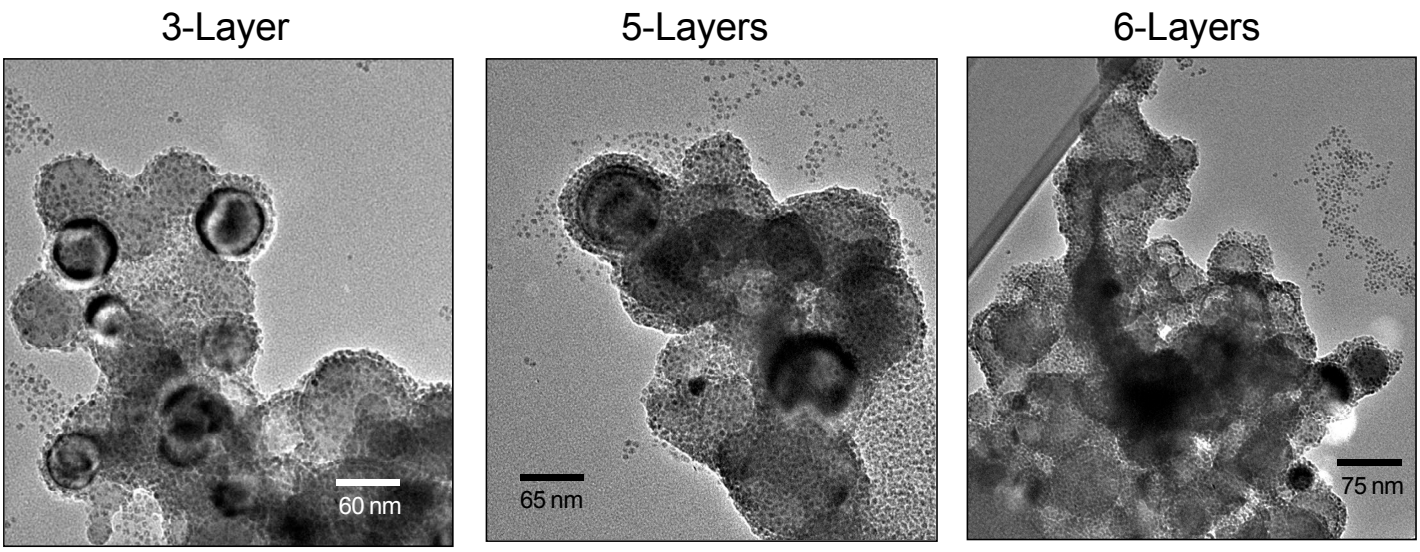

Figure S7. TEM images of 3-layer, 5-layer, and 6-layer FeO(OH) loaded ferritin on nAl.

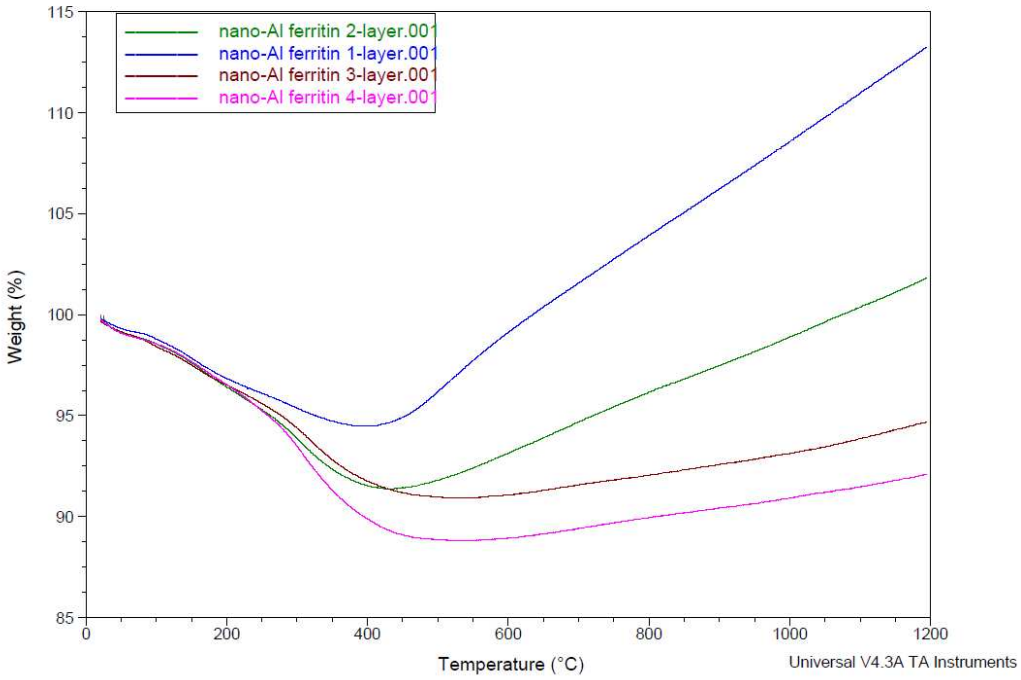

Figure S8. TGA profile showing mass loss of nAl with 1-4 layers of ferritin protein cages loaded with FeO(OH).

