# SUPPORTING INFORMATION

## Characterizing the Surface Coverage of Protein-Gold Nanoparticle Bioconjugates

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Contents:

**Figure S1:** TEM images and ImageJ analysis of synthesized AuNPs

**Figure S2:** Evidence for covalent binding of protein to AuNPs

**Figure S3:** Unstained SDS-PAGE conjugate gel

**Figure S4:** UV/Vis absorption spectra of conjugates

**Figure S5:** DLS of His tag containing DHFR-AuNP conjugates

**Figure S6:** SDS-PAGE of TEV cleaved protein

**Figure S7:** CD spectra of proteins

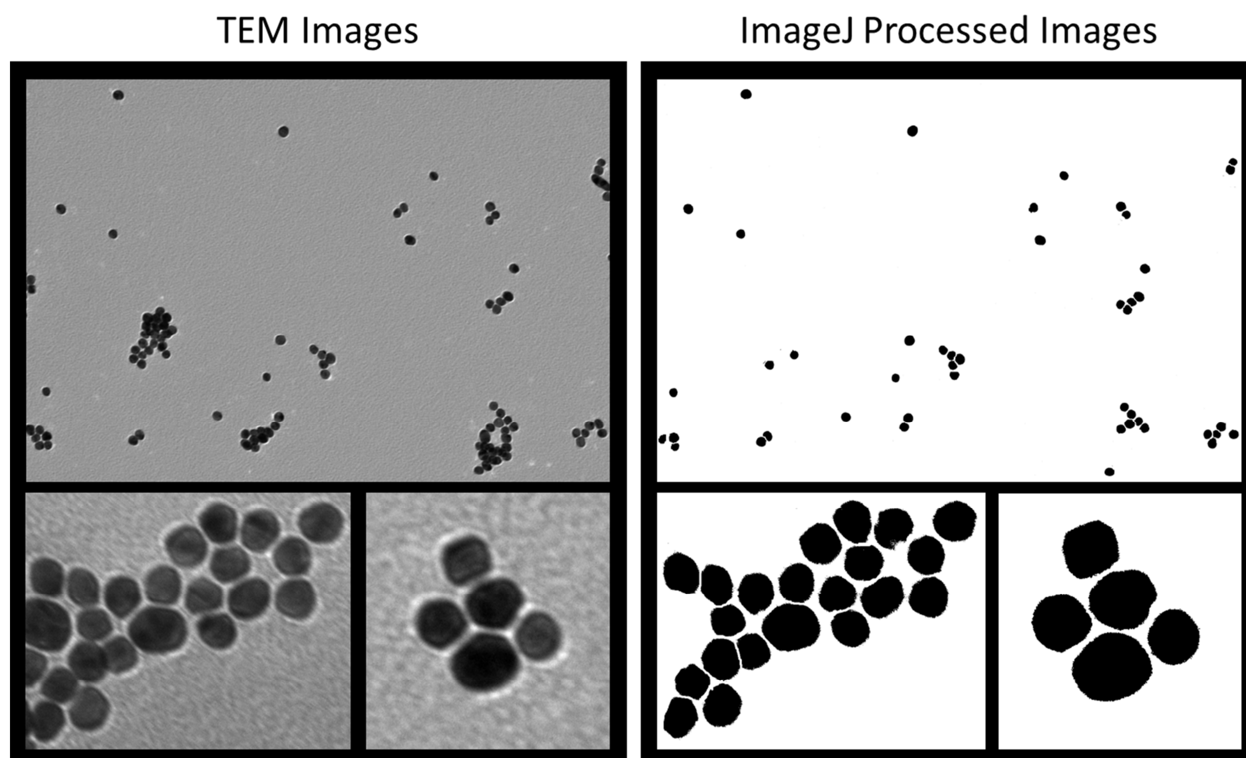


**Figure S1:** TEM images of synthesized AuNPs versus the same images processed in ImageJ for size analysis, each with different scale bars. The scale bar from each individual image was calibrated to set the scale in ImageJ for each respective image. The threshold in ImageJ was set to where the AuNPs were mostly saturated, but not bleeding into the background or each other, which turns the AuNPs black and the background white (in right images). Narrow lines between each AuNP were manually "erased" to keep them as separate particles for analysis. Any sections of aggregated AuNPs were completely erased because individual particles could not be distinguished. Particles were analyzed for area. The diameter was then determined from the area based on the area of a circle, $A = \pi(d/2)^2$. The average AuNP diameter and standard deviation of 75 analyzed particles were determined to be 14.1 ± 1.4 nm.

