SUPPORTING INFORMATION:

Colloidal Stability versus Self-Assembly of Nanoparticles Controlled by Coiled-Coil Protein Interactions

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Experimental Section

Protein synthesis, purification, and characterization. The protein sequences are shown in Supplementary Table 1. The proteins cysA, Bcys, nA, and nB have been previously reported,[1,2] and cysB was constructed in the Qiagen pQE9 expression vector. Each protein was expressed in the *E.coli* strain SG13009 under control of the bacteriophage T5 promoter and purified through nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity chromatography, as previously reported.[1,2] All the cysteinated proteins were purified in the presence of 1 mM TCEP to reduce disulfide bonds, and Bcys and cysB were purified in the presence of 1 mM PMSF to prevent protein degradation. Purified proteins were dialyzed against water, followed by lyophilization. The proteins were characterized by using SDS-polyacrylamide gel electrophoresis and MALDI mass spectrometry on a 5800 MALDI/TOF Mass Spectrometer (AB-SCIEx).

Synthesis and characterization of GNPs. The Frens-Turkevich method was used to synthesize GNPs.[3] Briefly, a solution of gold (III) trichloride (Sigma Aldrich) was prepared at a concentration of 0.1 mg mL^{-1} in water and boiled under continuous stirring with a condenser apparatus attached. A solution of

trisodium citrate (Sigma Aldrich) was prepared at a concentration of 11.4 mg mL^{-1} and added quickly to the boiling gold (III) trichloride solution at a volume ratio of 1.88:100. The mixture was boiled for an additional 8 minutes, resulting in citrate capped GNPs. The GNPs were characterized by using dynamic light scattering (DLS) and UV-Vis spectrophotometry.

Surface modification of GNPs with proteins. The surface of GNPs was modified with cysA and cysB (cysA-cysB GNPs) or cysA and Bcys (cysA-Bcys GNPs) through the gold-thiol reaction. The solution of each cysteinated protein (50 μM) was prepared in the presence of its non-cysteinated heterodimerizing partner (75 μM) to form heterodimers (cysA/nB, cysB/nA, Bcys/nA), so that proteins covalently conjugated on GNPs would not be closely packed. Each solution was prepared in 40 mM HEPES buffer containing 5 mM TCEP (pH 7.8) and incubated for 12 hours at 4°C. To modify GNPs, the solutions of cysB/nA and cysA/nB or the solutions of Bcys/nA and cysA/nB were mixed at a 1:1 ratio, and the mixture was added dropwise to the GNP suspension (1.53E12 GNPs mL^{-1}) while stirring at a 1:3 ratio, followed by stirring overnight. The protein conjugated GNPs were stored at 4°C until further use and were characterized by using dynamic light scattering (DLS) and UV-Vis Spectrophotometry.

Sample preparation for examining particle self-assembly. The protein conjugated GNP suspensions and PBS (HyClone, 1X or 0.0067M, pH 7.4) were filtered through a 0.2-micron filter (Acrodisc). A 2 mL aliquot of each protein conjugated GNP suspension was centrifuged at 5.6krcf for 1 hour, followed by removal of 1.7 mL of the supernatant and resuspension in 1 mL of PBS. Immediately following resuspension was the Day 0 time point. Particle self-assembly was characterized by using UV-Vis Spectrophotometry, Transmission Electron Microscopy (TEM), and Small Angle X-ray Scattering (SAXS).

Dynamic Light Scattering. Samples were filtered through a 0.2-micron filter, and DLS was measured using a 90Plus Particle Size Analyzer (Brookhaven) at 90° scattering angle. The average hydrodynamic diameter was determined using the method of cumulants. Polydispersity values lower than 0.08 were considered to be monodisperse.

UV-Vis Spectrophotometry. UV-Vis spectra scanned from 350 nm to 750 nm were collected at a 600 nm min^{-1} scanning rate and a 1 nm resolution on a Cary Win 300 UV-Vis spectrophotometer (Agilent Technologies).

