Ratiometric Quantum Dot Protein Sensors

BY

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B.S., Saint Xavier University, 2009

THESIS

Submitted as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate College of the University of Illinois at Chicago, 2014

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This thesis is dedicated to my family and friends.
ACKNOWLEDGMENTS

I would like to thank my thesis committee Preston Snee, Scott Shippy, Timothy Keiderling, Jordi Cabana, and Anne George. I would also like to thank the members of the Snee group.

I would also like to acknowledge the references I am reproducing in whole or part. All approvals from publishers are located in the Appendix. I am reproducing my first author research paper, reference 157, throughout Chapters 2 and 4. I performed all research and participated in the writing of the manuscript. My advisor, Dr. Preston Snee, also participated in the writing of the manuscript. I am reproducing my first author research paper, reference 157, throughout Chapters 2 and 4. I performed all research and participated in the writing of the manuscript. My advisor, Dr. Preston Snee, also participated in the writing of the manuscript. I am reproducing my first author research paper, reference 157, throughout Chapters 2 and 4. I performed all research and participated in the writing of the manuscript. My advisor, Dr. Preston Snee, also participated in the writing of the manuscript. I am reproducing my first author research paper, reference 157, throughout Chapters 2 and 4. I performed all research and participated in the writing of the manuscript. My advisor, Dr. Preston Snee, also participated in the writing of the manuscript. I am reproducing my first author research paper, reference 157, throughout Chapters 2 and 4. I performed all research and participated in the writing of the manuscript. My advisor, Dr. Preston Snee, also participated in the writing of the manuscript.

I am adapting a figure from my first author review paper, reference 45, in Chapter 1. I participated in the writing of the manuscript. My advisor, Dr. Preston Snee, also participated in the writing of the manuscript. This figure was originally adapted from reference 69, a review paper by Gill, et al. I am adapting a small excerpt from reference 66 in triplicate in Chapters 2, 3, and 4. This small excerpt, from a research paper by Liu, et. al. is added for completeness to explain how to process quantum dots for water-solubilization. Finally, our collaborators, Clare Rowland and Richard Schaller of Northwestern University and Argonne National Laboratory, sent us the experimental parameters for the time-correlated single photon counting experiment that they performed, and these parameters closely match the wording of the experimental parameters in one of their research papers. I am adapting this excerpt from this paper, reference 237, for completeness.
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<td>QD</td>
<td>Quantum dot</td>
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<td>QY</td>
<td>Quantum yield</td>
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<td>DHLA</td>
<td>Dihydrolipoic acid</td>
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<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
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<tr>
<td>MPEG CD</td>
<td>Methyl-polyethylene glycol carbodiimide</td>
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<tr>
<td>EMC</td>
<td>1-ethyl-3-(2-methoxyethyl)carbodiimide</td>
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<td>DMTMM</td>
<td>4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride</td>
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<td>Sulfo-SMCC</td>
<td>Sulfo-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate</td>
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<td>FRET</td>
<td>Fluorescence resonance energy transfer</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>DL</td>
<td>Detection limit</td>
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<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<td>TLC</td>
<td>Thin-layer chromatography</td>
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<td>HPLC</td>
<td>High pressure liquid chromatography</td>
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<td>RBpip</td>
<td>Rhodamine B piperazine</td>
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<td>RBpip—biotin</td>
<td>RBpip—lysine—biotin</td>
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<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<tr>
<td>BOP</td>
<td>Benzotriazole-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate</td>
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<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
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<td>BSA</td>
<td>Bovine serum albumin</td>
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<td>Indocin</td>
<td>Indomethacin</td>
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Cox-2        Cyclooxygenase-2
RBiso        Rhodamine B isothiocyanate
NHS          N-Hydroxysuccinimide
DCC          N,N'-dicyclohexylcarbodiimide
RBpip—Indocin RBpip—lysine—Indocin
TFA          Trifluoroacetic acid
TBA          Thrombin binding aptamer
Amino-TBA    5’-/Amino/TTTTTTTTTTTTTGTTGGTGGTTGG/TAMRA/-3’
Acrydite-TBA 5’-/Acrydite™/TTTTTTTTTTTTTTGTTGGTGGTGTTG/TAMRA/-3’
TCSPC        Time-correlated single photon counting
SUMMARY

This thesis focuses on the development of a QD sensor that selectively and ratiometrically responds to the presence of unlabeled proteins. The first investigation generated a model QD-based protein sensor for streptavidin, a bacterial form of an egg white protein. The second studied a QD protein sensor for cyclooxygenase-2, a protein related to inflammation and thus tumor metathesis. A third QD-based protein sensor was developed for thrombin, a protein in the blood coagulation cascade that, in high blood-borne concentrations, is a marker for several diseases. The detection limits of these sensors are in the low pmol/mL range, making them competitive with ELISA assays. These sensors are fast, homogeneous, have detection limits that are scalable with concentration, and are translatable to many other biologically relevant proteins with the use of different small molecular protein binding agents and aptamers. Finally, the synthesis of very bright ZnSe/CdS/ZnS type II QDs is discussed. The unprecedented stability and brightness of these materials allowed for them to be water-solubilized via two different methods. To demonstrate the possibility for sensing applications, an energy-transfer accepting organic dye was conjugated to the water-solubilized type II QDs. Efficient energy transfer was characterized via time-correlated single photon counting; this result ultimately expands the variety of quantum dots that may be used in analytical chemistry.
1. Introduction

1.1 Introduction

The rise in nanotechnology over the past 30 years has made it one of the fastest growing fields in science. Quantum dots (nanocrystals, or QDs) have been one of the largest thrusts of research since their discovery in 1983.\textsuperscript{1, 2} Since then, the amount of publications on QDs has increased exponentially. The many properties, types, synthesis methods, water-solubilization techniques, functionalization methods, and applications of QDs have been and are currently being published in the literature.

1.2 Properties of Quantum Dots

QDs have many interesting properties, the characterization of which comprised the bulk of the initial studies published about them.\textsuperscript{1-9} Many of the properties of QDs are size dependent; this is because of the quantum confinement of the materials.\textsuperscript{1} As QDs approach the size of the Bohr-Exciton radius, their energy levels become discrete as opposed to band-like in the bulk material.

When an electron in the valence band is excited, it moves to the conduction band. The hole in the valence band and the electron in the conduction band have attractive Coulombic forces that result in the formation of an exciton; the separation of the hole and electron is known as the Bohr-Exciton radius. If the QD is approximately the same size as the Bohr-Exciton radius or smaller, the excitation energy rises inversely proportional to QD size. Thus, a change in the size of the QD can affect the properties of the nanocrystal. The kinetic energy of the exciton changes due to the
Heisenberg uncertainty principle when the size of the QD determines the quantum confinement.\textsuperscript{1} Because of this, the smaller the QD, the wider the band gap energy between the valence and conduction bands. This confinement causes the energy levels to be quantized, as shown in Figure 1. These energy levels are atomic-like; QDs have even been called artificial atoms.\textsuperscript{1, 10-12} The chemical composition of the QDs can also affect the band gap, allowing for band gap engineering due to both size and material of the QD.

In addition to QDs having unique electronic properties different from their bulk material, the size-dependent optical properties of QDs have drawn great interest in the materials. QDs have size-tunable, narrow emission spectra and continuous absorption profiles; an example of this is shown in Figure 2.\textsuperscript{1, 2, 5-10, 13-16} In addition, well-made QDs are highly photostable and can achieve near unit quantum yields (QYs).\textsuperscript{17}

1.3 Types of Semiconductor Core/Shell Quantum Dots

Semiconductor core/shell QDs are categorized into two groups, type I and type II. These classifications are based on the position of the hole and the electron energy levels within the QD. While the band gaps of the core material and shell material define the position of the hole and electron as shown in Figure 3, the relative position of the charge carriers’ energy levels affect the overall electronic structure resulting in type I or type II systems.

Type I QDs have their hole and electron both located in either the core or the shell. A large amount of QD research has been performed using this type of QD, primarily the ubiquitous CdSe/ZnS.\textsuperscript{18, 19} Other type I QDs include CdS/ZnS,\textsuperscript{20}
Figure 1. Quantum dots (right) have discrete valence and conduction band energy levels compared to bulk semiconductors (left). The relative splitting of the electron and hole levels are also shown; the hole levels are closer together due to the higher effective masses.
Figure 2. Absorption and emission spectra of CdSe/ZnS quantum dots.
Figure 3. Different types of core/shell quantum dots. Type I have the hole and electron localized in the core (or depending on the material systems, both are localized in the shell). Type II have the hole localized in the core and the electron localized in the shell (or depending on the material systems, the electron is localized in the core and the hole is localized in the shell).
CdSe/CdS, CdS/Se, and InAs/CdSe. When both the hole and electron are located in the core, the confinement caused by the shell can reduce surface defects and deep trap which in turn can improve the QY and photostability over the core alone.

Type II QDs are nanocrystals in which the hole is in the core and the electron is in the shell (or vice versa) due to the relative alignment of the band edge. Previous reports have described the synthesis of various type II QDs including CdTe/CdSe, CdSe/ZnTe, CdTe/CdS, ZnTe/CdS, ZnTe/CdSe, ZnTe/CdTe, CdSe/ZnSe, CdSe/CdS, ZnSe/CdSe, CdS/ZnSe, ZnSe/CdS, CdS/Cu2S, and Cu2S/CdS. The spatial separation of the charge carriers in type II QDs can cause low QYs; non-optical applications are the most prevalent for type II QDs, such as in alternative energy generation where fluorescence is detrimental.

1.4 Synthesis of Quantum Dots

QDs were initially synthesized in water in polymer templates. Although this allows the materials to be readily used for biological purposes, the QDs were of very low quality. Other methods such as reverse-micelle synthesis were reported that improved QD surface passivation and crystallinity. While these newer methods were an improvement, there were still issues with stability and QY.

It was not until the rapid injection synthesis method was developed to produce QDs in hydrophobic organic solvents that the quality of the materials made them useful for the various applications seen today. This method of colloidal synthesis was first introduced by Murray et al. Semiconductor precursors are rapidly injected into a very hot organic solvent, as shown in Figure 4, which causes the supersaturated precursors
Figure 4. The rapid injection synthesis method for quantum dots. Precursors are injected into very hot coordinating solvents to nucleate and then grow to achieve high quality quantum dots. Used with permission from Preston Snee.
to nucleate. Then, controlled growth occurs as the solvent temperature decreases; this forms QDs with a narrow size distribution that are coated with surfactants in the coordinating solvent.

Many different QD core sizes can be synthesized using the rapid injection synthesis method by properly adjusting the conditions. To improve the stability and QY, QD cores can be overcoated with a shell of a different material. For example, CdSe QDs are typically overcoated with ZnS. This shell minimizes precipitation, passivates the surface of the QD, and increases the QY. QD shells are generally grown epitaxially, where overcoating precursors are slowly injected into the core solution at moderately high temperatures.

The use of highly purified starting materials such as oleic acid, tetradecylphosphonic acid, and trioctylphosphine oxide has even allowed near unit QYs for QDs. The purity of the reagents used is essential to achieve the best QDs in terms of not only optical properties but also in terms of reproducibility of the method. Wang et al. proved this with CdSe wires; different lots (but the same 99% purity level) of one of their reagents gave different results. The “magic bottle effect,” an extreme dependence on the identification of a lot of precursor or solvent chemicals, is extremely prominent in the field of QD synthesis, and only by avoiding this dependence issue can high quality QDs be consistently produced.

1.5 Water-Solubilization Methods for Quantum Dots

As the best QDs are produced using organic solvents, the QDs must be transferred from their growth medium to an aqueous solvent for biological
There are two main routes by which the QDs can become watersolubilized, as shown in Figure 5. One involves exchanging the organic ligands surrounding the QDs with hydrophilic ligands. The other involves the use of amphiphilic surfactants such as phospholipids and amphiphilic polymers to encapsulate the native ligands, leaving the hydrophilic ends exposed on the surface of the QDs.

### 1.5.1 Cap-exchange

Cap-exchange is a method of QD water-solubilization where the native ligands on the QD are replaced with caps that impart water-solubility. These new ligands are commonly mercapto-acids such as dihydrolipoic acid (DHLA). While these ligands allow the QDs to be soluble in water with a small hydrodynamic radius, the QY is typically reduced, they tend to be unstable, and the shelf life is normally on the order of days or less. The bidentate nature of DHLA allows for increased stability, and tridentate ligands have been reported which increase stability even more. Other reports have described methods that alter the typical procedures for cap-exchange to enhance the stability of the cap-exchanged QDs. One such example, developed by my group, uses zinc-coordinated DHLA caps; the zinc reacts with the native phosphonic acid caps, leaving the new DHLA caps in their place. These QDs were stable for 10 days at room temperature, which was a record for cap-exchanged QDs at the time.

A recent development in cap-exchange involves a cap-exchange with a near monolayer of silane. This procedure, which is a one-step method that my group developed, involves the use of zinc mentioned above. The replacement of the native ligands of the QD with zinc-coordinated silane, and the subsequent precipitation with
Figure 5. The various methods for water-solubilization of quantum dots. The methods are typically either cap-exchange, where native ligand are exchanged for water-soluble ligands, or encapsulation, where the native ligands are coated with amphiphilic materials that impart water-solubilization. Adapted from Ref. 69 with permission. Adapted from Ref. 45 with permission from the PCCP Owner Societies.
hexane, yields QDs with a condensed, cross-linked, near-monolayer silica coating that retains high quantum yields and is stable on the order of months under benchtop conditions. Thiol functionalities on the surface of these QDs allows for further functionalization.

1.5.2 Encapsulation

Encapsulation involves keeping the native ligands on the QD intact and overcoating them with encapsulants that leave the QDs water-soluble. This is typically performed using block copolymers, gallic acid derivatives, lipids, phospholipids, or amphiphilic polymers. The hydrophobic regions of these compounds interdigitate with the native organic ligands, leaving the hydrophilic regions exposed toward the outer regions of the QD. The specific encapsulant used can impart functional moieties that can allow for functionalization.

Encapsulated (and particularly polymer-encapsulated) QDs are much more stable than cap-exchanged QDs. They also retain significantly more of their QY, this is due to the native ligands remaining on the QD. As there is no harsh removal of the original protecting ligands, the positive qualities of the QDs remain unperturbed. The drawback of this method is that the encapsulant layer is very thick, creating a hydrodynamic diameter that is too large for many biological uses.

1.6 Functionalization of Quantum Dots

Once synthesized and water-solubilized, QDs are still not very useful. QDs must be functionalized with chemical or biological components to have any utility for various
potential applications. While methods such as nonspecific interactions, electrostatic interactions, and adsorption can attach such components to QDs, the stability of these methods is questionable.

As many QDs had carboxylic- or amine-terminated functional groups on their water-solubilized surface, there was a prevalence of the use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in early functionalization papers. It is now widely known that EDC causes irreversible loss of materials through QD precipitation.

