Supporting Information

Chaperone-like activity of the N-terminal region of a human small heat shock protein and chaperone-functionalized nanoparticles

Running title: *HspB1 N-terminal sequence chaperone-like activity*

Emily F. Gliniewicz[†], Kelly M. Chambers, Elizabeth R. De Leon[†], Diana Sibai[†], Helen C. Campbell[†], and Kathryn A. McMenimen*

From the Department of Chemistry, Mount Holyoke College, 50 College Street, South Hadley, MA 01075

[†] undergraduate student

*To whom correspondence should be addressed: Kathryn A. McMenimen: Department of Chemistry, Mount Holyoke College, 50 College Street, South Hadley, MA 01075; kamcmeni@mtholyoke.edu; Telephone: 1-413-538-3375; Fax. (413) 538-2327.

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Materials and Methods

Protein Expression and Purification. The N-terminus (residues 1-88) of HspB1 with an inserted N-terminal cysteine (HspB1NTR) was expressed from the pMCSG7 vector after ligation independent cloning. All recombinant sHsps were expressed in *E.coli* BL21(DE3) cultured in LB media containing 100μg/mL ampicillin or 50 μg/mL kanamycin (wildtype HspB1). Protein expression was induced with the addition of isopropyl thio-β-D-thiogalactoside to a final concentration of 0.5 mM at 25°C for 5 hours. Wildtype HspB1 was purified by methods previously described (Makley et al., 2015). Recombinant human HspB1 was expressed in E. coli BL21 (DE3) cells using plasmids kindly gifted by Dr. Jason Gestwicki (UCSF). Briefly, cells were spun down at 11,000RPM at 4°C for 15 minutes and resuspended in lysis buffer (20mM Tris, 100mM NaCl, 6M urea, 5mM β-mercaptoethanol, 15mM imidazole, pH 8.0) with the addition of a cocktail protease inhibitor. Resuspended cells were then sonicated for 6 intervals of 30 seconds with 30 second breaks in between. The lysed cells were then spun again at 11,000RPM and 4° C for 45 minutes. All proteins were purified from lysate using Ni^{2+}-affinity column. Resulting proteins were exchanged into buffer, 25mM sodium phosphate and 100 mM sodium chloride, (pH 7.4) and underwent further purification by size exclusion chromatography in PBS 7.4. Protein concentration was determined using the Thermo Scientific BCA assay kit.

Chaperone-like Activity Assay. To determine whether the N-terminal region of HspB1 modulates chaperone function, HspB1NTR, HspB1NTR-NPs, and corresponding control proteins and nanoparticles were confronted with two heat-denatured substrate proteins, MDH and CS. The MDH and CS aggregation assays were performed using indicated substrate amounts in 0.5 mL PBS (pH 7.4) at 45°C, in the absence and presence of HspB1NTR, HspB1NTR-NP, wildtype HspB1, or other indicated controls. The extent of aggregation was monitored at 340 nm in a Cary 100 UV-Vis spectrophotometer (Agilent) equipped with a multi-well holder, temperature controller, and constant stirring capabilities. Aggregation experiments

were also carried out in a 96-well plate using the Molecular Devices M5e multi-mode plate reader and scaled to a final volume of 100 μL.

Chaperone and Substrate Solubility Gel. Samples obtained from the chaperone-like activity assay (0.5 mL) were centrifuged at 13,000 rpm for 3 minutes (x 2). The soluble fraction and pelleted fractions were collected separately. Following this 1X non-reducing sample loading buffer was added to each sample. MDH samples and controls were run on 10% mini-PROTEAN TGX precast gels (Bio-Rad). CS samples and controls were run on 8-16% gradient mini-PROTEAN TGX precast gels (Bio-Rad). All gels were run for 40 minutes at 160 V. The gels were silver stained according to directions of the Pierce Silver Stain kit (Thermo scientific).

Size Exclusion Chromatography (SEC). Changes in protein complex size as a result of mixing B1NTS with substrate proteins were analyzed by size exclusion chromatography (SEC). Each sample consisted of either 15μM B1NTS+2.5μM substrate, 15μM B1NTS, or 2.5μM of MDH or CS in PBS pH 7.4. Each sample (1 mL) was heated at 45^{o}C for one hour, then cooled to room temperature. Following this, 200 μL of each sample was loaded on Superdex 200 10/300 column equilibrated with PBS (pH 7.4) attached to an AKTA FPLC (GE Healthcare, Pittsburgh, PA). The column was run at the flow rate of 0.3 mL/min at 4^{o}C. The column was calibrated with the following protein markers: thyroglobulin (669 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B_{12} (1.35 kDa).

