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Noncovalent Surface Adsorption of Nucleotides in Gold Nanoparticle DNA Conjugates: Bioavailability at the bio-nano interface

By

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Submitted to the Department of Biological Engineering on March 27, 2008 in Partial Fulfillment of the Requirements of the Degree of Doctor of Philosophy in Molecular and Systems Toxicology

ABSTRACT

The practical viability of biomolecule-nanostructure hybrids depends critically on the functional and structural stability of biomolecules in application environments. Noncovalent interactions of biochemical functional groups with nanostructure surfaces can significantly disrupt biomolecular structure and function. We report a systematic study of the effect of DNA sequence on the binding interaction between gold nanoparticles and thiolated DNA (AuNp-DNA). Base specific noncovalent nucleotide adsorption on gold surfaces can affect nucleotide bioavailability in solution. Systematic investigation of DNA oligonucleotide sequence, the location of specific sequence motifs, and the effect of nanoparticle size was performed. Sequence effects on DNA coverage and oligonucleotide adsorption affinities were studied by Langmuir isotherm analysis. The nanoparticle coverage at saturating concentrations of thiolated DNA varied with oligonucleotide sequence. Saturation coverages correlated well with complement hybridization efficiency. From this we concluded that noncovalent interactions between nucleotides and the particle surface effect both hybridization and DNA coverage and adsorption. This hypothesis was confirmed by chemical treatment of the particle surface to eliminate noncovalent interactions. Upon treatment the effect of sequence on hybridization efficiency was removed. The effect of sequence is not consistent across nanoparticle sizes. Different bases show the highest saturation coverages and hybridization efficiencies on different AuNp sizes. These results allow for sequence selection guidelines based on AuNp size for sizes ranging from 4-11nm. For smaller particles (<5nm) adenine rich sequences show the highest saturation coverage and hybridization efficiency. For mid-sized particles (~7.5nm), guanine sequences show the highest saturation coverage and hybridization efficiency. Larger particles (>10nm) show little sequence dependent behavior and are likely the best choice for uses where sequence choice is limited. Sequence selection based on these guidelines will provide AuNp-DNA conjugates with the highest possible oligonucleotide bioavailability, maximizing their utility in biotechnology applications.

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Acknowledgements

I would like to thank all the people who have been my non-scientific partners in this process for all you love and support. In particular, my mom, you possess more strength and wisdom than anyone I know, thank you for all you advice and guidance; my Dad, you are a pain to talk to about my work, but only because you challenge and inspire so well; and Doris, I can't imagine this process without your sense of humor, your courage and your wardrobe advise. To my bear, I can not ever really thank you for all your support and love and for being you. I love you. To all the monkeys, near and far, thank you for being you and being Reedies and being my dear friends. To the fabulous ladies of 3 mossland, thanks for art-night, for making everyday my favorite day to be roommates, and for generally being fabulous.

I would like to thank all the members of the Hamad-Schifferli lab, past and present, for scientific and occasionally psychological support. In particular, my boys Victor, Josh and Shahriar, for countless laughs, arguments, love and support. I will miss you.

I would also like to thank my advisor Kim Hamad-Schifferli. I have learned so much from your example. The past 6 years have been an intense and immensely educational experience, and you have given me a firm grounding in the process of scientific research from the beginnings of a lab through to its establishment. I would also like to thank the members of my committee for your advice and guidance throughout this process. In particular I would like to thanks Doug Lauffenburger for his enormous support, scientific, personal, and financial.
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Chapter 1: Introduction

Nanostructure research has greatly expanded over the past several decades. A significant factor in this increase is the many potential biological applications for nanostructures. Applications ranging from imaging to biochemical sensing have sparked interest in bio-nano hybrids as tools of great potential. The range of nanostructures available and the numerous potential applications create a complex range of possibilities and areas research. Realization of this potential requires a detailed understanding of biomolecular interactions with nanosurfaces at the bio-nano interface. The functional groups found in biological systems provide a wide range of potential interactions with nanostructure surfaces, which can easily interfere with biomolecule structure and function. While there is an enormous body of work detailing the use of nanostructures in biological settings, there are few studies exploring their affects on biomolecular structure and function. Applications in highly complex biological settings such as inside cells or in cell culture create a myriad of possible interactions. Effective use of bio-nano hybrids requires a detailed understanding of biochemical functional group interactions with nanostructures.

In this study we focus on one area of interest within the nanostructure community, nucleotide interactions with gold nanoparticles covalently linked to DNA oligonucleotides (AuNp-DNA). Previous reports have demonstrated sequence specific nucleotide interactions which can affect conjugate behavior. Here we report an in depth study of base-dependent surface interactions, and the implications for sequence selection in nano-based applications. Though the study is limited to a specific biomolecule and
nanostructure type, these results represent broader implications for bio-nano applications in general, as the analysis methods detailed here can be applied to other nanostructured systems. The AuNp-DNA conjugates studied provide a test case for analysis of biomolecule nanostructure conjugates.

1.1 Nanostructure applications in biology

The use of nanostructure based technologies in biology and medicine continue to expand. Uses range from fluorescent imaging of cells and tissues to the sensing of individual biomolecular events. The scope and utility of a given nanostructure is defined by the material composition and particle shape. The most common technologies make use of transition metal and semiconductor nanoparticles. These are generally spherical in shape, and their properties vary with size and material makeup. Here we will briefly outline two categories of nanoparticles in use in biological applications, semiconductor quantum dots and magnetic nanoparticles in order to place this work in the context nanoscience in general. We then give a detailed background on gold nanoparticles, the focus of this work.

1.1a – Semiconductor Quantum Dots

Much of the early interest in nanoparticles was centered on their use in imaging technologies. Semiconductor quantum dots (Qdots) are single crystal nanoparticle of several nanometers diameter. Their small size results in confinement of valence electrons within the particle, which is on the same size scale as their de Broglie wavelength. This in turn results in the valence electrons having quantized energy states (hence the designation “quantum” dots). Absorption of a photon with energy
above the particle band gap energy results in exciton formation. For particles below the Bohr exciton radius, exciton relaxation occurs by nonradiative recombination, leading to photon emission.\(^2\) Both the adsorption and emission spectra of Qdots are narrow and dependent on the crystal size and character. With careful selection of crystal size and elemental makeup, Qdots have been synthesized with emission photon wavelengths ranging from the ultra-violet to the near infrared.\(^3\) Because of the mechanism of fluorescence, Qdots do not bleach at the rates that organic fluorophores do.\(^4\) Qdots therefore provide tunable, stable and long lasting fluorescent molecules, with narrower emission spectra and longer lifetimes than conventional organic dyes.\(^3\)

Early use of Qdots mirrored that of organic dyes, with extensive use in immunofluorescence labeling. Staining of membrane proteins,\(^5\)--\(^7\) microtubules,\(^7\) and actin\(^8\) as well as fluorescence in situ hybridization on chromosomes\(^9\) and combed DNA\(^10\) have been reported. However, the most significant advance afforded by Qdots relates to their long lifetimes and resistance to bleaching.\(^4\) This has allowed for increased acquisition times, and construction of crisp 3-D images.\(^3\) Experiments in confocal microscopy, total internal reflection microscopy and epifluorescence microscopy\(^11\) have also been improved by the long lifetimes.

Qdot functionalization with peptides, protein and nucleic acids have allowed for use in live-cell experiments.\(^3\) Functionalization is accomplished through surface ligands,\(^9\) which can be selected for reactivity with functional groups on the biomolecule of interest. Functionalization with numerous proteins have been demonstrated, including streptavidin,\(^7\) epidermal growth factor,\(^12\) and both primary\(^13\) and secondary antibodies.\(^14\) These Qdots have been successfully used to detect cancer markers and
several types of receptors in culture. Peptide functionalization has also been successful, and has been used for live animal imaging, including imaging blood vessels and bone marrow in mice.  

1.1b – Magnetic Nanoparticles

Nanoparticles synthesized from magnetic materials have unique and interesting properties. Particle diameters below the size of the magnetic domain for a given substance result in unique magnetic properties, including superparamagnetism and spin-glass behavior. Single domain particles made from ferromagnetic materials such as Fe₃O₄ give rise to superparamagnetic nanoparticles. These particles are useful for a broad range of applications, from biotechnology to computing. Key to the biological utility of such particles is the ability to manipulate them by external magnetic fields. Cells and tissues can be penetrated by magnetic fields, facilitating a wide range of possible applications, including drug delivery, hyperthermia, and imaging techniques.

The earliest use of magnetic nanoparticles in biology centered on cancer therapy through hyperthermia. Hyperthermia treatment involves the injection of highly concentrated solutions of magnetic nanoparticles, called ferrofluids, into tumors. Magnetic fields were then used to induce heating of the ferrofluid by a combination of mechanisms including induction heating, Neel relaxation and Brownian relaxation. The heat generated is toxic to the tumor, as sustained exposure to temperatures at or above 42°C cause apoptosis in most cells. Careful selection of magnetic field strength and nanoparticle type allows heating of cancerous cell with little affect on the surrounding healthy tissue. There continues to be significant interest in this technique
as a tissue specific and minimally toxic treatment option for tumors. Improvements in nanoparticle size distribution, surface coating, and magnetic field targeting have increased the reach of hyperthermia.\textsuperscript{24} Modification with cell-specific biomarkers show promise for highly specific targeting of cancer cells.\textsuperscript{25-27} There remain limitations however, chief among them concerns over human exposure to high magnetic fields.\textsuperscript{28} Improvements in delivery to increase localized nanoparticle concentration will be key to effective hyperthermia treatment with low magnetic field strengths.\textsuperscript{28}

Magnetic nanoparticles have also been used for drug delivery.\textsuperscript{18, 19} As in hyperthermia, external magnetic fields can be used to control Np behavior. Co-encapsulation of drug molecules with magnetic nanoparticles inside micelles or lipid bilayers allow triggered release upon application of a strong magnetic field.\textsuperscript{29} The field triggers release by inducing heat in the nanoparticles, melting the lipid layers and releasing the drug into the surrounding solution. These techniques show great promise for future treatments, including time-controlled release of previously administered drugs.\textsuperscript{30}

Delivery can also be accomplished by using magnets to attract a drug tagged with a magnetic nanoparticle to a specific area of the body.\textsuperscript{31} This is accomplished with strong permanent magnets placed outside the body over specific sites. Nanoparticle-drug conjugates are administered intravenously and congregate in areas of high magnetic field strength. This technique is of particular interest for toxic treatments such as chemotherapy agents in order to reduce the toxic effect on healthy tissue.\textsuperscript{32, 33} There are significant limitations to this technique however, as the speed of blood flow can
greatly affect the efficiency of particle segregation, and to date this technique is thought to be effect only for areas of slower blood flow.\textsuperscript{34}

Magnetic nanoparticles, particularly superparamagnetic iron particles, show great promise for use as magnetic resonance imaging contrast agents.\textsuperscript{35} Because these nanoparticles are magnetically saturated at the normal working ranged of MRI scanners, they create local magnetic field variations and affect the magnetic fields “seen” by nearby water molecules.\textsuperscript{36} This in turn leads to changes in the relaxation times of water protons, altering the image.\textsuperscript{30} Differential tissue uptake due to nanoparticle size and coating allows visualization of specific tissue types.\textsuperscript{22} For example, iron particles coated with dextran are selectively taken up by reticuloendothelial cells in healthy tissue. In tumors, this cell type is compromised and Np uptake is affected.\textsuperscript{37-39} This creates visual distinctions between healthy and cancerous tissue in the MRI. Nanoparticle size effects can be used to select for liver and spleen uptake, as large nanoparticles are quickly cleared to these organs, while smaller nanoparticles remain in the blood stream longer.\textsuperscript{22} Since particle size affects the degree of contrast, tissue specific contrast is created. Similar techniques have also been used for visualization of malignant lymph nodes,\textsuperscript{38} liver tumors,\textsuperscript{39} and brain tumors.\textsuperscript{37} Biomolecule conjugation has allowed for targeting of specific cell types, both by cell surface receptor over-expression, and targeting of apoptotic cells.\textsuperscript{30} Such labeling techniques show great promise for increase sensitivity in cancer cell detection.

1.1c – Summary

The potential of biological nanoscience is immense. The above sections represent only a portion of what has been attempted in the field. The use of nanoparticles alone
has already yielded exciting results, but the future of the field lies in nanoparticle-biomolecule conjugates. While this topic has received significant attention, there are still numerous avenues of investigation open. The key factor that will govern the success of such applications will be the synthesis of nanoparticle conjugates in which biomolecules remain stable and active. There are numerous biochemical functional groups which have the potential to interact with nanoparticle surfaces and disrupt biomolecule structure and function. A detailed understanding of bio-nano interactions is essential to the realization of the potential of both Qdots and magnetic nanoparticles.

