A Simple Method for Preparing Spectrally Encoded Magnetic Beads for Multiplexed Detection

Robert Wilson,[†,*] David G. Spiller,[‡] Ian A. Prior,[§] Kees. J. Veltkamp,[⊥] and Alistair Hutchinson[∥]

[†]Department of Chemistry, Liverpool University, Liverpool L69 7ZD, U.K., [‡]Centre for Cell Imaging, School of Biological Sciences, Liverpool University, Liverpool L69 7ZD, U.K., [§]Department of Physiology, School of Biological Sciences, Liverpool University, Liverpool L69 7ZD, U.K., [⊥]Department of Earth and Ocean Sciences, Liverpool University, Liverpool L69 7ZD, U.K., and [∥]Defence Science and Technology Laboratory (DSTL), Fort Halstead, Sevenoaks, Kent TN14 7BP, U.K.

ABSTRACT This report describes a simple method for preparing encoded microspheres for use in multiplexed biological detection. In this method, hydrophobic trioctylphosphine oxide (TOPO)-capped CdSe@ZnS quantum dots (QDs) are assembled on polyamine-coated microspheres in chloroform and encapsulated in an outer shell of silica nanoparticles functionalized with a specific recognition surface. Because TOPO-capped QDs are assembled instead of their water-soluble equivalents, the microspheres are highly luminescent. The amount of QDs assembled depends only on the surface area of the substrate, and therefore, the photoluminescence intensity increased uniformly in proportion to the number of QD layers assembled. The outer shell of silica nanoparticles confers stability on the assembled QDs but has no effect on their photoluminescence because it is transparent to excitatory and emitted light. It was activated with aminosilane and functionalized with a recognition surface of protein antigens using disulfide exchange chemistry. Magnetic beads furnished with spectral codes of up to three colors of QDs matched to specific recognition surfaces were used as multianalyte sensors for serum proteins.

KEYWORDS: paramagnetic · layer-by-layer · covalent · self-assembly · nanoparticles · suspension array · immunoassay

The design of new materials based on nanoscale components is a major focus of current research. Many of these new materials require the structure to be organized into multilayer thin films with internal dimensions on the same scale as those of the individual components. Of particular interest is the preparation of thin films of photoluminescent semiconductor quantum dots (QDs) because of their potential applications in optoelectronics and sensing.[1,2] Such films can be prepared by spin-coating,[3] spin-casting,[4] controlled precipitation,[5] chemical deposition,[6] Langmuir–Blodgett transfer,[7] and Langmuir−Schaefer deposition,[8] but none of these techniques offer the same prospect of fine control and cost-effective simplicity provided by LBL (layer-by-layer) self-assembly.[9,10] LBL methods have been used to assemble QDs on planar[11−13] and spherical surfaces[14−19] for graded semiconductor films[11,12] and biological labels.[14−19] A common feature of all these reports is that they are based on water-soluble QDs capped with mercapto ligands assembled on surfaces coated with oppositely charged polymers in aqueous solutions. Quantum dots capped in this way have reduced luminescence intensities,[20,21] and although they are moderately stable in slightly basic solutions, the ligands slowly dissociate leading to precipitation.[22] Even before precipitation occurs, they may undergo changes that lead to variations in the amount of QDs that can be assembled on oppositely charged polymers. The amount of water-soluble QDs that can be assembled also depends on precise control over conditions such as pH and ionic strength. In this report, we show how these problems can be eliminated by assembling trioctylphosphine oxide (TOPO) capped QDs on polyamine-coated surfaces in an aprotic solvent. We also show how microspheres on which QDs have been assembled in this way can be coated with an outer shell of silica nanoparticles and provided with a recognition surface of covalently attached protein antigens. In order to demonstrate a practical application of these microspheres, we show how they can be encoded with up to three colors of QDs and used for multiplexed detection.

RESULTS AND DISCUSSION

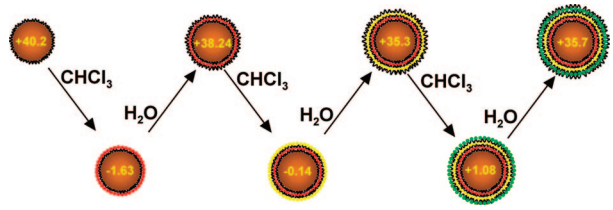
Assembly of Quantum Dots. In previous reports, when latex microspheres have been used as templates for the LBL self-assembly, they have been functionalized with sulfonate groups because the negatively charged surface groups provide a foundation for the deposition of oppositely charged polymers.[14,16] Magnetic microspheres functionalized with sulfonate groups cannot be made due to metal

*Address correspondence to R.Wilson@liv.ac.uk.

Received for review October 9, 2007 and accepted November 28, 2007.

