SCLM origin of these peridotites is the close spatial proximity of mantle with such widely varying extraction ages, when every other geochemical aspect of the peridotites suggests they are petrogenetically related (*9, 10*).

These Os isotope data indicate that unusually old depleted mantle is stranded within subduction zones. Our preferred interpretation is that the peridotites analyzed are from the oceanic mantle, which suggests that portions of the upper mantle are less radiogenic in Os isotopes than previously recognized.

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11. The surfaces of the rock samples were cleaned to remove any obvious alteration material. The samples were then milled in an agate Tema until a very fine powder was produced. Powder (2 g) was spiked and digested with aqua regia in a Carius tube. Os and Re were separated using a solvent extraction scheme and were purified using a microdistillation method and small anion columns, respectively. Os concentrations, $^{187}Os/^{188}Os$ ratios, and Re concentrations were measured as negative ions on a Finnigan 261 mass spectrometer. Analytical methods are presented in detail elsewhere [A. S. Cohen and F. G. Waters, *Anal. Chim. Acta* **332**, 269 (1996)]. Precision of the $^{187}Os/^{188}Os$ ratios, based on counting statistics, ranges from 0.03 to 0.13% (2σ). Standards run during the course of this work are reproduced at a precision of 0.2%. A full procedural replicate analysis of one sample agreed within 0.15%. Unfortunately, there was a considerable difference in the Os concentration between the replicates, suggesting some heterogeneity in distribution of Os in the sample. However, the fact that the replicates have identical $^{187}Os/^{188}Os$ ratios supports the notion that there is negligible Re in the peridotites. Procedural blanks for Os during the course of this work varied from 16 pg to less than 2 pg. Blank corrections ranged from 0.02 to 1.5%. Re concentrations were negligible (<10 ppt) and were swamped by the Re blank.
12. REE data from (*10*); the amphibole-bearing harzburgite has a smooth REE pattern increasing from La to Lu. By contrast, all the other samples have U-shaped patterns with variable light REE enrichment.
13. Seawater alteration and sea-floor weathering processes both add radiogenic Os to peridotites (*5*). Drilled peridotites that have not weathered on the sea floor are generally not affected by Os addition (*5*). Roy-Barman and Allègre leached an ODP-drilled peridotite with oxalic acid and found no difference in the $^{187}Os/^{188}Os$ ratio of the unleached and leached sample. The peridotites in this study are serpentinized but are all from deep sections of the drill core. No manganoan coating was observed on the peridotites. Moreover, it has been suggested that serpentinization of these peridotites is not by seawater alteration at a ridge crest, but by fluids from the underlying subducting plate [M. J. Mottl, *Proc. ODP Sci. Res.* **125**, 373 (1992)].
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15. Al_2O_3 data from (*10*).
16. In the model age calculation, the peridotite is assumed to contain no Re, and therefore the $^{187}Os/^{188}Os$ ratio of the peridotite has not changed because the Re was extracted during an ancient melting event. The age is calculated from the time in the past that the measured $^{187}Os/^{188}Os$ ratio intersects a chondritic Re-Os evolution curve with a present-day $^{187}Os/^{188}Os$ ratio of 0.12757 and a $^{187}Re/^{188}Os$ ratio of 0.3972 [R. J. Walker, R. W. Carlson, S. B. Shirey, F. R. Boyd, *Geochim. Cosmochim. Acta* **53**, 1583 (1989)]. Recent work suggests that the putative primitive upper mantle has an $^{187}Os/^{188}Os$ ratio of 0.1290 and a $^{187}Re/^{188}Os$ ratio of 0.423 [T. Meisel, R. J. Walker, J. W. Morgan, *Nature* **383**, 517 (1996)]. Using these parameters gives slightly older (100 Ma) model ages but does not affect our conclusions.
17. T_{RD} model ages from DMM are calculated in a similar fashion to (*16*) except that the $^{187}Os/^{188}Os$ ratio of modern-day DMM is taken to be 0.1246 (*5*) and that it is extracted from a chondritic mantle at 2500 Ma. This calculation depends on the age of extraction of the DMM reservoir from the primitive mantle, but T_{RD} from DMM always gives a younger model age than does T_{RD} from the chondritic mantle.
18. The refectory nature of the Leg 125 peridotites (highly forsteritic olivine, low Al_2O_3 contents) means that its density is still slightly lower than that of ambient fertile mantle, even when the peridotites have cooled substantially. Therefore, it is relatively buoyant and will become stabilized (see W. L. Griffin, S. Y. O'Reilly, C. G. Ryan, O. Gaul, D. A. Ionov, *AGU Spec. Monogr.*, in press).
19. Modeling of the amount of melt extraction is as follows: For each peridotite, an initial Re content is assigned using its Os content and the assumption that it has a chondritic Re/Os ratio of 0.8. The simplest approach is to assume that Os has a bulk partition value of 1, so the Os concentration does not change during melting. Using more realistic Os partition values does not markedly change the conclusion of the calculations. The Re concentration and therefore the $^{187}Re/^{188}Os$ ratio is changed such that the sample would evolve from the chondritic mantle curve (*15*) to its measured $^{187}Os/^{188}Os$ ratio for any given extraction date. The amount of melting is then calculated using the observation that Re and Al_2O_3 correlate linearly during partial melting and that Re is completely extracted after ~25% partial melting. Although modeling assumptions change the absolute values calculated, each calculation is internally consistent and the relative difference in partial melting between samples is also consistent.
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26. We thank J. Bartlett for assistance in the laboratory. I.J.P. is funded by a Natural Environment Research Council (NERC)/ODP Fellowship, and isotope facilities at the Open University are supported by NERC.