1.2 Gold Nanoparticles

Gold nanoparticles (AuNp) were the first nanoparticle type to be studied extensively by modern science.\textsuperscript{40} Studies of the nucleation and growth processes were published as early as 1951.\textsuperscript{41} The history of AuNps extends far beyond the twentieth century however. The optical properties of gold nanoparticles were exploited in the 4th and 5th century to color glass, and later in the middle ages for diagnosis and treatment of a variety of diseases.\textsuperscript{40} Recently gold nanoparticles have received a great deal of attention for the optical and electronics characteristics they display have also been of great interest due to their biocompatibility, ease of conjugation, and high stability.\textsuperscript{40}

1.2a Synthesis and Physical Properties

The physical properties of AuNps are dominated by their size, and are neither those of the bulk material nor those of individual molecules. They depend strongly on particle diameter, inter-particle distances, the chemistry of passivating ligands, and the particle shape.\textsuperscript{40} As seen in the Qdots describe above, there is a quantum size effect for
particles on the size scale of the de Broglie wavelength, where the particle behaves as a zero-dimensional quantum box. In the case of a metal such as gold, the freely mobile electrons trapped within this metal box show a characteristic oscillation frequency of the plasmon resonance. This gives rise to a characteristic absorption peak at ~520nm for AuNps in the 5-25nm size range. This absorption frequency is strongly dependent on inter-particle distance. When AuNps are brought close to one another, the absorbance frequency wavelength increases to ~650nm. This change can be seen by eye in the color change of a AuNp solution from red to blue upon particle aggregation, making AuNps excellent indicators of binding and hybridization events.

A wide variety of synthesis techniques and surface passivations are available for AuNps. Syntheses can be performed in aqueous, organic, and two phase systems, yielding particles with narrow size distributions from 1.5-30nm. AuNp solubility and reactivity is dominated by the ligand molecules passivating the surface. Particles can be synthesized with numerous surface ligands, including citrate, various alkane thiols, lipid molecules and polymers. Further choice of surface ligand is available through ligand exchange after synthesis. Covalent linkage to AuNp surfaces can be achieved both through direct bond formation with the surface Au atoms, and by bifunctional ligands which form bonds to the particle surface and to molecules in solution. This flexibility in surface chemistry, solubility, and covalent attachments means AuNps can be used in a wide variety of settings. AuNps have been linked to DNA for self-assembly and hybridization sensing, labeled with antibody for enhanced immunolabeling, and linked to proteins and peptides for cell imaging and bioassay applications. For the purposes of this study, we will focus on AuNps linked to DNA.
This system was chosen both for its frequent use in biological applications, and due to previous investigations of DNA nucleotides on gold surfaces.

1.2b Gold nanoparticles DNA conjugates

Early work in gold nanoparticle biocompatibility focused on conjugation to DNA, motivated by the promise of programmable self-assembly. By exploiting DNA complement recognition and specificity, DNA modified AuNps (AuNp-DNA) were shown to assemble in specific patterns based on the sequences of the DNA selected for conjugation. Aggregate formation is reversible, as it is based on nucleotide base pairing, and formation and dissolution are visible due to color changes related to interparticle plasmon effects. The potential of programmable nanoparticle arrangements sparked considerable interest in the area, and numerous reports have been generated regarding the isolation and characterization of Au-DNA. More recently, AuNp-DNA conjugates have been used in gene regulation. Antisense DNA, designed to block protein translation from mRNA was linked the gold nanoparticles and shown to be significantly more effective than the DNA alone.

Synthesis of AuNp-DNA conjugates is generally accomplished by covalent attachment of DNA modified with a 5' C6-thiol. Addition of the modification during oligonucleotide synthesis is simple, and provides a direct covalent link between the particle surface and the DNA. The Au-S bond is very stable, with a bond energy of ~89kJ/mol, and the synthesis is a spontaneous process. Isolation of the conjugates is straightforward and usually accomplished by agarose gel electrophoresis. The resulting conjugates can be made in a wide variety of DNA coverages, from 1 DNA/Np to up to 200 depending on the Np size used. These conjugates have been used in
numerous biological applications, including the above mentioned detection and gene regulations techniques, and also delivery,\textsuperscript{59} and DNA sequencing.\textsuperscript{6, 60}

Most of these applications for AuNp-DNA conjugates rely on effective complement hybridization. The efficiency of hybridization can be compromised by noncovalent interactions between the DNA bases and the gold surface.\textsuperscript{61, 62} The functional groups of DNA bases can adsorb to gold and several studies, both theoretical and experimental, have attempted to describe the precise interactions that take place.\textsuperscript{63-65} Figure 1 shows a summary of the predicted interaction geometries. Both modeling studies of 2-6 Au atom clusters\textsuperscript{64, 65} and SERS analysis of free base interactions with 13nm AuNps\textsuperscript{63} show DNA geometries perpendicular to the Np surface. The electron rich oxygens and nitrogens of the rings interact with the electron poor Au atoms on the Np surface.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1a.png}
\caption{Proposed structure of DNA base adsorption on AuNps: a) Adenine, b) thymine, c) guanine, d) cytosine.}
\end{figure}

These base specific interactions create differences in nucleotide affinity for the nanoparticle surface. This can in turn affect the availability of a given sequence to hybridize with its complement. Several studies have been conducted to elucidate the
sequence dependence of such adsorption behavior. Experiments studying the adsorption of free nucleotides onto 13nm AuNps by particle aggregation\textsuperscript{66, 67} have shown an affinity order of \( G > C > A > T \). Isothermal titration calorimetry of bases on 65nm AuNps give enthalpies of adsorption of \( C > G > A > T \).\textsuperscript{68} Thermal desorption studies of individual base behavior on gold thin films have determined affinities of \( G \geq A > C > T \).\textsuperscript{69} Homo-oligonucleotide competition assays also on thin films have shown relative affinities of \( A > G \geq C > T \).\textsuperscript{70} Related studies of oligonucleotide surface coverage have shown variations in DNA coverage per particle depending on sequence. PolyT spacers at the oligonucleotide-Au interface yield the highest coverages,\textsuperscript{71} while polyA spacers reduce the number of DNA per particle.\textsuperscript{72} This variety of results make definitive conclusions about sequence specific behavior difficult, though published results consistently indicate that T has significantly lower affinity than the other three nucleotides.\textsuperscript{66-69} High nucleotide affinity affects both oligonucleotide surface coverage and complement hybridization. There is a pressing need to develop strategies for oligonucleotide design for sequences coupled to AuNps. The development of such strategies requires a detailed understanding of nucleotide affinities for particle surfaces and the affect of varying affinity on DNA bioavailability. As detailed above, studies of nucleotide affinity have been conducted; however these have not been standardized. Consistency in gold formulations and surface ligand passivation are necessary to draft strategies for oligonucleotide design.

In this work we report a systematic study of the effect of DNA sequence and nanoparticle size on the behavior of AuNp-DNA conjugates. Measurement of adsorption behavior and complement hybridization allows analysis of the relative
nucleotide affinities for the AuNp surface. From these results we establish set of rough guidelines for DNA sequence selection. In a broader sense, the work presented here provides a demonstration of techniques which can be applied to bio-nano systems beyond AuNp-DNA. The specifics of this study will be presented in the next four chapters. Chapter 2 provides details of the experimental procedures used and the analysis techniques employed. Chapter 3 presents the results of DNA oligonucleotide conjugation to one size of AuNp, 7.5nm, and the effects of DNA sequence and sequence location on conjugate behavior. Chapter 4 describes the effect of AuNp size on conjugate behavior. Finally, chapter 5 details the development of an alternative model for the interpretation of DNA adsorption on AuNps. Chapter 6 provides a comprehensive summary of the results of these experiments and a proposed system for further study in a more complex and biologically relevent system.
Chapter 2: Materials and Methods

We were interested in evaluating the behavior of thiolated DNA covalently attached to gold nanoparticles (AuNps), which can effect conjugate behavior in biological applications.\textsuperscript{61,62} Noncovalent nucleotide adsorption will alter hybridization and change conjugate behavior as effective use of conjugates in biological applications requires careful study of adsorption behavior. Many applications allow for flexibility in selecting DNA sequences. Ideally, detailed study will also provide effective criteria for DNA sequence selection.

2.1 Laboratory Methods

Evaluating the behavior of AuNP-DNA conjugates for biological uses requires study of ensemble properties, rather than the individual conjugate level. To this end, the techniques used in this study measure average behaviors of large numbers of particles.

2.1a Nanoparticle Synthesis and analysis

Aqueous AuNP synthesis and isolation

AuNps of diameters 6nm and above were synthesized using a frequently sited aqueous method\textsuperscript{44}. Reduction of tetrachloroauric acid (HAuCl\textsubscript{4}) was accomplished with a mixture of tannic acid and sodium carbonate. 3mLs 1% HAuCl\textsubscript{4} was diluted with 237mLs water. This solution was then heated to 60\textdegree{}C. A separate solution of 12mLs 1% sodium citrate, and varying volumes of 1% tannic acid and 25mM sodium carbonate was diluted in water (60mL total volume) and heated to 60\textdegree{}C. The two solutions were
combined and agitated for 10 min at 60°C. The solution turns wine red immediately upon addition of the tannic acid/citrate solution. Particle size is controlled by the volume of tannic and sodium carbonate added. AuNp formation occurs in two steps, nucleation and growth. 41 The first step, nucleation, is controlled by the tannic acid, which reduces Au atoms from Au$^{3+}$ to Au$^{0}$ at a fast rate. The second step, growth, is controlled by the sodium citrate, which reduces Au atoms slowly over the 10 min of heating. Increased tannic acid volume leads to a greater number of quickly reduced Au atoms, leading to more nucleated particles. Since the total number of Au atoms in each synthesis is equal, high tannic acid volumes result in the atoms being distributed over a large number of particles. The size of an AuNp is determined by the number of atoms it contains, thus more nucleated particles yield smaller particle sizes. Sodium carbonate is added in a volume equal to that of tannic acid and acts as a buffer, as the solution pH can affect the reduction rate. Table 2-1 shows the volumes of tannic acid and sodium carbonate added for synthesis of several sizes of AuNps. After synthesis the solution was cooled to room temperature. Ligand exchange was performed overnight at room temperature using a large excess (2000x) of bis(p-sulfonatophenyl) phenylphosphine (BPS), 56 which displaces citrate molecules from the particle surface.

<table>
<thead>
<tr>
<th>Nanoparticle Diameter (nm)</th>
<th>Volume (mL) tannic acid/ Sodium citrate</th>
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<tbody>
<tr>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>7.5</td>
<td>1</td>
</tr>
<tr>
<td>10.6</td>
<td>0.5</td>
</tr>
<tr>
<td>12</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 2-1. Synthesis conditions for several nanoparticle sizes
BPS functionalized nanoparticles were isolated and dried for storage. Precipitation of Nps was accomplished by addition of excess sodium chloride (NaCl). Solid nanoparticles were collected by centrifugation and re-dissolved in water. Remaining salt was removed by precipitation with ethanol (C\textsubscript{2}H\textsubscript{6}O). Nanoparticles were then dried by vacuum and stored at room temperature.

Np sizes were determined from re-dissolved stored samples. Transmission electron microscopy (TEM) was performed on a JEOL 200cx scope. Digital photographs of AuNps were analyzed using photoshop and ImageJ programs.\textsuperscript{73} Size distributions were analyzed using a Gaussian fit to give average particle sizes and uncertainties. Sizes were determined by analysis of at least 200 individual nanoparticles and at a minimum of two different magnifications.

**Organic AuNp synthesis and isolation**

AuNps with diameters below 5nm were synthesized using an organic phase method developed by Jana et al.\textsuperscript{45} The reactions were performed in a solvent of 100mM didodecyl dimethyl ammonium bromide (DDAB) in toluene. Varying amounts of dodecylamine were added to 2.5mL of a 9.9 mM solution of gold (III) chloride (AuCl\textsubscript{3}) in 100mM DDAB. The gold salt solution was then reduced by 1mL 0.097M tetrabutyl ammonium borohydride (TBAB) with vigorous stirring. The solution turned red-brown immediately upon addition of TBAB. AuNp size is controlled by the amount of dodecylamine added to the gold (III) chloride solution. Au\textsuperscript{3+} atoms form complexes with the amines, and the number of these complexes determines the amount of nucleation that occurs upon the addition of TBAB. Table 2-2 shows dodecylamine amounts for the two sizes of nanoparticles used in this work.
Nanoparticle Mass
Diameter dodecylamine

<table>
<thead>
<tr>
<th>Diameter (nm)</th>
<th>Mass dodecylamine (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>4.4</td>
<td>90</td>
</tr>
</tbody>
</table>

**Table 2-2.** Organic AuNp synthesis conditions.

After synthesis, AuNp solutions were left at room temperature for 2 hours. AuNps were then precipitated with 5mL of a 50% methanol/50% Ethanol solution. Solid nanoparticles were collected by centrifugation and re-dissolved in 2mL toluene. Ligand exchange was achieved by addition of 0.1g BPS in 5mL methanol. The particles precipitated upon addition of the BPS solutions. The mixture was left for 2 hours and agitated every 30min. BPS functionalized particles were isolated by centrifugation and re-dissolved in 2mL water. The AuNps were then precipitated with 5mL ethanol and dried for storage. Particle sizes were determined by the same method used for larger particles. Figure 2-1 shows TEM images and radius distributions for several sizes.

