

Isolation and Characterization of Trioxyethylene-Encapsulated Gold Nanoclusters Functionalized with a Single DNA Strand

Sulay D. Jhaveri,[‡] Edward E. Foos,[†] Daniel A. Lowy,[‡] Eddie L. Chang,[†]
Arthur W. Snow,[†] and Mario G. Ancona^{*,†}

Naval Research Laboratory, Washington, D.C. 20375, and Nova Research,
Alexandria, Virginia 22308.

Received January 7, 2004; Revised Manuscript Received February 6, 2004

ABSTRACT

A scalable means of isolating significant quantities of trioxyethylene-encapsulated gold nanoclusters (GNCs) labeled with a single DNA strand is demonstrated. Quantitative characterization of the product is obtained using UV–vis absorbance spectroscopy. These methods of isolation and characterization of singly functionalized GNC–ssDNA conjugates are expected to aid development of nanoscience applications that use DNA molecular recognition for guiding self-assembly.

Gold nanoclusters (GNCs) have received considerable attention as potential building blocks for a variety of nanoscale applications, including chemical sensing, electronics, optics, and biology.^{1–6} A scalable method for isolating significant quantities of GNCs with a known number of DNA strands per GNC will have substantial impact in nanoscale applications dependent on using DNA molecular recognition in patterned self-assembly schemes, since DNA, with its highly specific base-pairing, is attractive as the basis of self-assembly of nanoclusters.^{7–11} Earlier work demonstrated the feasibility of this approach using GNCs coupled with multiple oligonucleotides; but to go beyond these assemblies, and for diagnostics based on the quantification of hybridization events, it is essential to work with GNCs bearing one and only one oligonucleotide strand.¹² Using a new kind of GNC that is encapsulated with trioxyethylene thiol, we report here a facile new extraction technique for isolating DNA–GNC conjugates that has a number of advantages over the gel electrophoresis technique employed previously. In addition, characterization by UV–vis absorbance spectroscopy demonstrates that the product isolated by this technique has a DNA/GNC ratio approaching 1:1.

Initial work on the preparation of DNA–GNC conjugates^{9–13} employed citrate-stabilized gold colloids that were subsequently passivated with a monolayer of anionic phosphine molecules^{9,10,12,13} in order to avoid nonspecific binding

of nucleic acids¹⁴ and overcome the tendency toward self-agglomeration and precipitation. Previously, we had developed another type of GNC, denoted as AuEO3 which consists of a 1.8 ± 0.2 nm gold core encapsulated by a monolayer of a methyl-terminated tri(oxyethylene) thiol, $\text{CH}_3(\text{OCH}_2\text{CH}_2)_3\text{SH}$.¹⁵ Being direct analogues of the well-known alkanethiol monolayer protected clusters, AuEO3 are very attractive for use in combination with biomolecules in that their synthesis exploits the well-understood Au–thiol chemistry yet these GNCs are water-soluble, charge-neutral, and show stability over a broad range of pH and ionic strengths.¹⁵ Charge neutrality is a particularly important attribute for cluster/biomolecule coupling since a ligand shell that contains ionic species or which is able to participate in hydrogen bonding may lead to self-agglomeration or nonspecific interactions with the DNA.¹⁴ The trioxyethylene shells are expected to suppress such unwanted processes much like similar polyethylene coatings on flat gold surfaces,¹⁶ and a recent report demonstrates that these AuEO3 clusters resist the nonspecific binding of both proteins and nucleic acids.¹⁷ Finally, from an electrical perspective these clusters are attractive because (i) they are small enough (<3 nm) to show Coulomb blockade effects at room temperature, and (ii) their trioxyethylene coating is not so thick as to block all electron transport between neighboring clusters.^{18,19}

The existing method for isolating conjugates containing both singly and multiply functionalized clusters from unreacted starting materials is via gel electrophoresis, followed by elution of the appropriate band.^{12,13} This is a tedious

* Corresponding author. E-mail: ancona@estd.nrl.navy.mil

[†] Naval Research Laboratory.

[‡] Nova Research.