10 July 1998; accepted 18 August 1998

Semiconductor Nanocrystals as Fluorescent Biological Labels

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Semiconductor nanocrystals were prepared for use as fluorescent probes in biological staining and diagnostics. Compared with conventional fluorophores, the nanocrystals have a narrow, tunable, symmetric emission spectrum and are photochemically stable. The advantages of the broad, continuous excitation spectrum were demonstrated in a dual-emission, single-excitation labeling experiment on mouse fibroblasts. These nanocrystal probes are thus complementary and in some cases may be superior to existing fluorophores.

Fluorescence is a widely used tool in biology. The drive to measure more biological indicators simultaneously imposes new demands on the fluorescent probes used in these experiments. For example, an eight-color, three-laser system has been used to measure a total of 10 parameters on cellular antigens with flow cytometry (*1*), and in cytogenetics, combinatorial labeling has been used to generate 24 falsely colored probes for spectral karyotyping (*2*). Conventional dye molecules impose stringent requirements on the optical systems used to make these measurements; their narrow excitation spectrum makes simultaneous excitation difficult in most cases, and their broad emission spectrum with a long tail at red wavelengths (Fig. 1A) introduces spectral cross talk between different detection channels, making quantita-

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tion of the relative amounts of different probes difficult. Ideal probes for multicolor experiments should emit at spectrally resolvable energies and have a narrow, symmetric emission spectrum, and the whole group of probes should be excitable at a single wavelength (*3, 4*).

In semiconductor nanocrystals, the absorbance onset and emission maximum shift to higher energy with decreasing size (*5*). The excitation tracks the absorbance, resulting in a tunable fluorophore that can be excited efficiently at any wavelength shorter than the emission peak yet will emit with the same characteristic narrow, symmetric spectrum regardless of the excitation wavelength (Fig. 1B). Variation of the material used for the nanocrystal and variation of the size of the nanocrystal afford a spectral range of 400 nm to 2 μm in the peak emission (Fig. 2), with typical emission widths of 20 to 30 nm [full width at half maximum (FWHM)] in the visible region of the spectrum and large extinction coefficients in the visible and ultraviolet range ($\sim 10^5$ M^{-1} cm^{-1}). Many sizes of nanocrystals may therefore be excited with a single wavelength of light, resulting in many emission colors that may be detected simultaneously.