**Nanoparticle Concentration Analysis**

AuNp concentration was determined from the characteristic absorbance peak at ~520nm. The extinction coefficient of this peak varies with particle size. From published extinction coefficients of several sizes of AuNps, we calculated a direct correlation between the number of gold atoms within a particle and the extinction coefficient. The number of atoms in an AuNp can be calculated from the volume and the density of gold. Figure 2-2 shows the relationship between extinction coefficients and the number of atoms in a particle. Table 2-3 shows calculated extinction coefficients for several sizes of AuNp used here. AuNp solution concentrations were determined from absorbance measurements at 520nm using the calculated extinction
coefficients. Figure 2-3 shows characteristic absorption curves for several sizes of AuNps collected on a Cary50 ultraviolet-visible light spectrophotometer.

Figure 2-1. Transmission electron microscopy images of three AuNp sizes: a) 4.4nm AuNp (red bars); b) 7.5nm AuNp (green bars); c) 10.6nm AuNp (blue bars); d) size distributions and Gaussian fits for the AuNp sizes.

Figure 2-2. Number atoms in an AuNp vs. natural log extinction coefficient.
AuNp Extinction diameter (nm) # atoms Extinction Coefficient (M⁻¹ cm⁻¹)
3.0 833 2.52E+06
4.4 2629 8.20E+06
7.5 13019 4.25E+07
10.6 36753 1.24E+08
12.5 60271 2.061E+08

Table 2-3. Calculated gold nanoparticle extinction coefficients

Figure 2-3. Ultraviolet-Visible absorbance spectra of three sizes of AuNp, 4.4nm AuNp (black line), 7.5nm AuNp (red line), and 10.6nm AuNp (blue line).

2.1b Gold nanoparticle DNA conjugate synthesis and analysis

Au-DNA synthesis and isolation

Covalent attachment of DNA to AuNps was accomplished using DNA oligonucleotides modified with a 5' C₆ thiol functional group. Table 2-4 shows the primary sequences used in this work, and their complement strands. Effective attachment of the oligonucleotides to AuNps requires reduction of any DNA dimers using dithiothreitol (DTT). This allows for significantly higher reactivity of the DNA for the nanoparticle surface.
Table 2-4. Oligonucleotide sequences.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence (5’ to 3’)</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>HS-AATTATACCGGCCGC</td>
<td>GCCCGGCTTCCTT</td>
</tr>
<tr>
<td>GC</td>
<td>HS-CCGCGCAATTATA</td>
<td>TATAATTGCGCCGG</td>
</tr>
<tr>
<td>A-near</td>
<td>HS-AATAATTTTTTTTTT</td>
<td>FAM-AAAAAAAAATTATT</td>
</tr>
<tr>
<td>A-middle</td>
<td>HS-TTTTTAATAATTTTT</td>
<td>FAM-AAAAAATTATTTAAAAA</td>
</tr>
<tr>
<td>A-far</td>
<td>HS-TTTTTTTTTTAAATAA</td>
<td>FAM-TTTTTAATAATTTAAAAA</td>
</tr>
<tr>
<td>G-near</td>
<td>HS-GGTGGTTTTTTTTTT</td>
<td>FAM-AAAAAAAAAACCCACC</td>
</tr>
<tr>
<td>G-middle</td>
<td>HS-TTTTTGCTTTTCTTTT</td>
<td>FAM-AAAAACCCACCAAAAA</td>
</tr>
<tr>
<td>G-far</td>
<td>HS-TTTTTTCTTTTCTTTT</td>
<td>FAM-CCACCAAAAAAA</td>
</tr>
<tr>
<td>C-near</td>
<td>HS-CCTCCTTTTTTTTTT</td>
<td>FAM-AAAAAAAAAGGAGG</td>
</tr>
<tr>
<td>C-middle</td>
<td>HS-TTTTTTCTTTTTCTTT</td>
<td>FAM-AAAAAGGAGGAAAAA</td>
</tr>
<tr>
<td>C-far</td>
<td>HS-TTTTTTTTTTTTCTCC</td>
<td>FAM-GGAGGGAAAAAAA</td>
</tr>
<tr>
<td>T-control</td>
<td>HS-TTTTTTTTTTTTTTTT</td>
<td>FAM-AAAAAAAAAAAAA</td>
</tr>
</tbody>
</table>

DNA sequences were incubated for ~16 hours at 4°C in 100mM DTT. The DTT was then extracted using multiple washes ethyl acetate (EtAc), as DTT will dissolve AuNps. The concentration of then isolated DNA was the determined by the characteristic absorbance peak at 260nm. Stored particles were dissolved into a high concentration solution (0.1-2μM), and the exact concentration determined from the 520nm absorbance peak. DNA and AuNps were combined in an appropriate ratio (between 2:1 DNA:AuNp and 60:1 DNA:AuNp). Combined samples were dried under vacuum and resuspended in 100ul 1X PBS (137mM sodium chloride, 2.7mM potassium chloride, 10mM phosphate buffer). Samples were left for ~16 hours at room temperature to react. The high salt concentration is required for effective synthesis due to the intermolecular charge repulsions between the negatively charged DNA oligonucleotides and the negatively charged BPS ligands on the particle surface. Excess salt and unreacted DNA was removed by repeated centrifugation. Isolated samples were volume adjusted.
with 0.5X TBE (0.0445M tris base, 0.0445M borate, 0.001M EDTA) to 0.9μM AuNp for 4.4nm samples, 0.11μM AuNp for 7.5nm samples, and 0.05μM for 10.6nm samples. Samples were stored at 4°C. Supernatant from centrifugal isolation were saved, their volume recorded, and stored at 4°C.

Double stranded DNA was also covalently attached to AuNp surfaces. Thiolated DNA was hybridized to complementary DNA using a slow annealing process. A solution of 20μM thiolated DNA and 22μM in 1x PBS was heated to 65°C and the temperature lowered 5°C every 5 min. Anealed samples were stored at 4°C. DNA attachment was performed as detailed above with the exception that samples were on ice. Figure 2-4 shows an agarose gel with bands of single and double stranded DNA attachment reactions.

![Figure 2-4](image)

Figure 2-4. 2% agarose gels of 11nm Au-DNA conjugatation; a) single stranded DNAconjugation, lane 1 - 11nm AuNp, lane 2 - 5:1 AT:AuNp, lane 3 - 5:1 GC:AuNp, lane 4 - 11nm AuNp; b) double stranded DNA conjugatation, lane 1 - 11nm AuNp, lane 2 - 5:1 AT:AuNp, lane 3 - 5:1 GC:AuNp, lane 4 - 11nm AuNp

AuNp-DNA Coverage measurements

The coverage of thiolated DNA per AuNp was determined by displacement of the DNA by mercaptohexanol (MCH). MCH has been shown to form a monolayer on both
gold nanoparticles and crystal surfaces. High concentrations of MCH will totally remove thiolated DNA from the particle surface. MCH coated nanoparticles were not soluble in water and can easily be removed by centrifugation. Prior to MCH treatment, AuNp concentration was calculated using UV-Vis spectroscopy of diluted AuNp-DNA solutions. MCH was then added directly to these solutions. DNA concentration can then be evaluated using a fluorescent stain SYBRgold (λ_{excitation} = 493 nm, λ_{emission} = 542 nm). This intercalating agent allows quantification of DNA strands by fluorescence spectroscopy on a Spex Fluoromax 3 fluorometer. Figure 2-5 shows calibration curves for each sequence used in this study. The sequence specificity of the calibration curves is due to nucleotide specific intercalation by the SYBR gold.

![Figure 2-5. SYBRgold treated thiolated DNA calibration curves](image)

**DNA Hybridization and Analysis**

The hybridization behavior of AuNp-DNA conjugates was studied by quantification of complementary DNA hybrid formation. Complementary DNA oligonucleotide
sequences were listed in table 2-4. Complement strands were purchased with 5' fluorescein modifications. These modifications allow quantification of complement concentration independent of thiolated DNA concentrations.

Isolated AuNp-DNA conjugates were annealed to their complements under mild conditions. Annealing solutions were prepared from stored AuNp-DNA solutions of 0.27$\mu$M AuNp for 4.4nm samples, 0.11$\mu$M AuNp for 7.5nm samples, and 0.06$\mu$M for 10.6nm samples. Complement concentrations added were adjusted to each size and coverage sample to yield ~2X the thiolated DNA concentration present. For example, for a 7.5nm 1.5 DNA/AuNp sample, 0.33$\mu$M complement DNA was added. Solution salt concentration was adjusted to 1X TBE to facilitate hybrid formation. Samples were heated to 30°C and held for 10min. Solution temperature was then lowered slowly to 4°C and held for 16 hours. Hybridized AuNp-DNA conjugates were isolated by repeated centrifugation at 4°C. Isolated samples were then diluted with 0.5X TBE to ~150ul. AuNp concentration was measured using UV-Vis spectroscopy. 50uL MCH was then added directly to the solution to displace the thiolated and complement DNA. Insoluble nanoparticles were removed by centrifugation. Complementary DNA concentrations were evaluated using the fluorescent signal from the 5' fluorescein modification ($\lambda_{\text{excitation}} = 495$ nm, $\lambda_{\text{emission}} = 517$ nm). Without addition of the intercalating agent, thiolated DNA is not detectable, and does not affect the complement concentration measurements. Figure 2-6 shows the calibration curves for quantification of complementary DNA concentrations.

The melting behavior of the annealed samples were collected using fluorescence spectroscopy (figure 2-7). DNA melting can be seen by the dequenching of
complement fluorophore with dehybridization. The fluorescent signal of the fluorescein is quenched by the particle when the DNA is fully hybridized, however melting causes the fluorophore to move out of the effective range of particle quenching. Unfortunately that melting curves are complex and yield little information. Figure 2-7 show the melting curves of the A and G sequences listed in table 2-4. The wide variation in melting behavior seen is attributed to changes in the optical behavior of the particles with temperature.

![Calibration curves for complementary DNA concentration](image)

**Figure 2-6.** Calibration curves for complementary DNA concentration

**MCH treatment**

The effect of noncovalent nucleotide surface affinity on the extent of complement hybridization was studied by partial surface functionalization with MCH. While high concentrations of MCH will totally displace thiolated DNA from nanoparticle surfaces, low MCH concentrations have more subtle effects. Low concentration exposures for short periods of time result in partial MCH surface coating without removing the
thiolated DNA. These conditions result in disruption of nucleotide surface adsorption without displacement of the thiol-gold bond. AuNp-DNA samples were treated with 1 µM MCH for 2 minutes. Excess MCH was then extracted with ethyl acetate. Samples were then hybridized, isolated and analyzed as described above.

![Figure 2-7. AuNp-DNA melting curves](image)

**Agarose gel electrophoresis**

Agarose gels were prepared by standard methods. Agarose of an appropriate weight for the desired gel percentage (1g/100mL for 1%) was added to 0.5X TBE. The suspension was then heated in a microwave to boiling. The agarose solution was then poured into a gel tray and allowed to cool. Samples were then loaded with 2ul glycerol to facilitate confinement within each well. Gel electrophoresis was performed for set periods of time (60, 90 and 120min). Applied voltage was measured at the conclusion of each run.
2.3 Analysis Methods

2.3a Ferguson Plots

Ferguson plot analysis allows for the evaluation of the size and surface charge of an aqueous particle. Analysis of a sample's mobility through agarose gels of varying fiber densities, when compared with standard particles of known size, allows for calculation of the sample's hydrodynamic radius. This analysis is based on the extended Ogston model, a statistical treatment of the migration of particles through a random meshwork of inert fibers. This model specifies that migration is dependent on particle collisions with gel fibers. Collisions can occur either along the fiber (cuts), or with the fiber ends (hits). Each interaction has a distinct probability based on fiber length, area, and density. Gel conditions in which cuts dominate particle migration were designated 1-D gels, while hit-dominated gel conditions were 0-D. Agarose gels were generally treated as 1-D systems. Under 1-D conditions, the fiber radius, \( r \), and the total length per unit weight of the gel matrix, \( l \), can be determined from the mobilities of particles of known radius \( R \). Standard particle mobilities (\( M, \text{cm}^2/\text{V*min} \)) were plotted as a linear function of gel percentage (\( T, \text{g/mL} \)) using equation 1.

\[
\log_{10}(M) = \log_{10}(M_0) - K_R \cdot T \quad (1)
\]

The slope is \( K_R \), the retardation coefficient, which depends on gel fiber dimensions and the size of the sample:

\[
\sqrt{K_R} = \sqrt{d} \cdot R + \sqrt{d} \cdot r \quad (2)
\]

\( K_R \) and gel fiber dimensions were calculated using AuNp's of known radii, determined from TEM analysis.
The use of Ferguson plots to evaluate AuNp-DNA size is essential to the preliminary work of this study. Evaluation of particle size by this method revealed differences due to DNA sequence and lead to the design of a systematic and detailed set of DNA oligonucleotides.

