

# Quantum Dots in Biophysical Applications

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## **ABSTRACT**

Quantum dots are relatively new developments in nanotechnology. With these dots it has been demonstrated to track cells in cancer patients (1) and it is hoped researchers will be able to use these dots for numerous medical advances. Quantum dots are semiconductor nanocrystals (2). They fluoresce when excited by light and have many advantages to traditional dyes. Our goal is to put the quantum dots in the cell's membrane and to probe physiological states of biological cells. The greatest challenge thus far is delivering the quantum dots into the cells membrane. Once we accomplish that, there are three main areas in which we are interested: making a voltage sensitive probe, a light gated channel, and a marker for synaptic vesicles.

Before attempting biological cells, we are trying to put the quantum dots into artificial vesicles made from phospholipids that mimic a biological environment. The concentration gradient of salts between the inside and outside of the vesicle and the selectively permeable membrane yields a voltage. We hope to see the change in color of the dots due to the Stark effect.

Under the fluorescent microscope, small fluorescent light coming from the vesicles is observed. However we believe this is either one of two possibilities: auto-fluorescence or somehow the vesicles are quenching the fluorescence of the dots. We explored the addition of different chemicals such as AOT, 1-butanethiol, and 1-octanethiol to better protect the quantum dots so that it is a more preferred state to be in the vesicle membrane rather than the glass tube wall.

Another area we are interested in is studying the fundamental properties of the quantum dot in an effort to determine possible applications of these nanocrystals. By running fluorescence correlation spectroscopy we can determine the diffusion coefficient and radius of our quantum dots.

## INTRODUCTION

Quantum dots are semiconductor nanocrystals ranging in size from 3-9nm (1). The quantum dots prepared at the University of Pittsburgh are made from cadmium selenide. Unless chemically altered, quantum dots are hydrophobic and kept in chloroform. “Quantum dots” receive their name from the fact that they are “dots” or particles that exhibit quantum behavior.

The fundamental properties of quantum dots vary depending upon the size of the dots, not the chemical composition. The size of the interior of quantum dot changes the density of the electronic energy levels and therefore their optical and electrical properties. For example, the melting temperature varies from 1600 down to 400°C (1). The emission and excitation properties also vary with the size. Quantum dots fluoresce when excited by light from wavelengths ranging from ultra violet to their emission wavelength. A dot of diameter 3nm fluoresces blue whereas a dot of 9nm fluoresces red (see figure 1). Our dots tend to be around 4nm and fluoresce green.

Probably the most valuable property of quantum dots is that even though they are inorganic solids, they may be coated with organic ligands (2). The surfaces of quantum dots affect virtually every property from light emission to solubility. By coating the quantum dots with various substances they are able to dissolve in various fluids, attached to an electrical

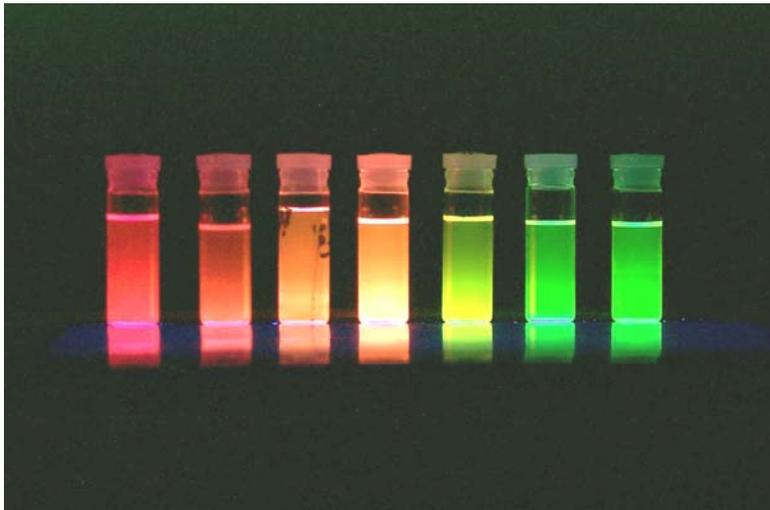


Figure 1: UV excitation of Quantum Dots. Dots on left ~9nm, dots on right ~4nm.

