The Adsorption of DNA onto Unmodified Gold Nanoparticles

by

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Curriculum Vitae

The author was born in Ann Arbor, Michigan on January 27, 1979. He attended Hamilton College from September 1997 to May 2001 and completed a bachelor’s thesis under the supervision of Professor Peter Millet. He graduated with a Bachelor of Arts degree with a major in Physics and a minor in Mathematics. He came to the University of Rochester in the fall of 2002 and began graduate studies in Physics. He earned the degree of Masters of Arts in Physics and Astronomy in the spring of 2005. He later pursued his research in experimental condensed matter/biological physics under the guidance of Professor Lewis Rothberg.
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Abstract

The remarkable observation that single-stranded (ss) oligonucleotides (DNA) adsorb onto like-charged gold nanoparticles (Au-np) while double-stranded DNA (dsDNA) does not was investigated by the use of binding kinetics. The transition enthalpy of the interaction between ssDNA and Au-np was calculated using binding rate data from time-dependent fluorescence quenching measurements. We propose a simple electrostatic model based on DLVO theory for the interaction. The model utilizes a van der Waals type attraction and predicts that ligands irreversibly bind to the surface of gold nanoparticles. Electrostatic screening is achieved via Debye-Hückel theory and the weak-overlap approximation. Experimental results are compared to the peak binding transition energy calculated for 1:1 and 1:2 type salts from the model and show acceptable predictions for ssDNA at low concentrations of salt (up to 20 mM) but not for dsDNA nor for high salt concentrations. Shortcomings to the model are discussed. Enthalpy-entropy compensation plots are used to investigate the role of the hydrophobic effect in the reaction. Exothermodynamic properties such as the change in entropy and the change in enthalpy are compared to the known hydrophobic properties of the ligands. A classification scheme for salt ions is proposed and the results roughly follow the Hofmeister series. Finally, binding kinetics using urea demonstrates an increase in transition enthalpy and entropy and an increase in the binding time constant consistent with a weaker hydrophobic interaction. These results demonstrate that the electrostatic interaction between DNA and gold nanoparticles is of little importance to the overall theory of interaction and the largest effects are from solvation forces, specifically the hydrophobic effect.
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Chapter 1

Introduction

1.1 DNA screening assays

For many years now, oligonucleotide (DNA) hybridization assays have been used increasingly more often in medicine and molecular biology to screen for whole genomic sequences and, possibly of greater importance, for more subtle anomalies such as single nucleotide polymorphisms (Stoneking and Chakravarti, 2001). The basic procedure common to most DNA screening assays is as follows: a known DNA sequence, referred to as the probe sequence, is exposed to an unknown sample of DNA called the target. Complementary sequences of probe and target hybridize, forming double-stranded DNA (dsDNA), which is detected using a variety of techniques. There are a wide range of assay detection methods using traditional methods. Of greater promise is the use of nanotechnology in DNA screening assays.

One of the earliest and possibly one of the most widely utilized methods of DNA detection is Southern blotting (Southern, 1975). This method separates target DNA sequences by molecular weight using agarose gel electrophoresis. Then, the DNA is transferred to a membrane and exposed to probe DNA sequences that hybridize to their complement. Southern blotting is considered the “industry standard,” but it is time consuming and resource intensive. It is precisely these reasons that have motivated research in faster, cheaper and more sensitive DNA screening technique.
CHAPTER 1. INTRODUCTION

It is nearly impossible to detect specific sequences of DNA without first amplifying the target. This is achieved, most commonly, by polymerase chain reaction (PCR), a technique to increase the number of DNA copies within a specific region of a DNA strand (Bartlett and Stirling, 2003). In a single PCR cycle, three temperature dependent steps are needed: denaturation, annealing and elongation. In denaturation the dsDNA is separated into two single-stranded DNA (ssDNA) sequences. Next, a primer is attached, or annealed, to the target DNA. A primer is a short ssDNA sequence, designed to define the region of amplification and provide a footing for the DNA polymerase, a variety of enzyme, which is used to elongate the primer the entire length of the target sequence. For each cycle of PCR the number of DNA copies is doubled assuming perfect efficiency. After 20-40 cycles, a sufficient number of DNA are available for genomic detection to be practical.

A more rapid and sensitive method for DNA detection is a variation of PCR where quantification and amplification occurs simultaneously and is commonly referred to as real-time PCR (Kubista et al., 2006). In one example of real-time PCR, ethidium bromide, which fluoresces in the presence of dsDNA, is used to quantify the PCR product (Higuchi et al., 1992). Other variations use reporter-probe complementary sequences that change their fluorescence after hybridization with the target sequence (Bassler et al., 1995, Livak et al., 1995, Swan et al., 1997). The real-time PCR method offers a sensitive and quantitative method for DNA amplification and detection. Its shortcomings are that it uses a large amount of reagents and the cost of capital equipment is significant and is therefore only used in high throughput applications.

1.2 Nanotechnology

Advances in material science, specifically in nanotechnology, have shown that metallic nanoparticles have a host of interesting properties and characteristics that make them extremely useful to biotechnology. These properties include large surface/volume ratio, great flexibility in shape-controlled synthesis (Wiley et al., 2005), chemically active surfaces that bind to amine (Leff et al., 1996, Sastry et al., 2001, Sel-
vakannan et al., 2003) and thiol (Templeton et al., 2000) groups and biocompatibility in that they are generally non-toxic (Shukla et al., 2005). In addition, they have useful optical properties such as strong, tunable visible absorption that depend on shape and size (Liz-Marzan, 2006, Hurst et al., 2006).

Nanotechnology is well suited for various medical technologies such as imaging, drug delivery and detection. Quantum dots are one such application of nanoparticles in biological imaging (Colvin et al., 1994, Bruchez et al., 1998, Gao et al., 2004). Traditional methods of intracellular imaging involve the use of fluorescent tags. Quantum dots are semiconductor nanoparticles small enough to experience quantum confinement and so readily fluoresce (Henglein, 1989). Compared with traditional fluorescent probes, quantum dots have a narrow, tunable emission spectrum and are photochemically stable (Bruchez et al., 1998). Much like fluorescent imaging, the quantum dot is attached to a ligand that makes specific binding to a target receptor. The ligand can be an antibody, protein or oligonucleotide.

Imaging is not the only field to benefit from nanotechnology. Along with targeted imaging of unhealthy tissue comes targeted drug delivery to those affected regions. Nanoparticles are ideally suited for packaging and delivering medicine. First, by their very nature, nanoparticles are small and so can reach the target region easily and unobtrusively. Second, because the drug is applied directly to the affected region, lower doses can be administered, which reduces overall toxicity to neighboring healthy tissue. There are many other promising and novel applications of nanotechnology currently in development that may offer breakthroughs in medicine and biotechnology (Caruthers et al., 2007).

One such area of interest is in the field of biomolecular detection. In particular, gold nanoparticles have been incorporated into a number of novel DNA sensor strategies (Foultier et al., 2005). These include DNA functionalized gold nanoparticle arrays (Mirkin et al., 1996, Mucic et al., 1998) and using a variety of schemes including colorimetry (Elghanian et al., 1997, Storhoff et al., 1998, Reynolds et al., 2000), fluorescence (Zhao et al., 2003, Xu et al., 2005), radioactivity (Weizmann et al., 2001), Raman spectroscopy (Cao et al., 2002, 2003), light scattering (Taton et al., 2000) and
electrochemical detectors (Park et al., 2002). These schemes generally use DNA functionalized gold nanoparticles as their building block which means that each assay will require distinct particles, a practical problem if the technology is to become pervasive.

In 2004, Li and Rothberg (2004c) discovered that ssDNA adsorbs onto citrate coated gold nanoparticles (Au-np) while dsDNA does not. This is a remarkable observation. First of all, both DNA and Au-np are negatively charged so by virtue of their electrostatic charge these particles should repel each other. Of course, the interaction is observed only in solutions containing salt ions. Presumably, the electrolyte is needed for screening the charge. Based on this information, it seems that the effect is electrostatic in origin. Intuition tells us that this may be a reasonable hypothesis - dsDNA has twice the linear charge density of ssDNA so while ssDNA may adsorb at a certain concentration of electrolyte, dsDNA may not.

By this logic though it should be possible to adsorb dsDNA onto Au-np by increasing the ionic strength of the electrolyte. In fact, experiments have shown (Li and Rothberg, 2004c) that ssDNA adsorbs for all concentrations of salt while dsDNA does not (the exception seems to be MgCl₂, which adsorbs dsDNA to Au-np as freely as ssDNA does). In addition, this view fails to take into account hybrid sequences - DNA sequences that are partially single-stranded and partially double-stranded. In experiments Li and Rothberg (2004b) found that if a substantial part of the sequence is single-stranded then the whole molecule adsorbs onto Au-np. What is certain in any case is that this interaction is more complex than it initially appears.

The attraction of like-charged particles is a problem of general importance. In the biological sciences, many interactions involve particles of the same charge. For example, DNA in the cell nucleus packs into condensed structures that form the basis of the chromosomes (Alberts et al., 1983). Bacteriophages contain large concentrations of oligonucleotide material in the capsid, or head of the virus (Johnson et al., 2007, Petrov and Harvey, 2008). The interaction between proteins, and proteins and oligonucleotides often occurs between particles of the same charge (Squires and Brenner, 2000). In colloid science, understanding the interaction between monodispersed suspensions of charged colloid is an important aspect of industrial processes and is
critical in the production of paint, toners, emulsions, cosmetics, pharmaceuticals, processed foods, soils and lubricants (Israelachvili, 1985). Understanding the fundamental forces in the interaction between DNA and Au-np would advance scholarship in these other systems as well.

### 1.3 Background

The discovery of preferential adsorption of ssDNA over dsDNA onto Au-np forms the basis of a number of simple DNA hybridization assays. The assays used either a fluorescence quenching (Li and Rothberg, 2004b) technique or a colorimetric (Li and Rothberg, 2004a) technique based on salt-induced aggregation of the Au-np.

The fluorescence-based method is shown schematically in Figure 1.1 and the steps are as follows: 

1. **Dye-modified probe DNA sequences** are exposed to target DNA sequences and allowed to incubate or hybridize. Double-stranded DNA is formed from complementary probe and target sequences.

2. The DNA sample, buffer solution, and gold colloid are mixed together and only ssDNA adsorbs onto the Au-np.

3. Fluorescence from the probe DNA is quenched due to the chromophore’s proximity to the gold surface. Any dsDNA, on the other hand, remains in solution and fluorescence is detected.

The colorimetric method associates hybridization with an identifiable color change due to colloid aggregation. The color of the colloid is a result of the coupling of the electromagnetic field to oscillations of conduction electrons on the metal and are called surface plasmon resonances (Liz-Marzan, 2006). When the dimensions of the metal are reduced, as is the case for nanoparticles, boundary and surface effects become important and small changes in shape can have large effects on the conduction electron oscillation resonance and absorption frequency. One way to change the shape of the nanoparticle, and its color, is to induce aggregation, typically with salt ions. When the stability of the nanoparticle is increased, after adsorption of DNA strands or by introducing an amphilic surfactant such as TWEEN (Sigma-Aldrich, St. Louis, MO) or polyethylene glycol, molecules that are both soluble and insoluble in water,
Figure 1.1: Schematic of the fluorescence based DNA screening assay (Li and Rothberg, 2004b). The three steps are: I) target and probe sequence hybridization, II) addition of Au-np, and III) detection.

it requires a greater concentration of salt ions to induce aggregation. As a result, it is possible to differentiate between bound and unbound DNA, simply by the color of the colloid following exposure to a quantity of salt.

The assay procedure is similar to the fluorescence assay and is depicted schematically in Figure 1.2 where the steps are as follows: I) probe and target DNA sequences are mixed together. II) Non-complementary or excess DNA is adsorbed by the gold, changing its properties. Complementary sequences remain in solution and have no effect on the stability of the colloid. III) Salt is added and the stabilized colloid retains its color while the Au-np in the samples that contained complementary sequences aggregate leading to a red shift in the plasmon resonance absorption frequency and a change in the colloid’s color from red to blue.

The benefit of this type of assay is that the nanoparticles are not functionalized and hybridization of the probes and targets is separated from detection. Recently, other proposed biosensors have been designed around this same principle including DNA aptamer based protein detectors (Wang et al., 2008, Wei et al., 2007) and potassium-ion detectors (Li et al., 2007). An aptamer is any oligonucleotide (DNA or RNA)
that binds to a specific target. When the aptamer binds to a target molecule, in some instances, it folds into a secondary structure similar to dsDNA and hence would have a similar interaction. An application of this technique, invented as part of this thesis research, is discussed further in Appendix A.

This phenomenon could be potentially applied as a “filter” to separate short sequences from longer ones, as a filter of PCR product for example. As mentioned earlier, PCR is one of the most popular tools in molecular biology. After the last cycle of PCR, the product contains many contaminants such as deoxynucleoside triphosphates (dNTPs - the building block of DNA), polymerase enzymes and unused primers. Before the amplified DNA can be used in clinical assays, these contaminants must be eliminated from the product. Currently, various technologies exist for PCR purification including micro-centrifuge spin columns and post-processing with chemicals (Dugan et al., 2002). These systems remove the short primers and nucleosides from solution, a process easily accomplished by the Au-np. This demonstrates another function of the ssDNA/Au-np adsorption interaction. Because the Au-np filters ss-DNA sequences from dsDNA the assay can be applied to unpurified PCR product.
Ultimately, the goal is to bypass amplification of the sample and test a genomic target directly and Au-np may offer the possibility to do such a thing. The fluorescence based assay is, in principle, a null experiment. The fluorescence of chromophore tagged DNA is strong and since nearly all ssDNA sticks to the nanoparticle, any detectable fluorescence above background constitutes a relevant result. Even so, measuring low concentrations of analyte is notably difficult so one must understand first how the surface charges and the salt concentration affects the interactions that lead to adsorption.

Figure 1.3: Cartoon of the dipolar interaction between ssDNA and Au-np. A dipole is formed at the surface of the Au-np between the negatively charged surface (- - -) and counter-ions ⊕ near the surface. This dipole interacts with a second dipole formed between DNA’s phosphate backbone and nearby counter-ions. The figure is not to scale.

Initial speculation as to why ssDNA and dsDNA have different bias for adsorption was based on the idea of dipolar interactions (Li and Rothberg, 2004a). Charged particles in an electrolyte exhibit double-layer screening which is well described by colloid science (Hunter, 1987). The double-layer forms a dipole that, in specific orientations and configurations of DNA, is net attractive. In normal circumstances, dsDNA and ss-DNA have helical secondary structure (Bloomfield et al., 2000) and in this configuration, the double-layer is uniformly distributed around the molecule. An isotropically
distributed double-layer does not form a net electrostatic dipole moment. In some circumstances however, it was speculated that ssDNA can make transient structural fluctuations which allow it to unwind. Since the nucleobases strongly exclude water, the double-layer cannot form a uniform distribution of counter-ions and this asymmetry leads to a net non-zero dipole moment which leads to attraction and adsorption. A cartoon depiction of the dipolar interaction model is given in Figure 1.3.

1.4 Structure of the Thesis

Selective adsorption of biomolecules is used in a number of technologies and is important for medical diagnosis. The goal of this work is to study the specific case of selective adsorption of single-stranded and double-stranded DNA on Au-np. Our initial hypothesis is that the interaction pertinent to the assay could be described by DLVO theory, named after Derjaguin and Landau (1941), and Verwey and Overbeek (1948). The basic assumption of the theory is that the interaction between suspensions of charged particles in electrolyte can be viewed as the sum of electrostatic and van der Waals forces. This theory, while originally intended for colloidal suspensions, has been applied successfully to biological systems including bacteria (Jacobs et al., 2007, Chen and Walker, 2007, Liu and Zhao, 2005) and proteins (Javid et al., 2007, Tcholakova et al., 2005).

In Chapter 2, time-dependent fluorescence quenching spectroscopy is used to measure the adsorption rate between ssDNA and Au-np. These data are used to determine the transition enthalpy and entropy of the reaction. Next, the transition enthalpy is compared to the results from a simple electrostatic model and shows good agreement at low salt concentrations. At high salt concentrations and for some divalent cations such as Mg$^{2+}$ the model fails. In addition, when the model is applied to ds-DNA, the results are inconsistent with observations. We conjecture that this failure is primarily due to solvation forces, which are not included in classic DLVO theory. Specifically, we believe that the hydrophobic effect (Kauzmann, 1959) plays a domi-
nant role in the interaction and set out to prove this assertion.

In Chapter 3 the hydrophobic effect is investigated. The hydrophobic effect causes an attractive interaction between hydrophobic particles in aqueous solutions that causes phase separations similar to the segregation of oil and water (Chandler, 2005). Because many biological particles such as DNA contain discrete, separated hydrophobic and hydrophilic groups, the hydrophobic effect is common to biological systems (Tanford, 1978). The thermodynamics of the system in a wide range of salt types is measured. The binding rate of the reaction as well as the degree in change of the thermodynamic parameters vary with the variety of salt used. This allows us to correlate the binding kinetics using different salt solutions with the often used Hofmeister series: a method to classify ions by their ability to change the intermolecular structure of water. The correlation between binding kinetics and solvation effects is strong evidence for the dominant role of hydrophobic forces within this interaction and is another aspect which cannot be rationalized by DLVO theory on its own. Enthalpy-entropy compensation plots demonstrate the linear dependence of the thermodynamic parameters and document the importance of salt ions in the adsorption. This allows us to measure the compensation temperature for the interaction, a temperature unique to hydrophobic processes and characteristic of particular ligands. In addition, the compensation temperature is separately determined by comparison of the thermodynamic parameters with quantitative differences in the physical properties of DNA.

This study, to investigate the interaction between DNA and gold nanoparticles, should be of great use to improving the Li and Rothberg DNA screening assay and its derivatives. In addition, since the interaction involves the attraction of liked-charged particles, this study may be prove useful in understanding the interaction between proteins, densely packed DNA and colloidal suspensions.

References


CHAPTER 1. INTRODUCTION


Chapter 2

The Electrostatic Model

In our experiments, binding kinetics were determined using fluorescent quenching spectroscopy. The transition state enthalpies for the adsorption of various fluorescently tagged ssDNA onto Au-np in colloidal solution were determined from binding rate data. The rate of adsorption was slower than predicted from diffusion limits. Van’t Hoff analysis is used to calculate the transition state enthalpy. These results are compared to a simple electrostatic model that is consistent with data for low salt concentrations but breaks down at concentrations relevant to the assay. In addition, the model cannot explain the behavior of dsDNA. We discuss these limitations as well as possible solutions.