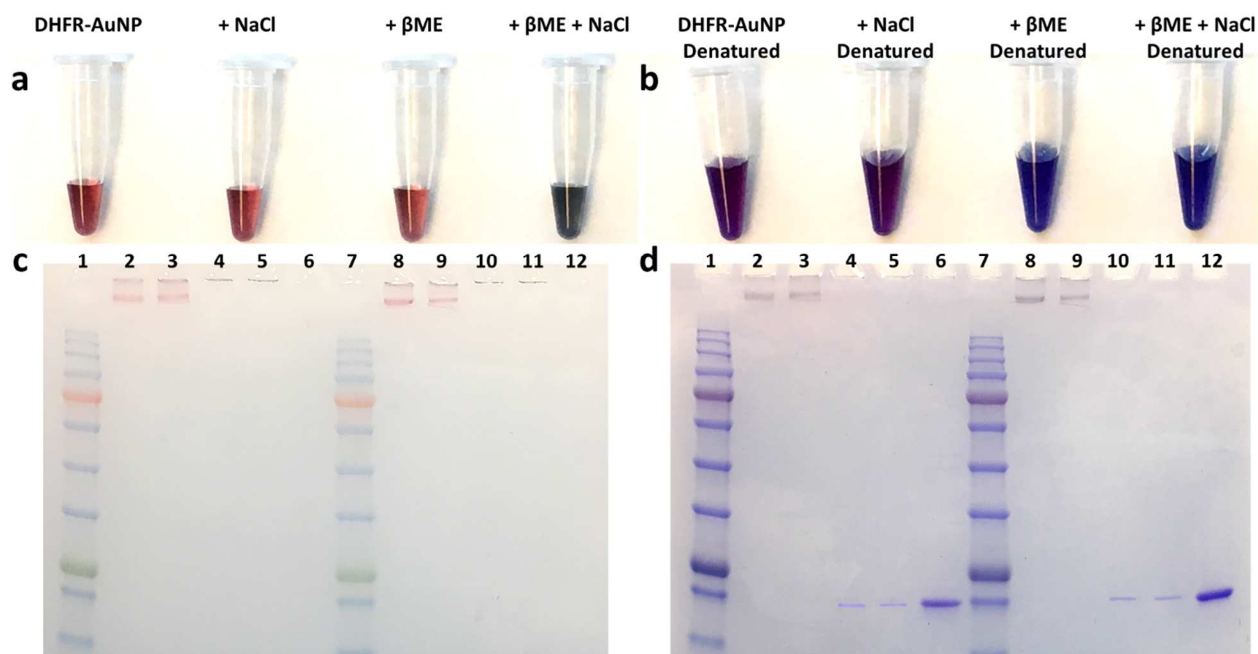


**Figure S2:** Evidence for covalent binding of protein to AuNPs. **a.** Pictures of DHFR-AuNP conjugates with the E120CΔCys mutant (FG loop), 1 hour after preparation. The first tube contains the standard DHFR-AuNP conjugates prepared according the method described in the methods section. The pink color indicates stable, non-aggregated AuNPs. Tube 2 contains the standard conjugates with 1 M NaCl added. In the absence of protein, addition of high salt causes the AuNPs to aggregate, changing the color to purple, but in this case DHFR conjugation stabilizes the AuNPs and no aggregation is observed. Tube 3 contains the standard conjugate preparation in 10 mM βME; it has the identical pink color of stable AuNPs, indicating βME does not induce their aggregation. Tube 4 is the standard conjugate preparation with 10 mM βME and 1 M NaCl. The purple color is clear evidence of aggregation, indicating the AuNPs are not stabilized by DHFR in this case. βME caps the gold binding sites on the AuNPs as well as the thiols on the protein, preventing covalent binding and formation of stable conjugates. Therefore, upon addition of both βME and high salt, the AuNPs aggregate because the protein is not bound in this case.

