Supplementary Information

Synthesis and Bioconjugation of 2 and 3 nm-diameter Gold Cluster Compounds

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S1. Details of particle synthesis. Chemicals were supplied by Sigma/Aldrich and used as supplied, with the exception of 4-mercaptobenzoic acid, which was supplied by TCI America. In a typical synthesis, a concentrated gold-thiol polymer[1] stock solution is first made. This solution is composed of 1mM $HAuCl_4$, 3.4mM 4-mercaptobenzoic acid, and 50% aqueous methanol solution with the pH adjusted to ~13 using sodium hydroxide. This solution is allowed to sit overnight at room temperature in a sealed vessel in order to allow full reaction of the Au(III) salt and thiol.

For the products shown in figure 1, for instance, the stock solution was diluted to the a final concentration of 27% methanol by addition of appropriate amounts of methanol and water to a final Au(III) concentration of 10μM. Sodium borohydride was added in an empirically determined excess, typically a ~3:1 molar excess of $NaBH_4$ to Au.

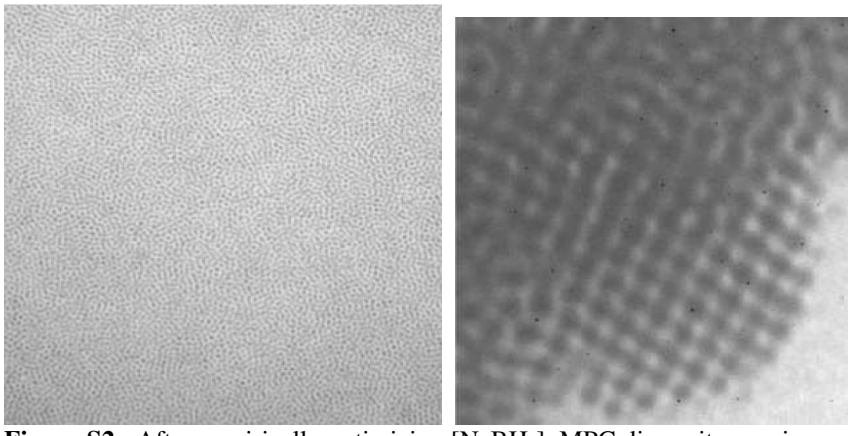


Figure S2. After empirically optimizing [$NaBH_4$], MPC dispersity was improved further. Left panel shows a TEM image of a thin film of the 2.0nm particles, right panel shows crystalline order from a drop cast solution of 3.0nm particles.

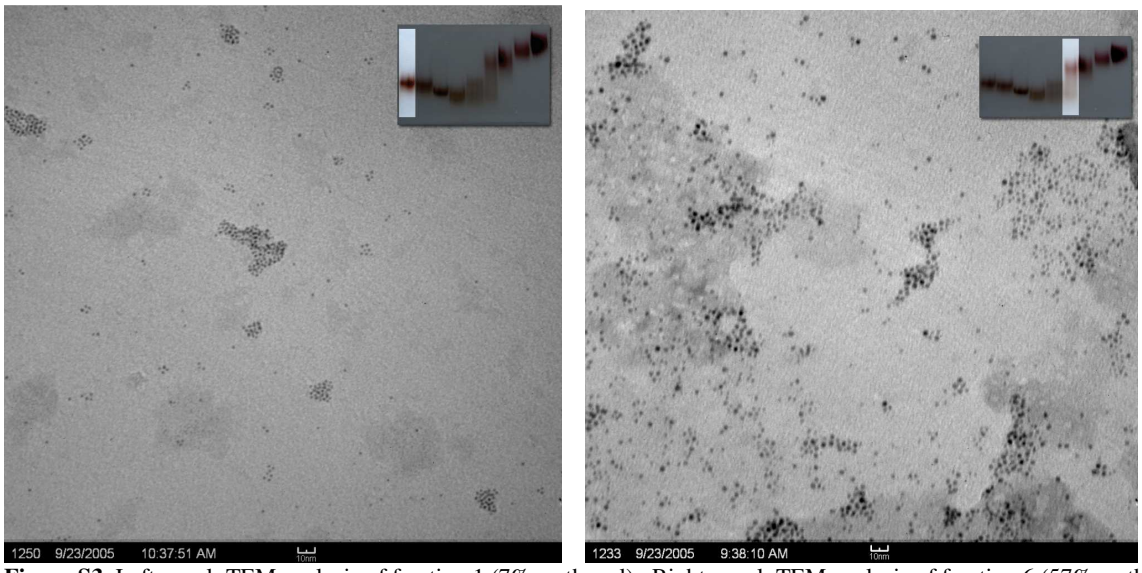


Figure S3. Left panel: TEM analysis of fraction 1 (7% methanol). Right panel: TEM analysis of fraction 6 (57% methanol)