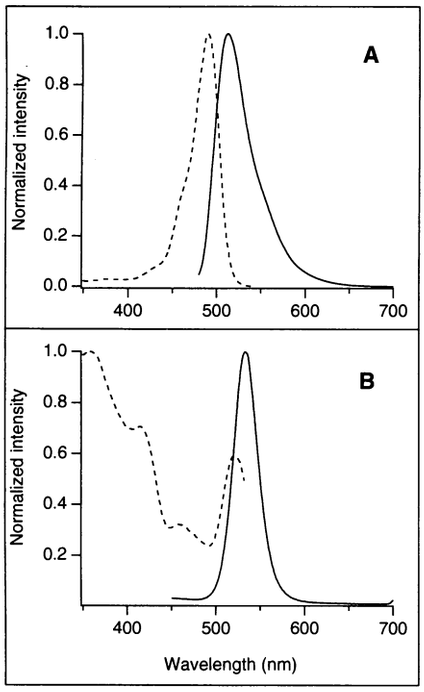

Fig. 1. Excitation (dashed) and fluorescence (solid) spectra of (**A**) fluorescein and (**B**) a typical water-soluble nanocrystal (NC) sample in PBS. The fluorescein was excited at 476 nm, and the NC at 355 nm. Excitation spectra were collected with detection at 550 nm (fluorescein) and 533 nm (NC) because of the difference in emission spectra. The nanocrystals have a much narrower emission (32 nm compared with 45 nm at half maximum and 67 nm compared with 100 nm at 10% maximum), no red tail, and a broad, continuous excitation spectrum.

Metallic and magnetic nanocrystals, with the appropriate organic derivatization of the surface, have been used widely in biological experiments (*6–11*). The use of semiconductor nanocrystals in a biological context is potentially more problematic because the high surface area of the nanocrystal might lead to reduced luminescence efficiency and photochemical degradation. Bandgap engineering concepts borrowed from materials science and electronics have led to the development of core-shell nanocrystal samples with high, room temperature quantum yields (>50%) (*12–14*) and much improved photochemical stability. By enclosing a core nanocrystal of one material with a shell of another having a larger bandgap, one can efficiently confine the excitation to the core, eliminating nonradiative relaxation pathways and preventing photochemical degradation. The synthesis of the semiconductor nanocrystals and the growth of the shell by methods from the literature yield gram quantities of a variety of materials with a narrow size distribution (5%), coated with a surfactant but soluble only in nonpolar solvents (*15, 16*).

Biological applications require water-soluble nanocrystals. We have extended the chemistry of the core-shell systems by adding a third layer of silica that makes the core-shell water soluble, similar to a procedure detailed for coating gold and cadmium sulfide nanocrystals (*17, 18*). This strategy has a number of advantages compared with strategies that use a single direct bond to the surface of the nanocrystal: the multivalency of an extensively polymerized polysilane ensures that the nanocrystals stay soluble in spite of the potential loss of bound thiol. Furthermore, the chemistry of silica is well characterized and widely used to functionalize supports for chromatography. Modification of the silica surface with different groups can be used to control the interactions with the biological sample. The core-shell nanocrystals prepared in this manner are soluble and stable in water or buffered solution, and they retain a fairly large quantum yield [up to 21%, comparable to some conventionally used fluorescent dyes that have yields between 14 and 71% (*3*)] (Fig. 2B). Further developments in the bandgap engineering of nanocrystals and modifications to the silanization chemistry are expected to result in higher quantum yields and improvement of other emission properties (*19*). This chemistry has been applied to a number of different sized core-shell nanocrystals, generating a spectrally tuned family of probes, all of which are amenable to the same modification chemistry (in contrast to organic dyes, for which specific chemistries must be developed on a case by case basis).