circuit, or bound to biological cells (2). We coat our dots with two layers of zinc sulfide to achieve brighter and longer lasting fluorescence. For water soluble dots we add either one of two copolymers: Dioctyl Sulfosuccinate (AOT) or n-poly(ethylene glycol) phosphatidylethanolamine (PEG-PE).

Quantum dots have many advantages to traditional dyes. They are highly resistant to chemical and metabolic degradation and have less photobleaching (3). Quantum dots have a high quantum yield so very low concentrations can be detected. When an electron de-excites from an excited (or higher energy level) state to a lower state, a photon is released. Every time this happens the photon has a probability of destroying the quantum dot. The decrease in fluorescence due to this process is called photobleaching. It has been estimated that each quantum dot has the potential to emit up to a million photons before being destroyed. Quantum dots also have wide excitation spectra, which enable efficient excitation, and narrow tunable emission spectra (3). Conversely, traditional dyes tend to have narrow excitation spectra and wide emission spectra. Finally, one can dress the outside of a quantum dot with arbitrary

functional groups to attach to DNA or proteins so that biological activities can be tracked inside or outside of a cell.

Quantum dots are inert to biological cells and have already served as excellent markers in cells. Quantum dots linked with immunoglobulin G (IgG) and streptavidin were used by researchers to label the breast cancer marker Her2, both fixed and live cancer cells (1). The dots proved to be brighter and more photostable than organic dyes and dots could be used with different emission spectra excited by a single wavelength.

### **VESICLES: Designing a Model Cell**

Phospholipid bilayer vesicles are our model cells. As already mentioned, quantum dots have numerous advantages to organic dyes and have already been used in tracking cancer cells and studying embryo development (1,4). Our eventual goal is to put quantum dots into the membrane of a cell, and more specifically, a neuron cell. We would use the quantum dots to label and hopefully through the Stark effect be able to follow the neuron action-potential path. Due to the color change in the quantum dots fluorescing we may be able to tell which cells are active.

Phospholipid vesicles are formed from a lipid with a phosphate group attached to one end of the lipid chain. The phosphate group is hydrophilic and the lipid tail is hydrophobic. When placed in an aqueous solution the phospholipids immediately seek a preferred state. The lowest energy state is simply a layer of phospholipids coating the surface of the water with the phosphate group submerged in the solution and the lipid tail protruding out of the solution into the air. The second preferred state is a vesicle. First the phospholipids form a bilayer, where the tails are in and the phosphate groups are out facing the aqueous solution. This double layer of phospholipids then curves around to form a vesicle.

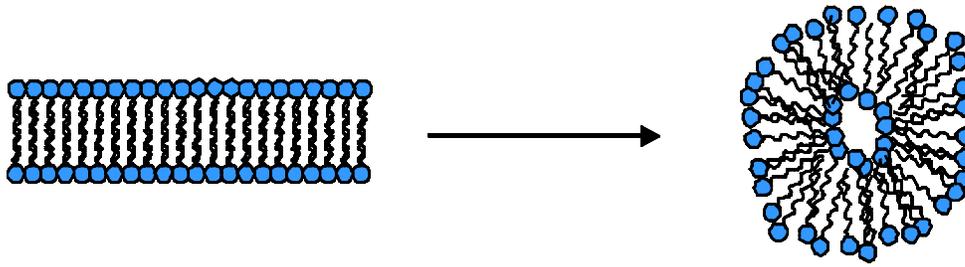


Figure 2: Formation of phospholipid vesicle from lipid bilayer. Since the phosphate group (or head of the lipid) is hydrophilic and the lipid chain (or tail) is hydrophobic, in an aqueous solution one of the preferred energy state for the phospholipids is a vesicle.

