

multilayer cases, there was another dip in a pH-adjusted Milli-Q water bath before returning to the polycation solution. The pH was kept constant between all the solutions and water baths. All polyelectrolytes were prepared as 10 mM solutions in Milli-Q water on a repeat unit basis. Ru(phen)<sub>3</sub><sup>+</sup> dye solution was prepared as a 5 mM solution. HCl and NaOH were used to adjust solution pH.

A Digital Instruments Dimension 3000 AFM was used in tapping mode to observe the topography of the different samples. Fluorescence micrographs were taken with Nikon fluorescent microscopy. A fluorescein isothiocyanate (FITC) optical filter, which was originally used to probe FITC dye emission, was used to observe the Ru(phen)<sub>3</sub><sup>+</sup> emission images, while a 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) optical filter, which was originally used to probe DAPI dye emission, was used to observe PPP<sup>-</sup> dye emission images. The photoluminescence (PL) spectrum was recorded with a SPEX Fluorolog scanning fluorimeter. The PL excitation wavelength was 350 nm.

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## Recognition Molecule Directed Interfacing Between Semiconductor Quantum Dots and Nerve Cells\*\*

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The creation of heterojunctions, which interface dissimilar materials (e.g., semiconductors or metals), has transformed the microelectronics industry and spurred the development of a variety of device applications, including lasers, photodetectors, computers, and solar cells.<sup>[1]</sup> The integration of biological systems and microelectronics offers a completely new strategy for next-generation heterojunction applications, such as neuronal memory devices and prosthetics that control cells directly. These new approaches will rely on the ability to interface biological molecules with each other and with inorganic substrates. While biochemists and biologists have learned much about biomolecular recognition and specificity, relatively little is known about direct interfacing of semiconductors with biological units. Here we demonstrate two routes to interface living neurons with semiconductor quantum dots (qdots). The first employs known antibody–antigen recognition, and the second, a new approach, utilizes peptide recognition groups. Both of these methods target receptors on the neuron surface, localizing semiconductor–biomolecule binding to the exterior of the cell.

Neurons are an ideal cell type for bioelectronic interfaces because they are similar to electrical components. Neurons produce electric fields that are sensed by the surrounding “biological circuitry”, evidencing the same physical principles as the transistor (i.e., applied electric fields change the response characteristics of the device). In fact, firing patterns<sup>[2]</sup> and growth characteristics<sup>[3,4]</sup> can be altered by external electrical stimuli. Additionally, there is an urgent need in the medical community for devices that can interface nerve cells with prosthetics. Nerve cell–semiconductor interfaces provide a first look at the future of these devices.

The interfacing of objects and cells using bio-recognition molecules, such as antibodies and peptides, has been investigated extensively. Recognition molecules have been utilized to attach a variety of objects to cells, including fluorescent dyes, enzymes, and radioactive labels.<sup>[5]</sup> However, none of these molecules is capable of interacting electrically with cells to provide stimulation. Although gold particles, which have

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also been attached to cells using recognition molecules, could be used in this way, their electrical properties have not been exploited to produce electrical interaction.<sup>[5]</sup> Our approach represents one of the first attempts to both attach an object to the cell and establish electrical interactions, particularly at the nanometer scale.

Previously, laboratories have attempted micrometer scale interactions between neurons and semiconductor surfaces, most notably by patterning electronic device structures with biocompatible features favoring neuron growth in desired locations.<sup>[6–11]</sup> This approach meets two major challenges: a) reproducible neuron/device interfacing is difficult due to uncontrollable neuronal growth, and b) the relatively large separation between the cell and the device (on the order of 1  $\mu\text{m}$ )<sup>[12]</sup> leads to poor electronic coupling.<sup>[13,14]</sup> These devices operate primarily through induction, the movement of separated charges. Coulomb's law, describing the field between separated charges, is dependant on the inverse of the radius squared. Thus, to achieve high fields, small separation distances are desired.

