ADVANCED FUNCTIONAL MATERIALS

Supporting Information

for

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Genetic Engineering of Heat Shock Protein

The *Methanococcus jannaschii* small heat shock protein (MjHsp16.5) was cloned into a pET30a(+) vector (Novagen) as previously described.[1] The MjHSP clone was then used as a template for Polymerase chain reaction (PCR) mediated site directed mutagenesis (Stratagene, San Diego, CA) to engineer the CoPt binding peptide sequence (KTHEIHSPLLHK) onto the N-terminus of the protein. Complementary forward and reverse primers were designed to modify the coding sequence of the N-terminus of MjHSP from the native sequence to the CoPt sequence. This mutagenesis resulted in the modification of amino acids 3 – 14 on the N-terminus of MjHSP. The primer, which includes an engineered *BspHI* restriction enzyme site (italics) to facilitate screening of the desired mutation was used (the ATG coding for the start of translation is indicated in bold): 5' ctttaagaaggagatatacat **atg** ttc aaa ac*t cat ga*a ata cat tca cca tta ttg cac aaa atg ttt aaa gag ttt ttt gca aca cc 3'. The entire sequence was confirmed by sequencing on an ABI 310 automated capillary sequencer using Big Dye chain termination sequence technology (Applied Bio Systems, Foster City, CA). The final construct (CP_Hsp) was expressed in *E. coli* and resulted in the production of the assembled small heat shock protein cage with the CoPt peptide on its N-terminus.

Identification of CoPt Peptide Sequence

The dodecapeptide specific for the L10 phase of CoPt (KTHEIHSPLLHK) was identified by phage display techniques as previously described.[2-3] Preparation of the CoPt substrate used in peptide screening involved the synthesis of CoPt nanoparticles using the protocol of Murray *et. al.*,[4] followed by dropcoating onto silicon wafers.

Annealing of these CoPt nanoparticle films under 5% H_2 at 600°C yielded continuous, $L1_0$ films of CoPt as determined by XRD. These films where cut into ~1cm^2 pieces and immediately used in biopanning experiments as described in the New England Biolabs phage display handbook. Interaction of the CoPt surface with the PhD-12 library for 1 hour at room temperature was followed by a 10-fold washing step with increasing amounts of Tween® to increase stringency. Acid elution using Glycine-HCL (pH 2.2) was used to elute the bound bacteriophage from the CoPt surface for amplification and sequencing. After five rounds of selection, the dodecapeptide (KTHEIHSPLLHK) emerged as the dominant sequence.

Purification and Characterization of Hsp

One liter cultures of *E. coli* (BL21(DE3) B strain) containing pET-30a(+) MjHsp16.5 plasmid were grown overnight in LB + kanamycin medium (37 °C, 220 rpm). Cells were harvested by centrifugation 3700 xg for 15 minutes and re-suspended in 30 mL of 100mM HEPES, pH 8.0. Lysozyme and DNase were added to final concentrations of 1 mg/mL and 1 mg/L respectively, incubated for 30 minutes at room temperature and sonicated on ice with a blunt tip (Branson Sonifier 250, power 4, duty cycle 50%, 3 x 5 minutes with 3 minute intervals). Cell debris was removed by centrifugation for 20 minutes at 12,000 xg. The supernatant was heated for 15 minutes at 60 °C, thereby denaturing many heat labile *E. coli* proteins. The supernatant was centrifuged for 20 minutes at 12,000 xg and the assembled CP_Hsp purified by gel filtration chromatography (Superose-6, Amersham-Pharmacia). The 16.5 kDa subunit molecular weight was verified by SDS poly-acrylamide gel electrophoresis (SDS-PAGE). The assembled protein was imaged by transmission electron microscopy (TEM, LEO 912

AB), stained with 2% uranyl acetate on formvar carbon coated grids and analyzed by dynamic light scattering (DLS 90 plus, Brookhaven Instruments). The masses of the engineered protein was determined by mass spectroscopy (Esquire3000, Bruker) and compared with its calculated mass. Protein concentration was determined by absorbance at 280 nm using the molar absorptivity $\varepsilon = 9322\ M^{-1}cm^{-1}$.