This membrane of the vesicle is removed from the aqueous water environment and since the quantum dots are hydrophobic, we hypothesized it would be a preferred energy state for them to go inside the vesicle membrane. By creating a semi-permeable membrane with valinomycin we want to set up a concentration gradient of sodium and potassium ions. The sodium would flow in and potassium would flow out and due to the concentration gradient an electric field will form. Due to the small thickness of the membrane, a very small voltage yields a large electric field. For example, if the thickness of the membrane is only  $\sim 10$  nm, and a voltage difference of 100 mV, the electric field,  $E = V/d$ , would be 100 kV/cm. We can create the voltage using the Nernst Potential:  $V = -(RT/F) \ln (K_{in}/K_{out})$ . Once the vesicles are made, the original concentration of potassium or sodium in the buffer is trapped inside the vesicle. By diluting the surrounding solution we can set up a situation very similar to human cells which have a potential difference of  $-70$  mV.

In a neuron cell, the potential difference and electric field would be initiated when the neuron fires its action-potential. The fluorescence of a quantum dot can be described by the particle in a box model where an electron confined in a 1-dimensional well with walls of infinite

height has energy levels given by:

$$E_n = \frac{n^2 \pi^2 \hbar^2}{2mL}, \text{ where } n = 1, 2, 3, \dots$$

Quantum dot's fluorescent properties change when the electric field changes. This could be observed by both the brightness and color of the quantum dot. From the measured emission spectrum we expect to see a shift in color due to the applied electric field. In the presence of an electric field spacing between energy levels shift and therefore the colors of the absorbed and emitted photon's shift. A first order term shifts all levels absolutely, and a second order term shifts the levels relative to each other. When the energy levels are closer together you would see a red shift because  $E = hc/\lambda$ , hence less energy equals a longer wavelength (red light is a longer wavelength).

Since phospholipid vesicles are the model cell, we attempted to first place the dots inside the vesicle. We synthesized giant vesicles, which are ~20-100  $\mu\text{m}$ . Then we endeavored to develop a protocol to position the quantum dots inside the vesicle lipid membrane.

## **MATERIALS AND METHODS**

### **Materials**

Phosphatidylcholine (egg) and phosphatidylglycerol (egg) were obtained from Avanti Polar Lipids Co. (Alabaster, AL). Cadmium selenide quantum dots, both uncoated and coated, were prepared according to the procedure developed by Hines and Guyot-Sionnest (5).

Deionized water was used in all experiments. Lipids were each dissolved at 10 mg/ml in chloroform and stored in bottles with a teflon cap. Teflon square cut with 1 cm per side and ~1

mm thick. Buffer made from 100 mM KCl and 0.1 M Sucrose in deionized water. Dioctyl sulfosuccinate (AOT), 1-ethanethiol, 1-propanethiol, 1-butanethiol, 1-hexanethiol, and 1-octanethiol were used in some preparations with a purity above 98%.

### Preparation of Giant Liposomes

Phosphatidylcholine (POPC) and phosphatidylglycerol (POPG) were mixed in a 9:1 ratio to make a final lipid solution at 10 mg/mL in chloroform. The solution was placed on a teflon square around one square centimeter in a larger 20 mL glass vial with a teflon cap. Just enough solution was used to cover the teflon square. Then it set out to dry until all of the chloroform had evaporated, usually one hour. A thin lipid film coated the teflon square. It was then dessicated in a vacuum for at least 4 hours to insure all organic solvent was removed (see figure 3). The dried lipid film was then slowly hydrated by water saturated nitrogen at 60°C for one hour (see figure 4). We gently pipetted 5-6 mL of the buffer so the surface of the teflon was submerged. It was placed in an incubator at 37°C for at least four hours, overnight for highest liposome yield. A cut pipette tip provided a wider and more gentle opening to harvest the vesicles. For a detailed protocol refer to Deborah Fygenon's work and K. Akashi's et. al. (6,7).