The use of semiconductor qdots, attached directly to cells via biological recognition molecules, eliminates the need for controlled cell growth on a substratum. In addition, by selecting recognition molecules with very short length scales, such as peptides, semiconductor qdots can be positioned within nanometers of the cell surface. This reduces the deleterious effects of cell membrane counter-ion charge screening. Patterning a surface with the nanometer-size features needed to interface electronic materials with specific cellular receptors is impossible using current technologies. Furthermore, the location of such receptors is varied, unpredictable, and dynamic from cell-to-cell. Qdots, on the other hand, provide a "smart" approach: wiring neurons directly with electronic structures through designated cell surface receptors.

Alivisatos et al. and Nie et al. were the first to label biological cells with luminescent semiconductor nanocrystals (i.e., qdots).<sup>[15,16]</sup> Qdot binding was achieved through non-specific interactions or through secondary binding to molecular receptors. In one case, nanocrystals were attached to cells through non-specific electrostatic/hydrogen bonding interactions or by using avidin and biotin molecules as intermediaries between the nanocrystal and the cell.<sup>[15]</sup> In another study, qdots were modified with transferrin protein for non-specific cellular uptake, or attached to Immunoglobulin G (IgG) secondary (2°) antibodies.<sup>[16]</sup> Indirect attachment methods (i.e., avidin–biotin systems and 2° antibodies), while specific, give rise to significant cell–qdot separation distances, on the order of 30 nm,<sup>[17]</sup> which reduce electrical interfacing and binding selectivity. Primary immunofluorescence, or direct qdot/1° antibody binding, could be employed in place of secondary immunofluorescence to reduce this separation. However, the use of single antibody molecules still produces ~15 nm separation distances, and the antibodies are difficult and expensive to manufacture. By using peptides, with the shortest sequence necessary for recognition, qdots can be placed in intimate contact with the desired docking location on the cell.

Cadmium sulfide (CdS) qdots (Fig. 1A) were selected for these studies because of their straightforward single-step preparation,<sup>[18]</sup> which was easily modified for recognition molecule addition. The synthesis yields nanocrystals coated with