A recent development in the field of carbodiimide coupling reagents showed that EDC causes precipitation due to charge cancellation between the cationic reagent and anionic QD. The resulting colloidal instability is what causes the loss of sample through aggregation and precipitation. Thus, reagents such as methyl-polyethylene glycol carbodiimide (MPEG CD) and 1-ethyl-3-(2-methoxyethyl)carbodiimide (EMC) were developed; these reagents are electrostatically neutral. DMTMM, which is commercially available, was also recently reported in the literature to effectively functionalize QDs. These reagents, shown in Figure 6, cause little to no precipitation of QD samples at loading ratios needed to achieve efficient functionalization. The reported efficiencies of these reagents can be as high at 95%, meaning that QDs can be robustly functionalized with minimal losses with these reagents.

Other reagents have been used to successfully conjugate QDs with other functional groups on their water-solubilized surface, such as sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) for thiol-functional QD surfaces. A more recent direction for conjugation is toward reagentless or “spring
Figure 6. Robust conjugation reagents. A: long poly(ethylene glycol)-carbodiimide (MPEG-CD), B: short poly(ethylene glycol)-carbodiimide (EMC), C: 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). These reagents replace the inefficient EDC.
loaded” conjugation chemistries that involve no external coupling reagents and produce no byproducts. These protocols remove some of the difficulty in functionalizing QDs as no purification steps are required after functionalization. Reagentless methods are very useful for tagging biologicals as they can be performed in vitro and in vivo if they are bioorthogonal. Bioorthogonal strategies can react with their targets without affecting the surrounding biological environment. This can allow for many biological studies that cannot be done with the current conventional methods of QD functionalization.

1.7 Applications of Quantum Dots

QDs have found utility in many areas due to their many interesting properties. Once properly synthesized, water-solubilized, and functionalized, these materials have applications ranging from solar cells, energy generation, QD-based lasers, and light-emitting devices to chemical and biological sensors and labels for biological tracking studies.

The applications of interest in this thesis revolve around the use of QDs in sensors. QDs are ideal for use in optical sensors over organic dyes due to the optical properties mentioned in section 2 of this chapter. Initial studies using QDs as sensors used materials that were so unstable that they would respond to anything. As QDs became highly stable due to robust surface passivation, they became insensitive to their environment.

A new method of sensing for QDs was first introduced by van Orden’s group using fluorescence resonance energy transfer (FRET) from a QD donor to a dye acceptor. With their continuous absorption profiles and narrow, size-tunable emission
spectra, QDs are ideal donors for FRET as long as the QD donor emission overlaps the dye acceptor absorption, as shown in Figure 7. The efficiency of the FRET interaction is dependent upon the spectral overlap as well as the distance between the donor and acceptor, among other factors.

Subsequently, Mattoussi’s group which described QD-based sensing of trinitrotoluene (TNT) via FRET; the displacement of a quencher by TNT caused an enhancement in QD emission. More singular “turn-on” or “turn-off” sensors were published soon after, but these sensors can be difficult to calibrate in complex environments, such as biological systems. The next development in QD FRET sensing involved the use of ratiometric QD FRET sensors. Ratiometric, or dual emissive, QD FRET sensors are self-calibrating due to the fact that the ratio of the emission intensity of the two emitters can be quantified regardless of the concentration of the QDs and the complexity of the environment of the QDs. The majority of this thesis describes such ratiometric QD FRET sensors for the detection of unlabeled proteins.

1.8 Overview of Thesis

This thesis focuses on the development of a QD sensor that selectively and ratiometrically responds to the presence of unlabeled proteins. The first investigation generated a model QD-based protein sensor for streptavidin, a bacterial form of an egg white protein. The second studied a QD protein sensor for cyclooxygenase-2, a protein related to inflammation and thus tumor metathesis. A third QD-based protein sensor was developed for thrombin, a protein in the blood coagulation cascade that, in high blood-borne concentrations, is a marker for several diseases. The detection limits of
Figure 7. CdSe/ZnS quantum dot donor emission and rhodamine B piperazine dye acceptor absorption. The spectral overlap (green) is a factor in determining the FRET efficiency of the donor and acceptor pair.
these sensors are in the low pmol/mL range, making them competitive with ELISA assays. These sensors are fast, homogeneous, have detection limits that are scalable with concentration, and are translatable to many other biologically relevant proteins with the use of different small molecular protein binding agents and aptamers. Finally, the synthesis of very bright ZnSe/CdS/ZnS type II QDs is discussed. The unprecedented stability and brightness of these materials allowed for them to be water-solubilized via two different methods. To demonstrate the possibility for sensing applications, an energy-transfer accepting organic dye was conjugated to the water-solubilized type II QDs. Efficient energy transfer was characterized via time-correlated single photon counting; this result ultimately expands the variety of quantum dots that may be used in analytical chemistry.
2. Ratiometric Quantum Dot Streptavidin Sensor

2.1 Introduction

The quantitative and selective detection of proteins is a significant research endeavor; this is especially true as many ailments such as HIV can be diagnosed by the presence of blood-borne antibodies against the infection. Unfortunately, methods of protein sensing can be complex due to the fact that unlabeled proteins are essentially optically inert. The ubiquitous enzyme-linked immunosorbent assay (ELISA)\textsuperscript{159} and Western Blot\textsuperscript{160} heterogeneous methods utilize optical detection through the use of secondary reporters that only indirectly target the analyte of interest. These secondary antibodies catalyze the formation of an absorptive stain or a fluorescent signature that results in a nonlinear and highly sensitive response to targeted analytes; however, nonspecific adsorption of the secondary antibody (or alternatively a weak binding affinity) is detrimental in terms of the detection limit (DL). In this chapter, a homogeneous novel architecture of ratiometric (or dual emissive or self-calibrating) sensing of unlabeled proteins using fluorescent semiconductor QDs that avoids the use of secondary antibodies is presented; this sensor has a specific response that is scalable by concentration and is fully quantitative.

As discussed in Chapter 1, fluorescent QDs have been utilized in a variety of biological applications due to their many desirable traits as imaging agents.\textsuperscript{8, 9} Highly photostable QDs have narrow and size tunable emission spectra and continuous absorption profiles.\textsuperscript{1, 2, 6, 7} Although QDs are often employed as markers in biological tracking studies, the use of nanocrystals for specific chemical and biological sensing is
a more difficult endeavor due to the surface passivation of QDs, which makes them unresponsive to the surrounding matrix.\textsuperscript{18, 19, 152} Many groups have demonstrated that the manipulation of FRET is an effective means of imparting a sensing response to highly passivated semiconductor QDs.\textsuperscript{113, 135-137, 161, 162} These systems of FRET manipulation function by either spatially or spectrally altering the efficiency of QD FRET donors to organic chromophore acceptors. These reports have shown that highly surface-passivated fluorescent semiconductor QDs, although inherently well-protected from their exterior, have utility in a variety of specific and quantifiable sensing applications.

Biological sensing applications have also been demonstrated with semiconductor QDs. Specifically, the dynamics of DNA replication have been examined,\textsuperscript{141} and the presence of HIV and other proteins has been quantified via QD FRET sensing.\textsuperscript{140, 142} However, the analytes of interest were prelabeled in these studies. In such a sensing scheme, extra processing of analytes is required to remove any unbound dyes that would interfere with the fidelity of the method. Recently, sensing unlabeled proteins with nanocrystals has been demonstrated via manipulation of the QD’s quantum yield via the nanocrystal–protein interaction; however, the singular response of fluorescence quenching\textsuperscript{163, 164} or brightening\textsuperscript{165, 166} is difficult to calibrate in a complex biological environment. Consequently, a self-calibrating method of specific protein detection, where the analyte of interest is in its native state, is highly desirable.

In this chapter, the development of a ratiometric QD sensor that selectively and ratiometrically responds to the presence of unlabeled proteins is discussed.\textsuperscript{157} A model QD protein sensor system for sensing the often-studied streptavidin is discussed. The
motif was inspired by FRET-based protein sensors that function by energy transfer from a donor chromophore to a nearby dye-labeled protein analyte, where the protein is tagged with a broad-band reactive dye. In this system, the sensing dye is simply moved from the analyte to the donor chromophore (the nanocrystal) directly; furthermore, initial studies on the system suggested that conjugation of the dye to the protein binding agent would improve the system’s response. A diagram of the QD protein sensor is shown in Scheme 1, in which the binding of the protein streptavidin to its agonist biotin differentially alters the emission intensities of the QD and the dye to create a ratiometric response. It was found that the binding is specific and quantifiable, and that the response is consistent with the known environmental dependence of rhodamine’s absorptive and emissive properties.\textsuperscript{167-171} The results indicate that the DL of the QD protein sensor is competitive with the averaged DLs from a set of ELISA assays surveyed from the literature\textsuperscript{172-193} and is in fact scalable with concentration; essentially, the DL can be lowered by diluting the sensor within reasonable limits. In addition, the presented assay is faster and easier than ELISA due to the fact that the platform is homogeneous.

2.2 Materials

2.2.1 Reagents

Commercial sources were used to obtain all chemicals. These chemicals were used as they were received except where indicated.
Scheme 1. CdSe/ZnS quantum dot conjugated to a RBpip—Lysine—Biotin Dye. Reprinted with permission from Ref. \(^{157}\). Copyright 2014 American Chemical Society.
2.2.2 Equipment and Instrumentation

\(^1\text{H}\) Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance DRX 400 NMR spectrometer using \(d_4\)-methanol as the solvent. A Varian Cary 300 Bio UV-vis spectrophotometer was used to obtain UV-vis absorbance spectra. A customized Fluorolog (HORIBA Jobin Yvon) modular spectrofluorometer was used to obtain fluorescence emission spectra. Inner-filtering effects were avoided by having an absorbance near or below 0.1 OD for all solutions at the excited wavelength. Thin-layer chromatography (TLC) was performed with Analtech UNIPLATE Silica Gel GF thin-layer chromatography plates. The TLC plates were rinsed in chamber solvent (the same solvent ratio that would be used to run the sample on the TLC plate) before use. To rinse a plate, the plate is placed in the TLC chamber with the chamber solvent, and the solvent is allowed to run to the top of the plate. The plate is then taken out of the chamber, and after thorough drying of the plate, the plate can be used to perform TLC. High-pressure liquid chromatography (HPLC) was performed on a Waters 600 using a Rainin Microsorb-MV column (C18, 3 μm, 100 Å, 86-200-E3).

2.3 Methods

2.3.1 Summary of Sensor Design

The initial work on QD FRET protein sensors began by exposing QD–dye FRET pairs to nonspecifically binding proteins; in this system, QD emission was enhanced with a simultaneous drop in the dye emission upon exposure to excess protein. These initial results indicated that a strong, ratiometric, and specific response may be observed if a protein binding agent is in close proximity to the FRET-accepting dye. As a
result, streptavidin was chosen as a model analyte due to its strong affinity for small molecular biotin ($K_d \approx 10^{-14}$ M).\textsuperscript{194} A method was developed to conjugate biotin to rhodamine B piperazine dye using lysine such that water-soluble CdSe/ZnS QDs could be functionalized with the couple. First, the succinimidal ester of biotin was synthesized and was then coupled to the free amine of singularly protected Boc—lysine—OH. Rhodamine B piperazine\textsuperscript{195} (RBpip) was then conjugated to the Boc—lysine—biotin using standard BOP chemistry. After deprotection using trifluoroacetic acid, the RBpip—lysine—biotin (RBpip—biotin) was conjugated to polymer-coated water-solubilized CdSe/ZnS QDs using my group’s recently developed methyl poly(ethylene glycol) carbodiimide chemistry.\textsuperscript{17} Full details on the synthesis, purification, and characterization are described below. Both QD and dye emission are observable when exciting the QD at 450 nm due to FRET, as supported by the PLE data shown in Figure 8.

2.3.2 Quantum Dot Synthesis

Previously published protocols\textsuperscript{83, 196-198} were used to synthesize core CdSe and core/shell CdSe/ZnS QDs. For completeness, an example CdSe/ZnS synthesis is described here.

For core CdSe, 6.02 g of trioctylphosphine oxide, 0.55 g of tetradecylphosphonic acid, 0.27 g of cadmium acetate, and 6 mL of trioctylphosphine are added to a round bottom flask and degassed at 180 °C. Next, the sample is heated to 260 °C under nitrogen for 10 min. Then, the sample is cooled to 180 °C and degassed again. The sample is then heated to 360 °C under nitrogen, and 2.5 mL of 0.1 M selenium in trioctylphosphine is swiftly injected. The sample is cooled immediately.
Figure 8. Photoluminescence Excitation (PLE) spectra of CdSe/ZnS-RBpip-Biotin and RBpip-Biotin with absorption of CdSe/ZnS; emission was monitored at 605 nm. The PLE of RBpip-Biotin and the absorption of CdSe/ZnS combine to form the PLE of CdSe/ZnS-RBpip-Biotin. This is the result of FRET between a QD donor and dye acceptor. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
To overcoat with ZnS, the cores are first precipitated with isopropanol and a minimal amount of methanol. The supernatant is discarded, and the sample is dissolved in hexane. The cores are then precipitated with isopropanol and a minimal amount of methanol. Next, the cores are dissolved in 4 mL of hexane. Then, 0.23 g of tetradecylphosphonic acid, 17 mg of cadmium acetate, 36 mg of zinc acetate, 3.38 g of trioctylphosphine oxide, and 3.4 mL of trioctylphosphine are added to a round bottom flask and degassed at 180 °C. Next, the sample is heated to 260 °C under nitrogen for 10 min and then cooled to 80 °C. The cores in hexane are then added to the round bottom flask, and the hexane is removed at 80 °C. Next, 2.8 mL of decylamine is added to the round bottom flask, and the sample is stirred for approximately 2.5 hours under nitrogen. Then, 62 mg of diethylzinc in 2.4 mL of trioctylphosphine and 84 mg of bis(trimethylsilyl)sulfide in 2.5 mL of trioctylphosphine are slowly dripped in at 160 °C.

2.3.3 Quantum Dot Water-Solubilization

“Samples were processed by addition of a small amount of isopropanol followed by methanol to [precipitate] the samples. The supernatant is discarded leaving behind a gel most likely composed of a decylamine–tetradecylphosphonic acid adduct used in the ZnS overcoating procedure. The CdSe/ZnS [QDs] were extracted from this gel by several washings of hexane; the washings were collected, from which the [QDs] were precipitated again through the addition of a few drops of isopropanol and [sufficient] methanol. The precipitate was collected by centrifugation and dried under reduced pressure.” (Reprinted with permission from ref 66. Copyright 2011 American Chemical Society.) 40% octylamine modified poly(acrylic acid) polymer76 (ref 70 has details on the
synthesis and characterization) was used to water-solubilize these QDs. The concentration of QDs was determined by the methods outlined in ref 83.