Transmission Electron Microscopy (TEM). The morphology of sHsp-nanoparticles and protein aggregates was monitored by transmission electron microscopy (TEM). Briefly, the protein mixtures MDH(2.5μM), MDH(2.5μM) +B1NTS(15μM), and MDH(2.5μM)+B1NTS-AuNP (OD2) were prepared in PBS (pH 7.4) and heated at 45^{o}C for 1 hr while being stirred. After 1hr, 4 μL of each sample was applied to a carbon-coated 150 mesh copper grid (Electron Microscopy Sciences, Hatfield, PA) at room temperature, stained with freshly prepared 4% uranyl acetate, and examined under a Phillips/FEI CM100 (100kV). The images were obtained using an AMT digital camera and analyzed with ImageJ.

Optical Density (OD) Measurements of BL21 *E. coli*. Competent cells were transformed, grown overnight at 37°C and induced with IPTG for 8 hours at 25°C. Optical density of the cells expressing wildtype HspB1 or HspB1NTR or no overexpressed protein (untransformed BL21 cells) were measured at 600 nm in a clear 96-well plate on a Spectramax M5e at 44°C or 55°C.

Supporting Data

A

sHsp N-Terminal Domain Sequences

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HspB1  MTERRVPFSLLRGPSWDPFRDWYPHSRLFDQAFGLPRLPEEWSQ  44
HspB5  M-DIAIHHPWIR----RPFFPFHSPSRLFDQFFGEHLLESDLFP  39

HspB1  WLGGSSWPGYVRPLPPAAIESPAVAAPAYSRALSRQLSSGVSEI  88
HspB5  -TSTSLSPFYLR-------------PPSFLRAPS-WFDTGLSEM  68
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B

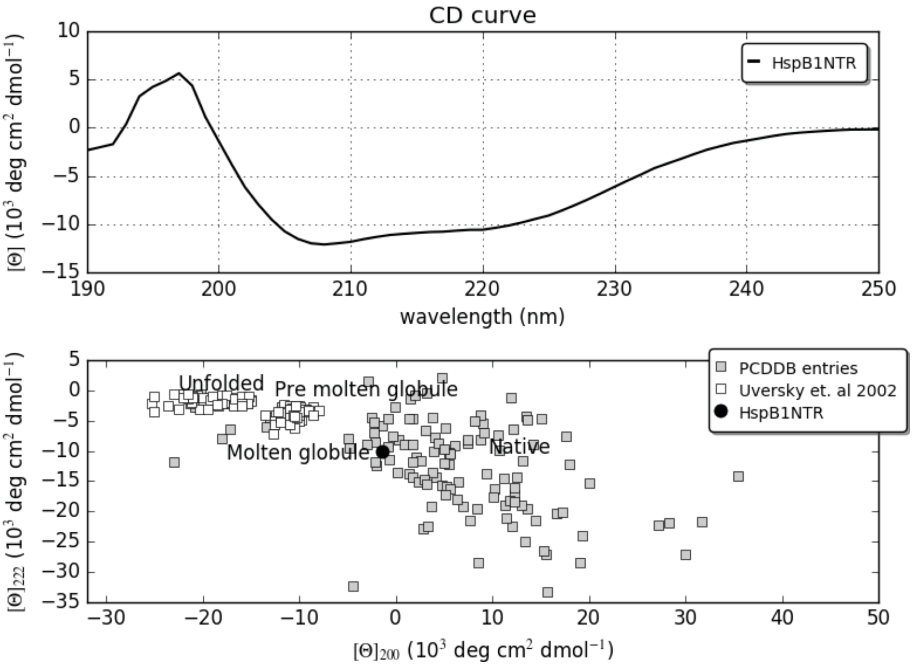

Figure S1. sHsps N-terminal region characterization. (A) N-terminal domain sequences from two human sHsps, HspB1 and HspB5. The sequence alignment depicts the highly un-conserved region of these sHsps for comparison. Alignment was performed using T-Coffee (Notredame, Higgins, & Heringa, 2000). Identity between HspB1 and HspB5 is 36% in the N-terminal region, calculated by T-Coffee. Identity between full-length HspB1 and HspB5 is 45%, calculated similarly. Previous studies have shown that substrate specificity is, at least in part, dictated by sHsp N-terminal regions (Arbach, Butler, & McMenimen, 2017; Jehle et al., 2011), therefore we would predict different activities for each sHsp NTR. (B) CD spectrum and predicted secondary structure folding of primarily irregular structure by CAPITO(Wiedemann, Bellstedt, & Görlach, 2013).