The model is based on DLVO theory which has been used in a number of systems including salt-induced aggregation of colloidal particles (Enustun and Turkevich, 1963, Kallay and Zalac, 2002), interaction between bacteria (Liu and Zhao, 2005, Jacobs et al., 2007, Chen and Walker, 2007) and proteins (Tcholakova et al., 2005, Javid et al., 2007), interactions between DNA and membranes (Sushko et al., 2006), and interactions between charged biological membranes (Sculley, 1991). The DLVO interaction is the sum of two primary forces: repulsion due to overlapping double-layers and attraction due to the van der Waals force. In this model, the double-layer interaction is determined by treating both the DNA molecule and the Au-np as hard spheres. The van der Waals interaction is calculated along similar lines. The
total DLVO interaction is used to calculate the peak interaction barrier energy similar to the activation energy in bimolecular reactions (Levin, 1983).

2.1 Background

The dipolar interaction model proposed by Li and Rothberg (2004a) and described in Chapter 1 treated the DNA molecule as a rigid rod. While this may be an accurate depiction of dsDNA, for ssDNA it is an oversimplification because of its considerable longitudinal flexibility. Here, we expand the original electrostatic model by including the influence from dispersion-type forces also known as van der Waals interactions. The salt ions also play an important role in pair-wise electrostatics, screening the repulsion between like-charged particles. The contributions from these two interactions form the basis of classic DLVO theory (Israelachvili, 1985).

The challenge in modeling biological molecules are the difficulties in representing key aspects of these complex systems including solvation, steric, electrostatic and van der Waals energies, hydrodynamic interactions, and conformational changes (Roth and Lenhoff, 1993). Of these, solvation and conformational effects are the most difficult to describe. Electrostatics and van der Waals forces are easier to model and well described by DLVO theory (Israelachvili, 1985). Proteins, for example, are extensively modeled using DLVO theory. The usual approach is to pattern the biomolecule after a hard sphere with the same size and charge as the biomolecule. This is the method used by Ruggiero et al. (1999) and others (Johnson et al., 1994, Curtis and Lue, 2006). While there is little direct experimental analysis using DLVO theory in biological systems, the interaction has been successfully measured between mica sheets in aqueous salt solutions at various temperatures (Pashley, 1981a,b), across soap films (Donners et al., 1977), and between metal surfaces (Smith et al., 1988). These studies are all in good agreement with DLVO theory and, on this basis, it is plausible to try to describe the interaction between DNA and Au-np.

In this study, binding kinetics were used to calculate the activation energy. When DNA, modified with a fluorescent tag, adsorbs onto Au-np, its fluorescence is quench-
ed by either nonradiative energy transfer or by out-of-phase dipole coupling (Soller et al., 2007). The orientation of a chromophore’s dipole and the Au-np surface determines whether the radiative rate, and consequently the luminescence, is increased or is decreased (Gersten and Nitzan, 1981). Orthogonally oriented dipoles suppress the radiative rate and increases the nonradiative rate. Both effects lead to quenching of the luminescence. Radially oriented dipoles increase both the radiative rate and the nonradiative rate (Dulkeith et al., 2002, 2005). Quenching occurs if the nonradiative energy transfer is dominant and luminescence increases if instead the radiative rate is dominant (Soller et al., 2007). Given that the dye-tag is small compared to DNA and the phenomenon seems to be independent of the choice of dye, it was assumed that the dye had no affect on the interaction.

### 2.2 Theoretical Model

In our electrostatic model, the DNA molecule is treated as a sphere that is hydrodynamically equivalent to a worm-like chain. This is an approach similar to the one taken by Ruggiero et al. (1999) to model proteins. The measure of the polymer chain length is the contour length $L_N = N b$, the distance between the ends of the chain measured along the helical axis for each segment of length $b$, and the end-to-end length, the vector sum of each segment length (see Figure 2.1). For a worm-like chain, the mean-square end-to-end length can be expressed as

$$
< R_N^2 > = 2 \ell ( L_N - \ell + \ell e^{-L_N/\ell} ),
$$

where $\ell$ is the effective persistence length defined as the length over which the projection along the initial direction of the chain is lost as $L_N$ tends to infinity (Bloomfield et al., 2000). The persistence length for a charged polymer is

$$
\ell \simeq \ell_0 + \ell_{OSF},
$$

where $\ell_0$ is the bare persistence length, 10 Å for ssDNA and 500 Å for dsDNA (Tinland et al., 1997), and $\ell_{OSF}$ is an electrostatic contribution from the Odijk-Skolnick-
CHAPTER 2. THE ELECTROSTATIC MODEL

Figure 2.1: The two measures of a polymer. The contour length $L_N$ is the distance between the two ends of the chain if the polymer were stretched out. The end-to-end length $R_N$ is the vector sum $R_N = \sum_{i} b_i$ where $b_i$ represents each numbered segment of length $b$ along a chain.

Fixman model (Netz and Andelman, 2003) which is

$$\ell_{OSF} = \ell_B f^2 / 4\kappa^2$$  \hspace{1cm} (2.3)$$

for the fractional number of charges per monomer length $f$. The Bjerrum length, $\ell_B = e^2 / 4\pi\varepsilon\varepsilon_0 k_B T$, is the length at which the electrostatic interaction between two point charges $e$ is equal to the thermal energy $k_B T$ (Netz and Andelman, 2003). The inverse Debye length $\kappa$ is the length at which salt ions screen the electric field. It is defined as

$$\kappa^2 = \frac{2e^2 (n_1 + 3n_2)}{\varepsilon\varepsilon_0 k_B T},$$  \hspace{1cm} (2.4)$$

where $n_1$ and $n_2$ are the bulk ion densities of the 1:1 (e.g. NaCl) and 1:2 or 2:1 (e.g. (NH$_4$)$_2$SO$_4$ or MgCl$_2$) electrolyte respectively. Importantly, the Debye length does not depend on the charge of the particle but on the properties of the electrolyte. At 298K, the Debye length $\kappa^{-1}$ of aqueous solutions of 1:1 salt is $\kappa^{-1} = 9.7$ nm at 1 mM and $\kappa^{-1} = 0.97$ nm at 100 mM. For 1:2 salts and 2:1 salts $\kappa^{-1} = 5.6$ nm at 1 mM.

When the contour length is much shorter than the persistence length $L_N \ll \ell$, as is the case for short sequences of dsDNA, Equation (2.1) approaches the limit
\[ <R^2_N> = L_N^2 \] which is the expected behavior for a rigid rod. When the contour length is much greater than the persistence length \( L_N \gg \ell \) then Equation (2.1) approaches \( <R^2_N> = 2\ell L_N \). The dependence of the root-mean-squared end-to-end length of ss-DNA and dsDNA on the contour length is plotted in Figure 2.2.

\[ \begin{align*}
\text{ssDNA} & \quad \text{dsDNA}
\end{align*} \]

**Figure 2.2:** Comparison between the root-mean-square end-to-end length \( <R^2_N>^{1/2} \) of ss-DNA (---) and dsDNA (- - -) and its contour length \( L_N \). For contour lengths much shorter than the persistence length \( L_N \ll \ell \) the end-to-end length approaches \( L_N \). For large contour lengths where \( L_N \gg \ell \) the end-to-end length approaches \( \sqrt{2\ell L_N} \). These limits are labeled accordingly in the plot.

The dynamics of flexible polymers in dilute solutions can be modeled by either the Rouse model (Rouse, 1953, Doi and Edwards, 1986) or by the Zimm model (Zimm, 1956, Doi and Edwards, 1986). The Rouse model ignores both the excluded volume interaction and the hydrodynamic interaction. The excluded volume interaction is a steric effect due to the fact that the interaction between segments is not limited to their closest neighbors but extends to more distant segments when the polymer is coiled. The Zimm model includes hydrodynamic interactions. Accordingly, the diffusion
coefficient $D$ can be expressed as

$$D = \frac{8k_BT}{3(6\pi^3)^{1/2}\eta_0 R_N},$$

where $\eta_0 = 8.90 \times 10^{-4} \text{ Pa} \cdot \text{s}$ is the viscosity of water at 298K (Lide, 2008). The Stokes-Einstein equation

$$D = \frac{k_BT}{6\pi \eta_0 R_H}$$

can be combined with Equation (2.5) to find the radius

$$R_H = \frac{3\sqrt{\pi}}{8\sqrt{6}} R_N \approx 0.271 R_N$$

of a sphere that is hydrodynamically equivalent to a worm-like chain where $R_N$ is the root-mean-square end-to-end length in Equation (2.1).

Single-stranded DNA forms single-helices with an axial rise per segment that depends strongly on the type of nucleotide. For homonucleotide sequences of adenine the axial rise per nucleotide is 2.82 Å. For thymine and random sequences the axial rise per nucleotide is 3.5 Å (Saenger, 1984). The hydrodynamic radius using Equation (2.7) for a series of random ssDNA sequences is given in Table 2.1 as well as the associated diffusion coefficient $D$ at that radius. For comparison, experimentally derived diffusion coefficients from Lapham et al. (1997) and Nkodo et al. (2001) are also included.

From these values, treating DNA as a sphere is accurate for long sequences of DNA and is increasingly less so for shorter sequences. This indicates that the theory underestimates the stiffness of short sequences of DNA which is most likely due to strong electrostatic repulsion between the phosphate groups and the large persistence length compared to the contour length of the shorter sequences.

The gold nanoparticle is also modeled as a sphere. The surface charge is assumed to be evenly distributed across the surfaces of both spheres.

### 2.2.1 The electrostatic double-layer interaction

The interaction potential between two charged surfaces in an electrolyte is directly related to the pressure when these surfaces approach one another from infinite sepa-
Table 2.1: Hydrodynamic radius $R_H$ and associated theoretical and experimental diffusion coefficient $D$ of random ssDNA sequences of length $L_N$ in 10 mM salt and temperature $T = 298$K.

<table>
<thead>
<tr>
<th>$L_N$ (bases)</th>
<th>$R_H$ (Å)</th>
<th>Theoretical $D$ ($\times 10^{-6}$ cm$^2$ s$^{-1}$)</th>
<th>Experimental $D$ ($\times 10^{-6}$ cm$^2$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>10.4</td>
<td>2.26</td>
<td>1.25$^a$</td>
</tr>
<tr>
<td>14</td>
<td>12.1</td>
<td>1.95</td>
<td>1.22$^a$</td>
</tr>
<tr>
<td>15</td>
<td>12.9</td>
<td>1.83</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>15.3</td>
<td>1.54</td>
<td>0.994$^b$</td>
</tr>
<tr>
<td>24</td>
<td>20.0</td>
<td>1.14</td>
<td>0.975$^a$</td>
</tr>
<tr>
<td>50</td>
<td>38.5</td>
<td>0.620</td>
<td>0.615$^b$</td>
</tr>
<tr>
<td>250</td>
<td>125</td>
<td>0.197</td>
<td>0.197$^b$</td>
</tr>
</tbody>
</table>

$^a$ extrapolated to 10 mM salt (Lapham et al., 1997).

$^b$ in 8 M urea (Nkodo et al., 2001).

The pressure between the surfaces is given by (Israelachvili, 1985)

$$P = 4k_BT \sinh^2 \left( \frac{e\psi_m}{2k_BT} \right) \left( n_1 + n_2 \left( e^{-e\psi_m/(k_BT)} + 2 \right) \right),$$

(2.8)

which depends on the bulk ion density $n_1$ and $n_2$, and on the electrostatic potential $\psi_m$ at the midpoint separation distance. The midpoint potential is assumed to be small so Equation (2.8) reduces to lowest non-zero order in $\psi_m$ to

$$P \simeq \frac{e^2(n_1 + 3n_2)}{k_BT} \psi_m^2.$$  

(2.9)

For an isolated surface, the electrostatic potential is determined from the Poisson-Boltzmann equation

$$\nabla^2 \psi(x) = -\frac{e}{\varepsilon\varepsilon_0} \sum_i z_i n_i \exp \left( \frac{\pm z_i e \psi(x)}{k_BT} \right)$$

(2.10)

for ions $i$ of valency $z$. This nonlinear second-order differential equation has no analytical solution in spherical coordinates but for low surface potentials, Equation (2.9)
can be linearized. Using the boundary conditions $\psi \to 0$ and $d\psi/dr \to 0$ as $r \to \infty$ and assuming that the electrostatic potential at the surface ($r = a$) is small, the linearized solution to the Poisson-Boltzmann equation for a sphere of radius $a$ is

$$\psi(r) = \psi_0 \frac{a}{r} e^{-\kappa(r-a)},$$

(2.11)

where $\psi_0$ is the electrostatic surface potential.

Unfortunately, neither the surface potential of DNA or Au-np is small so the linearized solution of the Poisson-Boltzmann equation is not entirely valid for this interaction. This necessitates a more general solution. For planar surfaces, the solution of the Poisson-Boltzmann equation valid for all surface potentials, is

$$\psi(x) = \frac{2k_B T}{e} \ln \left( \frac{1 + \gamma e^{-\kappa x}}{1 - \gamma e^{-\kappa x}} \right),$$

(2.12)

where $\gamma = \tanh(e\psi_0/4k_B T)$. To lowest non-zero order in $e^{-\kappa x}$, Equation (2.12) reduces to

$$\psi(x) \simeq \frac{4k_B T}{e} \gamma e^{-\kappa x},$$

(2.13)

and is known as Gouy-Chapman theory (Israelachvili, 1985). The midpoint potential is the contribution from each surface at the midpoint and is expressed as

$$\psi_m = \frac{4k_B T}{e} (\gamma_1 + \gamma_2) e^{-\kappa D/2},$$

(2.14)

for surfaces separated by a surface-to-surface distance $D$. The subscripts of $\gamma$ implies that each surface has a different surface potential $\psi_0$. The interaction free energy is determined by inserting Equation (2.14) the midpoint potential into Equation (2.9) and integrating the result with respect to $D$ giving

$$W(D) = \frac{16k_B T}{\kappa} (n_1 + 3n_2)(\gamma_1 + \gamma_2)^2 e^{-\kappa D}$$

(2.15)

per unit area.

The energy between two spheres was estimated from Equation (2.15) using the Derjaguin approximation (Israelachvili, 1985)

$$F(D) \simeq 2\pi \left( \frac{a_1 a_2}{a_1 + a_2} \right) W(D),$$

(2.16)
which estimates the force between two spheres from the energy per unit area of two flat surfaces at the same separation $D$. After further integration, the total interaction energy as a function of surface-to-surface separation distance is written as

$$W(D) = \frac{32\pi k_B T}{\kappa^2} (n_1 + 3n_2) \left( \frac{a_1a_2}{a_1 + a_2} \right) (\gamma_1 + \gamma_2)^2 e^{-\kappa D}$$  \hspace{1cm} (2.17)

and depends on the electrolyte concentration, the size and, through the constants $\gamma_1$ and $\gamma_2$, the electrostatic surface potential of each particle.

**Estimation of surface potential**

The surface potential of the Au-np is estimated from zeta potential measurements of colloidal gold. The zeta potential is the potential at the Stern layer, a narrow space, one or two angstroms thick that separates the charged surface from the diffuse double-layer (Israelachvili, 1985). While the zeta potential is not equivalent to the surface potential, for the purposes of this model the distinctions are not important. We found that the zeta potential was $\zeta = -50 \pm 10$ mV (a result consistent with other studies (Kim et al., 2005)) corresponding to a surface charge density of $-5.4 \text{ C m}^{-2}$ or equivalently, to one electronic charge per 0.03 nm$^2$. For a 13 nm nanoparticle, this amounts to roughly 8 electronic charges per nanoparticle.

The surface potential of the DNA “sphere” is calculated from the Grahame equation (Israelachvili, 1985)

$$\sigma^2 = 2\varepsilon \varepsilon_0 k_B T \left[ n_1 \left( e^{-e\psi_0/k_BT} + e^{e\psi_0/k_BT} - 2 \right) + n_2 \left( e^{+2e\psi_0/k_BT} + 2e^{-e\psi_0/k_BT} - 3 \right) \right],$$  \hspace{1cm} (2.18)

which depends solely on the surface charge density $\sigma$ of the particles and the bulk ion number density. The surface charge density of DNA is

$$\sigma = \frac{eN_b}{4\pi R_H^2},$$  \hspace{1cm} (2.19)

where $N_b$ is the number of bases that make up the DNA sequence. Using this value in Equation (2.18) the surface potential was determined by numerically solving for
ψ_m. In Table 2.2 the computed surface charge density and surface potential of 15 mer homonucleotide sequences of adenine in electrolyte are given. The temperature in these calculations is 298K.

Table 2.2: Surface charge density $\sigma$ and corresponding surface potential $\psi_0$ from the Grahame equation, Equation (2.18), for 15 mer single-strands of polyadenine in 1:1 and 1:2 salt. The temperature is 298K.

<table>
<thead>
<tr>
<th>Salt conc. (mM)</th>
<th>$\sigma$ (C m$^{-2}$)</th>
<th>$\psi_0$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 Salts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-0.155</td>
<td>-168</td>
</tr>
<tr>
<td>20</td>
<td>-0.164</td>
<td>-153</td>
</tr>
<tr>
<td>100</td>
<td>-0.219</td>
<td>-127</td>
</tr>
<tr>
<td>1:2 Salts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-0.159</td>
<td>-169</td>
</tr>
<tr>
<td>50</td>
<td>-0.243</td>
<td>-151</td>
</tr>
</tbody>
</table>

The results show, almost counterintuitively, that the surface charge density increases with the electrolyte concentration. To see why this is the case, consider again the initial assumptions. We assumed that the DNA would be treated as hard spheres with a uniform surface charge density. The radius of these spheres was determined by finding the hydrodynamically equivalent worm-like chain whose characteristic length is given by Equation (2.1). Since the average size of the chain coil decreases when the electrolyte concentration is increased, the radius of the hydrodynamically equivalent sphere will also decrease. A smaller radius in Equation (2.19) leads to a larger surface charge density. Of course, the increase in surface charge density is offset in Equation (2.18) by stronger electrolyte screening and leads to smaller surface potentials.

2.2.2 The van der Waals interaction

The attractive interaction between the two spheres is due to the van der Waals force. The interatomic pair potential between atoms separated by a distance $r$ takes the form $w(r) = -C/r^6$ where $C$ is a constant dependent on the system. The total interaction
potential was calculated by summing the energies of all atoms in one body with all the energies in the second body and is called the Hamaker summation method (Israelachvili, 1985). It is assumed that the interaction takes place on time scales much longer than the light propagation time scale. In this case, the interaction is said to be non-retarded, in other words, the speed of light is understood to be infinite. For small separation distances this is a valid assumption. Also, it is assumed that the van der Waals interaction is additive.

The attractive non-retarded van der Waals interaction calculated by pairwise additivity between two spheres is

\[
W(D) = -\frac{A_H}{12D} \left\{ \frac{\hat{a}}{D} + \frac{D}{1 + \frac{D}{\hat{a}} + \frac{D^2}{4a_1a_2}} \right\} + 2D \ln \left( \frac{D}{\hat{a}} \left( \frac{1 + \frac{D}{2(a_1 + a_2)}}{1 + \frac{D}{\hat{a}} + \frac{D^2}{4a_1a_2}} \right) \right), \tag{2.20}
\]

where \( \hat{a} = 2a_1a_2/(a_1 + a_2) \) and \( A_H \) is the Hamaker constant determined from Lifshitz theory (Israelachvili, 1985, Hunter, 1987). In the limit where \( D \ll a_1, a_2 \) Equation (2.20) reduces to

\[
W(D) \simeq -\frac{A_H}{6D a_1 + a_2} a_1a_2, \tag{2.21}
\]

and in the other limit, where \( D \gg a_1, a_2 \), the interaction energy becomes

\[
W(D) \simeq -\left( \frac{16A_H}{9} \right) \frac{a_1^3a_2^3}{D^6}, \tag{2.22}
\]

which has the characteristic long-range \( r^{-6} \) dependence between interacting atoms.