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE FEB 2004		2. REPORT TYPE		3. DATES COVERED 00-00-2004 to 00-00-2004	
4. TITLE AND SUBTITLE Isolation and Characterization of Trioxyethylene-Encapsulated Gold Nanoclusters Functionalized with a Single DNA Strand				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Research Laboratory, 4555 Overlook Avenue SW, Washington, DC, 20375				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT A scalable means of isolating significant quantities of trioxyethylene-encapsulated gold nanoclusters (GNCs) labeled with a single DNA strand is demonstrated. Quantitative characterization of the product is obtained using UV-vis absorbance spectroscopy. These methods of isolation and characterization of singly functionalized GNC-ssDNA conjugates are expected to aid development of nanoscience applications that use DNA molecular recognition for guiding self-assembly.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 4	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

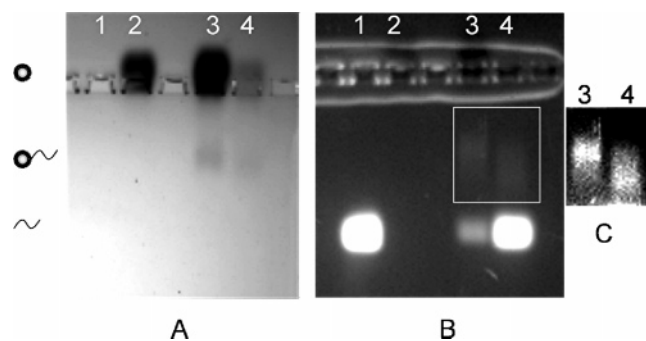


Figure 1. Results of gel electrophoresis of reactions of AuEO3 and DNA viewed under luminescence (A) and fluorescence (B) mode. Cropped image (C) shows product bands from lanes 3 and 4 viewed at higher sensitivity. Samples loaded are: lane 1, 28 pmol DNA 2; lane 2, 108 pmol AuEO3; lane 3, 8 μ L of reaction 2 (1.8 μ M DNA and 20 μ M AuEO3); lane 4, 8 μ L reaction 1 (5.6 μ M both DNA and AuEO3).

procedure that is not readily scaled up to larger volumes. Furthermore, it appears to be ineffective when the DNA lengths fall below about 50 bases.¹² This latter consideration is important because in order for a ssDNA to serve as a proper “address label” for a GNC, the length of the DNA must be comparable to the cluster size. In our case, this implies ssDNAs on the order of 15 bases long.²⁰ We have therefore developed a new protocol that stoichiometrically favors the formation of singly functionalized GNCs, which are then purified by exploiting solubility differences, as described below.

DNA-functionalization of the AuEO3 clusters is achieved via thiol exchange reaction, which takes place when AuEO3 is combined with thiolated DNA in water.²¹ This is a relatively slow reaction requiring several hours of incubation. To ensure that almost no multifunctionalized clusters are formed, we use an excess of clusters. In Figure 1, we show gel electrophoresis images that compare the products obtained with AuEO3/DNA ratios of 1:1 (reaction 1) and 11:1 (reaction 2), as seen in luminescence mode (Figure 1A) showing the reddish brown of the AuEO3 and through a fluorescence filter (Figure 1B) that highlights the fluorescein labeled DNA.²³ Lanes 3 (11:1) and 4 (1:1) show the products as running distinctly from the unreacted DNA (lane 1) and the unreacted AuEO3 (lane 2). Furthermore, while the product in lane 3 appears to be mostly a single band, the product in lane 4 yields a more diffuse band with a higher average mobility under the fluorescent filter, consistent with a scenario in which cluster products with both single and multiple DNAs attached are present in the mixture. We believe that, up to a certain point, additional short ssDNAs attached to AuEO3 will increase the electrophoretic mobility of the DNA_(n)–AuEO3 conjugate by increasing the negative charge density without appreciably adding to its hydrodynamic radius. The extent to which the additional DNA strands, which add both size and charge, affect the electrophoretic mobility of conjugates is currently under investigation. The existence of these bands suggests that the electrophoretic separation of DNA–GNC conjugates may also be limited by the ratio of the cluster size to DNA length and/or