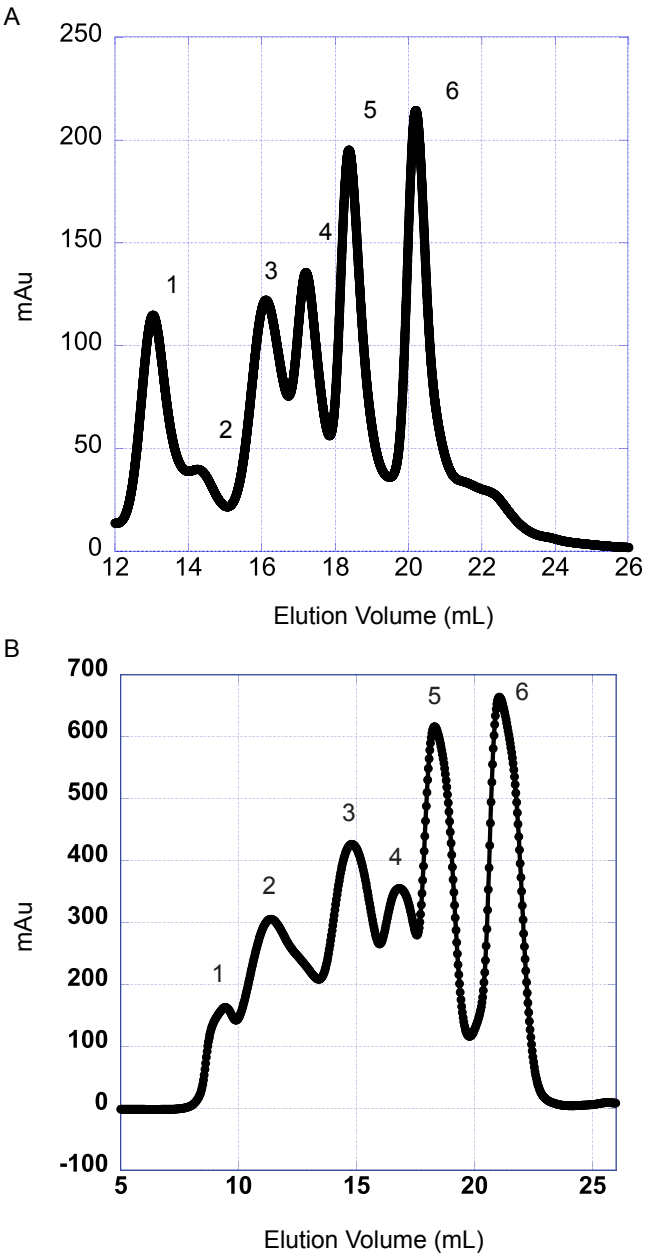

Figure S2. A) Size exclusion chromatography standards. Size exclusion chromatography of molecular weight standards eluted (0.3 mL/min.) from a Superose 6 increase 10/300 column attached to an AKTA FPLC for calibration. The peaks are assigned as follows: 1. aggregate peaks (>670kD), 2. thyroglobulin (669 kDa), 3. γ-globulin (158 kDa), 4. ovalbumin (44 kDa), 5. myoglobin (17 kDa), 6. vitamin B_{12} (1.35 kDa). B) Size exclusion chromatography standards. Size exclusion chromatography of molecular weight standards eluted (0.3 mL/min.) from a Superdex 200 10/300 column attached to an AKTA FPLC for calibration. The peaks are assigned as follows: 1. aggregate peaks (>670kD), 2. thyroglobulin (669 kDa), 3. γ-globulin (158 kDa), 4. ovalbumin (44 kDa), 5. myoglobin (17 kDa), 6. vitamin B_{12} (1.35 kDa).