The Hamaker constant is determined by Lifshitz theory. The basic idea is that the dipole moments within one medium establish an electric field that induces other dipoles in a neighboring medium. For two media of dielectric permittivity \( \varepsilon_1 \) and \( \varepsilon_2 \) interacting across a third medium with dielectric permittivity \( \varepsilon_3 \), the Hamaker con-
constant is approximately given by

\[ A_H \simeq \frac{3}{4} k_B T \left( \frac{\varepsilon_1 - \varepsilon_3}{\varepsilon_1 + \varepsilon_3} \right) \left( \frac{\varepsilon_2 - \varepsilon_3}{\varepsilon_2 + \varepsilon_3} \right) + \]

\[ + \frac{3h}{4\pi} \int_{\nu_n}^{\infty} \left( \frac{\varepsilon_1(i\nu) - \varepsilon_3(i\nu)}{\varepsilon_1(i\nu) + \varepsilon_3(i\nu)} \right) \left( \frac{\varepsilon_2(i\nu) - \varepsilon_3(i\nu)}{\varepsilon_2(i\nu) + \varepsilon_3(i\nu)} \right) d\nu, \]  

(2.23)

where \( \varepsilon(i\nu) \) is the value of \( \varepsilon \) at imaginary frequencies and \( h \) is Planck’s constant.

The absorption frequency \( \nu_n = (2\pi k_B T / h)n \) where \( n \) is the refractive index of the medium is \( \nu_n = 5 \times 10^{14} \, s^{-1} \) at 298K. The first term of Equation (2.23) gives the zero-frequency energy from dipole-dipole interactions and dipole-neutral interactions known as the Keesom effect and the Debye effect respectively (Hunter, 1987). The second term of Equation (2.23) contains the dispersion energy. For non-metallic particles such as DNA and proteins, the dielectric permittivity \( \varepsilon(i\nu) \) is given by the expression

\[ \varepsilon(i\nu) = 1 + \frac{(n^2 - 1)}{1 + (\nu / \nu_0)^2}, \]  

(2.24)

where \( \nu_0 \) is the main absorption frequency of the media. The DNA molecule has a maximum absorption at 260 nm or \( \nu_0 = 1.15 \times 10^{15} \, s^{-1} \). The main absorption frequency of water is \( \nu_0 = 3.0 \times 10^{15} \, s^{-1} \) (Israelachvili, 1985). For metallic particles, the dielectric permittivity is estimated by the plasma response function

\[ \varepsilon(i\nu) = 1 + (\nu_e / \nu)^2, \]  

(2.25)

where \( \nu_e^2 = n_e e^2 / 4\pi^2 m_e \varepsilon_0 \) is the squared plasma frequency of a free electron gas of number density \( n_e \) where \( m_e \) is the electron mass (Hunter, 1987). The number density of gold is \( n_e = 5.90 \times 10^{28} \, m^{-3} \) (Lide, 2008) giving a plasma frequency of \( \nu_e = 2.18 \times 10^{15} \, s^{-1} \).

The Hamaker constant scales the strength of the van der Waals force to the specific material properties of the system. For our system it was calculated by numerical integration with MATLAB using adaptive Gauss-Kronrod quadrature of Equation (2.23) with Equation (2.24) for DNA and water (media 1 and 3 respectively), Equation (2.25) for the Au-np (medium 2), and the physical properties from Table 2.3. The
Table 2.3: Physical properties used in the calculation of the Hamaker constant.

<table>
<thead>
<tr>
<th>medium</th>
<th>DNA</th>
<th>Au</th>
<th>H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>refractive index $n$</td>
<td>$1.6^a$</td>
<td>$-$</td>
<td>$1.33$</td>
</tr>
<tr>
<td>permittivity $\varepsilon$</td>
<td>$2.56^b$</td>
<td>$\infty$</td>
<td>$80$</td>
</tr>
</tbody>
</table>

$^a$ for proteins from Israelachvili (1985)

$^b$ $\varepsilon = n^2$

computation resulted in a Hamaker constant of $3.12k_BT$ and is a result consistent with the Hamaker constant between bovine serum albumin (BSA) proteins, found to be $3.10k_BT$ (Roth et al., 1996).

2.2.3 The DLVO interaction

The total DLVO interaction energy is calculated from the algebraic sum of the double-layer interaction, Equation (2.17), and the van der Waals interaction, Equation (2.20). These results are plotted against the separation distance. The model shows the presence and size of an equilibrium point, from which it is possible to determine if the interaction is attractive or repulsive. The first derivative of the energy represents the force on the particle. The point where the derivative changes sign represents an equilibrium point.

First, the role of the electrolyte on the DLVO interaction between 15 mer ssDNA and a 13 nm diameter ($a_2 = 6.5$ nm) Au-np was investigated. The total energy is plotted versus the separation distance in Figure 2.3 (left plot). The concentration of 1:1 electrolyte was increased from 10 mM up to 100 mM in 10 mM increments. The temperature was constant at 298K. We note that the barrier energy decreases when the salt concentration increases. This is due to the increased number density of counter-ions available to screen the electrostatic (Coulombic) repulsion between the like-charged particles.
Next, we calculated the DLVO interaction energy and varied the size (i.e. the number of bases) of the DNA sequence. The electrolyte concentration is 10 mM and the temperature is 298K. By changing the length of DNA, we are both increasing its physical size and, because each base of DNA carries one electronic charge, the surface charge density is increased as well. These results are plotted in Figure 2.3 (right plot). The lengths of DNA were 5 mer, 15 mer, 30 mer, 100 mer, 250 mer, 500 mer and 1000 mer. We find that the peak interaction energy barrier increases with the length of DNA. This is expected due to the increased surface charge density of DNA and the corresponding increase in electrostatic repulsion. It is interesting to note that while the peak interaction energy increases, the longer sequences seem to converge at or around 49 kJ mol\(^{-1}\). A likely explanation for this behavior is due to the geometry and its effect on the system. The double-layer interaction, Equation (2.17), is, to first-order, linearly dependent on the radius of the particles. The van der Waals interaction is also linearly dependent on the radius, Equation (2.21). So, to first order, increasing

**Figure 2.3:** The DLVO interaction energy between ssDNA and 13 nm diameter Au-np is calculated when increasing the 1:1 salt concentration from 10 mM up to 100 mM in 10 mM increments (left plot). The length of DNA is maintained at 15 mer. Also the interaction is calculated for increased DNA size (right plot). The lengths of DNA are 5 mer, 15 mer, 30 mer, 100 mer, 250 mer, 500 mer and 1000 mer. The temperature in both plots is 298K.
the radius of the particle only affects the surface charge density and the electrostatic interaction. Eventually though, increasing the surface charge will have increasingly smaller effects on the surface charge density as DNA swells in size. At that point, the system returns to a linear dependence on the particle radius and the overall interaction reaches an equilibrium state with the change in radius.

Finally, some specific situations pertinent to the assay are analyzed. The total DLVO interaction as a function of separation distance between 15 mer ssDNA and a 13 nm diameter Au-np is plotted in Figure 2.4a for 1:1 salts and Figure 2.4b for 1:2 salts. The solid lines indicate the results for homonucleotide sequence of adenine while the dashed lines are for thymine. The temperature in these calculations is 298K. The peak DLVO interaction energies, $\delta W/\delta D = 0$ and $\delta^2 W/\delta D^2 < 0$, for 1:1 and 1:2 salt are given in Table 2.4.

**Table 2.4:** The DLVO interaction energy between 15 mer ssDNA sequences of adenine and thymine and a 13 nm diameter Au-np in 1:1 and 1:2 salts.

<table>
<thead>
<tr>
<th>Salt conc. (mM)</th>
<th>Adenine</th>
<th>Thymine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 Salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21.67</td>
<td>24.02</td>
</tr>
<tr>
<td>20</td>
<td>16.45</td>
<td>17.66</td>
</tr>
<tr>
<td>100</td>
<td>7.33</td>
<td>7.33</td>
</tr>
<tr>
<td>1:2 Salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20.61</td>
<td>22.73</td>
</tr>
<tr>
<td>50</td>
<td>6.44</td>
<td>6.44</td>
</tr>
</tbody>
</table>

### 2.2.4 Caveats and limitations of model

A number of assumptions are made with Equation (2.17), which is referred to as the weak-overlap approximation (Israelachvili, 1985). First, it is assumed that the midpoint potential between the particles is small. This may not be valid for small separation distances. Second, it is assumed that there is little or no overlapping be-
Figure 2.4: The DLVO interaction between 15 mer ssDNA and 13 nm diameter Au-np as a function of separation distance in (a) 10 mM (red), 20 mM (blue) and 100 mM (green) of 1:1 electrolyte or (b) 5 mM (red) and 50 mM (blue) of 1:2 electrolyte. The solid lines (—–) are the results for homonucleotide sequences of adenine while the dashed lines (- - -) are the results for the thymine sequences. The surface potential of DNA is estimated from Equation (2.18) and the surface potential of the Au-np is estimated from zeta potential measurements as described in the text. The temperature in these calculations is 298K.
In regards to the van der Waals force, it was mentioned that the interaction between the media is instantaneous or non-retarded. Since the instantaneous dipole moment in one molecule induces a dipole in another molecule, if the propagation and absorption time between the molecules is large compared to the characteristic internal motion time then the dipole moments may have substantially altered their orientations in this time period. For separation distances \( D \ll 10 \text{ nm} \) the propagation can be regarded as instantaneous. Above 10 nm only the dispersion force suffers; the zero-frequency term (the first term in Equation (2.23)) is not affected (Hunter, 1987).

Another limitation of the van der Waals interaction is when taking into account the electrolyte. Electrostatic field propagation between molecules is screened due to polarization of salt ions in the medium. The screening affects the zero-frequency term in Equation (2.23) and reduces the overall interaction by a factor proportional to \( e^{-2\kappa D} \) (Mahanty and Ninham, 1976). The interaction is further complicated because it involves two charged surfaces where the distribution of ions in the medium is non-linear. The exact contribution to the van der Waals interaction for charged surfaces in electrolyte solutions has been calculated but under too restrictive of conditions to be of any use (Hunter, 1987). For the purpose of this theoretical model, the effects of the electrolyte on the van der Waals interaction are ignored.

### 2.3 Materials and Methods

#### 2.3.1 Materials

DNA sequences were synthesized by IDT (Integrated DNA Technologies, Inc., Coralville, IA) and used without further purification. The following sequences were used:

- 5’-AAA AAA AAA AAA AAA-3’ (referred herein as poly(A))
- 5’-TTT TTT TTT TTT TTT-3’ (poly(T))

The DNA sequences were modified with a Cy5 dye molecule (Ex: 645 nm, Em: 665 nm) at the 5’ end. The concentration of the DNA was confirmed using UV absorption spectroscopy. The salts: sodium chloride (NaCl), ammonium sulfate ((NH₄)₂SO₄), ammonium chloride, (NH₄Cl), potassium nitrate (KNO₃), sodium sulfate (Na₂SO₄), sodium phosphate dibasic (Na₂HPO₄) and
sodium phosphate monobasic (NaH$_2$PO$_4$) were obtained from Mallinckrodt Baker (Phillipsburg, NJ). For Au-np synthesis, hydrogen tetrachloroaurate(III) trihydrate (HAuCl$_4$·3H$_2$O) and trisodium citrate dihydrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O) were obtained from Alfa Aesar (Ward Hill, MA).

Au-nps were prepared according to the method used by Frens (1973) and Grabar et al. (1995). The synthesis procedure was as follows. First, in a rounded flask, 250 mL of 1 mM gold compound (HAuCl$_4$·3H$_2$O) was brought to a boil with stirring and a condenser. Next, 25 mL of 38.8 mM trisodium citrate was quickly added to the vertex of the solution. Within a minute of mixing with the citrate the solution changed color from pale yellow to a dark burgundy. This was indication that the nanoparticles were forming. The mixture was heated for an additional 10 minutes and then removed to cool for an additional 15 minutes while stirring was continued. After the solution returned to room temperature the colloid was transferred to amber colored storage bottles. This method yields particles with an absorption maximum at 520 nm and a particle size of 13 nm (Grabar et al., 1995).

In the subsequent kinetics experiments we needed a sufficient quantity of DNA to be easily detectable by the fluorimeter yet not large enough to completely cover the Au-np nor to stabilize the colloid from salt induced aggregation. For this reason, throughout the study unless noted otherwise, the DNA sequences were diluted with Milli-Q deionized water until their concentration was 5x the Au-np concentration of 17 nM.

2.3.2 Instrumentation

Fluorescence measurements

Fluorescence measurements were carried out on a HORIBA Yobin Jvon (Edison, NJ) FluoroLog-3 spectrofluorometer with temperature control provided through a digital circulating water bath manufactured by VWR Scientific (West Chester, PA). The procedure for fluorescence quenching measurements is as follows: the input and output monochrometers were set to the respective emission and excitation wavelength of Cy5.
dye. Then, 500 µL of gold colloid in a 1 cm path-length cuvette was placed in the sample holder and 500 µL of analyte (DNA and buffer) was added. Data acquisition began within 2 seconds of mixing the reagents but before the mixing was complete and typically ran to 300 seconds. For most DNA samples, quenching occurred within 180 seconds. For this reason, it was necessary to begin data collection as soon as possible after mixing so as to properly analyze the binding rates.

Time-dependent fluorescence quenching measurements were fit to a single exponential decay function using least-squares fitting to determine the characteristic binding rate. This is consistent with a single binding event in the dilute limit where changes in surface charge density or binding coverage over the course of the experiment had little effect on the binding rate. The quality of the fit was confirmed using the program *MemExp* which uses the maximum entropy method to analyze a time-dependent signal in terms of discrete or distributed lifetimes (Steinback et al., 1992, 2002, Steinback, 2002). In Figure 2.5 an example of this analysis method is shown.

**Figure 2.5:** Analysis by *MEMexp* of the binding kinetics data of poly(A) at 20°C in 10 mM of NaCl. The figure on the bottom left shows the raw kinetics data in arbitrary units (——) and best fit lines using either a distributed binding time constant (——) or a discrete binding time constant (- - -). The figure on the top left shows the residual of the best fit lines and the raw data. The residuals show that the distributed lifetimes (——) more accurately fit the data. It is not possible to apply van‘t Hoff analysis to distributed lifetimes so discrete lifetimes are used in the transition state calculations. The figure on the right shows the distributed binding time constant (——) and the discrete binding time constant determined by the program to be the most likely fitting parameter by the maximum entropy method.
The plot on the bottom left of the figure shows the fluorescence binding kinetics data as well as two best fit lines: one using a distributed binding time constant and one using a discrete binding time constant. The quality of each fit can be assessed by the residual of the best fit line plotted above the left plot. The figure on the right shows the most likely distributed binding time constant and the discrete binding time constant as determined by the program.

Zeta potential measurements

The surface potential of Au-np was estimated from zeta potential measurements of colloidal gold. Measurements were taken on a Brookhaven Instruments Corporation (Holtsville, NY) 90Plus Particle Size Analyzer. All measurements were taken at room temperature with no added electrolyte.

Data for these measurements are given in Table 2.5. We noticed that the pH of the colloid changed with age and that increased the stability of the colloid. For this reason, the zeta potential of various colloid samples were tested.

Table 2.5: The zeta potential $\zeta$ of 13 nm diameter Au-np. The temperature during the measurement was 298K.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>$\zeta$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td>$-42 \pm 9.4%$</td>
</tr>
<tr>
<td>434</td>
<td>$-60 \pm 5.8%$</td>
</tr>
<tr>
<td>791</td>
<td>$-47 \pm 18%$</td>
</tr>
<tr>
<td>950</td>
<td>$-51 \pm 9.1%$</td>
</tr>
</tbody>
</table>

| Mean      | $-50 \pm 5.2\%$ |

Next, we determined how the range of values for the zeta potential of Au-np in Table 2.5 can affect the interaction. The DLVO energy depends on the surface potential of both the DNA and the Au-np so it is reasonable to expect a range of possible interaction energies as well. Using Equation (2.17) and Equation (2.20) the DLVO
interaction energy is plotted in Figure 2.6 (left) for three zeta potentials of Au-np: -60 mV, -50 mV and -40 mV. In addition, the peak DLVO interaction energy barrier as a function of Au-np zeta potential is plotted in Figure 2.6 (right). From these two plots, we can see how the zeta potential affects the DLVO energy barrier. Over the range of values in Table 2.5 the theoretical peak DLVO interaction energy varies from 19.5 kJ mol\(^{-1}\) for \(\zeta = -42\) mV to 23.7 kJ mol\(^{-1}\) for \(\zeta = -60\) mV, a total range of 4.2 kJ mol\(^{-1}\). It is difficult to extend uniformity between synthesized batches of gold colloid. In addition, as the colloid ages, its characteristics change in ways that are not easy to predict. The strong dependence of the DLVO barrier energy on the zeta potential demonstrates that the physical properties of the colloid are important to the accuracy of the model.

