PERSPECTIVE

Curvature Effects in DNA:Au Nanoparticle Conjugates

Kristin B. Cederquist and Christine D. Keating*

Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania 16802

ABSTRACT DNA-coated Au nanoparticles have myriad applications as versatile building blocks in nanomaterials assembly, powerful amplification tags for bioanalysis, and promising new approaches to medical therapeutics. Characterization, control, and a thorough understanding of the DNA surface interface are essential in the development of these conjugates. A new paper in this issue explores the impact of nanosphere diameter on DNA adsorption and demonstrates that particle curvature plays an important role in controlling the DNA surface density. The study proposes a model that can be used to predict DNA packing on nonspherical particles and validates it using Au nanorods. This work paves the way for improved understanding of the DNA:Au interface in these versatile bioconjugates.

See the accompanying Article by Hill *et al.* on p 418.

*Address correspondence to keating@chem.psu.edu.

Published online February 24, 2009.
10.1021/nn9000726 CCC: $40.75

The properties of alkanethiol-protected Au nanoparticles, also known as monolayer-protected clusters (MPCs), are size-dependent. In addition to the well-known relationship between Au core size and physical properties such as optical absorbance, the molecular packing in the alkanethiol self-assembled monolayer (SAM) also varies with core size, leading to variations in the properties of the MPC.[1] Packing density of the monolayer decreases and disorder increases, moving away from the particle surface. This can be understood on the basis of the volume per molecule, which increases for spherical particles as the square of the radius with distance from the particle center (in the shape of a truncated cone), but does not change with distance from a plane.[2] Consequently, phase transitions can be observed in MPCs for high molecular packing densities, at which SAMs on planar Au surfaces are quasi-crystalline and do not exhibit these transitions.[3] Reduced steric hindrance due to the curved surface of the particle can also facilitate superior partitioning of solutes into the SAMs[4] and increased reactivity of the SAM molecules.[5,6] The MPC core size dependence is most important for few-nanometer-diameter particles, with dodecanethiol packing densities on larger core sizes (>10 nm) having properties more similar to planar Au.[3]

In this issue, Mirkin and co-workers demonstrate related curvature effects for monolayers of thiolated DNA on Au nanospheres 10−200 nm in diameter (Figure 1).[7] The DNA oligonucleotides differ from the alkanethiols introduced above both in their greater length (*e.g.*, a 25-nucleotide sequence has an estimated length of nearly 10 nm, as compared with <2 nm for dodecanethiol) and their greater intermolecular repulsions, due largely to the negative charge of the phosphodiester backbone. On planar Au surfaces, maximum coverages for single-stranded (ss) DNA are on the order of $\sim 5 \times 10^{13}$ molecules/cm^2 (2 nm^2/molecule),[8] as compared with 4.2×10^{14} molecules/cm^2 (0.2 nm^2/molecule) for dodecanethiol.[9]

DNA:Au nanoparticle conjugates have already found use as building blocks for nanomaterials and in a wide range of different analytical and bioanalytical detection strategies, including schemes based on their high density, their ability to bind large numbers of probe molecules, and their optical properties (*e.g.*, intense absorbance and scattering, surface-enhanced spectroscopies).[10] For example, DNA:Au conjugates have been used to detect prostate-specific antigen at attomolar levels[11] and recently were employed to detect telomerase activity in just 10 HeLa cells without PCR amplification.[12] In addition to signal amplification, these conjugates can provide improved selectivity due to their sharper melting curves, which result from multivalency on the particle surface.[13,14] Selectivity for perfectly matched sequences over those containing a single mismatch was improved threefold by substituting DNA:Au conjugates in place of fluorescently tagged DNA due to the cooperativity possible for the conjugates.[15] Antisense DNA:Au bioconjugates have been used to bind intracellular mRNA in living cells for gene regulation or RNA detection.[16,17] Careful characterization of the DNA environment on the particle surface is crucial to understanding and controlling the behavior of these conjugates at the high level required for ultimate use in medical diagnostics, gene therapy, or electronic applications.