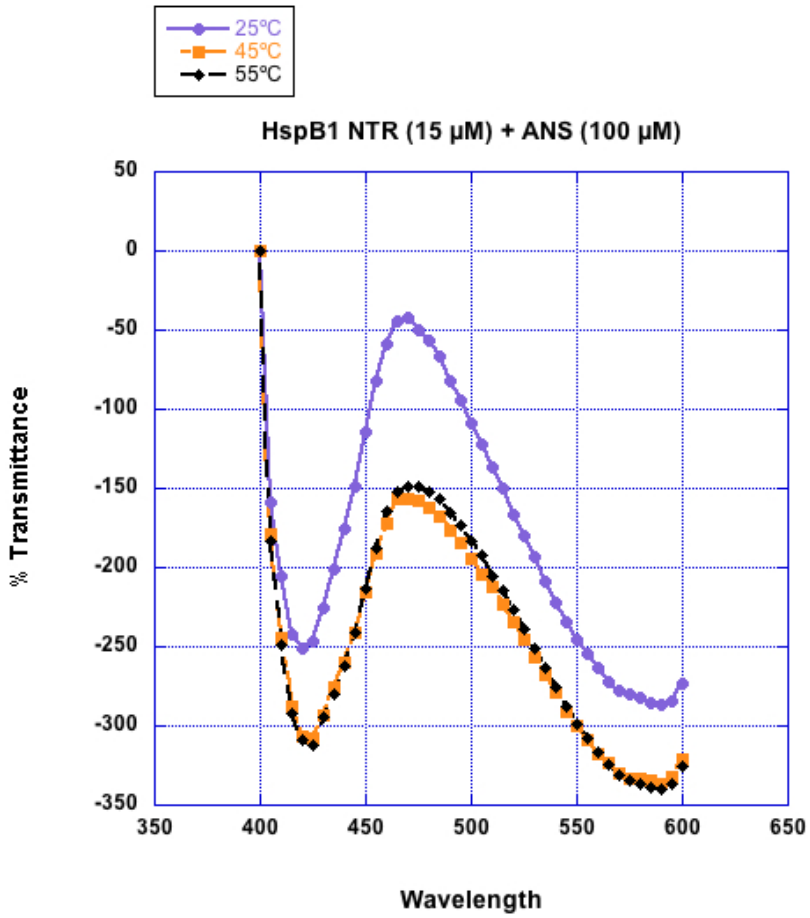

Figure S3. Temperature-dependent ANS binding to B1NTR. B1NTR (15 µM) and 1,8-ANS (100 µM) were mixed and incubated at 25°C, 45°C, 55°C for 1 hour before fluorescence readings were obtained. A decrease in ANS fluorescence was observed as temperature increased, indicating more ANS exchange with the solvent occurred upon heating.

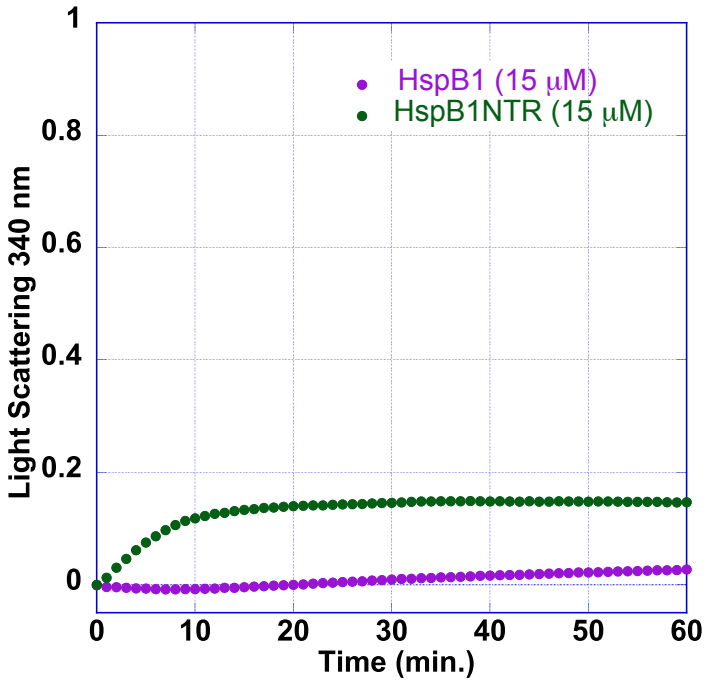

Figure S4. Wild type HspB1 and unconjugated B1NTR heat stability. Heat stability of HspB1NTR and HspB1. (A) No aggregation of HspB1 (15 μM) HspB1 or B1NTR (15 μM) is observed at 45°C. Each curve is an average of ≥ 5 replicates.

