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Biolabeling Through the Use of Water-Soluble Colloidal Quantum Dots

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Abstract: Nanomaterials continues to be a growing field of study due to their wide range of potential applications. Quantum dots are artificially synthesized crystalline clusters of atoms able to confine electron motion as a result of their incredibly small size. Recently, medical applications of nanomaterials have expanded greatly. Quantum dots are ideal for biolabeling due to their rather narrow photoluminescence emission peaks. By synthesizing quantum dots of a specific diameter, it is possible to predetermine the peak photoluminescence wavelength of a sample. Through ligand exchange and immunoconjugation of the quantum dots with proteins, it is possible to use the quantum dots as biolabels to study the inner machinations of the cellular world. These processes have a predictable effect on the properties of the quantum dots: most importantly, their photoluminescence peak wavelength. By understanding the ways in which these processes effect the quantum dots, it is possible to choose the correct quantum dots for a specific final emission wavelength. Further research is being conducted to perform bio-imaging using these processes and resolve some current limitations found therein.

Introduction

Nanomaterials continues to be a growing field of study due to their wide range of potential applications. From solar cells to water filtration systems, the applications are nearly endless due to their unique optical and electronic properties.[1,2] Quantum dots (QDs) are artificially synthesized crystalline clusters of semiconductive atoms able to confine electron motion as a result of their incredibly small size. Because the diameter of a QD is usually between 1-10 nm, the electrons within the nanocrystal experience a quantum confinement which makes the energy states of the electrons lose their continuity and become discrete.[3] The energy band-gap is therefore also quite large. As a result, the absorption and emission of the QD is size-dependent. Recently, medical applications of nanomaterials have expanded greatly.[4] QDs are ideal for biolabeling due to their rather narrow range of photoluminescence (PL). This PL wavelength is important, because it is the signal which will be used during biolabeling.[5] By being able to predict the final PL wavelength, it will be possible to choose the correct size of QDs required for the desired wavelength.

Experimental Section

Quantum Dot Synthesis. Inside a 3-neck flask, lead(II) oxide (PbO), oleic acid (OA), and 1-octadecene (ODE) are heated and magnetically stirred under a nitrogen (N_2) flow for 1 hour. PbO acted as the necessary lead precursor which was decomposed in a hot organic solvent. OA was used to serve as the QD's capping organic ligand, to improve luminescence efficiency and chemical functionality. The N_2 flow purges oxygen from the flask which can impede the necessary reactions. The temperature and OA:Pb molar ratio are determined by the emission wavelength that is desired. While this was heating, a sulfur precursor solution of hexamethyldisilathiane ($(TMS)_2S$) in ODE was prepared inside of a glove box to avoid oxygen contamination. The $(TMS)_2S$ solution was then injected into the 3-neck flask and allowed to react for 1 minute before the reaction solution was rapidly cooled with an ice bath to suppress particle

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growth while still under N_2 flow. The growth solution was then removed from the flask and added to a vial of hexane. At this point, the synthesis was finished and the only remaining task was to remove unreacted precursors and excess OA. The newly formed PbS QDs were precipitated using a minimum amount of methanol and butanol and then centrifuged. The clear supernatant was removed leaving behind only QDs. These were then re-dissolved in an inorganic solvent, such as toluene, and stored.