**b.** A similar effect of the addition of salt to βME containing conjugates is obtained when the protein is denatured in Laemmli (SDS) buffer. After boiling these samples for 5 minutes (followed by icing for 5 minutes), tubes 1 and 2 have a color characteristic of stable AuNPs, with a purple tint due to the dye present in the Laemmli buffer. Tubes 3 and 4 are blue in color, rather than purple, indicating aggregation of the AuNPs when βME is present under denaturing conditions. Again, the absence of a stable covalent attachment of the enzyme in the presence of βME destabilizes the AuNPs. Aggregation in the presence of βME alone under denaturing conditions likely means that semi-stable conjugates form even in the absence of covalent binding with the folded protein, possibly by electrostatic interactions, but not when it is unfolded. **c.** Unstained SDS-PAGE gel of the DHFR-AuNP samples. Laemmli sample buffer is added to all conjugate samples to denature the protein before running the gel. Lanes 1 and 7 are protein ladder. Lanes 2-5 are for FG Loop-15 nm AuNP conjugates, and lanes 8-11 are for Alpha Helix-15 nm AuNP conjugates. Lane 6 is free FG Loop DHFR, and lane 12 is free Alpha Helix DHFR. Lanes 2 and 8 are standard DHFR-AuNP conjugates, lanes 3 and 9 are DHFR-AuNP conjugates + NaCl, lanes 4 and 10 are DHFR-AuNP conjugates + βME, and lanes 5 and 11 are DHFR-AuNP conjugates + βME + NaCl. It is clear that stable conjugates are present in lanes 2-3 and 8-9, since the conjugate bands travel down the lanes whereas unconjugated AuNPs do not. Free protein is also not present in these cases, since all of the protein remains covalently bound to the AuNPs.  In contrast, the AuNPs have aggregated in lanes 4-5 and 8-9, indicating stable conjugates are not formed in the presence of βME, which blocks covalent binding. **d.** Stained gel of the samples. Lane assignments are described in **c.** Here, it is evident that there is protein bound to AuNPs in lanes 2-3 and 8-9, by the color of the conjugates turning from pink in **c** to

purple. Further, there is clearly free, unbound protein present in lanes 4-5 and 10-11, indicating that the presence of βME prevents DHFR binding to the AuNPs.