### Experimental Setup



Figure 3: Vacuum set up.



Figure 4: Water saturated nitrogen at 60°C

## Preparation of Giant Liposomes with Quantum Dots

Around 100  $\mu\text{L}$  of same lipid mixture and roughly 100  $\mu\text{L}$  of quantum dots, either coated or uncoated with zinc sulfide, were placed in a glass test tube. Teflon won't work because the quantum dots are highly attracted to the teflon and never leave it to go into the vesicles. Instead of letting it dry only one hour, we let it dry overnight so that all the chloroform is evaporated. It was desiccated in a vacuum for 4 hours and then slowly hydrated with water saturated nitrogen at 60°C for one hour. Only 2 mL of the buffer was used and then the solution was incubated overnight at 37°C.

## RESULTS

We have successfully made giant vesicles, but as to whether they are unilamellar or multilamellar will require further investigation. Most of our observations have shown vesicles within vesicles. We have also noticed a lot of tubes and once saw a tube expand into a circular vesicle. Many of the vesicles fluctuated in shape.

Under the fluorescent microscope, very faint fluorescence from the vesicles is seen. This

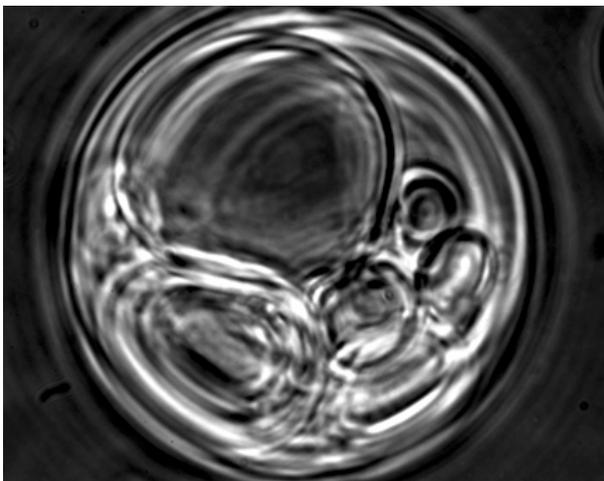


Figure 5: Red light image of multiple vesicles in vesicles.

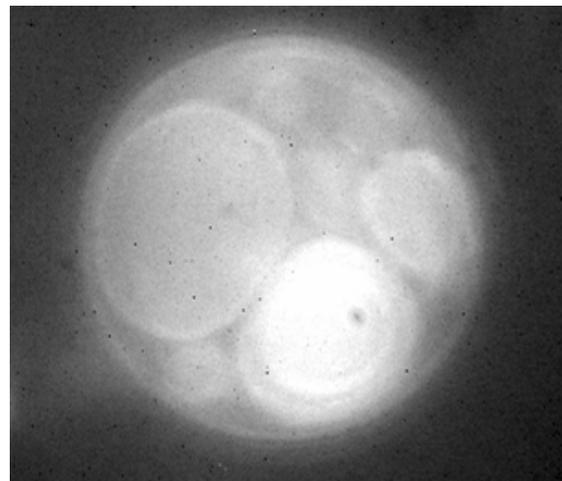


Figure 6: Autofluorescence. Exposure 60 sec.

could be due to autofluorescence, or if it isn't, the quantum dots could be quenched by the phospholipids.

Sometimes in preparation we would notice the quantum dots leave the glass tube to float in the solution, however they wouldn't be found in the vesicles. Determining where they went will require further investigation. Rarely we would see micelles, a molecular aggregate that constitutes a colloidal particle, but most of the time no other fluorescence is visible. So the question remains: Where did the quantum dots go? Sometimes however, it was obvious from observation that the quantum dots never left the glass surface.

