Inelastic Laser Light Scattering Spectroscopy and Functionalization of Semiconductor Quantum Dots with Peptides and Integrins of Cancer Cells for Biophotonic Applications

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Abstract. Results of our study of structural properties of the nanoscale integrated semiconductor quantum dots such as CdS and ZnS-capped CdSe, conjugated with biomolecules such as short peptides and cells are presented. Nanoscale functionalization of semiconductor quantum dots with biomedical structures is promising for many applications and novel studies of intrinsic properties of both constituent systems. We study CdS semiconductor quantum dots functionalized with peptides composed of the following amino acid chains: CGGRRGD, CGGRRVDS, CGGIKVAV, and CGGGLDV, where R is arginine, D is aspartic acid, S is serine, V is valine, K is lysine and L is Levine. As will be seen the cysteine (C) amino acid links to CdS semiconductor quantum dots via the thiol link. Furthermore, the GGG sequences of glycine (G) amino acids provide a spacer in the amino acid chain. At the same time the RGDS, RVDS, IKAV, and LDV sequences have selective bonding affinities to specialized transmembrane cellular structures known as integrins of neurons and MDA-MB-435 cancer cells, respectively. Since protein hydration is known to be a key factor affecting protein energy balance, we also studied a role that water and other bioenvironments may play in stability, surface properties, dynamical and structural characteristics of these systems. We found also the roles that the quantum confinement and functionalizing in the biomedical environments play in...
altering and determining the electronic, optical, and vibrational properties of these nanostructures as well as demonstrated the effectiveness to use the semiconductor quantum dots as integrin sensitive biotags.

1. Introduction

Semiconductor quantum dots (SQDs) have been investigated over the past years due to their very specific optical, electronic and catalytic properties. Created by quantum confinements of electrons and holes they exhibit unique spectroscopic properties with broad absorption, narrow and symmetric as well as tunable emission bands, resistance to photobleaching, strong luminescence and long luminescent lifetimes. These remarkable properties of fluorescence from SQDs have resulted in their use as an alternative to traditional protein fluorophores that is an emerging research area for numerous biomedical applications.

We have been developing SQDs based strategies for imaging, identification, simultaneous localization and tracking these assembly to protein complexes and cells with high special resolution. We studied a set of key issues underlying the nanoscale integration of semiconductor nanostructures with biomedical structures. Such functionalization of SQDs with biomedical structures is promising for many applications and novel investigations of intrinsic properties of both constituent systems though it is in an early stage of development. One intriguing application is the use of SQDs as biotags that has emphasized use of the semiconductor luminescence to determine the location where chemically functionalized SQDs bind to a biomedical sample. In the present study chemically prepared CdS SQDs are functionalized with peptides composed of the following amino acid chains: CCGGRGDS, CCGGRVDS, CGGIKVAV, and CCGGLDV. Since protein hydration is known to be a key factor affecting protein energy balance, we also studied a role that water and other biological environments may play in stability, surface properties, dynamical and structural characteristics of these molecular systems.

As will be seen the cysteine (C) amino acid links to SQDs (in the case of CdS) via the thiol link, the GGG sequences of glycine (G) amino acid, provide a spacer in the amino acid chain, so we were able to control and modify CdS SQDs surface with the very high surface-to-volume ratio. We will show that selected amino acid minimize surface defects and increase luminescence efficiencies. At the same time the RGDS, RVDS, IKAV, and LDV sequences have selective bonding affinities to specialized transmembrane cellular structures known as integrins of neurons and MDA-MB-435 cancer cells, respectively.

Since protein hydration is known to be a key factor affecting protein energy balance, we also studied a role that water and other bioenvironments may play in stability, surface properties, dynamical and structural characteristics of these systems. We found the roles that the quantum confinement and functionalizing in biomedical environments plays in altering and determining the electronic, optical, and vibrational properties of these nanostructures as well as demonstrated the effectiveness to use SQD as integrin sensitive biotags.

In particular, IKAV and LDV are known to bind preferentially to cellular integrins of neurons and cancer cells, respectively. Clearly, CGGGLDV- and CCGGIKVAV functionalized CdS nanocrystals have potential applications in the study of cancer and the neuronal currents in neurons. In addition to investigating RGDS and RVDS binding properties, this study investigates the interaction between colloidal CdS nanocrystals and the CCGGIKVAV and CGGGLDV peptides by measuring absorption and photoluminescence spectra (PL) for these SQD-peptide complexes. Particular emphasis is placed on determining the changes in the optical properties of the surface states as a result of being functionalized by the peptides.