Published online December 28, 2007. 10.1021/nn700289m CCC: $37.00

© 2007 American Chemical Society



Scheme 1. Assembly of QDs. TOPO-capped QDs were assembled on magnetic microspheres in $CHCl_3$, and then each layer of QDs was overcoated with a layer of PEI in H_2O. Numbers indicate ξ-potentials in millivolts at different stages in the assembly; each value is the mean of measurements made on four different samples.

sulfonate interactions, and therefore, we covalently attached a foundation layer of poly(ethylene imine) (PEI) to epoxy-functionalized microspheres. The microspheres were then dehydrated with methanol. If the surface was not dehydrated, the microspheres irreversibly aggregated when they were transferred to an aprotic solvent. After transfer, the microspheres were incubated with TOPO-capped CdSe@ZnS QDs in chloroform and then washed with chloroform and methanol. In early work, we assembled an intervening layer of PEI/PSS/PEI (PSS = poly(sodium 4-styrenesulfonate) between each layer of QDs as in aqueous LBL methods, but we later found that the same result could be achieved with only a single intervening layer of PEI. This is in contrast to the assembly of water-soluble quantum dots in aqueous solution where we found that assemblies based on single intervening layers of PEI were much less luminescent than microspheres with an intervening layer of PEI/PSS/PEI. In order to produce the same photoluminescence intensity, it was necessary to assemble three layers of water-soluble QDs for every layer of TOPO-capped QDs that was assembled in an aprotic solvent. The process is summarized in Scheme 1. Microelectrophoresis measurements showed that epoxy-functionalized microspheres had a slightly negative ξ-potential, which became positive after covalently attaching PEI and then approximately zero after assembling a layer of QDs. In previous work Nann has shown that highly luminescent water-soluble QDs can be prepared by adding PEI to TOPO-capped QDs in chloroform,[23] and Mulvaney and colleagues have shown that when microspheres coated with PEI are shaken with TOPO-capped QDs in chloroform, the QDs are transferred from the organic phase to microspheres in the aqueous phase.[24] In these methods, and the one reported here, it is suggested that some TOPO ligands are replaced by primary amines of PEI covalently bonded to the QDs. In order to assemble additional layers of QDs, each layer must be overcoated with a layer of PEI; in the absence of this layer, no further QDs can be assembled, but the deposition of PEI must be performed in aqueous solution because in chloroform the QDs are displaced by the PEI. We believe that this is because the assembled QDs are still mainly capped with TOPO so that in aqueous solution, but not chloroform, the structure is maintained by hydrophobic bonding while covalent bonds are formed between PEI and the QDs.

Commercial microspheres are encoded by internally doping them with different colors and concentrations of organic dyes. In this approach, all colors are incorporated at the same time, and therefore, it is necessary to exercise precise control over an increasing number of variables as the number of colors and intensities increase. This drawback imposes a low upper limit on the total number of codes that can be generated. In layer-by-layer approaches, each color is confined to a different layer, and therefore the only variable is the surface area of the microspheres. Provided that the microspheres are uniform in size and that the size distribution does not change as additional layers of QDs are assembled, it should be possible to generate a series of resolvable codes. In order to investigate whether this would be possible using the method reported here, we assembled five layers of QDs on planar and spherical surfaces. On planar surfaces, the luminescence increased linearly in parallel with the number of layers as shown in the Supporting Information, but the increase in intensities of the microspheres was a curve as shown in Figure 1B. When the intensities of the microspheres were plotted against the square of the layer number, however, a linear fit with a correlation coefficient of 0.998 was obtained. This suggests that the nonlinear increase is due to a gradual increase in surface area as additional layers of QDs are assembled. The intensities of the microspheres in the five groups shown in Figure 1A have coefficients of variation (CVs) of 5.5%, 6.34%, 6.4%, 6.98%, and 5.6%, respectively. These values are close enough to the manufacturer's values of 5% for the CV of the microspheres to suggest that most of the observed variation is due to their size distribution rather than imperfections in the assembly process.

Coating with a Specific Recognition Surface. In early work, we attempted to attach proteins to the microspheres by nonspecific adsorption or covalent attachment to an outer layer of polyamines, but microspheres functionalized in this way displayed relatively weak luminescence when they were interrogated with fluorescent (Cy-5 labeled) antibodies and often appeared diffuse in a way that suggested that the outer surface had begun to unravel in the presence of the nonionic detergents and protein-blocking proteins that are used in multiplexed detection. These problems were overcome by coating the microspheres with several layers of silica nanoparticles (SiNPs). This surface was then activated with an aminosilane and functionalized with protein antigens by disulfide exchange chemistry as shown in Scheme 2. Although we used an aminosilane to activate the SiNPs, a wide range of other silanes are available that could have been used to activate the surface of the microspheres with alternative chemical groups.