Ligand Exchange. The QDs were then made water-soluble using a ligand exchange process. This ligand exchange replaced the QDs' native hydrophobic OA ligand with hydrophilic molecules, which have a higher affinity for the sulfur on the QD surface. Water-solubility is necessary for the QDs to be introduced to organic samples since water is the most common organic solvent. QDs suspended in toluene were mixed with sodium hydroxide (NaOH) solution and a solution of monothiol ligands. Both 11-mercaptoundecanoic acid (MUA) and 8-mercaptooctanoic acid (MOA) were used.[6] It has been found that a 1:100 Pb:thiol molar ratio is ideal for this process. Too few thiol ligands prevent the QDs from becoming suspended colloidally, while too many turns the entire solution into a gel-like substance due to an overabundance of ligands cross-linking the entire solution. This mixture was stirred with a vortex mixture for approximately one minute to ensure a homogenous distribution. Sodium hydroxide (NaOH) was then added to maintain a basic pH level. The NaOH turns all of the free carboxylic acid groups into their salt forms (COO^-Na^+) to enhance stability and solubility of the thiol-capped QDs in water.[7] If the pH at the nanocrystal-ligand interface decreases below a certain value, the ligands are protonated and detach from the nanocrystals. This would destroy the nanocrystal-ligand complex, destabilizing the colloidal system and causing possible cytotoxic issues.[8] A 1 molar NaOH solution was used to minimize the amount of NaOH required, while still maintaining a pH above the necessary level. This solution was once again stirred in the vortex mixer for another minute to create a homogeneous distribution of QDs, thiol ligands, and NaOH. A centrifuge was then used to separate the now water-soluble QDs (WDs) from the residual byproducts.

Protein Attachment. The next step was to attach the QDs to proteins for the purpose of biolabeling.[9] For this portion of the experiment, bovine serum albumin (BSA) was used as the immunoconjugate and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrocholoride (EDC) was used to link the MUA and the BSA. The EDC solution activates the carboxylic acid groups on the end of the thiol ligands, forming covalent amide linkages with the amine groups of the BSA protein surface.[10] After adding the BSA and EDC solutions to the WDs, the vortex mixer was again used to create a homogenous solution. This final solution must then be allowed to sit undisturbed for a minimum of two hours to allow the intended reaction time to happen. For storage purposes, it is critical to keep the BSA conjugated quantum dots (BSA-QDs) refrigerated due to the fragile organic nature of the resulting molecules.

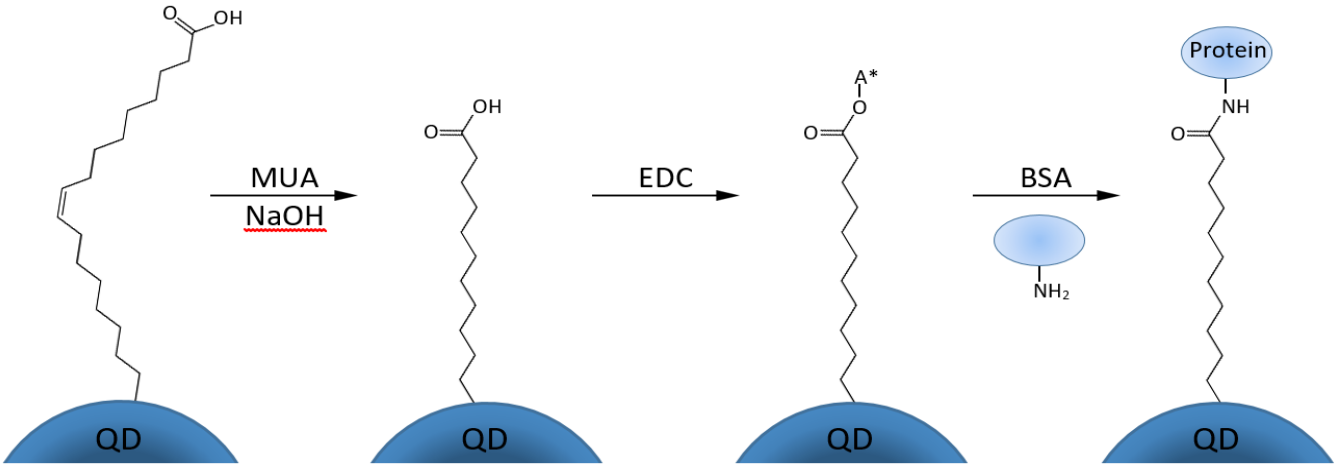

Figure 1. Schematic of a sequence of ligand exchange and protein attachment steps employed in the study.

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Results

Photoluminescence Spectroscopy. The different samples were excited with a high power laser. The QDs absorb energy which is then in turn released within the QDs' narrow emission band gap.[11] The peak emission wavelengths of the sample were found to experience a redshift at each stage of the process (Figure 2). Average redshifts of 105 nm and 20 nm were measured for the ligand exchange and protein attachment processes, respectively when using MUA. For MOA-capped QDs, the ligand exchange and protein attachment processes resulted in average redshifts of 96 nm and 76 nm, respectively.

