Biomedical Diagnostics Using Manmade Nanostructures Integrated With Biomolecules

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THESIS
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# LIST OF ABBREVIATIONS

<table>
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<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tr>
<td>QD</td>
<td>Quantum Dot</td>
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<tr>
<td>GNP</td>
<td>Gold Nanoparticle</td>
</tr>
<tr>
<td>SELEX</td>
<td>Systematic Evolution of Ligands by Exponential Enrichment</td>
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<td>MB</td>
<td>Molecular Beacon</td>
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<tr>
<td>PL</td>
<td>Photoluminescence</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>FRET</td>
<td>Förster (or Fluorescence) Resonant Energy Transfer</td>
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<tr>
<td>SERS</td>
<td>Surface Enhanced Raman Spectroscopy</td>
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<tr>
<td>TBA</td>
<td>Thrombin-Binding Aptamer</td>
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<tr>
<td>IDT</td>
<td>Integrated DNA Technologies</td>
</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl- 3- [3]-dimethylaminopropyl]carbodiimide hydrochloride</td>
</tr>
<tr>
<td>TCEP</td>
<td>Tris(2-carboxyethyl)phosphine</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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SUMMARY

There has been extensive research done on the applications of nanoparticles in biomedical engineering. This thesis continued that work and focused on the application of semiconductor QD (quantum dots) specifically as a nano-scale detector. By integrating QDs with functional deoxyribonucleic acid (DNA), nano-scale detectors may be fabricated. These nano-scale detectors have many advantages, such as making systematic studies on the molecular level possible. The targets of such nanobiosensors can be ions, cells, oligonucleotides, peptides or proteins. This thesis focuses on potassium ions as the detection targets due to its importance and prevalence in biological systems. This thesis investigated this novel nanobiosensor thoroughly from probe construction, performance evaluation, optical property characterization and possible quality control methods.

More specifically, QDs were conjugated with short single-stranded nucleic acids and their fluorescent response was studied and evaluated to determine the viability of using them in nanoscale detector applications. The electrophoresis behavior and Raman spectra of these conjugations are also studied to examine these conjugations. As will be discussed, monitoring their optical properties, these conjugations may be utilized as molecular
probes to detect ions and biomolecules. This thesis emphasizes the fabrication of quantum dot-based detectors for a wide range of targets by selecting appropriate aptamers as binding agents.
I. INTRODUCTION

A. Background

People did not fully understand the potential impact of nanoscience and nanotechnology until a talk given by the physicist Richard Feynman in Caltech in 1959. Ever since then, people started to realize the importance of understanding nanoscale phenomena, and recognized that we are surrounded by nanoscale structures and nanoscale events. In the endless search for truth, scientists found more and more critical evidence buried in a world at dimensional scales less than those of biological cells. The demand to understand and control materials and events on the nanoscale has continued to grow for decades.

For many years, people have been using antibodies and dyes to make manmade fluorescent probes to study biomolecules. Not until late in the 20th century, did people start to realize that DNA can do more than genetic information storage, and scientists began exploring the possibility of using aptamers as probes in the 21st century 1-9. Example of such devices include: insulin sensors, platelet derived growth factor (PDGF)-BB sensors 4, 10, lead (Pb^{2+}) sensors, cocaine sensors 5, 7, and cancer cell sensors. In this research, the emphasis is on replacing the traditional organic dyes with manmade quantum dots that have a variety of advantages. These advantages include: temporal stability, high brightness,
symmetric emission, multiple color for same quantum dot material, and excitation of
different QDs with the same optical excitation pump. Furthermore, this research exploits
the advantages of using aptamers in lieu of traditional antibodies.

1. **Motivation**

   In the endless search for truth, the necessity of understanding and controlling
   biological phenomena is evident. The emergence of nano-scale materials introduces
countless possibilities in all aspects of science. In the field of medicine and biology,
understanding biological systems at the molecular level is crucial for treatments and
diagnostics of higher quality. Utilizing this new class of nano-materials to build devices in
nano-size may be the solution; nano-devices make the investigation at the molecular level
possible; the engineering of subcellular components becomes practical. Research on
specific membranes, cytoskeletons, genetic materials and organelles may be conducted.
Nano-sensing devices will shed light on molecular biological science topic such as cell
growth, metabolism, cellular processes, and even the origin of cells. Moreover,
nanosensors are easy to carry and they result in minimal volumes for the assay needed for
measurement. This thesis therefore discusses and provides insights underlying the
potential for the development of self-diagnostics devices with the application of nanotechnology in biological diagnostics. New nanostructures need to be built and studied as new problems are recognized and as new aspects of diseases are discovered.

The importance of developing semiconductor nanocrystals as probes is illustrated by their popularity in medical research and in pharmaceutical research. There are also many examples of using quantum dots to study biological systems \(^{10-19}\). However, very little research integrates the two novel concepts to study QD-based aptamer beacons \(^{10,11}\) and explore the potential applications in biomedicine.

2. Specific Aims

Aim 1: To design novel molecular beacons (MBs) using DNA and semiconductor quantum dots. Also, to understand the variability in these QD-DNA nanocomplexes.

There have had many strategies used to functionalize QDs for hybridization with biomolecules. The method we used to construct the aptamer beacons with quantum dots is by adding EDC as the crosslinker. The carboxyl functioned QDs therefore bind to the aptamer sequences which have amide groups in the terminal. The QDs are the donors in the beacons; nanogold particle have been explored as quencher in the hybridization-based
assay, and have performed well as accepters\textsuperscript{20}. The nanogold used in this study has a monomaleimido group on each of the nanogold. The nanogold particles are then bound to aptamers by adding TCEP. The thiol groups on the 3 terminal of the aptamers are designed to link with the maleimide functional group through a disulfide bond.

Change in the composition of these QD-DNA nanocomplexes - such as a change in the number of DNA molecules bound to the QD - can result in different mobility. We have used agarose gel electrophoresis to study the compositional variability of these nanocomplexes.

\textbf{Figure 1.} \textit{The schematic presentation of molecular beacons constructed using DNA and semiconductor quantum dots.}
Aim 2: To test these molecular beacons in water-based electrolytes. To observe the luminescence with naked eye first and then with photoluminescence techniques.

A different amount of target present in the solution will result in a change of the fluorescence of the MB. The human eye can sense the difference between strong and weak light, but this does not give a quantitative determination of the fluorescence output. To have a quantitative sense of how much light the MB gives out, we must conduct PL (photoluminescence) measurements.

Aim 3: To engineer such molecular beacons to make them functional in specific concentration ranges.

The advent of nanotechnology offers numerous potential applications and study methods in the biological system. To further apply these QD-based aptamer beacons, it must first be decided what working concentration range is expected for each analyte. As an example, the human body uptakes 90 to 110 mmole/L of potassium on an average per day and exports 90% of the these potassium ions.

Importantly, the QD-based aptamer probe needs to be engineered to work for concentrations around 50 mM to 100 mM if the target of interest is the potassium ions in urine while the preliminary result shows the aptamer detector works in solutions with
potassium ion concentration in the range from 5 mM to 50 mM. Moreover, the aptamer detector will need to be engineered to work for the potassium ions concentration range from 3 mM to 5 mM if the detector is designed to work in human serum or plasma; other desired concentration ranges depend on the targets of interest and the specific application cases. In this research, the application of QD-based aptamer probes will be explored for the diagnosis of potassium levels in urine and for the potential diagnosis of the potassium level in human serum.

B. Materials and background

In this section, we discussed some major materials, theoretical background and techniques used and involved in this thesis.

1. Quantum dots

Quantum dots (QDs) are semiconductor particles having diameters typically in the range of 2-10 nm and have been used widely in applications ranging from nanoelectronics to photoelectronics to biotags. QDs have become an important photonic tool in recent years due to their unique properties, such as 100 times higher chemical stability, 20 times
brighter, lower photodegradation relative to dyes, and readily tunable optical properties \(^{21}\).

Other advantages of QDs include symmetric and one-third of the emission spectrum linewidth as compared with organic dyes \(^{21,22}\). These advantages have stimulated attention on using QDs as biological labels. As an example, Dahan \(^{19}\) used QDs to label neural cell membranes and to monitor the individual glycine receptors in living cells. Other applications of quantum dots in biological systems have been reported; these include: nucleic acid detection, urea detection, tissue and species differences detection in the inner ear and kidney \(^{16}\), and single biomolecule imaging \(^{14}\).

2. **Gold nanoparticles**

Gold nanoparticles, also named colloidal gold, are gold crystals with diameter less than about 100 nm. The gold nanoparticle that was used in this study is a 1.4-nm-diameter gold nanoparticle. Nanogold has been explored in many health and medical applications. One of the applications is its use as an acceptor in the donor-acceptor pair. As one example, gold nanoparticles may be used with QDs where the nanoparticles act as acceptors and the QDs act as donors. This is similar to the situation where debeyl is used as a quencher in sequence-specific DNA detection. Moreover, detectors using nanogolds as the quencher are appealing because they exhibit approximately 2-fold greater fluorescence increase
REMOVE (be relatively efficient quenchers) in the hybridization-based assay, compared with detectors using dabcyl as quenchers, while the donors are 7 nm QDs.

3. **DNA and aptamer**

Deoxyribonucleic acid (DNA) is known for its storage of genetic information, but in recent years, researchers have investigated a new class of DNA, called functional DNA, that has interests beyond the storage of genetics information. Specifically, DNA can also act like an enzyme (called DNAnzymes, catalytic DNA, or deoxyriboxymes). It can also function in specific ligand binding, in which case it is referred to as an aptamer, or even in the dual roles of enzyme and ligand binding, in which case it is called an aptazyme.

Aptamers are nucleic acids with specific binding affinities selected from nucleic acids in a random sequence pool in an iterative process consisting of a round of amplification and filtration; this process is known as “systematic evolution of ligands by exponential enrichment (SELEX)”. The chemically synthesized random sequence is mimicking a Darwinian type of process, which generate a random DNA sequence library consists of about $10^{13}$ to $10^{15}$ different motifs. Those selected nucleic acid sequences that manifest high affinity binding to their target that may be 10,000 times stronger than random sequences that are filtered out. The dissociation constant $K_d$, which is a parameter that
measures the affinity of two smaller components combined into one object, may reach the nanomolar regime and the targets range from ions, small molecules, peptides, proteins, organelles, viruses, and even entire cells 25.

Aptamers have many advantages compared with antibodies. First of all, they are cheap and easy to obtain; their identification and production do not require using animals. They are simple to manipulate in vitro; by chemical modification, the kinetic parameter such as on and off rate can be changed, which can not be achieved with antibodies. The selection and utilization of aptamers can also be optimized for working in different applications and conditions, e.g. non-physiological systems. Moreover, the quality of aptamers is generally less variable than that antibodies because they are synthesized chemically and purified. Aptamers exhibit higher stability in a wider pH range and at higher temperatures. This much higher control is, in part, the reason that a growing community of researches is using aptamers instead of antibodies. As an example, the aptamer that binds to prostate specific membrane antigen (PSMA) was conjugated to CdSe/CdTe nanocrystals and being engineered to label tumor cells that overexpressed PSMA 26. Therefore, aptamers are being studied for the utility in replacing antibodies in biosensors 21. The conformational change of an aptamer in the presence of a target has been utilized to develop many biosensors including, an insulin sensor 27, a platelet derived growth factor (PDGF)-BB sensors 104, a
lead (Pb^{2+}) sensor, and a cocaine sensor.

C. Fluorescence resonant energy transfer

Förster (or fluorescence) resonant energy transfer (FRET) is the phenomenon of energy transfer between a donor (while excited by a higher energy photon) and an acceptor when the distance between the pair is small enough. When the donor and acceptor are within a few nanometers of each other, FRET results in weaker signals from donors and stronger signals from the acceptors are detected. FRET is a nonradiative energy transfer process and is caused by a dipole-dipole interaction. In the Förster formalism, the rate of energy transfer is defined by Equation (1)

$$k_{DA} = \frac{B \times Q_p I}{\tau_D r^6} = \left(\frac{1}{\tau_D}\right) \times \left(\frac{R_0}{r}\right)^6 \ldots (1),$$

where $\tau_D$ = the excited-state radiative lifetime of the donor. In Equation (1), $R_0$ represents the Förster separation distance corresponding to a rate of FRET equaling the rate of radiative decay ($k_{DA} = 1/\tau_D$). $R_0$ can be expressed by Equation (2)

$$R_0 = \left(\frac{9000(\ln10)\kappa_p^2 Q_D I}{N_A \frac{1}{2} n_D^3 \pi^2 \sigma^2 I}\right)^{1/6} \ldots (2),$$
where \( n_D \) is the refractive index of the medium; \( N_A \) is the Avagadro number; \( Q_D \) is the donor PL quantum yield; \( \kappa_p \) is a parameter depends on the relative orientation of the donor and acceptor dipoles. In Equation (2), \( I \) is the quantitative measurement of the donor and acceptor wavelength overlap,

\[
I = \int J(\lambda) d\lambda = \int P(L_{D\text{-corr}}(\lambda) \times \lambda^4 \times \varepsilon_A(\lambda) d\lambda,
\]

where \( PL_{D\text{-corr}} \) is the donor normalized dimensionless emission spectrum; \( \varepsilon_A \) is the acceptor absorption extinction coefficient spectrum.