To establish the utility of nanocrystals for biological staining, we fluorescently labeled 3T3 mouse fibroblast cells using two different size CdSe-CdS core-shell nanocrystals enclosed in a silica shell (*20*). The smaller nanocrystals (2-nm core) emitted green fluorescence (maximum 550 nm, 15% quantum yield), the larger (4-nm core), red fluorescence (maximum 630 nm, 6% quantum yield). The surface of the nanocrystals may be tailored to interact with the biological sample either through electrostatic and hydrogen-bonding interactions or through a specific ligand-receptor interaction, such as the avidin-biotin interaction (*21*). As an example of the former, nanocrystals coated with trimethoxysilylpropyl urea and acetate groups were found to bind with high affinity in the cell nucleus. This nuclear binding could be suppressed with an anionic silane reagent [3-(trihydroxysilyl)propyl methylphosphonate] or by incubating with the nanocrystals in a 0.2% SDS solution. This property was used to "stain" the nucleus with the green-colored nanocrystals, relying on the silanized nanocrystal surface to control the binding.

The avidin-biotin interaction, a model

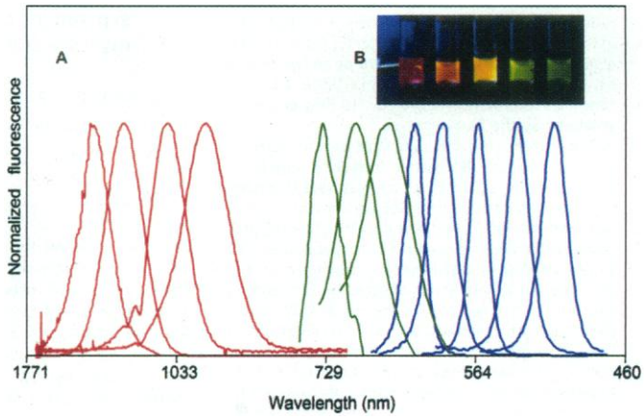

Fig. 2. (**A**) Size- and material-dependent emission spectra of several surfactant-coated semiconductor nanocrystals in a variety of sizes. The blue series represents different sizes of CdSe nanocrystals (*16*) with diameters of 2.1, 2.4, 3.1, 3.6, and 4.6 nm (from right to left). The green series is of InP nanocrystals (*26*) with diameters of 3.0, 3.5, and 4.6 nm. The red series is of InAs nanocrystals (*16*) with diameters of 2.8, 3.6, 4.6, and 6.0 nm. (**B**) A true-color image of a series of silica-coated core (CdSe)–shell (ZnS or CdS) nanocrystal probes in aqueous buffer, all illuminated simultaneously with a handheld ultraviolet lamp.

system for ligand-receptor binding, was used here to specifically label the F-actin filaments with red nanocrystal probes. Biotin was covalently bound to the nanocrystal surface (*22*), and the biotinylated nanocrystals were used to label fibroblasts, which had been incubated in phalloidin-biotin and streptavidin. One round of amplification was carried out by incubating the sample with streptavidin and then with red biotinylated nanocrystals once again.

The resulting samples were imaged with both conventional wide-field and laser-scanning confocal fluorescence microscopes. In contrast to conventional multicolor dye imaging, light from a mercury lamp with a fluorescein isothiocyanate excitation filter and a single long-pass detection filter (515 nm) were used with the wide-field microscope to see both colors at one time. The green and red labels were clearly spectrally resolved to the eye and to a color Polaroid camera. Nonspecific labeling of the nuclear membrane by both the red and the green probes resulted in a yellow color. The red actin filaments, however, were specifically stained. These filaments were not visible or were only very faintly visible in the control experiments lacking phalloidin-biotin. The penetration of the green probes into the nucleus and specific red staining of the actin fibers is readily visible in Fig. 3. Over repeated scans, the nanocrystal-labeled samples showed very little photobleaching, far less than with conventional dye molecules (Fig. 4).