DNA Surface Coverage and Curvature. DNA surface coverage is traditionally controlled *via* solution conditions during assembly (*i.e.*, ionic strength, DNA concentration, diluents) and is extremely important for many DNA:Au bioconjugate properties, including the stability of the conjugates, and the ac-

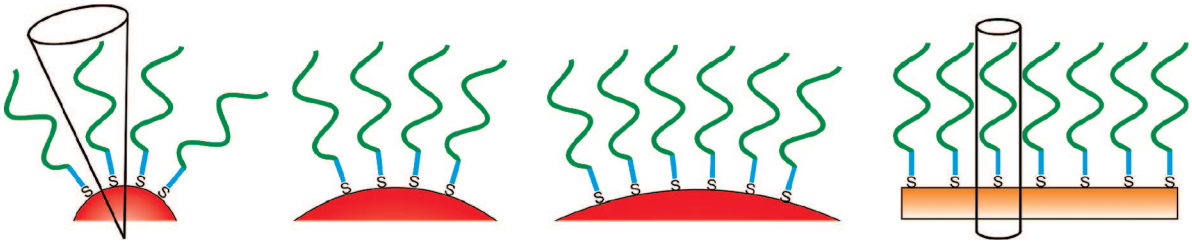

Figure 1. Depiction of thiolated DNA immobilized on 10, 30, and 60 nm Au nanoparticles and a planar Au surface. Blue sections represent the hexyl group linking the thiol to the DNA strand (green).

cessibility of bound oligonucleotides for hybridization to complementary strands, or enzymatic processing.[18,19] In this issue, Mirkin and co-workers highlight particle size as an additional key variable: as the curvature of the Au surface increases, it is possible to pack considerably more oligonucleotides into a given area. Because DNA coverage is strongly dependent upon electrostatic repulsion due to the negative charges on adjacent strands, Mirkin and co-workers attached their DNA at a relatively high salt concentration, 1 M, to screen the charges during DNA adsorption. They found that the molecular area occupied by each 25-nucleotide DNA strand decreased threefold from nanospheres with 200 to 10 nm diameters, from a maximum of 15 nm^2 ($6.8 \times 10^{12}/cm^2$) for the largest particles to only 5 nm^2 ($2.0 \times 10^{13}/cm^2$) for the smallest (Figure 2).[7] These DNA packing densities were observed for adsorption under identical conditions and can therefore be ascribed as resulting from differences in particle radius of curvature. The effect is most striking for the smallest diameter particles: molecular area increased more than twofold for particles between 10 and 30 nm, but essentially leveled off by 60−80 nm, where the DNA surface density already approaches that for planar surfaces.

> As the curvature of the Au surface increases, it is possible to pack considerably more oligonucleotides into a given area.

Bioconjugates in which thiolated DNA oligonucleotides coat Au nanospheres are particularly amenable to surface coverage characterization. Both the DNA oligonucleotides and the Au nanospheres are well-characterized, relatively stable, and commercially available in a variety of sizes. The attachment chemistry is straightforward, with the thiol−Au interaction dominating, facilitating single-point attachment of the DNA. Perhaps most importantly, thiolated DNA strands can be displaced from the Au surface by excess of a short-chain thiol (*e.g.*, 2-mercaptoethanol or dithiothreitol), releasing the DNA for analysis. Demers *et al.* introduced a method for determination of DNA coverage in which fluorescently labeled, thiolated DNA is attached to Au nanospheres and then quantified after its removal.[19] Removal prior to quantification is important because Au particles can effectively quench the fluorescence of bound fluorophores.[20] These measurements can be quite accurate because it is possible to know the Au surface area in solution based on the size of the particles, which can be prepared with <10% variability in diameter, and based on the number of particles in solution, which can be followed *via* their extraordinarily high extinction coefficient. Mirkin and co-workers were able to determine DNA coverage with standard deviations of *ca.* 10%, sufficient to enable robust conclusions about the role of particle curvature in determining maximum DNA packing densities.