These studies [1] emphasize the determination of the binding site rather than determination of how the interaction between the SQDs and the biological structure changes the electronic and optical properties of SQDs. Recent efforts to study the interaction between SQDs and biological structures have investigated the binding of peptide-functionalized colloidal SQDs to transmembrane proteins in
the bilipid membranes of cells [2]. In this study SQDs are bound to CCGGRGDS peptide through the thiol link between the cysteine (C) amino acid and the SQD.

**Experimental Procedures**

In the present study chemically prepared CdS SQDs are functionalized with peptides composed of the following amino acid chains: CCGGRGDS, CCGGRVDS, CCGGIKVAV, and CCGGGLDV. Since protein hydration is known to be a key factor affecting protein energy balance, we also studied a role that water and other biological environments may play in stability, surface properties, dynamical and structural characteristics of these molecular systems.

As will be seen the cysteine amino acid links to the CdS SQD via the thiol link, the GGG sequences of glycine (G) amino acids provide a spacer in the amino acid chain, and the RGDS, RVDS, IKAV, and LDV sequences have selective bonding affinities [3] to specialized transmembrane cellular structures known as integrins. In particular, IKAV and LDV are known to bind preferentially to cellular integrins of neurons and cancer cells, respectively. Clearly, CCGGGLDV- and CCGGIKVAV functionalized CdS nanocrystals have potential applications in the study of cancer and the neuronal currents in neurons.

In addition to investigating RGDS and RVDS binding properties, this study investigates the interaction between colloidal CdS nanocrystals and the CCGGIKVAV and CCGGGLDV peptides by measuring absorption, photoluminescence and vibrational inelastic laser light scattering spectra for these SQD-peptide complexes. Particular emphasis is placed on determining the changes in the optical properties of the surface states as a result of being functionalized by the peptides.

**Sample Preparation**

The investigated peptide-functionalized colloidal CdS nanocrystals were synthesized at room temperature using techniques of colloidal chemistry [4]. In particular, a 5mM solution of CdCl₂ (36.6 mg of CdCl₂ in 40 mL of H₂O) was titrated with mercaptoacetic acid until a pH of 2 was achieved. Concentrated NaOH was then added dropwise until a pH of 7 resulted. Upon mixing this solution with a 5mM solution of Na₂S·9H₂O, a stable yellow colloidal suspension of CdS was formed.

The functionalization of the colloidal CdS SQDs with peptides was accomplished by introducing 5 mg of CCGGRGDS in 2 mL of CdS suspension, 3 mg of CCGGRVDS in 2 mL of the CdS suspension, 2.4 mg of CCGGIKVAV in 5 mL of the CdS suspension, and 1.8 mg of CCGGGLDV in 5 mL of the colloidal CdS suspension.

**Results and Discussions**

We found in the absorption spectra of the CdS nanocrystal suspension with and without peptides for the cases of CCGGIKVAV and CCGGGLDV, respectively, that there is a strong absorption peak in each of these spectra at about 440 nm. The band gap of bulk CdS at 10K is 2.58 eV (480.6 nm) and the band gap increases to ~ 2.8 eV (439.9 nm) for a CdS SQD dot as a result of the quantum confinement.

By comparing the absorption edge at 440 nm with these scaling results, the diameter of the SQDs in the CdS suspension is estimated to be about 3 nm.

In previous studied of colloidal SQDs [5,6], a dominant feature of the PL found to be due the recombination from surface state in the gap of the semiconductor. To examine such states, PL spectra were taken using a 514.53 nm Ar⁺ laser source to ensure the sub-band gap excitation of the CdS-peptide complexes. As a result the strong PL feature is not present due to the recombinination of the electron-hole pair at the quantum confined state.

To determine the role of surface functionalization on the PL emission from the surface states, PL studies have been performed for a variety of peptide concentrations.

In Fig. 1 varying concentrations of CCGGIKVAV (denoted by IKVAV) are seen to affect the PL from surface states for higher concentrations. In each case, a 5 mL volume of the previously-describe CdS suspension was functionalized with the indicated mass of CCGGIKVAV peptide.
The emission bands near 620 nm are due to the presence of water. We also observed these bands’ characteristic for the high-frequency O-H stretching region at 2900-3700 cm\(^{-1}\) in Raman spectra obtained using the same excitation line of the Ar\(^+\) laser for the high purity water (with electrical resistivity of > 18 M\(\Omega\)·cm (Fig. 2) and 1 mM lysozyme solution (\(pH=7.0\)) [7,8]. In the latter spectra formation of water bridges with carbonyl oxygens of main chain on protein surface at 1660 cm\(^{-1}\) (Amide 1) in good agreement with results of molecular dynamics simulations for Lysozyme (3LZT) in an explicit periodic water box using AMBER 4.1 package and results of AB INITIO calculations using GAMESS 6.4 and GAUSSIAN 94W quantum chemical programs packets with 6-31\(^{*}\)G basis set.