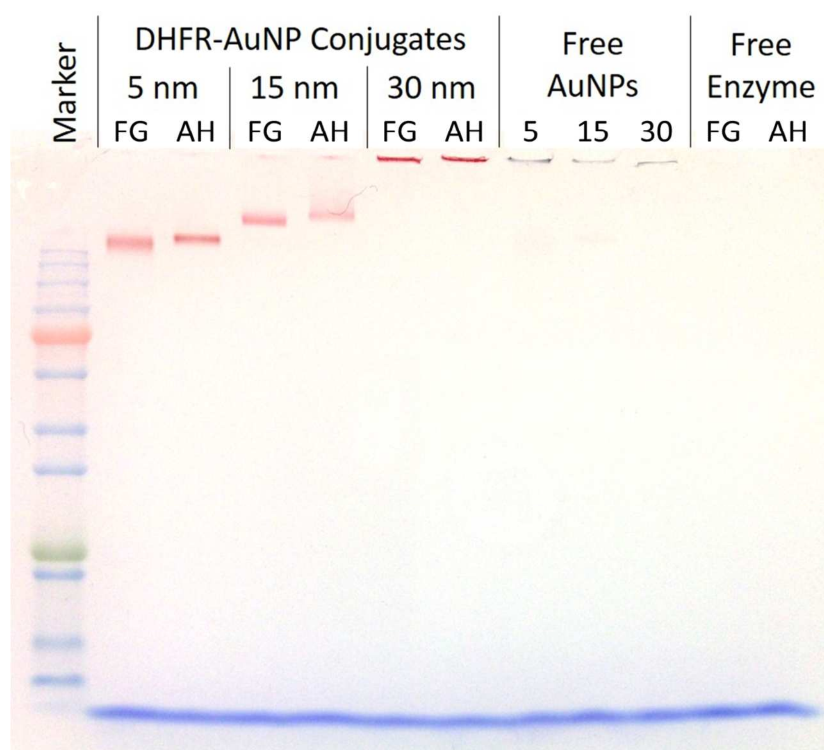


**Figure S3:** Unstained SDS-PAGE gel. Lane 1 is protein marker. Lanes 2-3 are DHFR-5 nm AuNP conjugates. Lanes 4-5 are DHFR-15 nm AuNP conjugates. Lanes 6-7 are DHFR-30 nm AuNP conjugates. Lanes 8-10 are free AuNPs: 5 nm, 15 nm, and 30 nm. Lanes 11-12 are free protein. The DHFR-AuNP conjugates are pink in color, indicating their stability. The smaller AuNP conjugates run faster on the gel than the larger AuNP conjugates. The free citrate

stabilized AuNPs are deep purple and did not leave the wells during the run cycle of the gel. These AuNPs aggregated immediately upon addition to the well, indicating that the AuNPs in lanes 2-7 are indeed bound to protein. The gel is unstained, so no sample is visible in the free protein lanes. FG = FG Loop mutant. AH = Alpha Helix mutant. In the samples run on the gel, the concentration of protein on the AuNPs is on average 900 ng, and the detection limit of the Coomassie Blue stain is known to be 30 ng.[1] Thus, the detection limit is approximately 3% of the AuNP bound protein concentration.

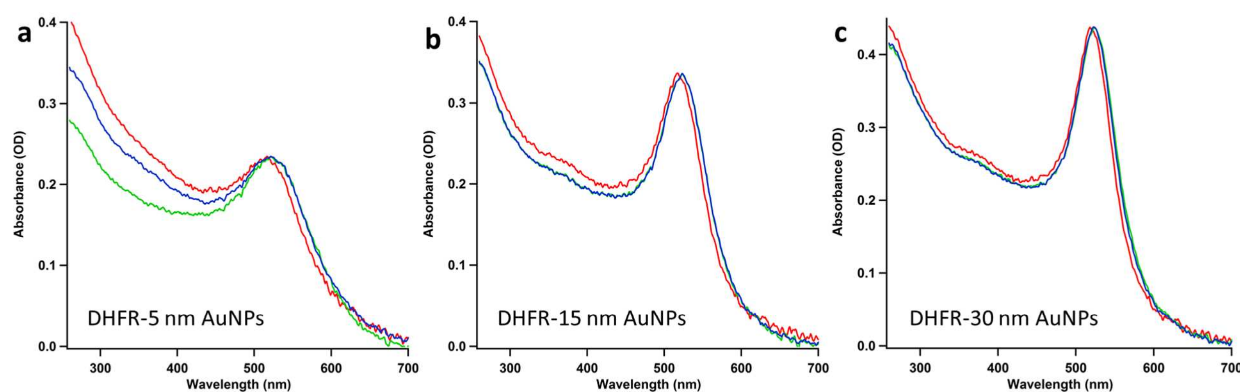


**Figure S4:** UV/Vis absorption spectra of DHFR-AuNP conjugates. **a.** 5 nm AuNP conjugates. SPR shift from 515 nm to 521 nm upon protein conjugation. **b.** 15 nm AuNP conjugates. SPR shift from 518 nm to 523 nm. **c.** 30 nm AuNP conjugates. SPR shift from 520 nm to 524 nm. Red = Free AuNPs. Green = FG Loop mutant conjugates. Blue = Alpha Helix mutant conjugates.