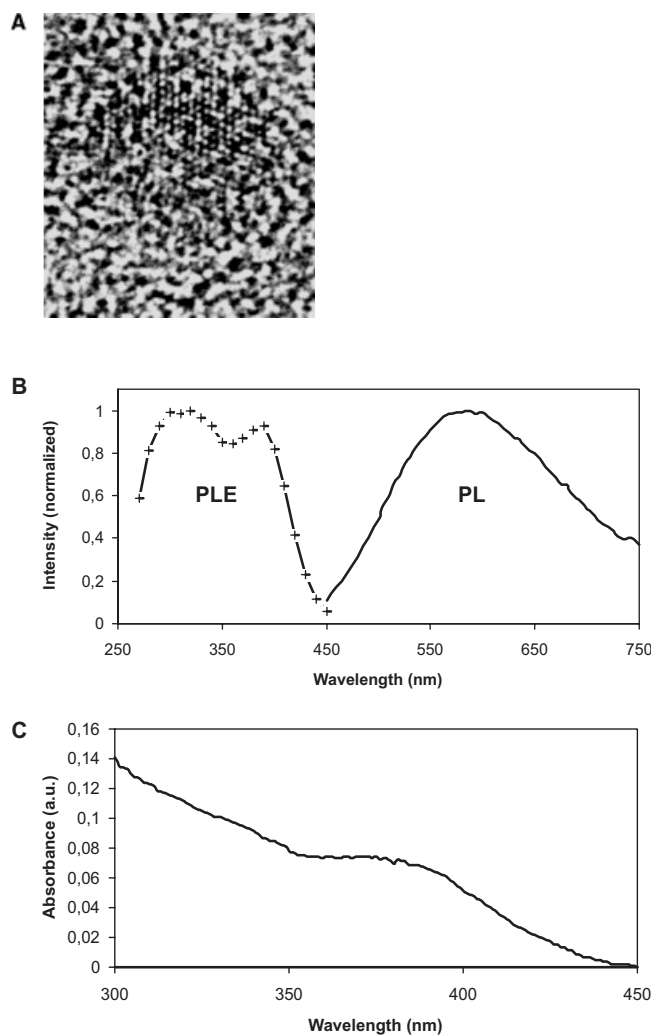


Fig. 1. Properties of CdS qdots. A) TEM of CdS qdots. The nanocrystals have an average diameter of ~30 Å with a 3.1 Å lattice spacing characteristic of wurtzite CdS. B) Room temperature PL ( $\lambda_{\text{exc}} = 400 \text{ nm}$ ) and PLE ( $\lambda_{\text{em}} = 600 \text{ nm}$ ) spectra of CdS qdots dispersed at pH 7.4 in PBS. C) Room temperature absorbance spectrum of an aqueous dispersion of CdS qdots. The exciton peak at 380 nm (3.6 eV) corresponds to an average particle size of ~30 Å [28].

exposed carboxyl groups on the particle surface, which provide water solubility and convenient linking sites for amine-terminated antibodies.<sup>[19]</sup> The CdS qdots exhibit robust photoluminescence (PL) in visible wavelengths with excitation energies (PLE) just above those that induce cell damage (Figs. 1B,C). To confirm that CdS qdots could be used successfully in conjunction with living cells, cells were exposed to qdot concentrations up to ten times in excess of that applied during labeling for five days. Cells were monitored for attachment and proliferation. No differences were seen from controls, suggesting that qdots were not toxic to cells over this time frame.

In order to extend previous work<sup>[15,16]</sup> to living cells, qdots were attached to SK-N-SH (American Type Culture Collection #HTB-11) cells using an indirect immunofluorescence approach without fixation.<sup>[20]</sup> SK-N-SH cells were chosen because they are the most prevalent human neuron studied in connection with nerve signaling. The integrin  $\alpha_v$  subunit, which binds both vitronectin and fibronectin in the form of  $\alpha_v\beta_1$  and  $\alpha_v\beta_3$  complexes, was chosen as the attachment site because of its location on the exterior of the cell<sup>[21]</sup> and the high expression levels of the  $\alpha_v\beta_1$  complex in the SK-N-SH cell type.<sup>[22]</sup> A primary antibody ( $1^\circ\text{Ab}$ ), targeting the  $\alpha_v$  portion of the receptor (anti-CD51, Accurate Chemical) was attached to the cell surface. Qdots were covalently bound to IgG secondary antibodies ( $2^\circ\text{Ab}$ ) and exposed to  $1^\circ\text{Ab}$ -labeled SK-N-SH cells (Fig. 2A). Attachment of IgG  $2^\circ\text{Ab}$  to qdots was confirmed through UV-vis absorbance spectroscopy (Fig. 3). Qdot–IgG conjugates exhibit absorbance peaks indicative of both antibody and qdots; controls do not. Attachment of the antibody complexes to the cells was verified through bright field and fluorescence optical microscopy images (Fig. 4A and B, respectively). Controls establishing the location of the  $\alpha_v$  subunits on the SK-N-SH nerve cells were performed in a series of separate experiments using IgG  $2^\circ\text{Ab}$

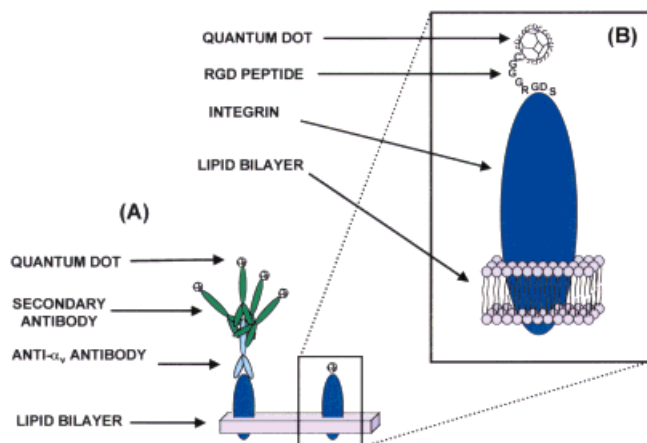


Fig. 2. Recognition molecule directed interfacing between qdots and nerve cells. A) Antibody technique. A primary antibody binds to specific cell surface receptors. Multiple secondary antibodies bind to the primary antibody. Qdots are attached to the secondary antibody through amine bonds. The expected separation distance between the qdot and the cell is  $\sim 30$  nm [17] assuming no cross-linking of secondary antibodies and a linear attachment. Actual distances may be larger. B) Peptide technique. Peptide recognition sequences bind to specific cell surface receptors. These sequences are embedded in the capping layer of the qdots producing primary attachment. Based on stoichiometric and chemical considerations, a monolayer of peptide and mercaptoacetic acid is expected. The maximum expected separation distance between the qdot and the cell is  $\sim 3$  nm [29] based on an octamer peptide recognition sequence.

