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Silicon Quantum Dots Conjunctive with Stable Radicals: Potential Application in Non-invasive Cancer Diagnostics

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Silicon Quantum Dots Conjunctive with Stable Radicals: Potential Application in Non-invasive Cancer Diagnostics

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in the Department of Physics and Astronomy

By

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ABSTRACT

Quantum Dots, and nanomaterials in general, are one of the most promising areas of current physical study, especially their potential medical uses. In this study we introduced Nitroxide free radicals into a Silicon Quantum Dot (SiQD) solution and conducted experiments on the modified SiQDs in order to characterize their physical properties. We gathered emission, excitation, absorption and electron spin resonance (ESR) spectra of both modified and unmodified SiQDs. Previous studies have shown the possibility of using proteins as markers for cancer detection. Our measurements show the potential of using the interaction between these protein markers and SiQDs as a form of non-invasive cancer diagnostics.

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1. Introduction

1.1 History and Characteristics of QDs

Quantum Dots (QDs) were first discovered by Alexey Ekimov, a Russian solid-state physicist, while he was working at the Vavilov State Optical Institute. Ekimov published his finding in a 1981 paper [(1)] where he first described QDs, yet the term "quantum dot" was not coined until 1986 by American physicist Mark Arthur Reed. Later, American physicist Louis E. Brus discovered QDs within colloidal solutions while he was working at AT&T Bell Laboratories.

QDs are particles with a size of only a few nanometers (SiQDs being around 2nm). Due to their small size they have physical properties which differ from larger particles due to quantum mechanics. Some possible uses for QDs include use in photovoltaic devices, light emitting diodes, quantum dot displays, photodetectors and in a variety of possible biological applications. In particular, QDs have unique optical characteristics. Larger QDs, expectedly, have longer more red wavelengths compared to their smaller counterpart whom tend to show more quantum effects. Yet the study of QDs is still relatively new and cutting-edge research is being done continuously in order to find more possible applications.

1.2 Free Radicals and QDs

A free radical is an atom / molecule which binds itself to another atom / molecule in order to fill the outer shell of an electron cloud. Generally speaking, free radicals are unstable due to their unfinished electron shell and therefor will react quickly with other materials in order to become stable. An easy example is most diatomic elements such as

oxygen which is naturally found as O2 because they pair with each other electrons. If the two oxygen atoms were to be separated, then they would both become free radicals.

QDs can be combined with a variety of different free radicals in order to change their physical properties. Previous research has been done pertaining to the use of Nitric Oxide (NO) as a free radical to be attached to QDs in order to create a fluorescent nanoprobe (Xu et al., 2011). This use of QDs as a nanoprobe will be further discussed in the upcoming section.

1.3 QDs as a Tool for Diagnostics

The basic principle used in using QDs as a nanoprobe revolves around the quenching caused due to the free radicals and the recovered quenching when the QDs interact with certain proteins. Xu and company[(2)] showed that they could create a QD probe that was sensitive to nonprotein thiols. They covalently combined CdTe QDs with 4-amino-2,2,6,6-tetramethylpiperdine oxide (QDs- AT) for their experiment and were able to gather promising results. They showed that when the nanoprobes interacted with nonprotein thiols they detected an increase in fluorescence intensity directly relating to the amount of nonprotein thiols. This proves that the thiols interact unquench the QDs-AT.

Another study conducted at the *Division of Immunology, Beckman Research Institute of the City of Hope* (Balendrian et al., 2004)[(3)] showed that Glutathione (GSH) (a naturally forming tripeptide found in almost all cells) is present in higher levels in many tumor cells. GSH is a natural way our bodies fight against cancer, yet at the same time the elevated levels of GSH may protect cancer cells against our current forms of chemo

and radiation therapies. Examples of possible cancerous cells containing high levels of glutathione are bone marrow, breast, colon, larynx, and lung cancers. GSH is also the most common non-protein molecule found in cells, meaning it's readily available in our bodies. The previously discussed research by Xu et al. used GSH as their nonprotein thiol for their experiments. This means that combining the technique of using QDs as nanoprobes and the knowledge that GSH is abundant in higher quantities in cancer cells means we could utilize QDs as a method for cancer detection.

1.4 Disadvantages and Advantages of Silicon QDs vs other QDs

Our research revolved around the use of Silicon QDs (SiQDs) which have been shown to have a multitude of possible benefits. Firstly, they are easy to produce in quantity. Second, silicon is non-toxic. Lastly, SiQDs are water soluble and protein binding. This last point may prove itself to also be a downside due to the fact that the SiQDs are more likely to bind with impurities due to their water solubility.

2. Method and Characterization

2.1 Sample Creation and Modification

We were provided with dry SiQDs from one of Dr.Li Ma's colleagues which were created using the following technique:

N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (DAMO) (98 %), trisodium citrate dihydrate (≥ 99.0%) were purchased from Sigma-Aldrich without any additional purification. The solutions were prepared using deionized water. The fluorescent SiQDs were synthesized by a one-step hydrothermal method, modified from a microwave preparation. In a representative preparation of 200 ml precursor solution, dissolved 11.16 g trisodium citrate dehydrate in 240 mL deionized water. After 20-min stirring, added 60

mL DAMO to above solution and continually stirred for 40 min. In order to remove dissolved oxygen, the mixed solution was bubbled with nitrogen gas. The precursor solution was then transferred into a teflon-lined stainless-steel autoclave and heated at 160 ºC for 12 hours. After cooling to room temperature naturally, SiQDs were purified by dialysis out residual reagents (MWCO 1 kDa) in 10 X deionized water for 20 h and freeze-dried. The reaction is pictured below.

