ORIGINAL ARTICLE

# Enhancing silicon quantum dot uptake by pancreatic cancer cells via pluronic® encapsulation and antibody targeting

**Jasmine L. May [1, 3], Folarin Erogbogbo [1], Ken-Tye Yong [4], Hong Ding [1], Wing-Cheung Law [1], Mark T. Swihart [1, 2], Paras N. Prasad [1, 3]**

1. Institute for Lasers, Photonics and Biophotonics. 2. Department of Chemical and Biological Engineering. 3. Department of Chemistry, University at Buffalo (SUNY), Buffalo, New York 14260-4200, United States. 4. School of Electrical and Electronic Engineering, Nanyang Technological University, Singapore.

**Correspondence:** Paras N. Prasad. Address: Department of Chemistry, University at Buffalo (SUNY), Buffalo, New York 14260-4200, United States. Telephone: 1-716-645-4148. Email: pnprasad@buffalo.edu

**Received:** March 2, 2012 **Accepted:** April 24, 2012 **Published:** June 1, 2012
**DOI:** 10.5430/jst.v2n3p24 **URL:** http://dx.doi.org/10.5430/jst.v2n3p24

## Abstract

**Objectives:** Silicon quantum dots (SiQDs) are of great interest for bio-imaging applications due to their tunable luminescence, low toxicity, unique surface chemistry, and high quantum yield. Most synthesis routes produce SiQDs that are not water-dispersible, making them unattractive for biological applications. Here, we show that Pluronic® block copolymers can encapsulate SiQDs to make them water dispersible and suitable for cancer imaging applications. **Methods:** Transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential, and temperature and pH stability measurements were used to evaluate these Pluronic®-encapsulated SiQDs (PSiQDs). The particles were also tested in targeted in vitro imaging and in vivo bio-distribution experiments.

**Results:** Encapsulation with Pluronic® polymers renders the SiQDs water dispersible, preserves their optical properties, protects them from oxidation, and prevents aggregation. Surface modification of the PSiQDs with pancreatic cancer targeting moieties, anti-claudin-4 and anti-mesothelin, led to enhanced uptake of these nanoconstructs in comparison to PSiQDs modified with folate as the targeting moiety.

**Conclusions:** The particles are stable at biological conditions, and show promise for targeted diagnostic applications without possessing elementally toxic components.

### Key words

Silicon quantum dots, Bioimaging, Pluronic®, Micelle encapsulation, Antibody

## Introduction

Research on quantum dot technology for biological applications aims to produce optical imaging agents that are useful for labeling, monitoring, and delivery [1, 2]. The unique advantages of QDs for these applications include bright luminescence, tunable emission spectra, broad excitation, and photostability [1-4]. Significant research contributions have been made towards the development [5-10] and biological applications [11-16] of QDs that are made from combinations of elements in

group II and VI of the periodic table (e.g. CdSe). Even though many successful demonstrations of the use of QDs in biological applications have been published, the elemental toxicity of heavy metal components of these QDs has raised concerns about their future clinical applicability [17-20]. Multiple approaches are being used to alleviate concerns related to releasing cadmium ions or other heavy metals, but extensive investigations are still required to fully understand QD toxicity [20, 21]. This has created a demand for non-heavy-metal-based QDs made from elements such as silicon; such QDs are much less toxic than Cd-based QDs [22]. In addition to the low elemental toxicity of silicon, its potential biodegradability to silicic acid, its abundance and low cost, and promising results of recent bio-imaging investigations indicate that SiQDs should be further investigated for biomedical applications [22-25]. Sailor's group has demonstrated passive tumor uptake with porous silicon; however, the structure of those particles differs from the free-standing SiQDs considered here. It has also been demonstrated that properly encapsulated SiQDs can be used for active targeting of cancer cells [26] and tumors in vivo [22]. A few other studies have demonstrated in vitro imaging [27]. Kauzlaurch has shown water dispersible SiQDs can be doped with Mn and used for multimodal imaging of macrophages [27]. The emission spectrum of their silicon, however, was limited to the blue region of the spectrum, peaking at 441nm. He et al. showed that HEK2-293T cells can take up acrylic acid terminated SiQDs [28]. Even though some biological applications have been demonstrated, synthesizing water dispersible SiQDs remains a challenge and a subject of ongoing investigation [29]. Water dispersible SiQDs with narrow emission spectra (FWHM of ~ 60nm) have been synthesized by Kang et al. [30]. Their method involves the oxidation of small particles, which can be difficult to control and reproduce. The Veinot group recently investigated the effect of organic chain length on water-dispersible SiQD surfaces to improve the understanding of SiQD properties [29].

Addressing the fundamental surface coating issue to enable biomedical application of SiQDs is important for a number of reasons: 1) The surface coatings affect the luminescence of particles; the ideal surface coating will maintain optical properties. 2) The coatings provide functional groups for conjugation reactions; however, amine groups may quench SiQD luminescence, so simple methods used for creating amine termination on other QDs may result in degraded optical properties. 3) Silicon is biodegradable, and different coatings will have differing effects on biodegradation rates.

Therefore, the main question being addressed in this article is, "What is the best surfactant, based upon the criteria given above, for utilizing SiQDs in medical applications?" Here, we present block copolymer coated SiQDs, (PSiQDs) that maintain the optical properties of SiQDs while providing reactivity needed for bio-conjugation. These are evaluated in targeted cancer cell imaging applications. Pluronic® block copolymers are used because of their biocompatibility, versatility as delivery vehicles for chemotherapeutic drugs and MRI agents, and their observed minimal effect on the optical properties of QDs.

# Materials and methods

## Materials

Silane ($SiH_4$, Voltaix, electronic grade, 99.999%), hydrofluoric acid (HF, Acros Organic, 48-51%), nitric acid ($HNO_3$, EMD, 68%-70%), ethyl undecylenate (Acros Organic, 99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Pierce Biotechnology, 98%-100%), anti-mesothelin (Clone 5B2, dilution 1:20; Novocastra, Newcastle Upon Tyne, United Kingdom), anti-claudin-4 (0.5mg/mL Invitrogen, Carlsbad, California), folic acid (Sigma-Aldrich, >98%), dimethyl sulfoxide (DMSO, Fisher Scientific, 99.9%), N-hydroxysulfosuccinimide (Sulfo-NHS, Fluka, ≥98.5%), and Pluronic® F127 NF Prill Surfactant (BASF Corporation) were all used as received if not otherwise noted.

## Preparation of silicon nanocrystals

A fine powder of non-luminescent silicon nanocrystals was formed through high temperature $CO_2$ laser pyrolysis of silane ($SiH_4$) gas, a method developed and described in detail previously [31]. The resulting product was collected without

exposure to the air in a glove box to prevent any oxidation of the particle surfaces. Primary particle diameters in the aggregated powder averaged about 10nm.

## Preparation of photoluminescent SiQDs

The non-luminescent particles were dispersed in methanol, 300mg per 30mL, using sonication. Then a 10:1 acid mixture, hydrofluoric acid (48 wt%, 100mL) and nitric acid (69 wt%, 10mL), was added to the SiQD suspension and the mixture was stirred for about 2 to 4 min. At the end of this mixing step, the particles were luminescent, but emitted at a wavelength longer than the desired wavelength. The solution was then added to 400mL of methanol to quench the reaction. A slight blue shift of the luminescence was observed during this quenching step. The SiQDs were then collected on a poly (vinylidene fluoride) (PVDF) membrane filter using a pressure filtration device (Millipore, 142mm diameter). The etched particles were washed four times with a water-methanol mixture (3/1, v/v), (500mL, 500mL, 50mL) to remove acid residue. Lastly, the particles were rinsed four times with 50mL of methanol. The filters upon which the particles were collected were sonicated in a vial containing ethyl undecylenate to remove the particles from the filter. The entire etching process was conducted in a glove box under a nitrogen atmosphere to prevent oxidation. This helps maintain the photoluminescence quantum yield (QY). This procedure is illustrated in Figure 1(A).