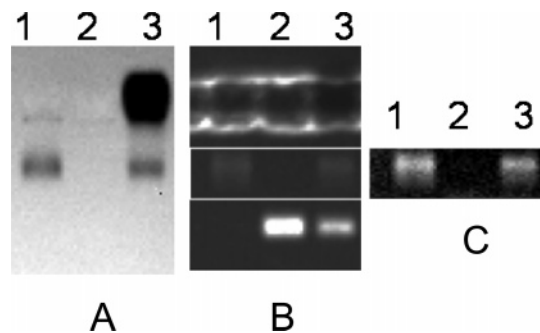


Figure 2. Results of gel electrophoresis showing isolation of product viewed under luminescence (A) and fluorescence (B) mode. Cropped image (C) shows product bands from lanes 1 and 3 viewed at higher sensitivity. Samples loaded are: lane 1, product isolated by extraction from reaction 2; lane 2, 11 pmol DNA; lane 3, 12 μ L of reaction 2 (1.8 μ M DNA and 20 μ M AuEO3).

by cluster charge and that the limit of 50 bases cited previously^{12,13} was merely a constraint associated with a different system and does not apply here.

Electrophoretic harvesting of product is both tedious and yields very little product. To enable scale-up, we exploit the unique solubility properties of the trioxyethylene clusters; it is very likely that this technique is specific for AuEO3 and would not work with the citrate clusters and their derivatives used by others. AuEO3, although water-soluble, preferentially partitions into the organic phase in a binary solvent system of water and CH₂Cl₂, whereas DNA prefers the aqueous phase. Furthermore, we find that ethanol, which is miscible in both CH₂Cl₂ and H₂O, facilitates the migration of the components to their preferred phases. When the AuEO3–DNA reaction mixture (in H₂O) is added to a 1:2 mixture of ethanol in dichloromethane, a large fraction of the unreacted AuEO3 is found to migrate into the ethanol/CH₂Cl₂ phase, whereas the GNC–DNA conjugates and the unreacted DNA are retained in the ethanol/water phase.²⁴ Apparently, when the AuEO3 cluster is functionalized with DNA its solubility is sufficiently influenced by the presence of the charged species such that it resists transfer to the organic phase. This process is repeated several times, and the final separation of the cluster–DNA conjugate from free DNA is achieved by salting out the conjugates into CH₂Cl₂. The solvent is then evaporated and the product resuspended in water. This final product, isolated by extraction from 300 μ L of reaction 2, is shown as Lane 1 in Figure 2. Only a single product band is seen (with no free clusters or free DNA), confirming that the extraction procedure has succeeded.

Previous methods for assessing the degree of DNA conjugation to GNCs make use of fluorescence measurements that depend on hybridization with fluorescent complementary strands²⁵ or that compare relative intensities of free fluorescent DNA in the presence or absence of nanoparticles when visualized on a gel.¹⁴ Both of these methods are indirect and have questions associated with the degree of hybridization and quenching.²⁶ Instead, we rely on simpler and more direct UV–vis measurements of AuEO3 and its DNA conjugate. Such a simple spectroscopic comparison is unique

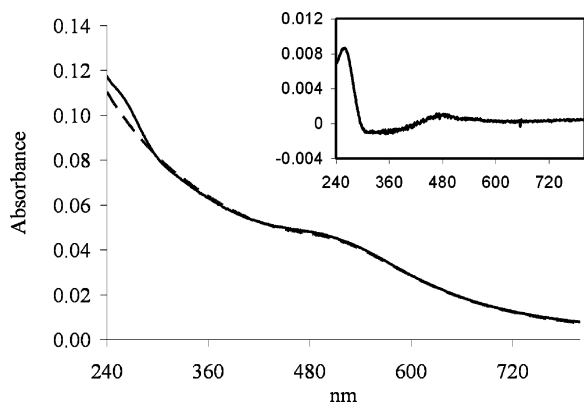


Figure 3. UV-vis spectra of solutions of (a) AuEO3 (---) and (b) AuEO3-DNA isolated by extraction (—). Inset: background subtracted spectrum (b – a) used for quantifying DNA.