### 2.3.4 Protein-Binding Ligand Synthesis

A general summary of the synthesis of RBpip—biotin dye is shown in Scheme 2. Biotin–NHS was synthesized and purified according to a previously published protocol. Approximately 273 mg of biotin—NHS was added to 197 mg of Boc–lysine–OH and 111 μL of triethylamine in 10 mL of dimethylformamide (DMF) and allowed to stir overnight under nitrogen. The Boc—lysine—biotin was purified with boiling ethyl acetate and centrifuged three times followed by recrystallization with hot ethanol.

Rhodamine B piperazine was synthesized according to a previously published protocol. The RBpip was then purified using HPLC, and stored in the refrigerator until use. It was found that HPLC purification is not necessary for these purposes as the final product must also be rigorously purified. Approximately 5 mg of Boc—lysine—biotin, 5 mg of benzotriazole-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (commonly known as BOP), and 3 μL of 2,4,6-collidine were stirred in DMF for 30 min. Initially this reaction was performed with EDC and 4-dimethylaminopyridine (DMAP), but BOP and 2,4,6-collidine appeared to afford much higher reaction yields according to TLC. Then, 5 mg of RBpip was added, and the reaction was allowed to continue overnight. After drying under vacuum, the RBpip—biotin was purified with TLC (90% dichloromethane/10% methanol solvent); the darkest band with an Rf value of ~0.5 was removed from the plate and extracted in a 67% dichloromethane/33% methanol
Scheme 2. Reaction scheme for the synthesis of RBpip—Biotin dye. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
solution. The resulting supernatant was dried by vacuum and then further purified by HPLC, with 100% water used to remove impurities and a mixture of 60% water and 40% methanol used to elute the RBpip—biotin.

The RBpip—biotin was deprotected with 1 mL of trifluoroacetic acid in 1 mL of dichloromethane. After stirring overnight, the RBpip—biotin was dried by vacuum and was used without further purification. The QY of the functionalized dye was determined to be 0.28. Although it was found that it was necessary to utilize extensive purification (prep-scale TLC followed by HPLC) to obtain essentially pure material for NMR analysis, surprisingly it was found that the crude product (without the HPLC purification step) can be used to synthesize the QD sensor for streptavidin. The only difference is that the magnitude of the response is not the same due to the presence of some dye-containing byproduct conjugated to the QD. The presence of a significant number of unidentifiable impurities was also observed in the NMR data. All data in this chapter were obtained with the rigorously purified RBpip—biotin QD ligand.

2.3.5 List of NMR peaks for Boc—lysine—biotin and RBpip—biotin

NMR was performed on the purified Boc—lysine—biotin; NMR data is shown in Figure 9. \( ^1H \) NMR (400 MHz, MeOD): \( \delta \) 1.39-1.82 (m, 21), 2.17-2.21 (t, 2), 2.67-2.71 (d, 1), 2.85-2.99 (dd, 1), 3.15-3.23 (3, m), 3.89-4.07 (1, m), 4.28-4.32 (1, m), 4.47-4.50 (1, m). Some residual ethanol remains after drying; further, an impurity is also seen in the range of 1.14-1.28 and 7.97-8.00 ppm.

HPLC purified, protected RBpip—biotin was used to take the NMR data shown in Figure 10. \( ^1H \) NMR (400 MHz, MeOD): \( \delta \) 1.28-1.32 (t), 1.36-1.73 (m), 2.15-2.19 (t),
Figure 9. $^1$H NMR spectra of Boc—lysine—biotin. Residual ethanol solvent from the recrystallization step is evident at 1.2 and 3.6 ppm. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 10. $^1$H NMR spectrum of RBpip—biotin. The region between 3.2 and 3.4 as well as 3.8 and 6.4 ppm is obscured by the solvent. The complex splitting pattern makes the protons labeled "9" difficult to assign in the 3.4 to 3.6 ppm region. Reprinted with permission from Ref. $^{157}$. Copyright 2014 American Chemical Society.
2.66-2.70 (d), 2.88-2.93 (dd), 3.12-3.13 (br s), 3.15-3.22 (m), 3.47-3.48 (t), 3.54-3.57 (m), 3.63-3.70 (m), 6.96-6.97 (d), 7.06-7.09 (dd), 7.27-7.30 (d), 7.50-7.53 (m), 7.73-7.78 (m). Some impurities in the sample were observed at 1.88, 3.40, 6.47, 8.08, and 8.54 ppm.

2.3.6 Conjugation of Ligand and Quantum Dot

Approximately 1 mL of water-solubilized CdSe/ZnS QDs was stirred for 30 min with 9 mg of methyl-polyethylene glycol carbodiimide (prepared with 2-methoxyethyl isothiocyanate instead of ethyl isothiocyanate),\textsuperscript{17} after which 3 mL of pH 8 phosphate buffer was added. Then, RBpip—biotin dye was added until the emission of the QDs and dye appeared approximately equal; other dye-to-quantum-dot ratios were not examined in this study. The reaction was allowed to stir overnight; the next day, \(\sim 3 \text{ mL}\) of 0.1 M NaOH was added, and the reaction was again allowed to stir overnight. Dialysis was performed using centrifugation filters to remove unreacted RBpip—biotin dye, with the QDs concentrated in pH 7 phosphate buffer upon completion. The QD protein sensor solution was then diluted in a 1.4 mL cuvette to a working concentration of \(4.19 \times 10^{-7} \text{ M}\), which was diluted to \(4.01 \times 10^{-7} \text{ M}\) by the addition of the streptavidin analyte. For the \(\sim\)one tenth dilute study, the QD protein sensor solution had a concentration of \(5.90 \times 10^{-8} \text{ M}\), which was diluted to \(5.44 \times 10^{-8} \text{ M}\) by the addition of the streptavidin analyte. Note that the dilution obviously lowers the total signal intensity; however, a ratiometric detection scheme is not affected by the signal strength (so long as a clear spectrum is obtainable).
The number of RBpip—biotin ligands per QD, 2.4 ligands/QD, was determined using UV−vis spectroscopy by measuring the ligand concentration based on the absorptivity of the dye component; the QD concentration was already known as discussed above. It was found that the signal was saturated when the streptavidin concentration was ∼one-fifth that of the RBpip—biotin ligands. As streptavidin has four biotin-binding sites, it would appear that the saturation was partially due to the interaction of the analyte with all available binding partners. However, I do not believe that this is the case as the samples would have aggregated if streptavidin was binding to multiple RBpip—biotin ligands (and therefore putting multiple QDs together in close proximity). I attribute this to the possibility that some RBpip—biotin ligands cannot bind to the protein due to the likely complexity of the environment of the QD polymer-coating layer. Some RBpip—biotin ligands may be buried in the polymer-coating layer in such a way as to prevent binding with streptavidin.

2.3.7 Streptavidin Preparation

One milligram of streptavidin lyophilizate (∼0.9 mg of streptavidin, ∼0.1 mg of NaCl) (MP Biomedicals, Solon, Ohio) was diluted in 400 μL of deionized water giving a concentration of $4.09 \times 10^{-5}$ M streptavidin. Another dilute solution at $1.74 \times 10^{-7}$ M was also prepared from this solution. Solutions were stored at 4 °C when not in use.
2.3.8 Control Protein (Bovine Serum Albumin) Preparation

Bovine serum albumin (BSA, 3.5 mg) was diluted in 200 μL of deionized water giving a concentration of $2.64 \times 10^{-4}$ M. BSA was stored at 4 °C, and solutions were prepared the same day used.

2.3.9 Detection of Streptavidin

All protein-sensing experiments were performed as follows: after a 1 μL scale volume of protein solution was added to the sensing solution, the mixture was very gently mixed by pipet and then immediately measured by excitation at 450 nm. The detection time from addition of protein to a complete fluorescence spectrum was approximately 5 min, with less than 1 min of sample preparation and just over 4 min of spectrum collection. As fluorimeters and their settings vary, it is possible for the detection time to be significantly less; for example, detection time could be significantly decreased if an array detector is used.

2.4 Results and Discussion

Streptavidin in deionized water was introduced into the QD—RBpip—biotin solution in μL scale quantities. As demonstrated in Figure 11, a ratiometric response is observed upon exposure to nanomolar concentrations of protein. As seen in the initial studies, the fluorescence intensity of the QDs increases concomitantly with a loss of dye emission. This creates a ratiometric response to the streptavidin, with an isosbestic point appearing at 570 nm. Given the strong interaction of streptavidin and biotin, and
Figure 11. Normalized emission of streptavidin-sensing CdSe/ZnS upon exposure to the protein. The left peak is the QD emission, and the right peak is the streptavidin-sensing dye emission. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
the response of the emission specifically to the protein, I believe that the close proximity of streptavidin to the dye alters the microenvironment in such a way as to cause this ratiometric response. This is supported by the observation that the free RBpip—biotin ligand is reversibly quenched by streptavidin as shown in Figure 12, which is consistent with the known loss of QY when rhodamine dyes are conjugated or exposed to proteins.\textsuperscript{170, 171, 201} As shown in Figure 13, the QD—RBpip—biotin construct has no response to BSA; a significant excess of this protein has to be added before the emission is perturbed. There is also no response to streptavidin in a QD—RBpip chromophore missing biotin as shown in Figure 14. The absorption spectrum of the QD—RBpip—biotin construct also shows little change before and after exposure to streptavidin, as can be seen in Figure 15.

The response of the QD protein sensor was quantified through the separation of the emission spectra shown in Figure 11 into QD and dye components using multiple Gaussian functions. This allows for the determination of the integrated emission of each chromophore; with these data, the percent change in QD/dye integrated emission ratio as a function of the protein concentration was then plotted as shown in Figure 16. The raw data is shown in Figure 17. After rising sharply, the QD/dye emission ratio appears to saturate at a streptavidin concentration of $\sim 200$ pmol/mL. I attribute this to protein binding to all available RBpip—biotin ligands on the surfaces of the QDs, especially as I calculated that the sample contained a similar concentration ($\sim 400$ pmol/mL) of QD sensors. This concentration of QD sensors translates to $\sim 960$ pmol/mL of RBpip—biotin ligands; therefore, the saturation occurs when the streptavidin concentration is approximately one-fifth that of the RBpip—biotin ligands. Interestingly, as seen in the
Figure 12. The emission of biotin-bound rhodamine B piperazine dye (RBpip—biotin) is quenched by the addition of streptavidin. Addition of excess biotin results in a significant return of the dye emission. Inset: Exposing rhodamine B piperazine dye to streptavidin under identical conditions results in a minimal change in the dye’s fluorescence intensity. Samples were excited at 530 nm. Note these spectra are dye samples only; no QDs are present in these samples. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 13. The streptavidin-sensing CdSe/ZnS exposed to a high concentration of bovine serum albumin. No response is observed. The left peak is the QD emission, and the right peak is the streptavidin-sensing dye emission. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 14. Emission spectra of CdSe/ZnS water-soluble QDs conjugated to rhodamine B piperazine before and after exposure to excess streptavidin; note that biotin is not conjoined to the dye nor the QD. The lack of a response demonstrates the necessity of biotin for ratiometric streptavidin sensing. The samples were excited at 450 nm. The left peak is the QD emission, and the right peak is the dye emission. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 15. The absorption spectrum of a QD streptavidin sensor displays little differences before and after exposure to excess streptavidin. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 16. Percent change in the ratio of the integrated emission of QD over RBpip as a function of streptavidin concentration; each point has been divided by the initial QD/dye ratio value, which is then subtracted from the data. The line is a guide to the eye; unprocessed data are shown in Fig. 17. Inset: Expansion of the same showing enhanced sensitivity at low protein concentrations (<1 pmol/mL). Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 17. Absolute change in the ratio of the integrated emission of QD over RBpip as a function of streptavidin concentration; these data are used in the calculation of the detection limit. The same data are divided by the initial data point, and then normalized by subtracting the first point from the remaining data, to generate the percent ratio change as shown in Fig. 16. The line is a guide to the eye. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
inset of Figure 16, there is an overall sharp rise in the QD/dye emission ratio at concentration levels of less than 1 pmol/mL of protein that is consistent with the picomolar order DL as discussed below.

To calculate the detection limit under various conditions, the bootstrap method\textsuperscript{202} was employed to generate 500 Monte Carlo sets of blank QD—RBpip—biotin emission spectra, as shown in Figure 18. This data set was then used to calculate the standard deviation of the blank response, from which the detection limit was determined as 3 times this standard deviation divided by the slope of the calibration curve shown in Figure 17 (the linear region between 1.2 and 75 pmol/mL was used). See Figure 19 for linear regression. From this, a DL of 3.06 ± 0.07 pmol/mL (3.06 × 10\textsuperscript{-9} M or 1.68 × 10\textsuperscript{-4} g/L) streptavidin was calculated. If the DL is calculated from the more responsive region below 1 pmol/mL of protein, which is more appropriate, the DL lowers to a total of 210 ± 70 femtomol/mL protein (2.10 × 10\textsuperscript{-10} M or 1.16 × 10\textsuperscript{-5} g/L).

The literature was surveyed to compare the QD streptavidin sensor’s DL against a representative set of ELISA assays that detected various proteins.\textsuperscript{172-193} The average detection limit of ELISA from the 22 references examined is 1.3 × 10\textsuperscript{-4} g/L (lowest 4.0 × 10\textsuperscript{-9} g/L and highest 3.0 × 10\textsuperscript{-3} g/L), corresponding to 3.8 × 10\textsuperscript{-8} M (lowest 5.7 × 10\textsuperscript{-14} M and highest 8.9 × 10\textsuperscript{-7} M) after the molecular weight of the proteins is accounted for. Thus, the QD protein sensor is highly competitive with ELISA; however, as noted above, ELISA DLs span several orders of magnitude. As such, I can only comfortably state that the method is as effective as ELISA.