Using their experimentally determined ssDNA coverages to calculate molecular footprints as a function of particle curvature, Mirkin and co-workers derived a formula to predict the surface coverage of thiolated ssDNA on the surface of nonspherical particles, in this case Au nanorods (35 nm diameter, 475 nm long).[7] Their calculated surface coverage on the nanorods was within the error of the experimental coverage measurement. The same basic approach should enable prediction of oligonucleotide coverages on other particle shapes. Generalizing to different DNA sequence lengths may require collection of additional footprint data for the various particle curvatures but should be relatively straightforward following the methodology introduced in this paper. Since on most surfaces the maximum coverage of thiolated ssDNA is dictated not by the density of surface reactive groups[9,21] but rather by steric and electrostatic repulsions between adjacent molecules, the approach introduced here should translate to other single-point DNA attachment chemistries and other nanoparticles, such as metal oxides.

Although DNA surface coverage is known to be important for DNA:Au bioconjugate properties, few studies have examined the role of curvature in these systems. In part, this stems from the fact that bioconjugates of larger diameter particles are more difficult to stabilize than their smaller diameter counterparts; preparations for particle diameters up to 250 nm require some changes in the DNA adsorp-

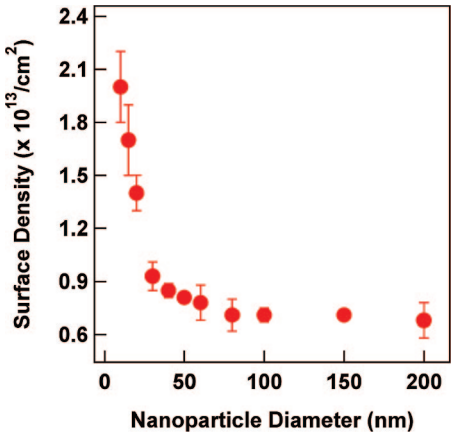

Figure 2. Impact of Au nanoparticle diameter on thiolated DNA surface coverage. Adapted from ref 7. Copyright 2009 American Chemical Society.

tion procedure.[22] Larger diameter Au nanoparticles have been shown to carry a substantially greater number of thiolated ssDNA strands on a per particle basis, making them attractive for biobarcode assays, in which these strands are ultimately released to serve as part of an amplification scheme.[22] Experiments that compare particle sizes have demonstrated that electrostatic repulsions during ssDNA adsorption are important in determining not only the DNA coverage but also its variation with particle curvature. When adsorbed at moderate salt concentrations (0.3 M NaCl), DNA coverage did not vary strongly with particle diameter in the 15 to 80 nm diameter range, in contrast to what was observed at 1 M NaCl.[7,22] Adsorption at 1 M NaCl is desirable for achieving high DNA coverages, which can facilitate cellular uptake of DNA:Au conjugates[23] and protect the bound DNA from enzymatic degradation by nucleases.[24]

Consequences of Curvature. The consequences of increased curvature and reduced surface coverage are related but distinct. For example, at constant DNA surface coverage, one might anticipate improved binding of complementary strands from solution on more highly curved surfaces, due to greater molecular volumes away from the particle surface. This should be especially true for sequences attached to the particles *via* long vertical spacers, which would enable the hybridization reaction to occur entirely in the lower molecular density region away from the particle surface. In contrast, for hybridization-induced assembly of two or more particles carrying cDNA strands, particle curvature might reduce the number of DNA duplexes that spanned the particles since the surfaces presenting the DNA strands curve away from each other (Figure 3). A recent article by Hill, Hurst, and Mirkin[25] elegantly demonstrated the importance of curvature in this type of two-particle hybridization system. They designed pairs of DNA:Au conjugates for which only the last few bases were complementary, and "slipping" these bases away from a perfect match would yield reduced stabilization *via* non-Watson−Crick interactions. For example, if the terminal bases were −CGCT on particle 1 and TGCG− on particle 2, slipped interactions between just the terminal CT/TG or even T/T could provide some additional stability, albeit less than the complementary CGC/GCG interaction. They probed the importance of the slipped interactions using a series of terminal sequences and found that those leading to more stable slipped interactions led to particle assemblies with markedly higher thermal stability. They then compared melting behavior for DNA:Au nanoparticle assemblies formed with spherical nanoparticles to those formed with prism-shaped nanoparticles and found that the nanoprisms, which have flat slides and thus less curvature, were not sensitive to slipping interactions. The nanoprism assemblies also exhibited higher melting temperatures, consistent with a greater number of fully complementary interactions due to the reduced curvature along their sides.[25]