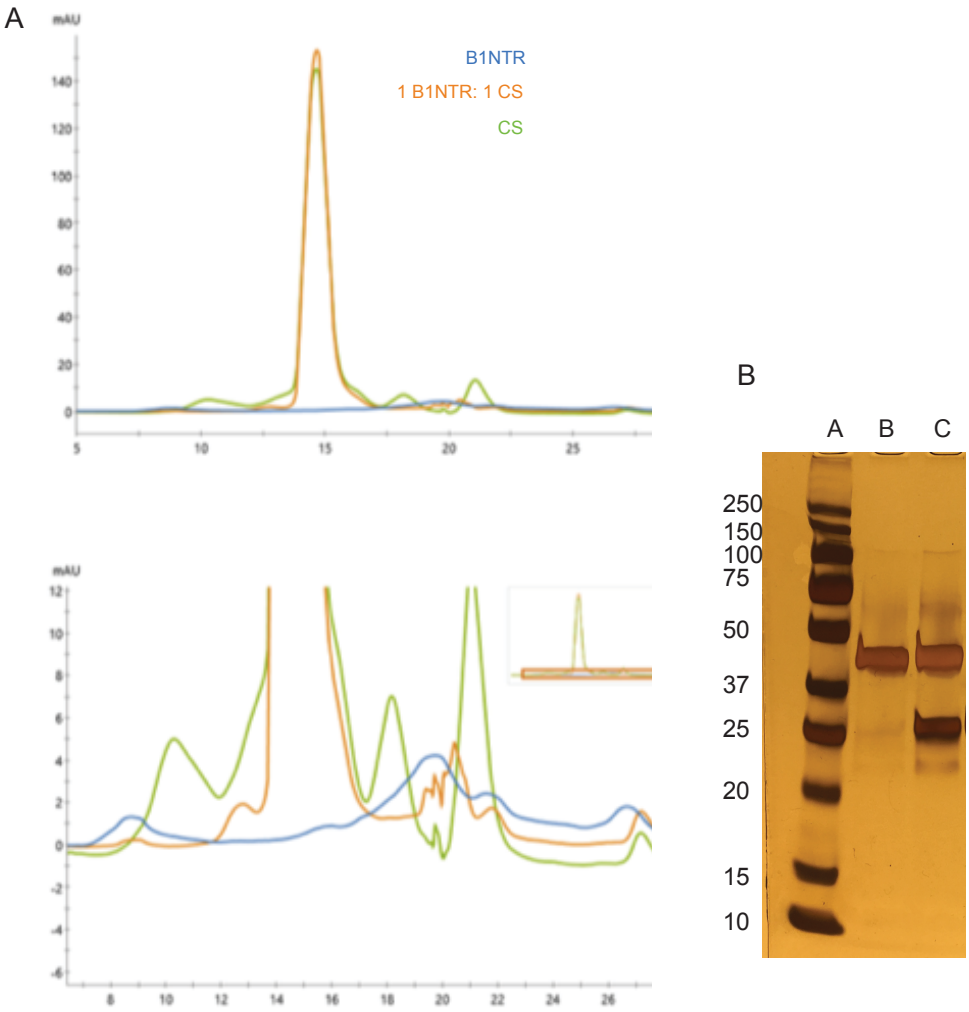

Figure S5. CS and B1NTR (1:1) complexes observed by SEC and SDS-PAGE. A) SEC profiles of B1NTR, blue curve (7.5 μM), B1NTR (7 mM) + CS (7 μM) orange curve, or CS, green curve (7 μM) only. B) SDS-PAGE analysis of major peaks eluted during SEC analysis. The molecular mass marker (A), CS only major peak (B), and CS + B1NTR mixture major peak are presented in each respective lane. All of these curves were obtained on a Superdex 200 10/30 column.