2.3b Langmuir Isotherm

The Langmuir isotherm was first designed to describe the behavior of gases when in contact with plane surfaces.\textsuperscript{80} First tested on glass, mica and platinum, the Langmuir isotherm lays out a theoretical analysis of adsorption phenomena. The theory has been adapted for use in numerous conditions. As the system under consideration here is an aqueous one, a brief derivation of the isotherm under such conditions follows.\textsuperscript{81}

If we consider an equilibrium adsorption reaction of the form

\[ C + S \rightarrow CS \]

where C represents an aqueous colloid having A surface sites, and S represents an absorbate molecule. Each molecule of S will occupy one surface site. The equilibrium constant for the above reaction will be:

\[ K_{eq} = \frac{[CS]}{[S_e][C_e]} \]  \hspace{1cm} (3)

\( S_e \) and \( C_e \) represent the respective concentrations of each reactant at equilibrium. If we define the total number of adsorbed S molecules as \( N \) then the density \( \Theta \) of S molecules on the surface will be:

\[ \theta = \frac{N}{A} = \frac{[CS]}{[C_e] + [CS]} \]  \hspace{1cm} (4)
or the fraction of surface sites filled with ligand. Substitution of $K_{eq}$ and rearrangement gives the usual form of the Langmuir isotherm

$$N = \frac{A * K_{eq} * \left[S_e\right]}{1 + K_{eq} * \left[S_e\right]}$$  \hspace{1cm} (5)

often rewritten as

$$q_e = \frac{Qb[S_e]}{1 + b[S_e]}$$  \hspace{1cm} (6)

Where $q_e$ is the particle coverage and $A$ is replaced by $Q$, the maximum number of $S$ molecules that can theoretically adsorb on the surface. $K_{eq}$ is replaced with $b$, designated as a binding efficiency term. Numerical fits of adsorption data yield values for $Q$ and $b$ which allow interpretation of adsorption behavior.

This model of adsorption is based on four assumptions about the system; all surface adsorption sites were equal, adsorbed molecules do not interact with one another, all adsorption occurs via the same mechanism, and only a monolayer of absorbate molecules is formed. When any of these conditions were violated more complex models were required for effective interpretation of the data. The Langmuir isotherm model of adsorption has been used extensively in the literature to describe adsorption of DNA on many surfaces, including gold.\textsuperscript{82–84} We use it here as a simple model for interpreting our data by a method consistent with previous reports.
3. Sequence Dependent nucleotide adsorption on 7.5nm gold nanoparticles

3.1 Introduction

As noted in chapter 1, AuNp-DNA conjugates have been utilized in many applications, including hybridization sensing, self assembly, and delivery. Covalent attachment of DNA to AuNps and thin films is straightforward and generally accomplished by thiol linkers. However, functionality of AuNp-DNA conjugates is complicated by non-specific adsorption of the nucleotides on the AuNp. DNA adsorption on the AuNp can impair conjugate ability to hybridize to complementary DNA, and can be problematic for applications of conjugates where proper hybridization is necessary. As noted previously, several studies have attempted to detail the adsorption of free nucleotides onto AuNps. These have resulted in a variety of affinity orders, making definitive predications of sequence-specific AuNp-DNA behavior difficult. Biological applications using AuNp-DNA conjugates put constraints on sequence choice as target oligonucleotides may require the presence of high affinity nucleotides.

Here, the effect of oligonucleotide sequence on noncovalent nucleotide adsorption is investigated. 7.5nm AuNps were conjugated to oligonucleotides differing in nucleotide composition and placement within the sequence. The effect of DNA coverage on oligo behavior was studied. Reactivity of the oligonucleotides toward the AuNp and the extent of hybridization of the conjugates varied with sequence.
3.2 Preliminary Data

Initial studies of the sequence dependent behavior of AuNp-DNA conjugates were performed on the two DNA sequences shown in Table 3-1.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>HS-AATTATACCGGCGC GCGCCGGTATAATT</td>
<td>GCGCCGGTATAATT</td>
</tr>
<tr>
<td>GC</td>
<td>HS-CCGGCGCAATTATATA TATAATTGCGCCGG</td>
<td>TATAATTGCGCCGG</td>
</tr>
</tbody>
</table>

**Table 3-1. Oligonucleotide sequences**

Placement of GC rich regions, which we predict will have a greater tendency to stick to Au surfaces, is either adjacent to or far from the thiol end. Sequences were analyzed by mfold and chosen such that self hybridization was not energetically favorable.

Figure 3-1 shows the obtained DNA:AuNp ratios for different reaction incubations. The AT oligo incubated at 10:1 results in a ratio of 3.5 DNA/AuNp, while an incubation ratio of 5:1 results in 2 DNA/AuNp. In contrast, the GC oligo incubated at 10:1 results in 5.5 DNA/AuNp and the 5:1 incubation ratio results in 3.5 DNA/AuNp. Despite the fact that both oligos link to the AuNp by thiol-gold chemistry, the GC oligo is evidently more reactive to the AuNp than the AT oligo. 3.5 DNA/particle coverage translates into roughly 1 DNA oligo for every 44 nm².

The hydrodynamic radius of the particle conjugates was obtained by subsequent agarose gel electrophoresis. Figure 3-2a shows an agarose gel of 2.5% (w/v) containing Lane 1, 7nm AuNps; Lane 2, AuNp-AT; Lane 3, AuNp-GC, Lane 4, 7nm AuNp. It is visible that the mobilities of the samples differ. In order to obtain a value for $R_{eff}$, $\log_{10}M$ is plotted as a function of $T$, and is fit according to the linear equation as detailed in chapter 2.

$$\log_{10}M = \frac{\log_{10}M_0 - K_R \cdot T}{78}$$

(1)
$K_R$ values were obtained from the mobilities of particles of known sizes, as detailed in chapter 2. Figure 2-2d shows the resulting $R_{\text{eff}}$ of the 10:1 AT and 5:1 GC samples. The AuNp-AT oligo has a larger $R_{\text{eff}}$ (4.8nm), compared to the GC oligo, $R_{\text{eff}} = 4.3$nm. This suggests that the conformation of the AT oligo is such that it points outward from the AuNp surface more than the GC oligo.

![Figure 3-1](image.png)

**Figure 3-1.** Coverage Analysis: Coverages in # DNA oligos/AuNp for samples. AT 10:1, AT 5:1, GC 10:1, GC 5:1. (DNA:AuNp incubation ratio). Inset: calibration curve of fluorescence as a function of AT and GC DNA concentration exposed to intercalating dye.
The amount of complementary DNA the AuNp-DNA conjugates can hybridize to was quantified. Figure 3-3 shows the amount of DNA on the AuNp-DNA conjugates which have been annealed with their complementary DNA. The black data is the amount of DNA before annealing to the complement, and the gray data is the amount with the complement added. AuNp-AT increases from 3.3 DNA to 5.7 strands upon
hybridization. AuNp-GC increases from 3.3 to only 4.1 oligos. Translating this into a percent capacity, where an increase of 3.3 oligo/NP upon annealing would be 100%, the AT oligo can hybridize 70% of its capacity while the GC oligo can hybridize only $\sim$25% of its capacity.

These results demonstrate the importance of local nucleotide sequence to both the conformation of DNA-AuNp conjugates in solution, and their ability to hybridize complementary DNA. GC rich regions adjacent to the particle surface appear to increase the reactivity of the DNA to the AuNp. This arrangement also has the effect of decreasing both the effective size of the conjugate and its ability to hybridize to its complement. In contrast, AT rich regions adjacent to the AuNp surface result in a less efficient reaction to the AuNp surface, but yield a AuNp-DNA conjugate that is more available for hybridization to its complement, and has a larger hydrodynamic radius in solution. Single stranded DNA has very short persistence lengths, reported anywhere from 0.2nm to 3 nm $^{89}$ and is quite flexible at the buffer concentrations used in these

![Figure 3-3. Hybridization capacity. Coverages of single-stranded (black) and annealed (gray) samples in # DNA/AuNp.](image)
experiments. Several geometries of DNA adsorption to the AuNp surface are possible (figure 3-4). The calculated hydrodynamic radii indicate that both the AT and GC oligonucleotides adsorb on the particle surface. The smaller size seen for the GC oligonucleotides indicates that it adsorbs with higher affinity than the AT oligonucleotide. For 14mers the topological length is approximately 5nm, and the observed increases in $R_{\text{eff}}$ for the AT samples is only 1nm, implying that neither oligonucleotide is fully extended off the particle surface.

![Figure 3-4. Hypothesized conformation of DNA oligos on AuNp surface. GC rich, red. AT rich, green.](image)

In conclusion, the conformation of DNA oligos adsorbed on AuNp surfaces depends on oligo sequence. Placement of high affinity nucleotides (G, C) relative to the thiol end influences noncovalent adsorption, reaction efficiency, and the ability to hybridize to targets. These results are limited however, by the mixture of oligos investigated. We can form not definitive conclusions about individual nucleotide affinities because of these limitations. Full understanding of nucleotide affinities requires a more systematic study.
3.3 Published Results

To investigate sequence and sequence location effects, ten oligonucleotides were compared (Table 3-2). Each of the high affinity nucleotides (A, G, C) were surrounded by poly-T stretches as a low affinity background, because T has consistently shown minimal adsorption on Au surfaces.\textsuperscript{61, 67} Four bases of the nucleotide of interest were placed at the 5' end near the thiol and AuNp (X-near), at the halfway point (X-middle), or at the 3' end (X-far). A poly-T oligonucleotide is the low affinity control. Mfold simulations\textsuperscript{88} verified that the oligonucleotides did not self-fold.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence (5’ to 3’)</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-near</td>
<td>HS-AATAATTTTTTTTTTT</td>
<td>FAM-AAAAAAAATTATT</td>
</tr>
<tr>
<td>A-middle</td>
<td>HS-TTTTTAATAATTTTTT</td>
<td>FAM-AAAAATTATTTAAAA</td>
</tr>
<tr>
<td>A-far</td>
<td>HS-TTTTTTTTTTTAAATAA</td>
<td>FAM-TTATTTTTTTTTTTTTTT</td>
</tr>
<tr>
<td>G-near</td>
<td>HS-GGTGGTTTTTTTTT</td>
<td>FAM-AAAAAAAACCCACC</td>
</tr>
<tr>
<td>G-middle</td>
<td>HS-TTTGTGGTTTTTTTT</td>
<td>FAM-AAAAACCACTAAAAA</td>
</tr>
<tr>
<td>G-far</td>
<td>HS-TTTTGGTTTTTTTGTGG</td>
<td>FAM-CCACCAAAAAAAAA</td>
</tr>
<tr>
<td>C-near</td>
<td>HS-CCTCTTTTTTTTTTTT</td>
<td>FAM-AAAAAAAAAAAAAGGAGG</td>
</tr>
<tr>
<td>C-middle</td>
<td>HS-TTTTTTCTCTCTCTTTT</td>
<td>FAM-AAAAAGGAGGAAAAA</td>
</tr>
<tr>
<td>C-far</td>
<td>HS-TTTTTTTTTTCTCTCC</td>
<td>FAM-GGAGGAAAAAAAAAA</td>
</tr>
<tr>
<td>T-control</td>
<td>HS-TTTTTTTTTTTTTTT</td>
<td>FAM-AAAAAAAAAAAAAAA</td>
</tr>
</tbody>
</table>

Table 3-2. Oligonucleotide sequences.

The reactivities of the oligonucleotides to the AuNp were compared. For a given reaction ratio, the number of oligonucleotides per AuNp, or coverage, was quantified. Figure 3-5a shows coverages resulting from 5:1 DNA:AuNp incubation. Coverage varied with sequence, ranging from 1.25 oligonucleotides for C-near to 3.4 oligonucleotides for G-mid. A- and G-oligonucleotides resulted in higher coverages.
compared to C-oligonucleotides and poly-T. Coverages also varied as a function of DNA:AuNp incubation ratio. Figure 3-5b compares results for the X-near oligonucleotides at ratios of 2, 5, 10, and 15:1 DNA:AuNp. To understand adsorption behavior, coverage was plotted vs. free DNA concentration using the Langmuir isotherm. Langmuir adsorption assumes that all adsorption sites are equivalent and that maximum coverage is a monolayer. For these coverages, there is ~one oligonucleotide per 100 to 700 nm$^2$, where coverages as high as ~1 oligonucleotide/2nm$^2$ are still considered to be monolayer. The coverage here was sub-monolayer. Coverage for X-far oligonucleotides as a function of DNA concentration (Figure 3-5c) was fit to the Langmuir equation described in chapter 2. Figure 3-5c shows the coverages for the X-far oligonucleotides. In general, coverage first increased linearly with DNA concentration and then leveled off, suggesting that the AuNp surface becomes saturated. In general, $b$ values (Table 2) were similar for a given nucleotide, indicating that the oligonucleotide sequence affects the adsorption affinities. Sequence also affects the saturation coverage ($Q$) on the AuNp surface (Table 2). $Q$ and $b$ also varied slightly with sequence placement. In general, $Q$ values are in the same range as those observed for thiolated DNA on gold surfaces and nanowires, with $Q$ ranging from $2-11\times10^{12}$ molecules/cm$^2$. Also, observed $b$ values are similar to those measured for thiolated DNA and alkanethiols on gold. G and T oligonucleotides on average have lower $b$ values, while values for A and C oligonucleotides are higher. Generally, lower $b$ values were correlated to higher $Q$ values. The Langmuir model is a simple one, and the $b$ values encompass both the thiol interaction and any noncovalent nucleotide interactions. Sequences are not
expected to affect thiol affinity for the AuNp. Differences in $b$ can thus be attributed to base specific affinities. Oligonucleotides with higher affinity nucleotides are more prone to noncovalently adsorb to the AuNp surface, effectively reducing the surface area available for adsorption and sterically hindering attachment of subsequent DNA molecules, resulting in a lower maximum DNA coverage. The A and C oligonucleotides demonstrate this behavior.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>$Q$ (DNA/AuNp)</th>
<th>$b$ ($\mu$M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-near</td>
<td>4.00 ± 0.16</td>
<td>6.04 ± 1.47</td>
</tr>
<tr>
<td>A-middle</td>
<td>5.53 ± 0.39</td>
<td>2.32 ± 0.63</td>
</tr>
<tr>
<td>A-far</td>
<td>5.26 ± 0.11</td>
<td>5.26 ± 0.53</td>
</tr>
<tr>
<td>G-near</td>
<td>7.70 ± 0.39</td>
<td>1.98 ± 0.32</td>
</tr>
<tr>
<td>G-middle</td>
<td>8.41 ± 0.58</td>
<td>3.82 ± 0.99</td>
</tr>
<tr>
<td>G-far</td>
<td>10.06 ± 0.53</td>
<td>2.11 ± 0.42</td>
</tr>
<tr>
<td>C-near</td>
<td>7.07 ± 0.57</td>
<td>9.02 ± 2.73</td>
</tr>
<tr>
<td>C-middle</td>
<td>5.34 ± 0.56</td>
<td>9.94 ± 3.92</td>
</tr>
<tr>
<td>C-far</td>
<td>5.23 ± 0.42</td>
<td>6.59 ± 2.02</td>
</tr>
<tr>
<td>T-control</td>
<td>6.44 ± 0.40</td>
<td>8.23 ± 1.87</td>
</tr>
</tbody>
</table>