The FRET efficiency, \( E \), which accounts for the fraction of excitons transferred from donor to acceptor nonradiatively is given by Equation (3)

\[
E = \frac{k_{DA}}{k_{DA}^* + k_{D}^{-1}} = \frac{R_0^6}{R_0^6 + r^6} \quad \ldots (3)
\]

When there are \( n \) acceptors surrounding a single donor, the efficiency equation can be extended as

\[
E(n, r) = \frac{nR_0^6}{nR_0^6 + r^6} \quad \ldots (4)
\]

D. **Micro Raman spectroscopy**

Raman spectroscopy is a spectroscopy technique measuring the vibration, rotational and other low-frequency mode of specific chemical bonds after excitation by a higher-
energy laser beam. The sensitivity and selective of Raman spectra provide a “fingerprint” of compounds, and therefore is widely used in chemistry and solid-state physics.

One of the major disadvantages of the Raman technique is its weak signal, resulting from the small scattering cross-section of the biomolecules. This disadvantage limited its application in biology until the discovery of the surface enhanced Raman spectroscopy (SERS) phenomenon. The phenomenon was first observed by Fleischmann et al. In 1974, and broaden the application of this technique. Subsequently, SERS was using in studying complicated biomolecules like protein and DNA with Raman spectroscopy. Based on the intensity enhancement resulting from SERS, the detection of a single biomolecule becomes possible. However, this is not easy compared with inorganic molecules, especially for huge biomolecules like DNA, comprised of multiple atoms and multiple chemical bonds.

There are many theories aimed to explain the enhancement (of $10^6$), but the exact mechanics is still not understood completely. The geometry needed to produce this “hot spot” for SERS optimization becomes an interesting topic. Herein, this thesis includes the discussion of two such geometries in the following sections.
E. **Application in food microbiology**

Food microbiology is the study of microbiology which inhibits, creates or contaminates foods. It contains two large subgroups, the microbial biopolymers and foodborne pathogen study. Microbial bacteria are “good” bacteria that are used in the food industry to produce polymers while there are some “bad” bacteria including enteric viruses, protozoan parasites, etc that are potentially harmful. These bacteria are microbes that contaminate food and cause illness. Herein, molecular beacons designed to detect Salmonella and Listeria monocytogen are considered; in this thesis, several such beacons were synthesized and tested.

F. **Photoluminescence measurements**

Photoluminescence is the optical emission from material when they receive energy from a higher energy beam, usually a laser. Two PL measurement set-ups are used in this study, one is, and the other is an LED with a USB4000FL spectrometer from Ocean Optics.

In this study, the QD-based aptamer beacons are therefore excited by the light source around 350 nm and emit light of lower energy, which is received by the spectrometer during the measurements.
II. EXPERIMENT SECTION

Potassium ion indicator

The importance of potassium ions

The potassium ion is one of the most abundant cations in the cellular fluid compartment. It regulates body fluid electrolyte balance, acid-base balance, muscle contraction, as well as the synthesis of proteins and nucleotide acids, and as an energy source. The distribution difference of potassium ions inside and outside of cells offers the fundamental properties and functions of nerve system and muscles cells. By controlling the flow of the potassium ions inward and outward through the permeable cellular membrane, neurons are able to conduct impulses and muscles are able to contraction. Therefore, the potassium level is also related to the pH value of biological systems and provides cells an environment in which they can work normally. Yet, it is difficult using traditional pH-based techniques to differentiate Na$^+$ and K$^+$ 32.

Potassium ions also affect the functionality of the heart. As the heart is the most important muscle in human body controlled by its own nerve impulses, potassium is extremely important for normal heart function. A slight change of blood potassium could change the electrocardiogram significantly and cause fetal problems. Abnormal potassium level has been shown to affecting the electrocardiogram and contractility of isolated rabbit
hearts. For the human heart, experiments also supported the theory that the inward and outward $K^+$ current of cardiac myocytes are critically associated with the congestive heart failure.

To maintain the potassium ion balance in human body, three physiological processes are involved: uptake, distribution and excretion. The human body uptakes 90 to 110 mmol/L potassium ions on an average per day through the intestinal tract, and excretes 90% of the potassium ions through kidney. The normal potassium ion level in the serum is between 3.5 and 5 mmol/L, while the normal plasma potassium may be lower. An abnormal potassium level usually indicates a disease in connection with the kidney and heart.

Hypokalemia is defined as low levels of serum potassium and can lead to chronic diarrhea or renal artery stenosis. Hypokalemia generally defines when a serum potassium level falls below 3.5 mmol/L, while some people use 3.0 mmol/L or even lower. According to Lawson’s survey, a third of hypokalemia patients are caused by drugs. Moreover, 19% of them are caused by diuretics, while diuretics are still one of the main drugs used to treat hypertension. Furthermore, for patients with hypokalemia also show higher risk of arrhythmias. Hyperkalemia is defined as serum potassium above 5.5 mmole/L and may lead to kidney failure and red blood cell destruction. While rennin-
angiotensin-aldosterone system (RSSA) inhibitors are the main strategy in treating hypertension, cardiovascular and renal disease, reports show this treatment increases the risk of hyperkalemia. Furthermore, patients with heart failure and chronic kidney disease are at a higher risk of hyperkalemia (5% to 10%) than those without.

**Traditional Potassium Analysis**

Potassium ion levels have been amenable to measurement by early chemical techniques since the 1920s. In the late 40s, by introducing a sample into a high-temperature, air-propane flame, it became possible to use flame photometer to provide an inexpensive way to determine wide ranges of potassium level. The emission intensity at 766 nm of the sample is, in most cases, proportional to the potassium quantity in the assay. Other potassium level measurement techniques used in physiology and medicine meeting different needs include ion-selective electrodes, coulometry, and spectrophotometry. However, all of these methods that give accurate readings but they do not offer on-site measurement capabilities and they require professional laboratory-type instruments and operations. As the need for early detection and self tests is growing, a new detection method that provides privacy, convenience, and speed is need. Furthermore, these instruments are good tools for determining ion levels in solutions, but they do not
qualitatively analyze potassium in cells and biological systems. Detectors of smaller size are needed and nanosensors have potential for filling the perceived need.

**Methods: Construction of QD-based aptamer beacons and test them in electrolyte solution**

Among several aptamers have higher affinity to potassium ions, the thrombin-binding-aptamer (TBA) is chosen in the study. The aptamers have been obtained from integrated DNA technologies (IDT) modified with an amide group with 12 extra carbon atoms on the 5’ end and a thiol group on the 3’ terminal. 1-Ethyl-3-[3]-dimethylaminopropyl]carbodiimide hydrochloride (EDC or EDAC) from Pierce served as the crosslinker to activate the carboxyl groups on the QD surface to bind with the amino group on DNA 5’ terminal. Tris(2-carboxyethyl)phosphine (TCEP), also from Pierce Protein Research Products, was used to break the disulfide bond on the DNA’s 3’ terminal. 10 μM carboxyl-functioned QDs from eBioscience, eFluor 605NC, were used in this study; they could be excited by 350–500-nm light and emit light with a wavelength of 605 nm. 1.4-nm-diameter monomaleimide nanogold have been obtained from Nanoprobes, Inc. The product was a frozen combination of 0.02 M sodium phosphate at pH 6.5 and 150 mM sodium chloride dissolved in 10% isopropanol/water. The nanogold particles were stored
in -20 °C. They were dissolved in 0.2 mL DI water to activate the nanogold. It is usually stable unless the temperature goes above 50 °C. Saturated KCl was obtained from Fisher Scientific. The synthesis process begins with the following steps: dissolve DNA with both zero-base and six-base spacers in DI water at a concentration of 0.83 nmol/μL; dissolve 41 nmol TCEP in 20 μL DI water and add into both DNA vials; wait for 30 minutes for the disulfide linkage on DNA to break, and then dissolved the 6-nmol Au nanoparticle in 50 μL DI water; and add 25 μL into the zero-base-spacer aptamer beacon vial and 25 μL into the six-base-spacer aptamer beacon vial. Each of the 200 μL solutions contained 3 nmoles of gold nanoparticles, 2 nmol of zero/six-base spacer aptamers, 41 nmol TCEP, and DI water as the solvent. Samples were stored in a refrigerator overnight. The concentration of the Au–DNA conjugation is expected to be 10 μM. Take 5 μL out of both 200-μL stock conjugation for use in experiments. Next, 5 μL of 10 μM carboxyl QDs are added into each of the 5 μL stock conjugation solution vials. Then 150 nmols EDC are added in each solution right after dissolving EDC in the DI water. Finally, both QD-based aptamer beacons are filtered with a 50-k membrane in 5000 g for 10 min to remove extra salts.

**Results**

The detection experiments are performed by QD-based aptamer beacons in 50 μL
phosphate-buffered saline (PBS); the concentration is expected to be 1 \( \mu \text{M} \). Next, 1, 10, and 100 \( \mu \text{L} \) of saturated potassium chloride are added in vials B, C, and D after all the four vial volumes are set to be 1112 \( \mu \text{L} \) by adding DI water. These vials are then imaged with the result shown in.

Results presented in Fig. 2 show that the fluorescent light emitted from QD-based aptamer beacons designed to detect potassium ions is obviously quenched when potassium ions are present. Indeed, as illustrated in Fig. 2, the quenching is so pronounced that it is readily seen with the naked eye after the potassium ion concentration reaches 512 mM. The fluorescence of the QD-based aptamer beacon with different concentrations of potassium ions is observed by the naked eye under 305 nm UV light in a UVP darkroom.

**Figure 2.** Each of the (A)(B)(C)(D) four vials contains 1 \( \mu \text{L} \) of 907 \( \mu \text{M} \) 6-base-spacer beacons. There are 0, 1 \( \mu \text{L}, 10 \mu \text{L}, 100 \mu \text{L}, 5.69 \text{M} \) saturated potassium chloride added from left to right while all of the volumes are designed to be equal as 1112 \( \mu \text{L} \). This photograph was taken in a dark room with a Panasonic LX3 1 minute after potassium ions were added into the beacon solution. The overall potassium ion concentrations are 0 mM (A), 5.11 mM (B), 51.1 mM (C) and 511 mM (D). The overall beacon concentrations of these four vials are the same, 907 \( \mu \text{M} \).
1. **Molecular beacon with a spacer vs. without the spacer**

Comparisons between aptamer probes, which one with a 6-base spacer, and one without the spacer

A comparison between the case with and without the 6 extra bases as the spacer on the aptamer is shown in Fig. 3. Aptamers, both with and without spacers having six extra bases, had been obtained from integrated DNA technologies (IDT). The picture illustrated in Fig. 3 was taken with a Panasonic LX3 digital camera while the sample was in a UVP darkroom being excited by 305 nm UV light. Aptamer beacons with a 6-base spacer exhibit a better on/off contrast.
Figure 3. Cases a) and b) are before and after adding potassium ions to the QD-based aptamer beacons without spacers for QD. Cases c) and d) are before and after adding potassium chlorides to the QD-based aptamer beacons with 6-base spacers for the QD on the aptamer end.

To have a quantification sense of the fluorescence and ion concentration, photoluminescence (PL) experiments on the QD-based aptamer beacon were further performed. For PL measurements, the two QD-based aptamer beacons are diluted in 0.2 μM in two separate vials. 500 μL borate buffer and 80 μL of beacon solution are added in each of the vials. Then saturated potassium chloride is added unit by unit. The exposure time for PL measurement is 5 seconds and the sample was excited with a 325 nm laser beam. The results are summarized in Figs. 4 and 5. Samples contain 500 μL borate buffer,
80 µL 0/6-spacer beacons at a concentration of 0.2 µM and saturated potassium chloride with concentration varying from 0 to 31 µL.