GNP size and concentration. The UV-Vis spectra revealed the size of GNPs and the concentrations of GNP suspensions according to Equations S1 and S2:[4]

$$d = \exp\left(B_1 \frac{A_{SPR}}{A_{450}} - B_2\right) \qquad \text{Equation S1}$$

$$N = \frac{A_{450} * 10^{14}}{d^2\left[-0.295 + 1.36 exp\left(-\left(\frac{d-96.8}{78.2}\right)^2\right)\right]} \qquad \text{Equation S2}$$

where d is the diameter in nanometers, N is the concentration of GNPs in particles mL^{-1}, A_{SPR} is the absorbance at the SPR peak (or maximum peak), A_{450} is the absorbance at 450 nm, and the constants B_1 and B_2 are 3.00 and 2.20, respectively.

Transmission Electron Microscopy. Samples were prepared by dropping 20 μL of a particle suspension onto a TEM grid (carbon type B, 300 mesh grid; TedPella), incubating for 20 minutes, and removing

excess liquid with a KimWipe. The TEM grids were further dried for 12 hours, and TEM images were collected on a Tecnai T12 instrument operated at 100kV.

Small angle x-ray scattering (SAXS). SAXS data were collected with a Cu K_α radiation source (operated at 60 kV and 0.3 Ma) at a wavelength of 1.5409 Å on SAXSlab Ganesha (Xenocs). After a sample was incubated at room temperature for the intended time, it was pipetted up and down to ensure uniform particle suspension, and loaded into a quartz capillary tube with an inner diameter of 1.3 cm. The tube was sealed with 5-minute epoxy (Loctite) and allowed to dry overnight. All spectra were collected with the x-ray beam centered near the bottom of the capillary tube, with a 2 mm centered beam stop using 2 aperture SAXS, and were transmission corrected and thickness corrected to the inner diameter of the tube. Filtered PBS buffer was used as the background for protein conjugated GNPs; the supernatant collected from a centrifuged, unmodified GNP sample was used as the background for unmodified GNPs. Collection times for SAXS measurements were 1 hour for test samples and 30 minutes for background samples. Background subtraction of all SAXS spectra was completed using JADE software.
The particle center-to-center distance d was determined from the scattering vector q according to Equation S3, where d is the interparticle distance and q is the scattering vector:[5]

$$d = \frac{2\pi}{q} \qquad \text{Equation S3}$$

The radius of gyration was determined by a Guinier plot fit to Equation S4:[6]

$$\ln(I) \approx \ln(I_o) - \frac{R_g^2 q^2}{3} \qquad \text{Equation S4}$$

where I is scattering intensity, I_0 is the background scattering intensity, q is the scattering vector, and R_g is the radius of gyration. PRIMUS software was used.[7] The hard sphere radius, R, was determined from the radius of gyration according to Equation S5.[8]

$$R = \sqrt{\frac{5}{3}} R_g \qquad \text{Equation S5}$$

Disassembly of self-assembled GNPs. GNPs self-assembled over 10 days were transferred to a low protein binding Eppendorf tube and heated at 80°C for 1 hour to denature the proteins. Samples were characterized using UV-Vis spectrophotometry before and after the treatment.

Effect of higher protein packing density on self-assembly of cysA-Bcys conjugated GNPs. It was expected that when the packing density of conjugated proteins on the GNP surface was too high for interparticle protein-protein heterodimerization to occur, self-assembly of cysA-Bcys conjugated GNPs would be inhibited. To test this hypothesis, surface modification of GNPs was conducted without non-cysteinated coiled-coil proteins (nA and nB), which were used to prevent an excessively dense packing. The nanoparticle behavior of these GNPs was examined over a time course of 10 days.