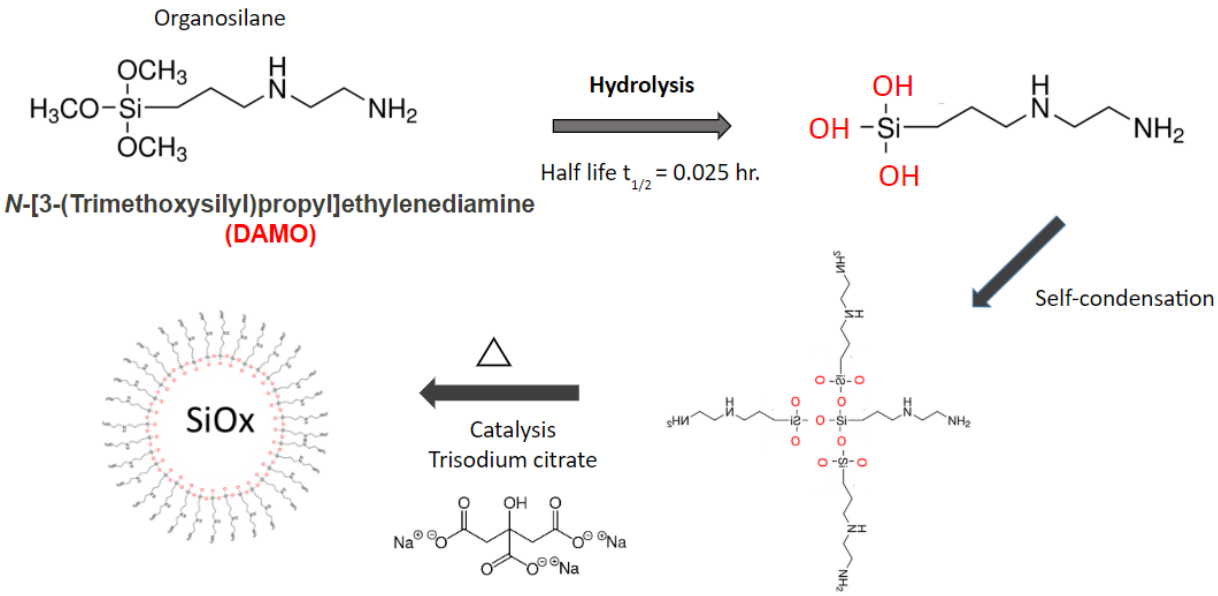


Figure 1: Reaction Model provided by Dr.Li Ma

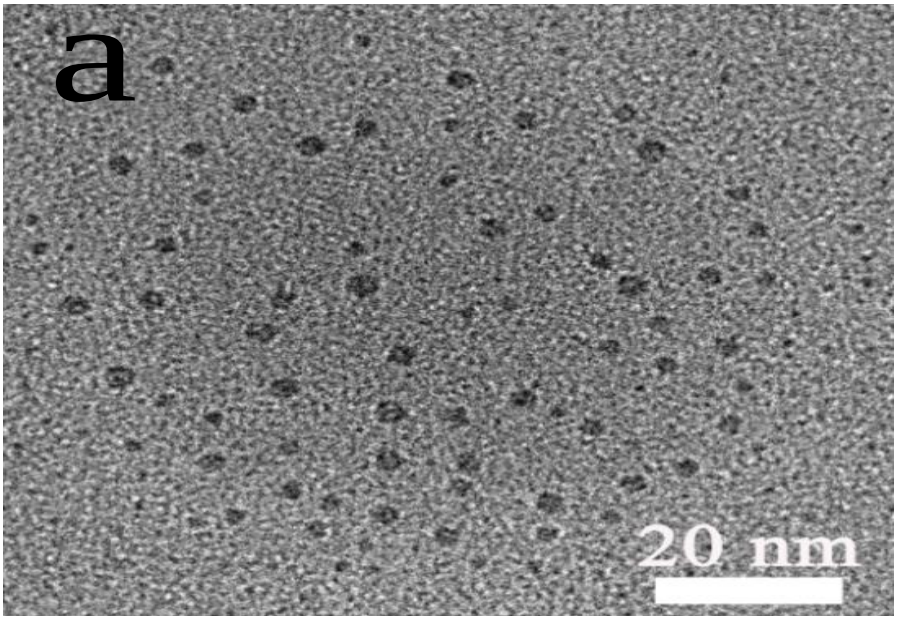


Figure 2: A Tunneling Electron Microscope image of the SiQDs provided to us. (Approx. size 2nm each)

For the modification process, we used Nitric Oxide (NO or Nitroxide) as our free radical. The process we used was first to place some of the dry SiQDs into a 50 milli-molar PB (7.4ph) and sonicated it to create a solution ($250mg\ SiQD + 10ml\ 50mili - molar\ PB$). Next, we would place our NO into the SiQD solution let the reaction occur at room temperature with a magnetic stirring stick. Once the reaction occurred, we would then pass the new modified solution through a particulate filter in order to remove any large impurities. The sample we used for all of our modified measurements were modified using 120mg of NO and then we removed the solids at the bottom from the liquid at the top using a syringe. This retrieved liquid was our sample.