Table 3-3. Langmuir adsorption model parameter fit results. Maximum surface coverage ($Q$) and bonding affinity ($b$)
Figure 3-5. Reactivity of oligonucleotides to AuNps. a) Coverage of 5:1 DNA: AuNp reaction ratio synthesis; b) Coverage of X-near sequences at 2:1, 5:1, 10:1 and 15:1 DNA:AuNp ratio syntheses, A-near (white), G-near (light grey), C-near (dark grey) and T control (black); c) coverage data (points) and Langmuir isotherm fits (lines) of X-far sequences. A-far (white squares, dashed line) G-far (stars, light grey line), C-far (triangles, dark grey), T-control (circles, black).
The ability of DNA on the AuNps to hybridize to complements was quantified. Since conjugate behavior is influenced by coverage, both AuNp-DNA conjugates with equivalent coverages were compared, as Figure 3-6a shows the number of complements hybridized by the AuNp-DNA conjugates with 1.5 ± 0.4 DNA per AuNp. These data are re-plotted in Figure 3-6b, along with similar data for 3.5 and 7 DNA/AuNp, as % complements hybridized (\# complements / \# DNA per AuNp \times 100). A-oligonucleotides had low hybridization (~10%), with little dependence on location. C-oligonucleotides also had a similar hybridization (~15%). However, G-oligonucleotides exhibited higher hybridization (24-38%), varying the most with location. T-control also exhibited a higher hybridization (37%). These differences were also observed at 3.5 and 7 DNA:AuNp (Figure 3-6b, gray and black, respectively). The extent of hybridization for G and poly-T oligonucleotides was higher at all coverages, with the location effects similar to those at 1.5 DNA / AuNp. A sequence location effect emerged at higher coverages for the C-oligonucleotides. The C-mid sequence exhibited higher hybridization than the C-near and C-far at 3.5 and 7 DNA/AuNp. A-oligonucleotides showed a similar trend, though less pronounced.

The differences observed in the extent of hybridization as a function of oligonucleotide sequence are not observed upon removal of non-specific adsorption. MCH displaces noncovalent adsorption of nucleotides on Au surfaces by thiol binding to free sites. Under controlled conditions, MCH can displace non-specific adsorption without displacing thiolated DNA. AuNp-DNA conjugates treated with MCH were tested for hybridization efficiency (Figure 3-6c), which increased to 30-45% uniformly for all
Figure 3-6. Extent of hybridization of 7.5nm AuNp- DNA conjugates. a) Coverages for 1.5 DNA/AuNp samples. # Thiolated DNA/AuNp (white), # complements/AuNp (grey); b) % hybridization for 1.5 DNA/AuNp (white), 3.5 DNA/AuNp (grey), and 7 DNA/AuNp (black); c) the extent of % hybridization for conjugates treated with MCH to remove non-specific adsorption. 1.5 DNA/AuNp (white), 3.5 DNA/AuNp (grey), and 7 DNA/AuNp (black).
sequences at all coverages. Furthermore, sequence effects were removed, suggesting that non-specific adsorption is responsible for the effect of sequence on hybridization. The statistical significance of these results for all coverages can be seen in the results of a paired t-test comparison of the % hybridization of treated and untreated samples. The p-values, listed in table 3-4, show that differences in treated and untreated sample hybridization are significant.

<table>
<thead>
<tr>
<th>DNA coverage</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 DNA/Np</td>
<td>0.0065</td>
</tr>
<tr>
<td>3.5 DNA/Np</td>
<td>0.0029</td>
</tr>
<tr>
<td>7 DNA/Np</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

Table 3-4. p-values for comparison of % hybridization of untreated AuNp-DNA and MCH treated AuNp-DNA.

3.4 Discussion and Conclusions

The behavior of DNA oligonucleotides on AuNp surfaces strongly depends on nucleotide composition and to a lesser extent on the position of non-T bases relative to the particle surface. Nucleotide adsorption is responsible for the differences in behavior, where A- and C-containing oligonucleotides have a higher affinity for AuNp surface than the G-containing and T-control oligonucleotides. Noncovalent adsorption results in a lower saturation coverage for a given incubation ratio, and seems to affect the reaction of the thiol with the AuNp surface. Also, noncovalent adsorption decreases the extent of hybridization, as previously observed.\(^62\)

The effect of sequence location, which seems to be secondary to the type of nucleotide present, is most likely due to the propensity of the oligonucleotide to adhere to the AuNp surface. Figure 3-7 illustrates a possible scheme for nucleotide surface adsorption. The X-near samples can adsorb adjacent to the AuNp (Figure 3-7a), and X-
far sequences can potentially wrap around the nanoparticle and adsorb at the far end (Figure 3-7b), while X-mid oligonucleotides adsorb in the center of the strand (Figure 3-7c). Oligonucleotide conformation and flexibility is known to affect base adsorption.\textsuperscript{92-94} Evidently an adsorbed 3' end inhibits hybridization to a larger degree, as this places most of the oligonucleotide face down on the AuNp surface. Placement of high affinity nucleotides close to the 5' end does not improve hybridization as much as placing it in the middle. These effects are more pronounced for G-containing sequences, suggesting that strong noncovalent adsorption inhibits hybridization independent of placement in the oligonucleotide.

![Figure 3-7. Non-specific adsorption of DNA oligonucleotides on AuNp s. a) X-near DNA. b) X-far DNA. c) X-middle DNA.](image)

Differences in nucleotide affinity can result in variations in the reactivity of thiolated DNA oligonucleotides to AuNps and hybridization. Sequences with A and C result in lower saturation coverages than those with T and G, and consequently lower hybridization. This is due to increased non-specific adsorption for A and C containing oligonucleotides. Location of high affinity sequences within an oligonucleotide also affects extent of hybridization. High affinity nucleotides in the center of a sequence are less detrimental to hybridization than when placed at the ends, likely due to the limited conformations available when adhesion occurs at the center of the oligonucleotide. These results demonstrate that noncovalent adsorption can greatly affect biomolecular
function in AuNp-DNA conjugates. Because these conjugates are utilized in numerous applications which rely on hybridization, the importance of rational sequence selection is underscored. It is important to note that these results are specific to the AuNp ligand (BPS) used in these experiments. The choice of this ligand was dictated by the high stability of AuNps passivated with this ligand. That stability makes BPS coated AuNps excellent particles for use in biology.
Chapter 4: Nanoparticle Size Effects in Sequence Specific Nucleotide Surface Adsorption

4.1 Introduction

As has been detailed above, there is a sequence dependence to noncovalent nucleotide adsorption on gold surfaces.\(^6\),\(^7\),\(^5\),\(^6\) The experiments described in chapter 3 show the impact of oligonucleotide sequence on AuNp-DNA behavior. There is also evidence for a nanoparticle size dependence on adsorption.\(^7\),\(^9\) Particle curvature effects on adsorption have been shown previously using bent and kinked DNA, with high oligonucleotide affinities corresponding to particles whose curvature matches the bend of the DNA.\(^7\),\(^9\) Further, protein adsorption and function on silica nanoparticles has been shown to depend on particle size. Significantly more denaturation of lysozyme is seen on large nanoparticles compared with small Nps. The greater surface curvature of small nanoparticles promotes the retention of protein structure and function.\(^9\) Both modeling studies and SERS measurements of nucleotide interactions with gold atoms indicate that each base interacts with a specific geometry.\(^4\),\(^5\),\(^6\) Thus we hypothesize that nanoparticle size may influence DNA adsorption behavior.

4.2 Preliminary data

Initial studies of AuNp size affects were conducted on the two sequences listed in table 4-1. Figure 4-1a shows the coverages resulting from DNA:AuNp reaction ratios of
<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>HS-AATTATACCGGCGGC</td>
<td>GCGCCGGTATAATT</td>
</tr>
<tr>
<td>GC</td>
<td>HS-CCGGCGCAATTATA</td>
<td>TATAATTGC GGCCG</td>
</tr>
</tbody>
</table>

**Table 4-1. Oligonucleotide sequences**

5:1 for three sizes of AuNps, 3nm, 5nm and 11nm. The DNA coverage per Np changes inversely with Np size. 3nm AuNp samples show significantly higher coverage than either 5nm or 11nm AuNps at the same incubation ratios. In addition, the relative reactivity of the two sequences is affected by particle size. For 3nm AuNp samples, the AT strand shows higher reactivity towards the particle surface, as indicated by the higher coverage. For both 5nm and 11nm AuNp samples, the GC strand is present at higher coverages, indicating a higher reactivity to the AuNp than the AT oligonucleotide. This change in sequence specific behavior with particle size implies that there is an important affect to AuNp size and curvature which changes the behavior of DNA sequences on the nanoparticle surface. Figure 4-1b shows the effect of particle size and DNA sequence on the hydrodynamic radius of a AuNp-DNA conjugate. The hydrodynamic radius of 3 and 5nm AuNps conjugated with 1 DNA molecule per particle was measured using the Ferguson plot analysis method detailed in chapter 2. For 3nm AuNps, the AT sample shows a hydrodynamic radius smaller than the GC sample. By contrast, the AT oligonucleotide on the 5nm AuNp results in a larger radius than that seen for the GC oligonucleotide. These data further indicate an affect of size on sequence specific AuNp-DNA behavior. The effects of sequence change with particle size, implying that rational sequence selection must incorporate AuNp size as a consideration. However, the oligonucleotides used for these experiments are not well designed for investigation of individual nucleotide affinities. These data prompted a
more thorough analysis of the effect of AuNp size on sequence specificity of DNA adsorption and behavior using the sequences shown in table 4-2.

![Graph showing nanoparticle size dependent coverage and size; coverage results for 5:1 DNA:AuNp reaction conditions at three Np sizes: 3nm AuNp (black), 5nm AuNp (grey), 11nm AuNp (white).]

**Figure 4-1.** Nanoparticle size dependent coverage and size; coverage results for 5:1 DNA:AuNp reaction conditions at three Np sizes; 3nm AuNp (black), 5nm AuNp (grey), 11nm AuNp (white).

### 4.3 Published results

To investigate NP size dependent sequence affects on DNA behavior, ten sequences were compared (Table 1). Each sequence contains a set of four high affinity nucleotides (A, C, G), surrounded by poly-T stretches to form a low affinity background. Affinity of T for Au surfaces has consistently been shown to be significantly lower than that of A, C, or G.61,67 The four bases of the nucleotide of interest were placed at the 5' end near the thiol and NP (X-near, X = A, C, or G), at the halfway point (X-middle), or at the 3' end (X-far). A poly-T oligonucleotide was the low affinity control. Mfold simulations88 verified that the oligonucleotides did not self-fold.