attached to a common fluorescent dye, Alexa-488 (Molecular Probes), as well as published results.<sup>[23]</sup> Qdot–IgG conjugates and Ab–Alexa-488 controls demonstrate binding only on the cell exterior, where integrin proteins are located.<sup>[21]</sup> Without  $1^\circ\text{Ab}$  tagging of the cell, no observable binding of IgG/CdS qdots occurred. Also, bare qdots (i.e., qdots coated with carboxyl groups only) did not attach to the cells. These results

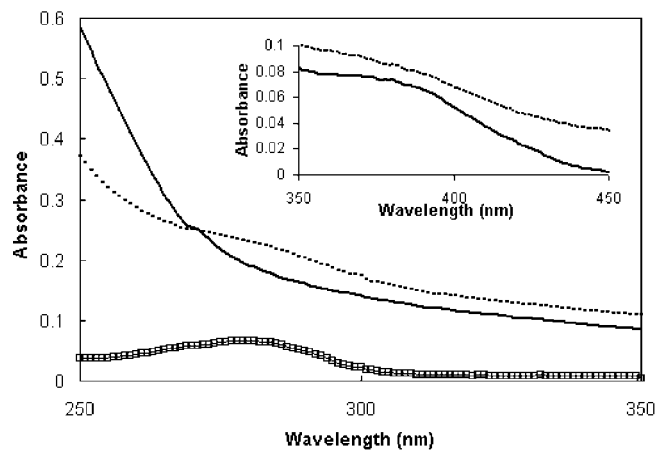


Fig. 3. Room temperature absorbance spectra of CdS qdots and CdS/antibody complexes. IgG absorbs at 280 nm (squares). After binding IgG, the qdot absorbance spectrum (dashed) also exhibits this feature, which is absent in bare qdots (solid). Inset: CdS qdots bound to IgG (dashed) exhibit the same exciton peak as bare qdots (solid) at 380 nm. The absorbance of antibody–qdot complexes is slightly reduced due to less than 100 % reaction yields. All materials were dispersed in PBS buffer (pH 7.4).

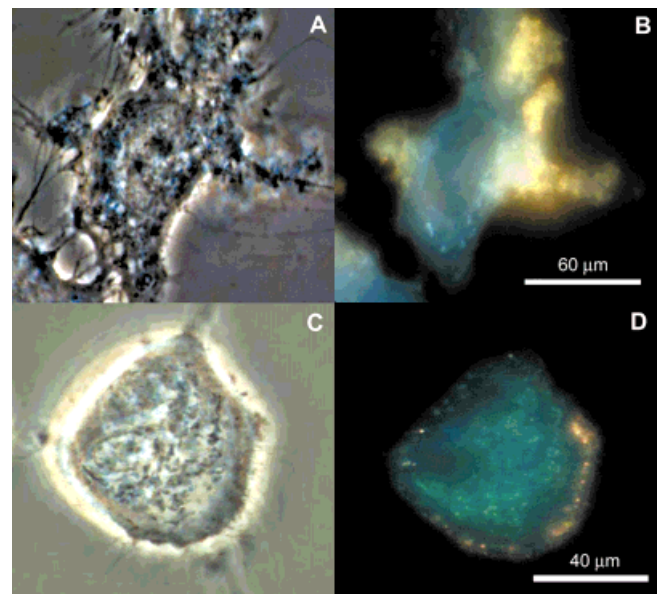


Fig. 4. Qdot attachment to neurons using antibody and peptide binding techniques. A,C) Bright-field and B,D) fluorescence ( $\lambda_{\text{exc}} = 388$  nm) optical microscopy images (Olympus IX-70 fluorescence microscope) of SK-N-SH human neuroblastoma cells labeled with CdS qdots functionalized with A,B) IgG ( $2^\circ$ ) and anti- $\alpha_v$  integrin subunit antibodies ( $1^\circ$ ) and C,D) CGGGRGDS. In (B) and (D) the blue color is the native auto-fluorescence of the cytoplasm of the cell – common in near UV excitation – and the yellow/orange color is CdS qdot luminescence.

further confirm that antibodies can be used to attach qdots to living cells.