## Functionalization

The cloudy dispersion of freshly-etched SiQDs in 20mL of ethyl undecylenate was placed into a vial containing a stir bar, which was then placed in a UV reactor under 254nm illumination. The double bond of the ethyl undecylenate reacts with the hydrogen-terminated surface of the SiQDs resulting in a Si-C bond. After reaction, the particles form stable dispersions in nonpolar solvents like chloroform, hexane, and toluene. The solution goes from cloudy to optically clear as the reaction goes to completion. The SiQDs were collected by centrifugation at 10,000 rpm.

## Preparation of F127COOH

The triblock copolymer (F127) was modified to have carboxylic acid termination using published methods [32-34]. To a solution of toluene (100mL), pyridine (5mL), and F127 (25.0g, 1.95mmoL), maleic anhydride (1.93g, 19.5mmoL) was added. The resulting solution was stirred for 8 hr at room temperature under nitrogen. The product was then purified by precipitation from excess diethyl ether thrice. Finally 21.3g (85% yield) of F127-COOH, a light-brown solid, was obtained [32-34].

## Micelle encapsulation

The SiQDs and the modified Pluronic® F127 were each suspended in chloroform and mixed. The chloroform was then removed in a rotary evaporator, leaving a film of SiQD/Pluronic® on the flask wall. HPLC water was added and the dispersion was sonicated to encapsulate the nonpolar SiQDs within micelles formed by the the amphiphilic Pluronic® polymer. We used a 1:20 mass ratio of SiQDs: Pluronic® at a total (Silicon plus Pluronic®) concentration of ~5mg/mL for all experiments described below.

## Bioconjugation

In separate experiments, the PSiQDs were conjugated to folic acid, anti-mesothelin, and anti-claudin-4. An EDC-coupling reaction was used to form an amide bond between the carboxyl groups on the PSiQDs, at the end of the hydrophilic segment of the Pluronic®, and a primary amine group on these reactants. Along with EDC, sulfo-NHS was used to stabilize the reactive intermediate of the EDC reaction, which is prone to react with water to re-form the carboxyl group. To conjugate the PSiQDs to anti-mesothelin or anti-claudin-4, we started from 1mL of the PSiQD sample. 8mg/mL solutions of EDC and sulfo-NHS were freshly made for each experiment using HPLC water. HPLC water solutions of anti-mesothelin and anti-claudin-4 were made at 2mg/mL (1.6mg of biomolecule in 0.8mL), and a DMSO solution of folic acid was made at 0.12mg of folic acid in 60μL. Then, 40μL of EDC was added to the PSiQD solution and stirred for 1 min. Then 40μL of sulfo-NHS was added while the stirring continued for 1hr. These steps were conducted at room temperature. Then the PSiQDs were pelleted out of the solution by adding 1mL of methanol and centrifuging at 10,000g for 15min. This

removed the EDC and sulfo-NHS. The particles were then redispersed in water and conjugated to folic acid or other targeted molecules which were added to the dispersion. The particles were washed with water and collected by centrifuging the sample three times.

## Cellular imaging

The bio-conjugated samples were imaged with human pancreatic cancer cells (Panc-1) which were maintained in DMEM medium, with 10% fetal bovine serum (FBS) and appropriate antibiotics. One day before treatment, cells were seeded in 35mm cell culture dishes. The next day, the cells, at a confluency of 70%-80%, were incubated with bio-conjugated SiQDs at 37ºC for two hours. Then, the cells were washed with PBS three times and imaged using a Leica laser scanning confocal microscope. The excitation wavelength used was the shortest available wavelength on the microscope, 405nm.

## Organ imaging

The animal was housed at 24ºC and was exposed to 12 hour light/dark cycles. He was fed ab libitum with water and standard laboratory feed. Animal experiments were conducted in compliance with the guidelines set by the University at Buffalo (SUNY) IACUC. The animal was acclimated to the animal facility at least 48 hours before the experiment was conducted. A Balb C mouse was injected intravenously with the micelle encapsulated SiQD formulation. After 24 hours the mouse was sacrificed and dissected. The heart, liver, spleen, lungs, kidneys, and brain were removed. These organs were then imaged with the Maestro in vivo optical imaging system (CRI, Inc., Woburn, MA) using blue excitation (with a 488nm short-pass filter), and a 580nm long-pass emission filter.

## SiQDs characterization

The silicon nanoparticles were characterized by transmission electron microscopy (TEM, JEOL 2010) before and after encapsulation. Photoluminescence spectra (Fluorescence spectra) were recorded with a Fluorolog-3 Spectrofluorometer (Jobin Yvon; fluorescence spectra). The excitation wavelength was set to 350nm and the emission cutoff filter was set to 400nm. In order to determine the QY, the PSiQDs dispersion emission was compared to that of rhodamine 6G dye solutions of matched absorbance. The hydrodynamic diameter was measured by dynamic light scattering (BIC, 90Plus). The zeta potential was measured with a BI-ZEL Electrode Assembly for aqueous and polar lipids (BIC 90Plus). The uptake of bioconjugated SiQDs into pancreatic cancer cells was confirmed by laser confocal imaging (Leica Microsystems Semiconductor GmbH, Wetzlerm Germany, laser excitation at 405nm).Temperature and pH studies were conducted using the Fluorolog-3 Spectrofluorometer mentioned above.

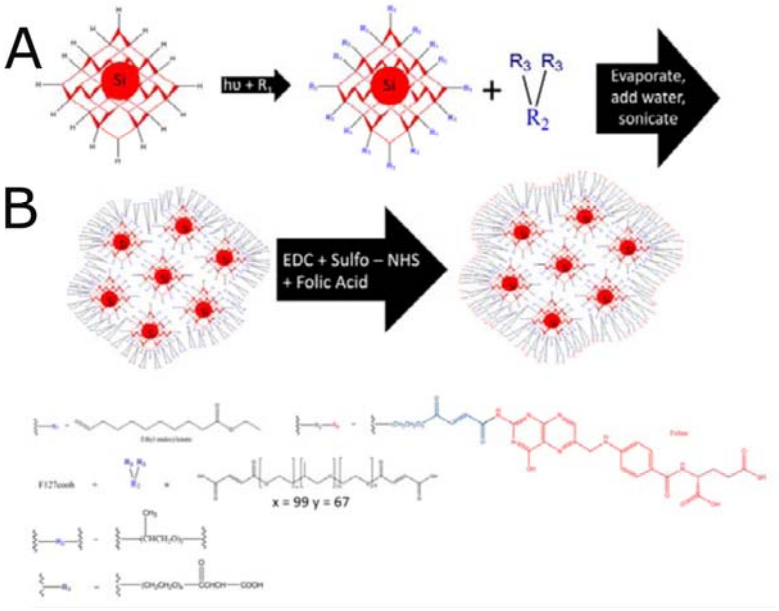


**Figure 1.** Schematic of SiQDs plus Pluronic formulation. A: Hydrophobic SiQDs coated with ethyl undecylenate (covalently bound to the SiQD surface) were encapsulated with pluronic block copolymer F127COOH. B: Folic acid was bioconjugated to the surface of the micelle.

## Results

Transmission electron microscopy (TEM) imaging of red-emitting (650nm peak wavelength) SiQDs cast from organic media [Figure 2(A)] shows that the average size of these functionalized QDs is about 5nm. The blue circle highlights lattice fringes that show the crystallinity of the SiQDs. The lighter shade that can be seen around the dark core is due to the organic layer formed by functionalizing the QD with ethyl undecylenate. TEM images of PSiQDs cast from aqueous media are shown in Figure 2(B). These are the same SiQDs that can be seen in Figure 2(A), except they are now within micelles formed by F127COOH. The micelles are formed by the interaction of the ethyl undecylenate layer around the SiQDs and the poly (oxypropylene) (PPO) section of the F127COOH. As a result the poly (oxyethylene) (PEO) and carboxylate groups at the ends of the F127COOH are pushed to the outside of the micelle, forming a hydrophilic shell and making the micelles dispersible in water and other polar solvents. The size of the micelles ranges from 20-200nm, and the number of SiQDs encapsulated within each micelle varies with the overall size. At the expense of reduced yield, the dispersions can be filtered to achieve sizes as low as 20nm. Upon close observation of the low resolution TEM image [Figure 1(B), left panel)] small micelles within this range can be observed.