The behavior of these oligonucleotides was investigated on three sizes of AuNps, 4.4nm, 7.5nm and 10.6nm. These particle sizes were selected to provide a range of
curvatures, from $0.18 \text{nm}^{-1}$ (10.6nm) to $0.45 \text{nm}^{-1}$ (4.4nm). For ssDNA of 15 base pairs with a persistence length of 5nm, these sizes will provide an effective range of representative curvatures.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence (5' to 3')</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-near</td>
<td>HS-AATAATTTTTTTTTT</td>
<td>FAM-AAAAAAAATTATT</td>
</tr>
<tr>
<td>A-middle</td>
<td>HS-TTTTTAATAATTTTT</td>
<td>FAM-AAAAAATTATTTAAAA</td>
</tr>
<tr>
<td>A-far</td>
<td>HS-TTTTTTTTTTTTAATAA</td>
<td>FAM-TTATTTTTTTTTTTTT</td>
</tr>
<tr>
<td>G-near</td>
<td>HS-GGTGGTTTTTTTTTTT</td>
<td>FAM-AAAAAAAAACCGACC</td>
</tr>
<tr>
<td>G-middle</td>
<td>HS-TTTTGTTGGTTTTTT</td>
<td>FAM-AAAAACCACAAAAA</td>
</tr>
<tr>
<td>G-far</td>
<td>HS-TTTTTTTTTTTTGTGG</td>
<td>FAM-CCACCAAAAAAAAA</td>
</tr>
<tr>
<td>C-near</td>
<td>HS-CCTCCTTTTNTTTTT</td>
<td>FAM-AAAAAAAAAGGAGG</td>
</tr>
<tr>
<td>C-middle</td>
<td>HS-TTTTTCCTCCTTTTT</td>
<td>FAM-AAAAGAGGAAAAA</td>
</tr>
<tr>
<td>C-far</td>
<td>HS-TTTTTTTTTTTCTCC</td>
<td>FAM-GGAGGAAAAAAAAA</td>
</tr>
<tr>
<td>T-control</td>
<td>HS-TTTTTTTTTTTTTTT</td>
<td>FAM-AAAAAACCCAAA</td>
</tr>
</tbody>
</table>

**Table 4-2. Oligonucleotide sequences.**

Oligonucleotide adsorption behavior was quantified by Langmuir isotherm analysis of DNA coverage. Figure 4-2a shows coverage data (molecules/cm²) and Langmuir fits of a selected sequence, G-middle, at the three AuNp sizes under investigation. Coverage for G-middle oligonucleotides as a function of DNA concentration (Figure 4-2c) was fit to the Langmuir equation described in chapter 2. Figure 4-2b shows the $Q$ values obtained for each oligonucleotide on the three NP sizes. The range of $Q$ values observed (0.02 to 0.1 molecules/nm²) agree with previous thiolated DNA coverages at similar salt conditions. For 4.4 nm NPs (black bars), the A sequences and T-control show higher $Q$ values than for G and C. In contrast, for 7.5 nm NPs (grey bars), G sequences show higher $Q$ values than the other sequences. $Q$ for 10.6 nm NPs exhibits no trend with sequence (white bars). Comparing $Q$ for sequences as a function of NP
size, A sequences and the T-control Q values are constant, while both G and C sequences vary significantly with NP size.

Looking at each set of sequences across the three AuNp sizes, A sequences and the T-control oligonucleotide Q values are constant, while both G and C sequences vary significantly with NP size. The fitted $b$ values and the corresponding free energies of adsorption are listed in table 4-3. For all three sizes of AuNps, the $b$ values cluster with sequence. For 4.4nm AuNp the general values are C > A, T > G. For 7.5nm AuNp the values show an affinity trend of C, T > G > A. For 10.6nm AuNp the trend is different, C, T > G > A.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>4.4nm AuNp</th>
<th>7.5nm AuNp</th>
<th>10.6nm AuNp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$b$ ($M^{-1}$)</td>
<td>$\Delta G$ (kcal/mol)</td>
<td>$b$ ($M^{-1}$)</td>
</tr>
<tr>
<td>A-near</td>
<td>1.37 ± 0.48</td>
<td>-8.37</td>
<td>6.04 ± 1.47</td>
</tr>
<tr>
<td>A-middle</td>
<td>1.10 ± 0.37</td>
<td>-8.24</td>
<td>2.32 ± 0.63</td>
</tr>
<tr>
<td>A-far</td>
<td>0.76 ± 0.27</td>
<td>-8.02</td>
<td>5.26 ± 0.53</td>
</tr>
<tr>
<td>G-near</td>
<td>0.86 ± 0.17</td>
<td>-8.09</td>
<td>1.98 ± 0.32</td>
</tr>
<tr>
<td>G-middle</td>
<td>0.78 ± 0.23</td>
<td>-8.03</td>
<td>3.82 ± 0.99</td>
</tr>
<tr>
<td>G-far</td>
<td>2.78 ± 1.37</td>
<td>-8.79</td>
<td>2.11 ± 0.42</td>
</tr>
<tr>
<td>C-near</td>
<td>3.58 ± 1.95</td>
<td>-8.94</td>
<td>9.02 ± 2.73</td>
</tr>
<tr>
<td>C-middle</td>
<td>3.17 ± 1.12</td>
<td>-8.86</td>
<td>9.94 ± 3.92</td>
</tr>
<tr>
<td>C-far</td>
<td>4.92 ± 1.42</td>
<td>-9.12</td>
<td>6.59 ± 2.02</td>
</tr>
<tr>
<td>T-control</td>
<td>1.57 ± 0.55</td>
<td>-8.45</td>
<td>8.23 ± 1.87</td>
</tr>
</tbody>
</table>

Table 4-3. Langmuir fit results for DNA oligonucleotide affinity ($b$) and the corresponding free energy values.
Figure 4-2. Reactivity of oligonucleotide to AuNps. a) coverage data (points) and Langmuir fits (lines) for the G-middle sequence. 4.4nm AuNp (black triangles), 7.5nm AuNp (grey circles), 10.6nm (white squares); b) Langmuir fit parameter Q, maximum DNA coverage. 4.4nm AuNp (black), 7.5nm AuNp (grey), 10.6nm (white).
The efficiency of complement hybridization was measured for each sequence. Sample with ~1 DNA/NP were selected to study the impact of nucleotide adsorption on the efficiency of hybrid formation. Selection of a low density coverage allowed investigation well below saturation coverage where nucleotide affinity for the NP surface will significantly impact conjugate behavior. Figure 4-3 shows hybridization of ~1 DNA/NP samples at each NP size in terms of the % complement hybridized (# complements / # DNA per NP × 100). All three NP sizes displayed similar results for this coverage. For 4.4 nm NP conjugates (Figure 4-3a), A sequences showed higher hybridization efficiencies (~25%) than either C sequences (~15%) or G sequences (~16%). The poly-T control sequence showed similar behavior to the A sequence (27%). 7.5 nm NP conjugates (Figure 4-3b) showed the highest hybridization efficiency with G sequences (~36%) and poly T (~33%), while A and C sequences showed lower hybridization (~21%). The hybridization efficiency of the 10.6 nm NPs exhibited no statistically significant dependence on sequence (Figure 4-3c). Assessing the highest hybridization efficiency for each size we see a trend of increasing hybridization efficiency with NP size, with 10.6 nm Au NP samples hybridizing the most complement and 4.4 nm Au NP samples hybridizing the least, as can be seen for the X-near sequences shown in Figure 4-3d. Figure 4-4 shows the correlation between the hybridization efficiency and the saturation coverage value (Q).

The effect of nucleotide adsorption was investigated by sample treatment to remove non-covalent surface adsorption. Under controlled conditions, exposure to MCH can displace non-covalent adsorption without displacing the thiolated DNA. NP-DNA conjugates treated with MCH were tested for hybridization behavior (Figure 4-5).
Hybridization efficiency increased for all sizes of NPs, to ~32% for 4.4 nm Au NP conjugates (Figure 4-5a), and to ~45% for both 7.5 nm Au NP (Figure 4-5b) and 10.6 nm Au NP (Figure 4-5c) conjugates. Sequence dependence of the hybridization efficiency was removed for all sizes.

**Figure 4-3.** Complementary DNA hybridization. a) 4.4 nm NP DNA conjugates 0.9 DNA/NP; b) 7.5 nm NP DNA conjugates, 1.1 DNA/NP; c) 10.6 nm NP DNA conjugates, 1.1 DNA; d) Hybridization efficiency oligonucleotides as a function of NP size, A-near (black squares), G-near (open circles), C-near (light grey triangles), T-control (dark grey stars).
Figure 4-4. Correlation of hybridization % with Q for a) 4.4nm NPs, b) 7.5nm NPs, and c) 10.6nm NPs.
Figure 4-5. Complement hybridization of mercaptohexanol treated NP-DNA conjugates; a) 4.4 nm NP DNA conjugates 0.9 DNA; b) 7.5 nm NP DNA conjugates, 1.1 DNA/NP; c) 10.6 nm NP DNA conjugates, 1.1 DNA/NP; d) Hybridization efficiency oligonucleotides as a function of NP size, A-near (black squares), G-near (open circles), C-near (light grey triangles), T-control (dark grey stars).

4.4 Discussion and Conclusions

The maximum number of thiolated DNA molecules that can bind to a AuNp per unit surface area depends on the size of the particle. For example, the coverage of G-near oligos is low at the smallest 4.4nm AuNps ($4 \times 10^{12}$ molecules/cm$^2$), higher for the 7.5nm AuNps ($8 \times 10^{12}$ molecules/cm$^2$) and low again for the 10.6nm AuNps ($4 \times 10^{12}$ molecules/cm$^2$).
molecules/cm\(^2\)). In addition, for the G-X and C-X sequences, the saturation coverage is not a monotonic function with size, with 7.5nm showing the highest coverage. By contrast, for CdSe Nps, the amount of the ligand trioctyl phosphine oxide (TOPO) that can fit on a NP per unit surface area increases with increasing curvature (decreasing size).\(^{100}\) This effect is due to decreasing steric hindrance between individual ligands with increasing surface curvature. The non-monotonic behavior of DNA on AuNps indicates that this steric effect does not dominate in this system. The behavior of the T-control oligonucleotide suggests this complex behavior is dominated by noncovalent nucleotide adsorption on the AuNp surface. The saturation coverages of the T-control sequence show no effect of AuNp size. As noted previously, polyT sequences have minimal affinity for gold surfaces. We thus conclude that the behavior seen with changing particle size and DNA sequence is a complicated relationship between the steric effect previously described for Nps in general, and the noncovalent nucleotide adsorption that occurs on AuNps.

The efficiency with which a AuNp-DNA conjugate can hybridize to its complement also depends on the size of the particle (Figure 4-3). For example, the A-near oligo hybridizes 27% of its total capacity on the 4.4nm AuNps. By contrast, on the 7.5 nm AuNps only 20% of the total hybridization capacity is reached. On the large 10.6nm AuNps the hybridization efficiency is higher, 40%. This indicates that the particle size can influence the biological function of AuNp-DNA conjugates, and should be accounted for when using these systems. These data indicate that the largest particles have the highest hybridization efficiency.
As seen for saturation coverage, hybridization efficiency does not vary monotonically with size for all sequences (Figure 4-4). Hybridization efficiency seems to be correlated to saturation coverage in that the higher the Q value, the better a given sequence can hybridize to its target. This correlation indicates that Q values can be used as a predictor of AuNp-DNA behavior. This is contradictory to what is expected in terms of simple steric arguments, in that higher coverage is expected to result in lower hybridization efficiencies due to crowding on the particle surface. However, at the coverage levels that these experiments were conducted, steric effects will not be the dominating effect.

The effect of nucleotide sequence on hybridization efficiency depends on the size of the AuNp. For example, A oligos hybridize well on the small 4.4 nm AuNps (25-30%), but hybridization is not as efficient on 7.5nm NPs (15-20%). By contrast, G oligos have the lowest hybridization efficiency on the 4.4nm NPs, and efficiency increases as the size of the AuNp is increased, up to ~50% for the 10.6nm AuNps. Placement of the nucleotides of interest did not significantly affect the hybridization capacity. The 10.6nm AuNp conjugates showed the least sequence dependence, with ~50% for all oligos, suggesting that non-specific adsorption on the biggest particles does not affect the behavior of the conjugated DNA.

The effect of non-specific adsorption is reduced by chemical treatment with MCH (Figure 4-5). Primarily, treatment with MCH removes all sequence effects for a given NP size, as all oligos have similar hybridization efficiencies after treatment. This reinforces the hypothesis that non-specific adsorption occurs via base binding to AuNp surface. The significance of the effect of MCH treatment can be measured by
performing a pair t-test of the treated and untreated results. The p-values for this analysis are listed in table 4-4. Both 4.4nm and 7.5nm AuNP samples show statistically significant differences between treated and untreated samples. This indicates an effect of noncovalent adsorption of these nanoparticle sizes. In contrast, the p-value for 10.6nm AuNP samples indicate not statistically significant difference between treated and untreated samples, indicating a minimal effect of noncovalent adsorption. Hybridization efficiency for all sequences after treatment approach the adsorption limit seem when minimal noncovalent adsorption occurs, as indicated by the T-control hybridization. From these data we can conclude that high Q values correlate to low adsorption affinities for a given sequence. The hybridization efficiency of the 10.6nm AuNP conjugates does not change much with MCH treatment, confirming that the 10.6nm AuNPs have the weakest noncovalent adsorption.

<table>
<thead>
<tr>
<th>AuNP</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4nm</td>
<td>0.0008</td>
</tr>
<tr>
<td>7.5nm</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>10.6nm</td>
<td>0.3910</td>
</tr>
</tbody>
</table>

Table 4-4. p-values for comparison of % hybridization of untreated AuNP-DNA and MCH treated AuNP-DNA.