The concentrations follow

$$[\text{probe}] = \frac{0.2(\text{mol/L}) \times 80(\mu\text{L})}{580(\mu\text{L})} = \frac{16}{580} \frac{\mu\text{mol}}{L} = 0.0276 \text{ mM} = 27.6 \text{ µmol c.c.},$$

$$\text{added K}^+ \text{ unit} = \frac{5.69(\text{mol/L}) \times 1(\mu\text{L})}{580(\mu\text{L})} = 9.81 \frac{\text{mmol}}{L} = 9.81 \frac{\mu\text{mol}}{\text{c.c.}},$$

overall potassium concentration $= 9.81 \text{ mM} < [\text{K}^+] < 304 \text{ mM}$.

Figure 4. (a) PL measurements of the aptamer probe without the 6-base-spacer. The on-off contrast is determined by the ratio of maximum intensity over the minimum intensity and is found to be 19.9. (b) PL measurements of the probes with a 6-base-spacer. The on-off contrast is 28.0.

The main difference between these two on–off contrasts comes from the higher
intensity of the beacon in the on state, which indicates that the spacers do provide 1.8-nm longer separation and results in less FRET.

![Figure 5. Plot of emitted 605 nm intensity by the conjugation versus potassium ion concentration.](image)

The FRET efficiency is defined as the fraction of energy transfer events occurring per donor excitation events. When the size of the donor and acceptor are about the same, the efficiency $E$ can be expressed as follows:

$$E = \frac{k_{DA}}{k_{D} + \tau_{D}} = \frac{R_0^6}{R_0^6 + r^6}$$

The Forster radius $R_0$ is assumed to be 5 nm. $r$ is the separation distance between the donor and the acceptor. In the case of the beacon with a six-base spacer, $r$ is about 6.3 nm. In the case of the beacon without the spacer, $r$ is 4.5 nm. However, when the size of
the QD is about 10 times that of the quencher in diameter, the FRET efficiency can be expressed as follows:

\[ E = \frac{R_0^4}{R_0^4 + r^4} \]

The estimated QD fluorescent intensity before FRET is calculated based on the model, where \( \beta_y = \text{pure QD intensity} \times (1 - E) \); see Table I. The estimated results from the two aptamer beacons are with a 13.9% difference.

### Table I. Comparison of estimated QD fluorescent intensities for both beacons.

<table>
<thead>
<tr>
<th></th>
<th>( r ) (nm)</th>
<th>on state intensity</th>
<th>off state intensity</th>
<th>( \beta_y )</th>
<th>( E (%) )</th>
<th>calculated pure QD intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>with spacer</td>
<td>6.3</td>
<td>25117</td>
<td>898</td>
<td>24219</td>
<td>0.28</td>
<td>33638</td>
</tr>
<tr>
<td>without spacer</td>
<td>4.8</td>
<td>18921</td>
<td>951</td>
<td>17970</td>
<td>0.54</td>
<td>39065</td>
</tr>
</tbody>
</table>

In conclusion, beacons with the six-base spacers exhibit better on–off contrast by a factor of 1.41 times those without. The fluorescence intensity of the quenched state for both beacons with and without the spacer is about the same for the particular aptamer-based probes considered in this paper, showing that the 1.8-nm spacer does not appreciably help the aptamer in binding better to the QD. The spacer therefore does not
result in a better quenching effect, but it produces a larger separation between the donor-acceptor pair and provides better contrast for the beacon. However, the introduction of the spacer also results in beacons exhibit a stronger dependence to $\text{Na}^+$ as expected. Aptamers with six spaces have a longer sequence and lead to more quenching as expected from the fact that DNA has a shorter persistence length as the ion concentration increases. Both the on/off ratio and the other ion effects such as persistence length should be taken into consideration for the selection of a successful potassium ion detection device.

2. **Dissociation constant determination**

Herein, the dissociation constant is calculated based on three assumptions. First, for the sake of definiteness it is assumed that two potassium ions bind to each beacon\textsuperscript{38}. The equilibrium equation can be written as

$$1 \text{ tetraplex complex} \leftrightarrow 1 \text{ probe} + 2 \text{ potassium ions.}$$

Second, it is assumed the concentrations of aptamer beacons and QDs are linear functions of the scattered light intensity they emit by a factor of $\gamma$ and $\beta$, respectively. So, on-state beacons produce a fluorescent intensity $\beta y$, while $y$ represents the initial concentration of on-state beacon, which can be determined by the maximum difference of the PL signal. Furthermore, $x$ represents the concentration of any flawed QDs not affected
by potassium level, and the intensity of light emitted by those dots is proportional to $\gamma x$.

Third, the light produced by the off-state beacon is assumed to be negligible. Then, the dissociation equilibrium constant is defined by a general equation

$$K_d = \frac{[\text{probe}][K^+]^2}{[\text{tetraplex-complex}]} = \frac{\gamma(1-\alpha)[K^+]^2}{\gamma\alpha} = \frac{(1-\alpha)[K^+]^2}{\alpha}$$

while the variable $\alpha$ represents the fraction of beacons that stay in the off-state.

![Graph](image)

**Figure 6.** Components that produce light in the on-state are “flawed” QDs and on-state beacons, and the intensity they contribute is $\gamma x + \beta y$. For the off-state, “flawed” QDs are the only components give out light $\gamma x$, while the light given out by the quenched beacon is negligible by the assumption. States in between can be represented by the variable $\alpha$, the fraction of beacons that is quenched.

$$\frac{\alpha}{1-\alpha} = \frac{1}{K_d} \cdot [K^+]^2$$

Furthermore, $\alpha$ can be determined from the PL results, the dissociation constants of the aptamer probe without and with a 6-base-spacer against potassium ions are obtained as
the slopes shown in Fig. 7. Aptamer probes without a 6-base-spacer bind with potassium ions with a dissociation constant of 0.0057 M$^2$, while aptamer probes with a 6-base-spacer bind with potassium ions with a $K_d$ value 0.0058 M$^2$. This result implies that aptamers with and without the 6-base-spacer have essentially the same affinity to potassium ions.

\begin{center}
\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure7}
\caption{Plot of the $\frac{\alpha}{1-\alpha}$ against the square of potassium ion concentration, where variable $\alpha$ represents the fraction of beacons that is quenched. This plot gives the dissociation constant $1/K_d$ as the slope.}
\end{figure}
\end{center}

3. **Tested in the urine**

In the hospital, patients are often asked to collect the 24-hour urine as a single urine sample for potassium level checking. The reason is that the urine potassium level varies
with the day and one’s diet. For a normal adult, 25 ~ 125 mmol/L of potassium is usually observed in urine.

Herein, the aptamer-based molecular beacon consisted of the thrombin-binding-aptamer (TBA) sequence without the 1.8 nm spacer is tested in DI water containing real urine with an unknown potassium level. The purpose of this test is to challenge the performance of the aptamer beacons with various pH values, molecules and ions present in the real urine sample.

In the results shown below, six aliquots of different urine percentage are excited with light of 380 nm from an LED for one second. A constant beacon concentration of 67 nM and uniform solution volume of 320 μL were used to test for the performance of the molecular beacon. A decreasing fluorescence trend is observed with some perturbation between 0 ~ 10 % of urine sample. A total of 30 % fluorescent quenching implies a urine potassium level of approximate 70 mM is obtained, after compared with Fig. 13 in section 2.1.6.
Figure 8(a). The PL measurement of six aliquots containing different levels of urine, where each of the aliquot has 67 nM beacon, 320 µL solution in total; Aliquots are exposed to an LED give out 380 nm light in wavelength, and signal recorded by Ocean Optics USB4000 spectrophotometer. (b) A plot of the quenching ratio of the beacon at 650 nm of its fluorescence against the urine percentage. A total of over 30% quenching implies a reasonable urine potassium of around 70 mM, after comparing with Fig. 13.

4. $K^+$ vs. $Na^+$

The differentiation of potassium ions and sodium ions has been a challenge for real-time detection. According to previous report, TBA binds with potassium ions with 30 times the affinities that it has for sodium ions. Herein, the response of the TBA beacon to potassium ions is compared to its response to sodium ions. Result shows that the fluorescence fluctuates with different levels of sodium ions, but shows a constant decrease with the increase of potassium ion level. These results indirectly agree with N. Kumar, S. Maiti, etc., and show promising potential in the ion indication application as an ion level indicator.
Figure 9. The fluorescence quenching of the beacon is plotted against the salt concentration, to compare the respond of the beacon to potassium ions and to sodium ions.

5. **TBA vs. AG3**

In this paragraph, TBA is compared with AG3 (5’ GGG TTA GGG TTA GGG TTA GGG 3’), which is a sequence that is found in the human chromosome telomere. It contains four GGG sequences and is observed to fold and be stabilized specifically by potassium ions because of its size and charge. It was therefore utilized to build the potassium sensing probes because of its high affinity to potassium ions \(^{40,41}\). The TBA and AG3 sequences are both sequences that are reported to be potassium aptamers. Herein, the performance of these two sequences is compared and the potential applications of these potassium probes are discussed.

AG3 and TBA, were identified in the early and late 1990s, respectively \(^{42-44}\). Both
experimental NMR results and molecular dynamics simulations were used in each case to determine the structures shown in Fig 10. There is evidence to indicate that DNA forms a quadruplex structure in meiosis \(^45\), in chromosome telomeres \(^46\), in immunoglobulin switch regions \(^47\), and in the upstream region of the insulin gene \(^48\). These different sequences are usually stabilized by different cations. Here in this work, we examine the quadruplex structures formed by TBA and AG3, when stabilized by potassium ions. Fig. 10 depicts the folding modes of the TBA and AG3 aptamers in the presence of K\(^+\) based on the work of Yeates \(^49\), Nagatoishi \(^50\) and Vorlickova \(^51\). In addition, this paper evaluates their potential applications as potassium ion detectors.

\textbf{Figure 10.} a) The structure of potassium stabilized TBA aptamer beacon tetraplex according to Bolton’s group determined by NMR and restrained molecular dynamics simulations \(^44\); b) The AG3 aptamer beacon tetraplex structure according to Patel’s group determined using NMR, distance geometry and molecular dynamics approach \(^43\).
Methods

Both TBA and AG3 were purchased from Integrated DNA Technologies, Inc. (Coralville, IA); the 5' terminal is functionalized with an amide group with 12 extra carbon atoms, and the 3’ end is functionalized with a thiol group. EDC or EDAC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride) from Pierce served as the crosslinker to activate the carboxyl group on the quantum dot surface to bind with the amino group on the DNA 5’ terminal. TCEP (tris(2-carboxyethyl)phosphine), also from Pierce Protein Research Products, was used to break the disulfide bond on the DNA 3’ terminal. 10 μM carboxyl functionalized QDs from eBioscience, eFluorTM 605NC, were used in this study; these QDs can be excited by 350-500 nm light and they emit light with a wavelength of 605 nm. 1.4 nm-diameter monomaleimide nanogold had been obtained from Nanoprobe, Inc. The product was a frozen combination of 0.02 M sodium phosphate at pH 6.5 and 150 mM sodium chloride dissolved in 10 % isopropanol/water.

The nanogold particles were stored at -20 °C. They were dissolved in 0.2 ml DI water to activate the monomaleimido functionalized nanogold. They are usually stable unless the temperature goes above 50 °C. Saturated KCl was obtained from Fisher Scientific.
The synthesis process begins with the following steps: dissolve both the TBA and AG3 DNA sequences in DI water at a concentration of 25 µM; add 15 µL of 0.5 M TCEP in both DNA vials; wait 30 minutes for the disulfide linkage on DNA to break, and then dissolved the 3 nmole Au nanoparticle in 100 µL DI water; and add 50 µL into TBA aptamer beacon vial and 50 µL into the AG3 aptamer beacon vial. Each of the 265 µL solutions contained 5 nmole of aptamer sequence, 3 nmole of gold nanoparticles and 7.5 µmole of TCEP. Samples were stored in a refrigerator overnight. Next, DI water is added to both conjugations and they are centrifuged with 10 K cutoff membranes at 5000 g for 15 minutes. The washing step is repeated 5 times. Then the conjugation is dissolved in borate buffer with pH 7.2. Next, 15 µL of 10 µM carboxyl functionalized QDs are added to each conjugation solution. Then 450 nmoles EDC are added right after dissolving EDC in the DI water. Afterwards, both solutions are put in a refrigerator overnight. Finally, the QD-based aptamer beacons are filtered with a 50 K cutoff membrane in 5000 g for 15 minutes followed by adding DI water to remove extra salts. The previous step is repeated 3 times and diluted in 1500 µL with DI water. The final beacon concentration is 0.1 µM. Beacons are then tested with the addition of potassium ions, while fluorescence responses are monitored using a USB4000FL spectrometer from Ocean Optics, Inc.
Results

The comparison is first performed for potassium levels in the range from 1 to 4 mM; see Fig. 11. Instead of fluorescence intensity, the ordinate represents the fluorescence intensity (I) ratio, I/I₀, where I₀ is the intensity when there are no potassium ions in the solution. The fluorescence intensity of the TBA aptamer beacons are measured three times with three different exposure times, which are 3 seconds, 4 seconds, and 5 seconds. In the range from 1 to 4 mM, the fluorescence of AG3 aptamer beacon exhibits a constant decrease with the increase in the potassium level while TBA aptamer beacon fluorescence intensity fluctuates when potassium level is between 2 mM and 3 mM. This result indicates that aptamer beacons made of AG3 shows more promise for potential applications as an ion sensor in this potassium level range, which is the level in the serum. This implies that AG3 serves better than TBA in the design of take-home diagnostic devices for hyperkalemia and hypokalemia.
Figure 11. The aptamer beacons are first compared for the potassium level range from 0 to 4 mM, at about the serum potassium range. In this working range, the fluorescence of AG3 aptamer beacons in response to the increase in the potassium level shows a constant decrease. This implies that the AG3 aptamer beacon has potential for applications as a hyperkalemia and hypokalemia diagnostic device.