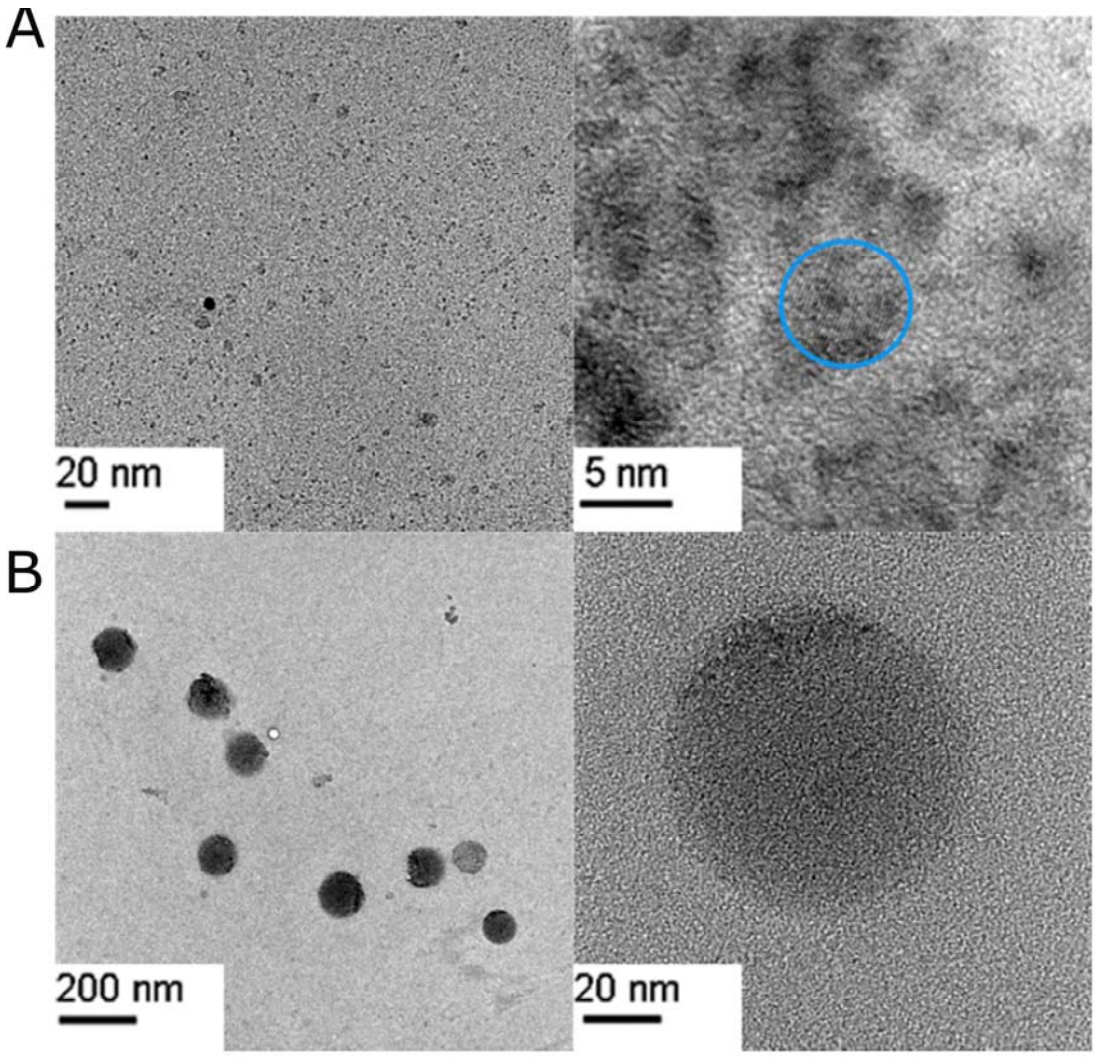


**Figure 2.** TEM images of SiQDs before A: and after B: encapsulation with Pluronic® F-127COOH. To demonstrate the hydrophilicity of the particles, a sample of PSiQD suspended in HPLC water was added to a vial containing only chloroform. When exposed to 350nm UV [Figure 3(A)] only the aqueous phase shows luminescence.

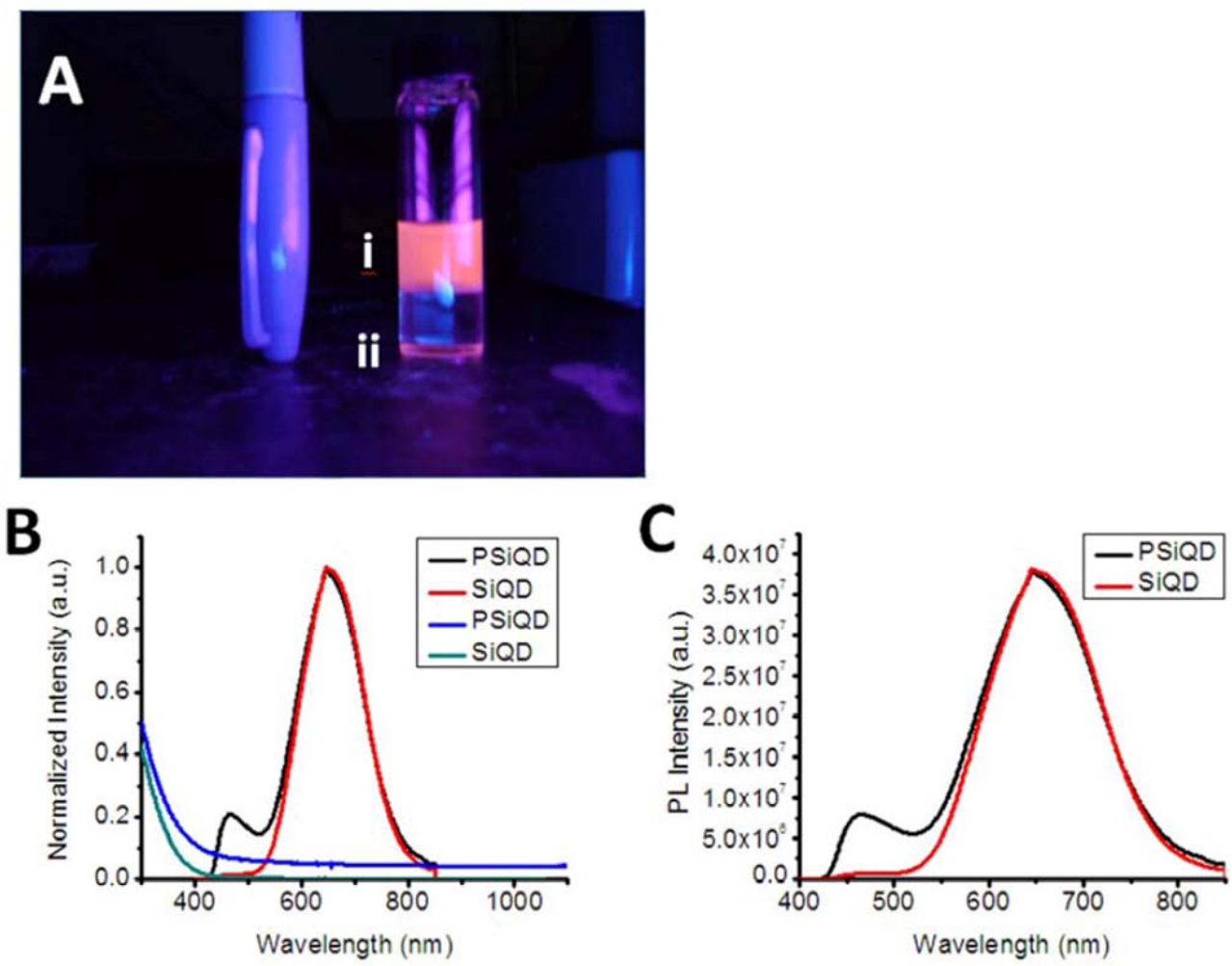


**Figure 3.** Photograph of sample and photoluminescence and absorbance spectra. A: PSiQDs suspended in (i) HPLC water, above (ii) chloroform; B: Normalized UV-vis absorption (blue and green) and PL emission (red and black) spectra, in chloroform (before encapsulation) and in water (after encapsulation) C: PL emission spectra (350nm excitation) unnormalized, of red-emitting SiQDs. Each sample contains 1mg of SiQDs in 3ml solvent. The optical properties of these SiQDs were examined by UV-vis absorbance and photoluminescence spectroscopy. Figure 3(C) shows the unnormalized PL spectra of the same samples shown in Figure 3(B).