Curvature in Other Nanobioconjugates. Peptides adsorbed to Au nanoparticles *via* cysteines or other thiol moieties also show curvature-dependent properties. For example, cellular uptake of peptide:Au conjugates in which the surface coverage of an arginine-rich peptide was held constant was more effective for 30 nm as compared with 60 nm Au particles.[26] Vibrational spectroscopy has been used to evaluate peptide secondary structure on Au nanoparticles for comparison with free peptides and those bound to planar surfaces. The effect of particle curvature on peptide secondary structure is not general for all peptide sequences. Mandal and Kraatz showed that the secondary structure of a leucine-rich peptide that was α-helical in solution adopted a primarily β-sheet conformation on 5 nm diameter particles and increasing α-helical character as particle size was increased.[27] They attributed this to differences in intermolecular interactions between adjacent molecules at different particle curvatures. In contrast, a different peptide, designed to adopt a 3_{10} helical structure, maintained a conformation similar to that in solution even on <3 nm particles, participating in both inter- and intramolecular hydrogen bonding interactions.[28] Still another report shows greater α-helical content for peptides bound to nanoparticles as compared to the free molecule.[29] The sensitivity of peptide secondary structure to local environment and the propensity for binding interactions between adjacent peptide molecules both point to sequence as an important

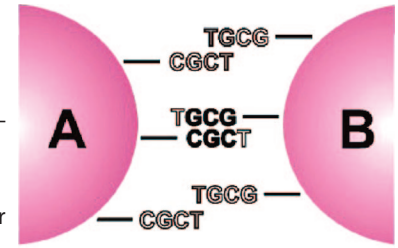

Figure 3. Illustration of base pair slipping during particle assembly due to hybridization of DNA on the Au nanoparticles. Adapted from ref 25. Copyright 2009 American Chemical Society.

Particle curvature effects can be less straightforward for proteins and peptides as compared to alkanethiols or ssDNA.

factor in the response of peptides to particle curvature.

A variety of more complex nanobioconjugates have been prepared in which various types of biomolecules (including not only DNA or peptides but also proteins and other biological or biologically active molecules) were bound to spherical and nonspherical particles of metals, polymers, metal oxides, or semiconductors.[30] For globular proteins, the general trend regardless of nanoparticle composition is that greater native-like structure and function is retained on smaller, more highly curved particles; however, this is not true for all systems.[31−34] Serum protein adsorption is thought to occur for nanoparticles of essentially any composition upon exposure to serum[35] and to mediate nanoparticle uptake by cells. Curvature-dependent differences in the adsorption of these proteins may help explain the unexpected observation of improved cellular uptake for 50 nm diameter citrate-capped Au than for either larger or smaller particles.[36] These examples underscore that particle curvature effects can be less straightforward for proteins and peptides as compared to alkanethiols or ssDNA, due to the greater number of ways in which these molecules can interact with the surface and/or each other. Nonetheless, particle curvature remains a key variable in the properties of the conjugates. In any application, the properties of nanobioconjugates will depend upon their composition. Successful transitions from the academic laboratory environment to diagnostic laboratories or other real-world applications will require that these composite materials have well-defined compositions and robust, reproducible behavior. Knowledge of and control over the biomolecule:particle stoichiometry is therefore critically important for realizing the promise of these materials.