Next, these two aptamer beacons are compared under the conditions that the potassium level in the range of 5 to 80 mM; this range corresponds to the urine potassium test application. As shown in Fig. 12, the TBA aptamer beacon manifests a better decrease with an increase in the potassium level. Again, the fluorescence data of the TBA aptamer beacon comes from the average of three measurements, while three exposure times of the spectrometer to the sample are employed, which are 3, 4 and 5 seconds. In this range, the AG3 aptamer beacon saturates and does not change much with the different potassium levels. This fluorescence response supports the conclusion that the TBA aptamer beacon will serves better than the AG3 beacon in the urine potassium test.
Figure 12. These aptamer beacons are compared in the urine potassium range. The TBA aptamer beacon exhibits a better decrease with increasing potassium level, while intensity from the AG3 aptamer beacon is almost saturated.

Furthermore, the fluorescence stability of these aptamer beacons are studied as a function of time. Specifically, fluorescence intensities are compared and it is found that there is less than 15 % variation in QD intensity from 2 to 24 hours for AG3 aptamer beacons, and less than 8 % fluorescence decrease for TBA aptamer beacons.

Conclusion

There have been many studies using TBA to construct potassium ion sensors, while very few papers utilize the AG3 aptamer to detect potassium. However, in this paper, we demonstrate that the AG3 aptamer beacon serves better as a disease detector compared with the TBA aptamer beacon. It responds stably to the potassium level change, and gives a more reliable reading in the hyperkalemia and hypokalemia detection application. On the
other hand, the TBA aptamer beacon can be utilized to test the urine potassium level and shows better contrast in responding to the potassium level changes.

More comparative studies need to be done for further specific applications. Depending on the applications, such studies might include the following: performance of these aptamer beacons to different ions, the specificity of these aptamer beacons, and performance characteristics for longer monitoring times to evaluate their potentials for disease diagnosis.

6. **Potassium ion indicator conclusion**

This is an overall comparison of fluorescent respond from two experiments discussed in Section 2.1.1 “molecular beacon with a spacer vs without a spacer” and Section 2.1.5 “TBA vs. AG3”. The two experiments were conducted for different purposes with different PL setups, different PL exposure times, different potassium level, and different measuring solution volume. Yet, the quenching effect is similar and comparable. The author therefore plots them together with the error bar, so that the fluorescence respond of the aptamer-based molecular beacon associative with the potassium level can be better described.
In conclusion, more comparative studies need to be done for further specific applications. Depending on the applications, such studies might include the following: performance of these aptamer beacons to different ions, the specificity of these aptamer beacons, and performance characteristics for longer monitoring times to evaluate their potentials for disease diagnosis.

**Figure 13.** An overall comparison of fluorescent respond from two experiment discussed in Section 2.1.1 “the potassium ion detector with a spacer vs. without a spacer” and 2.1.5 “TBA vs. AG3”.

### B. The Foodborne Pathogen Detector

Molecular beacons for foodborne pathogen detectors have also designed and constructed. The foodborne pathogen beacons are evaluated in this section for the case
where the conformational change in the antisense beacon is sensed through the monitoring of the luminescence of the quantum dot which depends on the distance-dependent fluorescence resonant energy transfer between the quantum dot and the quencher.

1. **Listeria monocytogenes detector**

The foodborne pathogen discussed in this section is the Listeria monocytogenes bacterium. Listeriosis, which is caused by *L. monocytogenes*, accounts for 0.02% of all illnesses caused by foodborne pathogens, but results in 27.6% of death in all deaths due to food contaminations. The high mortality causes *L. monocytogenes* to be the number one cause of death among all foodborne pathogens.

**Experimental Methods**

The molecular beacon designed to detect *L. monocytogenes* detects the *hlyA* gene of the bacteria. The *hlyA* gene has the sequence 5' /ACA TCG TCC ATC TAT TTG CCA GGT A/3', and accounts for synthesis of the toxin, listeriolysin O. The L. monocytogenes detectors are constructed and optimized by the sequence 5’-/5AmMC12/AAC CCC TAC ATG ACA AAT AGA TGG ACG ATG TGG GGT T/3BHQ_2/-3’. The synthesis process begins with the steps described in the following discussion. Dissolve the nucleic acid in DI water at a concentration of 100 µM. Next, mix
nucleic acid with 10 μL of 10 μM carboxyl functionalized QDs in a glass tube, so that the DNA to QD molar ratio is 20 to 1. 1.7 nmoles of QDs are used to synthesize the Salmonella beacon and the Listeria beacon, and 0.96 mg of EDC is used as the crosslinker to activate the carboxyl groups on the QDs. Then, gently stir the mixture in room temperature for two hours, followed by letting the tubes sit overnight in 4 °C. Finally, all of these conjugations are filtered with a 3K membrane in 7500 g for 15 minutes to remove extra salts. The filtration process was repeated five times to wash out extra ions.

L. monocytogenes beacons are synthesized by Tsai-Chin Wu from UIC and tested by Dr. Kellie Burris of the University in Tennessee at Knoxville in 9 % apple juice and 2 % milk. Fluorescence was greater in the presence of the *Listeria monocytogenes* genome DNA, which was extracted from *L. monocytogenes* strain Scott A by Dr. Kellie Burris.

2. **Salmonella detection**

The foodborne pathogen discussed in this paragraph is the *Salmonella* serotype typhimurium, one of the most common *Salmonella* serotypes in the U.S. About 142,000 Americans were infected with *Salmonella* enteritidis from chicken eggs and about 30 of them died. According to a report from the Center of Disease Control and Prevention
(CDC), approximately 3,600 illnesses were reported from 1st of May to 30th of November. About a billion eggs were recalled during the 2010 salmonella outbreak.

**Experimental Methods**

The stock culture of *Salmonella* Typhimurium DT104 strain 2765 were obtained from the Department of Food Science and Technology at the University of Tennessee, Knoxville. The culture were grown overnight at 35 °C, centrifuged at 150 rpm, and the DNA was extracted using GenEluteTM Bacteria Genomic DNA kit by Dr. Kellie Burris.

The antisense beacon sequence, CCC CCG TTG TAC CGT GGC ATG TCT GAG CGG GG, obtained from Integrated DNA Technologies (IDT) was designed to detect the *Salmonella invA* gene GCT CAG ACA TGC CAC GGT ACA ACG.

Probes to detect *Salmonella* were successfully constructed and tested with synthesized nucleic acid target. The result is shown below.
42

**Figure 14.** Eppendorf a) contains 100 µL 10nM of beacon solution and showed no fluorescence under a UV lamp in the UVP darkroom. Eppendorf b) contains 101 µL of solution, including the MB solution and the target sequence obtained from IDT. It gives out fluorescence emitted by eFluorTM 605NC quantum dots.

The Salmonella beacons were further tested by Dr. Kellie Burris with Salmonella genomic DNA extracted from *Salmonella* Typhimurium DT104 Strain 2756. These beacons exhibited a significant fluorescence increase when 234 ng and 437 ng of *Salmonella* genomic DNA were present.

3. **BtCry1AC detector**

**Experimental Methods**

*BtCry1AC*, the truncated *cry1AC* gene from *Bacillus thuringiensis*, is a 1859-bp-long linear DNA and is responsible for cry toxin generated by the soil-dwelling bacteria.
The molecular beacon for *BtCry1AC* was built successfully, based on the detection of 5’/CTC TCA ATG GGA CGC CTT TCT TGT AC / -3’, which lies in positions 219 to 244 in the *BtCry1AC* gene. The steps to synthesize the BtCry1AC beacon are the same as the steps to synthesize *L. monocytogenes* and *Salmonella* beacons, while the DNA to QD ratio is synthesized at 30 to 1; 0.3 nmole of QDs and 0.9 µmole of EDC are consumed. The beacon was again tested by Dr. Kellie Burris of the University of Tennessee at Knoxville and exhibited significant enhancement of fluorescence when the BtCry1AC genomic DNA present.

**C. The nano-complex variation issue**

During the procedure of synthesizing the molecular beacons, a complication needs to be considered; it is the fabrication-related variability of nano-complexes; shown below in Fig. 15. This variation occurs (for example) for different number of deoxyribonucleic acids on its surface. In the mixture solution, some quantum dots bind to only one ssDNA, some quantum dots bind to two ssDNAs, and some quantum dots are bound to three or more ssDNAs on their surface. The complication arises from the fact that the quantum dots obtained from eBioscience. Inc are functionalized with carboxyl groups all over their
surface. An approximation given by eBioscience is that each of the quantum dots has about 300 carboxyl group on its surface. This implies that each of the quantum dots might be able to bind up to 300 deoxyribonucleic acids on its surface.

![Figure 15](image)

**Figure 15. An illustration of the formation of variant nano-complex.**

1. **Molecular weight calculation**

   When dealing with the crosslinking issue, it is crucial to have a sense of the object size. Here an approximate calculation is made.

   \[
   \text{Phosphate} \quad \text{PO}_4 = 30.97 + 16 \times 4 = 94.97 \text{ Da} \\
   \text{Sugar} \quad C_7H_4O_3 = 5(12.01) + 7(1.01) + 3(16.00) = 115.12 \text{ Da} \\
   \text{Total} = 94.97 + 115.12 = 210.09 \text{ Da}
   \]

   adenine \( C_2H_4N_5 \) = 134.14Da \( \rightarrow \) 344.23Da

   Granine \( C_3H_4N_3O \) = 150.14Da \( \rightarrow \) 360.23Da

   Cytosine \( C_3H_5N_2O \) = 110.11Da \( \rightarrow \) 320.20Da

   Thymine \( C_3H_2N_2O_2 \) = 125.12Da \( \rightarrow \) 335.21Da

   average = 339.97Da
5nm QD, assume 25 atoms in diameter; \( \frac{4\pi}{3} (12.5)^3 = 8177 \) (atoms)

CdSe molecular weight: Cd = 112.41 Da; Se = 78.96 Da.; On average = 95.69 Da

One CdSe QD \( 95.69 \times 8177 = 782,465 Da \approx 2301 \) base ssDNA in molecular weight

This calculation is made without considering the coating of ZnS, polymer and functionalized groups on the surface of the QD. If we take all of those into consideration, the size of QD and ssDNA that is used in this study would be even more beyond comparison.

2. **Modeling nano-complex variations**

   After considering the size of the particles, the conjugation is then examined and discussed statistically. The process are modeling involves binding discrete integral numbers of DNA molecules to QDs. Therefore, a distribution function that models discrete processes is needed. The model chosen to apply in this case is the Poisson distribution, a discrete distribution, first introduced by a French mathematician S. D. Poisson (1781-1840). This model predicts the frequency of any specific event within a given interval/area with a known average rate. In our case, the specific event would correspond to the “crosslink happens”. This event is also independent of the last occurrence, since the
binding sites of each quantum dot has is approximately 300 ideally, while the DNA/QD ratio used in synthesis is less than 20/1.

The Poisson probability distribution with a random variable is given by

\[ p(y) = \frac{N^y e^{-N}}{y!} \]

where \( y \) is the number of DNA molecules attached to single quantum dot; \( N \) is the mean number of DNA attach to single QD. Here we assume \( N \) depends on the ratio of DNA and QD during the synthesis procedure; \( e = 2.71828\ldots \); mean \( \mu = N \); variance \( \sigma^2 = N \).