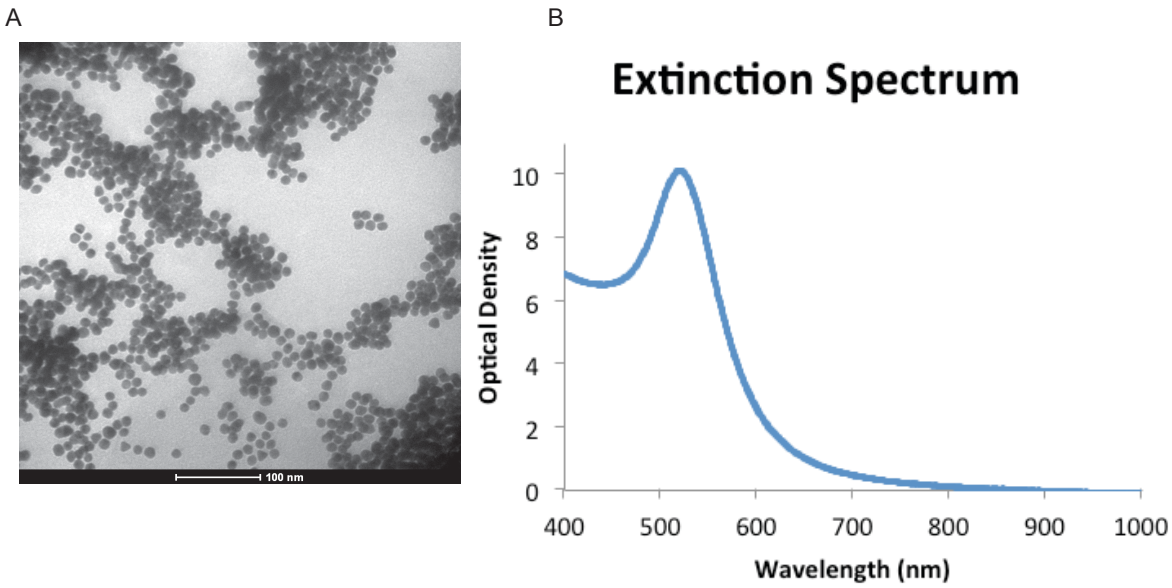

Figure S6. Description of conjugated nanoparticle species. (A) Transmission electron microscopy of HspB1NTR-AuNP in 1x PBS with 0.05% Tween 20. (B) Extinction spectrum measuring optical density with λ_{ODmax} = 521 nm. HspB1NTR-NPs are estimated to contain ≥10 HspB1NTR conjugates per particle. Characterization of nanoparticles was performed by Nano Hybrids, Inc (Austin, TX).

Table S1. Characterization of size, surface features, diameter (from DLS measurements), zeta potential, and approximation of peptides/particle at 25°C.

Particle	Peptide	Linker/Surface	DLS Size (nm)	Zeta potential (mV)	~ Peptides/particle
10 nm AuNP	none	citrate	16	-35	n/a
10 nm AuNP	none	PEG-CH3	n.d.	-4	n/a
10 nm AuNP	none	PEG-NHS	41	n.d.	n/a
10 nm AuNP	HspB1NTR	PEG	37	-6	> 10
10 nm AuNP	mini-αA	PEG	31	-6	> 100