2.2 Method

We measured five main characteristics of our modified SiQD sample during these experiments: 1) Absorption 2) Fluorescence 3) emission 4) Excitation and 5) Electron

Spin. Four of these characteristics are optical. The absorption was gathered using a spectrometer and we particularly focused in the wavelength ranging from 240nm to 440nm. We then used this absorption data and compared it with the absorption data from a sample of unmodified SiQDs. We then measured the Fluorescence, emission, and excitation spectra were all collected in the same fluorescence spectrometer. Similarly, these measurements of the modified SiQDs we compared to the unmodified measurements.

We used an Electron Spin Resonance machine in order to detect the spins. This device uses magnetic fields and a microwave wavelength scanner in order to read the spins of the molecules. This process is similar to Nuclear Magnetic Resonance readings, but the frequency of the microwave is different. Our machine fluctuated the strength of the magnetic field while keeping the frequency constant.

3. Results

3.1 Results of Optical Measurements

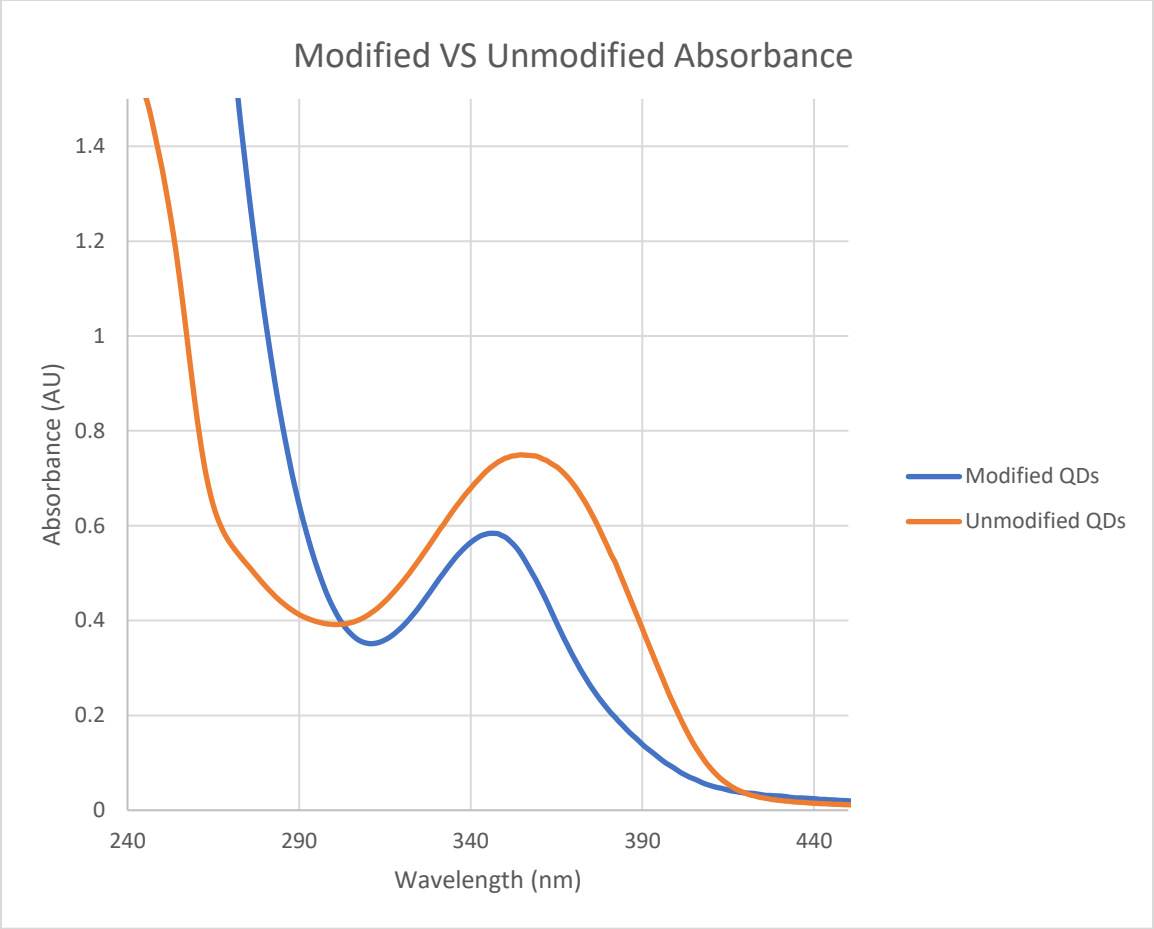
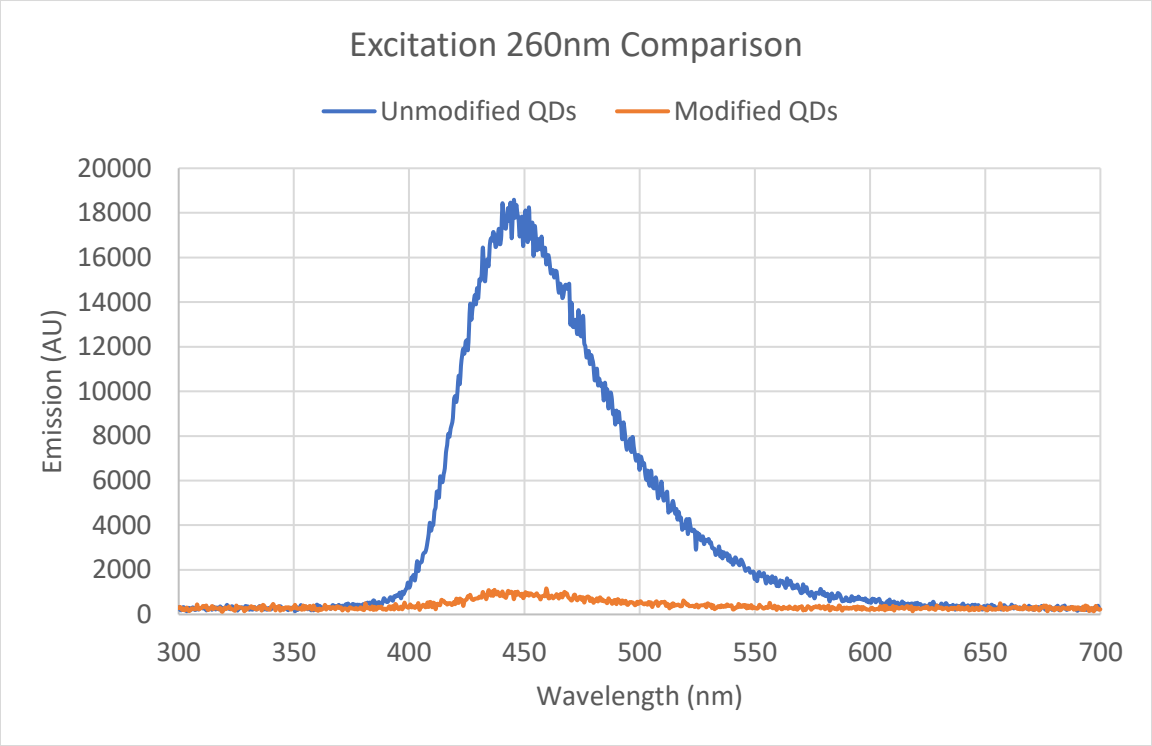