Herein, three circumstances with different mean conjugation numbers per QD are considered. The average binding number of each QD is assumed to be equal to the mixture ratio of DNA to QD.
TABLE II. The calculated probabilities of the nano-complexes according to the Poisson distribution. In the case of QD has only half the amount of the DNA available, 60% of the QDs would stay unbound. In the case of DNA has 10 times the amount of QDs, most nano-complexes present in the solution are QDs bound to between 4 to 17 ssDNAs on the surface.

<table>
<thead>
<tr>
<th>y, # of DNA attach to 1 QD</th>
<th>QD:DNA=2:1 N=0.5</th>
<th>QD:DNA=1:2 N=2</th>
<th>QD:DNA=1:10 N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.60653</td>
<td>0.13534</td>
<td>0.00005</td>
</tr>
<tr>
<td>1</td>
<td>0.30327</td>
<td>0.27067</td>
<td>0.00045</td>
</tr>
<tr>
<td>2</td>
<td>0.07582</td>
<td>0.27067</td>
<td>0.00227</td>
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<tr>
<td>3</td>
<td>0.01264</td>
<td>0.18045</td>
<td>0.00757</td>
</tr>
<tr>
<td>4</td>
<td>0.00158</td>
<td>0.09022</td>
<td>0.01892</td>
</tr>
<tr>
<td>5</td>
<td>0.00016</td>
<td>0.03609</td>
<td>0.03783</td>
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<tr>
<td>6</td>
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<td>0.01203</td>
<td>0.06306</td>
</tr>
<tr>
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<td>0.00000</td>
<td>0.00344</td>
<td>0.09008</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0.000086</td>
<td>0.11260</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0.00019</td>
<td>0.12511</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0.00004</td>
<td>0.12511</td>
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<td>19</td>
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<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0.00187</td>
</tr>
</tbody>
</table>
Figure 16. A plot of table II. Three calculated possibility distribution curve of the nano-complex with $N=0.5$, 1 and 10 according to the Poisson distribution.

3. **Electrophoresis of suspended particles**

Experimental Methods

To examine the distribution of these different configurations, a series of electrophoresis experiments are perform. The assumption is made that nanoconjugation of different configuration behave different in electrophoresically. The 27-base-ssDNA (5’ TTT TTA GGG TTA GGG TTA GGG TTA GGG 3’) had been obtained from IDT, modified with an amide group with 12 extra carbon atoms on the 5’ end and a thiol group on the 3’ terminal. EDC or EDAC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride) from Pierce served as the crosslinker to activate the carboxyl group on the quantum dot surface to bind with the amino group on DNA 5’ terminal. 10 M
carboxyl functioned QDs from eBioscience, e FluorTM 605\textsuperscript{NC} and 565\textsuperscript{NC} were used in this study; they could be excited by 350-500 nm light and emit light with a wavelength of 605 nm and 565 nm respectively.

The synthesis process begins with the following steps: Dissolve the ssDNA in DI water at a concentration of 100 \( \mu \text{M} \) and take out 10 \( \mu \text{L} \), 1 \( \mu \text{L} \) and 0.5 \( \mu \text{L} \) of solution into three glass tubes respectively. Next, 10 \( \mu \text{L} \) of 10 \( \mu \text{M} \) carboxyl QD are added into each of the solution tubes followed by 300 nmoles EDC added in each solution. The EDC solution should be used right after dissolving EDC powder in the DI water. These tubes were then sit overnight in 4 °C. Finally, all of these conjugations are filtered with a 3K membrane in 6000 g for 15 minutes to remove extra salts. The filtration process was repeated for five times to wash out extra ions. The conjugations are then run in agarose gel of 0.4 % ~ 1 % with EtBr, while 1X TBE buffer is used as the running buffer.

**Results and Discussion**

a. **The variation of the mobility of conjugations synthesized under different conditions are observed.**

Samples synthesized with different DNA to QD ratios exhibit electrophoresis
patterns with significant differences. The ssDNA (5’ TTT TTA GGG TTA GGG TTA
GGG TTA GGG 3’) used in this study is a sequence that is found in human chromosome
telomere, plus 6 bases (ATT TTT) on the 5’ end as a spacer. The nucleotide acids are
bound to orange QD emitting fluorescence 605 nm in wavelength in this experiment, result
shown in Fig. 17. The left lane is loaded with sample synthesized under DNA to QD ratio
1 to 2, and shows a wider band at the bottom that barely moves along the applied electric
field compared with the middle lane. This band might be comprised with those unbound
605^{NC} QD that can barely move due to the weak surface charge from carboxyl groups on
the 605^{NC} QD surfaces. The middle is loaded with sample that DNA is twice as much as
QD and shows an extra band on the top of the plot. This band should correspond to the
extra nucleotide acids. This study indicates that agarose-based electrophoresis provides a
potential separation method for further applications that demand specific conjugation
configurations.
b. **We observed that the binding of DNA to QD causes the conjugation to get trapped in the well**

It is found that binding to DNA causes the DNA-QD complexes to get stuck in the gel and decreases the mobility of the DNA-QD complexes. The QD used in this experiment is the green $565^{\text{NC}}$ QD manufactured by eBioscience. The gel experiments demonstrate that binding to DNA causes the mobility of the $565^{\text{NC}}$ quantum dots to decrease. Evidence shows that the more strands of DNA that bind to one QD, the more likely it is that this QD will get stuck in the gel and not be able to move freely in the
Results show that as more strands of DNA are bound to the QDs, the more likely it is that the conjugation is trapped in the gel as the DNA-QD complexes migrate. In the case of no DNA (Fig 18 far right lane), the QDs are not found to stick in the well along the migration. Also, Fig. 20(B) shows QDs with richer DNAs bound to their surface show lower mobility in the agarose gel.

**Figure 18.** *Taken by a UVP darkroom Epi camera, shows 0.5 % agarose gel results for conjugations synthesized under different DNA to QD ratios, with 100 Volts applied for 90 minutes. Samples loaded in lanes from left to right are synthesized under the conditions that the DNA to QD ratios, N, are 10 to 1, 1 to 1, 0.5 to 1, and 1 to none. This implies that the QD-DNA complexes in the far right lane are the richest in DNA.*

c. **The buffer in which the conjugation is suspended also changes its mobility.**

The migration distances of DNA-QD conjugations in different buffers (TE and
PBS) have been measured and the results are presented herein. Moreover, as an extended
Smoluchowski formulation is based on

\[ D_m = e^{-K_m} \exp \left( \frac{2\sigma f(\kappa)E_t}{3\eta}\frac{k_B T}{ez^+} \right) \frac{a}{\kappa^+ (N)} \ln \frac{K_m N}{10^{-3}\varepsilon AiZC} \]

for 0.4% agarose gel with EtBr, is used to model these experimental results. Theory
shows that the different buffer that nanocomplexes are suspended should reflect on their
migration distance. As \(10^{-3} e AiZC\) corresponds to the total electrolyte charge in the buffer,
the same nanocomplex should migrate differently when suspended in different electrolyte
under applied electric field.

These conjugations are synthesized under a TBA DNA to 605\(^{NC}\) QD ratio 1 to 1. A 50
V electric field is applied for 60 minutes. Fig. 19(A) is the top view taken with the UVP
darkroom Epi camera. These results indicate that the buffer changes the surface charge of
the nano-conjugations and therefore changes their mobility in gel.

The PBS buffer contains 137 mM of NaCl, 2.7 mM of KCl, 8.1 mM of
\(Na_2HPO_4\cdot2H_2O\) and 1.76 mM KH\(_2\)PO\(_4\) with a pH value at 7.4 has an ion strength around
10 times stronger compared with TE buffer which contains 10 mM of Tris and 1 mM of
EDTA. As a result, conjugations suspended in PBS buffer are encounter stronger screening
effect and exhibit lower conjugation mobility compared with conjugation suspended in TE
buffer. This result agrees with the Zeta potential theory. The higher ion strength leads to
smaller Debye length and weaker migrating force.

**Figure 19.** Taken by a UVP darkroom Epi camera shows 0.5 % agarose gel results for conjugations synthesized under different DNA to QD ratios, with 50 Volts applied for 60 minutes. Right lanes in contain DNA-QD conjugations suspend in PBS buffer. Left lanes in the figure contain conjugation suspended in TE buffer, the light they emit comes from the mixture of the 605Evitag quantum dots and the nucleic acid stain, EtBr. Most of the samples become stuck in well and not able to move, but conjugation ion TE buffer was able to move better along with the applied electric field.

d. **The bandwidth**

Fig. 20(A) and (B) present measurements of $D_m$. As illustrated by the measurement of migration distance distributions observed in the agarose electrophoresis, as presented in Fig. 20(A), a broader band is observed for a higher DNA/QD ratio, in agreement with the simulations on Fig. 16. Slightly different averaging mobility of conjugations of different DNA/QD ratio is also observed in Fig. 20(B).
Figure 20. The bandwidth of different DNA to QD ratio conjugations are examined in 0.5% agarose gel. Results for DNA to QD ratio, \( N \), equal to 10, 1 and 0.5 are shown in (A). The bandwidths of conjugations for \( N \) equal to 10 and 1 are measured to be 243 pixels and 209 pixels. The applied voltage is 100 volts for 30 minutes, taken by a Panasonic LX3 digital camera. These results are then used to fit the transport.

\( (A) \quad (B) \)

\( N=10 \quad N=0.5 \)

\( N=1 \)

\( (X, Y) = (723, 732) \)

\( D_m = 332 \) (pixel)

\( (890, 741) \quad D_m = 342 \)

\( (1052, 743) \quad D_m = 345 \)

Migration direction

\( e. \quad \text{Formula derivation} \)

Previous research has been conducted to study the electrokinetic charateristics of biomolecules \(^{52, 53}\). The formula commonly used to describe electrophoretic behavior is developed based on the double layer, as originally propounded by Quicke and Helmholtz.
The theory assumes that there is an interface between two fluid regions moving relative to each other having phases corresponding to an inner sheet and an outer sheet. The inner sheet attaches firmly to the rigid phase, called the Stern layer. Everything within the sheet moves with the rigid object. The moving outer sheet joins the inner sheet at an interfacial slipping plane. The most commonly accepted expression describing electrophoretic behavior in the community is based on Smoluchowski’s theory published in 1914, broadened by Debye and Huckel in 1924, and then solved by D. C. Henry in 1931. This model was claimed to be valid for a particle of any shape, as is frequently assumed.

Herein, we apply the model to examine experiments results reported herein as well as to discuss its utility.

Herein, we further investigate the formula of the migration distance of particles with QD-DNA complexes in electrophoresis. The quantitative study of moving particles in electrophoresis originated from Smoluchowski’s theory, which stated the velocity of moving particle is given by

\[ v_e = \left(4\pi\varepsilon_0\right) \frac{E\zeta}{4\pi\eta} = \frac{\varepsilon_0 E\zeta}{\eta}, \]

\( \varepsilon_0 \) and \( \eta \) are the dielectric constant and the viscosity of the medium that the particles are surrounded by; \( E \) is the applied electric field; \( \zeta \) is the potential across of the double layer.
between the two phases, named Zeta potential. This equation was then analyzed by Debye
and Huckel in 1924\textsuperscript{54}, which stated that the relation should be
\[
\nu_e = c \cdot \frac{4\pi \varepsilon_0 E \zeta}{\eta},
\]
where \(c\) depends on the shape of the electrophoretic particle. Specifically, \(c = \frac{1}{6\pi}\) for
spherical particles\textsuperscript{54}. Henry\textsuperscript{55} continued the work and determined the relation between the
mobility of the electrophoretic particle and its net charge \(Q\):
\[
Q = 4\pi \varepsilon_0 \varepsilon_r a \int_0^\infty \zeta dr = 4\pi \varepsilon_0 \varepsilon_r a (1 + \kappa a) \zeta;
\]
\(\kappa^{-1}\) is the the thickness of the double later.