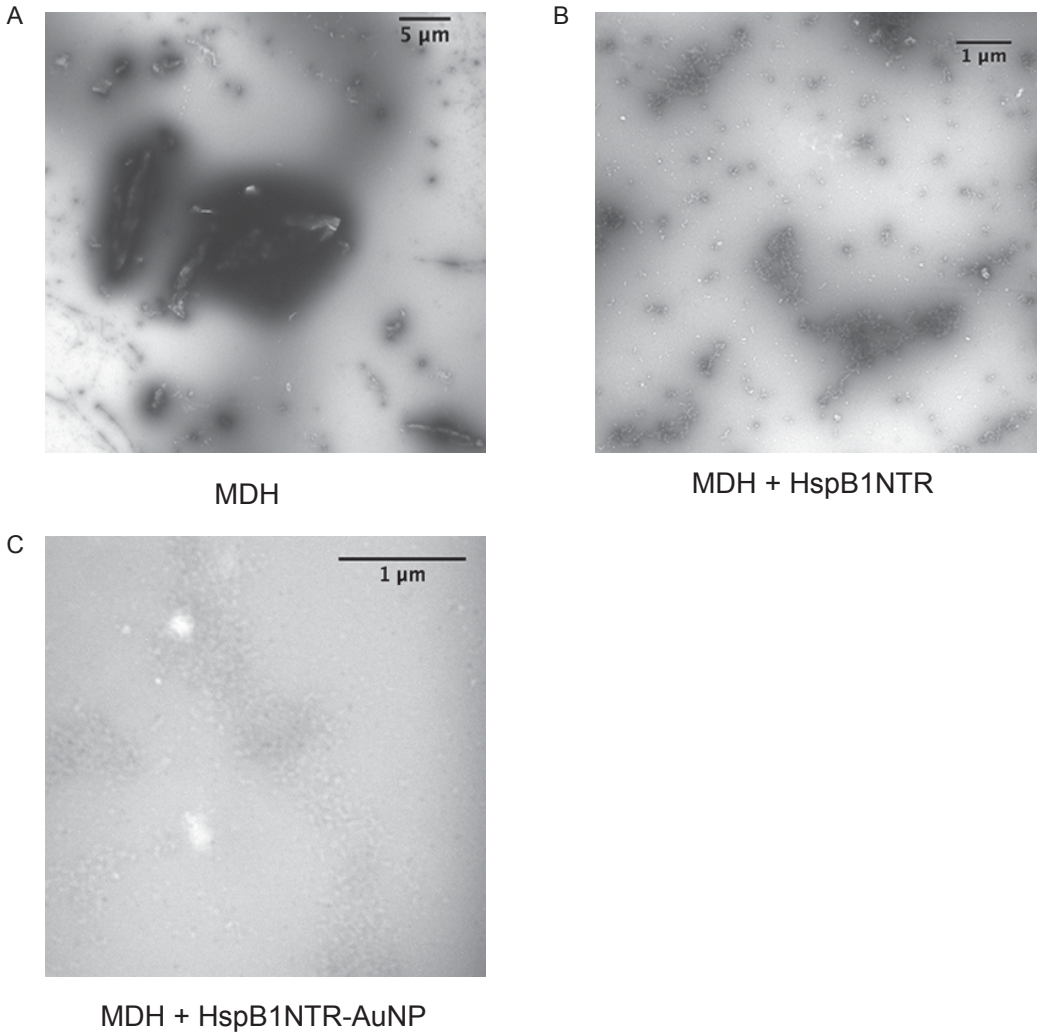

Figure S7. Transmission electron microscopy of MDH in the presence of conjugated and unconjugated HspB1NTR. The presence of HspB1NTR and HspB1NTR-NP changes the physical properties of the substrate, malate dehydrogenase. Transmission electron microscopy (TEM) images of the substrate malate dehydrogenase in the presence and absence of HspB1NTR. Briefly, the protein mixtures MDH(2.5µM), MDH(2.5µM) +B1NTS(15µM), and MDH(2.5µM)+B1NTS-AuNP (OD2) were prepared in PBS (pH 7.4) and heated at 45^{o}C for 1 hr while being stirred. After 1hr, 4 µL of each sample was applied to a carbon-coated 150 mesh copper grid (Electron Microscopy Sciences, Hatfield, PA) at room temperature, stained with freshly prepared 4% uranyl acetate, and examined under a Phillips/FEI CM100 (100kV). The images were obtained using an AMT digital camera and analyzed with ImageJ. (A) MDH (2.5 µM) after incubation at 45°C for 40 min, magnification 1450x. (B) MDH (2.5 µM) + HspB1NTR (2.5 µM) heated at 45°C for 40 min, magnification 5800x. (C) MDH (2.5 µM) + HspB1NTR-NP (14.9 nM) heated at 45°C for 40 min, magnification, 19000x. All samples were stained with 4% uranyl acetate.