Effect of lower protein packing density on colloidal stability of cysA-cysB conjugated GNPs. It was expected that when the packing density of conjugated proteins on the GNP surface was too low for intraparticle coiled-coil heterodimerization to occur, colloidal stability of cysA-cysB conjugated GNPs would be impaired. To test this hypothesis, surface modification of GNPs was conducted in the presence of 0, 5, 25, and 50 μM β-mercaptoethanol (βME), a small thiolate that competes with thiol-mediated

protein binding to the GNP surface, to reduce protein packing density. The nanoparticle behavior of the resulting GNPs was examined.

Table S1 The sequences of the proteins used in the study. The coiled-coil domains are highlighted in blue. The cysteine residues are highlighted in red.

Bcys	MRGSHHHHHHGSDDDDKWASGTSGDLKNKVAQLKRKVRSLKDKAAELKQEVSRLENEIEDLKAKIGDH VAPRDTSMGGC
cysB	MRGSHHHHHHGSDDDDKASSGSGCSGSGTSGTSGDLKNKVAQLKRKVRSLKDKAAELKQEVSRLENEE DLKAKIGDHVAPRDTSW
cysA	MRGSHHHHHHGSDDDDKASSGSGCSGSGTSGDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLK AEIGDHVAPRDTSW
nA	MRGSHHHHHHGSDDDDKASGDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLKAEIGDHVAPRDTSW
nB	MRGSHHHHHHGSDDDDKASGTSGDLKNKVAQLKRKVRSLKDKAAELKQEVSRLENEIEDLKAKIGDHVAPRDTSW

Figures:

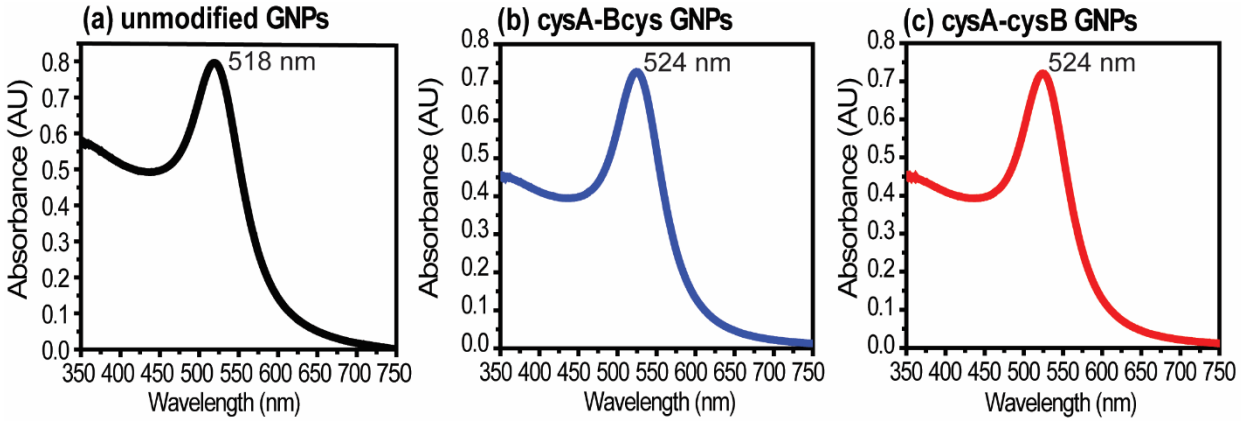

Figure S1 The UV-Vis spectra for unmodified GNPs (a), cysA-Bcys conjugated GNPs (b), and cysA-cysB conjugated GNPs (c) reveal a red-shift of the maximum SPR peak from 518 nm for unmodified GNPs to 524 nm for protein conjugated GNPs.