**Figure 2.6:** The effects of the Au-np zeta potential \(\zeta\) on the DLVO interaction energy. The DLVO interaction energy between ssDNA and Au-np as a function of separation distance for three zeta potentials of Au-np: -60 mV (---), -50 mV (---) and -40 mV (---) (left plot). The peak DLVO interaction energy barrier as a function of the Au-np zeta potential (right plot). Both figures use 15 mer ssDNA and 13 nm diameter Au-np in the calculations.
CHAPTER 2. THE ELECTROSTATIC MODEL

2.4 Results and Discussion

In transition rate theory (Levin, 1983, Laidler and King, 1983), a bimolecular reaction does not take place unless a certain minimum energy, called the activation energy, is reached. The distribution of energy states throughout the reaction follows the Boltzmann distribution so that the rate constant \(k\) can be expressed as

\[
k = \frac{k_B T}{h} e^{\Delta S^\ddagger / R} e^{-\Delta H^\ddagger / RT},
\]

where \(R\) is the gas constant and \(\Delta S^\ddagger\) and \(\Delta H^\ddagger\) are the transition state entropy and enthalpy of reaction respectively. On the right-hand-side of Equation (2.26) the term \((k_B T / h)e^{\Delta S^\ddagger / R}\) is a measure of the number of encounters between reactants that lead to a successful product (Levin, 1983). The rate constant is equal to the total collision rate times a probability of success determined from the entropy of the transition state. The bimolecular collision rate constant \(k_D\) for a diffusion limited reaction is

\[
k_D = 4\pi(D_1 + D_2)a_0,
\]

where \(D_1\) and \(D_2\) are the diffusion coefficients of DNA and Au-np respectively and \(a_0 = (a_1 + a_2)\) is the distance of closest approach of the molecular centers (Steinfeld et al., 1989). By the Stokes-Einstein equation the diffusion limited binding rate constant is then

\[
k_D = \frac{2RT}{3\eta} \left(2 + \frac{a_1}{a_2} + \frac{a_2}{a_1}\right)
\]

per molar concentration of reactant where \(R\) is the gas constant. The diffusion limited binding rate at 293K in an 8.5 nM colloidal solution of Au-np is \(k_D = 0.12 \text{ s}^{-1}\). This provides an upper limit to the experimental reaction. Taking into account entropic factors such as non-uniform surface reactivity and transition of DNA from closed helical form to open form lowers the overall probability of success and with it, the binding rate. Experimental binding results at 293K range in our measurements from \(k = 7 \times 10^{-3} \text{ s}^{-1}\) to \(60 \times 10^{-3} \text{ s}^{-1}\). Considering that not every encounter leads to adsorption these results are well within the limits of the diffusion limited model.
2.4.1 van’t Hoff analysis

The binding rate in Equation (2.26) can be expressed in linear form as

\[
\ln\left(\frac{k}{T}\right) = -\frac{\Delta H^\dagger}{R} \frac{1}{T} + \frac{\Delta S^\dagger}{R} + \ln\left(\frac{k_B}{h}\right)
\]  

(2.29)

signifying when \(\ln(k/T)\) is plotted against \(1/T\) as in standard van’t Hoff plots the slope of the resulting lines yields the transition state enthalpy \(\Delta H^\dagger\). The results of fluorescence quenching experiments are plotted in Figure 2.7 and the average transition state enthalpy for 1:1 and 1:2 salts are calculated from Equation (2.29) and given in Table 2.6.

![Figure 2.7: Van’t Hoff plots, \(\ln(k/T)\) vs 1000/T, for the interaction between Au-np and (a) poly(A) or (b) poly(T) in either 1:1 (\(a_1\) and \(b_1\)) or 1:2 (\(a_2\) and \(b_2\)) salts. The points are the binding kinetics measurements at each temperature. Linear fits through these points were taken from which the transition state enthalpy for the interaction was calculated.](image-url)
In Table 2.6 the experimental transition state enthalpy values are given. Comparing these values to the theoretical values in Table 2.4 it appears that DLVO theory underestimates the size of the activation energy barrier for poly(A) while showing reasonable agreement for poly(T) in the 10 mM and 20 mM 1:1 salt as well as the 5 mM 1:2 salt. In previous studies, it was found that thymidine (nucleoside composed of thymine base and pentose sugar) only weakly binds to Au-np (Storhoff et al., 2002). It was speculated that the amine group on adenine, which is not present on thymine, aids in adsorption due to chemical interactions with Au. Since this model is strictly electrostatic, chemical considerations such as the presence of amine groups are not accounted for by DLVO theory.

Table 2.6: The average experimental transition enthalpies $\Delta H^\ddagger$ and the theoretical values calculated from DLVO theory for poly(A) and poly(T). The theoretical transition state enthalpy was calculated at $T = 298K$.

<table>
<thead>
<tr>
<th></th>
<th>Transition state enthalpy $\Delta H^\ddagger$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>poly(A)</td>
</tr>
<tr>
<td>1:1 Salt</td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>22.7 ± 0.8</td>
</tr>
<tr>
<td>20 mM</td>
<td>12.6 ± 2.0</td>
</tr>
<tr>
<td>100 mM</td>
<td>–</td>
</tr>
<tr>
<td>1:2 Salt</td>
<td></td>
</tr>
<tr>
<td>5 mM</td>
<td>22.2 ± 0.9</td>
</tr>
<tr>
<td>50 mM</td>
<td>–</td>
</tr>
</tbody>
</table>

Typically, DNA screening assays are operated with at least 100 mM of salt buffer solution in order to stabilize hybridization of complementary sequences. We designed the experiments with lower concentrations of salt for two important reasons. First, at high buffer concentrations, adsorption of ssDNA is extremely rapid and fluorescence is short lived. Because fluorescence acquisition could not begin for at least 1–2 seconds after mixing due to operational complexities (fluorescence spectrometers are not
suited for fast reaction/slow mixing fluorescence measurements) much of the error in our exponential fitting is due to determining the initial baseline fluorescence. To minimize this shortcoming, experiments with longer reaction times were performed allowing for much greater accuracy in determining the binding rate. This necessitated low salt concentrations.

The second reason for the low concentrations of salt was to prevent instability of gold colloid in electrolyte. As mentioned earlier, salt causes the nanoparticles to aggregate, which changes the absorption frequency of the colloid. To demonstrate why this is significant, consider the excitation frequency of Cy5 dye - it has a peak absorption wavelength at 645 nm. The peak excitation absorption wavelength of 13 nm gold colloid is 520 nm (Grabar et al., 1995), or blue-green on the UV-spectrum. The rest of the incident light is either scattered or reflects which gives the colloid its characteristic red color. When the colloid aggregates, the peak absorption wavelength shifts due to a change in the surface plasmon resonance frequency of the nanoparticles. This shift, toward the longer wavelengths, causes the shorter wavelengths to be reflected and the colloid appears blue-violet. If a large amount of salt were used in the experiment, and this salt causes the colloid to aggregate before the reaction is completed, then the excitation intensity would decrease due to the colloid absorbing more photons after application of salt than at the beginning of the experiment. This would lead to a reduction in the fluorescence intensity that is not caused by chromophore quenching on the Au-np.

Our model suggests that at higher salt concentrations the barrier height is smaller than at low concentration and that leads to faster binding rates. Because of the previously stated reasons, it was not possible to test this aspect of the theory.

2.4.2 Model for dsDNA

For the DLVO model to be useful, it must not only describe the adsorption of ssDNA but it must also describe and predict the behavior of dsDNA. The experiments on DNA and gold colloid by Li and Rothberg (2004b) showed that dsDNA did not adsorb onto Au-np while ssDNA, at the same salt concentration, did adsorb. In DLVO theory, the
rate of adsorption is related to the interaction energy. This energy should therefore be considerably larger for dsDNA than it is for ssDNA.

The model for dsDNA, as was done for ssDNA, treats the particle as a hard sphere with a uniform charge density and a radius described by Equation (2.1). Double-stranded DNA differs from ssDNA by a longer persistence length and twice the linear charge density. This does not necessarily double the surface charge density of dsDNA. Double-stranded DNA is stiffer than ssDNA (see Figure 2.2) and so it has a longer end-to-end length for all but the shortest sequences. So while the number of electronic charges doubles, the charge is spread across a larger surface area.

Figure 2.8 plots the DLVO interaction energy as a function of separation distance between a 13 nm diameter Au-np and a random sequence of either 15 mer ssDNA (solid-line) or 15 mer dsDNA (dashed-line) in 10 mM and 20 mM of a 1:1 salt. The temperature in these calculations is 298K. It is clear that the theoretical peak interaction energy barrier between dsDNA and Au-np is not sufficient to deter dsDNA from adsorbing. Notice that the peak energy at 20 mM salt is smaller for dsDNA than it is for ssDNA in 10 mM salt. From this, we concluded that since ssDNA is observed to adsorb at 10 mM salt, dsDNA must therefore bind in 20 mM salt. In fact, as Li and Rothberg (2004b) have shown in experiments, no adsorption of dsDNA was observed at these salt concentrations (of course, this forms the basis for the hybridization assay discussed in the Chapter 1). This observation indirectly demonstrates the failure of the electrostatic model and is a major conclusion of this study. Accounting for the behavior of dsDNA is motivation for broadening our understanding of the interaction forces involved. Because ssDNA and dsDNA differ most noticeably in their solubilities, the interaction cannot be understood without first acknowledging the role of water as a solvent in the interaction. The lack of any consideration in DLVO theory for solvation forces is a major shortcoming of the theory (Israelachvili, 1985). This topic is discussed more thoroughly in Chapter 3.
Figure 2.8: The DLVO interaction between a 13 nm diameter Au-np and a random 15 mer ssDNA (---) or a random 15 mer dsDNA (- - -) sequence as a function of separation distance in 1:1 electrolyte. The salt concentration is 10 mM (red) and 20 mM (blue). The surface potential of DNA is estimated from Equation (2.18). The surface potential of the Au-np is estimated from the zeta potential. The temperature in these calculations is 298K.

2.5 Conclusions

We have assessed the validity of a simple electrostatic model to describe the adsorption of DNA onto Au-nps. In this model, the DNA molecule is treated as a sphere that is hydrodynamically equivalent to a worm-like chain. The surface charge density of the sphere is evenly distributed across its surface. The screened electrostatic interaction is described by Gouy-Chapman theory and the weak-overlap approximation. The attraction between DNA and the Au-np is described by the van der Waals energy. This forms the basis of DLVO theory, which has been applied to other biological systems such as the interaction between proteins. From this, a calculation of the peak interaction energy could be determined and was compared to experimental results.
The experimental transition state enthalpy between 15 mer homonucleotide sequences of adenine or thymine and Au-np was measured using time-dependent fluorescent quenching spectroscopy. The peak DLVO interaction energy barrier for thymine showed good agreement with experimentally derived interaction energy values at low salt concentrations. The DLVO interaction energy for adenine was greater than experimentally determined results. Unfortunately, the electrostatic model fails at high salt concentrations characteristic of biological environments. In addition, the model cannot explain the key observation that ssDNA adsorbs while dsDNA does not. The failure of this description and demonstrating a viable alternative model are the major results of this thesis.

In classic DLVO theory, no consideration is made for solvation forces but these can strongly affect interactions since there are large enthalpies and entropies associated with organization of water molecules around solutes and amongst themselves. These are referred to as hydration and hydrophobic effects and we will show conclusively in the next chapter that they are important in explaining the adsorption behavior of DNA on Au-np.

References


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Chapter 3

The Hydrophobic Effect

In the previous chapter, a simple electrostatic model was proposed. The model was based on DLVO theory which has already been used to describe the interaction between proteins and between DNA and membranes. While the theory was accurate for low concentrations of salt ions, it failed to accurately describe the interaction between dsDNA and Au-np; specifically, it failed to introduce a strong repulsive force that could keep dsDNA from adsorbing. In addition, the model began to fail under assay conditions when higher concentrations of salt were introduced. The challenge in modeling biological molecules are the difficulties in representing solvation, steric, electrostatic and van der Waals energies, hydrodynamic interaction, and conformational changes. DLVO theory is fundamentally an electrostatic model and does not take into account the characteristics of the solvent and therefore can only partially describe the system. In aqueous solutions the hydration of surfaces and the strong attraction of water for itself is a major contributor to the interfacial energy.

In this chapter, we propose an alternative point of view that the interaction is dominated by the hydrophobic effect, an attractive interaction between hydrophobic particles in aqueous solutions. We used binding kinetics to determine the thermodynamic properties of the surfaces and examine how the properties of the solvent affect the interaction. We document the strong dependence of binding rate on the type of salt, characteristic of hydrophobically regulated processes. In addition, we report on a
strong correlation in the thermodynamics commonly referred to as enthalpy-entropy compensation and use this to make the case for the dominance of the hydrophobic effect. Finally, we take some time to discuss isothermal titration calorimetry, a technique to measure the thermodynamic parameters directly from the reaction.

### 3.1 Introduction

Solvation forces arise because water cannot be treated as a continuous dielectric and is composed of molecules of well-defined non-zero size that have a propensity to organize. As charged surfaces approach one another and their electrical double-layers begin to overlap, water molecules in between begin to experience less mobility due to the reduction of accessible volume. The increase in molecular density between surfaces leads to a repulsive pressure as the water molecules struggle to conform to the space that is left to them.

When salt ions are introduced the situation gets more complicated. In experiments (Pashley, 1981a,b) conducted to directly measure the force between two mica surfaces in dilute 1:1 and 1:2 electrolyte the agreement with DLVO theory is remarkably good. At low electrolyte concentration, comparison with theory is possible at separations less than the Debye length (Eqn (2.4)). In addition, the surface potential $\psi_0$ inferred from the magnitude of the double-layer interaction agree with those measured independently by electrophoresis (Israelachvili, 1985). At high salt concentrations, counter-ions bind to the surfaces and originated a repulsive hydration force. The repulsive force was believed to be due to the additional energy required to dehydrate the bound ions and increase in strength with the hydration number, the number of water molecules that make direct hydrogen bonds with the ion. Because the force is characteristic of strongly hydrated surfaces, it is commonly referred to as hydrophilic hydration.

At hydrophobic surfaces, on the other hand, water molecules cannot bind to the surface. If the particle is small then water can arrange itself around the hydrophobic particle without breaking hydrogen bonds. It is therefore possible to dissolve small
CHAPTER 3. THE HYDROPHOBIC EFFECT

hydrophobic particles in water. For larger surfaces, water can no longer rearrange itself and some hydrogen bonds are lost. For this reason, there is a tendency to try to eliminate hydrophobic surface area from aqueous solutions and this leads to attractive forces between surfaces. This is referred to as the hydrophobic interaction. Experiments which measured the interaction between hydrophobic surfaces found that at separations below 6 nm, the attraction becomes much greater than can be expected from van der Waals forces (Pashley, 1981a). In addition, the force acts over much longer ranges than the van der Waals interaction. The hydrophobic effect is responsible for a number of biological interactions including protein (Dill et al., 2008, Dougan et al., 2008) and peptide (Daidone et al., 2007) folding, vesicle and lipid bilayer formation (Tanford, 1978) and drug-protein interactions (Cheema et al., 2008).

A number of features indicate that the hydrophobic effect is likely to be an important factor in the interaction between ssDNA and Au-np. First, a large fraction of the apolar surface area of ssDNA is exposed to water. The DNA molecule contains regions of hydrophilic and hydrophobic groups. The phosphate backbone is charged and binds to water readily, hence it is considered hydrophilic. The nucleotides, on the other hand, are relatively insoluble and repel water molecules (Bloomfield et al., 2000). Double-stranded DNA organizes to expose the phosphate backbone to water ensuring that no apolar surface area is exposed to water and the molecule, as whole, remains well hydrated.

The relative strength of the hydrophobic effect for nucleotides is dependent on the number of hydrogen bonds the nucleotides make with water. To some degree, the bases adenine, thymine and cytosine, all make a small number of hydrogen bonds with neighboring water molecules. Chemically, the carbonyl oxygens and the aromatic nitrogen are hydrogen-bonding acceptors while the amino and imino protons are hydrogen-bonding donor sites (Bloomfield et al., 2000). The nucleotide adenine has a total of five hydrogen-bonded water molecules in the first hydration shell, one each at N$_1$, N$_3$ and N$_7$ and two at the NH$_2$ site. Thymine has three bound water molecules in the first hydration shell, one for each O and one at the NH site. Cytosine binds directly to a total of four water molecules, one each at N$_3$ and O and two at the NH$_2$ site. Refer to Figure 3.1 for molecular numbering usage.
Figure 3.1: Molecular diagram of the nucleotides adenine, thymine and cytosine. Amino (NH$_2$) and imino (NH) protons provide hydrogen-bonding donors. Carbonyl oxygens (C=O) and aromatic nitrogen (numbered) provide hydrogen-bonding acceptors.

The features of DNA are only half the interaction. Gold nanoparticles synthesized by citrate reduction are stabilized by surface citrate anions (Grabar et al., 1995). The charge is not evenly smeared across the surface but is localized to discrete “islands” where preferential affinity to water is maintained through hydrogen bonds. The surface area of the Au-np that is not taken up by citrate ions is insoluble to water or, at a minimum, has a lower tendency to promote hydrogen bonds with neighboring water molecules than the citrate ions do (Chow and Zukoski, 1994).

To estimate the citrate surface coverage, consider the zeta potential measurement in §2.3.2 and the analysis in §2.2.1. We found that the zeta potential of the Au-np was $\zeta = -50$ mV, which corresponded to a surface charge density of 0.03 $e/nm^2$. Given the size of the nanoparticle, this amounts to roughly 8 electronic charges per nanoparticle. Each citrate ion has a -3 valency and assuming conservatively that one or two of these charges is taken up by the gold surface (Munro et al., 1995) it leaves between 4-8 citrate ions to contribute to the entire surface charge density of the nanoparticle. The dimensions of a citrate ion (see Figure 3.2) are 10 Å along the long axis and 6 Å along the short axis (Kaplan et al., 2000). This gives it a maximum surface area, if it rests flat on the gold surface, of 60 Å$^2$. The total surface area of a 13 nm diameter Au-np is $5.3 \times 10^4$ Å$^2$ signifying that the citrate ion coverage is between 0.45% and 0.90% of the nanoparticle surface. Therefore, most of the nanoparticle surface was insoluble in water and hydrophobic so, like DNA, water also seeks to eliminate Au-np from solutions.
In this chapter, the effects of solvation forces on the adsorption of ssDNA and Au-np are investigated. Time-dependent fluorescence quenching spectroscopy was used to determine the binding rate constant between ssDNA and Au-np. Data for a range of temperatures were used to determine the transition state enthalpy and entropy. Thermodynamic relationships are examined including enthalpy-entropy compensation, which is thought to be indicative of the hydrophobic effect (Lumry and Rajender, 1970, Vailaya and Horváth, 1996). Homonucleotide sequences of DNA, each with different solubilities, were tested and we find correlations between the thermodynamic parameters and ligand hydrophobicity. The analysis is used to determine the compensation temperature for each salt ion. These data are compared, with good agreement, to results obtained directly from van’t Hoff analysis. We go on to devise a qualitative system to classify and rank salts in terms of the variance from the group average and find good agreement with other classification methods such as the Hofmeister series. This unique innovation offers a practical strategy to compare quantitative measurements with empirically derived relationships. Based on this classification method it was possible to predict with some accuracy the binding rate of DNA using MgCl$_2$ as the electrolyte.

### 3.2 Background

The hydrophobic effect (Kauzmann, 1959) is an attractive solvation interaction between hydrophobic particles in aqueous solutions that causes phase separation similar to the segregation of oil and water (Chandler, 2005). The effect is dominated by the
role water has in its ability to hydrate solute particles. Because many biological particles such as proteins contain discrete hydrophobic and hydrophilic groups, the hydrophobic effect is common in biological systems (Tanford, 1978). One consequence of this attribute is the ability to organize biomolecules by their hydrophobicities and is done by a technique called hydrophobic interaction chromatography. In addition, because the hydrophobic effect is a non-DLVO force and consequently, not part of the electrostatic model, the behavior of hydrophobic particles relies heavily on the properties of the solvent in addition to the properties of the particles.