Transmission Electron Microscopy (TEM). TEM data were obtained on a Leo 912 AB, with Ω filter, operating at 100 keV. The samples were concentrated using microcon ultrafilters (Microcon YM-100) with 100 kDa cutoff and transferred to carbon coated copper grids. Samples were imaged unstained or stained with 2% uranyl acetate. Electron diffraction patterns were collected on samples and the d-spacings were calculated and compared with the powder diffraction file for the $L1_0$ phase of CoPt after calibrating with a Au standard.

Mass Spectrometry. Mass Spectrometry was performed on a Bruker Esquire 3000 Electrospray Ionization - Mass Spectrometer. DMF was added to the sample to the final concentration of 20% DMF prior to analysis.

Dynamic Light Scattering (DLS). DLS measurements were carried out at 25 °C using a 661 nm diode laser at 90°, and the correlation functions were fit using a non-negatively constrained least-squares analysis.[5]

Polyacrylamide Gel Electrophoresis (PAGE). PAGE was performed under native conditions using 5% polyacrylamide gels. Gels were stained using Coomassie blue.

Size Exclusion Chromatography (SEC). 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.

UV-Vis Spectroscopy. UV-Vis spectroscopy experiments were carried out with Ocean Optics S2000 spectrometer at 25 and 65 °C using a temperature controlled cuvette cell (Quantum Nortwest).

Magnetic Characterization. All magnetic characterizations were performed on a Physical Properties Measurement System (Quantum Design). Static and dynamic magnetic measurements were carried out using a vibrating sample magnetometer (VSM) and alternating current magnetic susceptibility (ACMS) options, respectively.

Thin Sectioning. CP_Hsp particles were centrifuged and slightly resuspended in 2% noble agar (Difco). After hardening, the agar was cut into small pieces and the pieces divided between two glass vials, one of which was fixed with 5% glutaraldehyde in 0.1M potassium phosphate buffer, pH 7.2, overnight. CP_Hsp agar pieces in both vials were then rinsed twice, 10 minutes each, with buffer before being dehydrated in a series of 50%, 70%, 95%, and 100% ethanol and propylene oxide. Spurr's low-viscosity resin was then used for embedding.[6] 'Thick' sections were cut from the embedded CP_Hsp using a glass knife in a Reichert OMU2 ultramicrotome. Thin sections, 60-90nm, were then cut with a Dupont diamond knife. Sections were then viewed with a LEO 912AB transmission electron microscope.