An interesting aspect concerning ratiometric sensors is that the signal (i.e., chromophore/chromophore integrated emission ratio) is dependent on the ability to
Figure 18. Bootstrap method for generating Monte Carlo blanks. A real blank spectrum (blue) is fit with multiple Gaussian functions (red dash). The difference spectrum (purple) of the two is calculated, multiplied with a random number at every point (black), and added back to the fit to generate the Monte Carlo blank spectrum (green dash).
Figure 19. Absolute change in the ratio of the integrated emission of QD over RBpip as a function of streptavidin concentration; the red line is the linear regression for the points between 1.2 to 75 pmol/mL. The slope, y-intercept, and R squared value are listed above the inset which shows a close up of the linear range used. This data was used to create Figure 16 as described in Figure 17. The blue line is a guide to the eye. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
observe a change in the emission over the background of QD sensors that are not bound to proteins. As such, diluting the sensor should increase the slope of the calibration curve and lower the DL; although doing so simultaneously lowers the total signal strength which adds noise and increases the DL. Thus, a balance must be found where the increase in slope (decrease in DL) outweighs the increase in noise (increase in DL) to achieve the optimal DL. This balance will be different depending on the quality of the materials and the instrument used. To examine the concentration-dependent response, the QD sensors were diluted to ∼one tenth of the original concentration and were exposed to increasing levels of streptavidin. Figure 20 shows the emission spectra of the diluted sensor, and the percent change in QD/dye integrated emission ratio as a function of the protein concentration is shown in Figure 21. As before, an initial sharp rise in the response which occurs at a concentration of less than 400 fmol/mL of analyte was observed. The QD/dye emission ratio then becomes linear and then saturates after exposure to ∼45 pmol/mL of streptavidin. The slope of the response was greater than previously observed, whereas noise levels were not significantly increased due to the performance of the detector used. As a result, the DL of this study lowered to 400 ± 20 fmol/mL of streptavidin (4.00 × 10^{-10} M or 2.20 × 10^{-5} g/L) over the range of 0.4 to 14 pmol/mL; however, using the enhanced response at low levels of protein below 0.4 pmol/mL affords a DL of 16 ± 9 fmol/mL (1.6 × 10^{-11} M or 8.8 × 10^{-7} g/L). These results demonstrate that the DL is scalable with concentration; as such, it may even be possible to ratiometrically detect one protein using single chromophore imaging techniques. The sensor also responds to streptavidin when the experiments are performed under the typical serum dilution of 1:100 as shown in Figure 22. Both the
Figure 20. Emission spectra of a highly dilute QD streptavidin sensor (~70 pmol / 1.2 mL) as a function of streptavidin concentration. The samples were excited at 450 nm. The left peak is the QD emission, and the right peak is the streptavidin-sensing dye emission. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 21. Percent change in the ratio of the integrated emission of QD over RBpip as a function of streptavidin concentration for a highly dilute QD streptavidin sensor. The absolute change in ratio is similar to the data presented in Fig. 17. The line is a guide to the eye. Inset: Magnified emission ratio below a streptavidin concentration of 2.5 pmol/mL. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 22. Emission spectra of QD streptavidin sensor responding to streptavidin in serum and without serum (control). Aside from the absence of serum in the control, conditions were identical. The left peak is the QD emission, and the right peak is the streptavidin-sensing dye emission.
sensor in serum and the sensor without serum have a similar response to equal concentrations of streptavidin.

2.5 Conclusion

A model fluorescent QD protein sensor which can specifically and ratiometrically respond to proteins with DLs as low as a few pmol/mL has been created. The DLs are competitive with ELISA assays; furthermore, the measurement technique is significantly less time-consuming and simpler compared to ELISA or backscattering interferometry. This is due to the method being homogeneous. By implementing a similar architecture with other small molecular protein-binding agents and aptamers and studying the responses of the new systems, I believe that a variety of proteins associated with many diseases could be ratiometrically quantified with this method. I also hope that this method may allow for widescale screening for infectious diseases in areas where the cost and time for implementing ELISA and Western blot assays are prohibitive. The sensing system will also likely find utility in cellular imaging studies as examining protein–protein interactions with FRET requires the use of multiply labeled species. Using ratiometric protein-sensing QDs obviates the need for the target analyte to be genetically modified with GFP or tagged with another dye. Furthermore, the identities of unbound and bound probes are distinguishable with the method.
3. Ratiometric Quantum Dot Cyclooxygenase-2 Sensor

3.1 Introduction

After the success of the ratiometric quantum dot streptavidin sensor discussed in Chapter 2, the next logical step is to modify the sensor to detect a more biologically relevant protein. The most important thing to further this research is finding a small, functionalizable agent that can bind to a biologically relevant protein and also be conjugated to a QD. Thus, the protein binding agent needs to have a QD-coordinating functional group that could be modified without affecting the binding to the target protein. Luckily, several of the binding agents found met these criteria.205-207

One of the first binding agents examined was folic acid, also known as vitamin B9, the agonist for folate receptor. Furthermore, there were many references detailing procedures on how to conjugate folic acid without removing its ability to bind to folate receptor protein, which is over-expressed on the surfaces of cancer cells.205 The ability to create a ratiometric QD protein sensor for folate receptor would be very useful as it could be used for cancer detection. Based on previous research, folic acid could be bound to the sensing platform on the QD through one of its carboxylic acid groups.205, 212-214 However, upon attempting to repeat previously reported procedures involving folic acid, it was found that the material degraded very quickly and the reaction yields were very low even under the reported reaction conditions. Despite vigorous protection from light and an air-free atmosphere, the first reaction step always resulted in NMR peaks that suggested many side reactions were occurring and that folic acid was degrading.
After exhausting every reported method to conjugate folic acid with no positive results, research into this binding agent and target protein pair was stopped.

The next binding agent studied was indomethacin (Indocin), which is a nonselective cyclooxygenase inhibitor. It binds to two of the three cyclooxygenase isoenzymes; one of these, cyclooxygenase-2 (Cox-2), is present at inflammation sites and tumors. Thus, this compound is used as a drug to reduce inflammation and relieve pain associated with tumors and cancer. A previous report by the Marnett group showed that the amide form of Indocin is selective towards Cox-2.$^{215}$ In another report, Indocin was conjugated to a 5-ROX dye via a linker; this report showed that the specific binding to Cox-2 was significantly higher than other compounds tested.$^{206}$ Given that their report described a very similar conjugation scheme to what was previous done with the ratiometric QD streptavidin sensor, this binding agent and target protein pair seemed like an ideal system to create a new ratiometric QD protein sensor for a biologically relevant protein.

Reported in this chapter is the research completed toward the development of a ratiometric QD Cox-2 sensor. While this project was ultimately stopped before publishable results were obtained, much work had been completed before all possible avenues had been exhausted. The information presented describes the work done for the two different protein sensing architectures that were designed. These sensing platforms were very similar to the one described in Chapter 2 for the detection of streptavidin. All the parameters that were modified in the hopes of designing a publishable sensor, such as the type of water-solubilization of the QDs, the ligand
syntheses, and the conjugation of the ligand and QD, will be discussed. Finally, the best results that were obtained from one of the samples will be presented.

3.2 Materials

3.2.1 Reagents

Commercial sources were used to obtain all chemicals. These chemicals were used as they were received except where indicated.

3.2.2 Equipment and Instrumentation

$^1$H NMR spectra were recorded on a Bruker Avance DRX 400 NMR spectrometer using $d_4$-methanol as the solvent. A Varian Cary 300 Bio UV–vis spectrophotometer was used to obtain UV–vis absorbance spectra. A customized Fluorolog (HORIBA Jobin Yvon) modular spectrofluorometer was used to obtain fluorescence emission spectra. Inner-filtering effects were avoided by having an absorbance near or below 0.1 OD for all solutions at the excited wavelength. TLC was performed with Analtech UNIPLATE Silica Gel GF thin-layer chromatography plates. The TLC plates were rinsed in chamber solvent (the same solvent ratio that would be used to run the sample on the TLC plate) before use. To rinse a plate, the plate is placed in the TLC chamber with the chamber solvent, and the solvent is allowed to run to the top of the plate. The plate is then taken out of the chamber, and after thorough drying of the plate, the plate can be used to perform TLC. HPLC was performed on a Waters 600 using a Rainin Microsorb-MV column (C18, 3 μm, 100 Å, 86-200-E3) and a Supleco Ascentis column (C18, 5μm, 25 cm × 21.2 mm, 581347-U).
3.3 Methods

3.3.1 Summary of Sensor Design

Given the success of the QD streptavidin sensor discussed in Chapter 2, the same design was used for the QD Cox-2 sensor as shown in Scheme 3. The linker was lysine, the dye was rhodamine B piperazine, and the QD was CdSe/ZnS. The only difference was that the protein binding agent was now Indocin instead of biotin so that the QD protein sensor would be selective towards Cox-2. Given the $K_d$ of amide-modified Indocin and Cox-2 is $1.7 \times 10^{-6}$ M, which is about eight times higher than the $K_d$ of biotin and streptavidin, it was expected that this QD protein sensor would have a different response to the target protein. After several experiments with this sensor architecture were tried, it became clear that there were some problems associated with this new QD protein sensor that were unexpected. The first was that the conjugation of the RBpip—lysine—Indocin—QD construct proved more difficult; precipitation of the samples was common. The second was that there sometimes appeared to be significant photobleaching of the dye upon excitation, which negatively affected the results obtained from experiments. In an attempt to circumvent these issues, many different avenues were explored, including different sets of CdSe/ZnS QDs and different water-solubilization techniques. When these experiments did not yield publishable results, a different dye was used. This new dye, rhodamine B isothiocyanate (RBiso), required a different protein sensing architecture for the QD protein sensor. The proposed architecture involved switching the position of the QD and dye on the lysine linker. Full details on the synthesis, purification, and characterization of all constructs are described below.
Scheme 3. CdSe/ZnS quantum dot conjugated to a RBpip—Lysine—Indocin Dye.
3.3.2 Quantum Dot Synthesis

As mentioned in Chapter 2, previously published protocols\textsuperscript{83,196-198} were used to synthesize core CdSe and core/shell CdSe/ZnS QDs. See Chapter 2 for an example of the synthesis of CdSe/ZnS.

3.3.3 Quantum Dot Water-Solubilization

Many different types of polymer-encapsulation and cap-exchange water-solubilization methods were used for this project. See Section 1.5 for information about the differences between polymer-encapsulation and cap-exchange for the water solubilization of QDs. For polymer-encapsulation, a 40\% octylamine modified poly(acrylic acid) polymer\textsuperscript{76} (see ref \textsuperscript{70} for details on the synthesis and characterization) and a 6\% PEG modified 40\% octylamine modified poly(acrylic acid) polymer were used. For some polymer samples, PEG 750-amine was also conjugated to the QDs after water-solubilization to improve stability. For cap-exchange, DHLA\textsuperscript{55} using the zinc method, carboxylic acid-PEG 400-DHLA, 50/50 carboxylic acid-PEG 400-DHLA/methyl-PEG 750-DHLA, and silane\textsuperscript{68} were used. For some silane samples, PEG 750-amine was also added after water-solubilization to improve stability.

CdSe/ZnS QD “samples were processed by addition of a small amount of isopropanol followed by methanol to [precipitate] the samples. The supernatant is discarded leaving behind a gel most likely composed of a decylamine–tetradeccylphosphonic acid adduct used in the ZnS overcoating procedure. The CdSe/ZnS [QDs] were extracted from this gel by several washings of hexane; the washings were collected, from which the [QDs] were precipitated again through the
addition of a few drops of isopropanol and [sufficient] methanol. The precipitate was *then* processed differently depending on the water-solubilization method. The concentration of all these water-solubilized QDs was determined by the methods outlined in ref 83.

For 40% octylamine modified poly(acrylic acid) polymer or 6% PEG modified 40% octylamine modified poly(acrylic acid) polymer QDs, the precipitate was collected by centrifugation and dried under reduced pressure. These QDs were water-solubilized with either a 40% octylamine modified poly(acrylic acid) polymer or 6% PEG modified 40% octylamine modified poly(acrylic acid) polymer. To improve stability, PEG 750-amine was sometimes added to 40% octylamine modified poly(acrylic acid) polymer QDs by reacting the QDs with PEG 750-amine in a basic solution in the presence of DMTMM. After stirring overnight, the QDs were dialyzed to remove unreacted PEG 750-amine.

For QDs water-solubilized with DHLA using the zinc method, the precipitate was collected by centrifugation and dried under reduced pressure. Briefly, lipoic acid was reduced to DHLA, purified via liquid-liquid separations with toluene and water, and dried. DHLA in methanol was reacted with sodium hydroxide and zinc nitrate hexahydrate under heat and nitrogen. Once clear, the solution was cooled and stirred overnight with precipitated QDs under nitrogen. Then, the QDs were precipitated, dissolved in basic water, and dialyzed.

For QDs water-solubilized with carboxylic acid-PEG 400-DHLA, the precipitate was collected by centrifugation and dried under reduced pressure. Carboxylic acid-PEG
400-thiolic acid was reduced to carboxylic acid-PEG 400-DHLA, purified via liquid-liquid separations in chloroform and acidic water, and dried according to a published procedure. The precipitated QDs and carboxylic acid-PEG 400-DHLA were stirred overnight in methanol with heat under nitrogen. The next day, the samples were precipitated, dissolved in deionized water, and dialyzed via dialysis tube.

For QDs water-solubilized with 50/50 carboxylic acid-PEG 400-DHLA/methyl-PEG 750-DHLA, the precipitate was collected by centrifugation and dried under reduced pressure. Carboxylic acid-PEG 400-thiolic acid was reduced to carboxylic acid-PEG 400-DHLA, purified via liquid-liquid separations in chloroform and acidic water, and dried. Methyl-PEG 750-thiolic acid was reduced to methyl-PEG 750-DHLA, purified via liquid-liquid separations first in diethyl ether and deionized, then in dichloromethane and water, and then dried. The precipitated QDs and equal amounts of carboxylic acid-PEG 400-DHLA and methyl-PEG 750-DHLA were stirred overnight in methanol with heat under nitrogen. The next day, the samples were precipitated, dissolved in deionized water, and dialyzed via dialysis tube. Some samples were water-solubilized in isopropanol with potassium hydroxide instead of in methanol. Other samples were water-solubilized in methanol with potassium hydroxide and zinc nitrate hexahydrate instead of just methanol.

For the QDs water-solubilized with silane, the precipitate was collected by centrifugation and dried under ambient conditions. These QDs were water-solubilized with a nearly one monolayer silane. To improve stability, PEG 750-amine was sometimes added to silane QDs by reacting the QDs with sulfo-SMCC in deionized water for 30 min. Then a desalting column was used on the sample to remove
unreacted sulfo-SMCC. Finally, the QDs were diluted in pH 8 phosphate buffer and PEG 750-amine was added. After stirring overnight, the QDs were dialyzed to remove unreacted PEG 750-amine.

3.3.4 Protein-Binding Ligand Synthesis

Several different methods were tried in an attempt to prepare a functional protein-binding ligand. To make boc—lysine—Indocin, Indocin and boc—lysine were either directly reacted together or Indocin—NHS was first synthesized and then reacted with boc—lysine. Several synthesis routes as well as purification methods were taken to make these compounds. To make RBpip—lysine—Indocin, two different levels of purity of the RBpip dye were tried; many different methods of purification for the RBpip—lysine—Indocin were also analyzed to determine the best method. RBiso—lysine—Indocin was also synthesized to determine if the RBpip dye was degrading. Finally, Indocin-PEG 400-amine was synthesized and conjugated to QDs separately from RBpip dye.