For steady motion, the total electrical force on a smooth sphere is given by the Stokes
frictional resistance
\[
f_v = -6\pi \eta \nu_e,
\]
which is generalized herein by,
\[
f_v = -6\pi \eta \kappa^{-1} \nu_e = -\frac{6\pi \eta \nu_e}{\kappa}
\]
to take into account deviation from the smooth sphere approximation, where \(\kappa^{-1}\) is the
effected thickness of the double layer that causes the Stokes friction. The applied force
\[
Q \times E = 4\pi \varepsilon_0 \varepsilon_r E a \int_0^\infty \zeta dr
\]
\[
= E \int_0^\infty 4\pi r^2 \rho dr = 4\pi \varepsilon_0 \varepsilon_r E a (1 + \kappa a) \zeta
\]
should be zero. Therefore,

$$\frac{-6\pi\eta v_0}{\kappa} + 4\pi\varepsilon_0\varepsilon_r E a \int_0^\infty \tilde{\zeta} dr = 0$$

or

$$v_0 = \frac{4\varepsilon_0 E}{6\eta} \left( \frac{a}{\kappa^{-3}} \right) \int_0^\infty \tilde{\zeta} dr$$

while

$$\tilde{\zeta} = \frac{\partial \psi}{\partial r} + \lambda \alpha^3 r \int_{r}^{\infty} \frac{1}{r^2} \nabla^2 \psi dr$$

$$v_0 = \frac{4\varepsilon_0 \tilde{\zeta}}{6\eta} a \left( 1 + \frac{\kappa^2 a^2}{16} + \frac{5\kappa^4 a^4}{48} + \frac{\kappa^4 a^4}{96} + \frac{11}{96} e^\varepsilon \int_0^\infty e^{-\varepsilon t} dt \right)$$

for $\kappa a < 5$.

$$v_0 = \frac{4\varepsilon_0 \tilde{\zeta} a}{6\eta} \left( \frac{2}{3} (1 + \kappa a) \right) = \frac{4\varepsilon_0 \tilde{\zeta} a}{6\eta} \left( \frac{2}{3} f(\kappa a) \right)$$

Here $f(\kappa a)$ is called the Henry’s function, which gives value from 2/3 to 1 when $\kappa a$ goes from 0 to infinity. The Zeta potential $\zeta$ can be described as the Nernest potential. It can be expressed as

$$\zeta = \frac{k_B T}{Z e} \ln \frac{K_e N}{10^{-3} eAIZC}$$

Where $A$ is the Avagadro’s number, and the nanocomplex is suspended in a system with $i$ types of ions of valence $Z$ and concentration $C$. By applying an electrical field $E$, the migration distance of the particle in the gel is

$$D_n = v_0 t = \mu_e E t = \frac{2\varepsilon_0 a}{\kappa^{-3} f(\kappa a) E t}{3\eta}$$

$$= \left( \frac{2\varepsilon_0 a}{\kappa^{-3} f(\kappa a) E t} \right) \left( \frac{k_B T}{Z e} \ln \frac{K_e N}{10^{-3} eAIZC} \right)$$
Following Ref. 60, this formulation can be extended to be a function of the agarose gel concentration factor, $G$, by introducing the retardation factor $K_{r-N}$.

$$D_m = e^{-\kappa^{-1}} e^{G} \left( \frac{2\varepsilon f'(\kappa a) ET}{3\eta} \right) \left( \frac{k_B T}{ze} \right) \frac{a}{\kappa^{-1}(N)} \ln \frac{K_r N}{10^{-3} e^AIZC}$$

Although $\kappa^{-1}$ is a function of $N$, Henry calculated that $f(\kappa a)$ gives value only from $2/3$ to 1. The first term is still constant. As $a$ is the size of the nanoparticles and since it changes little as DNA is bound to the nanoparticle, it is approximately constant. So, under the same gel concentration 0.5%, the migration distance of the electrophoretic particles is therefore a $\ln N$ dependent function $^{58,59}$ if all the nanocomplexes have the same thickness of the double layer. Therefore, $D_m$ may be represented by

$$D_m = s \ln(N) + M_0.$$ 

![Graph](image)

**Figure 21.** The plot is made based on the result of Fig. 20(B). The constant are calculated for this figure $s = -4.3404$, $M_0 = 342$. 

The result implies that \( e^{-K_{r,s} G} \cdot \frac{a}{\kappa^{-1}(N)} \) is a function independent of \( N \), but only dependent on the fabrication of QDs, surrounding buffer, etc. Herein, we define the constant \( f_{QD-buffer} \) through:

\[
e^{-K_{r,s} G} \cdot \frac{a}{\kappa^{-1}(N)} = f_{QD-buffer}
\]

or,

\[
\frac{a}{\kappa^{-1}(N)} = \frac{f_{QD-buffer}}{e^{-K_{r,s} G}}.
\]

where \( f_{QD-buffer} \) is independent of \( N \) but may vary depending on the QDs and the buffer.

From these results,

\[
\kappa^{-1}(N) = \frac{a}{f_{QD-buffer} e^{K_{r,s} G}} = \frac{a}{f_{QD-buffer}} e^{-K_{r,s} G}.
\]

Accordingly,

\[
f_v = 6\pi \eta v \cdot f_{QD-buffer} a e^{-K_{r,s} G}.
\]

The thickness of the double layer as a function of the number of DNA on one QD is derived. The Stokes friction as a function of \( N \) is also calculated. This derivation indicates that the introduction of retardation factor is directly caused by the Stokes friction.

\[
D_n = e^{-K_{r,s} G} \left( \frac{2\eta f (\kappa a) E T}{3\eta} \right) \frac{k B T}{2e} \frac{a}{\kappa^{-1}} \ln \frac{K_v N}{10^{-3} e A I Z C} = f_{QD-buffer} \left( \frac{2\eta f (\kappa a) E T}{3\eta} \right) (\kappa B T) \eta \ln N + \ln \frac{K_v}{10^{-3} e A I Z C}
\]
Fitting this result to $D_m = s \ln N + M_0$, result in,

$$s = f_{QD-buffer} \left( \frac{2\kappa (\kappa u)Et}{3\eta} \right) (\frac{k_B T}{ze}) = -4.3404$$

$$M_0 = f_{QD-buffer} \left( \frac{2\kappa (\kappa u)Et}{3\eta} \right) \frac{k_B T}{ze} \ln \frac{K_p}{10^7 eAiZc} = 342$$

$$\frac{M_0}{s} = \ln \frac{K_p}{10^7 eAiZc} = \frac{342}{-4.3404} = -78.79$$

$$\frac{K_p}{eAiZc} = 1000 e^{-78.79}$$

An essential feature of this formulation is that $D_m$ is a function of $\ln N$. Indeed, it is the $\ln N$ dependence of $D_m$ that is evident in our experimental results as illustrated by Fig. 21.

Herein, results of measurements of the migration distances of DNA functionalized QDs for varying numbers of DNA molecules bound to the QDs are presented. The measured migration distance exhibit a $\ln N$ dependence where $N$ is the number of DNA molecules per QD. In parallel with these measurements, an extended Smoluchowski equation has been formulated. In the formulation, the gel concentration factor and the lack of a smooth spherical surface, resulting in a modification of the Stokes formula, are combined in the factor $f_{QD-buffer}$ which may vary depending on the QDs and the buffer.
4. **Conclusions and Discussion**

As discussed previously, the introduction of $f_{QD-buffer}$ is motivated by several phenomena. First, an earlier generalized Smoluchowski equation was originally derived by D. C. Henry without considering the double layer thickness $55$. Then he approximated the outer layer with the exponential potential function $\psi = \psi_a \left( \frac{a}{r} \right) e^{-\kappa (r-a)}$, which is approximately the “Debye-Huckel” ionic atmosphere model. According to Henry, the approximation is least reliable with small $a$, which is our case. This effect is treated by the $(a/\kappa^{-1})$ factor in $f_{QD-buffer}$. Second, Smoluchowski claimed his model can be used on arbitrary rigid insulator objects, yet, Henry predicted this might not be applicable to colloidal particles having echinate surface according to Osborne Reynolds’ calculation. It is when $\frac{U_0 a \delta}{\eta}$ is not small compared with the unity, the failure of this model is pronounced. In that case, the surface is taken to be locally flat and thus the particle shape does not appear explicitly in the solution $61$. Third, although many experimental results indicate that insulator and non-insulator behave without noticeable differences, the rigidity limitation might still affect the appropriateness of applying Smoluchowski’s equation on our ssDNA-QD conjugation. Based on these known effects, the extended Smoluchowski model of the paper introduces $f_{QD-buffer}$. As described previously, this new factor will of course, depend on the QD surface coating, manufacturing method and the buffer system. As described in
the extended Smoluchowski equation of this paper agrees the measured value of
$D_m$ reported herein.

D. **Surface-enhanced Raman spectroscopy (SERS) on EBeam deposition of silver on microsphere substrates**

An independent means of determining when DNA is present in a given experiment is desirable. The technology affects application ranging from diagnostic device in clinical medicine to forensic DNA profiling in law enforcement. To have an easier and more efficient method to detect the compositions of nucleotide acid, the use of Raman spectroscopy to detect biomolecules is explored. Raman spectroscopy is a powerful spectroscopy technique for measuring the vibrational, rotational and other low-frequency mode of specific chemical bonds after excitation by a higher-energy laser beam. The sensitivity and selectivity of Raman spectra facilitate providing a “fingerprint” of compounds, and therefore is widely used in chemistry and solid-state physics.

One of the major disadvantages of the Raman technique is its weak signal, resulting from its small scattering cross-section of biomolecules. This disadvantage limited its application in biology until the discovery of the surface enhanced Raman spectroscopy
The phenomenon was first observed by Fleischmann et al. in 1974. Subsequently, SERS was used in studying complicated biomolecules like protein and DNA with Raman spectroscopy. Based on the intensity enhancement resulting from SERS, the direct detection and identification of a single biomolecule without labeling with dyes is possible. Yet, the detection of biological species are not easy compared with inorganic molecules, especially for huge biomolecules like DNA, comprised of multiple atoms and multiple chemical bonds. Moreover, there are many theories aimed at explaining the enhancement (of $10^6$), but the exact mechanisms are still not understood completely. These studies are mostly carried out by using Raman-active dyes, such as HEX, FAM, Cy3, Cy5, TAMRA, rhodamine 6G and methylene blue. The geometry needed to produce the “hot spot” for SERS optimization is an interesting topic, which deals with different kinds of nanostructures. Herein, this paper includes the discussion of two such geometries.

The object we include with SERS in this study is the thrombin-binding aptamer. Thrombin-binding aptamer (TBA, 5' GGT TGG TGT GGT TGG 3') is a naturally existing aptamer found to have high affinity to thrombin in 1993, and has been involved in many applications. It binds to alpha-thrombin and plays important roles in the blood-clotting process. TBA is also the first aptamer that was studied widely. This sequence is reported to
bind with potassium ions and form a tetraplex structure with 30 times affinity that it has for sodium ions \(^{39}\). Therefore, there are many studies that utilize TBA as a potassium probe \(^{32, 40, 69, 72-74}\).

Experiment shows that Raman spectra enhancement sometimes changes upon the addition of target molecules to aptamers \(^{75}\). This is caused by the increase of the randomization of DNA secondary structures. Herein, we specifically examine the response of Raman spectra of potassium ions on single strand TBA.

The procedure is started by preparing the platform for the biomolecules to sit on. Glass coverslips of 18 mm in diameter, are obtained from Fisher Scientific. The synthesis procedure is started by cleaning the glass substrate by Piranha solution (1:3 30%H\(_2\)O\(_2\):H\(_2\)SO\(_4\)) at 80 °C for 30 minutes, to remove any organic impurities on the surface of the glass coverslips. The substrates are then rendered hydrophilic by using 5:1:1 (H\(_2\)O:NH\(_4\)OH: 30% H\(_2\)O\(_2\)) with sonication for 1 hour, followed by DI water washing and compress air drying.

The spectra are obtained using a Renshaw microRaman 2000 with an argon ion laser (514.5 nm), maximum power of 24 mW. A 50X objective lens from Olympus is used to focus the laser beam down to a size of approximately 0.04 mm\(^2\).
1. **Substrate 1**

The short single strand deoxyribonucleic acid here immobilized on the substrate is the TBA (thrombin-binding aptamer) with a six-base-spacer (5' GGT TGG TGT GGT TGG ATT TTT 3'). A silver layer evaporated on nanosphere has found to produce the SERS effect \(^{76}\) and is discussed below. The substrate (schematic representation shown in Fig. 22) consists of a layer of silver with thickness varying from 100 to 200 nm, coated on polystyrene latex nanospheres of 390 nm diameter on the glass coverslips \(^{77}\).

![Figure 22](image.png)

**Figure 22.** The cross-section of the substrate that the TBA is drop coated on: a layer of silver with thickness varying from 100 to 200 nm, coated on polystyrene latex nanospheres of 390 nm in diameter.

The 21-base-deoxyribonucleic acid is drop coated on the silver. Then two samples are compared, one is the 5 μM TBA sequence with potassium chloride, and one is 10 μM TBA sequence alone. The spectra (shown below) are acquired with a Renishaw microRaman apparatus, with a power of 2.4 mW, at 10 % of the maximum power. The
reproducible and significant peaks are then compared; some known Raman lines are listed in Table III.