to AuEO3 and would not be possible with citrate- or phosphine-stabilized GNCs; the EO3SH is essentially transparent at 260 nm ($\epsilon = 1600 \text{ L mol}^{-1} \text{ cm}^{-1}$, compared to $\epsilon = 10,000 \text{ L mol}^{-1} \text{ cm}^{-1}$ for triphenylphosphine)²⁷ and thus allows us to consider the absorbance of the conjugate as the sum of the absorbances of the DNA and the AuEO3 in this critical region where DNA has its signature peak. In Figure 3 we show UV-vis spectra for 40 μL of the DNA-AuEO3 and for a solution of unconjugated AuEO3 to be used as a baseline. The difference spectrum is shown in the inset in Figure 3. Two peaks are visible in the difference spectrum, the first being the signature DNA peak at 260 nm and the second at 494 nm being associated with the DNA's fluorescein tag. A trace of a peak is also visible near 530 nm which is believed to be due to a slight increase in the surface plasmon absorbance of the cluster upon addition of the DNA. Such a change is not surprising given the sensitivity of plasmon absorption to the dielectric constant of the medium surrounding the gold nanocluster core.^{28,29} Based on the difference spectrum, we estimate the concentration of the DNA to be 56 nM. The concentration of AuEO3 based on A_{507} is estimated to be 75 nM,²¹ indicating that on average 75% of the AuEO3 was labeled with DNA for this sample. This measurement was repeated four additional times with conjugates isolated from separate reactions and differing in initial concentrations of DNA, while still maintaining a ratio of AuEO3 to DNA greater than 10. While the concentration of attached DNA determined from the background subtracted spectrum in those isolated products ranged from 121 nM to 48 nM, the number of DNAs per GNC, averaged from all five samples measured, was 0.82 ± 0.13 . For three separate reactions in which the initial amount of the limiting reagent, the DNA, in the reaction was 480 pmol, the amount of product recovered, in terms of DNA, was $131 \pm 11 \text{ pmol}$, leading to a percent yield for the entire process of 27%. This number is a reflection on both the reaction efficiency itself as well as the efficiency of the extraction process, since a significant fraction (30%)³⁰ of the DNA remains unattached even in the presence of an 11-fold excess of AuEO3, as seen in lane 3 of Figure 2.

As a check on whether the plasmon absorbance of AuEO3 is changed in the presence of DNA, we performed a control

experiment in which a single sample of AuEO3 was split into two fractions. Both fractions were incubated for 16 h at 35 °C, one sample containing equimolar thiolated DNA while the other was diluted to an identical concentration with H_2O . It should be noted that the first fraction is essentially identical to the sample in lane 4 in Figure 1 and thus most of its DNA is uncoupled to clusters. The UV-vis spectra for the two fractions were similar to those in Figure 3, except that the peak at 494 nm associated with fluorescein was more pronounced. There was no observed change in the absorbance of AuEO3 due to the presence of DNA, and the ratio of DNA/AuEO3 for the sample with DNA was calculated to be very close to the expected value of 1 (0.95).

A final question that should be addressed is why the measured molar ratio of DNA molecules to gold clusters in the isolated conjugates, although very close to unity as expected for $\text{DNA}_{(1)}\text{-AuEO3}$, was slightly lower at 0.82 ± 0.13 . The fact that the gel in Figure 2 (lane 1) shows no evidence of either free DNA or free gold clusters indicates that, to within the resolution of the gel, all of the DNA was attached to clusters. This would lead us to expect a ratio of at least one, and perhaps greater if a small amount of $\text{DNA}_{(2)}\text{-AuEO3}$ had remained in the isolate. That this is not seen suggests that the discrepancy is instead associated with an effect of the coupling of the DNA to the cluster on the UV-vis absorbance. In particular, it could be that there is a moderate broadening and consequent height reduction of the 260 nm DNA absorption peak due to its attachment to the gold cluster, or perhaps an overall reduction in the 260 nm extinction coefficient of the attached DNA relative to that for free DNA in solution. Support for the latter explanation comes from the fact that such reductions are known to occur with other adsorbents (mostly dyes), especially when the interaction with the gold surface is strong.³¹

In summary, by employing a gold nanocluster encapsulated with a trioxethylene thiol we have demonstrated a new extraction procedure for isolating DNA-GNC conjugates that improves on current methodologies by being more straightforward and better suited to volume production. In addition, our conjugates have been shown to allow standard UV-vis characterization to be used for a quantitative assessment of the isolated product. We are currently pursuing the construction of arrays of nanoclusters via the hybridization of these ssDNA-tagged GNCs to ssDNA templates.³²

Acknowledgment. The authors thank K. Pilobello for assistance in the electrophoresis and the Office of Naval Research and NRL's Institute for Nanoscience for funding support.