The first route to prepare boc—lysine—Indocin involved reacting Indocin and boc-lysine together. In the first method, Indocin, boc—lysine, EDC, and DMAP were stirred overnight in DMF under nitrogen. In the second method, boc—lysine was first dissolved in a minimal amount of deionized water before being added to Indocin, EDC, and DMAP in DMF. This reaction was stirred overnight under nitrogen. To purify boc—lysine—Indocin using either method, liquid-liquid separations in ethyl acetate and water were performed, after which the compound was dried. When NMR results were not clean, the boc—lysine—Indocin was dissolved in diethyl ether, precipitated with hexane,
and dried. When NMR results were again not pure, a recrystallization was attempted with a solution of hexane and ethyl acetate. When no crystals were formed when the sample was chilled, work on this method to prepare boc—lysine—Indocin was stopped.

The next route to prepare boc—lysine—Indocin started with preparing Indocin–NHS first. The first method used Indocin, EDC, and N-Hydroxysuccinimide (NHS) in DMF, which was stirred overnight under nitrogen. Recrystallization was attempted in ethanol, but no precipitate was formed when chilled. Recrystallization was also attempted in deionized water, but the solution gained a brown discoloration and very little precipitate was formed when chilled. The second method used Indocin, N,N'-dicyclohexylcarbodiimide (DCC), and NHS in DMF, which was also stirred overnight under nitrogen. The sample was chilled, and the solid by-product that formed was removed by first concentrating the sample under vacuum, centrifuging the sample, and extracting the yellow liquid. After drying the liquid, the NMR results showed a much better spectrum than was achieved using EDC. This Indocin—NHS product was dissolved in DMF and reacted with boc—lysine in deionized water and triethylamine overnight under nitrogen. After drying, the boc—lysine—Indocin was then purified using a prep-scale TLC plate with a 20% methanol/80% dichloromethane solvent mix. Several bands were extracted with DMF, dried, and examined by NMR, including a yellow band. The yellow band proved to be the target compound and had a clean NMR, shown in Figure 23.

Once pure boc—lysine—Indocin was obtained, the next step was conjugating it to RBpip. First, the boc—lysine—Indocin was dissolved in DMF, and BOP and 2,4,6-collidine were added. After stirring for 30 min, RBpip was added to the solution, and the
Figure 23. $^1$H NMR spectra of Boc—lysine—Indocin.
reaction was stirred overnight under nitrogen. The next day, the sample was concentrated, and was purified using a prep-scale TLC plate with a 20% methanol/80% dichloromethane solvent mix. Several of the red bands were extracted with a mixture of 33% methanol/67% dichloromethane, dried, and analyzed by NMR. The NMR spectra of these extracted dye bands still had many impurities that made verification of the target compound difficult. Therefore, silica columns with 10% methanol/90% dichloromethane solvent mix were used to further purify the extracted dye bands. The main bands from the columns were dried and then analyzed by NMR as well to determine which was the target RBpip—lysine—Indocin (RBpip—Indocin) compound.

When none of these compounds could be positively identified as the pure target, the initial reaction with boc—lysine—Indocin, BOP, 2,4,6-collidine, and RBpip in DMF was scaled up to allow for more robust purification. A portion of this sample was concentrated and separated via a large silica column with a 15% methanol/85% dichloromethane solvent mix. The two bands that resulted were separately dried by rotary evaporation and analyzed by NMR; both bands still had too many impurities to determine which band was the product. Then, the smaller of the two dye bands was purified by a silica column with 43% methanol/57% toluene solvent mix. The main band from this column was dried, and an NMR still showed impurities. The larger of the two dye bands was dissolved in ethyl acetate. Then, the dye was precipitated using hexane, and the liquid was discarded. The solid was dried and separated via a large silica column with 15% methanol/85% dichloromethane solvent mix. The two bands that resulted were dried and analyzed by NMR. Both bands had impurities, and were separately dissolved in ethyl acetate and precipitated with hexane two times each.
Then, the samples were dissolved in chloroform and centrifuged; the clear red liquids were filtered to remove any solid particulates. Next, HPLC was performed for the samples. After the method for each sample was optimized with an analytical HPLC C18 column, a prep-scale HPLC C18 column was used to further purify both of the dye samples. The solvents used were acetonitrile and deionized water. Both samples had their main dye peaks elute at the same time. These main peaks were separately dried, and NMRs were taken. Both of these dye samples still had impurities and missing peaks that hindered positive identification of the target compound. Another portion of the scaled up RBpip—Indocin reaction was separated via a large silica column with 15% methanol/85% dichloromethane solvent mix. After NMRs were taken of the bands, the most promising band was separated via a small silica column with 20% methanol/80% dichloromethane solvent mix. The darkest band from this column was dried, and the NMR taken was still inconclusive.

Finally, another large batch of RBpip—Indocin was prepared again from boc—lysine—Indocin, BOP, 2,4,6-collidine, and RBpip in DMF. After drying, a large silica column with 20% methanol/80% chloroform solvent mix was used to separate the sample. The three dark bands that resulted included a faster moving band and two slow moving bands. The faster moving band was dried, and a prep-scale TLC plate with a 10% methanol/90% dichloromethane solvent mix was performed. The dark top band was extracted first with dichloromethane, which did not extract most of the dye. This extract was discarded, and the band was extracted with DMF. After the sample was dried, the NMR spectrum in Figure 24 was obtained. This spectrum is the cleanest obtained for RBpip—Indocin.
Figure 24. $^1$H NMR spectrum of RBpip—Lysine—Indocin. All aromatic assignments are best estimates.
In addition to the above method to create pure RBpip—Indocin ligand, other methods were attempted. In the first method, immediately after synthesis of the RBpip—lysine—Indocin, the dye was deprotected with trifluoroacetic acid (TFA). Then, similar purification steps as those described above were performed. The second method involved repurifying the RBpip alone several times before conjugating it to the boc—lysine—Indocin as described above and purifying the compound using similar steps as described above.

The third method involved a slightly different dye, rhodamine B isothiocyanate, to be conjugated to the boc—lysine—Indocin. This new dye required an alteration in the protein sensing architecture as seen in Scheme 4. The dye would be added to the deprotected amine on the lysine, and the carboxylic acid of the lysine would be conjugated to diamino tetraethylene glycol. Boc—lysine—Indocin prepared as described above was deprotected with TFA under nitrogen overnight. Then, the sample was dried, a small portion of chloroform was added, and the sample was again dried. This addition of chloroform and redrying of the sample was performed four more times to ensure all TFA was removed. Next, lysine—Indocin, RBiso, and triethylamine were dissolved in DMF and reacted overnight under nitrogen. Then, a portion of the sample was separated using a prep-scale TLC plate with a 20% methanol/80% dichloromethane solvent mix. The two most prominent bands were separately extracted and dried. NMR results showed impurities and missing peaks, but the best band was purified further using a small silica column with a 20% methanol/80% dichloromethane solvent mix. NMR results, shown in Figure 25, of the best band again showed some impurities and at least one missing peak. At this point, the RBiso was examined itself by NMR. The
Scheme 4. CdSe/ZnS quantum dot conjugated to an RBiso—Lysine—Indocin—Diamino Tetraethylene Glycol Dye. This sensor scheme was never completed.
Figure 25. $^1$H NMR spectrum of RBiso—Indocin. All aromatic assignments are best estimates.
results showed that the RBiso itself was slightly impure and at least one peak seemed to be completely missing. A prep-scale TLC plate with a 20% methanol/80% dichloromethane solvent mix was performed for the RBiso, and several distinct bands were seen. After numerous types of NMRs, including proton, carbon, and COSY NMR, were performed on the unpurified and purified RBiso, it was determined that the dye had most likely degraded. Thus, no more work was performed with this dye.

The fourth and final method involved creating a separate Indocin ligand from the RBpip dye. This method was attempted to see if conjugating the dye to the Indocin compound was what was causing the NMR spectra to lose peaks as well as the poor protein sensing results described below. The RBpip would be separately conjugated to the QDs, but the Indocin compound needed to be modified for conjugation to the QDs. Indocin—PEG 750—azide was synthesized using amine—PEG 750—azide, Indocin, and DCC. The DCC was filtered out, and the product was dried by vacuum. Liquid-liquid separations were performed with sodium bicarbonate saturated deionized water and diethyl ether, followed by the same sodium bicarbonate saturated deionized water layer and ethyl acetate. The ethyl acetate layer was dried, and after NMR analysis showed impurities, the Indocin—PEG 750—azide was reacted with triphenylphosphine, tetrahydrofuran, and deionized water to reduce the azide to an amine. Spot TLC confirmed little azide remained. Liquid-liquid separations were performed with ethyl acetate and pH 1 HCl solution. Then, the pH of the water layer was raised to pH 4 with the addition of 0.1 M NaOH solution and separated with fresh ethyl acetate, and finally the pH of the water layer was raised to pH 8 with the addition of sodium bicarbonate.
and separated with chloroform. The chloroform layer was dried, and the resulting NMR analysis confirmed the desired product was formed.

### 3.3.5 Conjugation of Ligand and Quantum Dot

Several different methods were utilized to conjugate the ligands described above to several different types of water-solubilized QDs. Control RBpip was also conjugated to the QDs in a similar manner. In the first method, DMTMM was used to react polymer-encapsulated QDs and RBpip—Indocin in pH 8 phosphate buffer over 2 days. Dialysis was performed using pH 8 phosphate buffer and centrifugation filters. In the second method, the polymer-encapsulated QDs and RBpip—Indocin were just mixed together in deionized water and stirred overnight. As dyes tend to nonspecifically stick to polymer-encapsulated QDs, this method was attempted as a way to minimize instability in the QDs due to conjugation methods. The next day, dialysis in pH 8 phosphate buffer using centrifugation filters removed most of the dye, showing that this method failed. The third method involved doing everything in the first method described above, but after the QDs, ligand, and DMTMM had been reacting for a while, PEG—amine and 0.1 M NaOH solution were added. This was tried in another attempt to minimize QD instability. The fourth method involved the separate conjugation of Indocin—PEG 750—amine and RBpip. Polymer-encapsulated QDs were reacted with Indocin—PEG 750—amine diluted in pH 8 phosphate buffer and DMTMM diluted in deionized water and stirred for 2 days. Then, dialysis using centrifugation filters was performed with deionized water and pH 8 phosphate buffer. Next, these Indocin—PEG 750—QDs were reacted with DMTMM diluted in deionized water and RBpip diluted in
deionized water. The next day, dialysis using centrifugation filters was performed with deionized water and pH 8 phosphate buffer. Another variation of this method had the QDs reacting with DMTMM in deionized water, Indocin—PEG 750—amine diluted in pH 8 phosphate buffer, and RBpip diluted pH 8 phosphate buffer at the same time. Dialysis was then performed using centrifugation filters with deionized water and pH 8 phosphate buffer.

The fifth method involved 6% PEG modified 40% octylamine modified poly(acrylic acid) polymer-encapsulated QDs. These QDs were reacted using EDC diluted in deionized water and RBpip—Indocin diluted in DMF. This EDC method conjugated much more RBpip—Indocin than DMTMM. The sixth method involved cap-exchanged QDs. These QDs were reacted overnight with RBpip—Indocin diluted in DMF and DMTMM diluted in deionized water. Dialysis using centrifugation filters was performed with deionized water and pH 8 phosphate buffer. Another variation of this method had the QDs reacting with MPEG CD at pH 6 and then reacting with RBpip—Indocin diluted in DMF at pH 8. Dialysis using centrifugation filters was performed with deionized water and pH 8 phosphate buffer. The seventh method involved silane-coated QDs. These QDs were reacted with sulfo-SMCC at pH 6.5 and then reacted with RBpip—Indocin diluted in pH 8 phosphate buffer at pH 8. Dialysis was performed against deionized water using a dialysis tube.

3.3.6 Cox-2 Preparation

Cox-2 was purchased from Sigma Aldrich at a concentration of $3.0 \times 10^{-6}$ M in 80 mM Tris-HCl, pH 8, containing 0.1% Tween 20 and 300 μM diethyldithiocarbamate.
A second purchase of the same came from Cayman Chemical. A diluted Cox-2 solution was made by taking 5 μL of this solution and diluting it to 70 μL with pH 8 phosphate buffer. The concentration of this solution was $2.1 \times 10^{-7}$ M. Solutions were kept on ice when in use and kept at -80 °C when not in use.

3.3.7 Control Protein (Bovine Serum Albumin) Preparation

BSA (3.5 mg) was diluted in 200 μL of deionized water giving a concentration of $2.64 \times 10^{-4}$ M. BSA was stored at 4 °C, and solutions were prepared the same day used.

3.3.8 Control Solution Preparation

The control solution was 80 mM Tris-HCl, pH 8, containing 0.1% Tween 20 and 200 μM DDC. Solutions were kept on ice when in use and kept at 4 °C when not in use.

3.3.9 Detection of Cox-2

As in Chapter 2 for streptavidin detection, all protein-sensing experiments were performed as follows: after a 1 μL scale volume of protein solution was added to the sensing solution, the mixture was very gently mixed by pipet and then immediately measured by excitation at 450 nm. The detection time from addition of protein to a complete fluorescence spectrum was approximately 5 min, with less than 1 min of sample preparation and just over 4 min of spectrum collection. As fluorimeters and their settings vary, it is possible for the detection time to be significantly less; for example, detection time could be significantly decreased if an array detector is used.
3.4 Results and Discussion

Although much work had been completed before all possible avenues had been exhausted, this project was ultimately stopped before publishable results were obtained. As described above, work was done for two different protein sensing schemes. The type of QD, water-solubilization procedure, ligand synthesis and purification, and ligand conjugation were all varied in an attempt to optimize the sensor. Many other parameters including concentrations of the QD sensors and protein and fluorimeter cuvette sizes were modified to design a sensor that could be published.

It was seen in many samples that the QD sensors were unstable upon conjugation with the RBpip—Indocin ligand. As such, many different samples from many different batches of CdSe/ZnS QDs were tested to see if this instability was a result of a specific batch of QDs. When this did not reduce the instability, different water-solubilization techniques were studied. Many cap-exchanged samples would precipitate without provocation, and some polymer-encapsulated QDs would precipitate in the presence of experimental concentrations of Cox-2. After the several types of water-solubilized QDs discussed in section 3.3.3 were tried; the next step tried was desalting the Cox-2 protein prior to use. As the Cox-2 was not stable for long outside of the matrix it was purchased in, desalting via a desalting tube was performed immediately before the protein was used. The results obtained from the experiments that used desalted Cox-2 showed no improvement.

Another method to improve the Cox-2 sensor was to modify the RBpip—Indocin ligand. The RBpip—Indocin ligand was a poor FRET acceptor and photobleached often
when fluorimeter measurements were made. First, various levels of purity of the ligand were tested in an attempt to determine if the purification steps were affecting the performance. Then, several different conjugation techniques were explored to improve conjugation to the QDs. Next, a different rhodamine dye, RBiso, was tested, but this second sensor scheme was abandoned before the ligand was completed due to NMR analysis showing the dye was potentially degraded. Finally, the RBpip dye and Indocin were separately conjugated to the QDs. None of these attempts improved the sensor design.