The strength of the hydrophobic effect is subject to the number of hydrogen bonds lost by the water molecule at the solute-water interface. Typically, the loss of hydrogen bonds at the surface is compensated for by an increase in the number of hydrogen bonds between water molecules in the bulk. Large, extended hydrophobic surfaces make it impossible for water molecules to maintain a hydrogen-bonding network with their neighbors. A fraction of hydrogen-bonding opportunities are lost near a hydrophobic surface and results in water molecules moving away from these surfaces and into the bulk (Chandler, 2005). Processes that require reorganization of the hydrogen-bonding network are characterized by increased entropy while processes that result in water losing hydrogen bonds are characterized by an increase in enthalpy. The Gibbs free energy of the system determines whether the reaction is energetically favorable.

There are a number of thermodynamic characteristics common to hydrophobic interactions. The enthalpy of the system is strongly correlated to the temperature: exothermic at low temperature and endothermic at high temperature (Mikheev et al., 2007). There is a large negative entropy change due to increased order around the water molecule. This leads to a reduction in the molar concentration as the water molecules adapt to the reduced solubility and forms cavities where the solute can fit. In addition, the low solubility of the hydrophobic molecules leads to an increase in the chemical potential. Finally, the hydrophobic effect produces an increase in the heat capacity of the system (Gutmann, 1991).
Electrolytes affect the strength of inter-molecular hydrogen bonding between water molecules and between water molecules and the solute particles by altering whether a water molecule finds it more favorable to associate with itself or to hydrate the ions. The effectiveness of any particular salt to hydrate solutes was famously investigated by Frenz Hofmeister (1888). He classified salt ions by their ability to precipitate albumen proteins (Zhang and Cremer, 2006, Cacace et al., 1997). While the results are often of empirical use, recent research using neutron diffraction (Leberman and Soper, 1995), molecular dynamics simulations (Brown et al., 2005), Raman spectroscopy (Smith et al., 2007) and vibrational sum frequency spectroscopy (Chen et al., 2007) among others (Collins et al., 2007, Skipper and Neilson, 1989, Kiriukhin and Collins, 2002) are beginning to observe water rearrangements directly and to understand the hydrophobic forces at a microscopic level.

### 3.3 Materials and Methods

We used the same oligonucleotide sequences as in Chapter 2 for this part of the study. In addition, a third ssDNA sequence, 5′-CCC CCC CCC CCC CCC-3′ (referred herein as poly(C)), was synthesized by IDT and used without further purification. The DNA sequences were modified with a Cy5 dye molecule covalently bound to the 5′ end. The concentration of the DNA was measured using UV absorption spectroscopy. The DNA sequences were diluted with Milli-Q deionized water until the concentration was 85 nM or, 5x the Au-np concentration.

These sequences of DNA were chosen for this study because each, as mentioned earlier, has a different hydration number. In other words, each sequence binds to a different number of water molecules. Because, in essence, this study attempts to measure the hydrophobic effect, which depends strongly on solvation forces, it is important to have the greatest variation in the solubility of the sequences. We did not use a homonucleotide sequences of guanine because of the difficulty in maintaining this sequence free of any secondary structure. Guanine rich sequences tend to fold in monomer, dimer and tetramer G-quartet quadruplex structures in electrolyte
solutions (Macaya et al., 1993). In that configuration the sequence has the same characteristics as dsDNA and would not adsorb onto Au-np. Guanine rich sequences and G-quadruplexes are discussed more in Appendix A.

In addition to differing solubilities, the bases have other fundamental differences. One of these is that two of the bases, adenine and cytosine, have a much stronger documented affinity to gold than thymine does (Storhoff et al., 2002, Kimura-Suda et al., 2003). There is no clear consensus on the cause but the amine group on adenine and cytosine, which is lacking on thymine, appears to be a relevant.

Another factor is the ability of the sequences to undergo base-stacking. The base adenine (and guanine) is a purine made up of a six sided (pyrimidine) ring fused to a five sided (imidazole) ring and is hence larger than the pyrimidines thymine and cytosine (see Figure 3.1). The purine bases undergo base-stacking when two or more of these bases are adjacent to one another on a sequence (Goddard et al., 2000). Base-stacking is characterized by higher rigidity and greater enthalpies. This further sets to distinguish the sequences apart.

The salts used for this part of the study were the same as in Chapter 2. Again, these were: \( \text{NH}_4\text{Cl}, \text{NaCl}, \text{NaH}_2\text{PO}_4, \text{KNO}_3, (\text{NH}_4)\text{SO}_4, \text{Na}_2\text{SO}_4 \). The choice of salt gave us a mixture of 1:1 salts and 1:2 salts and a mixture of kosmotrope/chaotrope contact ion pairs (\( \text{NaCl} \) and \( (\text{NH}_4)\text{SO}_4 \)), purely kosmotrope contact ion pairs (\( \text{NaH}_2\text{PO}_4, \text{Na}_2\text{SO}_4 \) and \( (\text{NH}_4)\text{SO}_4 \)), and purely chaotrope contact ion pairs (\( \text{KNO}_3 \) and \( \text{NH}_4\text{Cl} \)). The terms kosmotrope and chaotrope describe the affinity of the salt ions to the water molecule and is an important factor in hydrophobic systems. These terms are discussed in more detail later on in the text. In addition, for a secondary experiment, urea ((\( \text{NH}_2 \))\text{CO}) and \( \text{MgCl}_2 \) were both obtained from Mallinckrodt Baker (Phillipsburg, NJ).

The Au-nps were synthesized using the Frens (1973) method as described in Chapter 2. Refer to §2.3.1 for specifics on the methodology and experimental procedure.
3.4 Results and Discussion

3.4.1 van’t Hoff plots

Standard van’t Hoff plots, $ln(k/T)$ versus $1/T$, are used to determine the transition enthalpy and transition entropy. Figure 3.3 shows the binding kinetics between ss-DNA and Au-np in 1:1 and 1:2 salt solutions. The DNA sequences are (a) poly(A), (b) poly(T) and (c) poly(C). The 1:1 salts, at a concentration of 10 mM, are NH$_4$Cl, NaCl, NaH$_2$PO$_4$, and KNO$_3$. The 1:2 salts, at a concentration of 5 mM, are (NH$_4$)$_2$SO$_4$, Na$_2$SO$_4$, and Na$_2$HPO$_4$. The transition enthalpy $\Delta H^\ddagger$ is determined from the slope of the linear fit to the temperature data points. The transition entropy $\Delta S^\ddagger$ is calculated from the intercept $y_0 = ln(k_B/h) + \Delta S^\ddagger/R$. A full description of van’t Hoff analysis is given in §2.4.1.

3.4.2 Enthalpy-entropy compensation

Relationships between the thermodynamic parameters are used to study the role of molecular structure in binding processes (Vailaya and Horváth, 1996). Theses correlations, commonly called linear free energy relationships (LFERs), are based on the assumption that the free energy of a process is the sum total of contributions from individual structural elements. In processes involving linear van’t Hoff plots, a linear dependence of the enthalpy on the entropy when experimental variables are changed is a form of LFERs called enthalpy-entropy compensation (EEC). Plots of enthalpy versus entropy are linear with a slope called the compensation temperature $T_C$, which is characteristic of the process. Typically, interactions between small molecules in aqueous solutions display a high degree of linear correlation between the thermodynamic parameters signifying that EEC is an important tool for investigating the hydrophobic effect (Lumry and Rajender, 1970, Vailaya and Horváth, 1996).
Figure 3.3: Van’t Hoff plots, \( \ln(k/T) \) versus \( 1000/T \), for the interaction between Au-np and (a) poly(A), (b) poly(T) or (c) poly(C) in either 1:1 (a₁, b₁ and c₁) or 1:2 (a₂, b₂ and c₂) salts. The data for poly(A) and poly(T) are the same as in Figure 2.7. The points are determined by binding rate kinetics at each temperature. Then, linear fits through these points are taken from which \( \Delta H^\ddagger \) and \( \Delta S^\ddagger \) for the interaction is calculated by Equation (2.29).
CHAPTER 3. THE HYDROPHOBIC EFFECT

For a typical hydrophobic interaction the EEC can be expressed in general form as

$$\Delta H^\ddagger = T_C \Delta S^\ddagger + \Delta G_{T_C}^\ddagger$$  \hspace{1cm} (3.1)

where $\Delta G_{T_C}^\ddagger$ is the Gibbs free energy at the compensation temperature. Figure 3.4 shows the thermodynamic parameters calculated from Figure 3.3 plotted against one another as an EEC plot. The compensation temperature is the slope of the best fit line through the data points.

![Figure 3.4: The EEC plot for the interaction of ssDNA and Au-np using the thermodynamic data from Figure 3.3. Each data point is a separate experiment using the labeled salt and either poly(A) (red), poly(T) (blue) or poly(C) (green).](image)

The thermodynamic quantities $\Delta H^\ddagger$ and $\Delta S^\ddagger$ may be written for a group of molecules differing by certain quantifiable properties as

$$\Delta H^\ddagger = \alpha_H X_i + \beta_H$$

$$\Delta S^\ddagger = \alpha_S X_i + \beta_S$$  \hspace{1cm} (3.2)

where $\alpha_H$ and $\alpha_S$ are the thermodynamic change per unit value of the molecular property $X_i$ and $\beta_H$ and $\beta_S$ are the thermodynamic change that is common to all of the
molecules. The molecular property $X_i$ can be any quantifiable measure characteristic of the substance under investigation such as the number of carbon atoms or the number of hydrogen atoms as long as the property is independent of temperature (Vailaya and Horváth, 1996). This technique has been adopted in the analysis of processes involving the hydrophobic effect (Lee, 1991). In various experiments (Chotia, 1974, Hermann, 1972, 1975, Reynolds et al., 1974), processes involved in the transfer of particles into water have been described by Equation (3.2). In these studies, the apolar surface area $A_0$ exposed to water was taken as the molecular parameter $X_i$.

The changes in enthalpy and entropy as a function of apolar surface area $A_0$ are plotted in Figure 3.5 for each salt. The apolar surface area of the DNA sequences are 89 Å$^2$ for poly(A), 115 Å$^2$ for poly(T) and 79 Å$^2$ for poly(C) (Madan and Sharp, 2001). Combining Equations (3.1) and (3.2), the compensation temperature is expressed as the ratio of the slopes of each thermodynamic parameter versus apolar surface area,

$$T_C = \frac{\alpha_H}{\alpha_S}. \quad (3.3)$$

Obviously, the plots in Figure 3.5 do not have strong linear dependence as predicted by the relationships in Equation (3.2) but, regardless, the data trend is consistent among the sequences: as the apolar surface area increases, $\Delta H^\ddagger$ and $\Delta S^\ddagger$ both increase. It is not known which, if any, of the sequences in Figure 3.5 are not described by Equation (3.2). Consequently, three best fit lines are taken for each salt, one through each pair of DNA sequences. The slopes of these lines are $\alpha_H$ and $\alpha_S$ and the compensation temperature follows directly from Equation (3.3). This gives us three compensation temperatures, one for each DNA pair. To determine which, if any, of the three is valid, we must first determine the compensation temperature by some other means. Specifically, the compensation temperature can also be measured directly from the van’t Hoff plot.
Figure 3.5: The transition enthalpy $\Delta H^\ddagger$ (□) and the transition entropy $\Delta S^\ddagger$ (○) as a function of apolar surface area per nucleotide for each salt as labeled. Here poly(A) = 89 Å$^2$, poly(T) = 115 Å$^2$ and poly(C) = 79 Å$^2$. Lines connect pairs of nucleotides and have slope $\alpha_H$ or $\alpha_S$. The compensation temperature $T_C$ for each salt is calculated by the ratio $\alpha_H / \alpha_S$ (see Equation (3.3)). Nucleotide hydrophobic surface area data from Madan and Sharp (2001).
CHAPTER 3. THE HYDROPHOBIC EFFECT

The binding rate for two molecules involved in the same process is equal at a single temperature. This temperature, by Equation (2.26) is

\[ T = \frac{\Delta H_i^\ddagger - \Delta H_j^\ddagger}{\Delta S_i^\ddagger - \Delta S_j^\ddagger}, \] (3.4)

where \( i \) and \( j \) are each molecule, in this case, DNA sequences. This relationship is the slope, or the compensation temperature of the EEC plot signifying that two processes will have the same binding rate at their compensation temperature \( T_C \). Table 3.1 shows the compensation temperature calculated with Equation (3.3) and the data in Figure 3.5, labeled \( T_{C} \), and the compensation temperature calculated from the intersection of each salt’s van’t Hoff plot, labeled \( T_{C}^{\ddagger} \). The sequence pairs are labeled \( AT \) for poly(A) and poly(T), \( TC \) for poly(T) and poly(C) and \( AC \) for poly(A) and poly(C). The asterisk (*) highlights data in disagreement with the other DNA sequence pairs and should not be considered in the analysis. From this, we conclude that poly(T) and poly(C) are most closely related to each other by Equation (3.2). The AT pair relationship shows the largest variation in the values and the largest disparity from the other DNA sequence pairs.

3.4.3 Relative rank of salt ions

Next, we turn our attention to the ability of the salt ions to hydrate and dehydrate hydrophobic surfaces. This is an important aspect of processes driven by the hydrophobic interaction. In order to assess the relative effectiveness of each salt in inducing thermodynamic changes that lead to hydrophobic adsorption it was necessary to invent a method to rank the salts against one another. We describe in this section the ranking method and compare it to another more well known scheme, the Hofmeister series.

First, in order to rank the salts, the mean \( \Delta H_i^\ddagger \) and \( \Delta S_i^\ddagger \) are calculated for each DNA sequence and the variance from the mean is applied as the rank. Unfortunately, as we
Table 3.1: The compensation temperature $T_C$ for each salt calculated from Equation (3.3) for the DNA sequence pairs in Figure 3.5, and the compensation temperatures $T_C^\dagger$ determined from the intersection of the van’t Hoff plots, Figure 2.7. Values marked by an asterisk (*) indicate when the temperatures $T_C$ and $T_C^\dagger$ are not within experimental uncertainty.

<table>
<thead>
<tr>
<th>Salt</th>
<th>AT</th>
<th>TC</th>
<th>AC</th>
<th>AT</th>
<th>TC</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4$Cl</td>
<td>589</td>
<td>303</td>
<td>260</td>
<td>1459</td>
<td>303</td>
<td>258</td>
</tr>
<tr>
<td>NaCl</td>
<td>369</td>
<td>318</td>
<td>257</td>
<td>376</td>
<td>319</td>
<td>259</td>
</tr>
<tr>
<td>KNO$_3$</td>
<td>522</td>
<td>317</td>
<td>283</td>
<td>620</td>
<td>318</td>
<td>283</td>
</tr>
<tr>
<td>NaH$_2$PO$_4$</td>
<td>398</td>
<td>320</td>
<td>268</td>
<td>392</td>
<td>323</td>
<td>265</td>
</tr>
<tr>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>-373</td>
<td>365</td>
<td>254</td>
<td>-546</td>
<td>379</td>
<td>247</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>535</td>
<td>320</td>
<td>278</td>
<td>899</td>
<td>323</td>
<td>278</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>587</td>
<td>351</td>
<td>303</td>
<td>-227</td>
<td>357</td>
<td>301</td>
</tr>
</tbody>
</table>

Have already shown, the relationship between $\Delta H^\ddagger$ and $\Delta S^\ddagger$ is linearly correlated for hydrophobic interactions so any variance from the mean would be one-dimensional. In other words, since an increase in $\Delta H^\ddagger$ leads to an increase in $\Delta S^\ddagger$ and vice versa the variance for both would change in the same direction. More useful is to find a relationship where one thermodynamic quantity changes in one direction while the other changes in the opposite direction. Applying this method, the mean is calculated for only one of the thermodynamic parameters, $\Delta S^\ddagger$. Then, since the reactions share a common temperature, the mean compensation temperature $T_{\mu}$, the corresponding $\Delta H^\ddagger$ is calculated from Equation (3.1) where $\Delta G^\ddagger_{\mu}$ is measured directly from the $y$-intercept of the EEC plot. Figure 3.6 shows the results of this calculation and plots the variance from the mean enthalpy ($\Delta H^\ddagger - \Delta H_\mu$) as a function of the variance from the mean entropy $T_{\mu}(\Delta S^\ddagger - \Delta S_\mu)$. The relative rank of the salts is defined as the $y$-intercepts of the best-fit line through each salt in Figure 3.6. The rank can be described another way as the variance from the mean enthalpy given an “average” salt with no
variance from the mean entropy, or $\Delta S^\ddagger = \Delta S_\mu$. Based on this system, the salts are ranked as

$$\text{NH}_4\text{Cl} - \text{NaCl} - (\text{NH}_4)_2\text{SO}_4 - \text{KNO}_3 - \text{NaH}_2\text{PO}_4 - \text{Na}_2\text{SO}_4 - \text{Na}_2\text{HPO}_4$$

from the least enthalpic $\Delta H^\ddagger < \Delta H_\mu$ to the most enthalpic $\Delta H^\ddagger > \Delta H_\mu$.

To determine if the ranking is indication of solvation forces it is necessary to compare the ranking to other systems known to be involved in hydrophobic processes. In interactions involving hydrophobic particles the Hofmeister series is typically employed. In addition, because the Hofmeister series is grounded in Jones-Dole theory and is therefore a relatively quantifiable ranking system, comparisons should allow us to predict the behavior of other salt ions.

![Graph showing the variance from the mean enthalpy versus the variance from the mean entropy for the interaction between ssDNA and Au-np. Each salt, as labeled has three data points, one for each DNA sequence. The color of each point denotes either kosmotrope/kosmotrope contact pairs (red), mixed kosmotrope/chaotrope contact pairs (blue) or chaotrope/chaotrope contact pairs (green). The rank of the salt is defined as the the y-intercept of the best-fit line through each salt triplet.]

**Figure 3.6:** The variance from the mean enthalpy *versus* the variance from the mean entropy for the interaction between ssDNA and Au-np. Each salt, as labeled has three data points, one for each DNA sequence. The color of each point denotes either kosmotrope/kosmotrope contact pairs (red), mixed kosmotrope/chaotrope contact pairs (blue) or chaotrope/chaotrope contact pairs (green). The rank of the salt is defined as the the y-intercept of the best-fit line through each salt triplet.
3.4.4 The Hofmeister series

In early experiments (Jones and Dole, 1929), the viscosity of aqueous solutions depended on the interaction between the individual ions and water. The viscosity $\eta$ of dilute solutions of electrolytes of concentration $c$ is described by the Jones-Dole equation

$$\frac{\eta}{\eta_0} = 1 + A c^{1/2} + B c$$  (3.5)

where $\eta_0$ is the viscosity of pure water, $A$ is related to long-range interionic electrostatic forces and can be ignored for low salt concentrations, and $B$ is a direct measure of the interaction between the ions and the solvent (Collins, 2004). From Equation (3.5) the affinity between salt ions and the water molecule is determined from the Jones-Dole viscosity $B$ coefficient. The sign of the $B$ coefficients separate the ions into two groups with positive $B$ coefficients for strongly hydrated ions and negative $B$ coefficients for weakly hydrated ions. The magnitude of the $B$ coefficients indicate the strength of the interaction. The point at which the $B$ coefficients change sign indicate when the strength of the interaction is equal to the strength of water-water interactions in the bulk. Strongly hydrated ions form tight hydration shells around the water molecule. This decreases the overall mobility of adjacent water molecules and affects the bulk viscosity of the liquid. Additionally, since the degree of hydrogen bonding between water molecules drives hydrophobic processes it is common practice to rank the efficacy of the salt ions by their $B$ coefficient. The relative rank of the salt ions using this method is commonly referred to as the lyotropic series (Israelachvili, 1985) and is a direct descendent of the Hofmeister series.