![Raman Spectra](image)

**Figure 23.** The Raman spectra of TBA sequences on the substrate. The dashed line is the single stranded deoxyribonucleic acid only. The dotted line is the spectra of TBA with the addition of potassium ions. The two major differences of these two spectra are pointed out with boxes.

The addition of potassium does not significantly shift all peaks. However, one of the most noticeable differences is found at frequency 1096 cm$^{-1}$, which corresponds to the phosphate backbone peak of DNA. This indicates the bending of the backbone in order to form tetraplex structure causes its vibrational frequency to change. The purine
band at 1575 cm$^{-1}$ and guanine ring at 1662 cm$^{-1}$ are also observed, which may imply the stacking interaction between guanine bases are changed to form the tetraplex structure.

The measurements were taken multiple times with different exposure time, to acquire a complimentary understanding of DNA peaks. Measured vibrational modes are listed in Table 1 and compared to known peaks, mainly from Ref. 78. Fig. 24 shows that the results are reproducible under different integration time settings.

The two most significant peaks of adenine are around 732 cm$^{-1}$ and 1330 cm$^{-1}$ 78. Surprisingly, they are also found in some of the measurements, while the 21-base-deoxyribonucleic acid (5' GGT TGG TGT GGT TGG ATT TTT 3') consists only one adenine in the spacer. As adenine is the only base that has a Raman line around 732 cm$^{-1}$, this line has been used as an identification of adenine base 62.
TABLE III. A list of peaks from multiple measurements, and are compared with known peaks.

<table>
<thead>
<tr>
<th>w/ K</th>
<th>w/o K</th>
<th>wavelength</th>
<th>Possible Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>488</td>
<td>531</td>
<td>512</td>
<td>Guanine$^{78}$</td>
</tr>
<tr>
<td>634</td>
<td>625</td>
<td>632</td>
<td>Thymine N$_1$C$_2$O+N$_3$C$_4$O$^{78}$</td>
</tr>
<tr>
<td>636</td>
<td>633</td>
<td>656</td>
<td>Guanine in-phase ring stretching of the six-membered ring except C$_4$C$_5$ $^{64, 78}$</td>
</tr>
<tr>
<td>714</td>
<td>715</td>
<td>732</td>
<td>Adenine ring stretching$^{78}$</td>
</tr>
<tr>
<td>780</td>
<td>771</td>
<td>776</td>
<td>Thymine Ring breathing mode$^{64, 78}$</td>
</tr>
<tr>
<td>782</td>
<td>773</td>
<td>852</td>
<td>Guanine N$<em>7$C$</em>{5s}$-N$_1$C$_3$N$_3$ $^{78}$</td>
</tr>
<tr>
<td>857</td>
<td>846</td>
<td>590</td>
<td>Guanine N$_9$R$^4$+N$_3$C$_7$ $^{78}$</td>
</tr>
<tr>
<td>952</td>
<td>946</td>
<td>1000</td>
<td>Thymine C$_5$-Me$^6$, Guanine$^{78}$</td>
</tr>
<tr>
<td>1002, 1003</td>
<td>1000</td>
<td>1000</td>
<td>Thymine C$_5$-Me$^6$, Guanine$^{78}$</td>
</tr>
<tr>
<td>1073</td>
<td>1073</td>
<td>1078</td>
<td>Guanine$^{63}$</td>
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<td>1100</td>
<td>1096</td>
<td>1098</td>
<td>Backbone (PO$_2$ st)$^{64}$</td>
</tr>
<tr>
<td>1136</td>
<td>1128</td>
<td>1154</td>
<td>Guanine C$_8$N$_7$+N$_9$R$^4$-C$_4$N$_3$ $^{78}$</td>
</tr>
<tr>
<td></td>
<td>1184</td>
<td></td>
<td>Thymine C$_8$H+C$_2$N$_4$ $^{78}$</td>
</tr>
<tr>
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<td>1158</td>
<td>1206</td>
<td>Thymine$^{63}$</td>
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<td></td>
<td>1273</td>
<td>1266</td>
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<td>1316</td>
<td>1317</td>
<td>1330</td>
<td>Adenine, Thymine$^{62, 64}$</td>
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<td>1392</td>
<td>1389</td>
<td>1372</td>
<td>Thymine$^{78}$</td>
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<td>1394</td>
<td>1396</td>
<td>Thymine$^{78}$</td>
</tr>
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<td>1457</td>
<td>1454</td>
<td>1442</td>
<td>Thymine C$_5$-Me$^78$</td>
</tr>
<tr>
<td>1472</td>
<td>1463</td>
<td>1504</td>
<td>Thymine NH$^{64, 78}$</td>
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<td>1476</td>
<td></td>
<td>Thymine NH$^{64, 78}$</td>
</tr>
<tr>
<td>1476</td>
<td>1517</td>
<td>1514</td>
<td>Guanine C$_4$C$_3$-C$_4$N$_3$ $^{78}$</td>
</tr>
<tr>
<td>1575</td>
<td>1570</td>
<td>1580</td>
<td>Guanine N$_3$C$_4$-C$_5$ $^{63, 78}$</td>
</tr>
<tr>
<td></td>
<td>1582</td>
<td></td>
<td>Thymine N$_3$C$_4$+N$_1$C$_5$C$_2$+C$_4$C$_5$ $^{63, 78}$</td>
</tr>
<tr>
<td>1602</td>
<td>1600</td>
<td>1602</td>
<td>Thymine$^{63}$</td>
</tr>
<tr>
<td>1619</td>
<td>1627</td>
<td></td>
<td>Thymine$^{63}$</td>
</tr>
<tr>
<td>1633</td>
<td>1638</td>
<td>1636</td>
<td>Cytosine$^{63}$</td>
</tr>
<tr>
<td>1648</td>
<td>1659</td>
<td>1650</td>
<td>Thymine C$_4^2$=O$^+$+C$_8^a$C$_6^g$ $^{63, 78}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1680</td>
<td>Guanine C$_6^d$=O$^+$C$_5^c$C$_4^g$ $^{78}$</td>
</tr>
<tr>
<td>1662</td>
<td>1681</td>
<td>1691</td>
<td>Thymine C$_6^d$=O$^+$C$_5^c$ $^{78}$</td>
</tr>
<tr>
<td>1821</td>
<td>1837</td>
<td></td>
<td>Thymine$^{63}$</td>
</tr>
<tr>
<td>1953</td>
<td>1956</td>
<td></td>
<td>Thymine$^{63}$</td>
</tr>
</tbody>
</table>

Abbreviations used: r refers to rocking; s is stretching; b is bending. Use has been made of Ref. [23].
Figure 24. The reproducibility of spectra evaluated under different integration time (A) TBA spectrum; (B) TBA spectrum with K⁺ present.

2. **Substrate 2**

It is known that the space between a nanoparticles and thin film may support a hot spot and produce a high SERS enhancement 65, 80, 81 (schematic representation shown in Fig. 25). Gold is chosen in this study because gold has been report to be a good substrate to immobilize monolayer on. The high order of the molecule immobilization to the surface and the ease of the process both make gold a popular substrate for monolayer formation 82. To prepare for gold thin film substrates, the glass coverslips were then brought to clean room for gold deposition. 0.5 nm of Cr was deposited before the 50 nm gold thin film for the stabilization of the gold.

The sample intended to put on top of the substrate is synthesized by the following
steps: bind 2 nmole of monomaleimide functioned GNP (obtained from Nanoprobe, Inc.) to 5 nmole of thiol-functionalized TBA antisense sequence with TCEP as the crosslinker. After sitting in room temperature overnight, the conjugation was filtered with 10 k membrane for 10 minutes at 5000 g, to filter out the extra antisense TBA. After the filtration, the sample was mixed with 2 nmole TBA sequence, heated to 50 °C, and cooled in room temperature environment overnight. This step is intended to promote the formation of the double strand TBA, while keeping GNPs stable (under 55°C degree).

![Figure 25](image)

**Figure 25.** *This depicts the structure of the sample, double stranded TBA sequences sandwiched between 1.4 nm gold nanoparticles (GNP) and a 50 nm gold thin film.*

The GNP-dsTBA is then drop coated on the gold thin film substrate with the addition of TCEP to activate the thiol groups on the 3’ end of TBA sequences. The drop sat overnight in room temperature to dry before the microRaman measurement was conducted.
Figure 26. The top solid line is the spectrum of the TBA between the gold thin film and a GNP (schematic representation shown in Fig.25). The dotted line in the middle is the spectrum of the control samples that synthesized according to Fig.25 but without the GNP. The dashed line at the bottom is simply the spectra of the gold thin film substrate.
TABLE IV. The observed Raman peaks of the TBA dsDNA

<table>
<thead>
<tr>
<th>Raman measurement</th>
<th>Known peaks</th>
<th>Possible Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>427</td>
<td>430</td>
<td>Cytosine (^78)</td>
</tr>
<tr>
<td>489</td>
<td>490</td>
<td>Thymine (-C_2N_1C_6+N_3C_4C_5) (^78)</td>
</tr>
<tr>
<td>547</td>
<td>548</td>
<td>Adenine (-C_2C_4N-C_2C_6N_1) (^78)</td>
</tr>
<tr>
<td>586</td>
<td>586</td>
<td>Thymine (N_1C_2N_3-C_2N_3C_4) (^78)</td>
</tr>
<tr>
<td>640</td>
<td>642</td>
<td>Z form DNA (^77)</td>
</tr>
<tr>
<td>661</td>
<td>656</td>
<td>Guanine in-phase ring strengthening of the six-membered ring except (C_2C_5) (^78)</td>
</tr>
<tr>
<td>700</td>
<td>709</td>
<td>Adenine ring stretching modes (^78)</td>
</tr>
<tr>
<td>723</td>
<td>732</td>
<td>Adenine ring stretching mode (^78)</td>
</tr>
<tr>
<td>755</td>
<td>776</td>
<td>Thymine ring breathing mode (^78)</td>
</tr>
<tr>
<td>823</td>
<td>812</td>
<td>Thymine (N_1C_2+N_1R^s+C_3C_4^s+N_1C_6^s+N_3C_4^s) (^78)</td>
</tr>
<tr>
<td>850</td>
<td>852</td>
<td>Guanine (-N_2C_5-N_1C_2N_3) (^78)</td>
</tr>
<tr>
<td>928</td>
<td>925</td>
<td>Backbone (^78)</td>
</tr>
</tbody>
</table>

3. **Conclusion and Discussion**

This work demonstrates two successful methods to obtain spectra of short DNA sequence. The seven strongest Raman peaks of base thymine are at 776 cm\(^{-1}\), 812 cm\(^{-1}\), 992 cm\(^{-1}\), 1216 cm\(^{-1}\), 1275 cm\(^{-1}\), 1372 cm\(^{-1}\), and 1652 cm\(^{-1}\) \(^78\). This study has identified five of the corresponded peaks listed in Table III. The characteristic spectrum of guanine base is primarily one peak at 656 cm\(^{-1}\) and four minor peaks at 1300 cm\(^{-1}\) to 1550 cm\(^{-1}\) region. These Raman lines are all found and listed in Table III. Moreover, Raman lines from adenine are also observed while each ssDNA molecule has only one adenine base. Based
on the demonstrated data, an argument is made that SERS has offered a promising
detecting method to identify single short nucleic acid sequence.

E. **Other design and calculation**

1. **Calcium ions detector**

   The thesis provides a model to build detectors with a wide range of targets by
   choosing the appropriate aptamer or linker. Here is an example as a calcium ion detector.

   The designed molecular beacon for calcium ions will consist of a gold nanoparticle
   (GNP) as the quencher, calmodulin (CaM), the calmodulin-binding peptide (M13), and a
   red quantum dot as the donor. Here, the author choose CaM-M13 as the linker, while CaM
   is the most versatile protein member in the calcium-binding family, naturally existed and
   expressed in all eukaryotic cells. It is a small acidic protein about size of 148 amino
   acids long, 17 k Dalton. M13, a synthetic peptide, is a 26-residue calmodulin-binding
   domain of skeletal muscle myosin light-chain kinase. It has the sequence of H-
   KRRWKKNFIAVSAANRFKKISSSGAL-OH, and wraps around the CaM in this detector
   when calcium ion present in the solution. In this case, QD and GNP are brought together
and induces FRET. In the absence of calcium ion, the CaM-M13 conjugation will be as normal biopolymer and forms random coil to separate the QD and the gold nanoparticle.