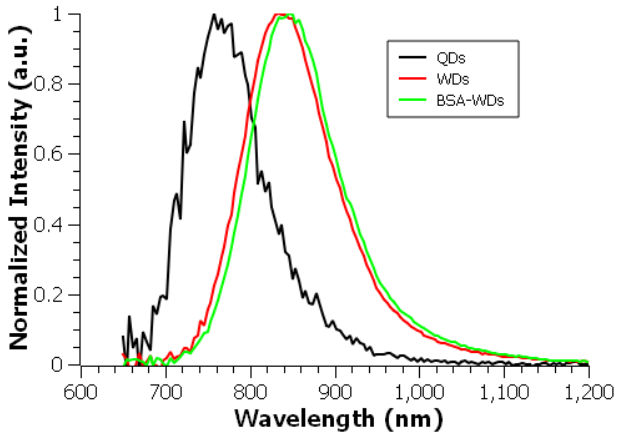

Figure 2: Normalized PL measurements taken throughout the process for a single sample of QDs utilizing MUA as the thiol ligand.

Photoluminescence Lifetime. After ligand exchange, measurements were taken to determine the lifetime of the fluorescence of the WDs. Decays of QDs MUA- and MOA-capped WDs are shown in Figure 3. The decays cannot be modeled by a single exponential function. A sum of two exponential-decay terms fits the data well. Biexponential decay of fluorescence from QDs has been observed previously. The first term has a time constant of approximately 200 ns and the second has a time constant between 20 and 40 ns. This data compares quite favorably with other QD fluorescence lifetimes observed, signifying that these WDs will indeed have a long enough signal lifetime to act as reliable biolabels.

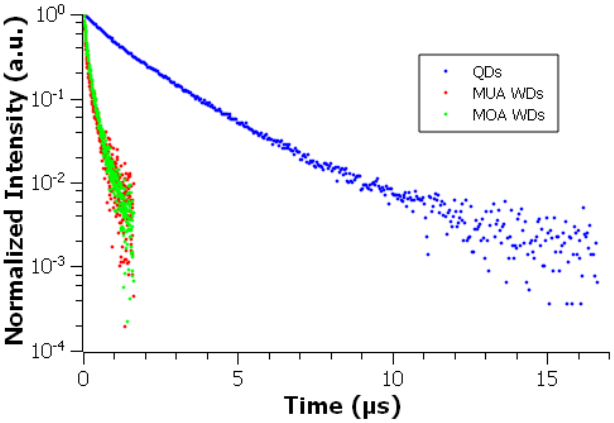

Figure 3: Normalized lifetime data of QDs, MUA WDs, and MOA WDs.

TABLE 1: Parameters of Fits to Fluorescence Decays [a]

	A_1	T_1 (ns)	A_2	T_2 (μs)
QDs	0.0099	714	0.015	2.0
MUA WDs	0.0060	33	0.0012	0.2
MOA WDs	0.010	42	0.0043	0.2

[a] Decays are fit to the function $A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$.

Scanning Electron Microscopy. The BSA-QDs immunoconjugates were introduced to an organic substrate of glutaraldehyde, $CH_2(CH_2CHO)_2$. This substrate allowed a scanning electron microscope (SEM) to be used to test the success of the BSA-QDs' biolabeling applications. By using the backscatter electron detector, an SEM image could be formed showing the lead in the QDs as bright spots (Figure 4).