The hydrodynamic diameter and zeta potential of PSiQDs were measured by dynamic light scattering. The average size of the original sample was 175nm and the zeta potential was -37mV [Figure 4(A)]. Upon dilution, the size of the micelles decreased slightly, down to 168nm after 100x dilution with water and 163nm after 100x dilution with saline solution. Note that after this dilution, the pluronic concentration is decreased from about $3.9 \times 10^{-4}$ M to $3.9 \times 10^{-6}$ M. The latter value is much closer to the critical micelle concentration (CMC), which according to the literature is $4.7 \times 10^{-7}$ M, but it is not below it [32]. The zeta potential became more positive upon dilution, -30mV in 100x water and ~ 0mV in 100x NaCl. The average hydrodynamic diameter could be reduced to about around 20nm by filtration with a membrane with 200nm nominal pore diameter and centrifugation.

The PL of a PSiQD sample was then measured under varying conditions of pH and temperature. The pH of the PSiQD sample was changed by the addition of HCl or NaOH. For pH values from 2 to 9, the intensity remained rather stable [Figure 4(B)] at about 80% of its highest intensity, obtained at pH 1. The intensity dropped dramatically to 40% of the pH

1 intensity at pH 10, to 20% at pH 11 and essentially disappeared at pH 12. The PL spectrum of the emission observed at pH 13 was different from that that at lower pH, suggesting that it did not come from the PSiQDs. Also, at pH 13 a white precipitate became visible after ~1 hour. The PL intensity of the sample was nearly constant with increasing temperature [Figure 4(C)]. Despite some irregularities in the overall trend, these results show that the intensity stays above 70% of its value at ambient conditions under physiological conditions.

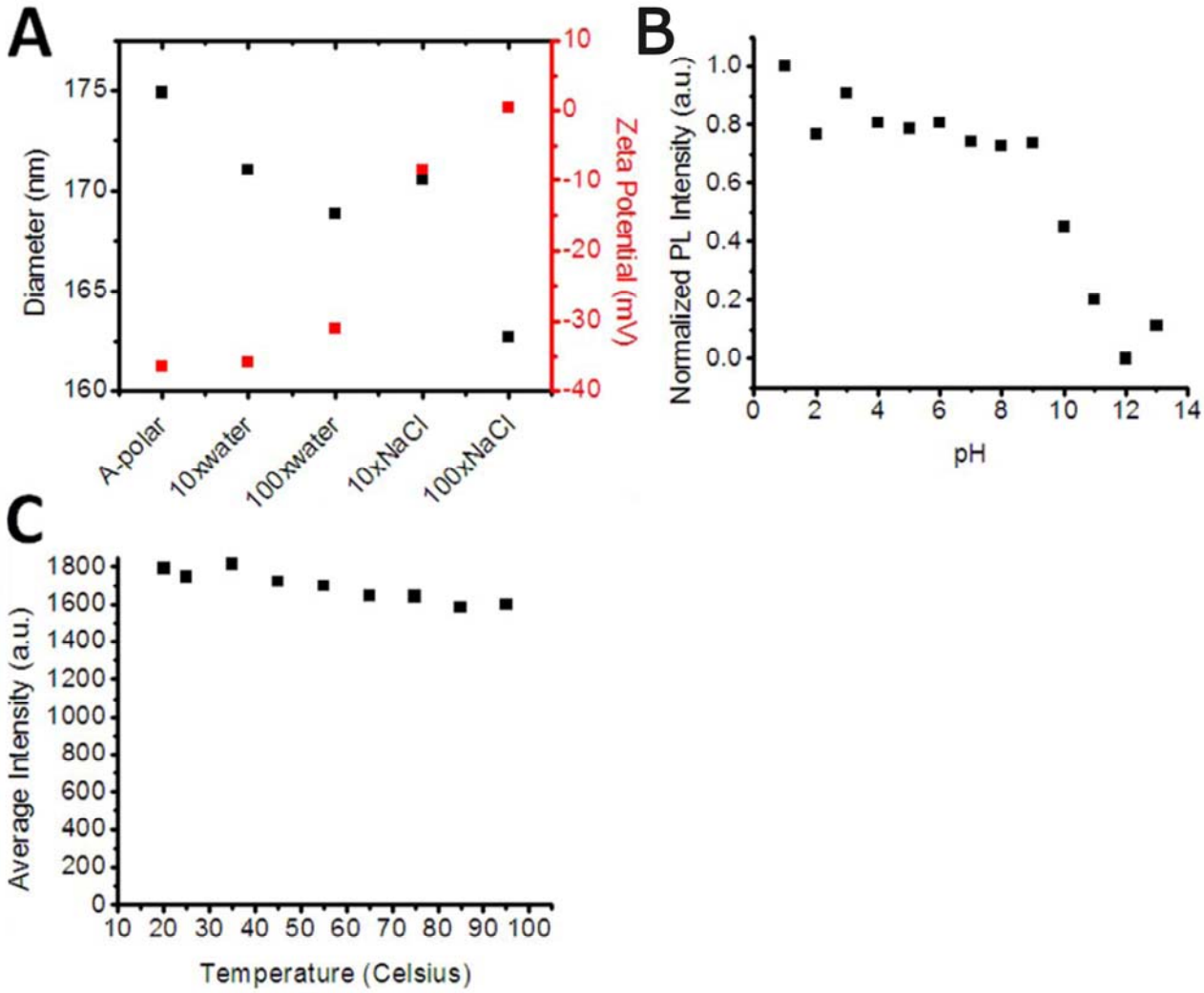


**Figure 4.** Physiological analysis results. A: Zeta potential and the effective hydrodynamic diameter of several different dilutions of a polar red-emitting SiQD sample. A-polar is the as-prepared PSiQDs, 10x water is the A-polar sample diluted 10x with water, others are A-polar diluted 100x with water (100xwater), A-polar diluted 10x with 10 mM NaCl (10x NaCl), A-polar diluted 100x with 10mM NaCl (100x NaCl). B: PL intensity as a function of pH. C: PL intensity as a function of temperature

Laser confocal imaging (Figure 5) of Panc-1 cells incubated with folate-conjugated PSiQDs showed clear luminescence from the SiQDs however it was sparsely distributed. In order to test the uptake method the Panc-1 cells were saturated with 3.5mM folic acid (FA) solution before adding the folate conjugated SiQDs. Due to competitive inhibition very little uptake of the particles was seen within the cells.