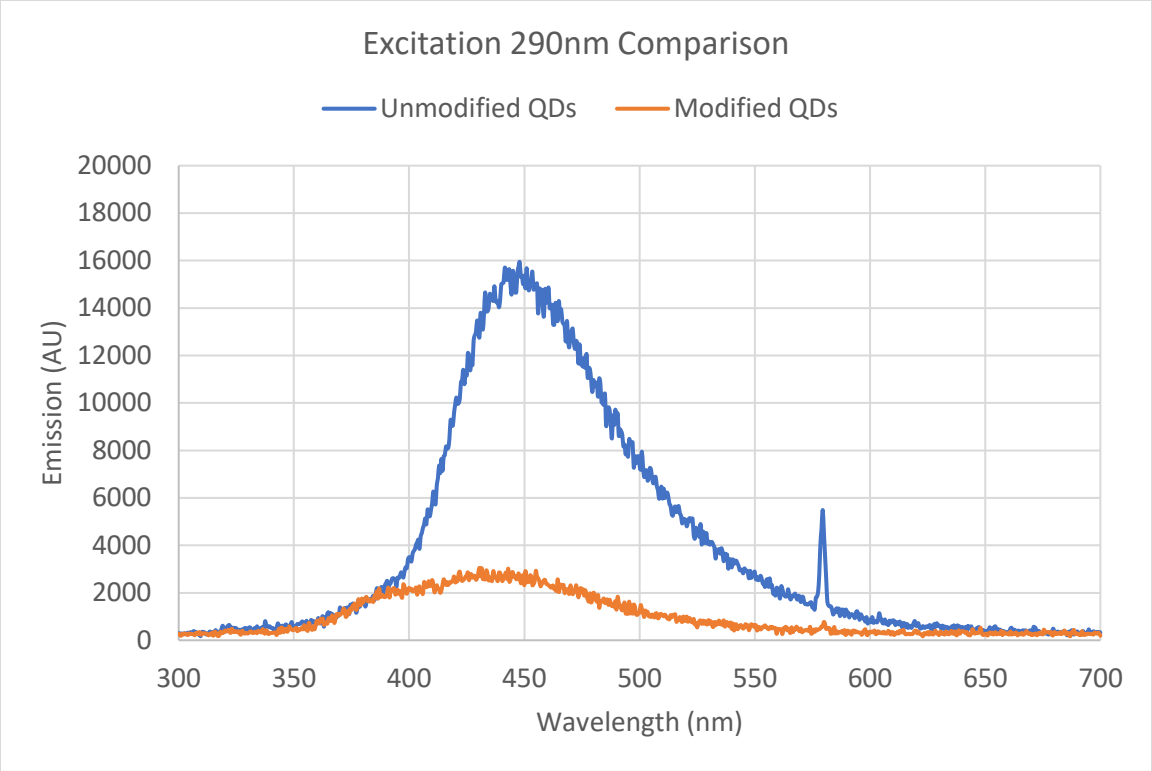
Figure 3: Modified VS Unmodified Absorption Spectra