Originally, the Hofmeister series ranked salt ions by their ability to precipitate relatively apolar molecules from aqueous solutions – a process termed “salting-out,” which is dominated by the hydrophobic effect. The position of ions relative to one another on the series depends on the magnitude of hydration; weakly hydrated ions are called chaotropes, from the Greek term chaos- or disorder-making, while strongly hydrated ions are referred to as kosmotropes, meaning order-making (Dill et al., 2003). As mentioned earlier, individual ions can be classified as strongly hydrated or weakly hydrated by the sign of the Jones-Dole viscosity $B$ coefficient.
The properties of selected ions including surface charge density and Jones-Dole viscosity $B$ coefficient are shown in Table 3.2. The surface charge density is calculated from the ionic radii (Marcus, 1997). While the Hofmeister series and the lyotropic series are similar, there are some differences between the two ranking methods. For example, multivalent cations are high on the lyotropic series scale but have low salting-out effectiveness and are consequently low on the Hofmeister series (Curtis and Lue, 2006). The differences between the two ranking systems are not important for this study.

To determine the relative effect of each salt in inducing adsorption, the sum total contribution of each ion to the viscosity of water is plotted in Figure 3.7 versus the variance from the mean enthalpy, the $y$-intercepts of Figure 3.6. Assuming complete dissociation of the salt, the viscosity of water is determined through the $B$ coefficient as the $\sum B_i c_i$, where $c_i$ is the concentration of the ion species $i$. In reality, the degree of dissociation is species dependent and is influenced by the makeup of the individual ion contact pairs (Collins, 2004) but we assume that this effect is small compared to the hydration of the salt ions as a whole.

In Figure 3.7, the salts that cause weak $\Delta H^\ddagger$ compared to the average $\Delta H_\mu$ (more negative values on $x$-axis) contain at least one chaotropic ion (i.e. NH$_4^+$ or Cl). The salts that exhibit strong $\Delta H^\ddagger$ compared to the average $\Delta H_\mu$ (more positive values on
**Figure 3.7:** Ion-water affinity (Jones-Dole viscosity $B$ coefficient) *versus* the variance from the mean enthalpy. Values on the positive $x$-axis indicate enthalpic processes while values on the negative $x$-axis indicate entropic processes. The best fit line (- - -) through the data points, excluding $\text{NaH}_2\text{PO}_4$ and $\text{KNO}_3$, shows a qualitative correlation between the Hofmeister series and our ranking system.

$x$-axis) are strong kosmotropes. Comparing $\text{NH}_4\text{Cl}$, $\text{NaCl}$, $(\text{NH}_4)_2\text{SO}_4$ and $\text{Na}_2\text{SO}_4$ in Figure 3.7, the chaotropic cation ($\text{NH}_4$) is less enthalpic (or more entropic) than the kosmotropic cation ($\text{Na}$) paired with either anion ($\text{Cl}^-$ or $\text{SO}_4^{2-}$). In regards to $\text{KNO}_3$ and $\text{NaH}_2\text{PO}_4$, these salts do not appear to be classified correctly based on a lack of linearity with the other ions. Specifically, it seems that $\text{NaH}_2\text{PO}_4$ is more entropic while $\text{KNO}_3$ is more enthalpic than the Hofmeister series predicts. The hydration of oppositely charged contact pairs not only depends on the affinity of each ion to water but also on the magnitude of disassociation. The energy required to break up kosmotrope-kosmotrope pairs is sometimes greater than the energy gained when the kosmotrope binds to water. Similarly, the energy required to break up water-water bonds is greater than the energy gained when water forms a contact pair with a chaotrope. Both kosmotrope-kosmotrope and chaotrope-chaotrope salt pairs do not
disassociate as easily as mixed kosmotrope/chaotrope salts do (Collins et al., 2007). For the case of NaH$_2$PO$_4$, it is likely that, because H$_2$PO$_4^-$ (B = 0.34 dm$^3$mol$^{-1}$) binds to water more strongly than Na$^+$ (B = 0.085 dm$^3$mol$^{-1}$) does, the salt disassociates as easily as a mixed kosmotrope/chaotrope salt would. In regards to KNO$_3$, the salt does not easily disassociate due to the relatively low affinity of either K$^+$ or NO$_3^-$ to water, hence requiring a large enthalpic cost to dehydrate bound surface water molecules.

As mentioned earlier, the desolvation ranking of the salt ions is determined from the y-intercepts of Figure 3.6. Again, that ranking is

$$\text{NH}_4\text{Cl} - \text{NaCl} - (\text{NH}_4)_2\text{SO}_4 - \text{KNO}_3 - \text{NaH}_2\text{PO}_4 - \text{Na}_2\text{SO}_4 - \text{Na}_2\text{HPO}_4$$

from the least enthalpic to the most enthalpic. According to the Hofmeister series, estimated from the Jones-Dole viscosity $B$ coefficients in Table 3.2, the approximate predicted rank is:

$$\text{NaCl} - (\text{NH}_4)_2\text{SO}_4 \parallel \text{NaH}_2\text{PO}_4 - \text{Na}_2\text{SO}_4 - \text{Na}_2\text{HPO}_4 \parallel \text{KNO}_3 - \text{NH}_4\text{Cl}$$

where the salts are separated into three groups by their likelihood to disassociate. The left-most group is comprised of salts with mixed kosmotrope/chaotrope contact ion pairs. The middle group is comprised of salts with two kosmotrope contact ion pairs and finally, the last group is comprised of salts with two chaotrope contact ion pairs. The experimental results (first series) match the theoretical results (second series) at all but two locations: NH$_4$Cl and KNO$_3$ (emphasized in bold). An explanation of this may be that chaotropic cations increase the solubility of the bulk solvent more efficiently than is expected solely from the $B$ coefficient. The rationale for this can be interpreted by looking first at the counter argument: when a kosmotropic cation approaches a hydrated surface, water molecules near the surface are conflicted between solvating the surface or the ion. Thus, the solution becomes a poorer solvent and the surface attempts to minimize its solvent exposed surface area. In contrast, a chaotropic cation does not compete for hydrogen bonds with solvating water molecules and the solution becomes a better solvent (Collins, 2004). In this case, the surface attempts to maximize its solvent exposed surface area, which is followed by a large change in entropy.
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Figure 3.8: The binding time constant at 293K between ssDNA and Au-np in each salt versus the variance from the mean enthalpy. Strong enthalpy contributing salts (+x-axis) display slower adsorption than weak enthalpy contributing salts (−x-axis). The lines are a guide to the eye.

In Figure 3.8, a comparison is made between the binding time constant between DNA and Au-np and the rank of the salt in the experiment. The plots show that, within each DNA sequence, a roughly linear relationship exists between the binding time constant and the variance from the mean enthalpy - the rank of the salts. Typically, the binding rate and thermodynamics need not be correlated. To understand why this is the case, consider first the bimolecular reaction depicted graphically in Figure 3.9. The reaction consists of three discrete populations: a reactant \( R \) population consisting of unbound ssDNA and Au-np, a product \( P \) population consisting of adsorbed ssDNA on Au-np and an intermediate \( I \) population. The emergence of a product population depends on the ability to create an intermediate population at the top of the energy barrier. We argue that there are three distinct processes necessary to reach the intermediate state and therefore contributes to the activation barrier. These are: the desolvation of the ssDNA, the desolvation of the Au-np and transition of the
oligonucleotide from closed helix to an open form more conducive to adsorption. The desolvation of the oligonucleotide will depend on the concentration and solubility of the electrolyte as well as the makeup of the sequences. As mentioned earlier, the type of salt has a large effect on the hydration of the bases and since each nucleotide has a different hydration number, the enthalpy cost for desolvation varies for each sequence. Likewise, the desolvation of the Au-np is also dependent on the type of salt. The third process, unwinding of the DNA helix, is strongly dependent on the nucleotide makeup of the sequence with the purines (adenine and guanine) undergo base stacking and necessitate an enthalpic cost to unwind while the pyrimidines do not (Goddard et al., 2000). Therefore, we can systematically investigate the solvation contributions by varying salt type in the Debye limit and the unwinding contributions by varying the base sequence. We address the former throughout this chapter and examine the latter limited to three homonucleotide sequences: poly(A), poly(T) and poly(C).

**Figure 3.9:** Graphical representation of the the energy level system in the adsorption reaction between ssDNA and Au-np. The three population states are reactants $\mathcal{R}$ composed of unbound ssDNA and Au-np, products $\mathcal{P}$ composed of adsorbed ssDNA on Au-np and an intermediate $\mathcal{I}$ state. A change in enthalpy $\Delta H^\ddagger$ is necessary for transitions between the $\mathcal{R}$ and $\mathcal{I}$ state.
3.4.5 Predicted effect of salt ion on adsorption

From Figure 3.7 and Figure 3.8 it may be possible to predict how other salts affect the adsorption of ssDNA onto Au-np. For example, using the values from Table 3.2 and the results from Figure 3.7, 1 mM of MgCl$_2$ should have a variance from the mean enthalpy ($\Delta H^\ddagger - \Delta H_\mu$) of about -1.0 kJ mol$^{-1}$. This value in Figure 3.8 predicts that poly(A) in 1 mM of MgCl$_2$ should have a binding time constant of about 4.6 s. Figure 3.10 shows normalized fluorescence quenching data for a 15 mer sequence of poly(A) in 1 mM of MgCl$_2$. The binding kinetics data are fit to a single exponential using non-linear least squared fitting as was done with the other salts. In fact, the binding time constant for poly(A) in 1 mM MgCl$_2$ is between 8.34 s at 10°C and 2.48 s at 30°C indicating that the prediction was accurate. While it may be premature to assume that it is possible to predict the binding time constant for every salt ion based solely a qualitative ranking of the salts as deduced from the Jones-Dole viscosity theorem, these results show that, at least, some relationship exists. As is the case with most empirical relationships, much more data are necessary before a definitive conclusion can be made.

3.4.6 Effect of urea

Urea, (NH$_2$)$_2$CO, is a molecule with interesting physical properties, which have been exploited in interactions involving hydrophobic particles (Pace, 1986, Alonso and Dill, 1991, Schellman, 2002). Depending on the circumstances, urea can be considered either a weak chaotrope (Hayashi et al., 2007) or a weak kosmotrope (Stumpe and Grubmüller, 2007). Research on aqueous solutions of urea at very high concentrations determined that the structure of water molecules around the urea molecule looks identical to the structure around pure liquid water showing that urea has negligible effect on the intermolecular hydrogen bonding network between water molecules (Rezus and Bakker, 2006). This is similar behavior to a chaotropic salt. In another study, Stumpe and Grubmüller (2007) found that approximately one water molecule is strongly immobilized by urea. This is the behavior of a kosmotropic salt. Urea
Figure 3.10: Normalized fluorescence of poly(A) in 1 mM MgCl$_2$ and Au-np at three temperatures: 10°C, 20°C and 30°C. The binding time constant $\tau$ for each experimental temperature is determined by fitting data to a single exponential.

 acts on hydrophobic surfaces indirectly, by perturbing water-mediated hydrophobic interactions (Frank and Franks, 1968). It is also believed that urea also acts on water directly and solvates hydrophobic groups more easily than water, thereby weakening the hydrophobic interaction (Kreschek and Scheraga, 1965, Zou et al., 1998, Das and Mukhopadhyay, 2008). These studies demonstrated that overall, urea weakens the hydrophobic effect.

Consequently, studies of urea’s effect on ssDNA adsorption should be able to corroborate the role of the hydrophobic effect in the adsorption of ssDNA onto Au-np. Furthermore, since urea is non-ionic, the electrostatic contributions to the transition state energy barrier, which were discussed in Chapter 2, are not affected. As was done with the various salts, binding kinetics are used to calculate $\Delta H^\ddagger$ and $\Delta S^\ddagger$. The goal here is to ascertain if urea slows down the reaction or leads to overall weaker binding compared with no urea. The results of the binding kinetics experiments using urea are shown in Figure 3.11. In Figure 3.11a, the van’t Hoff plot for the samples
Figure 3.11: Data for the adsorption of poly(A) onto Au-np with 10 mM NaCl and small amounts of urea. (a) The van’t Hoff plot of the interaction. The concentrations of urea are 0 mM (red), 10 mM (blue) and 50 mM (green) urea. The 0 mM urea data is the same data as in Figure 3.3. The lines are best-fits through the data from which the thermodynamic parameters are determined. (b) The EEC plot for the interaction involving urea. The lines are best-fits through the data points. The linearity of the data suggests that urea has the same effect on the interaction as increasing the hydrophobic area of the ligand. This is an indication of the hydrophobic effect.

containing 10 mM urea and 50 mM urea is shown. All samples contained 10 mM NaCl. The 0 mM urea sample is the same data as is in Figure 2.7 and Figure 3.3. As was done previously, the best-fit lines to the data are used to determine $\Delta H^\dagger$ and $\Delta S^\dagger$. Figure 3.11b shows the EEC plot of the same three samples. The linear relationship between $\Delta H^\dagger$ and $\Delta S^\dagger$ in the EEC is evident and indicates that urea has the same effect on the interaction that increasing the apolar surface area of the ligand does and is an indication of the hydrophobic effect.

In the most general terms, urea seems to be useful at dampening hydrophobic interactions between ssDNA and Au-np. Since it is non-ionic, urea can also be used with other ions whose properties are beneficial to the assay. For example, Mg$^{2+}$ is often used in many assays to stabilize RNA secondary structure. Unfortunately, Mg$^{2+}$ destabilizes colloids too quickly to be useful in a solution based colloidal assay. Urea could be used in these applications to weaken the interaction between the colloidal particles yet maintain the important stabilizing characteristics of the ions.
3.4.7 Isothermal titration calorimetry

The thermodynamics of an interaction can be measured directly using a technique called isothermal titration calorimetry (ITC). ITC is a quantitative method to measure the binding equilibrium by determining the heat produced upon association of a ligand with its binding partner (Pierce et al., 1999). In a single experiment, the binding constant $K_a$, the stoichiometry $n$ and the enthalpy $\Delta H$ are determined. ITC has been used to study a diverse assortment of systems including DNA-protein interactions (Kunne et al., 1998, Peters et al., 2004), large molecule interactions (Garidel and Hildebrand, 2005, Wu and Tabata, 2004, Dai et al., 2004, Talhout et al., 2004), polymer interactions (Thongngam and McClements, 2004, Sinn et al., 2004, Arnaud and Bouteiller, 2004), and protein interactions (Thompson et al., 1998, Leavitt and Freire, 2001, Saro et al., 2004, Kolobe et al., 2004). It also was reported that the binding of DNA and peptide nucleic acid (PNA) bases to gold nanoparticles was studied using ITC (Gourishankar et al., 2004). In this section, we report on our attempts to use ITC to characterize the thermodynamics of the interaction between short sequences of ssDNA and Au-np.

An ITC instrument (VP-ITC Isothermal Titration Calorimeter, MicroCal, Northampton, MA) consists of two identical cells composed of a highly efficient thermally conductive material enclosed by an adiabatic jacket (see Figure 3.12). Thermocoupling circuits detect temperature differences between the sample cell and the reference cell containing buffer solution or water. Heaters are activated when the cells are not in thermal equilibrium with each other or with the adiabatic jacket. In an ITC experiment, DNA solution is titrated into the sample cell containing gold colloid. Because the interaction between ssDNA and Au-np is exothermic, a small amount of heat is released for each ssDNA ligand that binds to gold. This raises the temperature of the sample cell with respect to the reference cell. The observed quantity measured by an ITC is the time dependent power required to maintain the cells in thermal equilibrium as heat is released by the interaction (Pierce et al., 1999).

Figure 3.13 shows the results of an ITC experiment. The experiment consisted of 48 injections of 6 $\mu$L each of a 600 nM 15 mer poly(C) solution in 10 mM NaCl buffer.
The DNA was injected into a 1.4 mL sample cell containing 17 nM gold colloid and 10 mM NaCl. The operational temperature of the ITC was 300K and the sample cell was stirred at 300 rpm. In Figure 3.13a shows the raw heat flow data. The decrease of power for each injection indicates that the sample cells had increased in temperature with respect to the reference cell requiring that heat be removed. The heat flow versus time is integrated for each injection to determine the total heat change per injection $Q$ and this data is fit using routines in the data analysis software ORIGIN (MicroCal, Northampton, MA) to the single site binding model given by (Pierce et al., 1999)

$$Q = \frac{nM_0 \Delta H V_0}{2} \left[ 1 + \frac{L_0}{nM_0} + \frac{1}{nK_a M_0} \right] \sqrt{\left( 1 + \frac{L_0}{nM_0} + \frac{1}{nK_a M_0} \right)^2 - 4L_0 \frac{1}{nM_0}} \tag{3.6}$$

where $n$, $K_a$ and $\Delta H$ are the fitting parameters. The other terms in Equation (3.6) are the cell volume $V_0$, the bulk DNA concentration $L_0$ and the bulk Au-np concentration $M_0$. The results of the integration and the fitting are plotted in Figure 3.13b.

The heat change plot in Figure 3.13b shows the characteristic S-shaped curve seen in many ITC experiments, especially those involving biological material (Leavitt and Freire, 2001). At low concentrations of ligand, before the sample cell becomes
saturated by ssDNA, every injection releases about the same amount of heat. This is represented by the flat region of the plot at low molar ratio. As the concentration of ssDNA increases more and more of the Au-np becomes covered by DNA and less heat is released each injection. Finally, at or near a molar ratio of 6 the Au-np has reached its capacity to release heat and the plot flattens out again.

Figure 3.13: Calorimetric titration of poly(C) and Au-np in 10 mM NaCl. The experiment consisted of 48 injections of 6 µL each of a 600 nM DNA solution. The sample cell contained 1.4 mL of 17 nM gold colloid. (a) Heat flow as a function of time. (b) Heat change per mole of injectant determined by integrating the heat flow for each injection. The solid line is a best fit line obtained by fitting Equation (3.6) to the data.
In Figure 3.13b, the best fitting of the integration data results in $\Delta H = -10.42 \times 10^3 \text{kJ mol}^{-1}$ which is at least three orders of magnitude greater than the $\Delta H^{\ddagger}$ values determined from binding kinetics. The two kinds of enthalpy are not strictly equivalent. The former refers to the final state enthalpy, the $\mathcal{P}$ state in Figure 3.9, while the latter refers to the transition state enthalpy. A major conclusion that can be made of the large negative enthalpy change is that a negative enthalpy change is caused by stronger hydrogen bonds at the surface of the solute and is a firm indication of the hydrophobic effect (Chandler, 2005).