References

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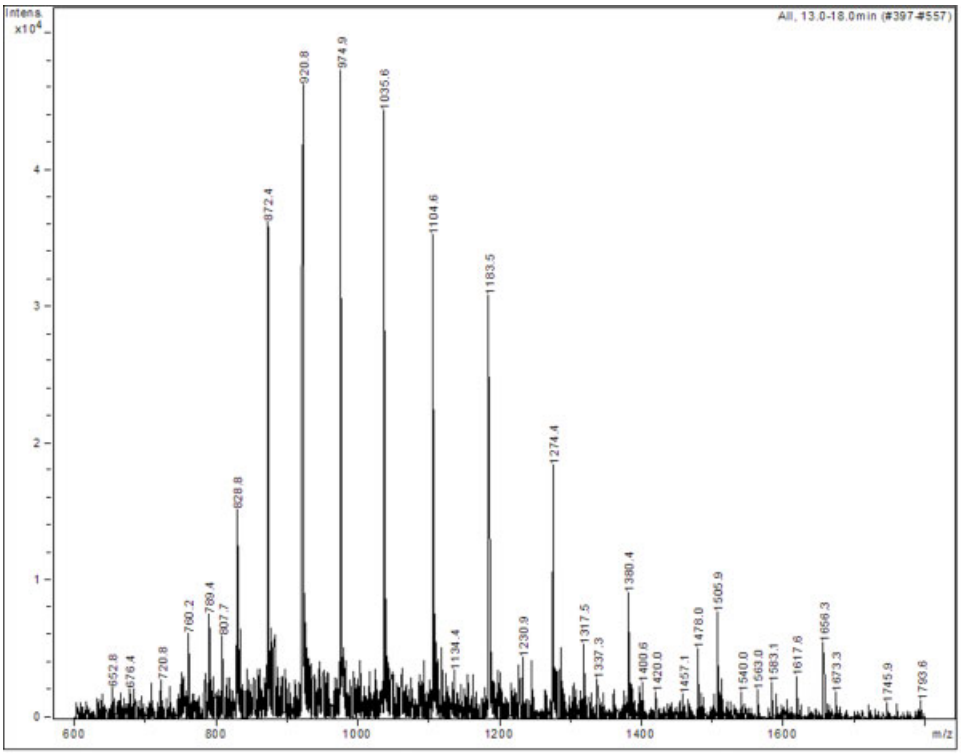

Figure S1. Mass spectrum of CP_Hsp. Deconvolution of the charge states yielded 16555.2(7) mass units, which is in agreement with the 16553 mass units predicted from the protein sequence.

