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Near-infrared peptide-coated quantum dots for small animal imaging

Gopal Iyer, Jack J. Li, Fabien Pinaud, James M. Tsay, Laurent A. Bentolila, Xavier Michalet, and Shimon Weiss^a

Department of Chemistry & Biochemistry, University of California at Los Angeles 607 Charles E. Young Drive E., Los Angeles, CA 90095, USA

Abstract

We have synthesized high quality type-II CdTe/CdSe near infrared quantum dots using successive ion layer adsorption and reaction chemistry. Transmission electron microscopy reveals that CdTe/CdSe can be synthesized layer by layer yielding quantum dots of narrow size distribution. Excitation and photoluminescence spectra reveal discrete type-II transitions, which correspond to energy lower than type-I bandgap. We have used a peptide coating technique on type-II and commercial near infrared quantum dots for delivery in live animals and cultured cells.

Keywords

Quantum dots; near infrared; peptide; fluorescence; type-II; SILAR

1. Introduction

Quantum dots (QDs) in the far-red and near-infrared (NIR) spectra are of great interest for in vivo biological imaging, diagnostics and possible therapeutics, due to separation from autofluorescence background and increased penetration of excitation and emission of light through thick tissues. NIR radiations transmit well through biological tissue between the absorption peaks of hemoglobin (> 650 nm) and the absorption of water (< 850 nm) [1]. Detection of folate-receptor targeted in tumor mice models based on optically quenched near infrared fluorescence (NIRF) probes have enhanced the detection of tumors [2]. Experiments using optically quenched NIRF probes were able to generate strong NIRF signal after enzyme activation by tumor-associated proteases in vivo [3,4]. However, NIRF probes rely extensively on the attachment of organic fluorophores. A potential application of QDs as fluorescent contrast reagent for tumor target imaging can be envisaged in the NIR spectrum. The advantage of using NIR QDs in surface/subsurface and deep imaging of living tissue is obvious due to relative low absorbance and scatter of living tissue in the NIR spectrum. In order to exploit the lack of autofluorescence in the NIR spectrum, the choice of wavelength of the NIR qdot and the scatter of living tissue should be studied to synthesize an optimal biocompatible NIR qdot. For instance, in rat skin, scatter is proportional to $\lambda^{-2.8}$ [5] suggesting a strong wavelength dependence [6] while in postmenopausal human breast, the

^a weiss@chem.ucla.edu; Ph: 310-794-0093; fax: 310-267-4672.

scatter is proportional to $\lambda^{-0.6}$ [7] suggesting weak wavelength dependence [6]. It is evident from published data that a working knowledge of the thickness of tissue, photon attenuation (sum of attenuation due to absorbance and scatter) is a prerequisite to optimize the appropriate wavelength of NIR QDs for imaging of tumor tissues. Since color tuning is achieved by controlling the qdot size, the chemical synthesis and conjugation chemistry of the different color probes is identical, vastly simplifying matching colors to an application.

Here, we describe the synthesis of high quality NIR type-II QDs using successive ion layer adsorption and reaction (SILAR) [8]. This method has been used with half monolayer accuracy to synthesize type-I core/shells and quantum well QDs [9] with small size distribution and high quantum yield. We characterize the photophysical properties of these QDs with each monolayer of shell grown using absorption, photoluminescence excitation, and conclude with solubilization of these QDs with peptides and large molecular weight polyethylene glycol (PEG) combined with live cell and nude mice studies.