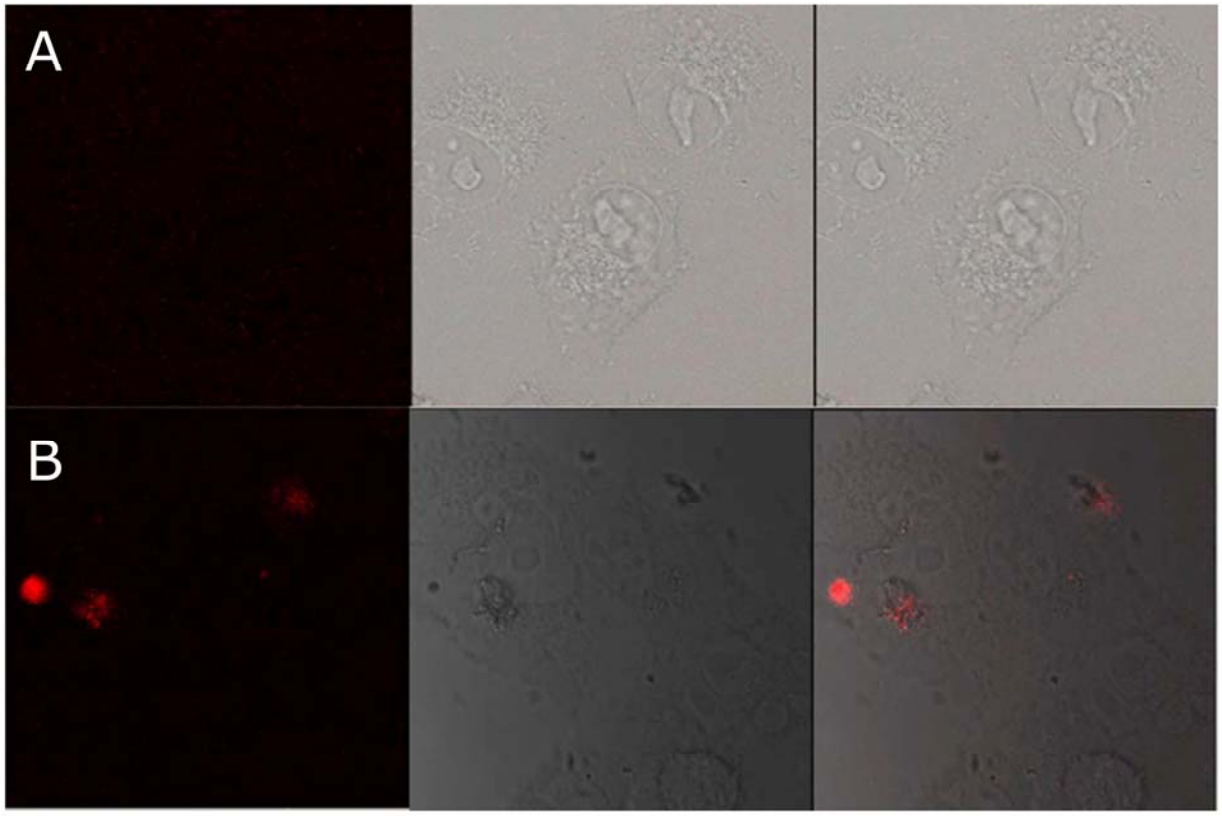


**Figure 5.** Confocal microscopy of pancreatic cancer cells treated with folate conjugated red-emitting SiQDs. Left Panel: Fluorescence; Middle Panel: Transmission; Right Panel: Overlay A: Cells after FA saturation with no uptake B: Cells with FA-conjugated PSiQDs showing some uptake

The confocal images shown in Figure 6 show the targeting effect of bioconjugated micelle encapsulated SiQDs. With both anti-mesothelin [Figure 6(B)] and anti-claudin-4 [Figure 6(C)] there is a high concentration of the luminescent SiQDs on the surface of the cells, thereby labeling them. In the control images, no luminescence can be seen. This confirms that labeling occurs through receptor-mediated attachment.

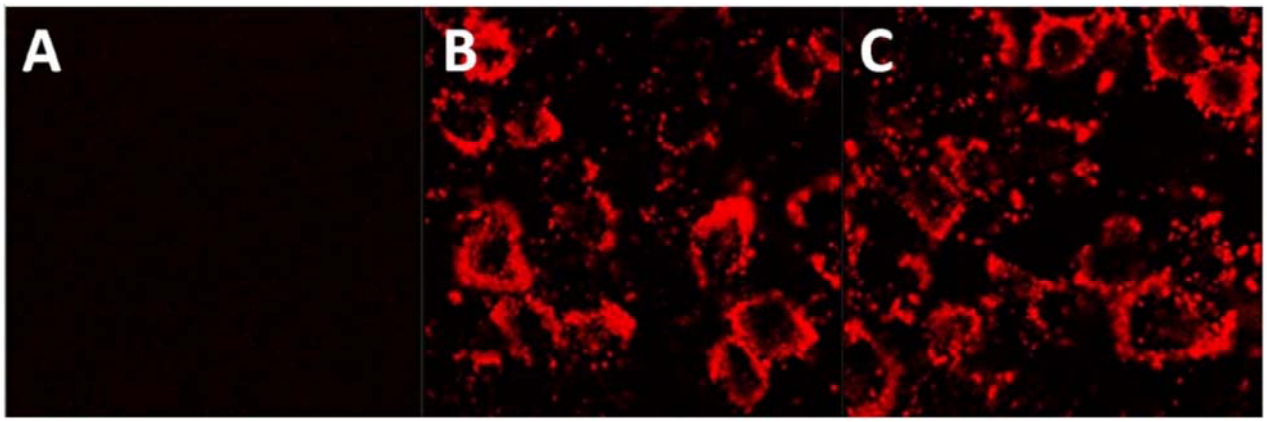


**Figure 6.** Confocal microscopy images of pancreatic cancer cells treated with antibody-conjugated PSiQDs. A: Control - unconjugated. B: Anti-mesothelin conjugated, and C: Anti-claudin-4 conjugated red-emitting SiQDs

In addition to the in vitro imaging the micelle encapsulated SiQDs were injected intravenously into a Balb C mouse which was then sacrificed after 24 hours. The mouse was dissected and the organs were imaged to observe where the SiQDs were concentrating in the body (Figure 7). The particles concentrated preferentially in the spleen with some seen in the liver. The particles are not seen in the heart, lungs, kidneys, or the brain.