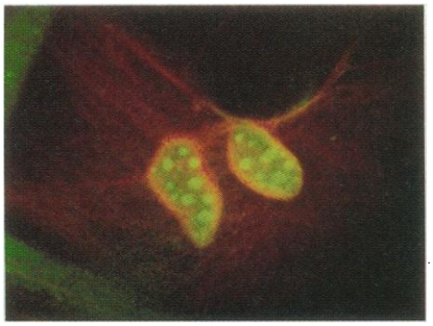

Fig. 3. Cross section of a dual-labeled sample examined with a Bio-Rad 1024 MRC laser-scanning confocal microscope with a 40× oil 1.3 numerical aperture objective. The mouse 3T3 fibroblasts were grown and prepared as described in (*27*). A false-colored image was obtained with 363-nm excitation, with simultaneous two-channel detection (522DF 35-nm FWHM narrow-pass filter for the green, and a 585-nm long-pass filter for the red). Image width: 84 μm.

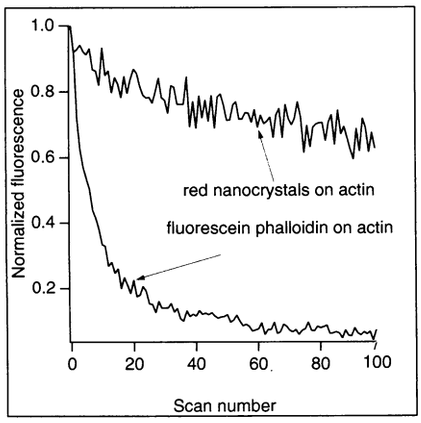

Fig. 4. Sequential scan photostability comparison of fluorescein-phalloidin–labeled actin fibers compared with nanocrystal-labeled actin fibers. Fluorescein was excited at 488 nm and the nanocrystals at 363 nm by a laser scanning confocal microscope with a 12-μs dwell time and ~20-mW power for each laser. The average intensity of four pixels was followed in each sample through 100 successive scans and normalized to its initial value. The intensity of the fluorescein drops quickly to autofluorescence levels, whereas the intensity of the nanocrystals drops only slightly.

The development of nanocrystals for biological labeling opens up new possibilities for many multicolor experiments and diagnostics. Further, it establishes a class of fluorescent probe for which no small organic molecule equivalent exists. The tunability of the optical features allows for their use as direct probes or as sensitizers for traditional probes. These nanocrystals also have a long fluorescence lifetime (hundreds of nanoseconds) (*23*), which can allow for time-gated detection for autofluorescence suppression (*24*). Further developments such as direct immunolabeling, in situ hybridization, and incorporation into microspheres will be important for applications such as cytometry and immunocytobiology. In addition, nanocrystal probes may prove useful for other contrast mechanisms such as x-ray fluorescence, x-ray absorption, electron microscopy, and scintillation proximity imaging, and the use of far red– or infrared-emitting nanocrystals (InP and InAs) as tunable, robust infrared dyes is another possibility.