Our measurements from the absorption spectrum were promising and also surprising. As you can see in the diagram above, our absorption peak broadened in the modified sample, which is expected, meaning that our modified solution has at least kept some of the NO but we were unaware at the time if the NO had bonded with the SiQDs or if they were simply suspended in the liquid with the SiQDs. Further measurements we gathered would resolve this confusion. Interestingly we measured that the peak for our modified SiQDs had decreased in wavelength. This goes against general understanding that larger molecules will read larger wavelengths. We are not exactly sure what may have caused this shift, but one possible explanation is electron interaction between the SiQDs and NO

free radicals caused some physical change in the size. For our unmodified sample we place 2mL of 5mili-molar PB(7.4ph) and 0.02g of dry SiQDs. For our modified sample we combines 1µL of our modified SiQDs and 2mL of distilled water.

Our fluorescence, excitation and emission measurements were primarily used to show the quenching of our SiQDs due to the introduction of NO. Our measurements show a clearly lower intensity in our modified SiQDs which agrees with previous research conducted. Alongside the intensity readings we see that certain excitation wavelengths show a much larger difference in intensity between modified and unmodified SiQDs, especially at approximately 440nm. This data can be seen in the graphs below.





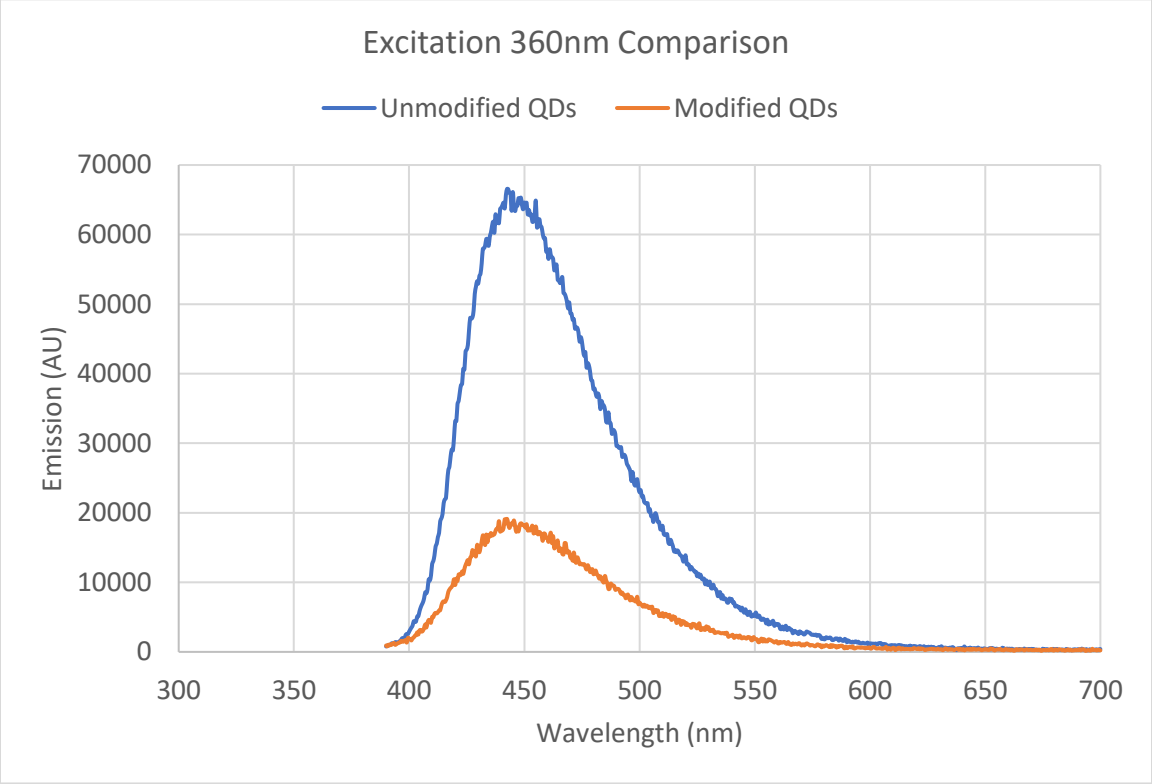


Figure 4: Comparison between modified and unmodified SiQDs to show the quenching effect in action. Note how the modified QDs show much less emission. We hope that with the introduction of GSH we will get back our fluorescence

Another important set of data Is the actual ratio between the peaks for each of the different excitation wavelengths. This data should help us understand which wavelength would be most efficient for measuring quenching return when we move onto further studies involving the introduction of GSH.

	260nm	290nm	360nm
Modified:	1160	3050	19130
Unmodified:	18590	15930	66540
% Difference:	6.24	19.15	28.75

3.2 ESR Measurements

Our ESR measurements seen below show uneven peaks in the modified sample compared to the symmetrical peaks in the unmodified sample. We collected both integrated and unintegrated data. Both showed distinctly uneven peaks like we were hoping.