Theoretically, quantum dots would want to be inside the phospholipid membrane since they are hydrophobic, but such had not been unambiguously observed with unaltered quantum dots. We believe that there are a couple of possibilities to explain this. Either the membrane is too small for the quantum dots and the energy for deformation is outweighed by a cluster or micelle formation, or the membrane doesn't protect the quantum dots enough. For the first problem, we would need smaller dots. We tried the procedure with uncoated quantum dots, which reduces the size by 1nm without success. Unfortunately, we don't have a protocol yet for dots smaller than 4nm. To address the latter problem we coated the quantum dots with dioctyl sulfosuccinate sodium salt (also known as aerosol-OT or AOT). However, the AOT prohibited any vesicles from forming at all.

To resolve the problem of protecting the dots from water while still allowing vesicles to form, the chemicals 1-butanethiol and 1-octanethiol were used. The quantum dots were coated with 1-butanethiol and 1-octanethiol using W. Chan and Shuming Nie's procedure (8). When the dots coated with 1-butanethiol were used the vesicles fluoresced very brightly, and it is sure the

quantum dots are indeed in the vesicle membrane. As you can see the fluorescence image below (Figure 8) which was exposed 2 seconds is much brighter than the fluorescence image above (Figure 6) that was exposed 60 seconds.

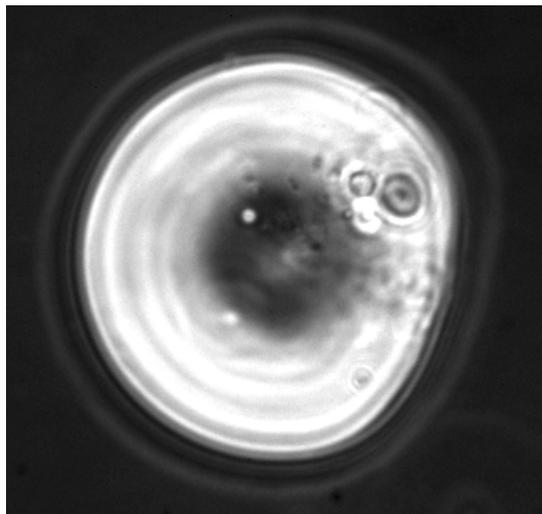


Figure 7: Red light image of vesicle.

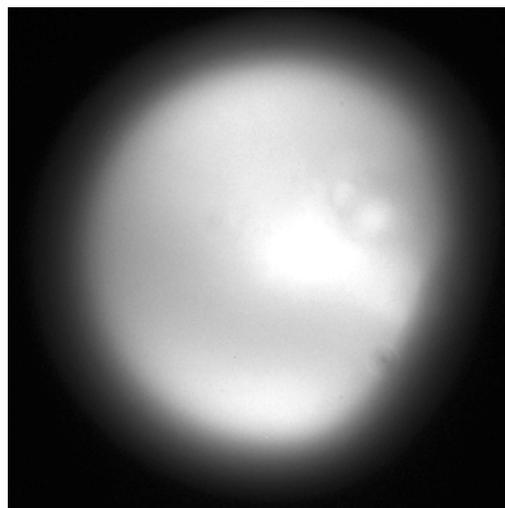


Figure 8: Fluorescence image of vesicle with 1-butanethiol quantum dots. Exposure 1 sec.

However, the vesicles formed were ten times smaller. When 1-octanethiol was used the vesicles fluoresced, but much dimmer than the 1-butanethiol. Further investigation will be required to determine if the quantum dots are being quenched at all, how to maintain their size, and if there is a preferred carbon length chain of the thiol coating. We plan to test 1-ethanethiol, 1-propanethiol, and 1-hexanethiol.