To reduce qdot–neuron separation distances beyond those achieved with the antibody method, peptide recognition sequences were attached to the CdS qdots. The peptide sequence, RGD (Arg–Gly–Asp), is known to bind the  $\alpha_v\beta_1$  and  $\alpha_v\beta_3$  integrins, and was chosen as the recognition molecule.<sup>[24]</sup> The actual sequence used, CGGGRGDS, included the tetra-

mer, RGDS, which has been found to bind the receptor more effectively than the trimer. The terminal cysteine residue was added to covalently attach the recognition group to the particle surface through exposed surface atoms. The three intermediate glycines serve as molecular spacers to reduce steric hindrance to binding resulting from the mercaptoacetic acid groups and the nanocrystal itself (Fig. 2B). The CdS nanocrystals were synthesized by performing a single-step arrested precipitation in the presence of a 1:10 molar ratio of peptide (UT Protein Microanalysis Facility) and mercaptoacetic acid. The qdots were coated with a mixture of mercaptoacetic acid and CGGGRGDS because the mercaptoacetic acid stabilizes the qdot size and prevents unwanted particle aggregation, while the peptide groups supply sites for cell surface receptor binding. Mercaptoacetic acid and the peptide both bind to the surface of the CdS particle through thiol linkages. There are no additional binding molecules exposed, and no chemical activators added. Additionally, the peptide is added in a low stoichiometric ratio (i.e., 1:10). As such, a mixed monolayer of peptide and mercaptoacetic acid is expected to form. Additional peptide ratios were not examined, but since peptide and mercaptoacetic acid compete for binding sites, it is expected that a minimum value for binding exists.

The resulting nanocrystals coated with a mixture of peptide and stabilizing ligands exhibit PL spectra identical to those of qdots made without peptide. Peptide binding to the nanocrystals was confirmed using anisotropic fluorescence spectroscopy (or tumbling fluorescence spectroscopy), which measures changes in fluorescence between species that result from differing rotational rates in solution. Heavier molecules rotate less frequently, and therefore exhibit more anisotropy in their fluorescence. Qdots functionalized with CGGGRGDS peptide demonstrated statistically higher anisotropy than mercaptoacetic acid coated qdots ( $p < 0.0001$ , Fig. 5) indicating peptide attachment.

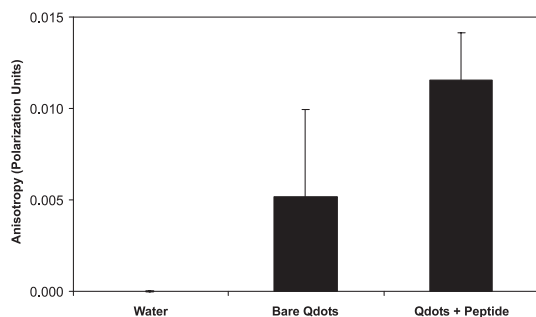


Fig. 5. Fluorescence anisotropy of CdS qdots. Peptide–qdot conjugates demonstrate a greater degree of anisotropy than bare qdots (i.e., qdots coated with only mercaptoacetic acid). This occurs due to the bulkier size of the CGGGRGDS peptide relative to mercaptoacetic acid. Peptide–qdot complexes displayed an anisotropy of  $(1.16 \pm 0.3) \times 10^{-2}$  polarization units, versus  $(5.17 \pm 4.79) \times 10^{-3}$  polarization units for the bare qdots ( $p < 0.0001$ ), confirming peptide attachment to the qdots ( $p < 0.0001$ ). Anisotropy measurements were collected with a Sim Aminco Bowman Series 2 luminescent spectrophotometer.