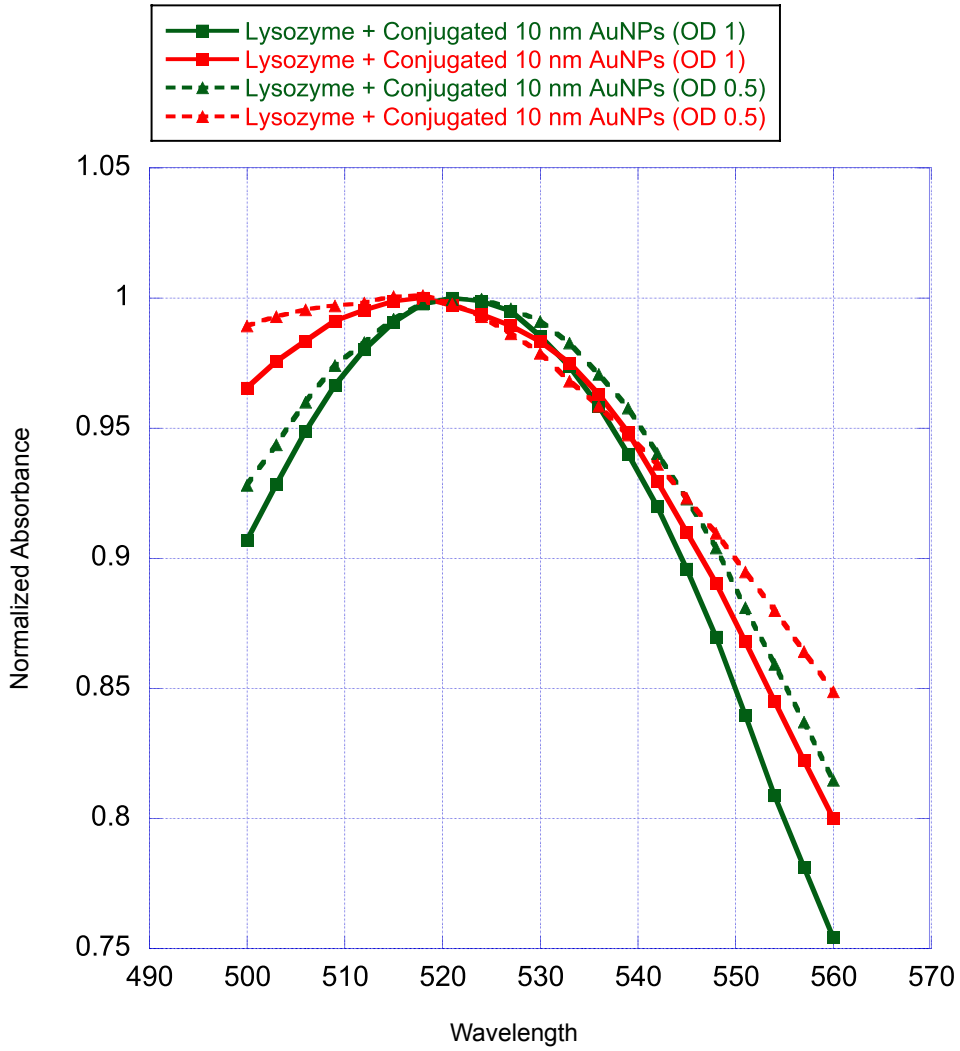

Figure S8. Absorbance of lysozyme with 10 nm B1NTR-AuNPs. Absorbance of lysozyme (35μM) + 10 nm B1NTS was observed between 500-560 nm. DTT (20mM) was added to induce substrate aggregation immediately prior to reading absorbance values. Green curves indicate absorbance before heating at 37°C**.** Red curves indicate absorbance after incubation for 90 minutes at 37°C**.** Each curve represents an average from 3 trials. Data was normalized by dividing each absorbance measurement by the maximum absorbance at each wavelength.

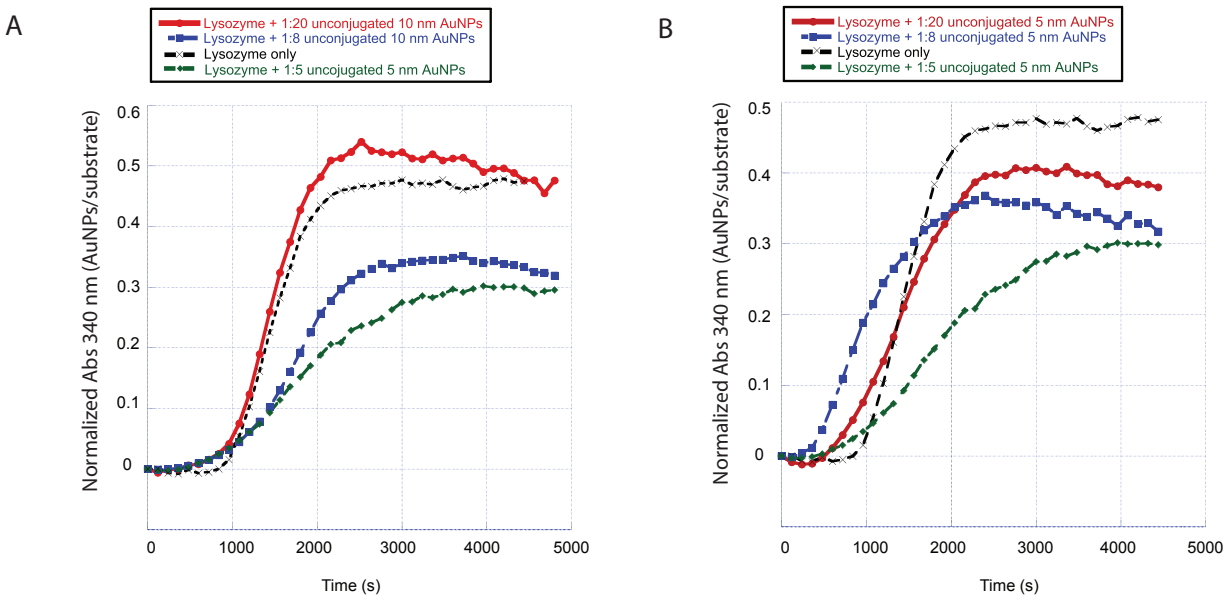

Figure S9. Aggregation of lysozyme with unconjugated 5 & 10 nm citrate-capped AuNPs. A) Concentration-dependent aggregation of lysozyme (35μM) with 10 nm citrate-capped AuNPs (OD 1 initial concentration) diluted to 1:5 (green), 1:8 (blue), and 1:20 (red). As concentration of unconjugated citrate AuNPs is increased, a corresponding decrease in Lys aggregation is observed. B) Aggregation of Lys (35 μM) is not concentration-dependent in the presence of 5 nm citrate-capped AuNPs (OD 1 initial concentration) diluted to 1:5 (green), 1:8 (blue), and 1:20 (red). Data was normalized to the maximum absorbance of the averaged Lys curves. Each curve is the average of ≥ 3 independent trials.

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