2. SILAR chemistry for type-II QDs

The development of SILAR chemistry for type-II QDs was motivated by two factors: the desire to use stable precursors as alternatives to highly toxic and pyrophoric organometallic compounds such as dimethyl cadmium, and the need for well-controlled and precise deposition of shells which results in narrow size distribution and photoluminescence emission width. In accordance to recently developed “greener” approaches, CdO was used as the Cd source, replacing dimethyl cadmium in both the synthesis of CdTe cores and CdSe shells. CdTe core nanocrystals were made by injecting Te-TBP (tributylphosphine) into a hot solution composed of Cd-TDPA (tetradecylphosphonic acid) and 1-octadecene (ODE) [10]. These cores were purified to remove unreacted tellurium before shell overcoating. In order to have precise deposition of shells, type-II CdTe/CdSe core/shell colloidal quantum dots were synthesized with a layer-by-layer colloidal epitaxy method [8]. Briefly, purified CdTe cores were mixed with two components: the coordinating ligands (oleic acid, octadecylamine or trioctylphosphine oxide), as a single component or their combination and a noncoordinating solvent (ODE) which was used as a substitute for TOPO. The reaction flask containing CdTe cores, ligands, and solvent was pumped down under vacuum at room temperature for 30 min. Then, the temperature was increased to 50 °C, and nitrogen was flowed into the reaction flask and used for the rest of the reaction. At a designated temperature, ca. 200 °C, one precursor solution, with the Cd (Se) source, was injected into the flask in a calculated amount corresponding to an overcoating of all the CdTe cores at once. The epitaxial growth of the single component rendered a red shift of both the absorption and the photoluminescence. Both absorption and emission spectra of aliquots were recorded to monitor the reaction and determine the next injection point. After a redshifted emission was no longer observed in an interval of 5 min, the injection of a second precursor solution, this time with the Se (Cd) source, was carried out. The full reaction of two successive injections (one for each cation/anion precursor) adds one monolayer of shell on top of the cores. Alternating injections were repeated until the desired number of CdSe layers was obtained. Because the injection amount is stoichiometric, no free precursor is left over. Due to the lack of coexistence of both precursors, there is no homogeneous nucleation even at high temperatures. Layer-by-layer colloidal epitaxy of heterostructured colloidal

quantum dots with extremely high quality could be produced by SILAR, in a way very analogous to MBE. This method is especially useful for the synthesis of more complex heterostructures, such as CdTe/CdSe/CdS. The CdS type-I shell effectively increases the QY and photostability of type-II structures.

3. Size Characteristics of TYPE-II QDs by conventional technique

Transmission Electron Microscopy

Transmission electron microscopy (TEM) was used to evaluate the size and shape of the CdTe/CdSe core/shell samples. After evaluating the TEM results, we found that the average diameters of the QDs with varying CdSe layers are consistent with the calculations for the amount of precursors injected (Fig.1). Each injection of precursors corresponding to one monolayer of shell increases the diameter of the particles by 0.7 nm, in agreement with the lattice spacing of CdSe. In a previous study using the SILAR technique, it was found that TEM results of shell thickness agreed with the X-ray photoelectron spectroscopy (XPS) depth profile, which strongly suggested that the growth of the shell is indeed epitaxial[8].

4. Photophysical Properties of Type-II NIR Quantum Dots

Absorption and Emission Spectroscopy

To study the effects of shell growth on fixed size cores, CdTe cores of 5.9 nm diameter were coated with 1, 2, or 3 monolayers of CdSe shell. As shown in Fig. 2, there are clear differences in the absorption spectra of CdTe with additional monolayers of CdSe. The absorption peaks continually redshift and lose their definition after each additional injection of shell precursors. After the addition of 3 shell monolayers, the excitonic features are completely lost. The spatially indirect nature of type-II transitions is responsible for the weaker absorption at the band edges. The transitions between the quantum levels of the valence band of CdTe and the conduction band of CdSe cannot be effectively detected in absorption, in contrast to type-I CdTe and CdSe QDs. The photoluminescence emission spectra of CdTe/CdSe were also taken with the same series of different shell thickness samples from a single reaction (Fig.2). As more layers of CdSe are deposited on CdTe cores, the emission of the QD redshifts, originating from the spatially indirect band edge transition. This redshifted emission gives a direct measure of the energy difference between the valence band edge of CdTe and the conduction band edge of CdSe. The high QY and narrow emissions indicate that the NIR emission from SILAR prepared CdTe/CdSe is not from trap state photoluminescence. Large redshifts of both the absorption profile and photoluminescence emission with additional layers of CdSe provide evidence that type-II QDs have been indeed synthesized.