The procedure for attaching peptide-coated qdots to the cell resembles that used for the antibody labeling method,<sup>[20]</sup> the key difference being that only a primary incubation was needed, as peptide-coated qdots do not require an intermediate linker. Bright-field and fluorescence microscopy images of the cells following exposure to RGDS-labeled qdots (Figs. 4C and D, respectively) demonstrate interfacing between qdots and cells. A yellow/orange layer of CdS qdots coats the blue autofluorescent cell. The qdots surround the exterior of the cell as expected, given the surface location of the integrin proteins.<sup>[23]</sup> Compared to nerve cells labeled with IgG-functionalized qdots (Fig. 4B), peptide-coated qdots (Fig. 4D) experience significantly lower aggregation. Antibody-labeled qdots were more susceptible to aggregation than the peptide–qdot conjugates because of multiple secondary antibody binding and chemically induced cross-linking. This resulted in increased particle clustering compared to the peptide approach. Both antibody and peptide conjugated qdot solutions demonstrate the same PL spectra intensity. Therefore, we conclude that the reduced overall luminescence intensity from the peptide-coated qdots compared to the antibody labeled qdots results from primary, specific binding (i.e., lower signal amplification), and not from differing material properties of the qdots. To ensure that the peptide sequences indeed recognize specific receptors on the cell surface, CdS nanocrystals were synthesized with a non-binding control peptide sequence,<sup>[24]</sup> CGGGRVDS (UT Protein Microanalysis Facility), and then exposed to the nerve cells. Qdot binding was not observed in this case, as expected.

In conclusion, we have attached semiconductor qdots to living neurons utilizing both antibody and peptide recognition molecules. Unlike previous work interfacing nerve cells and electronic materials, these techniques do not require directed neuronal growth. Instead, the electronic materials are brought to the nerve cell for interfacing with specific cell surface targets. Furthermore, peptide recognition molecules provide nanometer-scale control over the targeting and separation distance between the qdot and the cell. The ability to design devices with specific, known attachment sites and controllable nanometer-length separation distances opens the door to developing future bioelectronic devices. Qdots experience an electron–hole separation<sup>[25]</sup> when optically activated, thus producing electric fields. If an electric field is strong enough, an induction response may elicit changes in the local cell potential. Previous groups have used induction to both create responses in nerve cells and measure existing signals, leading to microarray devices that communicate with the cell.<sup>[26]</sup> Future qdot-based devices could include prosthetics that control the neuron directly (e.g., through voltage inputs or electric fields), or neuronal memory devices that use the nerve’s ion channels to convey a message (e.g., saturating the ion channels in either an open or closed position). Additionally, previous work (i.e., Chan and Nie<sup>[16]</sup>) has demonstrated the ability of qdot complexes to enter the interior of the cell. Thus, it is possible that optically activated qdots could be used as intracellular probes, to study internal electrochemical reactions. To test these hy-

potheses, we are examining different qdots and receptor sites, as well as attempting to measure the electrical interaction with the cell. The future of bioelectronic devices hinges upon the ability to make controlled, specific interfaces, such as those described here, between biological components and semiconductor devices.

## Experimental

**Synthesis of CdS Quantum Dots (and Peptide Coated CdS Dots):** Dots were synthesized using previously published methods [27]. Briefly, carboxyl-stabilized CdS nanocrystals were synthesized by arrested precipitation at room temperature in an aqueous solution using mercaptoacetic acid as the colloidal stabilizer. All chemicals were used as obtained from Sigma Chemical Co. (St. Louis, MO). Nanocrystals were prepared from a stirred solution of 0.036 g CdCl<sub>2</sub> (1 mM) in 40 mL of pure water. The pH was lowered to 2 with mercaptoacetic acid, and then raised to 7 with concentrated NaOH. Then, 40 mL of 5 mM Na<sub>2</sub>S·9H<sub>2</sub>O (0.023 g) was added to the mixture. The solution turned yellow shortly after sulfide addition due to CdS nanocrystal formation. For peptide coated dots, peptide (0.041 mg/mL final solution) was added to the solution until dissolved in the initial step (with the CdCl<sub>2</sub>). Additional peptide densities were not tested, but will be the focus of future studies aimed at improving binding between the cell and the qdots.