The concentrations of the QD sensors and Cox-2 protein solution were adjusted for several experiments, but none of the concentrations studied yielded acceptable results. For this project, cuvettes with path lengths less than 1 cm were also tried to reduce the volume of QD sample in the cuvette. Cuvettes with path lengths of 0.4 and 0.2 cm were used, reducing the volume of samples from approximately 2.5 mL to 1.4 and 0.7 mL, respectively. While these smaller cuvettes allowed the use of higher concentrations of Cox-2 protein relative to QD sensor concentration which did improve the results of the experiments, publishable results were still not obtained.

The best results from the work on the QD Cox-2 sensor came from DHLA cap-exchanged QDs where the RBpip—Indocin ligand, which was purified via a large silica column, prep-scale TLC, and HPLC, was conjugated with DMTMM. Figure 26 shows the normalized emission spectra upon introduction of Cox-2 to the QD sensors. Before Cox-2 was added, the emission of the blank QDs was measured twice. As can be seen, the QD emission increased, and the dye emission decreased, signifying photobleaching of the dye. The addition of Cox-2, giving a concentration of 12.8 pmol/mL Cox-2 in the
Figure 26. Emission spectra of QD Cox-2 sensor as a function of Cox-2 concentration. The left peak is the QD emission, and the right peak is the Cox-2-sensing dye emission. Note that two blank spectra were taken (blue and green). The increase in QD emission and decrease in dye emission shows that the sample is photobleaching; addition of Cox-2 (red) gives the opposite response (decrease in QD emission and increase in dye emission). This shows that while the QD Cox-2 sensor is sensing the Cox-2, the photobleaching of the dye is negatively affecting the results.
cuvette, showed the opposite response; the QD emission decreased, and the dye emission increased. Figure 27 again shows a similar response for this sensor. A second emission spectra of the QD sensor solution with 12.8 pmol/mL Cox-2, with no additional protein or any other changes, shows the same response seen with the duplicate blanks; the QD emission increases, and the dye emission decreases. When Cox-2 is added so the concentration in the QD sample is 25.6 pmol/mL, the opposite response is again seen. This shows that while the QD sensor is sensing the Cox-2, the photobleaching of the dye is negatively affecting the results. Without the photobleaching, the response of the QD sensor to Cox-2 would be much higher as the response for each emission spectra taken is negatively affected by the photobleaching. While it would be possible to mathematically determine the amount of photobleaching per emission spectra taken, and correct the response of the sensor accordingly, this approach would be difficult as it would produce overly analyzed and questionable results.

3.5 Conclusion

While much effort was put into the development of a QD Cox-2 sensor, the ultimate results obtained were not publishable. This sensor, similar to the one described in Chapter 2 for streptavidin, would have been useful as Cox-2 is a protein found at inflammation and tumor sites. The potential of this sensor for detection of tumors and cancer made it an ideal biologically relevant protein to explore. Many different methods of solving the issues with the QD Cox-2 sensor were explored. These included changing the QD, the water-solubilization of the QD, the Indocin-dye ligand, and the conjugation method for the QD and ligand. The concentration of the QD sensor relative to protein
Figure 27. Emission spectra of QD Cox-2 sensor as a function of Cox-2 concentration. The left peak is the QD emission, and the right peak is the Cox-2-sensing dye emission. Note that two spectra were taken at 12 pmol/mL (red and green), and the red spectrum is the same red spectrum as in Figure 26. The increase in QD emission and decrease in dye emission shows that the sample is photobleaching; addition of more Cox-2 (blue) gives the opposite response (decrease in QD emission and increase in dye emission). This shows that while the QD Cox-2 sensor is sensing the Cox-2, the photobleaching of the dye is negatively affecting the results.
concentration was also modified with varying sized cuvettes. Even the best results for this sensor were plagued with issues that could not be easily corrected, and all possible options for method improvement were exhausted. Therefore, this project was ended so that a new QD protein sensor system could be explored.
4. Ratiometric Quantum Dot Thrombin Sensor

4.1 Introduction

After successfully designing a QD streptavidin sensor, described in Chapter 2, and being unable to create a QD Cox-2 sensor, discussed in Chapter 3, the next step was to try a different biologically relevant protein to show that the protein sensing paradigm demonstrated with streptavidin could be used with other proteins. After reviewing the possible small molecular binding agents that had been found in the literature, it was decided to try a different type of binding agent altogether. A protein binding aptamer was chosen in an attempt to show that the QD protein sensor architecture could be easily modified to use this type of binding agent. If successful, many other protein binding aptamers that exist could potentially be used with in QD protein sensor to sense an even wider range of proteins. These aptamers are molecules that are made up of either peptides or oligonucleic acids.

After looking through the possible aptamers in the literature, a report by the Hamad-Schifferli group at MIT reported on the development of a thrombin binding aptamer (TBA) that was conjugated to a gold nanorod.\textsuperscript{207} Thrombin is important as it participates in the blood coagulation cascade; it also has a role in thrombosis and hemostasis.\textsuperscript{216} Several diseases have symptoms that include high levels of thrombin in the blood.\textsuperscript{216} Thus, the ability to make a QD protein sensor for thrombin would have significant biological relevance. The TBA and thrombin protein binding pair appeared to be thoroughly studied in the MIT report.\textsuperscript{207} One of the aptamer sequences used in the report was a chain of a dye, the TBA, a 15 T spacer, and a thiolated end meant for
conjugation to their gold nanorod. This aptamer, which is an oligonucleotide aptamer, was reminiscent of the protein sensing architecture previously used for the QD protein sensor; as such, the ability of this aptamer to be translated to the QD protein sensing system seemed hopeful. The only modification needed would be to exchange the thiolated end for another conjugation end that could bind to the surface of CdSe/ZnS QDs.

Reported in this chapter is the research that was performed for the QD thrombin sensor. This project was executed in a similar manner to the first two sensors described in Chapters 2 and 3, with the main differences being a change in the protein binding agent and dye. Another difference for this QD protein sensor is the use of silane-coated QDs for the data obtained. These QDs required specialized conjugation methods that will be described below. The methods, data, and results will be discussed below for the final QD protein sensor created for this thesis.

4.2 Materials

4.2.1 Reagents

Commercial sources were used to obtain all chemicals. These chemicals were used as they were received except where indicated.

4.2.2 Equipment and Instrumentation

$^1$H NMR spectra were recorded on a Bruker Avance DRX 400 NMR spectrometer using $d_4$-methanol as the solvent. A Varian Cary 300 Bio UV–vis spectrophotometer was used to obtain UV–vis absorbance spectra. A customized Fluorolog (HORIBA Jobin
Yvon) modular spectrofluorometer was used to obtain fluorescence emission spectra. Inner-filtering effects were avoided by having an absorbance near or below 0.1 OD for all solutions at the excited wavelength.

4.3 Methods

4.3.1 Summary of Sensor Design

TBA is a 15 oligonucleotide base sequence that binds to thrombin protein with a reported $K_d$ of $10^{-7}$ to $10^{-9}$ M, significantly higher than the biotin–streptavidin dissociation constant. To ensure space for proper folding of the TBA and binding to thrombin, a spacer of 15 T oligonucleotide bases was added on the Acrydite™ end that conjugates to the QD surface, and a TAMRA dye was attached to the other end. The final sequence was conjugated with silane-coated water-solubilized CdSe/ZnS QDs prepared according to a recently reported procedure. A diagram of this construct is shown in Scheme 5. The TBA sequence was also originally studied with an amino-modifier on its conjugation end as shown in Scheme 6. This sequence was conjugated to polymer-encapsulated and silane-coated QDs, but difficulty with sensor response led to the use of the Acrydite™ linker with silane-coated QDs.

4.3.2 Quantum Dot Synthesis

As mentioned in Chapter 2, previously published protocols were used to synthesize core CdSe and core/shell CdSe/ZnS QDs. See Chapter 2 for an example of the synthesis of CdSe/ZnS.
Scheme 5. A silane-coated QD conjugated with a dye-labeled thrombin binding aptamer. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Scheme 6. A polymer-encapsulated or silane-coated QD conjugated with a dye-labeled thrombin binding aptamer.
4.3.3 Quantum Dot Water-Solubilization

“Samples were processed by addition of a small amount of isopropanol followed by methanol to [precipitate] the samples. The supernatant is discarded leaving behind a gel most likely composed of a decylamine–tetradecylphosphonic acid adduct used in the ZnS overcoating procedure. The CdSe/ZnS [QDs] were extracted from this gel by several washings of hexane; the washings were collected, from which the [QDs] were precipitated again through the addition of a few drops of isopropanol and [sufficient] methanol.” (Reprinted with permission from ref 66. Copyright 2011 American Chemical Society.) For polymer-encapsulated QDs, the precipitate was collected by centrifugation and dried under reduced pressure. 40% octylamine modified poly(acrylic acid) polymer76 (ref 70 has details on the synthesis and characterization) was used to water-solubilized these QDs. For the silane-coated QDs, the precipitate was collected by centrifugation and dried under ambient conditions. These QDs were water-solubilized with a nearly one monolayer silane coating according to a recently developed procedure.68 Silane-coated QDs were sometimes further stabilized by addition of PEG-silane in basic (pH 13). After stirring overnight, the samples were dialyzed via dialysis tubes. See Section 1.5 for information about the differences between polymer-encapsulation and silane-coating (a type of cap-exchange) for the water solubilization of QDs. The concentration of QDs was determined by the methods outlined in ref 83.

4.3.4 Detailed Description of Protein-Binding Ligand

The protein binding ligand used for the thrombin sensor involved an oligonucleotide aptamer as the protein binding agent. As my lab is not familiar with the
synthesis and functionalization of compounds of this nature, it was decided to purchase TBA that was already conjugated to a dye and a conjugation linker. A 15 T spacer was also included to ensure the TBA was far enough away from the QD to allow for proper folding and binding to the target protein. 5'-/Amino/TT TTT TTT TTT TGG TTG GTG TGG TTG G/TAMRA/-3' (amino—TBA) and 5'-/Acrydite™/TT TTT TTT TTT TGG TTG GTG TGG TTG G/TAMRA/-3' (Acrydite—TBA) were purchased from IDT (Coralville, Iowa). The amino—TBA was purchased first for use with polymer-encapsulated and silane-coated QDs, but after issues with the sensor, the Acrydite—TBA was purchased. The silane-coated QDs used have free thiols on their surface to covalently react with the Acrydite™ by simply mixing the QDs with the dye-labeled aptamer overnight. The conjugation was verified by the fact that dye features remain after dialysis.

4.3.5 Conjugation of Ligand and Quantum Dot

For the amino—TBA, several conjugation methods were explored. First, polymer-encapsulated QDs were stirred with methyl-polyethylene glycol carbodiimide (prepared with 2-methoxyethyl isothiocyanate instead of ethyl isothiocyanate), for approximately 12 min, after which the amino—TBA was added, and the sample was stirred overnight at pH 8. It was determined that the TAMRA dye emission was very weak using this method even with a large amount of amino—TBA used. Next, silane-coated QDs were stirred with sulfo-SMCC for approximately 30 min, after which the QDs were put through a desalting column to remove unreacted sulfo-SMCC. The QDs were then reacted with the amino—TBA and stirred overnight. This method still had issues with weak TAMRA
dye emission even with large amounts of the amino—TBA. The sulfo-SMCC was also reacted with the amino—TBA first, put through the desalting column, and reacted with the QDs overnight, but this too saw no improvement in dye emission. The reasoning for the weak dye emission is discussed in Section 4.4 below. The procedure used for the amino—TBA results in Figure 28 involved the sulfo-SMCC reacted first with the dye, desalted, reacted with PEG-stabilized silane-coated QDs overnight, and dialyzed the next day.

For the Acrydite—TBA, approximately 2.5 mL of water-solubilized (with silane) CdSe/ZnS QDs were adjusted to pH 9 with 0.1 M NaOH. Then, a portion of Acrydite—TBA was added so that TAMRA emission was visible (approximately one-sixth of the QD emission). This solution was stirred overnight. The next day, the solution was dialyzed against a dilute NaOH solution (pH 8) using a dialysis tube. The sample was dialyzed for approximately 24 h. The QD protein sensor solution was then diluted in a 0.7 mL cuvette with a pH 8 (by NaOH) KCl solution (13.3 mM KCl concentration in cuvette) to a working concentration of $1.07 \times 10^{-6}$ M, which was diluted to $9.67 \times 10^{-7}$ M by the addition of the analyte. Cationic ions, specifically potassium ions, are necessary for proper folding and stability of the thrombin-binding aptamer.\textsuperscript{218, 219} The fluorescence was measured as a function of addition of thrombin solution as performed in the streptavidin study, although the samples were excited at 440 and 545 nm.
Figure 28. Normalized emission of thrombin-sensing silane-coated CdSe/ZnS QDs upon exposure to the protein. These QDs used the amino—TBA. The left peak is the QD emission, and the right peak is the thrombin-sensing dye emission. The inset shows the normalized emission of thrombin-sensing silane-coated CdSe/ZnS QDs upon exposure to the control solution described in Section 4.3.7. For the inset, the pmol/mL values are the concentration of thrombin that would have been present if the thrombin solution had been used instead of the control solution.
4.3.6 Thrombin Preparation

Thrombin (human) was purchased in a 50 mM sodium citrate, pH 6.5, containing 0.01% PEG and 20 mM sodium chloride solution at a concentration of 2000 NIH units/mg and 1000 units/mL (Cayman Chemical, Ann Arbor, Michigan). The solution was kept on ice when in use, and the solution was kept at -80 °C when not in use.

4.3.7 Control Solution Preparation

The control solution was 50 mM sodium citrate, pH 6.5, containing 0.01% PEG and 20 mM sodium chloride solution. The solution was kept on ice when in use, and the solution was kept at 4 °C when not in use.

4.3.8 Control Protein (Bovine Serum Albumin) Preparation

BSA (5 mg) was diluted in 5.6 mL of deionized water. BSA was stored at 4 °C, and solutions were prepared the same day used.

4.3.9 Detection of Thrombin

As done for streptavidin and Cox-2 detection, all protein-sensing experiments were performed as follows: after a 1 μL scale volume of protein solution was added to the sensing solution, the mixture was very gently mixed by pipet and then immediately measured by excitation at 450 nm followed by 545 nm. The detection time from addition of protein to a complete fluorescence spectrum was approximately 5 min, with less than 1 min of sample preparation and just over 4 min of spectrum collection. As fluorimeters
and their settings vary, it is possible for the detection time to be significantly less; for example, detection time could be significantly decreased if an array detector is used.

4.4 Results and Discussion

The initial study on streptavidin sensing with a QD—RBpip—biotin couple, discussed in Chapter 2, was performed to verify the efficacy of the technique of the QD protein sensor. As this study validated the methodology, a new system with a similar platform to sense thrombin using a dye-labeled DNA oligonucleotide aptamer that binds to thrombin was designed. TBA coupled to a TAMRA dye was conjugated with both polymer-encapsulated water-solubilized CdSe/ZnS QDs and silane-coated water-solubilized CdSe/ZnS QDs using amino—TBA and Acrydite—TBA.