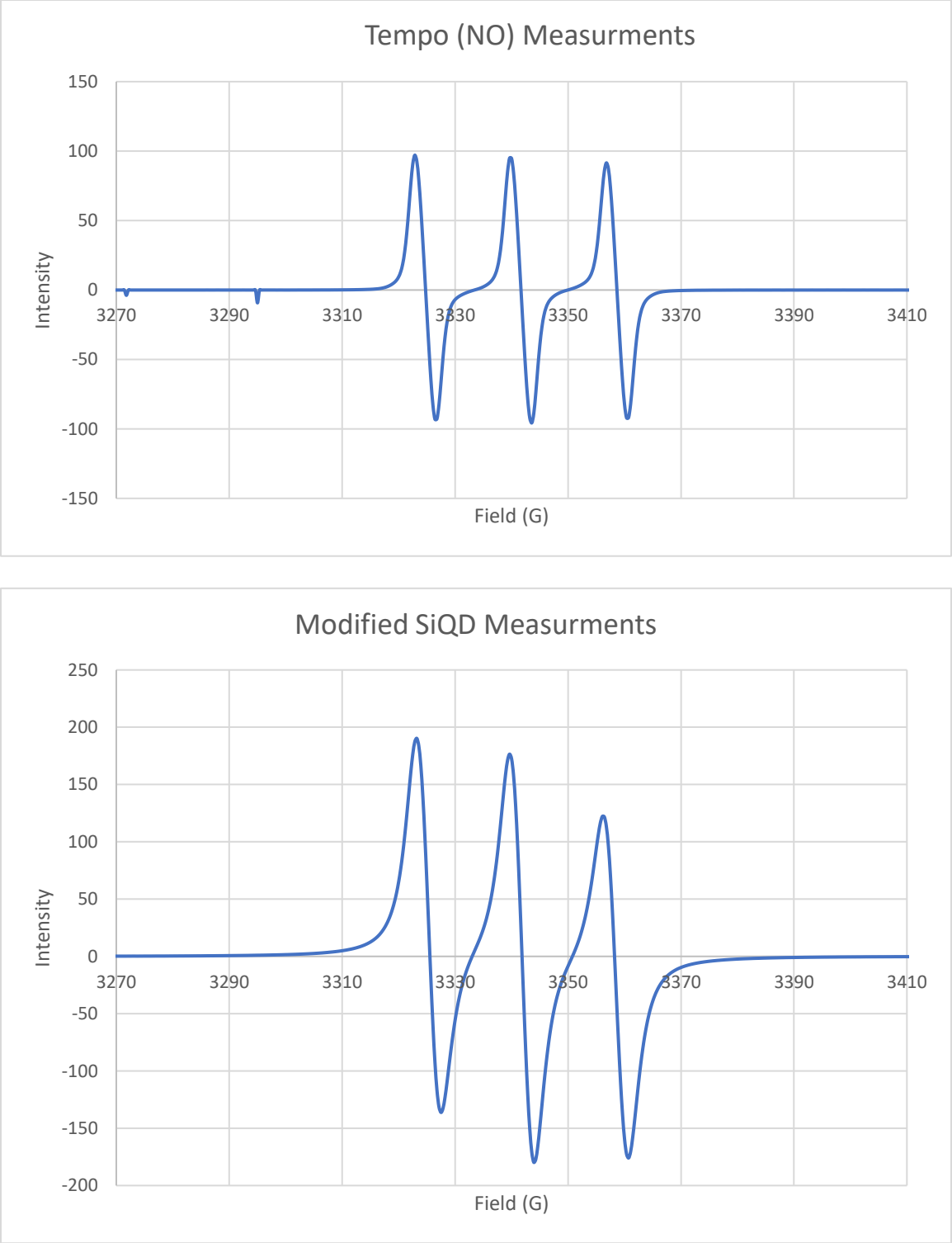
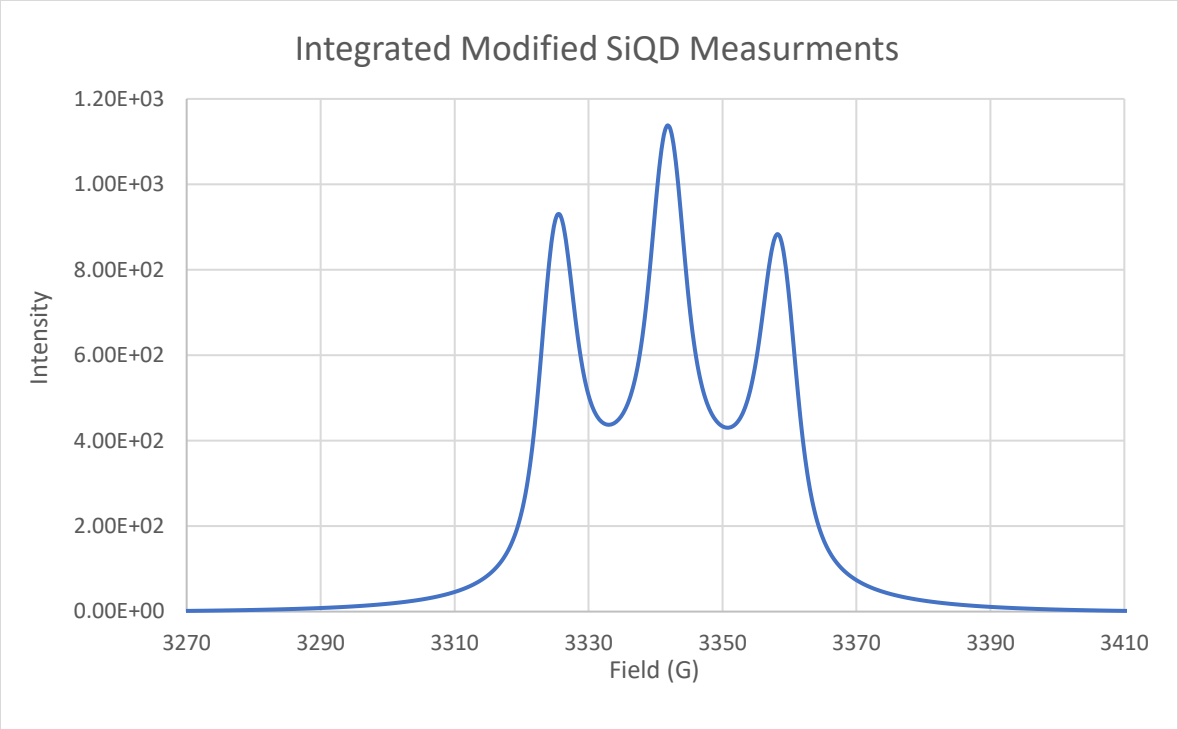