REFERENCES AND NOTES

1. Daniel, M.-C.; Astruc, D. Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology. *Chem. Rev.* **2004**, *104*, 293–346.
2. Tambasco, M.; Kumar, S. K.; Szleifer, I. Quantitatively Modeling the Equilibrium Properties of Thiol-Decorated Gold Nanoparticles. *Langmuir* **2008**, *24*, 8448–8451.
3. Heinz, H.; Vaia, R. A.; Farmer, B. L. Relation between Packing Density and Thermal Transitions of Alkyl Chains on Layered Silicate and Metal Surfaces. *Langmuir* **2008**, *24*, 3727–3733.
4. Lucarini, M.; Franchi, P.; Pedulli, G. F.; Gentilini, C.; Polizzi, S.; Pengo, P.; Scrimin, P.; Pasquato, L. Effect of Core Size on the Partition of Organic Solutes in the Monolayer of Water-Soluble Nanoparticles: An ESR Investigation. *J. Am. Chem. Soc.* **2005**, *127*, 16384–16385.
5. Templeton, A. C.; Hostetler, M. J.; Kraft, C. T.; Murray, R. W. Reactivity of Monolayer-Protected Gold Cluster Molecules: Steric Effects. *J. Am. Chem. Soc.* **1998**, *120*, 1906–1911.
6. Kell, A. J.; Donkers, R. L.; Workentin, M. S. Core Size Effects on the Reactivity of Organic Substrates as Monolayers on Gold Nanoparticles. *Langmuir* **2005**, *21*, 735–742.
7. Hill, H. D.; Millstone, J. E.; Banholzer, M. J.; Mirkin, C. A. The Role Radius of Curvature Plays in Thiolated Oligonuceotide Loading on Gold Nanoparticles. *ACS Nano* **2009**, *2*, 418–424.
8. Steel, A. B.; Levicky, R. L.; Herne, T. M.; Tarlov, M. J. Immobilization of Nucleic Acids at Solid Surfaces: Effect of Oligonucleotide Length on Layer Assembly. *Biophys. J.* **2000**, *79*, 975–981.
9. Dubois, L. H.; Nuzzo, R. G. Synthesis, Structure, and Properties of Model Organic Surface. *Annu. Rev. Phys. Chem.* **1992**, *43*, 437–463.
10. Rosi, N. L.; Mirkin, C. A. Nanostructures in Biodiagnostics. *Chem. Rev.* **2005**, *105*, 1547–1562.
11. Nam, J. M.; Thaxton, C. S.; Mirkin, C. A. Nanoparticle-Based Bio-Bar Codes for the Ultrasensitive Detection of Proteins. *Science* **2003**, *301*, 1884–1886.
12. Zheng, G.; Daniel, W. L.; Mirkin, C. A. A New Approach to Amplified Telomerase Detection with Polyvalent Oligonucleotide Nanoparticle Conjugates. *J. Am. Chem. Soc.* **2008**, *130*, 9644–9645.
13. Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A.; Schatz, G. C. What Controls the Melting Properties of DNA-Linked Gold Nanoparticle Assemblies. *J. Am. Chem. Soc.* **2003**, *125*, 1643–1654.
14. Dillenback, L. M.; Goodrich, G. P.; Keating, C. D. Temperature-Programmed Assembly of DNA:Au Nanoparticle Bioconjugates. *Nano Lett.* **2006**, *6*, 16–23.
15. Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. Scanometric DNA Detection with Nanoparticle Probes. *Science* **2000**, *289*, 1757–1760.
16. Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K. R.; Han, M. S.; Mirkin, C. A. Oligonucleotide-Modified Gold Nanoparticles for Intracellular Gene Regulation. *Science* **2006**, *312*, 1027–1030.
17. Seferos, D. S.; Giljohann, D. A.; Hill, H. D.; Prigodich, A. E.; Mirkin, C. A. *J. Am. Chem. Soc.* **2007**, *129*, 15477–15479.
18. Nicewarner-Peña, S. R.; Raina, S.; Goodrich, G. P.; Fedoroff, N. V.; Keating, C. D. Hybridization and Enzymatic Extension of Au Nanoparticle-Bound Oligonucleotides. *J. Am. Chem. Soc.* **2002**, *124*, 7314–7323.
19. Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A., III; Letsinger, R. L.; Elghanian, R.; Viswanadham, G. A Fluorescence-Based Method for Determining the Surface Coverage and Hybridization Efficiency of Thiol-Capped Oligonucleotides Bound to Gold Thin Films and Nanoparticles. *Anal. Chem.* **2000**, *72*, 5535–5541.
20. Maxwell, D. J.; Taylor, J. R.; Nie, S. Self-Assembled Nanoparticle Probes for Recognition and Detection of Biomolecules. *J. Am. Chem. Soc.* **2002**, *124*, 9606–9612.
21. Wang, R.; Wunder, S. L. Thermal Stability of Octadecylsilane Monolayers on Silica: Curvature and Free Volume Effect. *J. Phys. Chem. B* **2001**, *105*, 173–181.
22. Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. Maximizing DNA Loading on a Range of Gold Nanoparticle Sizes. *Anal. Chem.* **2006**, *78*, 8313–8318.
23. Giljohann, D. A.; Seferos, D. S.; Patel, P. C.; Millstone, J. E.; Rosi, N. L.; Mirkin, C. A. Oligonucleotide Loading Determines Cellular Uptake of DNA-Modified Gold Nanoparticles. *Nano Lett.* **2007**, *7*, 3818–3821.