According to the Nobel-prize-winner, Dr. Roger Tsien, the boundary regions between CaM-M13 and the green protein of his cameleon plays an important role in optimizing the performance, and therefore spent much effort on finding the optimized sequence \(^{84}\). The reason that the boundary region is critical is due to the directional of the green protein, which was first extracted from the Pacific Northwest jellyfish *Aequorea Victoria* in 1962 and determined structure in 1996 \(^{85,86}\). According to Dr. Roger Tsien’s review paper, the orientation of fluorescent protein has been a significant unknown perturbation. However, in our case, since nanoparticles are spheres, FRET will be able to happen in all direction, and the boundary amino acid sequence may be simply Gly as the spacer.

2. **Watson-Crick pair free energy change**

To have a better understanding of the molecular beacon, herein we consider the energy change of the TBA sequence associate to its complimentary sequence and potassium ions.
For every guanine-cytosine pairs, the free energy of the system decreases 21 kcal/mol. For the adenine-thymine pair, it is 13 kcal/mole \(^8^7\). As a result, the free energy change of the 15-base-pair TBA-antisense, \(\Delta G\), is 267 kcal/mole after the Watson-Crick bases pair up. In the case of TBA and potassium ion,

\[
\Delta G(p,T) = \Delta H - T\Delta S.
\]

\(T\Delta S\) is unknown, but the tetraplex formation is mainly driven by enthalpy change \(^3^9,^8^8\). \(\Delta H\) takes up around 17\textendash}38 kcal/mol according to Dr. Maiti’s result \(^3^9,^8^8\).

An experiment was then conducted (results not shown) and showed that the addition of potassium ions did not change the fluorescence respond of the double-strand TBA labeled with QD and GNP, which is in agreement with the calculation.

3. **Potassium ion detector with positive signal**

When there are potassium ions present, a positive detection signal is always desirable. To do this, a different detecting mechanics needs to be designed.

Based on the calculation of free energy change, design 1 is made. The probe will consist of two aliquots. First aliquot will be added first, which contains the TBA labeled with GNP and QD. In the case of potassium ions present, we will first see quenched signal
from the solution (as shown in the Figure 27a) if potassium ions are present. In this case, the addition of the second aliquots, which contain the antisense of TBA, will amplify the fluorescence signal (Fig. 27b).

In the case when potassium ions are absent, the TBA will form a random coil in the solution. The end-to-end distance of the random coil is calculated to be 4.24 nm based on the assumption that the ssDNA is a freely-jointed chain (ideal chain). According to the theory, the root mean square end-to-end distance of the ssDNA would be \( R_{\text{rms}} = b \sqrt{N} = 4 \sqrt{(15 \cdot 0.3/4)} = 4.24 \text{ nm} \), where \( b \) is the statistical segment, and \( N \) is the total number of the segments in the sequence. This statistical segment is considered as the persistent length of a single-strand DNA, which is 4 nm in 10^{-2} M electrolyte \(^{89}\). Since every 4 nm segments of the sequence is flexible enough to be considered to be statistically independent, the 15-base-TBA sequence contains 15x0.3/4 segments.

After adding the second aliquot, the double-strand-TBA sequence will formed at a length of 15x0.3 nm, which is 4.5 nm.
**Figure 27.** An illustration of the structure of the potassium detector with positive signal in the cases of potassium ions present (a) before adding the second aliquot and (b) after the addition. The addition of the second aliquot will enhance the fluorescence signal if potassium ions are present in the solution.

The end-to-end distance change of the random coil after binding to its complimentary sequence is shown in Table V. It is assumed that each QD has 10 TBA sequence with GNP successfully bound to its surface, and the Forster radius is 8 nm. This minor difference causes only 1.9% the energy transferred. Therefore, both before and after adding the second aliquot, the fluorescence respond change will not be able to be distinguished by the naked eye.

**Table V.** In the absent of potassium ion, the energy transfer rate caused by the conformation change. The length difference is 0.26 nm, and the energy transfer rate change is 1.8%. This minor difference would not be able to be distinguished by naked eye.

<table>
<thead>
<tr>
<th>Beacon conformation</th>
<th>End-to-end distance r (nm)</th>
<th>Energy Transfer rate E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tetraplex</td>
<td>10.00</td>
<td>72.39</td>
</tr>
<tr>
<td>Random coil</td>
<td>14.24</td>
<td>23.92</td>
</tr>
<tr>
<td>TBA dsDNA</td>
<td>14.50</td>
<td>22.00</td>
</tr>
</tbody>
</table>
III. CONCLUSIONS AND FUTURE WORK

As nanotechnology has grown rapidly in 1980s and is widely applied in fields such as plastics, medicine, energy, electronics and aerospace, this thesis contributes in the investigation of applications in biomedical diagnostics. In this research, foodborne pathogens and potassium ions are chosen as the primary targets in the development of nanosensors described in this thesis; due to their importance in the food industry and the biological sciences, respectively. The introduction of spacers and nanosensors synthesized with two different aptamer sequences are performed to optimize potassium nanosensors. The potassium nanosensors are then tested with different ions and in real biological fluids; the results indicate that these nanosensors have promising applications in self test devices and molecular biological science research.

In efforts to achieve better control over the proportional compositions of the entities that form these nanostructures, agarose electrophoresis is investigated as a separation method. Several electrokinetic behavior dependencies on the nanoconjugations are observed and examined with double layer theory. In particular, quantum dots functionalized with different numbers, N, of DNA molecules are studied. The results show that nanocomplex migrate distances as a function of Log N provide an an
approximate indicator of nanoconjugation compositions. A separation method that offers higher resolution is still needed and should be the subject of additional research as a means of realizing future applications of such nanocomplexes. This thesis has provided a model to fabricate detectors for a wide range of analytes by choosing an appropriate aptamer or another linker. Specific contributions and conclusions are listed below.

A. **Conclusions**

1. Molecular beacons are built using fluorescent nanoparticles and show promising potential for applications in self-administrated diagnostics.

2. Targets of interest included in this thesis that have been successfully synthesized as MBs are: potassium ion, *Salmonella* genome, *Listeria monocytogen* genome and *Bacillus thuringiensis* genome.

3. It is found that a 1.8 nm spacer helps increase the separation between donor-acceptor pair and gives better on/off contrast.

4. The performance of the potassium beacon is tested in urine samples and shows potential for applications in clinic diagnostics.

5. Two K\(^+\)-aptamer-based molecular beacons are compared and it was found that each has optimal performance for different potassium levels.
6. A stronger response of beacons to $K^+$ compared with $Na^+$ is observed.

7. The dependency of the electrokinetics characteristics on the DNA-QD complex variability is observed; such characteristics include the migration distance, bandwidth, and the solution the complex suspended in.

8. An extended Smoluchowski equation is formulated and proposed based on the results of the experiments.

9. Reproducible Raman spectra of the TBA are obtained using SERS, and therefore successfully identified the minor shifts of the peaks after the addition of $K^+$.

10. This thesis provides a model to fabricate detectors for a wide range of targets by choosing an appropriate aptamer or another linker.

11. Using the techniques demonstrated herein, it is possible to fabricate high sensitivity detectors by selecting aptamers that have high specificity for binding to specific analytes. Our results support the conclusion that the performance of the MB will be highly dependent on the affinity of the aptamer to its target.
B. **Future work**

The findings and results of this thesis have opened the way to a variety of possible future research projects. In this section, these possible new directions are discussed.

**Direction 1:** *To optimize these molecular beacons and apply them as probes to study biological system in molecular level.*

By conjugating biomolecules to fluorescent nanoparticles, nanoscale probes may be fabricated. With nanoscale sensors, it is possible to study the interactions of biological systems at the molecular level. To optimize these biosensors, physical behaviors of biomolecules need to be investigated. By understanding the underlying physical phenomena it may be possible to find new medicines, new drug delivery pathway, new diagnostics methods and new treatment methods.

**Direction 2:** *Develop nanoscale detecting devices.*

Furthermore, studying the optoelectronic and mechanical properties of biomolecules including DNA, RNA, peptides and proteins, may result in insights that lead to new classes of materials for nanostructure construction. As the high productivity
and high specificity of biomolecules present in the biological system are always desirable, by integrating man-made semiconductor nanoparticles with biomolecules, it may be possible to reproduce man-made devices and materials as fast and as highly-specific as in biological systems.

Direction 3: **Determine the Raman spectra of biomolecules using techniques that enhance the spectral intensity.**

The fingerprinting of biomolecules, using Raman technique is becoming more practical due to the surface-enhanced Raman scattering. Future research may focus on the reproduction of the SERS (surface-enhanced Raman scattering) effect with DNA sequences that have simple bases patterns. By comparing spectra with the known spectra for individual DNA bases, these Raman spectra facilitate the determination of the relative abundances of A, T, C, and G within a particular DNA strand. The SERS substrates studied will mainly be gold and silver based on previous experiments. These investigations should include synthesizing biomolecule-nanostructure complexes, understanding the plasma physics, setting up mathematical models, and simulating solutions.
Direction 4: *Study the electrophoretic behavior of DNA-QD conjugations.*

The study of the electrodynamics of these biomolecule-nanoparticle nanostructures will lead to new insights. With the unique shapes and charges of biomolecules and nanomaterials, the Smoluchowski equation describing the electrophoretic velocity is generally not appropriate. The modification of this model to better predict the electrophoretic behavior of these nanostructures is needed. The success of the modified model should lead to a better method of separating these nanocomplexes, and people will therefore have better control of structures on the nanoscale.
References


38. Ueyama H, Takagi M, Takenaka S. A novel potassium sensing in aqueous media with a synthetic oligonucleotide derivative. fluorescence resonance energy


58. A quantum dot based nanoassay for quantifying gene copy number with ultrahigh resolution. 14th international conference on miniaturized systems for chemistry and life sciences; 3-7 October 2010; ; 2010. .


Education

**Ph.D., Dept. of Bioengineering / Nanotechnology, Chicago IL, US [2006 ~ Apr. 2012]**
- Univ. of Illinois at Chicago (UIC), Advisor: Michael A. Stroscio GPA: 3.66/4.00
- Dissertation: Integration of Semiconductor Nanoparticles with Biomolecules

**B.S., Dept of Physics / Bioinformatics, Taipei, Taiwan [2002 ~ 2006]**
- National Taiwan Normal University (NTNU) GPA: 3.33/4.00

Professional Experience

- Worked closely with PhD students from electric engineering, physics and bioengineering
- Developed folding-based sensors, such as potassium ion indicators, Salmonella and Listeria monocytogen optical biosensors and tested them in vitro
- Synthesized molecular beacons by polymer crosslinking protein/DNA to 20 nm CdSe/ZnS nanoparticles and 1.4 nm gold nanoparticles
- Study the electrokinetic characteristics of the conjugations in agarose electrophoresis
- Familiar with carboxyl-to-amine and thiol-to-maleimide bioconjugation processes
- Analyzed nanocomplex compositions using UV-VIS, micro Raman spectroscopy (through SERS), agarose electrophoresis and photoluminescence
- Conceived project plans, summarized experiment data, form conclusions, and made presentations in conferences and classes

**Teaching Assistant, UIC Dept. of Bioengineering [2007 ~ 2008]**
- Trained students to examine and build operational amplifier circuits for basic analog signal processing, and record digital data with LabVIEW.
- Coached students for ability to process biopotential signal, applied circuits with thermistor, solid their own photoplethysmograph signal detector, and use thermodilutions to measure flow rate.
- Solved various problems, answered questions in laboratory class, taught materials and graded homework

Skills

**Professional Instruments Operation**
- Ocean Optics USB4000FL
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- Beckman DU 640 UV-VIS Spectrophotometer
- Varian Evaporation System (Clean Room Experiences)
- Micro Raman Spectrometer (Renishaw Raman 2000)
- UVP Epi Chemi II Darkroom (Software: Labworks)

**Software**
- Matlab signal analysis tool
- Matlab plotting tool
- Labview recordubg tool
- Proficient in Microsoft office

**Lab Techniques**
- Polymer crosslinking
- Agarose electrophoresis
- pH meter calibration / pH value titration
- Buffer preparation
- Steam sterilization
- Substrate (glass overslips) cleaning

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- Biomedical Optics
- Biomedical Imaging
- Biostatistic I, II
- Biomaterials
- Imaging System for Tissue

Language
- Fluent in Mandarin

(Continued on the back of this page)
Publication


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- President, UIC Taiwanese Students Association [2009 ~ 2010]
- Co-leader, worship leader, UIC Chinese Christian Fellowship [2008 ~ present]

Honor and Awards

- Chancellor’s Student Service & Leadership Awards [2009]

Conference Proceedings