It was found in the initial studies using amino—TBA conjugated to silane-coated QDs (see Figure 28) that the FRET efficiency between the QD and TAMRA chromophores is low, most likely due to the long space between them or the fact that TAMRA has a low extinction coefficient that is approximately one-third that of RBpip. As such, when repeating the studies with Acrydite—TBA and silane-coated QDs, the QD and dye were excited separately through judicious choice of excitation wavelengths to measure their emission spectra separately. Upon μL scale additions of thrombin protein solution, the emission spectra show that dye emission increases while QD emission decreases, as shown in Figure 29; note that this is in contrast to the streptavidin-sensing QD system. In this case, I believe that the dye becomes brighter in the presence of thrombin that is binding to the aptamer to which it is conjugated.
Figure 29. Normalized emission of thrombin-sensing silane-coated CdSe/ZnS QDs upon exposure to the protein. These QDs used the Acrydite—TBA. QD and dye were excited separately due to decreased FRET efficiency due to the long distance between the QD and dye. The two resulting spectra are shown here in the same figure for clarity. The left peak is the QD emission, and the right peak is the thrombin-sensing dye emission. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Unfortunately, it was noticed that sometimes the QD solutions became unstable and precipitated while performing the sensing experiments with thrombin. As it is known that salts can cause QD instability, one source of this observation may be the exposure of the QD solution to potassium ions that are necessary for the TBA to function. Other sources of instability include the sodium citrate, sodium chloride, and PEG that are also necessary components of the protein solution. Thus, control experiments were performed where the QD sensors were exposed to the buffered solution without any protein content. These experiments showed that μL scale additions of the buffered solution alone caused similar changes in the emission spectra (see Figure 30), where the dye emission is enhanced over the QD emission.

As in the previous study of streptavidin sensing, the emission spectra were integrated and divided to determine the ratiometric response to protein and buffer exposure, as shown in Figure 31. Although the control experiments show that the QD sensor’s response is not entirely due to thrombin, overall there is a response to the presence of the protein. By subtracting the average of the slopes of the control experiments from the slope of the thrombin linear response, the “working” DL of thrombin sensing was found to be 40 ± 10 pmol/mL (4.0 × 10⁻⁸ M or 1.5 × 10⁻³ g/L). This DL is high compared to standard thrombin ELISA assays; however, the result demonstrates that the QD-based protein sensor design presented in this thesis is generalizable and may be optimized to achieve better DLs in the future.
Figure 30. Emission spectra of the thrombin silane-coated QD protein sensor (~590 pmol / 0.55 mL) as a function of control solution (A and B). QD and dye were excited separately due to decreased FRET efficiency due to the long distance between the QD and dye. The two resulting spectra for each control are shown here in the same figure for clarity. The pmol/mL values are the concentration of thrombin that would have been present if the thrombin solution had been used. The samples were excited at 440 nm and 545 nm. The left peak is the QD emission, and the right peak is the thrombin-sensing dye emission. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
Figure 31. Percent change in the ratio of the integrated emission of dye over QD as a function of thrombin concentration; each point has been divided by the initial QD/dye ratio value, which is then subtracted from the data. Also shown are the responses to replicated blank solutions. The lines are guides to the eye. Reprinted with permission from Ref. 157. Copyright 2014 American Chemical Society.
4.5 Conclusion

A fluorescent QD protein sensor which can specifically and ratiometrically respond to a biologically relevant protein with a DL as low as a few pmol/mL was created. The DL of this sensor is competitive with general ELISA assays, but the DL is high compared to ELISA assays for thrombin. However, the measurement technique is significantly less time-consuming and simpler compared to ELISA or backscattering interferometry. The success of this QD protein sensor shows that the QD protein sensor architecture described in Chapters 2 and 3 can be translated to detect proteins using DNA oligonucleotide aptamers. It also shows that the QD protein sensor is viable for detecting biologically relevant proteins such as thrombin which is an indicator of several diseases. By implementing a similar architecture with other small molecular protein-binding agents and aptamers, and studying the responses of the new systems, a variety of proteins associated with many diseases could be ratiometrically quantified with this method. As there are many small molecular protein-binding agents and aptamers that bind to biologically relevant proteins, including those related to infectious diseases, these sensors may have use in areas where the cost and time for implementing ELISA and Western blot assays are prohibitive. This sensing system will also likely find utility in cellular imaging studies as examining protein–protein interactions with FRET requires the use of multiply labeled species. Using ratiometric protein-sensing QDs obviates the need for the target analyte to be genetically modified with GFP or tagged with another dye. Furthermore, the identities of unbound and bound probes are distinguishable with this method.
5. Synthesis and FRET Characterization of Very Bright ZnSe/CdS/ZnS Quantum Dots

5.1 Introduction

Reported in this chapter is the synthesis of brightly fluorescent ZnSe/CdS/ZnS type II QDs.\textsuperscript{221} QYs as high as 61% have been achieved in the QD organic growth media and QYs as high as 37% have been achieved after the QDs are water-solubilized. These QYs represent the best results to date for any type II QD to the best of my knowledge. Finally, a rhodamine dye was conjugated to the surface of the water-solubilized QDs, and it was shown with time-correlated single photon counting (TCSPC) that FRET is occurring from the donor QD to the acceptor dye with a FRET efficiency of 0.67 ± 0.03. To the best of my knowledge, this is the first report of FRET involving ZnSe/CdS or ZnSe/CdS/ZnS.

As previously discussed, QDs have been a significant research interest since their discovery.\textsuperscript{1} This is because fluorescent QDs have interesting properties such as photostability, continuous absorption profiles, and size tunable emission spectra.\textsuperscript{1, 2, 6, 7} Many applications for these materials have been reported over the years such as chemical and biological sensors,\textsuperscript{135-137} labels for biological tracking studies,\textsuperscript{8, 9, 96} solar cells,\textsuperscript{121, 123, 124, 222} QD-based lasers,\textsuperscript{127-130} and light-emitting devices.\textsuperscript{131-134}

As discussed in Chapter 1, there are two types of core/shell QDs. Type I QDs, which include the ubiquitous CdSe/ZnS,\textsuperscript{18, 19} have both their electron and hole located in either the core or the shell. A large amount of QD research has been performed using this type of QD. Type II QDs are nanocrystals in which the hole is in the core and the
electron is in the shell (or vice versa) due to the relative alignment of the band edge. Previous reports have described the synthesis of various type II QDs including CdTe/CdSe, CdSe/ZnTe, CdTe/CdS, ZnTe/CdS, ZnTe/CdSe, ZnTe/CdTe, CdSe/ZnSe, CdSe/CdS, ZnSe/CdSe, CdS/ZnSe, ZnSe/CdS, CdS/Cu2S and Cu2S/CdS. The most promising property, of many, of type II QDs is their use for photovoltaics and photocatalysis, which have driven an increase in interest for these type II materials. While they have utility in these applications, brightly emissive type II QDs would be very useful as they have much longer fluorescent lifetimes than type I. Very emissive type II QDs could be used in time-gated detection studies as well as in other optical detection methods were a reduction in toxic cadmium is desired.

The Zamkov group, to the best of my knowledge, first described the synthesis of ZnSe/CdS type II QDs. They reported QYs of up to 20% after their materials were purified, which is the best QY to date to the best of my knowledge. In the case of ZnSe/CdS, the hole is located in the ZnSe core and the electron is confined to the CdS shell. As type II QDs are known to have low QYs, a method of synthesizing high quantum yield type II QDs would be a new development.

One common method to increase the QY of QDs is to add a passivating shell. The shell passivates the surface by reducing surface trapping of the charge carriers; preventing this trapping increases the QY of the QD. This effect has been previously shown with core/shell/shell type II QDs such as CdSe/ZnTe/ZnS, CdSe/CdS/ZnS and CdS/ZnSe/ZnS. With a second shell of ZnS, the first shell of these QDs is better protected from its environment, reducing the instability and loss of
QY over time. Thus, a method of synthesizing ZnSe/CdS with a second shell of ZnS is described in this chapter.

5.2 Materials

5.2.1 Reagents

Commercial sources were used to obtain all chemicals. These chemicals were used as they were received except where indicated.

5.2.2 Equipment and Instrumentation

A Varian Cary 300 Bio UV–vis spectrophotometer was used to obtain UV–vis absorbance spectra. A customized Fluorolog (HORIBA Jobin Yvon) modular spectrofluorometer was used to obtain fluorescence emission spectra. Inner-filtering effects were avoided by having an absorbance near or below 0.1 OD for all solutions at the excited wavelength. For TCSPC performed by Clare Rowland and Richard Schaller of Northwestern University and Argonne National Laboratory, QDs, dye, and QDs with dye in water were contained in 1 mm quartz cuvettes. “For time-integrated PL and [time-resolved PL] (trPL) derived from TCSPC, samples were [photo-excited] using a [35]-ps pulsewidth, [450] nm diode laser operated between [1] and [2.5] MHz. PL was collected with a quartz lens and [focused] into a fiber optic [running] to a 300 mm grating spectrograph. Static PL spectra were collected using a [thermoelectrically] cooled CCD.”237 (Reprinted with permission from ref 237. Copyright 2014 American Chemical Society.) Time-resolved PL dynamics were produced using a single-photon sensitive avalanche photodiode detector and time-to-amplitude converter.
5.3 Methods

5.3.1 ZnSe Core Synthesis

9.4 g of hexadecylamine was added to a round bottom flask and degassed under vacuum at 110 °C. The flask was purged with nitrogen and degassed again several times. Next, the solvent was heated to 310 °C under nitrogen until it became clear. Then, a mixture of 4 mL of trioctylphosphine, 1 mL of 0.1 M selenium in trioctylphosphine, and 0.1 g of diethylzinc was quickly injected into the flask at 310 °C. The pale yellow sample was baked at 270 °C for 25 min and then allowed to cool to room temperature. An absorbance peak at 344 nm was observed; this absorbance peak verifies that ZnSe QDs have been synthesized. Five representative samples (denoted as Samples A, B, D, E, and F) made using these cores will be discussed below.

It was found that the amounts of hexadecylamine and trioctylphosphine could be reduced to 7.9 g and 1.5 mL respectively, with the baking temperature reduced to 140 °C. This produced cores with an absorbance peak at 331 nm; the smaller wavelength compared to above means that smaller QDs were synthesized. One representative sample (denoted as Sample C) made using these cores will be discussed below.

5.3.2 CdS Shell Synthesis for ZnSe Core

For a typical CdS shell overcoating (adapted from ref 30), 1.1 g of unprocessed ZnSe cores and 5.3 mL of octadecene were added to a round bottom flask and degassed at 100 °C. The flask was purged with nitrogen and degassed again several times. Next, the sample was heated to 240 °C under nitrogen, and a mixture of 0.6 mL of 0.034 mmol/mL cadmium stock solution and 0.06 mL of 0.29 mmol/mL sulfur stock
solution was slowly dripped in using a syringe by hand over approximately 55 min (see below for more details on preparation of the cadmium and sulfur stock solutions). The sample was then baked at 240 °C for 5 min and then cooled to room temperature.

It was found that the amount of octadecene added in the round bottom flask could be increased to as much as 6.9 mL. The mixture of cadmium and sulfur could be increased to as much as 2.0 mL of a 0.064 mmol/mL cadmium stock solution and 0.26 mL of a 0.30 mmol/mL sulfur stock solution. The length of time spent dripping in the cadmium and sulfur mixture varied between 18 to 82 min. The temperature during the addition of the cadmium and sulfur mixture could be varied between 200 °C to 270 °C; some samples were exposed to slowly increasing temperatures in this range during the dripping. The baking of the sample could also be varied between 0 min and several hours, with the baking temperature ranging between 200 °C to 270 °C. The exact differences between the six representative samples discussed below are shown in Table I.

5.3.2.1 Cadmium Stock Solution Preparation

A typical preparation of the cadmium stock solution involved adding 63.2 mg of cadmium oxide, 0.8 g of tetradecylphosphonic acid, and 7.9 mL of octadecene to a round bottom flask and heating to 300 °C. Once the solution was clear, the sample was cooled to 100 °C and degassed. This solution was stored under nitrogen and was gently rewarmed and degassed until it melted for use.
Table I. The variations of six representative samples of ZnSe/CdS. All other parameters where kept constant.

<table>
<thead>
<tr>
<th>CdS Shell Variations</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octadecene (mL)</td>
<td>6.9</td>
<td>5.3</td>
<td>5.3</td>
<td>6.4</td>
<td>6.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Cadmium Stock Solution (mL)</td>
<td>0.6</td>
<td>0.6</td>
<td>1.0</td>
<td>1.0</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Cadmium Stock Solution (mmol/mL)</td>
<td>0.06</td>
<td>0.03</td>
<td>0.03</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Sulfur Stock Solution (mL)</td>
<td>0.07</td>
<td>0.06</td>
<td>0.13</td>
<td>0.13</td>
<td>0.16</td>
<td>0.26</td>
</tr>
<tr>
<td>Sulfur Stock Solution (mmol/mL)</td>
<td>0.30</td>
<td>0.29</td>
<td>0.29</td>
<td>0.32</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Cadmium and Sulfur Drip Length (min.)</td>
<td>50</td>
<td>55</td>
<td>82</td>
<td>27</td>
<td>45</td>
<td>18</td>
</tr>
<tr>
<td>Cadmium and Sulfur Drip Temp. (°C)</td>
<td>200</td>
<td>240</td>
<td>240-260</td>
<td>240</td>
<td>270</td>
<td>270</td>
</tr>
<tr>
<td>Baking Length (min.)</td>
<td>196</td>
<td>5</td>
<td>5</td>
<td>24</td>
<td>11, then 165</td>
<td>174</td>
</tr>
<tr>
<td>Baking Temp. (°C)</td>
<td>200</td>
<td>240</td>
<td>240-260</td>
<td>240</td>
<td>270, then 200-240</td>
<td>270</td>
</tr>
</tbody>
</table>
5.3.2.2 Sulfur Stock Solution Preparation

A typical preparation of the sulfur stock solution involved adding 23.3 mg of sulfur and 2.5 mL of dodecylamine to a small vial with a septum. The solution was degassed as it was heated by an oil bath set to 80 °C (actual temperature of the sample was approximately half of the set temperature). This solution was stored under nitrogen, and was gently rewarmed and degassed until it melted for use.

5.3.3 ZnS Shell Synthesis for ZnSe/CdS Quantum Dot

A typical ZnS shell overcoating involved the sample being transferred to a centrifuge tube and precipitated with isopropanol. After centrifugation, the slightly colored supernatant was discarded. The precipitate was mixed with hexane by shaking and sonication and then precipitated with ethanol. After centrifugation, the slightly colored supernatant was discarded. The sample was then transferred to a round bottom flask with 4 mL of oleylamine and 5 mL of trioctylphosphine. After degassing at 80 °C, the sample was purged with nitrogen and degassed several more times. 71.3 mg of zinc acetate was added, and the sample was degassed again. Then, the sample was heated to 160 °C and 85 mg of bis(trimethylsilyl) sulfide in 5 mL of trioctylphosphine was slowly dripped in, with the temperature of the sample being increased to 175 °C over the course of the addition. The sample was then cooled to room temperature.