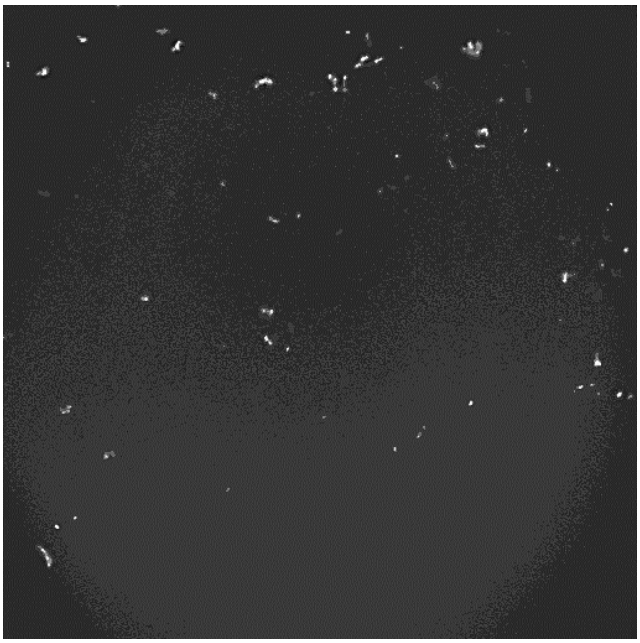

Figure 4: Preliminary SEM image shows some BSA-QDs on a glutaraldehyde substrate.

Another sample was then used to take more PL measurements to independently confirm the presence

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of the BSA-QDs by searching for an emission peak (Figure 5).

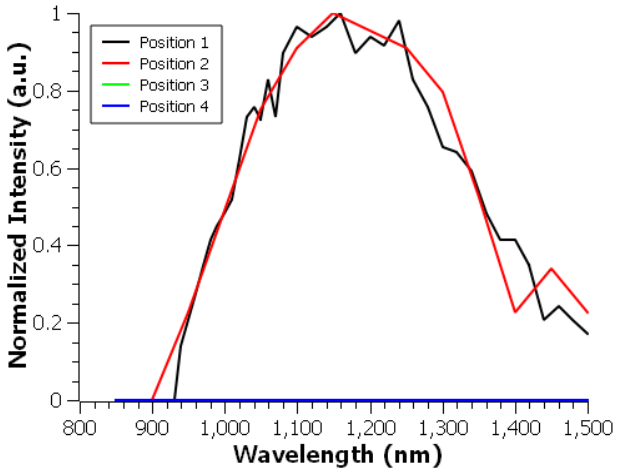

Figure 5: Normalized PL data showing the signal of BSA-QDs on a glutaraldehyde substrate. (Position 1) was located on the border of the BSA-QDs and a section of glutaraldehyde. (Position 2) was located amidst the BSA-QDs. (Position 3) was amidst the glutaraldehyde. And (Position 4) was on the wafer only.

Conclusion

I have presented a simple and rapid protocol for transferring PbS QDs into water and then conjugating proteins to the WDs to aid in biolabeling. Unfortunately for biological research, QDs are commonly composed of materials like PbS and cadmium sulfide (CdS) which are potentially toxic and environmentally hazardous.[12] There are two options for how to tackle this issue. One, is to begin using core-shell QDs with CdS at the core, wrapped in a protective zinc sulfide (ZnS) shell instead of the PbS QDs already used. These CdS-ZnS core-shell QDs would not only be nontoxic, but the core-shell structure would also increase quantum confinement, therefore shrinking the PL peak wavelength of the initial QDs allowing for a much broader spectrum of visibly emissive BSA-QDs. This method could also be extrapolated to use core-shell-shell QDs with PbS cores wrapped in a layer of CdS and then another layer of ZnS. These PbS-CdS-ZnS core-shell-shell QDs would have the same beneficial qualities as the CdS-ZnS core-shell QDs, but may also have some very novel applications due to their unique composition and structure. The second option, is to begin using carbon dots (or C-dots) in place of the PbS QDs. Carbon is non-toxic and would be the perfect material for *in vivo* biolabeling.[13,14] Also, recent research indicates that two-photon excitation using near-infrared light can be used to make C-dots fluoresce in the visible range (Cao *et al.*, 2007).[15] Because the excitation wavelength is further removed from the emission wavelength range, more precise imaging and data will be possible. These results are promising for more than biolabeling applications. Were this to be applied to nanosheets, it may be possible to perform electrolysis of the water solvent itself. Further research is being conducted to perform successful conjugation of BSA-QDs into a living cell, improve upon the current SEM images, as well as pursue the previously mentioned options for improvement.

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