**CdS Morbidity Studies:** SK-N-SH neuroblastoma cells (American Type Culture Collection #HTB-11) were incubated with CdS dots at concentrations of  $3 \times 10^{-11}$ ,  $1.5 \times 10^{-11}$ , and  $0.75 \times 10^{-11}$  M in Dulbecco's minimum essential medium (DMEM) cell culture medium (Sigma). These concentrations reflect multiples of the relative number of qdots added to the cells in the attachment procedure, up to ten times in excess. After adjustment to biocompatible salt concentrations (9 g/L), cell death did not occur with CdS qdot addition. Cells were studied for five days for proliferation and attachment. No differences from controls were observed.

**Conjugation of Quantum Dots to IgG Antibody:** Goat IgG antibody (Jackson Immunochemistry) was covalently linked to CdS qdots at the carboxyl terminus of the qdot capping ligands. Antibody was added to MES (2-(*N*-morpholino)ethanesulfonic acid, Sigma) 50 mM buffer at a concentration of 0.3 mg/mL. Then, an equal volume of 1.2 μM (80 mL batch diluted to 480 mL) qdots was added to the solution. After a 15 min incubation period, EDAC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, Sigma) was added at 4 mg/mL. Next, the pH was adjusted to pH 6.5 ± 0.2. Following 2 h in an orbital shaker, the reaction was quenched using glycine at 7.5 mg/mL. Conjugated qdots were isolated via repeated centrifugation (3000 g) and stored in phosphate buffered saline (PBS) at pH 7.4. Control experiments exposing qdots to antibody without EDAC revealed noticeable physisorption of IgG on qdots, as evidenced by pellet formation during centrifugation. However, absorbance measurements of these nanocrystals revealed substantially less IgG binding than in the presence of EDAC. Raising the pH above 6.5, the physisorbed antibody dissociated from the nanocrystals. Therefore, prior to all nerve cell labeling experiments, the nanocrystals were transferred to 1 mL of PBS (pH 7.4). As a result of the crosslinking chemistry in the qdot–antibody conjugation step, some agglomeration of particles occurred; however, the aggregates were small enough to remain suspended in solution.

**Attachment of Quantum Dot-Complexes to Cells:** Qdot-complexes were attached to cells using standard immunocytology techniques [20]. Briefly, cells were placed on 22 × 22 mm no. 1 thickness coverslips using imaging chambers (Sigma) to retain fluid. Cells were cultured in DMEM media (Sigma) at 37 °C and 5% CO<sub>2</sub> in sterile conditions. After the cells attained ~70% confluency, cells were washed with 10 mM PBS (pH 7.4) five times. Then, the cells were blocked with 5% bovine serum albumin (BSA) in PBS (BSA–PBS) for 30 min at 4 °C. Following blocking, cells were washed five times in PBS.

For antibody attachment, primary antibody was added at 10 μg/mL in BSA–PBS and incubated for 30 min at 4 °C. Cells were then washed five times with PBS. Then, antibody–qdot conjugate was added to cells to fill the imaging chamber (~0.25 mL/chamber). Cells were incubated for 30 min at 4 °C then washed with PBS five times. For peptide attachment, the imaging chamber was filled with peptide–qdot conjugate solution (~0.25 mL/chamber) taken from the 80 mL batch described above. Cells were incubated for 30 min at 4 °C, then washed five times with PBS. Following staining, cells were stored in Dulbecco's PBS (with Ca<sup>2+</sup> and Mg<sup>2+</sup>, GIBCO) at 4 °C.

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## Supramolecular Octupolar Self-Ordering Towards Nonlinear Optics\*\*

By Hubert Le Bozec, Thomas Le Bouder, Olivier Maury, Arnaud Bondon, Isabelle Ledoux,\* Sandrine Deveau, and Joseph Zyss

Dendrimers are particularly interesting because of their nanoscopic dimensions and their regular, well-defined, and highly branched three-dimensional architecture.<sup>[1–3]</sup> In contrast to polymers, these new types of macromolecules can be

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