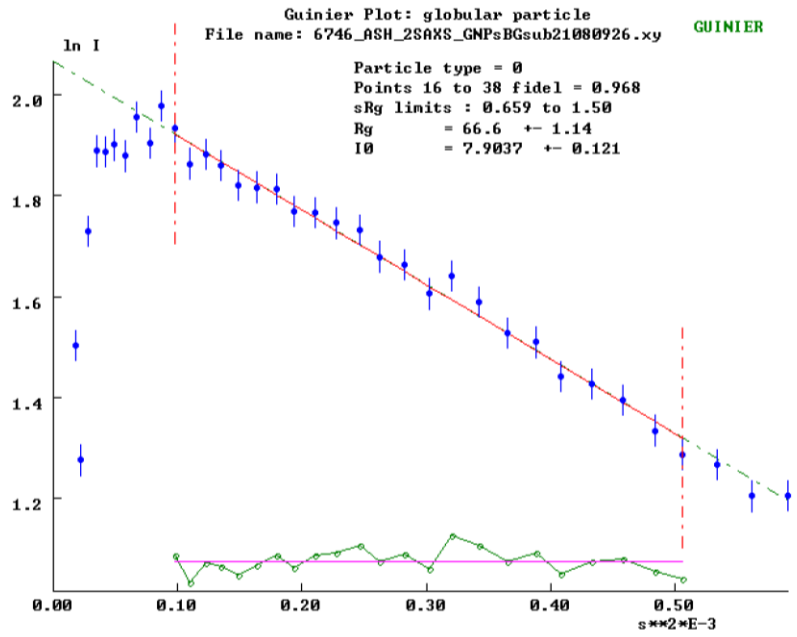

Figure S2 Guinier plot for the SAXS data collected from unmodified GNPs.

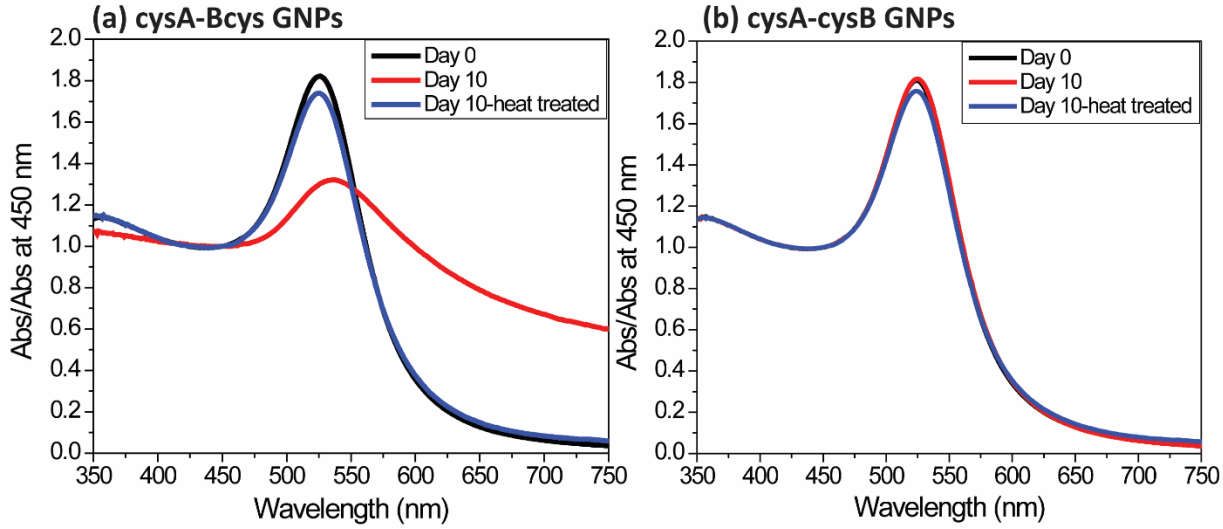

Figure S3 UV-Vis spectra of protein conjugated GNPs on day 0, day 10, and day 10 followed with heat treatment at 80°C for cysA-Bcys GNPs (a) and cysA-cysB GNPs (b). After heat treatment, broadening of the SPR peak of cysA-Bcys GNPs disappears and the maximum SPR peak is consistent with that before assembly, suggesting that self-assembled cysA-Bcys GNPs can disassemble upon protein denaturation.