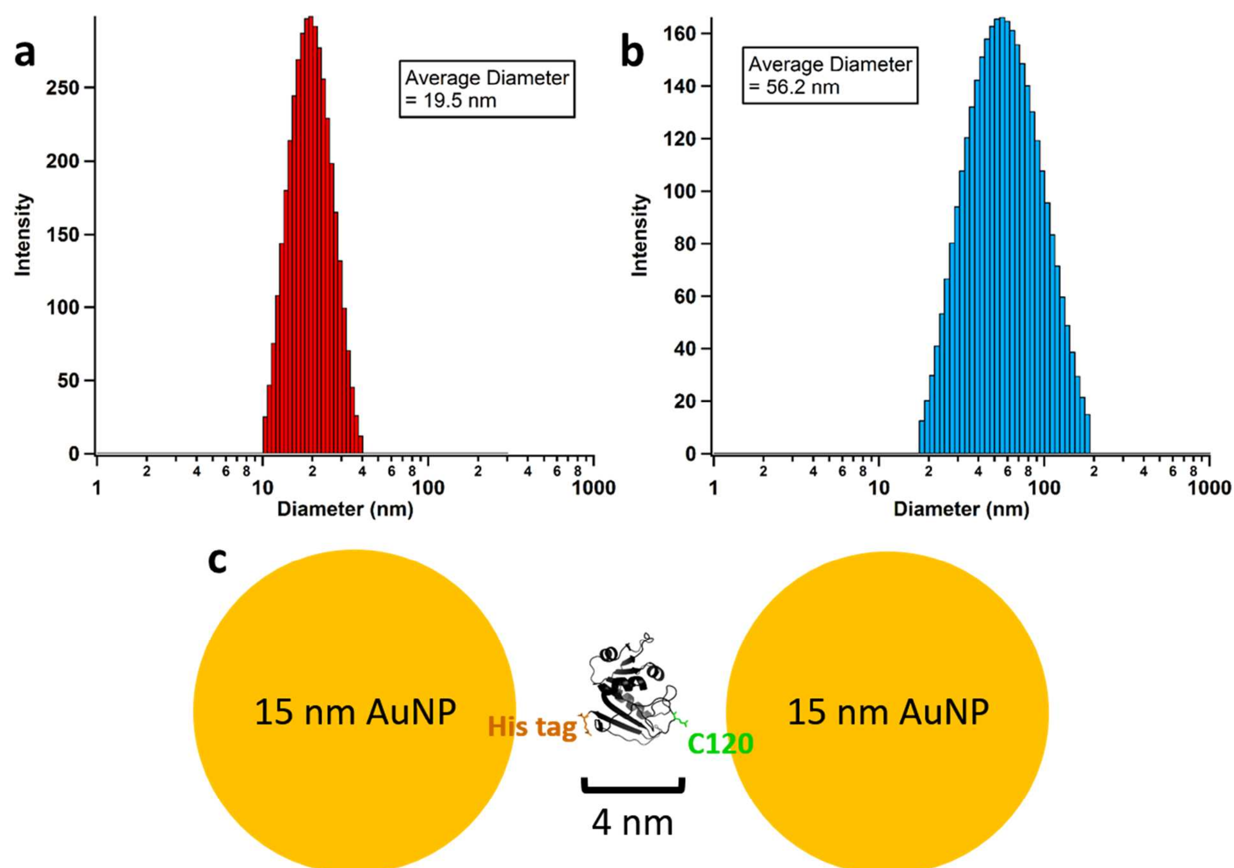


**Figure S5:** DLS comparisons of FG Loop mutant of DHFR prior to His tag being cleaved. **a.** Free, unbound 15 nm AuNPs. Diameter obtained is 19.5 nm. **b.** FG Loop mutant with His tag still present. Diameter obtained is 56.2 nm. **c.** Schematic of a potential scenario. One DHFR molecule is 4 nm at its longest, so the expected diameter of the conjugates is free AuNPs plus one full layer of protein, 8 nm maximum, which would be 27.5 nm here. However, 56.2 nm is the observed diameter, meaning there is likely some kind of interaction of the His tag with the AuNPs, potentially a bridging interaction, as depicted here.