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20. For the synthesis of water-soluble core-shell nanocrystals, 2 to 10 mg of CdSe-CdS (*13*) and CdSe-ZnS (*25*) core-shell nanocrystals were homogeneously dispersed in 2 ml of degassed *n*-butanol then precipitated with anhydrous methanol; addition of a roughly equal mass of trioctylphosphineoxide and 1 hour of heating at 100°C were necessary to disperse the CdSe-CdS. The precipitate was dissolved in 120 ml of a solution of 0.17% (v/v) (3-mercaptopropyl)trimethoxysilane in 25% dimethyl sulfoxide (DMSO) in methanol, the pH of which was adjusted to 10 to 11 with $(CH_3)_4NOH \cdot 5H_2O$. After overnight stirring, the solution was diluted with 100 ml of methanolic $(CH_3)_4NOH \cdot 5H_2O$, at pH 10 and gently refluxed at 69°C for 25 to 30 min. After the preparation was cooled, 200 ml of a 10% water and methanol solution containing 400 μl of trimethoxysilyl propyl urea and 40 μl of 3-aminopropyltrimethoxysilane was added, stirred for 2 hours, then heated to reflux at 69°C for less than 5 min and cooled. Next, 40 ml of a 10% chlorotrimethylsilane solution, basified with $(CH_3)_4NOH \cdot 5H_2O$, was added, the preparation stirred for another 2 hours, then concentrated partially at 60°C in vacuo to an oily solution. This solution was precipitated to a greasy solid with 50% acetone in isopropanol. This solid was redissolved in water and buffered solution.
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23. The emission from the nanocrystals is due to a transition that would be spin forbidden in a system of light atoms. Because of strong spin-orbit coupling and quantum confinement effects, the emission has a radiative rate that is on the order of 10^7 s^{-1}. In this report, we refer to this emission as the nanocrystal fluorescence [M. Nirmal *et al.*, *Phys. Rev. Lett.* **75**, 3728 (1995)].
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27. Mouse 3T3 fibroblasts were grown on fibronectin-treated, formvar-coated gold grids and fixed with 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.5% Triton at room temperature. Specimens were then treated successively with unbiotinylated green nanocrystals, phalloidin-biotin (Molecular Probes) (15 min), streptavidin (25 μg/ml) (30

min), a ~100 nM solution of biotinylated red nanocrystals (30 min), again streptavidin (30 min), then once more with the red biotinylated nanocrystals (30 min). At least three PBS or Superblock-PBS (Pierce) rinse steps (5 min each) were performed between each incubation. Samples were mounted on microscope slides in PBS.

28. We would like to thank X. Peng, J. Gray, C. Bertozzi, and P. Schultz for many helpful discussions. The photoluminescence spectra of InAs nanocrystals, InP nanocrystals, and CdSe nanocrystals in Fig. 2 were provided by J. Wickham, U. Banin, and X. Peng. Supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Materials Sciences, of the U.S. Department of Energy under contract number DE-AC03-76SF00098. M.B. acknowledges support from a NSF graduate research fellowship.

27 May 1998; accepted 20 August 1998

Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection

Warren C. W. Chan and Shuming Nie*

Highly luminescent semiconductor quantum dots (zinc sulfide–capped cadmium selenide) have been covalently coupled to biomolecules for use in ultrasensitive biological detection. In comparison with organic dyes such as rhodamine, this class of luminescent labels is 20 times as bright, 100 times as stable against photobleaching, and one-third as wide in spectral linewidth. These nanometer-sized conjugates are water-soluble and biocompatible. Quantum dots that were labeled with the protein transferrin underwent receptor-mediated endocytosis in cultured HeLa cells, and those dots that were labeled with immunomolecules recognized specific antibodies or antigens.

The development of sensitive nonisotopic detection systems has substantially impacted many research areas, such as DNA sequencing, clinical diagnostics, and fundamental molecular biology (*1*). These systems aim to solve the problems of radioactive detection (for example, health hazards and short lifetimes) and open new possibilities in ultrasensitive and automated biological assays. Current nonisotopic detection methods are mainly based on organic reporter molecules that undergo enzyme-linked color changes or that are fluorescent, luminescent, or electroactive (*1*). We have developed a class of nonisotopic detection labels by coupling luminescent semiconductor quantum dots (QDs) to biological molecules. In this design, nanometer-sized QDs are detected through photoluminescence, and the attached biomolecules recognize specific analytes, such as proteins, DNA, or viruses. These nanoconjugates are biocompatible and are suitable for use in cell biology and immunoassay. At the present level of development, however, the QDs are not sufficiently monodisperse, and intermittent photon emission could cause statistical problems at the single-dot level.