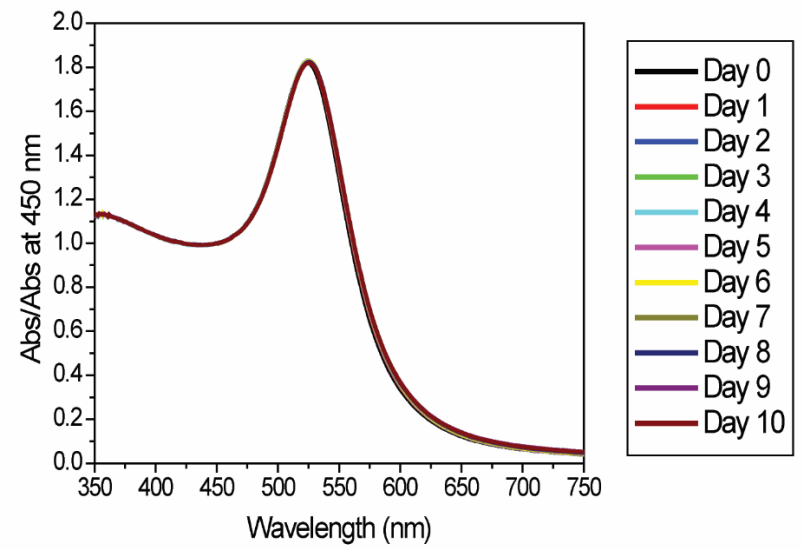

Figure S4 UV-Vis spectra of cysA-Bcys conjugated GNPs prepared without non-cysteinated proteins examined over 10 days. The UV-Vis spectrum does not show broadening or a shift of the maximum SPR peak for both types of GNPs.

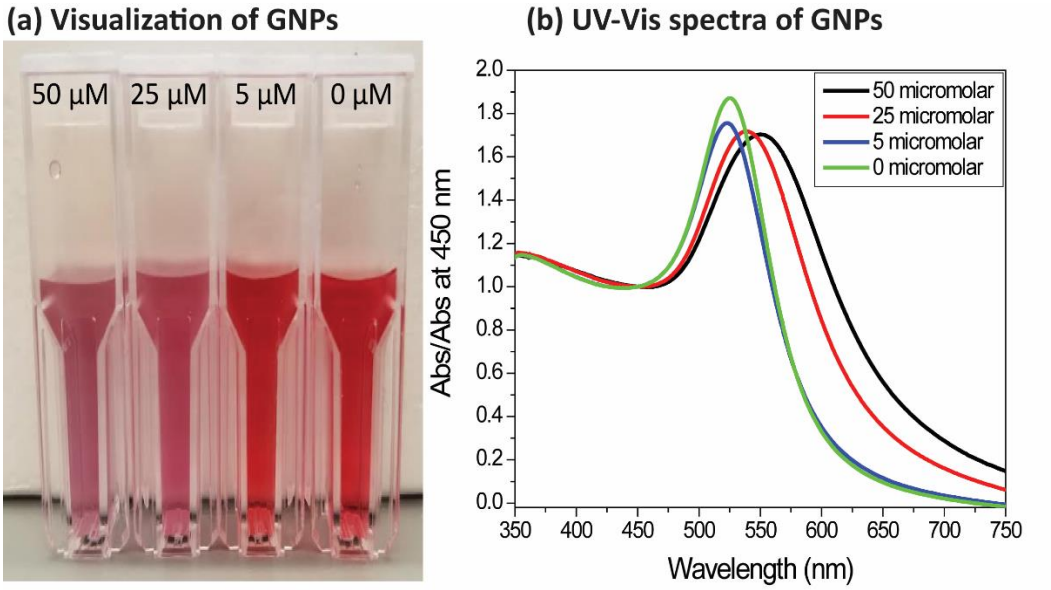

Figure S5 The particle behavior of cysA-cysB GNPs prepared with varying concentrations of β-mercaptoethanol (βME) on day 0. The samples prepared with high concentrations of βME showed unstability quickly, as revealed from the color of the particle suspensions (a) and the broadening and shift of the SPR peak (b).

References

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