References

- (1) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128–4158.
- (2) Lowy, D. A.; Jhaveri, S. D. In *Encyclopedia of Colloid and Surface Science*; Somasundaran, P., Ed.; Marcel Dekker: New York, 2003; Vol. 4.
- (3) Shipway, A.; Katz, E.; Willner, I. *Chem. Phys. Chem.* **2000**, *1*, 18–52.
- (4) Wohltjen, H.; Snow, A. W. *Anal. Chem.* **1998**, *70*, 2856–2859.
- (5) Bailey, R. C.; Nam, J. M.; Mirkin, C. A.; Hupp, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 13541–13547.

- (6) Parak, W. J.; Gerion, D.; Pellegrino, T.; Zanchet, D.; Micheel, C.; Williams, S. C.; Boudreau, R.; Le Gros, M. A.; Larabell, C. A.; Alivisatos, A. P. *Nanotechnology* **2003**, *14*, R15–R27.
- (7) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607–609.
- (8) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849–1862.
- (9) Loweth, C. J.; Caldwell, W. B.; Peng, X. G.; Alivisatos, A. P.; Schultz, P. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1808–1812.
- (10) Zanchet, D.; Micheel, C. M.; Parak, W. J.; Gerion, D.; Williams, S. C.; Alivisatos, A. P. *J. Phys. Chem. B* **2002**, *106*, 11758–11763.
- (11) Alivisatos, A. P.; Johnsson, K. P.; Peng, X. G.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G. *Nature* **1996**, *382*, 609–611.
- (12) Zanchet, D.; Micheel, C.; Parak, W.; Gerion, D.; Alivisatos, A. P. *Nano Lett.* **2001**, *1*, 32–35.
- (13) Taton, T. A. *Curr. Protocol. Nucl. Acid Chem.* **2002**, *2002*, Unit 12.2.
- (14) Sandstrom, P.; Boncheva, M.; Akerman, B. *Langmuir* **2003**, *19*, 7537–7543.
- (15) Foos, E. E.; Snow, A. W.; Twigg, M. E.; Ancona, M. G. *Chem. Mater.* **2002**, *14*, 2401–2408.
- (16) Roberts, C.; Chen, C. S.; Mrksich, M.; Martichonok, V.; Ingber, D. E.; Whitesides, G. M. *J. A. Chem. Soc.* **1998**, *120*, 6548–6555.
- (17) Zheng, M.; Davidson, F.; Huang, X. Y. *J. Am. Chem. Soc.* **2003**, *125*, 7790–7791.
- (18) Foos, E. E.; Snow, A. W.; Twigg, M. E. *J. Cluster Sci.* **2002**, *13*, 543–552.
- (19) Jhaveri, S. D.; Lowy, D. A.; Foos, E. E.; Snow, A. W.; Ancona, M. G.; Tender, L. M. *Chem. Commun.* **2002**, 1544–1545.
- (20) The DNA oligomer (5′HO-(CH₂)₆-S-S-(CH₂)₆-ACACACACACA-CACA-fluorescein-3′) was synthesized and HPLC purified (Integrated DNA Technologies). The disulfide on the 5′-terminus was first reduced with a freshly prepared 75 mM dithiothreitol (Pierce) solution, and the resulting monothiolated oligo was purified on a 15% acrylamide gel (Bio-Rad). The oligo from the excised gel slices was eluted into 1 mL distilled, deionized water, ($R = 18 \text{ M } \Omega \text{ cm}^{-1}$). The concentration of the DNA was determined by measuring A_{260} using a molar extinction coefficient of $172\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$ (provided by the manufacturer).
- (21) Reaction 1 consisted of 300 μL of 5.6 μM DNA and 5.6 μM of AuEO₃, and reaction 2 consisted of 300 μL of 1.8 μM DNA and 20 μM of AuEO₃. Both were conducted at 35 °C for 16 h. Prior to DNA conjugation, the solution of AuEO₃ was subjected to further purification on a P-6 size exclusion column (BioRad) to remove any residual EO₃SH or AuEO₃ with residual alkane thiol (which were insoluble in water). The concentration of the AuEO₃ solution was estimated using a molar extinction coefficient of $5 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$. This value was obtained by measuring an extinction coefficient at 512 nm of $9.7 \text{ L g}^{-1} \text{ cm}^{-1}$ for a CHCl₃ solution of the clusters, coupled with a molecular weight estimate of $52\,000 \text{ g mol}^{-1}$. The molecular weight estimate is based on the average core size of the nanoclusters as determined by TGA [Snow, unpublished results] using a previously published model of gold core atomic packing and thiol ligand surface coverage (ref 22).