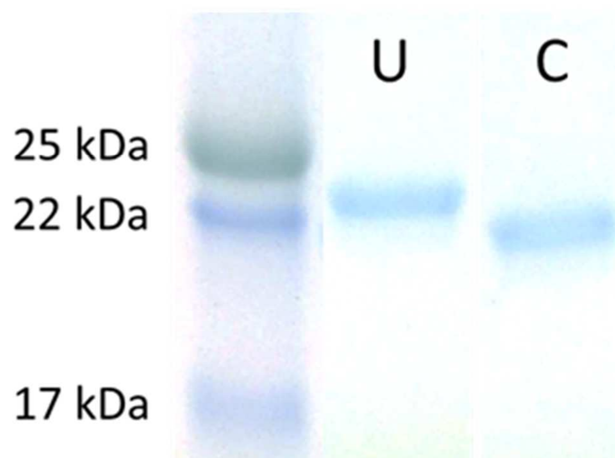


**Figure S6:** SDS-PAGE gel of the FG Loop mutant before (Lane 2) and after (Lane 3) TEV cleavage. Uncleaved protein (U) runs at 23 kDa. Cleaved (C) protein runs at 21 kDa. The Alpha Helix mutant also shows the same gel profile. Protein ladder is in lane 1.

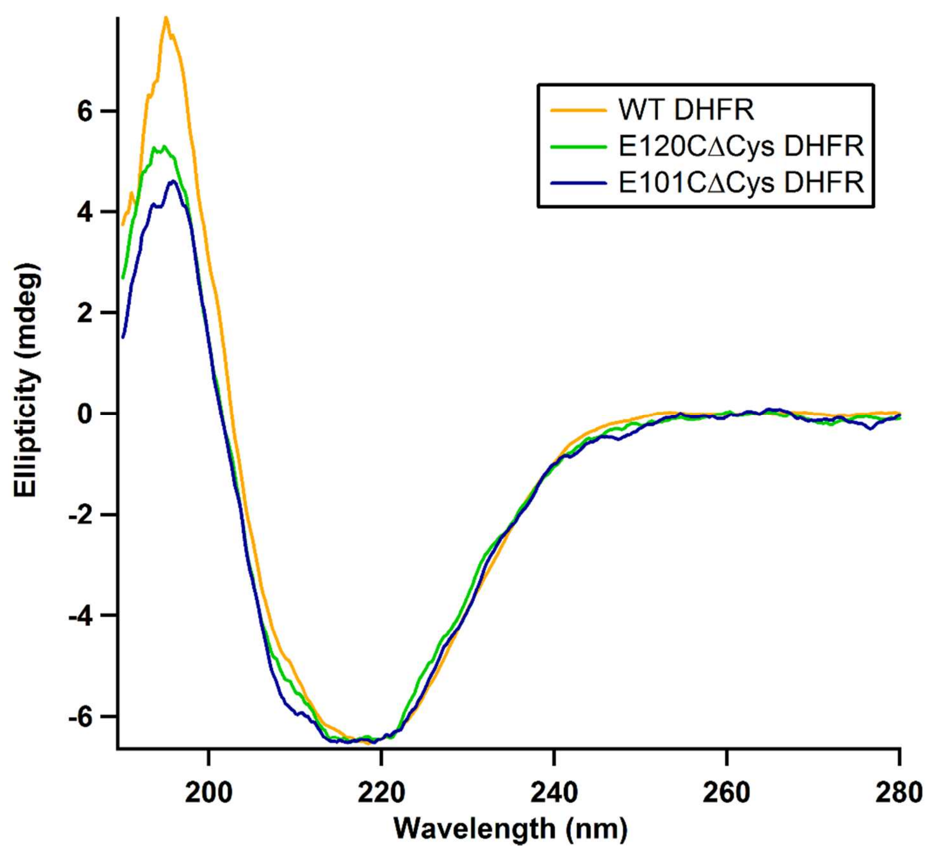


**Figure S7:** CD spectra of 5 μM free WT, E120CΔCys, and E101CΔCys DHFR in 10 mM sodium phosphate buffer, pH 7. There is very little change in the ellipticity of the mutants in comparison to WT DHFR, indicating that the secondary structure of the mutants is similar to that of WT DHFR; the mutants are properly folded.

Supporting Information Reference:

(1) Gauci, V. J.; Wright, E. P.; Coorssen, J. R., Quantitative proteomics: assessing the spectrum of in-gel protein detection methods. *Journal of Chemical Biology* **2011,** *4* (1), 3-29.