A secondary effect of MCH treatment is seen in the value of the hybridization efficiencies measured. While the conjugates to the 10.6nm and 7.5nm AuNPs approach 50%, likely the limit for hybridization efficiency under these conditions (annealing temperature and salt conditions), the conjugates to 4.4nm AuNPs only reach 30% efficiency. Taking into account the coverage, the 4.4nm AuNPs have 1 DNA / 60nm², which is quite low in comparison to the 7.5nm AuNPs (1 DNA / 176nm²) and the 10.6nm
AuNp (1 DNA / 353nm$^2$). This may indicate that the MCH treatment cannot remove all the noncovalent adsorption in the smallest particles.

The values of Langmuir isotherm affinity term $b$ (table 2) do not show a clear trend. Values are clustered by sequence but do not show the same trends as $Q$ and the hybridization efficiencies. This is likely because the Langmuir model is a simple one, and the $b$ values calculated for this system encompass both the thiol adsorption on the gold surface and the noncovalent nucleotide adsorption. Sequence specific interactions should not significantly affect the energetically more favorable thiol-gold bond formation. Thus differences in oligonucleotide affinity can be attributed to noncovalent, base specific nucleotide-gold interactions. However, $b$ values are related to the rate constants of adsorption and the free DNA concentration in solution. A high $b$ value corresponds to high coverages at a low concentration of adsorbate, but will not necessarily correspond to a high saturation coverage $Q$. As an example, G sequences on 7.5nm AuNps appear to adsorb strongly at low concentrations, resulting in low $b$ values, but are out-competed by the thiol at high concentrations, yielding high $Q$ values. This leads to a gradual increase in DNA coverage with increasing free DNA concentration. In contrast, C sequences on 7.5nm AuNps reach saturation at a lower free DNA concentration, yielding a high $b$ value. This in effect means that once a certain number of C sequences are adsorbed on the surface, additional thiols can no longer displace the adsorbed nucleotide and increasing free DNA will have no effect. It is difficult to make definitive statements as the meaning of the $b$ values in this case as they are a complex amalgamation of effects.
Both oligonucleotide sequence and nanoparticle size affect the behavior of Au NP DNA conjugates. These effects are complex and require careful study. Sequence effects do not remain consistent across nanoparticle sizes, indicating a possible curvature effect on the strength of base-specific adsorption. These data indicate the strength of using adsorption to assess the behavior of bio-nano conjugates. Large AuNps show the least amount of noncovalent nucleotide adsorption, and can fit more thiolated DNA on their surfaces. These particles also show the highest hybridization efficiencies. Removal of any sequence specific hybridization efficiency with MCH surface treatment indicates that these effects are due to noncovalent nucleotide adsorption on the gold surface. From these data we can conclude that high Q values correlate to low adsorption affinities for a given sequence and can be useful in sequence selection for a specific application. This has important implications for using AuNp-DNA conjugates in applications requiring high nucleotide bioavailability, such as hybridization sensing or gene regulation.

The methods detailed here will likely be effective for other biomolecule-nano systems. Noncovalent amino acid adsorption can affect protein structure and function, and adsorption behavior may effectively predict sequence specific behavior. Thus we believe that similar methodology will be useful in evaluating the bioactivity of proteins on nanoparticle surfaces. Finally, these results indicate that noncovalent base adsorption is a complex phenomenon, and may require a more nuanced adsorption model to analyze it effectively. Separation of thiol and nucleotide adsorption will increase the accuracy of the fit parameters and their analysis. However, the Langmuir model is effective for simple analysis of noncovalent adsorption affects on DNA bio-availability.
5. Adsorption Models for Noncovalent DNA Adsorption

5.1 Introduction

The adsorption data in chapters 3 and 4 have been fit to the Langmuir isotherm, consistent with previous literature reports.\textsuperscript{82, 84, 96} These fits have yielded useful saturation coverage values \((Q)\), but the affinity constants \((b)\) are difficult to interpret effectively. This is due in part to the limitations of the Langmuir model in describing noncovalent nucleotide interactions of thiolated DNA with gold. The Langmuir model assumes a simple system, as designated by the four system requirements detailed in chapter 2. The physical reality of the AuNp-DNA system is more complex, as shown in figure 5-1. The overall behavior of DNA adsorbing on the particle surface is a combination of two mechanisms (figure 5-1a); the covalent thiol-gold bond formation, with a bond strength of \(-89\text{kJ/mol}\),\textsuperscript{40} and the comparatively weaker noncovalent nucleotide-gold adsorption with a strength of 1-3 kJ/mol.\textsuperscript{69} At low DNA coverages both types of adsorption can take place,\textsuperscript{95} however the relative strengths of the interactions dictate that the primary mechanism governing the DNA coverage will be the energetically more favorable thiol adsorption.\textsuperscript{56} Nucleotide adsorption is a secondary interaction and is not predicted to occur in significant mounts in the absence of a thiol-gold bond.\textsuperscript{84} When DNA coverage is increased (figure 5-1b), formation of additional thiol-gold bonds requires displacement of nucleotide adsorption. It is here that the strength of the nucleotide affinity for the AuNp surface will impact the conjugate behavior. The thiols of new DNA strands chemisorbing to the particle surface will
compete with the nucleotides already adsorbed. The Langmuir isotherm does not account for the two adsorption interactions, nor the competitive binding behavior.

Figure 5-1. Mechanisms of DNA adsorption on 7.5nm AuNps; a) adsorption of a single DNA molecule; b) adsorption at high DNA coverage.

This limitation of the Langmuir model is illustrated in the data shown in Figure 5-2. The G-far data shown is representative of systematic effort in adsorption fits. Similar error
can also been seen in the literature data. A full understanding of the adsorption behavior of DNA on AuNps requires a more nuanced model, which incorporates the complexity of the system under study. The following sections detail the derivation of an alternative adsorption model for this system, and its applicability to other bio-nano systems.

![Graph](image)

**Figure 5-2** - G-far adsorption on 7.5nm AuNps.

### 5.2 The Competitive Prebinding Model Derivation

To create a model that accurately reflects the physical reality of AuNp-DNA conjugate adsorption we combined concepts from two previously existing adsorption models; the prebinding model and the competitive adsorption model. The prebinding model describes a system in which two substrates, X and Y, can bind to a particle, but Y can bind only if X is already bound.
\[ P + X \xrightarrow{K_x} PX \quad \text{and} \quad PX \xrightarrow{K_y} PXY \]

For AuNp-DNA we must add another interaction to describe the competition between adsorbed nucleotides and thiols.

\[ \begin{align*}
P + X & \quad \xrightarrow{K_x} PX \\
PX + Y + X & \quad \xrightarrow{K_z/K_Y} PX_2 \\
PX & \quad \xrightarrow{K_y} PXY
\end{align*} \]

Summing over all possible states gives a binding polynomial \( \Theta \)

\( \Theta = 1 + K_x X + K_x K_y X + K_x K_z X^2 \) \quad (1)

Each site on the particle can be free (statistical weight 1), bound to X (statistical weight \( K_x X \)), bound to both X and Y (statistical weight \( K_x K_y X \)) or bound to two molecules of X (statistical weight \( K_x X K_z X \)). Note that for the PXY binding term, there is no Y concentration value, because for AuNp-DNA, this adsorption is intramolecular and will be dependent only on the X concentration. This also means that \( K_Y \) is a unitless quantity. The value of \( K_z \) will be the ratio of affinities \( K_x \) and \( K_y \) such that:

\( K_z = \frac{K_x}{K_y} \) \quad (2)

\( K_z \) thus takes into account the competition between X and Y that will take place with the binding of a second X molecule.

If we take \( N \) to represent the total number of X molecules bound, and \( A \) to represent the total available sites on a given P, then the density, \( \theta \), of X on P is:
We can thus write an equation that relates the DNA coverage \((q_e)\) to the free DNA in solution at equilibrium \((C_e)\):

\[
q_e = Q \frac{K_X C_e + K_X K_Y C_e + \left( \frac{K_X}{K_Y} \right) C_e^2}{1 + K_X C_e + K_X K_Y C_e + \left( \frac{K_X}{K_Y} \right) C_e^2}
\]  

(4)

Where \(Q\) is the saturation coverage of DNA and \(K_X\) and \(K_Y\) are the affinity terms for the thiol and nucleotide adsorption respectively.

5.3 Results and Discussion

The model described by equation 4 fits the adsorption data noticeably better than the Langmuir isotherm. Figure 5-3 shows both models fitted to the X-far data for 7.5nm AuNp. The C-P model improves the fits, mainly by allowing a sharper transition between the free DNA concentration range of increasing particle coverage and the range of saturated coverage. The Langmuir fits underestimate the coverage at this transition (figure 5-3b insert). This in turn leads to an over-estimation of the \(Q\) value.

Based on visual inspection, the C-P model appears a better choice for analysis of AuNp-DNA adsorption data; however the numerical results, shown in table 5-1, show the limitations of this new model. The error values for \(K_X\) and \(K_Y\) are so high as to render the fits meaningless. The high error values are due to the fact that equation 4
does not have a unique set of solutions, there are multiple pairs of $K_X$ and $K_Y$ values which will fit the data.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>$Q$ (molecules/cm²)</th>
<th>$K_X$ (µM⁻¹)</th>
<th>$K_Y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-near</td>
<td>3.82 ± 0.14</td>
<td>2.53 ± 0.84</td>
<td>0.47 ± 0.79</td>
</tr>
<tr>
<td>A-middle</td>
<td>4.91 ± 0.25</td>
<td>0.39 ± 1.10</td>
<td>0.02 ± 0.14</td>
</tr>
<tr>
<td>A-far</td>
<td>5.01 ± 0.13</td>
<td>2.26 ± 0.42</td>
<td>1.17 ± 0.59</td>
</tr>
<tr>
<td>G-near</td>
<td>6.69 ± 0.20</td>
<td>0.85 ± 0.23</td>
<td>0.17 ± 0.15</td>
</tr>
<tr>
<td>G-middle</td>
<td>7.70 ± 0.37</td>
<td>0.98 ± 1.24</td>
<td>0.07 ± 0.21</td>
</tr>
<tr>
<td>G-far</td>
<td>8.92 ± 0.14</td>
<td>2.42E-04 ± 0.36</td>
<td>7.92E-09 ± 2.37E-05</td>
</tr>
<tr>
<td>C-near</td>
<td>6.43 ± 0.47</td>
<td>3.66 ± 2.96</td>
<td>0.24 ± 0.66</td>
</tr>
<tr>
<td>C-middle</td>
<td>4.90 ± 0.48</td>
<td>3.76 ± 3.96</td>
<td>0.23 ± 0.73</td>
</tr>
<tr>
<td>C-far</td>
<td>4.82 ± 0.25</td>
<td>0.00 ± 4.97</td>
<td>2.67E-07 ± 7.56E-04</td>
</tr>
<tr>
<td>T-control</td>
<td>5.82 ± 0.23</td>
<td>1.84 ± 2.07</td>
<td>0.05 ± 0.12</td>
</tr>
</tbody>
</table>

Table 5-1 – fit parameter for C-P fits of 7.5nm AuNp-DNA coverage data.
Figure 5-3. Langmuir fits (dashed lines) and C-P fits (solid lines) for X-far 7.5nm AuNp-DNA; a) A-far coverages; b) G-far coverages; c) C-far coverages.
If this model is to be use effectively, restraints must be placed on the values to keep them within the realm of physical reality. In the case of AuNp-DNA, there are several studies which give values for the thiol-gold affinity constant, $K_X$ in the C-P model. Values for both thiolated DNA and alkanethiol adsorption give a value of $K_X = 0.2$ M$^{-1}$.

Figure 5-4 shows three fits of the G-far 7.5nm AuNp coverages. The unrestricted C-P model fit (blue line) is nearly indistinguishable from the fit with a fixed $K_X$ value (red line). Table 5-2 shows the results of fits of all ten oligos under consideration in chapters 3 and 4.