5. Near Infrared-Emitting (NIR) Quantum Dots as Image Contrast Probes

The synthesized type-II NIR QDs are readily solubilized and functionalized with synthetic or recombinant peptides developed in our lab. To broaden our panel of available NIR probes we also use our peptide-coating chemistry to solubilize commercially available NIR QDs emitting at 800 nm and we have functionalize them with high molecular weight PEGs. Although these commercial NIR QDs (12-14 nm) are larger than CdTe particles (7-8 nm)

synthesized in our laboratory, they will be of interest to study how the size of the QDs and the nature of the amphiphilic coating (polymer coating for commercial material and peptides for our material) affects the bio-distribution and clearance in animals. Solubilization of QDs with peptides has been comprehensively reviewed [11] and we show evidence of peptide coating NIR QDs (pc-QDs) in live mice and HeLa cells in culture.

Live cells and Nude Mice Studies of NIR pc-QDs

Although we have mainly used pc-QDs emitting in the visible part of the spectrum for live cell fluorescence imaging (CCD cameras used in our laboratory are more sensitive in the visible range), we have also been able to image CdTe/CdS/ZnS pc-QDs in live cells. Fig. 4 shows that upon endocytosis of NIR 850 nm pc-qdot visualization and tracking of single endocytic vesicles in live HeLa cells are possible. In addition, we have successfully demonstrated in vivo imaging with peptide-coated NIR QDs using an IVIS-100 imaging system. A 1.1 μ M NIR 830 nm pc-qdot solution was injected through tail vein on a three-month-old nude mouse (Fig. 3). The light source for the fluorescence is a 150-watt quartz halogen lamp. Light is delivered from the lamp via fiber-optic cable and a band pass excitation filter (710-760 nm) to a light distribution plate. The emission is collected through a bandpass emission filter (810-875) by a back-thinned, back-illuminated Grade1 CCD camera cooled down to -120°C . Images were taken immediately following the injection.

6. Perspectives

We have exploited the layer by layer synthesis of type-II CdTe/CdSe QDs using the SILAR technique enables us to study wavefunction engineering in the quantum size regime. Photoluminescence excitation spectra can resolve excitonic states in type-II structures that are hidden in the absorption spectra. The degree of bandgap and wavefunction engineering demonstrated here could prove useful in the development of near-infrared fluorescent probes of different colors and different lifetimes, possessing ideal properties for in vitro and in vivo biological and medical applications. Towards these efforts, the solubilization of QDs is critical for further development of targeting in live cells and animals. The peptide coating technology developed in our lab allows for solubilization and functionalization with important macromolecules such as antibodies and DNA. Pc-QDs bring various benefits concerning targeting and specificity. The large number of surface attachment groups on QDs can be used to “graft” different functionalities to these probes. The peptide “toolkit” approach gives us considerable latitude to modulate parameters such as antibody density (number of single chain fragments or diabodies per qdot), overall charge, hydrophilicity/hydrophobicity, attachment of tumor-binding peptides and incorporation of moieties such as long PEGs to help increase biocompatibility, circulation time and to decrease immunogenicity.

Acknowledgments

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Fig. 1.

TEM images of CdTe/CdSe type-II core/shell samples with successive layers of CdSe grown on a 5.9 nm CdTe core. These samples were synthesized by layer-by-layer colloidal epitaxy in a single reaction. The right figure corresponds to a superlattice of CdTe/CdSe with 3 monolayers of CdSe.

Fig. 2.
Absorption and photoluminescence spectra of CdTe QDs with varying CdSe shell thickness.

Fig. 3. Nude mice injected with 1nmol of NIR pc QDs, imaged with IVIS 100. Exc: 710-760 nm; Em: 810-875 nm. The image was corrected for background with a control (un injected) animal.

Fig. 4. Fluorescence imaging of NIR QDs (850 nm) in living HeLa cells. (a-e) Endocytosis of NIR peptide -coated CdTe/CdSe/CdZnS QDs. Single vesicle trafficking is easily visualized. Frames (b-e) present the movement of a single endocytic vesicle at $t=0s$, 3s, 7.5s and 14s respectively. Scale bar 5 μm .