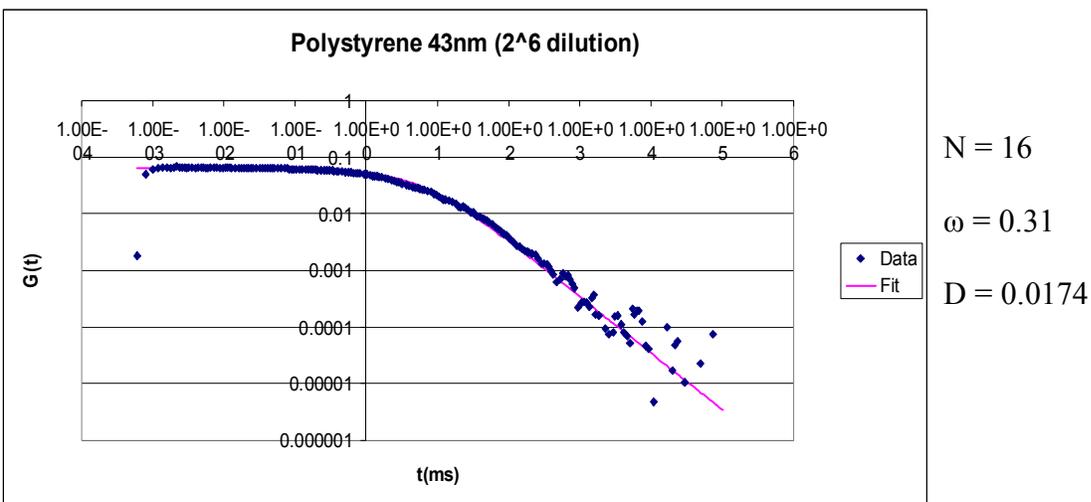
## FLUORESCENCE CORRELATION SPECTROSCOPY

To further understand the properties of our quantum dots, fluorescence correlation spectroscopy was used to determine their radius and diffusion coefficient. Through the Stokes-Einstein relation,  $D = \frac{K_B T}{6\pi\eta R}$ , the diffusion coefficient,  $D$ , of polystyrene spheres with a known diameter can be determined, where  $K_B T$  is the thermal energy,  $\eta$  is the viscosity of the solvent,

and R is the radius of the particles. We used the Lorentzian Intensity Autocorrelation Function,

$$G(\tau) = \frac{\frac{1}{T} \int_0^T I(t)I(t + \tau)dt}{\langle I(t) \rangle^2} = \left( \frac{1}{N} \right) \left( \frac{1}{1 + \frac{D\tau}{\omega^2}} \right)$$

to fit fluorescence correlation spectroscopy data for our argon laser's Gaussian radius,  $\omega$ . In the above equation  $I(t)$  is the intensity as a function of time,  $N$  is the number of particles in the light box,  $D$  is the diffusion coefficient, and  $\tau$  is time.



Ten concentrations of polystyrene spheres were used. The lower concentrations fit the model much better than the larger. The average Gaussian radius (of the lower concentrations) of our light box equaled  $0.26 \mu\text{m}$ . Next, we ran a fluorescence correlation spectroscopy on the quantum dots and fit the data with the Lorentzian, this time solving for the diffusion coefficient. Our average diffusion coefficient was  $0.045 \mu\text{m}^2/\text{ms}$ . Finally we plugged the diffusion coefficient back into the Stokes-Einstein relation and solved for the radius of our quantum dots. Our calculated radius of our hydrophilic quantum dots was  $4.8 \text{ nm}$ .

## **CONCLUSION**

This project is far from over. Quantifying the intensity of the fluorescence along with developing a protocol with the optimal carbon length thiol is the next step. Afterward, seeing the Stark effect requires producing a semi-permeable membrane and a concentration gradient. Finally, the quantum dots would be most advantageous in the biological cell membrane. Hopefully in neuron cells we would be able to track the action potential path by seeing which cells are active from their fluorescence.

Quantum dots have many useful applications, both biological and computational. Making lasers, quantum computers, voltage probes, tracking devices, and fluorescing for switches are just a few of the potential benefits from studying this exciting field.

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