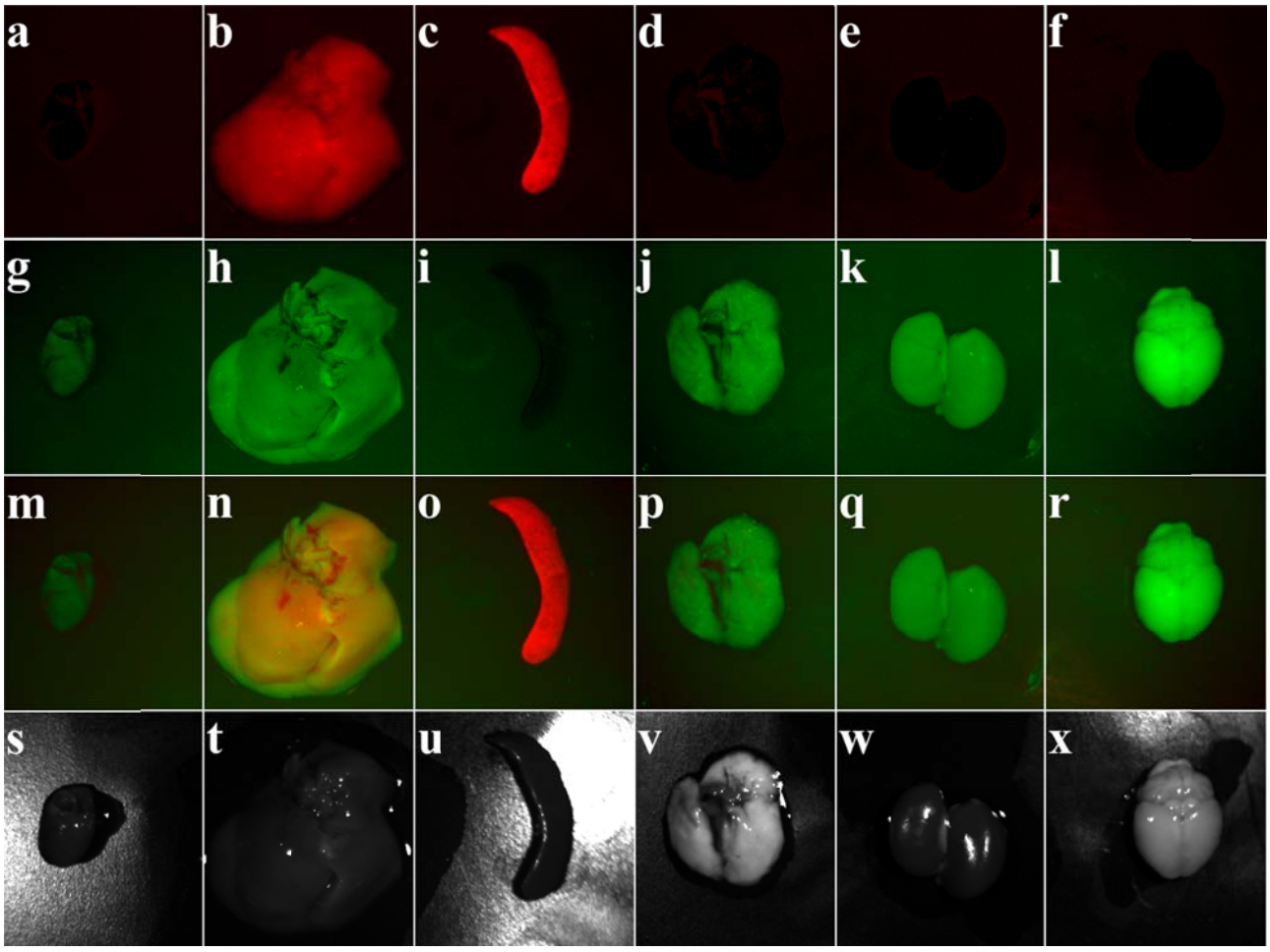


**Figure 7.** Images of organs of a Balb C mouse 24 hours after injection of the PSiQDs. A, G, M, S: Heart; B, H, N, T: Liver; C, I, O, U: Spleen; D, J, P, V: Lungs; E, K, Q, W: Kidneys; F, L, R, X: Brain. Top Row: Fluorescence; Second Row: Autofluorescence; Third Row: Overlay; Fourth Row: White light picture

## Discussion

SiQDs were investigated for use as nontoxic functional imaging agents in cancer diagnostics due to their low toxicity, low cost, high quantum yield, and biodegradability. A biocompatible polymer was used to encapsulate them, and the formed micelles were tested for their dispersibility in water, and the stability of the SiQD luminescence. The biocompatible polymer was terminated with carboxyl (COOH) groups, allowing for bioconjugation to targeting moieties.

One major limitation was finding a biocompatible polymer that could effectively encapsulate the Si QDs, making them water dispersible, but that did not quench their luminescence. Also, the protective coating must be stable enough to prevent any oxidation of the SiQDs that can cause a blue shift in their luminescence. This would decrease their visibility in a biological system, due to omnipresent green autofluorescence.

Thus the main question is how to stabilize the SiQDs in an aqueous solution, and make them biocompatible while maintaining their optical properties. To encapsulate the SiQDs, they are suspended in chloroform with the Pluronic® and stirred. After the chloroform is removed, the Pluronic® SiQD film is redispersed in HPLC water. Polymer micelles are formed due to hydrophobic and hydrophilic interactions between the water, the hydrophobic particles, and the amphiphilic Pluronic®.

The TEM images of the particles before and after encapsulation confirmed the presence of the particles within the Pluronic® The micelle diameters observed in TEM ranged from 20-150nm, a range that has previously been reported to be appropriate for use in vivo[22, 26]. Thus, the Pluronic® effectively encapsulated the particles in micelles within a reasonable size range.

The suspension test is another experiment done in order to assess how effectively the Pluronic® encapsulated the particles. Upon excitation of the particles, any non-encapsulated SiQDs that were only dispersible in organic solvent would have luminesced in solution (ii). Since all of the luminescence is observed in the top solution (i), the aqueous solution, we conclude that all of the particles have been encapsulated. Hydrophilicity is an important step in making SiQDs more biocompatibile and thus useful as in vivo imaging agents.

The absorbance and PL spectra of red-emitting SiQDs before encapsulation and after [Figure 3(B)] show little difference. These spectra are similar to those seen in other studies [35]. The slight increase in apparent absorbance from the PSiQDS can be attributed to scattering from the micelles. The expected structure of the PL spectra can be seen, with a peak at 645 nm. Figures 3(B) and Figure 3(C) show that the encapsulation process does not cause a shift of the PL spectra, nor does it decrease the intensity. Therefore, we conclude that the encapsulation process does not alter the optical properties of the SiQDs. The QY of the particles is estimated to be 25%. This is a great improvement over previously report QY values for SiQDs [36].

DLS measurements of hydrodynamic diameter were generally consistent with observations from the TEM images. The average hydrodynamic diameter of 175nm is somewhat larger than that observed in TEM. However, in the high vacuum environment of the TEM, the micelles are expected to collapse and all volatile components are removed. In solution, the hydrophilic domains of the polymer extend into the solvent, leading to a larger effective diameter. The degree of difference between DLS and TEM observations of size is typical of previous studies [37]. Even after dilution, the particles remained stably dispersed, because the concentration of the Pluronic® was still above the very low CMC of the polymer. Thus, these particles would be stable in aqueous environments typical of biological systems.

Zeta potential was also measured to quantify the surface charge of the particles, since the Pluronic® being used had terminal carboxylic groups, which at biological pH should have a negative charge. This hypothesis was confirmed with a measured zeta potential of -37mV initially, and -30mV after 100x dilution with water. The negative value is attributed to the carboxylic groups added to the normally neutral F127 Pluronic®. In previous studies using pluronic to encapsulate SiQDs, the zeta potential was 0mV [38]. The addition of carboxylic groups is a very important difference, because these groups provide a chemical “handle” that can be further utilized for bio-conjugation either through electrostatics or through covalent bonding.

The micelles proved to be quite stable over a broad range of pH and temperature, as evidenced by the maintenance of PL intensity and wavelength (Which would be degraded if the SiQDs were exposed to water). The PSiQDs maintained at least 80% of their initial intensity from pH 2 - 10. This stability has been observed for other water dispersible SiQDs [37] and is even greater than another formulation that utilized carboxylic acid terminated surfactants [39]. This could be the result of the particles being further separated from the surrounding media. In the temperature study the particles proved to be stable at physiological temperature and higher. The stability is even greater than the formulation previously reported using phospholipid-PEG encapsulation [26].

After the above-described characterization, particles were studied in vitro for their ability to target and label pancreatic cancer cells. With the terminal carboxylic acid functional group a peptide bond can be formed with a cancer targeting moiety that has a terminal amine group like folic acid, anti-claudin-4, or anti-mesothelin. With these targeting moieties there was a vast improvement in the cellular uptake. Similar results have previously been observed with heavy metal based quantum dots [40]. With this new formulation the clear visibility of the labeled cells is maintained without the toxicity concerns of heavy metal materials.

The particles were then studied in vivo in a single animal to determine qualitatively where the particles collected within the body. As can be seen from the overlay image the particles are concentrated in the spleen and the liver. These results were expected due to the role that these organs play in the reticuloendothelial system (RES). RES capture is the dominant mode of removal from circulation for nanostructures of this size range. This experiment confirmed that the particles did not migrate to any unexpected areas like the heart, brain, or lungs. Previous papers have also reported concentration of larger nanoparticles in the liver and spleen 24 hrs post injection [41, 42].

# Conclusions

This study showed that carboxyl-terminated Pluronic® can form effective coatings for creating stable aqueous dispersions of SiQDs. These amphiphiles do not quench or shift the luminescence of the particles while making them water dispersible and suitable for biological applications. These PSiQDs are also stable at physiological pH and temperature, comparably so to previous studies, upon some of which there are improvements with this formulation. The surface can readily be modified to incorporate cancer targeting molecules using the carboxylic groups, which also bestow electrostatic characteristics. The in vivo experiments showed no concentration of the particles in the heart, lungs, or brain. The micelles were only in the liver and spleen, as a result of RES clearance of the particles. This work moves SiQD in the direction of high quantum yield aqueous formulations that can be competitive with organics and useful for tumor targeting in cancer applications.

## Acknowledgement

This study was supported by grants from the NCI RO1CA119397, the John R. Oishei Foundation, and the University Honors College Research and Creative Activities Fund.

## Conflict of interest

The authors declare that they have no competing interests.

# References

[1] Prasad, P. N., Biophotonics. Wiley-Interscience: Hoboken, NJ, 2003.