![Figure 5-4. Fits of G-far 7.5nm AuNp-DNA (black circles); Langmuir fit (black line), C-P fit (blue line), C-P with $K_X$ value fixed at 0.2 M$^{-1}$ (red line).]
<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Q (molecules/cm²)</th>
<th>K_Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-near</td>
<td>3.78 ± 0.13</td>
<td>0.0013 ± 0.0004</td>
</tr>
<tr>
<td>A-middle</td>
<td>4.90 ± 0.23</td>
<td>0.0023 ± 0.0005</td>
</tr>
<tr>
<td>A-far</td>
<td>4.77 ± 0.14</td>
<td>0.0010 ± 0.0002</td>
</tr>
<tr>
<td>G-near</td>
<td>6.55 ± 0.19</td>
<td>0.0059 ± 0.0009</td>
</tr>
<tr>
<td>G-middle</td>
<td>7.66 ± 0.34</td>
<td>0.0054 ± 0.0016</td>
</tr>
<tr>
<td>G-far</td>
<td>8.94 ± 0.14</td>
<td>0.0059 ± 0.0006</td>
</tr>
<tr>
<td>C-near</td>
<td>6.30 ± 0.37</td>
<td>0.0004 ± 0.0001</td>
</tr>
<tr>
<td>C-middle</td>
<td>4.79 ± 0.37</td>
<td>0.0003 ± 0.0001</td>
</tr>
<tr>
<td>C-far</td>
<td>4.82 ± 0.22</td>
<td>0.0009 ± 0.0002</td>
</tr>
<tr>
<td>T-control</td>
<td>5.75 ± 0.19</td>
<td>0.0004 ± 0.0001</td>
</tr>
</tbody>
</table>

**Table 5-2.** Fit parameters for C-P model of 7.5nm AuNp-DNA coverage data with K_X value fixed at 0.2M⁻¹

As was seen for the Langmuir fit parameters, the affinity values cluster with sequence, however at first glance the values themselves are counterintuitive to the Q values and the hybridization efficiencies measured in chapters 3 and 4. From those measurements the predicted K_Y value order would be C > A > G, T. In fact we see an order of G > A > C,T. This order seems to imply that G sequences have the highest affinity for the particle surface. The Q values and hybridization data show that the opposite is true. This apparent discrepancy can be explained by the fact that K_Y and K_X/K_Y values actually describe the relationship between free DNA concentration and coverage. A low K_Y value indicates that the system will reach saturation coverage at a lower free DNA concentration than one with a higher K_Y. This is best illustrated by considering the adsorption of the C and G sequences. The C sequences reach saturation at a lower relative free DNA concentration than the G sequences (figure 53b and c). This indicates that once a certain number of C sequences have adsorbed to the surface, additional thiols can no longer displace the adsorbed nucleotide leading to a low relative K_Y value. By contrast, G sequences show a less immediate rise in coverage with free DNA concentration, indicating that as additional thiols are added to
the solution, there is continuing competition between the thiols and the nucleotides adsorbed on the particle surface. Such an interpretation of the system is consistent with the increased hybridization seen for G sequences.

5.4 Conclusions

The C-P model fits the adsorption data of AuNp-DNA conjugates better than the Langmuir model, removing the systematic error in the fits at "medium" free DNA concentrations and improving the accuracy of Q. This model has the potential to be useful in systems with multiple distinct but related adsorption mechanisms. Specifically, it may prove useful for analysis of biomolecule-nanostructure systems, as they generally comprise a primary covalent link between the biomolecule and the nanostructure, in addition to numerous potential secondary noncovalent interactions. It is important to note that the use of this model requires a certain level of knowledge of the system under consideration. Specifically, a value range for the primary adsorption mechanism will allow for more physically meaningful fits.
6. Summary, Conclusions, and future directions

The functional groups found in biological systems provide a wide range of potential interactions with nanostructure surfaces, which can easily interfere with biological structure and function.\(^{40}\) Previous reports have demonstrated sequence specific nucleotide interactions which can affect conjugate behavior.\(^{63, 68, 101, 102}\) Here we have reported an in depth study of base-dependent surface interactions, and their implications for sequence selection in nano-based applications. The AuNp-DNA conjugates studied provide a test case for analysis of biomolecule nanostructure conjugates and the development of an improved model of bio-nano adsorption behavior.

6.1 Summary and conclusions

DNA sequence affects the behavior of AuNp-DNA conjugates. These effects primarily take the form of differences in saturation coverages of DNA and the efficiency of complement hybridization. DNA sequence effects appear limited to base character, and the location of high affinity sequence motifs within DNA oligonucleotides have only a small affect on the adsorption behavior or bioavailability of DNA.

AuNp size influences the behavior of AuNp-DNA. DNA base affinities are not consistent across AuNp size but change their strength relative to one another. For 4.4nm AuNps, A sequences show both the highest saturation coverage and the highest hybridization efficiency, and we thus conclude that these sequences have the highest bioavailability. By contrast, G sequences show the highest bioavailability on 7.5nm
AuNp. Sequences containing any of the four DNA bases appear to have similar affinities to 10.6nm AuNps, displaying little difference in saturation coverage and hybridization behavior.

The agreement between the saturation coverage results calculated by both the Langmuir and competitive-prebinding (C-P) model and the hybridization efficiencies measured indicate that analysis of adsorption behavior can be an effective metric for evaluating conjugate behavior. Fits of adsorption data using the C-P model remove the systematic error seen in Langmuir Isotherm fits of the same data. This model has the potential to provide an effective tool for the analysis of noncovalent adsorption in bio-nano systems beyond AuNp-DNA conjugates studied here. The affinity constants for the secondary nucleotide adsorption interaction agree with the trend of the saturation coverage and help further explain the system.

From these data we can conclude some simple guidelines for rational sequence selection based on the size of the AuNp needed for a given application. As such, this work expands on the existing literature base and allows for more definitive sequence selection guidelines than have been possible from the information in previous reports. Having such tools for matching DNA sequence with AuNp size will improve the effectiveness of AuNp-DNA conjugates in biological applications. Improvements in the bioavailability of DNA will increase the likelihood of success for a given application. In addition, gaining a full understanding of nucleotide interactions with AuNps will allow use of the conjugates in more complex systems than previously tried. The guidelines we have determined from this study are outlined below.
Large particles (>10nm) yield higher bioavailability and more consistent coverage across all sequences than smaller Nps (<10nm). Based on particle curvature, we predict that high bioavailability will persist to particles up to 20nm, as the curvature does not change significantly in this size range compared to the contour length of the DNA. This size range is thus the best choice for applications with limited sequence choice. For applications requiring the use of particle diameters below 10nm, sequences must be selected to match with the AuNp size used. G or T rich sequences are a good choice for Np sizes ~7nm, as they will provide a higher degree of bioavailability than A or C rich sequences. For particle sizes ~4nm, A and T rich sequences will provide the highest bioavailability, and thus make the best choice. Such size specific selection guidelines are necessary for the future of nano-bio applications. Nanoparticle size selections can be limited by a number factors, including the material properties of the particle. This can be seen in the case of semiconductor particles, where the fluorescence wavelengths are determined by Np size. Size selection can also be limited in biological applications, large Nps can be inappropriate for certain applications. For examples, the pores created by electroporation are <10nm in diameter. As such, only Nps smaller than the pore radius are suitable for this application.

It is important to note that there are variables of the system that were not explored in these experiments. The specifics of these experiments were designed to mimic biological systems in terms of buffer choice and concentration. Salt concentration can have a large impact on the behavior of DNA on gold surfaces, and will change the affinities and adsorption behavior of DNA on gold. Peterson et al. have shown that higher salt concentrations lead to increased DNA coverage on 2-d gold surfaces, and it
is likely that this trend will hold true for AuNps. Changes in the Debye length of a solution will change the adsorption behavior of DNA and thus the results of Langmuir and C-P fits. While an analysis of such effects would be interesting, the salt concentrations here are sufficient for behavior prediction in biological settings.

The material properties and surface passivations have both shown to influence the behavior of proteins. Thus we must conclude that the results of this study are specific AuNps coated with the BPS ligand used. Gold nanoparticles were selected for study due to their wide use as substrates for active biomolecules. Nanoparticles of other materials must be evaluated individually, and this work provides an effective scheme for such evaluation. BPS was chosen as the ligand because it provides a high level of stability and is used often for AuNps in biology. As such, it is an ideal choice for evaluating AuNp-DNA behavior for biological systems.

6.2 Future directions

Most AuNp-DNA conjugate applications rely on effective complement hybridization. As we have shown, the efficiency of hybridization can be limited by noncovalent interactions between the DNA bases and the gold surface. The next step in evaluating the effects of sequence specific AuNp-DNA behavior will be direct application in a biological system. The controlled system used here provides an excellent initial system, but AuNp-DNA behavior must be evaluated in the significantly more complex biological systems. This final section details preliminary work using AuNp-DNA As antisense strands in an in vitro translation system.
6.2a – Antisense

Antisense gene silencing is a post transcriptional technique that involves short DNA oligonucleotides complementary to sections of mRNA coding for a specific protein. Hybridization of these oligonucleotides to the mRNA creates a double stranded region within the mRNA. The translational machinery of the ribosome can be disrupted by such regions, interrupting normal protein synthesis.

The ribosome has mechanisms which can uncoil double stranded regions, and as a result antisense can be an inefficient means of gene silencing. Increases in efficiency have been achieved by algorithms for rational sequence selection.\textsuperscript{106-108} Jayaraman et al. list 10 potentially effective antisense sequences for the silencing of human lactate dehydrogenase. There is an increase of 40% with the use of antisense sequences selected by their algorithm.\textsuperscript{106} Such rational antisense oligonucleotides selection yields several possible sequences for silencing a given gene. This provides an ideal test system for the analysis of sequence dependent AuNp-DNA behavior in biological applications. Here we show preliminary work on AuNp-DNA gene silencing of green fluorescent protein (GFP) in vitro. GFP was chosen as a model system because of its ease of detection. GFP synthesis can be monitored and quantified by fluorescent spectroscopy. Numerous commercial vectors for synthesis of the protein are available, and amplification of the gene is easily accomplished by bacterial transfection. Details of the experimental procedures are outlined in Appendix B.

An antisense oligonucleotide covering the ribosome binding site (RBS) and start codon was designed as an initial test strand. The antisense sequence and a control nonsense sequence are shown in table 6-1.
CustalW analysis$^{109}$ of the antisense sequence against the GFP mRNA sequence showed no complementary regions beyond the RBS. Similar analysis of the nonsense sequence showed no regions of complementarity. Figure 6-2a shows fluorescence spectra of diluted translation reactions. The amount of GFP can be quantified by the emission peak at 510nm.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antisense</td>
<td>HS-CTTGCTCACCATGGT</td>
</tr>
<tr>
<td>Nonsense</td>
<td>HS-TTTTCCGCCGTAAA</td>
</tr>
</tbody>
</table>

**Table 6-1. Oligonucleotide sequences**

Figure 6-1 – Antisense Mechanism
The antisense and nonsense oligonucleotide effects on protein synthesis were tested prior to the introduction of DNA modified with AuNps. Figure 6-2b shows the effect of increasing DNA oligonucleotide concentration on GFP production. The nonsense oligonucleotide had no effect on protein production. By contrast the antisense oligonucleotide suppressed protein synthesis by up to 40% of control levels. AuNp conjugates of both DNA oligonucleotides were tested (figure 6-2c). The effect of nonsense on GFP production is minimal, but AuNp-antisense significantly reduces protein production. It is interesting to note that AuNp-DNA antisense is significantly more effective than antisense DNA alone. Higher suppression of protein synthesis is achieved with significantly lower DNA / mRNA ratios. This has been seen in vivo as well, and may make AuNp-antisense an attractive technology in the future.

Here we have shown that AuNp-DNA antisense effectively shuts down in vitro protein synthesis, and that fluorescence quantification of protein synthesis is possible. Future work analyzing the effect of antisense DNA sequence on the efficiency of translational regulation of protein synthesis will provide an important practical analysis of the work described in this thesis. Using the antisense selection criteria outlined in the literature, several sequences can be chosen. Changes in the hybridization efficiency of antisense strands conjugated to AuNps will greatly affect the efficiency of an antisense strand. Such studies will highlight the importance of the bioavailability of molecules conjugated to nanostructures when used in biological applications.
Figure 6-2. GFP synthesis with antisense and nonsense DNA: a) Fluorescent spectra of GFP (red line) and blank translation solution (black line): b) effect of antisense (black squares) and nonsense (red circles) DNA on GFP synthesis: c) effect of AuNp-antisense (black squares) and AuNp-nonsense (red circles) on GFP synthesis.
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Appendix A

Chemicals Suppliers

**Alfa Aesar**
- Agarose
- Dithiothreitol
- Dodecylamine
- Mercaptohexanol (MCH)
- Sodium Carbonate
- Sodium Citrate
- Sodium Chloride
- Tannic Acid
- Tetrachloroauric acid (HAuCl₄)

**Strem Chemicals**
- bis(p-sulfonatophenyl) phenylphosphine (BPS)

**Sigma-Aldrich**
- Didodecyl dimethyl ammonium bromide (DDAB)
- DNA
- ethyl acetate (EtAc)
- ethanol (C₂H₆O)
- Gold (III) chloride (AuCl₃)
- methanol
- Tetrabutyl ammonium borohydride (TBAB)
- toluene

**EMD Chemicals**
- 1x PBS (137mM Sodium Chloride, 2.7mM potassium chloride, 10mM phosphate buffer)
- 0.5x TBE (0.0445M Tris Base, 0.0445M Borate, 0.001M EDTA)

**Invitrogen**
- SYBRgold™
Appendix B

GFP synthesis

GFP DNA was amplified from the Clonetech pEGFP-C1 vector amplified using transfected E.coli. DNA was isolated from E.coli cells using a Qiagen Maxiprep kit, and a standard T7 promoter was cloned into the liberalized DNA. Cloned DNA was isolated using a Qiagen PRC prep kit. Isolated DNA was stored in 1µg quantities at -80°C. Protein synthesis was accomplished using a coupled transcription/translation kit (Ambion). 0.1 µg DNA was added to transcription reaction components and incubated for 1 hour at 30°C. The resulting mRNA was isolated using a Qiagen RNeasy kit and stored at -80°C. GFP was synthesized using translation components of the Ambion kits with 0.1 ug isolated mRNA incubated at 30°C. GFP protein levels were measured using fluorescence spectroscopy (λ excitation = 495 nm, λ emission = 510 nm).

Details of kit procedures are available at the Qiagen and Ambion websites. All procedures used are standards for each kit.

http://www.ambion.com/

http://www1.qiagen.com/