It was found that excessive purification of the ZnSe/CdS beyond that described above was not necessary, but extra precipitation steps did not seem to affect the QDs. One such excessive purification involved, after the first two precipitation/centrifugation steps above, mixing the precipitate with hexane and a minimal amount of isopropanol.
Then the sample was centrifuged, after which the yellow supernatant was transferred to a new centrifuge tube. After the supernatant was split into fractions, one fraction was precipitated with ethanol and centrifuged. After the clear supernatant was discarded, a fraction of the yellow supernatant was added, along with ethanol to precipitate. After centrifugation, the clear supernatant was discarded, and this process was repeated for all fractions until all the precipitate was obtained. The sample was then used as above.

Another variation involved, after the first two precipitation/centrifugation steps described above, the precipitate being mixed with hexane, centrifuged, and drained. Next, the precipitate was mixed with hexane and a minimal amount of isopropanol, centrifuged, and drained. In both cases, the precipitate was then used as described above.

It was also found that the amount of zinc acetate used could be varied between 71.3 to 83.7 mg. The zinc acetate could also be added to the round bottom flask with the precipitated ZnSe/CdS, oleylamine, and trioctylphosphine and degassed at 80 °C. The amounts of bis(trimethylsilyl) sulfide and trioctylphosphine could also be reduced to 81 mg and 2 mL, respectively.

Other variations on the synthesis involved the oleylamine, trioctylphosphine, and zinc acetate being degassed at 90-120 °C in a round bottom flask (without ZnSe/CdS precipitate). Then, the flask was cooled to 43 °C, and the ZnSe/CdS precipitate was added with a minimal amount of hexane. The hexane was removed by reduced pressure, and the sample was degassed at 80 °C. Other variations on this synthesis involved the zinc acetate and the precipitate in a minimal amount of hexane being added at the same time. The hexane was removed, and the sample was degassed at
80 °C. The exact differences between the six representative samples discussed below are shown in Table II.

5.3.4 Quantum Dot Water-Solubilization

The ZnSe/CdS/ZnS QDs were processed by adding hexane to precipitate the byproducts in the sample solution. After shaking, sonication, and centrifugation, the now almost optically clear colored supernatant was transferred to a new vial. A small amount of isopropanol followed by methanol was used to precipitate the QDs. After shaking, sonication, and centrifugation, the clear supernatant was discarded and the precipitate was dried under reduced pressure. The sample was water-solubilized with a 40% octylamine modified poly(acrylic acid) polymer. ZnSe/CdS/ZnS QDs were also water-solubilized according to a recently reported procedure where the QDs are coated with approximately one monolayer of silane.

5.3.5 Conjugation of Rhodamine B Piperazine and Quantum Dot

~0.9 mL of water-solubilized (with polymer) ZnSe/CdS/ZnS QDs was stirred with 8.5 mg of methyl-polyethylene glycol carbodiimide (prepared with methoxyethyl isothiocyanate instead of ethyl isothiocyanate) for 10 min. Then, rhodamine B piperazine dye in pH 8 buffer with a very small amount of methanol was added until the dye emission was approximately 80% of the QD emission. After stirring overnight protected from light, the sample was concentrated to under 1 mL with a centrifugation dialysis filter. The sample was then dialyzed overnight against deionized water using a dialysis tube.
<table>
<thead>
<tr>
<th>ZnS Shell Variations</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra Purification of ZnSe/CdS*</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Zinc Acetate (mg)</td>
<td>77.3</td>
<td>71.3</td>
<td>72.3</td>
<td>76.2</td>
<td>75.5</td>
<td>83.7</td>
</tr>
<tr>
<td>Zinc Acetate Added‡</td>
<td>after</td>
<td>after</td>
<td>before</td>
<td>before</td>
<td>after</td>
<td>after</td>
</tr>
<tr>
<td>Dots Added‡</td>
<td>after</td>
<td>before</td>
<td>before</td>
<td>after</td>
<td>after</td>
<td>after</td>
</tr>
<tr>
<td>Initial Solvent Degas Temp. (°C)</td>
<td>90</td>
<td>80</td>
<td>80</td>
<td>120</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>Bis(trimethylsilyl) Sulfide (mg)</td>
<td>83</td>
<td>85</td>
<td>81</td>
<td>81</td>
<td>83</td>
<td>84</td>
</tr>
<tr>
<td>Triocylphosphine in Drip (mL)</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2.8</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table II. The variations of six representative samples of ZnSe/CdS/ZnS. All other parameters where kept constant. *see Section 5.3.2 ‡ before or after initial degas of solvents in round bottom.
5.4 Results and Discussion

As can be seen in Figure 32, ZnSe/CdS/ZnS QDs were synthesized with emissions spanning from blue to red. Samples were measured to have peaks centered as low as 469 nm and as high as 632 nm. The QYs for QDs in their organic growth media spanned from 43 to 61%, a significant improvement over previous ZnSe/CdS QYs of ~20%. Table III summarizes the characteristics of six representative samples.

The absorption spectra of some representative ZnSe/CdS/ZnS QDs shown in Figure 33 have broad peaks followed by continuous absorption into the UV. The broad peak shifts as the QD increases in size. The emission of these representative samples are shown in Figure 34. The full width at half maximum (FWHM) for all the samples was between 53 nm and 69 nm, as seen in Table III; significant broadening occurred during the overcoating of CdS that appeared to be unavoidable. As can be seen with the emission spectrum of the blue ZnSe/CdS/ZnS sample in Figure 34, some samples were prone to tailing. Careful monitoring of the heating of the sample and addition of the cadmium and sulfur mixture during the CdS overcoating helped to eliminate making such varying sized QDs. Our addition of the second shell of ZnS allows for commonly used water-solubilization methods that were developed for CdSe/ZnS to be used for ZnSe/CdS/ZnS. Two water-solubilization methods were successfully employed, one with a 40% octylamine modified poly(acrylic acid) polymer coating and one with a monolayer silane coating. Both methods’ yields for ZnSe/CdS/ZnS were on par with yields of these water-solubilization methods for CdSe/ZnS; little if any sample was lost during water-solubilization. Figure 35 shows the absorption and emission of a
Figure 32. A: ZnSe/CdS/ZnS samples showing the range of emitting wavelengths. Samples are shown in the dark excited by UV light. B: The best blue, green, and red emitting samples synthesized. Samples are shown in the dark excited by UV light.
Table III. Percent quantum yield, full width at half maximum, and peak of representative ZnSe/CdS/ZnS samples. Six representative organic samples and two aqueous samples are shown.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% QY</th>
<th>FWHM (nm)</th>
<th>Peak (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A in Hexane</td>
<td>43</td>
<td>53</td>
<td>469</td>
</tr>
<tr>
<td>Sample B in Hexane</td>
<td>57</td>
<td>69</td>
<td>482</td>
</tr>
<tr>
<td>Sample C in Hexane</td>
<td>51</td>
<td>69</td>
<td>522</td>
</tr>
<tr>
<td>Sample D in Hexane</td>
<td>54</td>
<td>58</td>
<td>528</td>
</tr>
<tr>
<td>Sample E in Hexane</td>
<td>61</td>
<td>55</td>
<td>623</td>
</tr>
<tr>
<td>Sample F in Hexane</td>
<td>49</td>
<td>53</td>
<td>632</td>
</tr>
<tr>
<td>Sample 1 in Water (from Sample D)</td>
<td>23</td>
<td>54</td>
<td>526</td>
</tr>
<tr>
<td>Sample 2 in Water (from Sample D)</td>
<td>37</td>
<td>52</td>
<td>526</td>
</tr>
</tbody>
</table>
Figure 33. Absorption spectra of blue, green, and red ZnSe/CdS/ZnS samples.
Figure 34. Emission spectra of blue, green, and red ZnSe/CdS/ZnS samples.
Figure 35. Absorbance and emission spectra of aqueous green ZnSe/CdS/ZnS.
representative water-solubilized ZnSe/CdS/ZnS QD. After water-solubilization, a reduction was seen in the QY and FWHM of the samples. The smaller FWHMs were most likely due to the inherent size selection involved in processing QDs carefully. The aqueous QYs were between 23 and 37% and the aqueous FWHMs were between 52 and 54 nm.

As the QDs were now water-solubilized with familiar materials, a rhodamine B piperazine dye could now be conjugated to the QD using the same reagents used for CdSe/ZnS conjugations. The absorption and emission of the QD-dye are shown in Figure 36. While dye emission could be seen when only the QD was excited, as in the inset of Figure 36, TCSPC performed by Clare Rowland and Richard Schaller of Northwestern University and Argonne National Laboratory on the QD-dye conjugates was used to confirm that FRET was actually occurring between the QD and dye. As seen in Figure 37, when exciting the QD, the fluorescence lifetime at 20 ± 1 ns of the QD alone is longer than the fluorescence lifetime at 6.64 ± 0.02 ns of the QD-dye. The inset of Figure 35 shows that when exciting the dye, the fluorescence lifetime at 5.19 ± 0.13 ns of the QD-dye is longer than the fluorescence lifetime at 2.36 ± 0.08 ns of the dye alone. The fact that the QD has a longer decay alone than in the QD-dye, and the dye in the QD-dye has a longer decay than the dye alone, proves that FRET is occurring between the QD and dye. From this information, a FRET efficiency of 0.67 ± 0.03 was calculated.
Figure 36. Absorbance and emission spectra of ZnSe/CdS/ZnS QD-dye. In the emission spectrum, the left peak is the QD emission, and the right peak is the dye emission.
Figure 37. Top: TCSPC data showing the QD alone has a longer exponential decay than the QD-dye. Bottom: The QD-dye has a longer exponential decay than the dye alone. Data taken by Clare Rowland and Richard Schaller of Northwestern University and Argonne National Laboratory.
5.5 Conclusion

In conclusion, I have successfully synthesized very bright ZnSe/CdS/ZnS type II QDs with QYs higher than previously reported for ZnSe/CdS and other type II QDs. These unprecedentedly bright materials have been water-solubilized via two different methods, and a dye has been conjugated to the water-solubilized QDs. The evidence that FRET is occurring between the QD and dye, proven via TCSPC, lends to the potential of ZnSe/CdS/ZnS being used in FRET-based chemical and biological sensing applications. The fact that these QDs are type II, and thus have longer fluorescent lifetimes than type I QDs, makes them an ideal material for use in time-gated detection studies. One of the benefits of creating high QY ZnSe/CdS/ZnS is the reduced cadmium content (and thus reduced toxicity) compared to the high-cadmium containing brightly emitting QDs such as CdSe/ZnS that are currently widely used for biological applications.
6. Conclusion

A significant area of current research is on the use of QDs for biological applications such as sensing and imaging. In addition, QDs are also useful for solar cells, QD-based lasers, and light-emitting devices among other uses. My research performed in the Snee group during the last 5 years at the University of Illinois at Chicago has produced novel advancements for the QD field. This thesis described the most prominent projects completed during my graduate career. The research included in this thesis has opened up the possibilities for unlabeled protein detection with QDs as well as introduced a new type II QD capable of FRET.

Ratiometric QD protein sensors were developed that relied on variations in FRET between the QD and protein-binding dye ligand. These sensors were designed to specifically and ratiometrically respond to the unlabeled target protein. A model sensor for the protein streptavidin was discussed in Chapter 2 that showed that the theory behind the sensor was correct. The QD streptavidin sensor had a DL as low as a few pmol/mL, which is competitive with ELISA assays, and this DL is scalable with the sensor solution concentration. The method is fast and homogeneous. While this QD protein sensor proved that the method worked, streptavidin, the detected protein, is not very biologically relevant.

The next ratiometric QD protein sensor discussed was a QD Cox-2 sensor. Cox-2 is a biologically relevant protein as it is found at inflammation and tumor sites; a Cox-2 sensor has the potential to detect tumors and cancer. Many different approaches were taken in an attempt to design a functional QD Cox-2 sensor. The QD sample, the water-
solubilization of the QD, the Indocin-dye ligand, the conjugation method for the QD and ligand, and the concentration of the QD sensor relative to protein concentration were all modified in the studies done with this sensor. Ultimately, the research performed for this project did not produce publishable results.

A third ratiometric QD protein sensor for the biologically relevant protein thrombin was described that showed the generality of the QD sensor platform and also proved that aptamers could be used in the sensing system. Thrombin is biologically relevant due to its participation in the coagulation cascade as well as the fact that high levels of thrombin in the blood is indicative of many diseases. This QD protein sensor proved that the sensing method is translatable to biologically relevant proteins. As many small molecular protein-binding agents and aptamers exist, many more relevant proteins can potentially be detected using this method. Ratiometric QD protein sensors have a potential utility to detect infectious diseases in places where ELISA and Western blot assays are prohibitive. They also have the potential to be useful in studying protein-protein interactions with FRET. The ratiometric QD protein sensor discussed in this thesis is a new paradigm for unlabeled protein detection.

Finally, the synthesis of very bright ZnSe/CdS/ZnS QDs was discussed which greatly improved upon the reported QY for this material system and type II QDs in general. High QY type II QDs have utility in time-gated detection studies as their fluorescent lifetimes are much longer than type I QDs. These highly emissive QDs were successfully water-solubilized, allowing these materials to be usable for biological applications. A dye was conjugated to the surface of these QDs, and the construct was
proven to participate in FRET. All of these results point to ZnSe/CdS/ZnS being highly useful for FRET-based chemical and biological sensing applications.
CITED LITERATURE


61. Dif, A.; Boulmedais, F.; Pinot, M.; Roullier, V.; Baudy-Floc'h, M.; Coquelle, F. M.; Clarke, S.; Neveu, P.; Vignaux, F.; Le Borgne, R.; Dahan, M.; Guerou, Z.; Marchi-Artzner, V. Small and stable peptidic PEGylated quantum dots to target


84. Smith, A. M.; Duan, H.; Rhyner, M. N.; Ruan, G.; Nie, S. A systematic examination of surface coatings on the optical and chemical properties of semiconductor quantum dots. Physical Chemistry Chemical Physics 2006, 8, 3895-3903.


89. Hanaki, K.; Momo, A.; Oku, T.; Komoto, A.; Maenosono, S.; Yamaguchi, Y.; Yamamoto, K. Semiconductor quantum dot/albunin complex is a long-life and


172. Human IL-18 ELISA Kit. MBL International Corporation **2003** Code No. 7620.


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BACK  CLOSE WINDOW
A primer on the synthesis, water-solubilization, and functionalization of quantum dots, their use as biological sensing agents, and present status


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