- (22) Hostetler, M. J.; Wingate, J. E.; Zhong, C. J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17–30.
- (23) Agarose gels, 2%, were made in TBE (Tris-borate-EDTA buffer, Sigma) and run in 0.5× TBE on a horizontal gel electrophoresis apparatus. All samples were treated with 0.2 vol of 30% glycerol prior to loading. The samples were run on the gel under an applied potential of 100 V for 20 min or until the fluorescent DNA ran past the middle of the gel. The gels were viewed using a Kodak Image Station 440 digital camera.
- (24) The DNA–AuEO₃ reaction (300 μL) was mixed with 300 μL of ethanol and 600 μL of CH₂Cl₂. The tube was vigorously shaken and then spun on a benchtop centrifuge for 10 s to separate the two phases. The top phase, along with the opaque film between the phases, was transferred to a fresh tube. To this mostly aqueous phase, 300 μL of ethanol and 600 μL of CH₂Cl₂ were added, and the aqueous phase was isolated again. This was repeated three more times, and the volume of the aqueous phase diminished with every step due to incremental amounts of water becoming more and more miscible in the ethanolic organic phase. At this point, the aqueous phase bore a dark reddish-brown color indicative of AuEO₃, and the organic phase bore no color. A final transfer of the aqueous phase into a fresh tube containing the ethanol/dichloromethane mixture resulted in total immersion and an apparent loss of phase separation. The five collected organic phases were subsequently rinsed with the same aliquot of 200 μL DIW to recover DNA that may have partitioned in the organic phase. The aqueous phase from the collected washes contained free DNA and AuEO₃ attached to DNA. To this sample, 50 μL of a 4 M NaCl solution, buffered with 20 mM sodium phosphate, pH 7, and 200 μL of CH₂Cl₂ were added. The tube was shaken and spun to separate the phases as before, and the AuEO₃ modified with DNA was extracted into the organic phase. The organic phase was subsequently washed with an additional 200 μL of a 1 M NaCl solution, buffered with 5 mM sodium phosphate, pH 7. The CH₂Cl₂ showed a light reddish-brown color, and when viewed under a fluorescent light, the salt-containing aqueous phase indicated the presence of the fluorescein-labeled DNA.
- (25) Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A.; Letsinger, R. L.; Elghanian, R.; Viswanadham, G. *Anal. Chem.* **2000**, *72*, 5535–5541.
- (26) Attempts to perform fluorescence measurements as a characterization method for our DNA–GNCs were inconclusive because when the fluor is in such a close proximity to the nanoparticle, it is quenched. However, the intensity of signal for the DNA labeled with fluor, on its own, displayed only a nominal decrease upon the addition of 10-fold excess AuEO₃.
- (27) Phillips, J. P.; Bates, D.; Feuer, H.; Thyagarajan, B. S., Eds.; *Organic Electronic Spectral Data*; John Wiley and Sons: New York; Vol. 24.
- (28) Kreibitz, U.; Vollmer, M., Eds. *Optical Properties of Metal Clusters*; Springer: New York, 1995.
- (29) Bohren, C. F.; Huffman, D. R. *Absorption and Scattering of Light by Small Particles*; Wiley: New York, 1983.
- (30) Following Sandstrom et al. (ref 14), an aliquot of reaction 2 was compared with a sample containing an equal amount of DNA in the absence of AuEO₃, both run side-by-side on the same gel. The amount of unreacted DNA was determined by the relative fluorescence intensities of the bands corresponding to unreacted DNA.
- (31) Franzen, S.; Folmer, J. C. W.; Glomm, W. R.; O’Neal, R. *J. Phys. Chem. A* **2002**, *106*, 6533–6540.
- (32) Jhaveri, S.; Foos, E. E.; Ancona, M. G.; Snow, A. W.; Twigg, M. E.; Chang, E.; Pilobello, K.; Goldman, E.; Lowy, D. *Mater. Res. Soc. Symp. Proc.* **2003**, *735*, 159.

NL0499591