Figure 5: Above we see the Tempo(NO) vs. Modified SiQD measurements. Note the uneven peaks representing a chemical bonding between our free-radical and the SiQDs



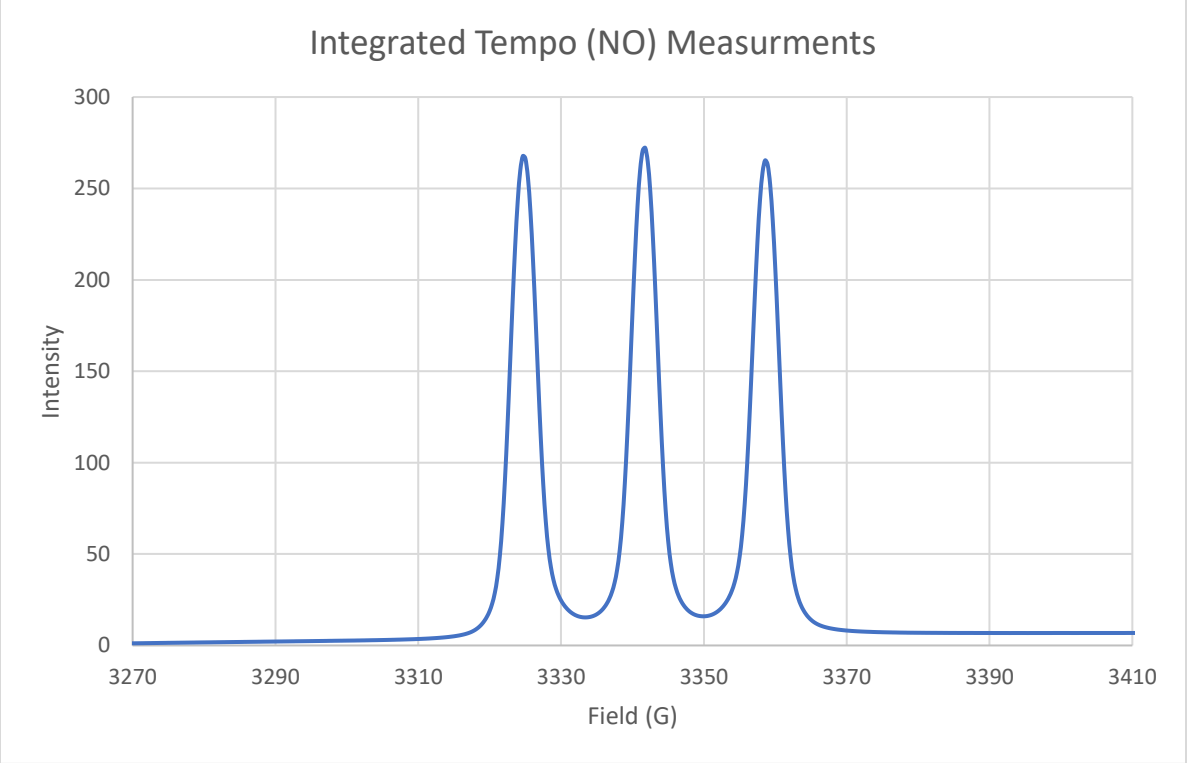


Figure 6: The integrated ESR measurements to better show the difference within the peaks

4. Interpretation of Results

4.1 Interpretation of Optical Results

Our absorption results show that the SiQDs and NO were both within our solution and caused a change when compared to pure SiQD measurements. This means that we had not lost out NO when filtering the solution through our particulate filter and also that some of the NO was within the liquid solution. We are still not fully sure why the peaks seem to have shifted to smaller wavelengths. Further research should be done to find the possible reasons for this measurement. Our absorption measurements also showed the peaks to be more narrow after modification which represents uniformity of some kind, weather that is uniformity of electron density or size is unknown.