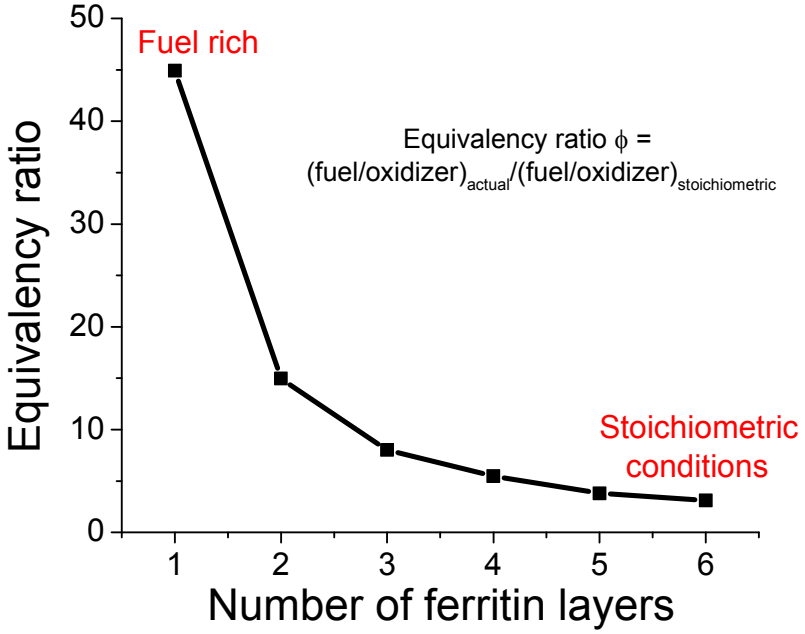

Figure S9. Plot of the equivalency ratio for biothermite reaction vs amount of iron oxide loaded ferritin on nAl. The actual fuel/oxidizer ratio was determined by using the amount of iron oxide in each protein layer from QCM plot in Figure 3b and the amount of nAl (10 mg).

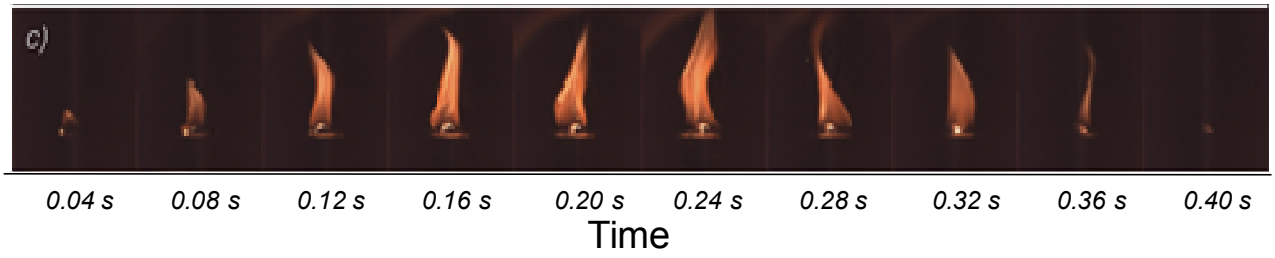

Figure S10. High speed camera digital image of 12-layer iron oxide loaded ferritin on nAl.

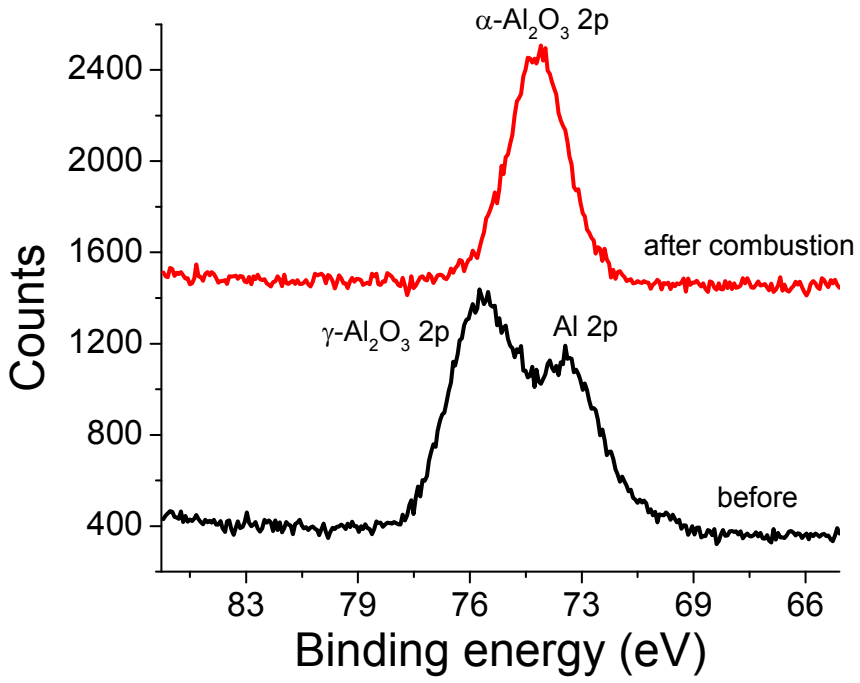

Figure S11. High resolution XPS spectrum of 4-layer FeO(OH) loaded ferritin on nAl showing Al 2p peaks before and after combustion.