[2] Prasad, P. N., Nanophotonics. Wiley-Interscience: Hoboken, NJ, 2004. http://dx.doi.org/10.1002/0471670251

[3] Alivisatos, A. P. Semiconductor Clusters, Nanocrystals, and Quantum Dots. Science 1996;271:933-37. http://www.sciencemag.org.gate.lib.buffalo.edu/content/271/5251/933.full.pdf http://dx.doi.org/10.1126/science.271.5251.933

[4] Dubertret, B.; Skourides, P.; Norris, D. J.; Noireaux, V.; Brivanlou, A. H.; Libchaber, A. In Vivo Imaging of Quantum Dots Encapsulated in Phospholipid Micelles. Science 2002;298:1759-62. http://web.ebscohost.com.gate.lib.buffalo.edu/ehost/detail?sid=f512e7e4-6b33-4dd3-a252-94b60302de0a%40sessionmgr15&vid=1&hid=18&bdata=JnNpdGU9ZWhvc3QtbGl2ZSZzY29wZT1zaXRl - db=mnh&AN=12459582

[5] Murray, C. B.; Norris, D. J.; Bawendi, M. G. Synthesis and Characterization of Nearly Monodisperse CDE (E = S, SE, TE) Semiconductor Nanocrystallites. Journal of the American Chemical Society 1993;115:8706-15. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja00072a025?source=chemport& http://dx.doi.org/10.1021/ja00072a025

[6] Dabbousi, B. O.; RodriguezViejo, J.; Mikulec, F. V.; Heine, J. R.; Mattoussi, H.; Ober, R., et al. (CdSe) ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. Journal of Physical Chemistry B 1997;101:9463-75. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/jp971091y

[7] Manna, L.; Scher, E. C.; Alivisatos, A. P. Synthesis of soluble and processable rod-, arrow-, teardrop-, and tetrapod-shaped CdSe nanocrystals. Journal of the American Chemical Society 2000;122:12700-06. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja003055%2B http://dx.doi.org/10.1021/ja003055+

[8] Mattoussi, H.; Mauro, J. M.; Goldman, E. R.; Anderson, G. P.; Sundar, V. C.; Mikulec, F. V., et al. Self-assembly of CdSe-ZnS quantum dot bioconjugates using an engineered recombinant protein. Journal of the American Chemical Society 2000;122:12142-50. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja002535y http://dx.doi.org/10.1021/ja002535y

[9] Peng, Z. A.; Peng, X. G. Formation of high-quality CdTe, CdSe, and CdS nanocrystals using CdO as precursor. Journal of the American Chemical Society 2001;123:183-84. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja003633m PMid:11273619 http://dx.doi.org/10.1021/ja003633m

[10] Kim, S.; Fisher, B.; Eisler, H. J.; Bawendi, M. Type-II quantum dots: CdTe/CdSe(core/shell) and CdSe/ZinTe(core/shell) heterostructures. Journal of the American Chemical Society 2003;125:11466-67. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja0361749 PMid:13129327 http://dx.doi.org/10.1021/ja0361749

[11] Pinaud, F.; King, D.; Moore, H. P.; Weiss, S. Bioactivation and cell targeting of semiconductor CdSe/ZnS nanocrystals with phytochelatin-related peptides. Journal of the American Chemical Society 2004;126:6115-23. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja031691c PMid:15137777 http://dx.doi.org/10.1021/ja031691c

[12] Gao, X. H.; Cui, Y. Y.; Levenson, R. M.; Chung, L. W. K.; Nie, S. M. In vivo cancer targeting and imaging with semiconductor quantum dots. Nature Biotechnology 2004;22:969-76. http://web.ebscohost.com.gate.lib.buffalo.edu/ehost/pdfviewer/pdfviewer?sid=c7547267-6fb9-4b6f-b9bc-5394feead80e%40sessionmgr12&vid=2&hid=18 PMid:15258594 http://dx.doi.org/10.1038/nbt994

[13] Gao, X. H.; Yang, L. L.; Petros, J. A.; Marshal, F. F.; Simons, J. W.; Nie, S. M. In vivo molecular and cellular imaging with quantum dots. Current Opinion in Biotechnology 2005;16:63-72. http://www.sciencedirect.com.gate.lib.buffalo.edu/science/article/pii/S0958166904001648 PMid:15722017 http://dx.doi.org/10.1016/j.copbio.2004.11.003

[14] Clapp, A. R.; Medintz, I. L.; Mauro, J. M.; Fisher, B. R.; Bawendi, M. G.; Mattoussi, H. Fluorescence resonance energy transfer between quantum dot donors and dye-labeled protein acceptors. Journal of the American Chemical Society 2004;126:301-10. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja037088b PMid:14709096 http://dx.doi.org/10.1021/ja037088b

[15] Chan, W. C. W.; Nie, S. M. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 1998;281:2016-18. http://web.ebscohost.com.gate.lib.buffalo.edu/ehost/detail?sid=e6a3cccc-e992-4a56-9f53-08811b987cff%40sessionmgr12&vid=2&hid=18&bdata=JnNpdGU9ZWhvc3QtbGl2ZSZzY29wZT1zaXRl - db=mnh&AN=9748158

[16] Chan, W. C. W.; Maxwell, D. J.; Gao, X. H.; Bailey, R. E.; Han, M. Y.; Nie, S. M. Luminescent quantum dots for multiplexed biological detection and imaging. Current Opinion in Biotechnology 2002;13:40-46. http://www.sciencedirect.com.gate.lib.buffalo.edu/science/article/pii/S0958166902002823 http://dx.doi.org/10.1016/S0958-1669(02)00282-3

[17] Derfus, A. M.; Chan, W. C. W.; Bhatia, S. N. Probing the cytotoxicity of semiconductor quantum dots. Nano Letters 2004;4:11-18. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/nl0347334 http://dx.doi.org/10.1021/nl0347334

[18] Hardman, R. A toxicological review of quantum dots: Toxicity depends on physicochemical and environmental factors. Environmental Health Perspectives 2006;114:165-72. http://web.ebscohost.com.gate.lib.buffalo.edu/ehost/pdfviewer/pdfviewer?sid=f8b9759c-ef82-459b-b13a-648eec111e34%40sessionmgr15&vid=2&hid=18 PMid:16451849 http://dx.doi.org/10.1289/ehp.8284

[19] Kirchner, C.; Liedl, T.; Kudera, S.; Pellegrino, T.; Javier, A. M.; Gaub, H. E., et al. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. Nano Letters 2005;5:331-38. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/nl047996m PMid:15794621 http://dx.doi.org/10.1021/nl047996m

[20] Hauck, T. S.; Anderson, R. E.; Fischer, H. C.; Newbigging, S.; Chan, W. C. W. In vivo Quantum-Dot Toxicity Assessment. Small 2010;6:138-144. http://onlinelibrary.wiley.com.gate.lib.buffalo.edu/doi/10.1002/smll.200900626/pdf PMid:19743433 http://dx.doi.org/10.1002/smll.200900626

[21] Rzigalinski, B. A.; Strobl, J. S. Cadmium-containing nanoparticles: Perspectives on pharmacology and toxicology of quantum dots. Toxicology and Applied Pharmacology 2009;238:280-88. http://pdn.sciencedirect.com.gate.lib.buffalo.edu/science?_ob=MiamiImageURL&_cid=272376&_user=681891&_pii=S0041008X09001604&_check=y&_origin=article&_zone=toolbar&_coverDate=01-Aug-2009&view=c&originContentFamily=serial&wc

hp=dGLbVlB-zSkWz&md5=edde8d7aeb009030b936df73c3910bd0/1-s2.0-S0041008X09001604-main.pdf PMid:19379767 http://dx.doi.org/10.1016/j.taap.2009.04.010

[22] Erogbogbo, F.; Yong, K.-T.; Roy, I.; Hu, R.; Law, W.-C.; Zhao, W., et al. In Vivo Targeted Cancer Imaging, Sentinel Lymph Node Mapping and Multi-Channel Imaging with Biocompatible Silicon Nanocrystals. ACS Nano 2010;5:413-23. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/nn1018945 PMid:21138323 http://dx.doi.org/10.1021/nn1018945