Our fluorescence, excitation, and emission measurements showed that there was in fact quenching in our modified sample. This means that the introduction of NO had caused a lessening of intensity. Xu et al. research has shown that GSH removes this quenching and therefore we could use this difference in intensity between quenched and unquenched SiQDs as a form of nanoprobe for cancer diagnostics. We were not able to conduct experiments with GSH but the principle is similar to the previous research done with QDs-AT. Alongside the intensity, we gathered info that an excitation wavelength of 260nm is most effective for comparison between quenched and unquenched states. We believe the free radical may be causing some free electrons to jump to it rather than the electron jumping down to lower energy states within the SiQD which is what gives us the quenching. Introducing the GSH would kick these electrons back to the SiQDs and bring back our emission readings. This also explains why the higher energy 260nm excitation

range gives us a higher difference in quenching. The higher energy allows for a band-gap jump which provides more free electrons to jump to the free radical.

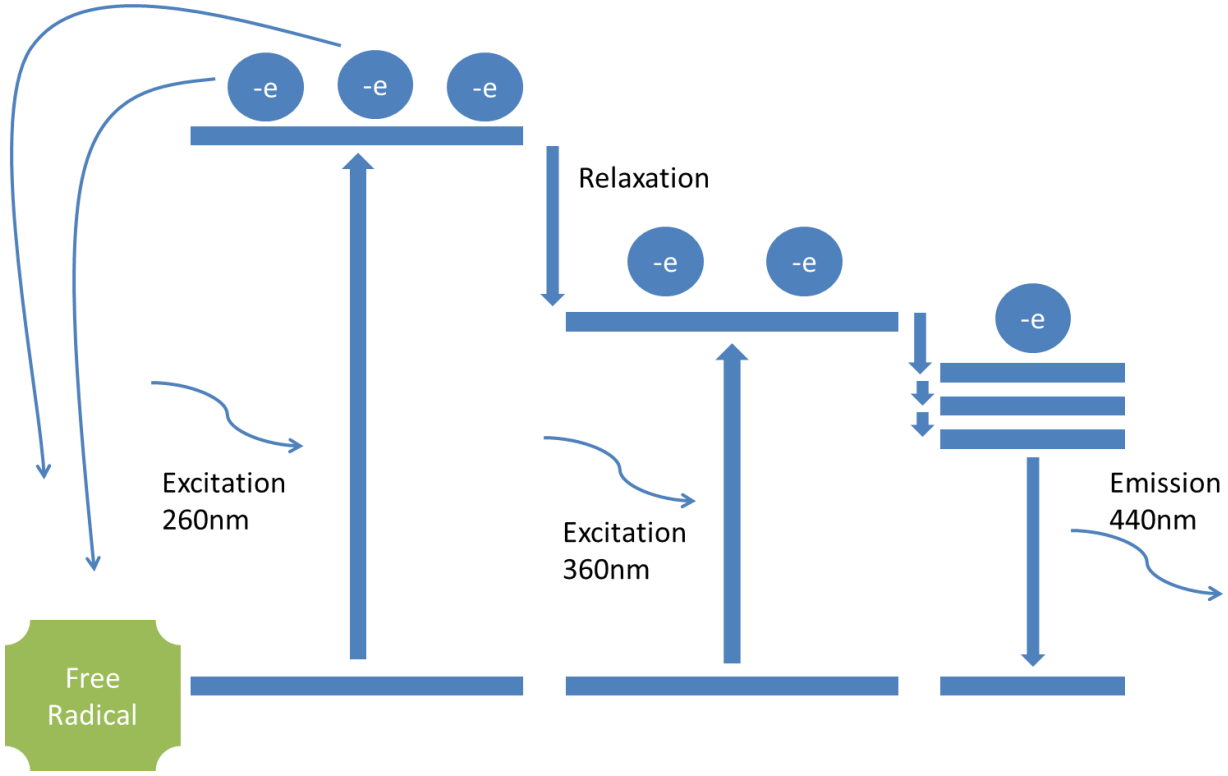


Figure 7: Illustration created by me to represent the electrons jumping to the free radicals after excitation

4.2 Interpretation of ESR Results

Our ESR measurements show distinctly uneven peaks. These results answer our previous question of whether or not the NO was actually bonding with the SiQDs. Data shows that the free radicals had covalently bonded directly with our SiQDs.

5. Conclusion and Further Research

Our research shows that SiQDs show great promise as possible detectors for cancer. A simple non-invasive blood test could possibly provide the necessary information for

cancer diagnoses. Future research should be conducted in order to see if the modified SiQDs interact with GSH and if the fluorescence intensity returns. As well as possible research into the most efficient way to combing SiQDs and NO in order to create samples on a larger scale. In-vitro study of nanomaterials in general is needed in order to make sure that the reliability, safety, and efficacy of SiQDs (and other nanomaterials) is present within the body. Preliminary results are promising.

6. Acknowledgments

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Main Citations

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3) Balendiran, Ganesaratnam K., et al. "The Role of Glutathione in Cancer." Cell Biochemistry and Function, vol. 22, no. 6, 2004, pp. 343–352., doi:10.1002/cbf.1149.

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