24. Seferos, D. S.; Prigodich, A. E.; Giljohann, D. A.; Patel, P. C.; Mirkin, C. A. Polyvalent DNA Nanoparticle Conjugates Stabilize Nucleic Acids. *Nano Lett.* **2009**, *9*, 308–311.
25. Hill, H. D.; Hurst, S. J.; Mirkin, C. A. Curvature-Induced Base Pair "Slipping" Effects in DNA-Nanoparticle Hybridization. *Nano Lett.* **2009**, *9*, 317–321.
26. Sun, L.; Liu, D.; Wang, Z. Functional Gold Nanoparticle−Peptide Complexes as Cell-Targeting Agents. *Langmuir* **2008**, *24*, 10293–10297.
27. Mandal, H. S.; Kraatz, H.-B. Effect of the Surface Coverage on the Secondary Structure of Peptides Adsorbed on Nanoparticles. *J. Am. Chem. Soc.* **2007**, *129*, 6356–6357.
28. Fabris, L.; Antonello, S.; Armelao, L.; Donkers, R. L.; Polo, F.; Toniolo, C.; Maran, F. Gold Nanoclusters Protected by Conformationally Constrained Peptides. *J. Am. Chem. Soc.* **2006**, *128*, 326–336.
29. Higashi, N.; Kawahara, J.; Niwa, M. Preparation of Helical Peptide Monolayer-Coated Gold Nanoparticles. *J. Colloid Interface Sci.* **2005**, *288*, 83–87.
30. De, M.; Ghosh, P. S.; Rotello, V. M. Applications of Nanoparticles in Biology. *Adv. Mater.* **2008**, *20*, 4225–4241.
31. Vertegel, A. A.; Diegel, R. W.; Dordick, J. S. Silica Nanoparticle Size Influences the Structure and Enzymatic Activity of Adsorbed Lysozyme. *Langmuir* **2004**, *20*, 6800–6807.
32. Shang, W.; Nuffer, J. H.; Dordick, J. S.; Seigel, R. W. Unfolding of Ribonuclease A on Silica Nanoparticle Surfaces. *Nano Lett.* **2007**, *7*, 1991–1995.
33. Roach, P.; Farrar, D.; Perry, C. C. Surface Tailoring for Controlled Protein Adsorption: Effect of Topography at the Nanometer Scale and Chemistry. *J. Am. Chem. Soc.* **2006**, *128*, 3939–3945.
34. Lundqvist, M.; Sethson, I.; Jonsson, B-H. Protein Adsorption onto Silica Nanoparticles: Conformational Changes Depend on the Particles' Curvature and the Protein Stability. *Langmuir* **2004**, *20*, 10639–10647.
35. Lundqvist, M.; Stigler, J.; Elia, G.; Lynch, I.; Cedervall, T.; Dawson, K. A. Nanoparticle Size and Surface Properties Determine the Protein Corona with Possible Implications for Biological Impacts. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 14265–14270.
36. Chithrani, B. D.; Ghazani, A. A.; Chan, W. C. W. Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. *Nano Lett.* **2006**, *6*, 662–668.