Molecular conjugates of luminescent QDs are expected to offer substantial advantages over organic dyes. The properties of QDs result from quantum-size confinement, which occurs when metal and semiconductor particles are smaller than their exciton Bohr radii (about 1 to 5 nm) (*2–4*). Recent advances have resulted in the large-scale preparation of relatively monodisperse QDs (*5–7*), the characterization of their lattice structures (*4*), and the fabrication of QD arrays (*8–12*) and light-emitting diodes (*13*, *14*). For example, CdSe QDs passivated with a ZnS layer are strongly luminescent (35 to 50% quantum yield) at room temperature, and their emission wavelength can be tuned from the blue to the red wavelengths by changing the particle size (*7*, *15*). However, these luminescent QDs are prepared in organic solvents and are not suitable for biological application. Additionally, it is unclear how to attach biomolecules to a QD and still maintain their activity.

We have solved these problems by using mercaptoacetic acid for solubilization and covalent protein attachment. When reacted with ZnS-capped CdSe QDs in chloroform, the mer-

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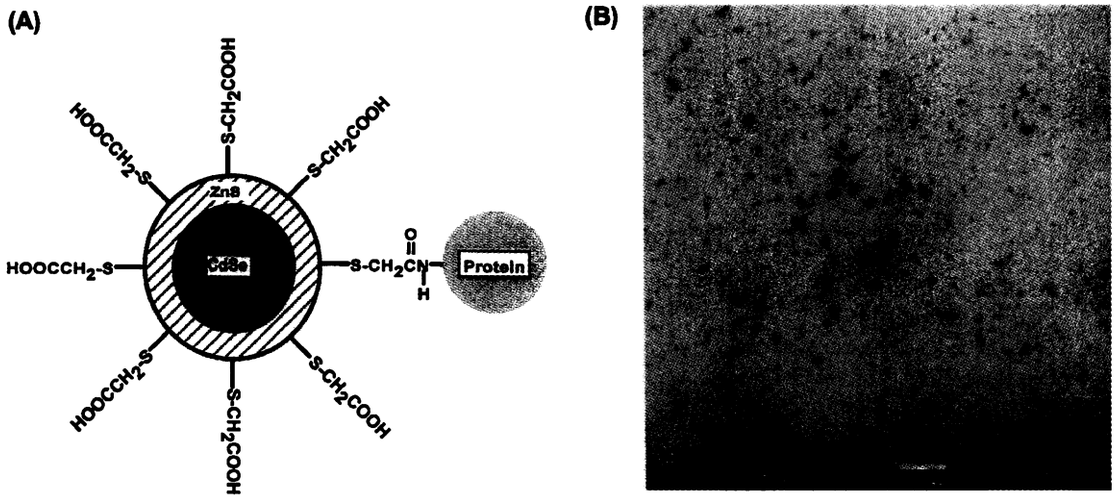

Fig. 1. (**A**) Schematic of a ZnS-capped CdSe QD that is covalently coupled to a protein by mercaptoacetic acid. (**B**) TEM of QD-transferrin (an iron-transport protein) conjugates. Scale bar, 100 nm. Clusters of closely spaced particles were mainly formed by sample spreading and drying on the carbon grid and not by chemical cross-linking. ZnS-capped QDs with a CdSe core size of 4.2 nm were prepared according to the procedure developed by Hines and Guyot-Sionnest (*7*). The colloidal QDs were dissolved in chloroform and were reacted with glacial mercaptoacetic acid (~1.0 M) for 2 hours. An aqueous phosphate-buffered saline (PBS) solution (pH 7.4) was added to this reaction mixture at a 1:1 volume ratio. After vigorous shaking and mixing, the chloroform and water layers separated spontaneously. The aqueous layer, which contained mercapto-coated QDs, was extracted. Excess mercaptoacetic acid was removed by four or more rounds of centrifugation. The purified QDs were conjugated to transferrin and IgG with the cross-linking reagent ethyl-3-(dimethylaminopropyl)carbodiimide. Standard protocols were followed (*16*), except the excess proteins were removed by repeated centrifugation. The purified conjugates were stored in PBS at room temperature.