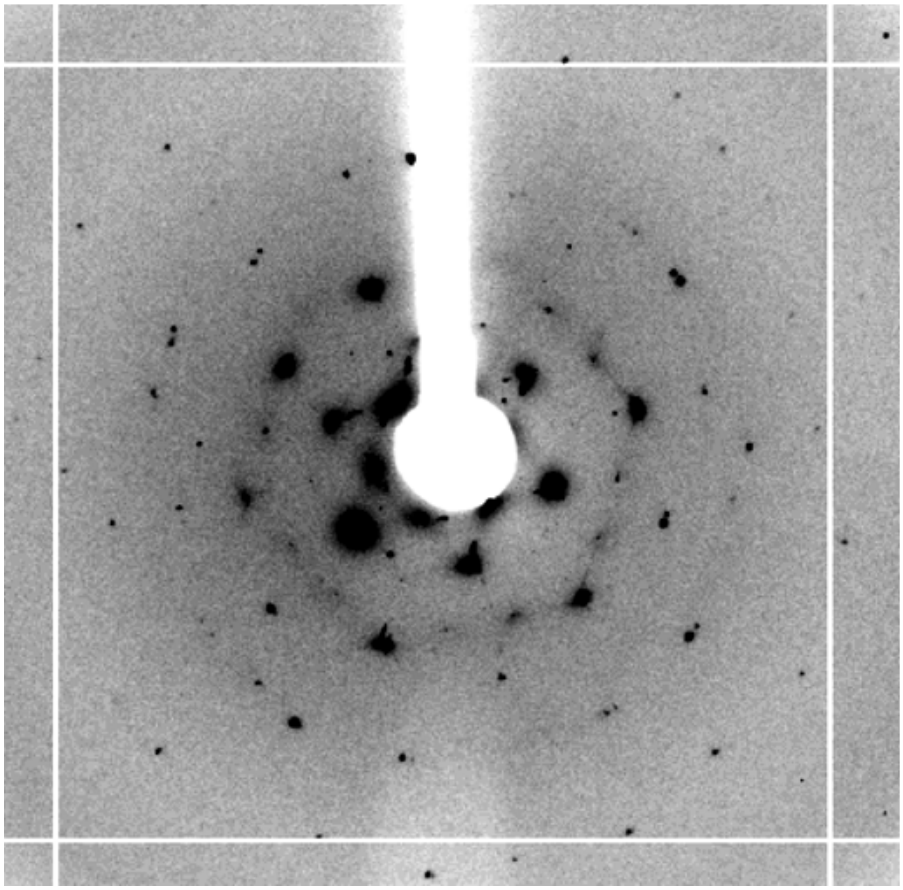
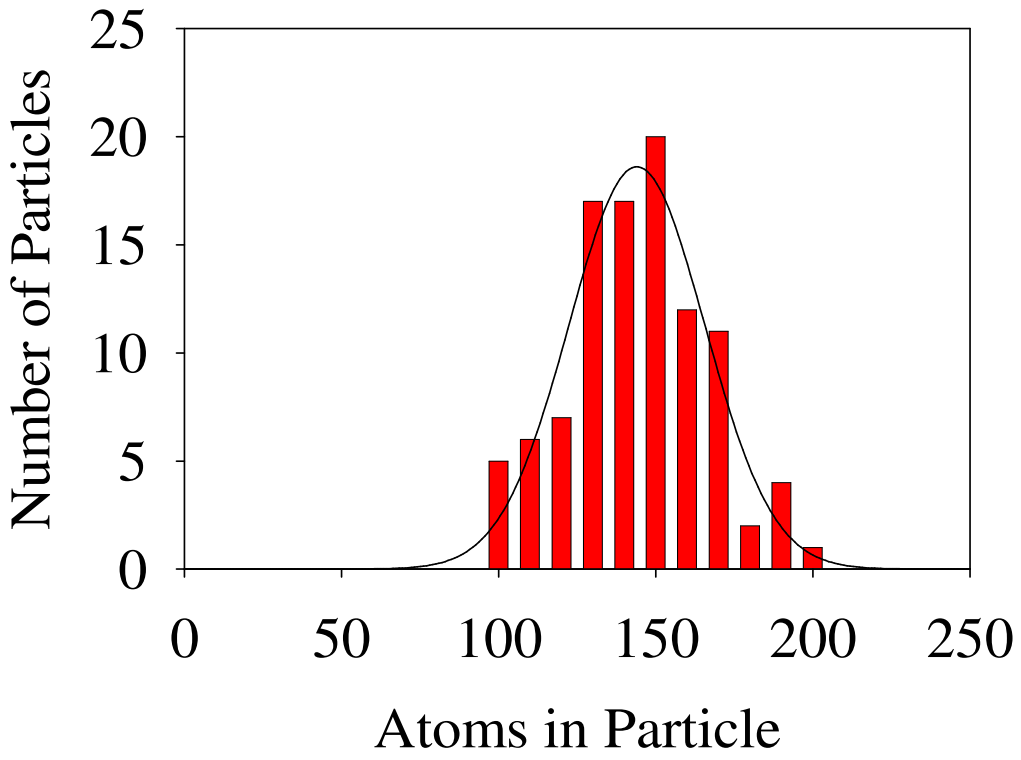