From comparison of the spectra in Fig. 1, Fig. 2 and Fig. 3 it is clear that the wings on the both sides of the characteristic O-H stretching bands in the spectra of CdS suspension are due to the additional luminescence in de-ionized water used. As is apparent form spectra in Fig. 1, the suspension exhibits significantly reduced luminescence for the case of relatively high peptide concentrations of 2 mg in 5 mL of suspension.

This finding is analogous of [5] where it was shown that increasing the concentration of DNA oligomer resulted in decreased surface state emission; a possible mechanism for this quenching effect may be the presence of a surface absorbed DNA (peptides in our case) that results in greater surface localization of charges. Anyway, the relative change in the PL intensity as a function of surfactant concentration portends applications in the determination of the concentrations of biomolecules in the SQD suspension.

In parallel with these experimental studies of luminescence for SQD suspensions, the role of the observed linewidth broadening has been examined. In particular, the linewidth broadening due to acoustic-phonon-assisted transitions is expected [1] to contribute satellite lines to the PL spectra that are downshifted by the acoustic phonon energies. Within the elastic continuum approach, the phonon mode frequencies sensitive to the boundary conditions at the surface of the quantum dot were calculated.

Figure 3 depicts the frequencies of the breathing mode — the lowest-order spherical acoustic mode for CdS for a selection of different matrix materials surrounding the CdS SQDs. As illustrated, free-standing, plastic encased, ZnS coated, water immersed, SiO\(_2\) coated coated dots are modelled. As is evident from Fig. 2, the mode frequencies differ by as much as a factor of three for a given SQD radius.

![Fig. 1. Room temperature photoluminescence of CdS SQD suspensions for different concentrations of IKVAV peptides.](image-url)
Fig. 2. Raman spectrum of double distilled deionized water with conductivity $< 0.05 \mu S\cdot cm$ for parallel polarizations of the incident and scattered light. The spectral resolution is $3 \text{ cm}^{-1}$ at the 514.453 nm excitation line.

Fig. 3. Calculated energy of the frequencies of the breathing mode — the lowest-order spherical acoustic phonon mode for CdS QD as function of CdS QD radius $R$ for several cases of interest.
Fig. 4. Image of MDA-MB-435 cells under illumination by white light.

Fig. 5. Image of MDA-MB-435 cells functionalized with the CGGGRDGS-based SQD-peptide complexes as illuminated with 360-nm radiation and light was collected through a filter with a window in the 435-nm to 490-nm band.
To examine the role of peptide selectivity in the binding of QDP complexes to cells, the binding of CGGGRGDS-functionalized and CGGGRVDS-functionalized quantum dots to cancer cells the MDA-MB-435 cell line has been used and examined by direct observation under a fluorescence microscope to determine the presence or absence of quantum dots bound to the cells.

Figure 4 shows MDA-MB-435 cells as viewed with white light exposure. The general features of the cells are clearly visible. The image of this same group of CGGGRGDS-functionalized cells was then illuminated with 360-nm radiation and light was collected through a filter with a window in the 435-nm to 490-nm band. As seen in the associated image in Fig. 5, the CGGGRDGS-based SQD-peptide complexes are bound to the MDA-MB-435. The binding is indicated by the collection of radiation near the 440-nm emission line of the lowest state of the CdS quantum dots with diameters of approximately 3 nm. On the other hand, similar images of cells exposed to CGGGRVDS-based QDP complex show no sign that the QDP bind to the cells. These results demonstrate peptide selectivity in the binding of QDP complexes to MDA-MB-435 cells. In addition, these finding demonstrate the utility of using peptides to link quantum dots the transmembrane proteins and to use quantum dots as integrin sensitive biotags.

Conclusions
We have addressed the roles that functionalizing CdS semiconductor quantum dots with biomolecules play in altering and determining the electronic, optical, and vibrational properties of these nanostructures. Our findings indicate that optical studies of semiconductor quantum dots in biological environments provide essential information on the interaction between quantum dots and their environments. We have identified amino acid sequences capable of interacting with biomaterials. And these results have demonstrated the integrin-selective binding of CdS QDP complexes to integrins of MDA-MB-435 cells. Clearly, these results portend many uses of semiconductor quantum dots going beyond those traditionally associated with biotags. The demonstrated sensitivity of the optical spectra opens a new way to a wide range of studies of how integrated semiconductor quantum dots -biological structures acquire modified properties as a result of their mutual interactions.

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