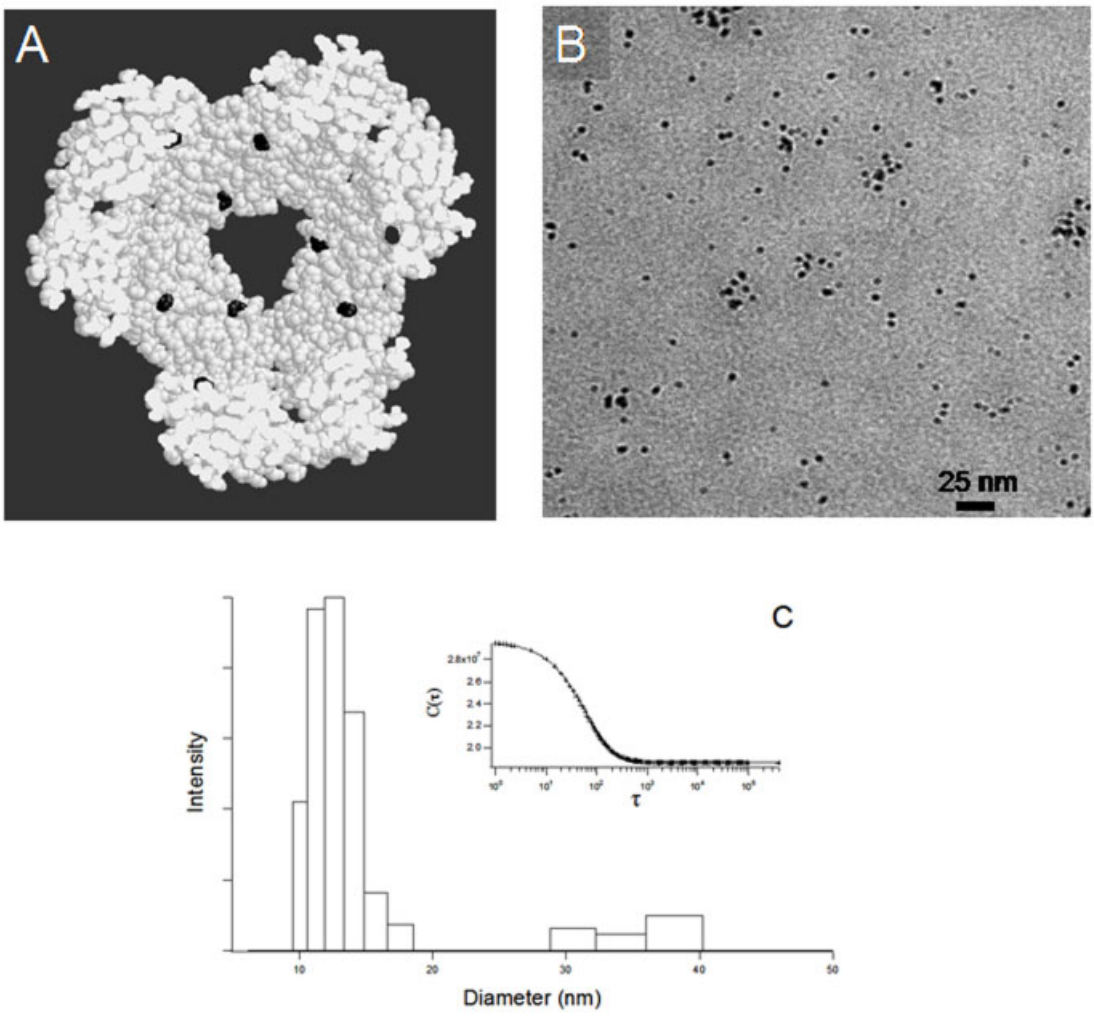

Figure S2. (a) Cutaway view of MjHsp showing the interior with the positions of the cysteine residues in G41C site indicated in black. (b) Transmission electron micrograph of mineralized G41C_Hsp. (c) Dynamic light scattering of CoPt mineralized G41C_Hsp indicating a diameter of 12 nm (inset is the corresponding correlation function).

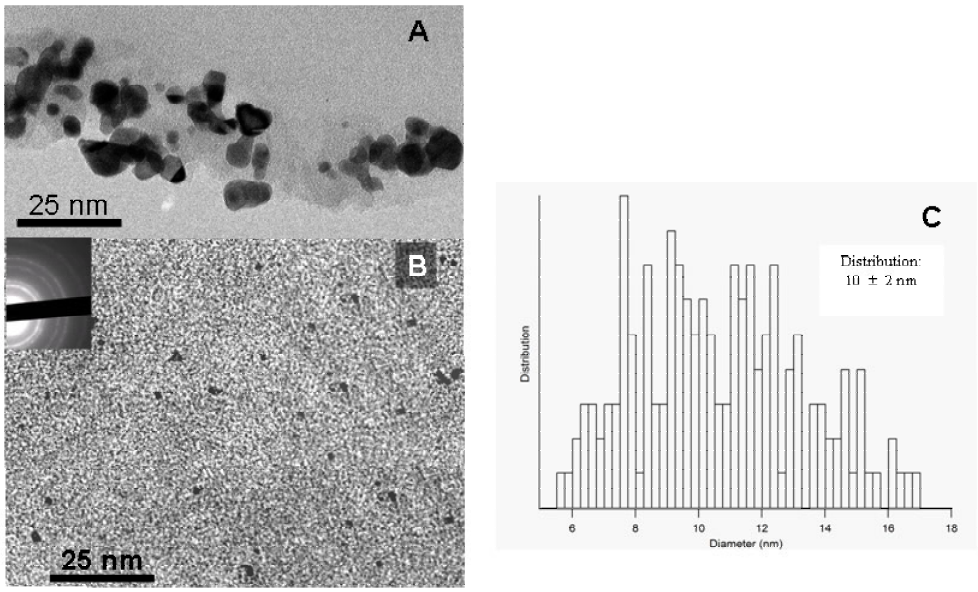

Figure S3. (a) Thin-section electron micrograph through a “bulk” section of annealed CP_Hsp. (b) Thin-section electron micrograph away from the “bulk” of annealed CP_Hsp with electron diffraction in the insert. (c) Size distribution of nanoparticles in annealed CP_Hsp.