Figure S4. X-ray diffraction pattern from single crystal grown from 2.0nm particles.



S5. HAADF-STM Analysis. High Angle Annular Dark Field - Scanning Transmission Electron Microscopy was performed by Laruent Menard at the University of Illinois - Urbana Champaign. For a total of 102 clusters analyzed, the average partile size was determined as 2.0±0.2nm. Average core size is 144±22 atoms. This dispersion compares favorably with the atomically defined Au_{13} particle which was used to establish the techique.

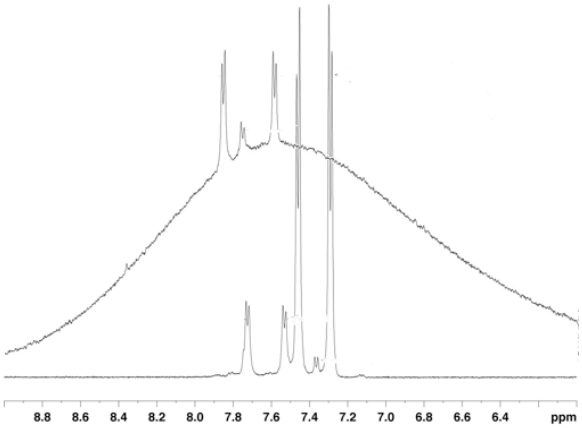
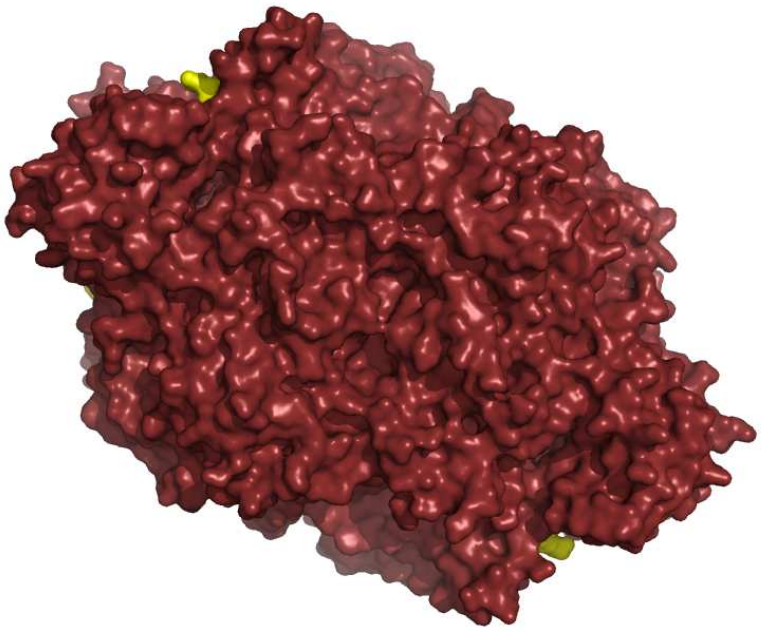


Figure S6. ^{1}H NMR spectra of the 2nm 4-mercaptobenzoic acid protected MPC (top trace) and as-supplied 4-mercaptobenzoic acid (bottom trace). The major product appears oxidized as disulfide in the as-supplied material. Peak broadening and downfield chemical shift is observed for nanoparticle bound 4-mercaptobenzoic acid. ^{1}H NMR data were acquired on a 500 MHz Bruker AVANCE instrument with a Bruker 5 mm ^{1}H triple resonance Z-PFG CryoProbe. The data indicates that the 4-mercaptobenzoic acid is intact on the MPC surface.