[23] Alsharif, N. H.; Berger, C. E. M.; Varanasi, S. S.; Chao, Y.; Horrocks, B. R.; Datta, H. K. Alkyl-Capped Silicon Nanocrystals Lack Cytotoxicity and have Enhanced Intracellular Accumulation in Malignant Cells via Cholesterol-Dependent Endocytosis. Small 2009;5:221-28. http://onlinelibrary.wiley.com/doi/10.1002/smll.200800903/full PMid:19058285 http://dx.doi.org/10.1002/smll.200800903

[24] Choi, J.; Wang, N. S.; Reipa, V. Conjugation of the Photoluminescent Silicon Nanoparticles to Streptavidin. Bioconjugate Chemistry 2008;19:680-85. http://pubs.acs.org/doi/abs/10.1021/bc700373y PMid:18290602 http://dx.doi.org/10.1021/bc700373y

[25] Park, J.-H.; Gu, L.; von Maltzahn, G.; Ruoslahti, E.; Bhatia, S. N.; Sailor, M. J. Biodegradable luminescent porous silicon nanoparticles for in vivo applications. Nat Mater 2009;8:331-36. http://web.ebscohost.com.gate.lib.buffalo.edu/ehost/pdfviewer/pdfviewer?sid=c8259af2-772a-43a6-8d81-fbc224ab4e89%40sessionmgr12&vid=2&hid=21 PMid:19234444 http://dx.doi.org/10.1038/nmat2398

[26] Erogbogbo, F.; Yong, K.-T.; Roy, I.; Xu, G.; Prasad, P. N.; Swihart, M. T. Biocompatible Luminescent Silicon Quantum Dots for Imaging of Cancer Cells. ACS Nano 2008;2:873-78. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/nn700319z PMid:19206483 http://dx.doi.org/10.1021/nn700319z

[27] Tu, C.; Ma, X.; Pantazis, P.; Kauzlarich, S. M.; Louie, A. Y. Paramagnetic, Silicon Quantum Dots for Magnetic Resonance and Two-Photon Imaging of Macrophages. Journal of the American Chemical Society 2010;132:2016-23. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja909303g PMid:20092250 http://dx.doi.org/10.1021/ja909303g

[28] He, Y.; Kang, Z. H.; Li, Q. S.; Tsang, C.; Fan, C. H.; Lee, S. T. Ultrastable, Highly Fluorescent, and Water-Dispersed Silicon-Based Nanospheres as Cellular Probes. Angewandte Chemie International Edition 2009;48:128-32. http://onlinelibrary.wiley.com.gate.lib.buffalo.edu/doi/10.1002/anie.200802230/pdf PMid:18979474 http://dx.doi.org/10.1002/anie.200802230

[29] Clark, R. J.; Dang, M. K. M.; Veinot, J. G. C. Exploration of Organic Acid Chain Length on Water-Soluble Silicon Quantum Dot Surfaces. Langmuir 2010;26:15657-64. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/la102983c PMid:20815392 http://dx.doi.org/10.1021/la102983c

[30] Kang, Z.; Liu, Y.; Tsang, C. H. A.; Ma, D. D. D.; Fan, X.; Wong, N. B., et al. Water-Soluble Silicon Quantum Dots with Wavelength-Tunable Photoluminescence. Advanced Materials 2009;21:661-64. http://onlinelibrary.wiley.com.gate.lib.buffalo.edu/doi/10.1002/adma.200801642/pdf http://dx.doi.org/10.1002/adma.200801642

[31] Li, X.; He, Y.; Swihart, M. T. Surface Functionalization of Silicon Nanoparticles Produced by Laser-Driven Pyrolysis of Silane followed by HF−HNO3 Etching. Langmuir 2004;20:4720-27. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/la036219j

[32] Ding, H.; Yong, K.-T.; Law, W.-C.; Roy, I.; Hu, R.; Wu, F., et al. Non-invasive tumor detection in small animals using novel functional Pluronic nanomicelles conjugated with anti-mesothelin antibody. Nanoscale 2011;3:1813-22. http://pubs.rsc.org.gate.lib.buffalo.edu/en/content/articlepdf/2011/NR/C1NR00001B PMid:15969188 http://dx.doi.org/10.1021/la036219j

[33] Hsu, Y.-C.; Chang, Y.-H.; Yang, C.-M. Swelling-agent-free synthesis of siliceous and functional mesocellular foam-like mesophases by using a carboxy-terminated triblock copolymer. Adv. Funct. Mater. 2008;18:1799-808. http://onlinelibrary.wiley.com.gate.lib.buffalo.edu/doi/10.1002/adfm.200701096/pdf http://dx.doi.org/10.1002/adfm.200701096

[34] Bali, D.; King, L.; Kim, S. Syntheses of new gramicidin a derivatives. Aust. J. Chem. 2003;56:293-300. http://www.publish.csiro.au.gate.lib.buffalo.edu/?paper=CH02142 http://dx.doi.org/10.1071/CH02142

[35] Wang, Q.; Ni, H.; Pietzsch, A.; Hennies, F.; Bao, Y.; Chao, Y. Synthesis of water-dispersible photoluminescent silicon nanoparticles and their use in biological fluorescent imaging. Journal of Nanoparticle Research 2011;13:405-13. http://web.ebscohost.com.gate.lib.buffalo.edu/ehost/pdfviewer/pdfviewer?sid=be2bd285-e2e0-4ef1-8e8e-2c4b40483b83%40sessionmgr11&vid=2&hid=21 http://dx.doi.org/10.1007/s11051-010-0047-7

[36] Warner, J. H.; Hoshino, A.; Yamamoto, K.; Tilley, R. D. Water-Soluble Photoluminescent Silicon Quantum Dots. Angewandte Chemie 2005;117:4626-30. http://onlinelibrary.wiley.com.gate.lib.buffalo.edu/doi/10.1002/anie.200501256/pdf http://dx.doi.org/10.1002/ange.200501256

[37] He, Y.; Zhong, Y.; Peng, F.; Wei, X.; Su, Y.; Lu, Y., et al. One-Pot Microwave Synthesis of Water-Dispersible, Ultraphoto- and pH-Stable, and Highly Fluorescent Silicon Quantum Dots. Journal of the American Chemical Society 2011;133:14192-95. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ja2048804 PMid:21848339 http://dx.doi.org/10.1021/ja2048804

[38] Shen, P.; Ohta, S.; Inasawa, S.; Yamaguchi, Y. Selective labeling of the endoplasmic reticulum in live cells with silicon quantum dots. Chemical Communications 2011;47:8409-11. http://pubs.rsc.org.gate.lib.buffalo.edu/en/content/articlepdf/2011/CC/C1CC12713F PMid:21698318 http://dx.doi.org/10.1039/c1cc12713f

[39] Ge, J.; Liu, W.; Zhao, W.; Zhang, H.; Zhuang, X.; Lan, M., et al. Preparation of Highly Stable and Water-Dispersible Silicon Quantum Dots by Using an Organic Peroxide. Chemistry - A European Journal 2011;17:12872-76. http://onlinelibrary.wiley.com.gate.lib.buffalo.edu/doi/10.1002/chem.201102356/pdf PMid:21984350 http://dx.doi.org/10.1002/chem.201102356

[40] Yong, K.-T.; Ding, H.; Roy, I.; Law, W.-C.; Bergey, E. J.; Maitra, A., et al. Imaging Pancreatic Cancer Using Bioconjugated InP Quantum Dots. ACS Nano 2009;3:502-10. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/nn8008933 PMid:19243145 http://dx.doi.org/10.1021/nn8008933

[41] Yong, K. T.; Swihart, M. T.; Ding, H.; Prasad, P. N. Preparation of Gold Nanoparticles and their Applications in Anisotropic Nanoparticle Synthesis and Bioimaging. Plasmonics 2009;4:79-93. http://www.springerlink.com.gate.lib.buffalo.edu/content/w26568733vh62415/ http://dx.doi.org/10.1007/s11468-009-9078-2

[42] Tu, C. Q.; Ma, X. C.; House, A.; Kauzlarich, S. M.; Louie, A. Y. PET Imaging and Biodistribution of Silicon Quantum Dots in Mice. ACS Med. Chem. Lett. 2011;2:285-88. http://pubs.acs.org.gate.lib.buffalo.edu/doi/pdf/10.1021/ml1002844