The results of the fitting determined an equilibrium constant of $K_a = 1.185 \times 10^9 \text{M}$, which is used to calculate the Gibbs free energy by way of the van’t Hoff relationship

$$\Delta G^{\ddagger} = -RT \ln K_a$$

and yields a free energy change of $\Delta G = -3.48 \text{kJ mol}^{-1}$. By the standard thermodynamic relationship $\Delta G = \Delta H - T\Delta S$, the large change in enthalpy and the smaller (less negative) change in free energy signals that $\Delta S$ is also negative. We reason that this is due to the increased order in the surrounding water Chandler (2005) and is another indication that the interaction is dominated by the hydrophobic effect.

Beyond the conclusions of this single experiment there were too many problems with ITC and the other sequences for specific conclusions to be made. First, an ITC requires a large amount of reagent. While it is possible to obtain DNA in higher concentrations it is not nearly as feasible to increase the concentration of gold colloid in such a way as to not cause stability issues. In addition, we have already shown that the adsorption and the thermodynamics of the DNA/Au-np interaction are affected by the salt concentration and by the salt type, so, for ITC to be a useful technique the stability issues with Au-np must first be resolved. In previous binding kinetics experiments the relatively quick data collection times for fluorescent quenching minimized the amount of gold that aggregated during the experiment. For the ITC, given that nearly 500 minutes are needed to complete a single run, it would be impossible to maintain a stable dispersion. If these limitations can be overcome, ITC remains an effective and proven (Gourishankar et al., 2004) way to directly measure the thermodynamics of DNA and Au-np adsorption.
3.5 Conclusions

The basic goal of DNA hybridization assays is to distinguish between ssDNA and dsDNA. The Li and Rothberg assay uses gold nanoparticles to filter ssDNA from dsDNA and is a useful method to detect DNA hybridization. For the purpose of optimization, the fundamental interactions of the assay should be understood. Having discovered that DLVO theory could not explain differential adsorption of ssDNA and dsDNA, we set out to determine whether the interaction between ssDNA and Au-np is caused by the hydrophobic effect. We found a linear correlation between the change in enthalpy $\Delta H^\ddagger$ and the change in entropy $\Delta S^\ddagger$, which is characteristic of the hydrophobic effect. In addition, we found a relationship between systematic changes in the thermodynamic parameters with known, quantifiable differences in the hydrophobic surface area of sequences of adenine, thymine and cytosine. Next, we classified the salts by their propensity to induce either strong or weak enthalpy changes when compared to the average enthalpy and compared these results to the Hofmeister series. In particular, kosmotropic cations showed larger changes in enthalpy than chaotropic cations in agreement with their postulated role in desolvation of the surfaces. These results showed that the hydrophobic effect was a major contributor to the interaction.

In regards to the Li and Rothberg DNA screening assay, the results of this study suggest a number of ways of altering fundamental parameters to achieve desired assay characteristics. The binding rate, for example, is sensitive to salt concentration, but more interestingly, the type of salt has an effect on the speed as well. Salts, which we classified as having a strong propensity to induce entropy changes, are salts with low preferential affinity to water, display the fastest binding rate. More precisely, the individual makeup of the salt is an important factor. Salts with a strong chaotropic cation increase the binding rate compared to weaker chaotropic or kosmotropic cations. So the binding rate can be adjusted not only by the ionic strength but by the type of ion as well.

While there is typically little opportunity or need to vary the makeup of the DNA sequence in the assay, the nucleotides also affect the speed of the adsorption reaction; the nucleotides adenine and cytosine have similar binding rates and adsorb faster than
thymine. This may not be a product of the thermodynamics and could simply be due to the chemical similarity of the bases, both of which contain amine groups.

Finally, another interaction, which will not be discussed here in too much detail but may have a bearing on the assay, especially in the later stages when a significant amount of single-stranded sequences have already adsorbed onto the gold, are steric forces. These forces arise from already adsorbed polymers and, as more DNA is adsorbed, are compressed further onto the surface. The adsorbed polymer resists compression and leads to a repulsive force. While the concentration and length of the ssDNA used in this study are not significant to contribute any significant steric repulsion, this force may be a factor in the general assay, specifically in the colorimetric method of detection where colloidal dispersions are forced to aggregate by the addition of a suitable amount of counter-ion additive. If the colloidal gold is already coated with ssDNA the colloid remains stable even after the electrolyte concentration is increased. According to DLVO theory, this is due to an increased surface charge density. However, any bound, semi-flexible polymer will also resist compression and contribute a repulsive force (Israelachvili, 1985).

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Chapter 4

Conclusion

We were motivated by the possibility to understand the fundamental interaction between ssDNA and unmodified gold nanoparticles. This is the basic interaction in a number of DNA screening assays (Li and Rothberg, 2004a,b,c) as well as a class of DNA/nanoparticle-based sensors (Wei et al., 2007, Wang et al., 2008). The assays are based on the remarkable observation that ssDNA sticks to like-charged gold nanoparticles while dsDNA does not (Li and Rothberg, 2004c). At its foundation, this is a study of the attraction between like-charged particles, which is considered a problem of general importance.

This study sought to expand upon a theory proposed by Li and Rothberg (2004b) in which DNA adsorption is a direct result of a dipolar interaction between Debye double-layers on both the Au-np and the DNA surfaces. While this is a common method to model biological systems (Liu and Zhao, 2005, Tcholakova et al., 2005, Sushko et al., 2006, Jacobs et al., 2007, Chen and Walker, 2007, Javid et al., 2007), the proposed theory omitted contributions from dispersion forces such as the van der Waals force.

Our first task was to develop a more robust electrostatic model that took into account both the geometry and the electrolyte concentration of the system. The theory is based on DLVO theory and included the van der Waals interaction for the necessary attractive force. The Au-np and the DNA molecule were treated as uniform
spheres with uniform charge distributions. This is similar to the DLVO-based theoretical models for protein interactions (Ruggiero et al., 1999, Johnson et al., 1994, Curtis and Lue, 2006). Because of the high degree of longitudinal flexibility exhibited by ssDNA, the DNA molecule was treated as a worm-like chain that folds into a “Gaussian blob” (Netz and Andelman, 2003) that takes into account nucleotide and the electrolyte concentration dependent self-avoidance.

In our model, the electrostatic repulsion between liked-charged molecules was screened by electrolyte. This idea is well understood in the context of Debye-Hückel theory. The interaction potential between DNA and Au-np in an electrolyte is calculated from the Poisson-Boltzmann equation. In most biophysical applications, the surface potential of the particles is relatively low and an analytic solution can be computed from the linearized Poisson-Boltzmann equation. Additionally, the solution can be computed for a wider range of geometries including spheres or cylinders, useful for modeling such molecules such as proteins or polymer. Unfortunately, the surface potential of both DNA, based on the number of phosphate groups relative to its size, and Au-np, confirmed via zeta potential measurements, is too large for the linearized approximation method. Then again, the full solution to the Poisson-Boltzmann equation is no longer analytic in spherical or cylindrical geometry so some level of approximation is necessary.

For this system, we solved the full Poisson-Boltzmann equation in planar geometry and extended the solution to spherical coordinates using the Bjerrum approximation (Israelachvili, 1985). This method allows for a large degree of flexibility in temperature, electrolyte concentration, ion type, DNA size and sequence, and Au-np size.

The double-layer interaction constituted the screened repulsion between the DNA and Au-np. The attractive portion of the interaction was described by the van der Waals force between two spheres. The Hamaker constant, which describes the dispersion forces, was calculated using the physical properties of DNA (assumed to be equivalent to protein) and gold. The total DLVO interaction, the sum of the double-layer interaction and the van der Waals interaction, is calculated for a range of salt concentrations, ion types and DNA sequences.
The next step was to validate this model. We used time-dependent fluorescent spectroscopy to determine the binding time constant for the reaction and used these data to calculate the transition entropy and enthalpy of the reaction. The model could describe the energy barrier when the concentration of salt was small but failed for higher concentrations typical of the assay. This is believed to be due to shortcomings of DLVO theory which makes no consideration for solvation forces. Moreover, the strong dependence on salt type discovered in this thesis is inconsistent with an electrostatic picture. These factors led us to propose an alternate model that included solvation forces.

Using some of the same binding kinetics data, linear relationships between the change in enthalpy and the change in entropy were used to show that the hydrophobic effect is dominant. We found that transition thermodynamics also varied predictably with DNA’s physical parameters including the stiffness of the sequence, the hydration of the nucleobases and the hydrophobic surface area. A method to classify the relative “strength” of various salts in inducing adsorption of ssDNA was also outlined. This method ranked salt ions by their propensity to induce changes in enthalpy or changes in entropy and we found good agreement when these results were compared to the Hofmeister series. Specifically, salts containing kosmotropic cations showed the largest changes in enthalpy and we interpreted this as signifying that these salts strongly dehydrate the surface. This is reasonable considering that kosmotropes are known to break hydrogen bonds between water molecules and the hydrated surface. The opposite was found to be true for chaotropic cations, which displayed larger changes in entropy and is consistent with water molecules increasing hydrogen-bonding opportunities in the bulk. In general, theses results confirmed our hypothesis that the hydrophobic effect plays a dominant role in the interaction.

Finally, we investigated urea in the interaction. We found that urea slowed the adsorption rate and revealed a linear relationship between the change in enthalpy and the change in entropy. This is consistent with the idea that urea reduces the hydrophobic effect. Not only does this confirm that the hydrophobic effect is dominant, or it at least, that it plays an important role, it has interesting implications for the assay. In
many assays, Mg$^{2+}$ is used because it is an effective phosphate buffer and oligonucleotide hybridization can be maintained. Unfortunately, even low concentrations of MgCl$_2$ (5 mM) cause total collapse of the gold colloid leading to nearly everything adsorbing onto Au-np including dsDNA and would not be useful in the employment of the Li and Rothberg free-solution assay. The Jones-Dole viscosity B coefficient of Mg$^{2+}$ reveals that the ion is a strong kosmotrope and amplifies the hydrophobic effect between DNA and the Au-np as well as severely compromising the stability of the gold colloid. In circumstances that require Mg$^{2+}$, urea may be useful in reducing the hydrophobic effect and slow the kinetics of the system to a rate that allows for accurate detection. In addition, the stability of the colloid is maintained and limits Au-np aggregation which is also useful to the assay.

Since unmodified Au-nps contain hydrophobic and hydrophilic groups and because the electrostatic interaction at physiological salt concentrations is negligible we feel that the results of this study are sufficiently general to be useful in a range of sensor technologies. There is a large amount of freedom in choosing a salt that can provide DNA sequence stability while improving binding affinity. In particular, salts with strong chaotropic cations hydrate the surface of DNA and the Au-np because these ions do not actively strip water molecules from the surfaces. These systems are characterized by large changes of entropy compared to other, more equally balanced salts. On the other hand, kosmotropic cations actively strip weakly hydrated water molecules from the surfaces of DNA and Au-np and decrease the solubility of water. These ions breakup hydrogen bonds and these systems are characterized by larger enthalpy changes than with other salts. Binding kinetics and thermodynamics are not typically correlated but we found a relationship, which was roughly linear, between the binding rate and the propensity to induce either large changes in entropy or changes in enthalpy. This may be useful when designing an assay by allowing for large freedom in varying the binding rate. In addition, since the reaction follows the Arrhenius relationship, the temperature becomes a variable that can be varied with predictable results.
Implications of study

The promise of miracle treatments which are tailored to the individual as well as the ability to pre-screen or assess the risk of developing adult-onset disorders such as Hutchinson’s disease, Alzheimer’s disease or cancer has driven biotechnology research. Most agree on the importance of understanding the role of genes, hence the enormous effort put into decoding the human genome. With the culmination of the Human Genome Project scientists finally had a complete picture of the entire sequence of the genome but understanding which genes are responsible for what is another matter all together and remains the focus of ongoing research. The field of genomics has benefited from advances in biotechnology, a term that describes a range of technological innovations where biological material, be it DNA, proteins, bacteria, viruses etc, are used in new and unintended ways. In particular, DNA screening assays are a way to separate and detect specific sequences of DNA rapidly and at low cost.

There are a number of factors to consider when designing a clinical assay. These include sensitivity and specificity, selectivity to contaminants, quantifiability, speed and material cost. For these reasons understanding the fundamental interactions in a biosensor is of general importance and leads to optimization and a wider use of the technology.

The basic role of DNA screening assays is the same: detect specific DNA sequences by introducing known probes and determining when hybridization occurs. There are already a large number of existing technologies that accomplish this ranging from Southern blotting to more rapid methods such as real-time PCR. Research into nanotechnology has revealed that nanoparticles have a number of characteristic features that makes them a great choice for biosensor designs. Already a number of nanoparticle based biosensors have emerged including colorimetric arrays (Elghanian et al., 1997, Storhoff et al., 1998, Reynolds et al., 2000), fluorescence arrays (Zhao et al., 2003, Xu et al., 2005) and electrochemical detectors (Park et al., 2002). Most of these assays use probe ligands that are covalently attached onto the nanoparticle surface. In a different technique, Li and Rothberg, developed a number of assays that use
unfunctionalized gold nanoparticles (Li and Rothberg, 2004a,b). The benefit of this type of assay is that hybridization of the probes and targets is separate from detection so each step can occur at optimum conditions for the assay.

**Future work**

The electrostatic interaction model has a wide degree of flexibility in describing the interaction in terms of the salt concentration, ion type, and DNA sequence and size. We were able to confirm some aspects of the model using binding kinetics including the dependence on salt concentration and ion type. What was not investigated were the effects of the DNA size on the interaction. A series of similar experiments using longer sequences of DNA could confirm this particular feature of the model.

In regards to the thermodynamic description, since the interaction is dependent on the hydrophobic surface area, using longer sequences would accentuate the differences between the different nucleotides. This would give us more than just three data points in which to calculate the compensation temperature. As it stands now, our description is rather empirical in nature. We know that the hydrophobic effect is a factor. What is not known is a quantitative relationship between salt concentration, apolar surface area and the thermodynamic parameters. Experiments with longer ssDNA sequences could fill in gaps in this picture.

The change in enthalpy and the change in entropy is calculated from van’t Hoff plots. While the slope of the best-fit line, corresponding to the change in enthalpy is simple enough to calculate and yields low error, the change in entropy is determined from the \( y \)-intercept and can cover many orders of magnitude so even small errors in the slope are compounded for the change in entropy. It is not uncommon to see errors of 20% in even the most meticulously measured experiments. To minimize this effect, many binding kinetic experiments are performed over a much larger range of temperatures. Unfortunately, because this interaction takes place in an aqueous solution, the range of viable temperatures is somewhat limited. Regardless, better data, encompassing a wider range of temperatures would form a stronger quantitative description and help confirm both the electrostatic and the thermodynamic picture.
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Another likely improvement in the accuracy of the binding kinetics measurements is in calculation of the binding rate constant. Currently, fluorescence data was taken within 1-2 seconds of mixing. There are two problems with this approach. First, the start time in experiments varies from experiment to experiment and depends really on the user. Another factor is that the experiments were done on 1 mL solutions of reactant. At this volume there is a finite non-zero mixing time. Both these considerations have the result of lowering the accuracy of the binding rate data. An improvement to the rate data would be to both begin data collection immediately after or even just before mixing and to reduce the size of the sample size so as to minimize the diffusion and mixing time of the reactants. These can both be achieved using a device such as a stopped flow fluorimeter. Initial attempts with this apparatus yielded mixed results. One of the biggest problems encountered was in regards to the stability of the colloid. Because of the small 80 µL sample chamber of the stopped-flow (SF-2000, KinTek Corporation, Austin, TX) and the already dilute nature of the reactants the fluorescence signal strength was too low to be useful.

The results of this study have general implications for biosensor technology. Already, other biosensors have been designed around the principle of selective adsorption of DNA onto Au-np including aptamer based protein detectors (Wang et al., 2008, Wei et al., 2007) and a potassium-ion detector (Li et al., 2007). Because, in some circumstances, an aptamer folds into a secondary structure that resembles dsDNA when it binds to a target molecule, the aptamer is protected from adsorption to Au-np much the same way as dsDNA was. When there is no suitable target molecule, the aptamer remains unfolded and single-stranded. An example of an aptamer-based detector is discussed with greater detail in Appendix A. The aptamer-based detector is one example of the type of sensor that could benefit from the results of this study.

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Appendix A

DNA Aptamer Based Potassium Ion Detector

We describe a DNA-based potassium ion detector. The technique uses a DNA aptamer that is known to have a strong affinity to bind potassium ions. When the aptamer binds to the ion it folds into a secondary structure that protects it from adsorption to Au-np. The detector exhibits high signal contrast between samples that contain no or small quantities of potassium ions. The high degree of selectivity of potassium over other ions is also demonstrated. This is an extension and possible future application of the Li and Rothberg (2004c) assay described in Chapter 1.

A.1 Background

Potassium has many physiological functions in the human body; it plays a role in the regulation of blood pressure, it aids in muscle contraction, and it maintains healthy nerve and kidney function (Alberts et al., 1983). A potassium detector, to be effective, must have a number of basic requirements including: high selectivity of potassium ions (K\(^+\)) against sodium ions (Na\(^+\)) or any other monovalent or divalent cation, high sensitivity, water solubility, and rapid detection. Various highly sensitive potassium detection technologies currently exist that use fluorescent dyes including potassium-
APPENDIX A. POTASSIUM ION DETECTOR

binding benzofuran isophthalate (Minta and Tsien, 1989), coumarin diacid cryptand [2.2.2] (Crossley et al., 1994) and γ-cyclodextrin (Yamauchi et al., 1999, 2000). Despite being highly sensitive to low concentrations of K\(^+\), these schemes show poor selectivity of K\(^+\) to Na\(^+\) and remain relatively insoluble in water. These limitations limit their practicality in clinical settings.

Recently, a number of DNA-based potassium detectors have been developed with higher selectivity than previous molecular based methods (Nagatoishi et al., 2005, Ueyama et al., 2002, He et al., 2005). The sensors are designed around guanine-rich DNA sequences, which have the tendency to form highly-stable quadruplex structures (Simonsson, 2001, Dapić et al., 2003, Gilbert and Feigon, 1999). The stability of the G-quartet (see Figure A.1) is salt dependent; K\(^+\) can fit into the pocket formed by two co-planar quartets whereas Na\(^+\) is not able to conform and is easily displaced by K\(^+\) (Risitano and Fox, 2005, Lu et al., 1992, Hud et al., 1996, Marathias and Bolton, 1999).

**Figure A.1:** Molecular structure of G-quadruplex made up of 4 guanine bases (left). Structure of thrombin-binding aptamer with 2 co-planar quadruplexes (right).