S7. K560C Catalase mutant. Mutant residues are rendered in yellow, and despite being solvent exposed, do not react to form covalent bonds with the AuNP. This is presumably due to the recessed position of the cysteine residue.

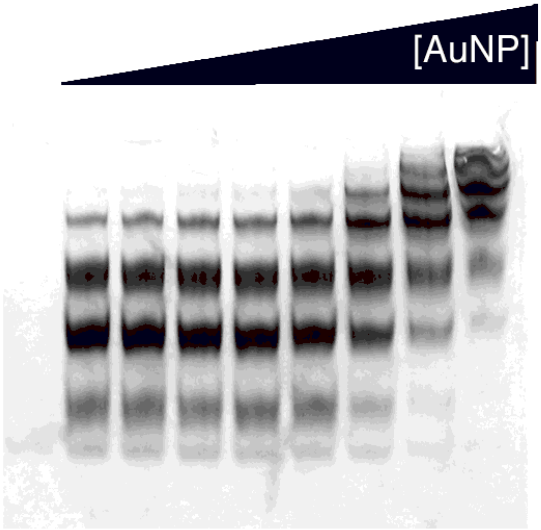
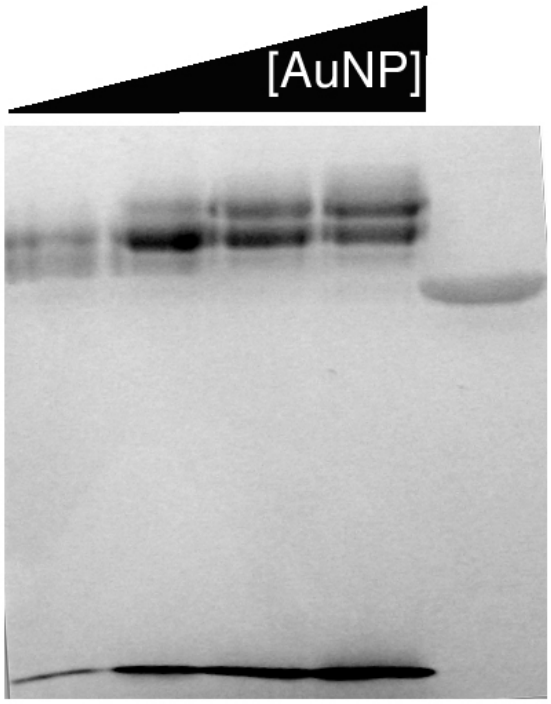


Figure S8. PIC DNA labeling. Shown is an image of "phosphor shadow" a 10T/5C Acrylamide gel showing product bands which result from conjugation of DNA to the 2.0nm particle. This imaging modality is very sensitive to DNA and less sensitive to the AuNP. Free DNA ran off the bottom of the gel. Free AuNP is the bottom most band, which is the only band present in the leftmost lane. Following from the leftmost lane are increasing concentrations of DNA, from approximately a 5:1 molar excess of DNA to particle in the second lane from the left, increasing to a 50:1 molar excess of DNA :AuNP in the rightmost lane.



S9. Actin labeling. 12T/3.3C SDS-PAGE gel showing gel shifted bands due to conjugation of one or more 2.0nm MPCs to rabbit muscle actin. In the rightmost four lanes of this gel, from left to right, are increasing concentrations of the 2.0nm MPC, mixed against a constant concentration of actin. In the rightmost lane is the same amount of actin. Up to three product bands are observed, indicating reaction to up to three of the seven cysteine residues of this protein.