Here, we describe a DNA-based potassium ion detector that relies on fluorescence quenching by gold nanoparticles. The technique is an extension of DNA screening assays developed by Li and Rothberg (2004a,b,c) and relies on the remarkable observation that ssDNA adsorbs onto like-charged gold nanoparticles while dsDNA does not. The primary interaction between DNA and Au-np is the hydrophobic effect. Wa-
ter molecules can only make spurious bonds with apolar groups and find that making hydrogen-bonds with bulk water both decreases the enthalpy and increases the entropy. The secondary structure of dsDNA ensures that no apolar groups are exposed to water and as a whole, dsDNA is well hydrated and does not adsorb onto Au-np. In the presence of $K^+$, a G-rich sequence forms a stable quadruplex structure that exposes its backbone in a similar manner to dsDNA. Again, apolar groups are restricted to the interior of the structure and cannot interact with water molecules. When no potassium is present the quadruplex reverts back to a single helix and adsorbs onto Au-np.

We use the guanine-rich sequence known as the thrombin-binding aptamer (TBA) (Bock et al., 1992, Macaya et al., 1993), which is modified with a fluorescent tag. When TBA is exposed to samples containing potassium a quadruplex is formed that does not adsorb to Au-np. We can confirm that non-specific binding does not take place by using a random sequence of DNA, which does not fold into a quadruplex. The random sequence when exposed to potassium remains single-stranded and adsorbs to gold and its fluorescence is quenched by the metal. If no potassium is present in the sample then TBA remains unfolded and adsorbs onto Au-np where its fluorescence is also quenched.

The detection results from samples contain potassium chloride and sodium chloride are shown. In addition, we show the ability of the sensor to detect low concentrations of potassium in solutions containing other ions. We believe this technique can be extended to other metallic ions or any ligand, such as some proteins, that have the tendency to bind to an oligonucleotides.

A.2 Materials

DNA sequences were synthesized by MWG Biotech (Huntsville, AL). The following sequences were used: $5' -$GGT TGG TGT GGT TGG$-3'$ (referred to as TBA) and the random $5' - $CGG GAG ATG CAG GAG$-3'$ (RDNA). The DNA sequences were modified with a Cy3 dye molecule (Ex: 552 nm, Em: 570 nm) at the 5’ end.
Sodium chloride (NaCl) and potassium chloride (KCl) were obtained from Mallinckrodt Baker (Phillipsburg, NJ). Au-np was synthesized according to the Frens (1973) method. The procedure is described in Chapter 2. The concentration of Au-np was 17 nM. The fluorescence spectrometer and the experimental procedure is the same as in previous experiments. Refer to §2.3.1 for specifics.

A.3 Results and Discussion

Time-dependent fluorescence of RDNA and TBA in various concentrations of NaCl and KCl are shown in Figure A.2. The samples contained either 50 mM NaCl, 25 mM NaCl and 25 mM KCl, 50 mM KCl or no salt at all. Quenching of the fluorescence signal is caused by energy transfer of the chromophore to the metallic surface and indicates adsorption of the oligonucleotide to the Au-np. Both sequences did not quench and maintained a constant fluorescence with no salt in the sample. Fluorescence of RDNA was quenched for all concentrations of salt and for both types of salt. Fluorescence of TBA was not quenched when the sample contained any amount of KCl. These results form the basis of a potassium ion detector. By using a combination of a random sequence of ssDNA and the TBA we should be able to determine if a sample contains KCl or not.
Figure A.2: Normalized fluorescence of (a) RDNA and (b) TBA when exposed to Au-np and either: no salt (black), 50 mM NaCl (red), 25 mM NaCl and 25 mM KCl (green), or 50 mM KCl (blue). Fluorescence quenching is due to energy transfer of the chromophore to the metallic surface and indicates adsorption of the oligonucleotide to the Au-np. Fluorescence of RDNA was quenched for all types and concentrations of salt ion. Fluorescence of TBA quenched only when the sample did not contain any KCl.

To determine the lowest detectable concentration of $K^+$ in solutions containing other salt ions, namely NaCl, the concentration of KCl was lowered while maintaining the final ion concentration constant at 50 mM. In other words, if the solution contained 5 mM of KCl then 45 mM of NaCl was added for a final salt concentration of 50 mM. The Figure A.3 shows the results when we reduced the KCl concentration from 5 mM to 0 mM. The results show that the potassium detector is able to detect a lower concentration of 1 mM KCl with a selectivity of 49:1 Na$^+$ to K$^+$. 
Figure A.3: Normalized fluorescence of TBA when exposed to Au-np and electrolyte solution. The KCl concentration in each sample is listed in the graph. An appropriate quantity of NaCl was added to each sample to raise the final salt concentration to 50 mM. Quenching of fluorescence due to adsorption of the nucleotide to the Au-np and chromophore energy transfer of the metallic surface. The increase in fluorescence is due to slow colloid aggregation. When the colloid aggregates, the excitation path length increases, i.e. the distance a photon travels before experiencing scattering or absorption. This causes both more light to reach the chromophores and it allows more emitted light to exit the sample.

A.4 Conclusion

We described an application of differential adsorption of ssDNA over dsDNA to Au-np to detect potassium ions in aqueous solutions containing other cations. The results show that there is a high degree of signal contrast between a positive and a negative result. In addition, the detector shows high sensitivity and is able to detect at least 1 mM of K⁺ in solutions containing high concentrations of NaCl. Because the detector uses a DNA aptamer, we believe the technique can be extended to other systems including protein detection or other metallic ion detection.
References


Appendix B

Detecting $\alpha$-Thrombin by the Selective Adsorption and Fluorescence Quenching of DNA on Gold Nanoparticles

We demonstrate an extension of the Li and Rothberg (2004) DNA hybridization assay, which is modified for the detection of proteins. The detector used a dye-tagged DNA aptamer that is known to bind to the target. When the aptamer binds to a target its secondary structure changes so that it is protected from adsorption onto the Au-np. Similarly, random sequences of DNA do not bind to the target but instead adsorb onto the Au-np where its fluorescence was quenched by the gold. From the difference in the fluorescence signal between the two sequences we are able to infer the presence of the thrombin in the sample. In this study, we tested samples containing $\alpha$-thrombin but the technique is sufficiently general to be applicable with any target depending on the availability of a suitable aptamer. We achieved similar results and conclusions to previously reported studies by Wei et al. (2007) and Wang et al. (2008).
APPENDIX B. THROMBIN DETECTOR

B.1 Introduction

Antibodies are used to detect most proteins through a technique called Enzyme-Linked Immunosorbent Assay (ELISA). While this technique is widely used, it requires several rinse and incubation steps that are time-consuming. Furthermore, two non-overlapping antibodies that do not obstruct one another are needed for every target protein. Even with extensive antibody libraries (Holt et al., 2000) the ELISA protocol is limited to multivalent antigens such as proteins and polysaccharides (Davies and Cohen, 1996). Recently, a different class of molecular detector has been developed around molecules called aptamers.

Aptamers are DNA or RNA oligonucleotides that have been shown through combinatorial chemistry in a technique called SELEX (systematic evolution of ligands by exponential enrichment) to bind with high affinity to specific target molecules (Tuerk and Gold, 1990, Ellington and Szostak, 1990, Osborne and Ellington, 1997, Famulok et al., 2000). Typical aptamers vary in length between 15 and 100 nucleotides and have a binding affinity in the nM range. Aptamers can bind to a wide range of targets including proteins (Bock et al., 1992), small organic and inorganic molecules (Famulok, 1999), antibiotics (Maurel et al., 2002), peptides (Ylera et al., 2000), red blood membranes (Morris et al., 1998), viruses (Pan et al., 1995), cells (Davis et al., 1998) and even the cocaine molecule (Ulrich et al., 1998). Aptamers can be used in traditional sensor schemes such as sandwich assays, ELISA or in western blotting (Jayasena, 1999). Of greater significance and functionality is the use of aptamers to directly report and transduce into an optical signal the existence of analyte. Given the wide range of possible targets, an aptamer-based sensor is a very flexible biosensing platform (Liu and Lu, 2004).

Currently, there are a number of aptamer-based protein detectors. One system, called an aptamer beacon (Hamaguchi et al., 2001), uses oligonucleotides containing complementary segments at either end. Each end segment is labeled by either a fluorophore or a quencher molecule. In normal circumstances, the aptamer folds into a hairpin loop and the fluorescence is quenched. When the aptamer binds to a target molecule, the hairpin loop is broken and the fluorescence increases. This technique
is rapid but occasionally suffers from false signals due to inactive chromophores or quenchers.

Electrochemical aptamer-based techniques have also been developed to detect proteins (Xiao et al., 2005, Ikebukuro et al., 2005). These methods use DNA self-assembled metallic substrates which are both sensitive and reusable. In one method (Xiao et al., 2005) a methylene blue modified aptamer is immobilized onto a gold electrode. When no target is present, the oligonucleotide can unfold giving the unattached end the freedom to couple with the gold surface and transfer an electron. As the aptamer binds to a target molecule it makes a conformational change, which increases the distance between the electrode and the methylene blue tag and leads to a current drop across the electrode.

In this section we describe the use of aptamers in an application of the Li and Rothberg (2004) DNA hybridization assay outlined in Chapter 1. The procedure is modified for the detection of biological molecules. The sensor relies on the selective adsorption of DNA onto Au-np, specifically, ssDNA adsorbs onto ionically stabilized Au-np while dsDNA does not. The aptamer is modified by a dye-tag and the detection method is by fluorescence quenching. When the aptamer binds to the target it is protected from adsorption to the Au-np and behaves like dsDNA. In the absence of a target molecule, the aptamer remains single-stranded and adsorbs onto the Au-np. Fluorescence measurements can be taken and determine the presence or absence of the target molecule.

This detection method is sufficiently general to be applicable to a wide range of target molecules contingent on the availability of a suitable binding aptamer. Here, we apply the technique to the detection of α-thrombin, a protein critical to blood coagulation (Walz et al., 1986). The aptamer, which binds to thrombin is DNA-based, short and has a strong binding affinity (Bock et al., 1992, Macaya et al., 1993). When the thrombin-binding aptamer attaches to its target it folds into a G-quadruplex that exposes the hydrophilic phosphate backbone. In this configuration the aptamer has the same outward appearance as dsDNA and does not bind to Au-np. The structure of the G-quadruplex and its interaction with Au-np is discussed with greater detail in Appendix A.
This technique was previously reported as a colorimetric based sensor (Wei et al., 2007) and later on, as a fluorescence based sensor (Wang et al., 2008). It is such an interesting and relevant extension of the Li and Rothberg DNA hybridization assay that we independently report the results of our trials.

B.2 Materials

Human $\alpha$-thrombin was purchased from Haematologic Technologies Inc. (Essex Junction, VT). Two DNA sequence: the thrombin-binding aptamer (TBA), \[5' - \text{GGT TGG TGT GGT TGG} - 3'\], and a random DNA sequence, \[5' - \text{CGG GAG ATG CAG GAG} - 3'\], were purchased from MWG Biotech (Huntsville, AL). Both DNA sequences were modified with a Cy3 dye molecule (Ex: 552 nm, Em: 570 nm) at the 5' end and used without further purification. Sodium chloride (NaCl) was obtained from Mallinckrodt Baker (Phillipsburg, NJ). Au-np was synthesized according to the Frens (1973) method in the procedure described in Chapter 2. The synthesized concentration of Au-np was 17 nM. The fluorescence spectrometer is the same as in previous experiments. Refer to §2.3.1 for specifics.

The experimental procedure is as follows. First, solutions containing either 20 pmol, 10 pmol or 5 pmol of $\alpha$-thrombin were mixed with 10 pmol of either TBA or the random sequence. Electrolyte was added to give the sample solutions an initial concentration of 100 mM NaCl. After a period of incubation, the sample solutions were mixed with an equal volume of Au-np and the fluorescence was immediately measured. The experiments were performed at room temperature.

B.3 Results and Discussion

In Figure B.1 the normalized fluorescence signal of the DNA sequences are plotted for both TBA (——) and the random sequence (-----) as the fluorescence is quenched by the gold. The sample containing TBA in 20 pmol of thrombin (Figure B.1a), representing a 2:1 protein to DNA ratio, show increased fluorescence after mixing with Au-np.
This is an indication that the aptamer is binding to the thrombin and not to the Au-np. The sample containing the random DNA sequence shows rapid quenching of the fluorescence, indicative that the DNA sequence has adsorbed onto the Au-np.

In Figure B.1b the results for the samples containing 10 pmol of thrombin are plotted. Again, the fluorescence of the TBA increases after the sequence is initially combined with Au-np. The increase is not as pronounced nor does not last for as long a duration as the fluorescence of the 20 pmol sample. The increase in fluorescence is followed by quenching and the fluorescence signal becomes indistinguishable from that of the random sequence.

Finally, in Figure B.1c the results for samples containing 5 pmol of thrombin are plotted. In these samples, the concentration of the probe sequences were greater than the concentration of thrombin. The increase in the fluorescence of the TBA can also apparent in this plot but more subdued than the plots in Figure B.1a and B.1b.

These results suggest that when the concentration of thrombin is higher than the concentration of the probe sequence the difference between the fluorescence signal of the aptamer sequence and the non-binding sequence can be used to detect the presence of α-thrombin in the sample. When the concentration of the protein is less than the concentration of the probe sequence the results are less conclusive but still substantive. We conclude that the efficacy of the detector depends on the smallest detectable concentration of DNA. Because the fluorescence of chromophore dyes is very strong, high sensitivity to thrombin may be attainable.
Figure B.1: Normalized fluorescence (100% at $t = 0\ s$) of TBA (---) or random DNA (-----) when exposed to Au-np and either: (a) 20 pmol, (b) 10 pmol or (c) 5 pmol of $\alpha$-thrombin. The salt concentration in these experiments was 100 mM NaCl. The experiments were conducted at room temperature.
B.4 Conclusions

We presented an application of the Li and Rothberg DNA hybridization assay modified to detect proteins. The technique used a dye-tagged DNA aptamer that is known to bind to the target protein. When the aptamer bound to the target it was protected from adsorption onto the Au-np and its fluorescence was detected. Similarly, other random sequences did not bind to the target but instead adsorbed onto the Au-np and their fluorescence was quenched by the gold. We achieved similar results and conclusions to previously reported studies by Wei et al. (2007) and Wang et al. (2008).

Even though we anticipate that the secondary structure of most aptamers would prevent them from absorbing onto the Au-np, it is possible to use target specific conditions in our proposed assay. For example, it may be possible to work at temperatures, salt concentrations or pH where the secondary structure is nearly “single-stranded” but can be stabilized by interaction with the target molecule. If the molecule-aptamer complex is stable, we can also try to “clean up” unbound aptamer probes by hybridizing them with thiol-tagged complementary oligonucleotides to ensure that they will bind to Au-np regardless of secondary structure of the aptamer. This would quench fluorescence except that from the aptamers protected from absorption by remaining bound to the target.

In general, this method offers an alternative to antibody-based protein sensors (ELISA, western blotting, etc.) and their analogues; a method that is both sensitive to small concentrations of analyte and is inexpensive to execute. This detector has a low limit of detection but the exact detection concentration is target dependent and as yet undetermined. We have still to demonstrate that the technique can be quantitative but this deficiency may be corrected by careful calibration and the judicious use of control substances. The ability to qualitatively evaluate the presence of target in a sample is practical if merely the existence of a target molecule is of importance to an assay. For example, a detector of E. coli in water samples, bovine spongiform encephalopathy (mad cow disease) in cattle or even human chorionic gonadotropin (beta hCG), an indicator of pregnancy, would not necessarily need an exact concentration to reveal important information - possessing the ability to detect the target’s existence at any
concentration is satisfactory for many functions. In addition, because this detector is much faster than more established techniques it can be used as a pre-screener to determine if more costly and quantitative tests are warranted.

References


## Appendix C

### Data

**Table C.1:** Binding time constant $\tau$ of the adsorption interaction between ssDNA and Au-np.

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Table C.2: Transition state enthalpy and transition state entropy for the interaction between ssDNA on Au-np

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<td>3.59</td>
<td>9.70</td>
<td>-267.71</td>
<td>33.41</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>7.20</td>
<td>0.22</td>
<td>-252.61</td>
<td>0.74</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>4.65</td>
<td>2.80</td>
<td>-266.38</td>
<td>9.51</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>7.86</td>
<td>6.11</td>
<td>-258.04</td>
<td>21.08</td>
</tr>
</tbody>
</table>

poly(C)

<table>
<thead>
<tr>
<th>Salt</th>
<th>∆H‡ (kJ mol⁻¹)</th>
<th>σ_{ΔH‡}</th>
<th>∆S‡ (J K⁻¹ mol⁻¹)</th>
<th>σ_{ΔS‡}</th>
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</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>5.26</td>
<td>2.26</td>
<td>-257.81</td>
<td>7.65</td>
</tr>
<tr>
<td>NaCl</td>
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<td>KNO₃</td>
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<td>6.15</td>
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<tr>
<td>(NH₄)₂SO₄</td>
<td>7.20</td>
<td>0.22</td>
<td>-252.61</td>
<td>0.74</td>
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<tr>
<td>Na₂SO₄</td>
<td>4.65</td>
<td>2.80</td>
<td>-266.38</td>
<td>9.51</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>7.86</td>
<td>6.11</td>
<td>-258.04</td>
<td>21.08</td>
</tr>
</tbody>
</table>
### Table C.3: Binding time constant $\tau$ of ssDNA with urea

<table>
<thead>
<tr>
<th>Urea$^a$</th>
<th>$\tau$ (sec)</th>
<th>$\sigma_\tau$</th>
<th>$\tau$ (sec)</th>
<th>$\sigma_\tau$</th>
<th>$\tau$ (sec)</th>
<th>$\sigma_\tau$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>40.42</td>
<td>0.44</td>
<td>27.48</td>
<td>0.32</td>
<td>23.40</td>
<td>0.31</td>
</tr>
<tr>
<td>10 mM</td>
<td>64.66</td>
<td>0.73</td>
<td>42.25</td>
<td>0.58</td>
<td>29.00</td>
<td>0.36</td>
</tr>
<tr>
<td>50 mM</td>
<td>90.82</td>
<td>2.40</td>
<td>51.27</td>
<td>0.85</td>
<td>30.37</td>
<td>0.46</td>
</tr>
</tbody>
</table>

$^a$ urea also includes 10 mM NaCl

### Table C.4: Transition state enthalpy and transition state entropy for the interaction of ssDNA and Au-np with urea

<table>
<thead>
<tr>
<th>Urea$^a$</th>
<th>$\Delta H^\ddagger$</th>
<th>$\sigma_{\Delta H^\ddagger}$</th>
<th>$\Delta S^\ddagger$</th>
<th>$\sigma_{\Delta S^\ddagger}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>17.34</td>
<td>2.85</td>
<td>-214</td>
<td>9.76</td>
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<tr>
<td>10 mM</td>
<td>26.19</td>
<td>0.42</td>
<td>-187</td>
<td>1.45</td>
</tr>
<tr>
<td>50 mM</td>
<td>36.52</td>
<td>0.27</td>
<td>-153</td>
<td>0.92</td>
</tr>
</tbody>
</table>

$^a$ urea also includes 10 mM NaCl