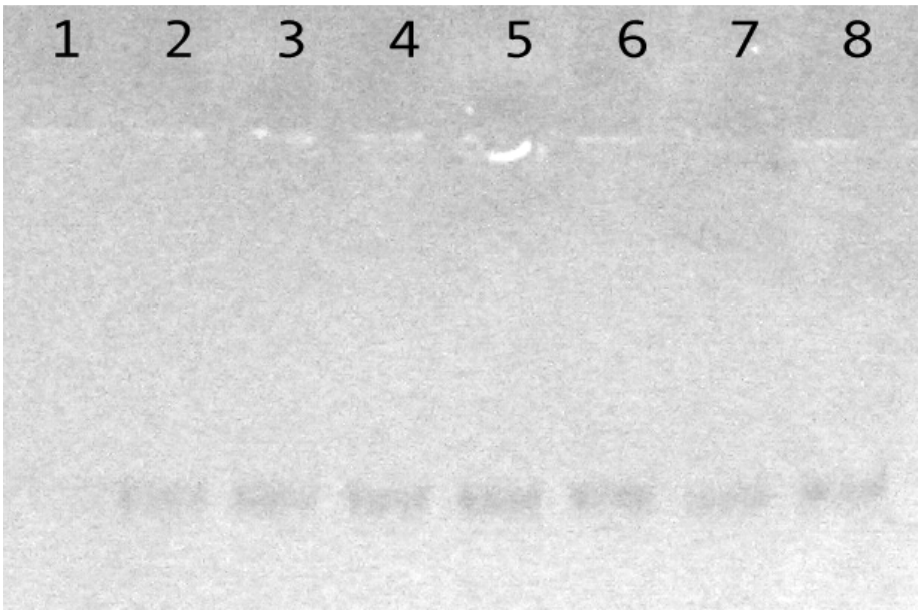


Figure S10. To render the pMBA MPCs non-reactive to subsequent exchange onto proteins, the MPCs were "re-passivated" with glutathione – a ligand we know from previous studies will render MPCs essentially no-reactive. Shown above is a a 10T/5C SDS-PAGE gel the result of challenging an scFV/MPC conjugate with glutathione in working concentrations of 16mM, 4mM, 1mM, 0.25mM, 62.5μM, 15.6μM, and 3.9μM and 0μM. The result of each experiment is shown from left to right. The absence of a gold-band in the leftmost lane indicates that the concentration of glutathione was sufficiently high to cleave the scFv/MPC bond.

The feed-ratio of glutathione used in a preparative scale repassivation experiment was determined empirically by titration of glutathione against pMBA/MPC/scFv conjugate. At high concentrations of GSH, the scFv/gold conjugate may be broken, as GSH displaces the scFv on the gold nanoparticle surface. At low concentrations of GSH, the MPC may retain reactivity. Typical concentrations of glutathione used in preparative scale repassivation are 4-fold below the lowest GSH concentration which disrupts the MPC/scFv bond, such as shown in lane 3. Since the pMBA appears to be a more 'labile' ligand than others we have attempted in exchange reactions, we anticipate that most or all pMBA is exchanged from the MPC before the scFv is exchanged.

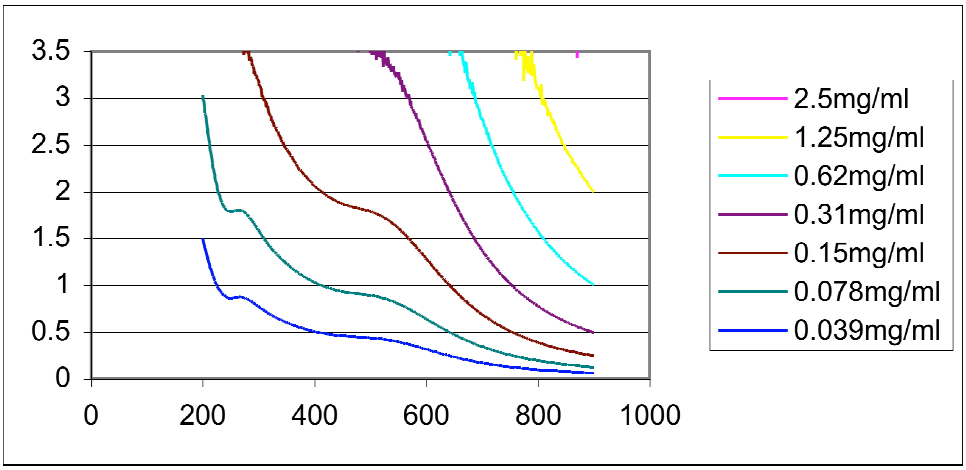


Figure S11. Optical electronic spectra were obtained for various concentrations of the 2.0nm particles. The surface plasmon absorbance peak at 510nm is apparent. If a formula of $Au_{144}(p\text{-MBA})_{52}$ is assumed for this compound a molar extinction coefficient of 4.35×10^5 M^{-1} cm^{-1} at 510nm is calculated for this MPC.

1. Bau, R., Crystal Structure of the Antiarthritic Drug Gold Thiomalate (Myochrysine): A Double-Helical Geometry in the Solid State. *Journal of the American Chemical Society* **1998,** 120, (36), 9380-9381.