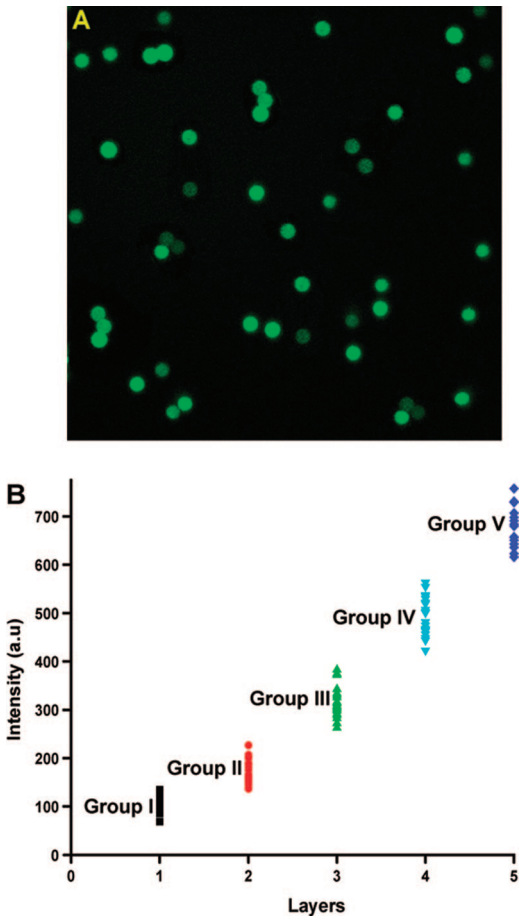


Figure 1. (A) Confocal image of approximately equal amounts of microspheres encoded with from one to five layers of green QDs and (B) graph showing the intensities of individual microspheres encoded with from one to five layers of green QDs. The CV of the intensities within each group is similar to that of the original magnetic microspheres suggesting that it could be decreased by starting with microspheres having a lower CV.

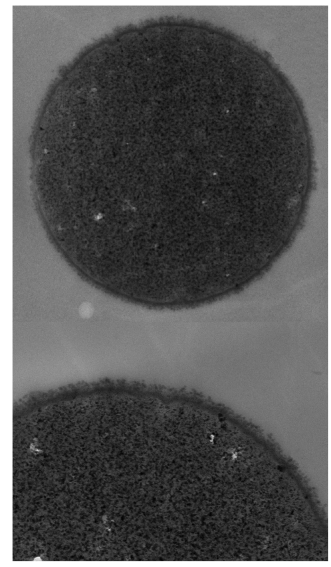
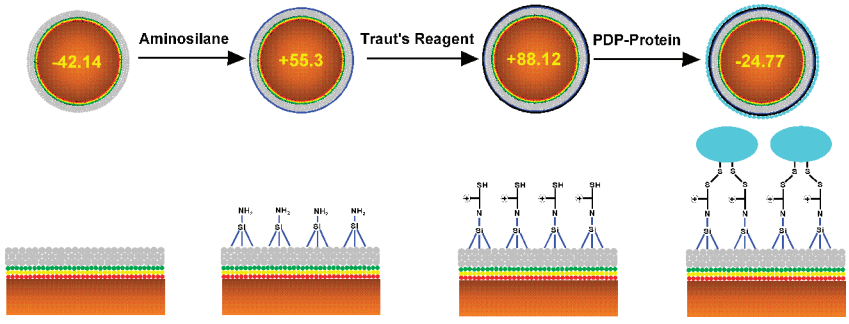


Figure 2. TEM images showing ~70 nm thick sections of microspheres embedded in epoxy resin. The core of the microspheres is made of cross-linked polystyrene packed with iron oxide nanoparticles (visible as dark specks) that allow rapid concentration of the microspheres when a small permanent magnet is applied. The inner shell (visible as a dark band) is composed of five layers of QDs that render the microspheres photoluminescent. The outer shell is composed of SiNPs functionalized with protein antigens.

The main problem that we encountered when developing a high-quality surface coating was irreversible aggregation of the aminosilanized microspheres when they were reacted with common bifunctional reagents such as disuccinimidyl suberate (DSS) and succinimidyldithiopropionate (SPDP), but we found that this could be avoided by using 2-iminothiolane (Traut's reagent).[25] This reagent simultaneously introduces a terminal thiol group and a charged side chain when it reacts with primary amines. Microelectrophoresis measurements showed that microspheres functionalized with Traut's reagent had a ξ-potential of +88 mV compared with ξ-potentials of close to zero produced by reagents such as DSS and SPDP. This difference suggests that Trauts's reagent may prevent aggregation by electrostatic repulsion. The terminal thiols are able to participate in disulfide exchange reactions that result in the formation of stable disulfide bonds between the microspheres and proteins that have be functionalized with SPDP.[26] UV/vis and photoluminescence spectra of the functionalized surface in a quartz cuvette showed that it did not have any effect on the absorbance or emission properties of the underlying QDs. Electron micrographs (Figure 2 and Supporting Information) show that microspheres have a well defined core–shell structure in



Scheme 2. Surface chemistry. Microspheres with an outer shell of SiNPs were activated with aminosilane and then reacted with Traut's reagent. This reagent introduces terminal thiol (−SH) groups and positively charged side chains that appear to be important in preventing irreversible aggregation of the microspheres. The thiol groups form disulfide covalent bonds with PDP-proteins (proteins that have been functionalized with SPDP). Numbers indicate ξ-potentials in millivolts.

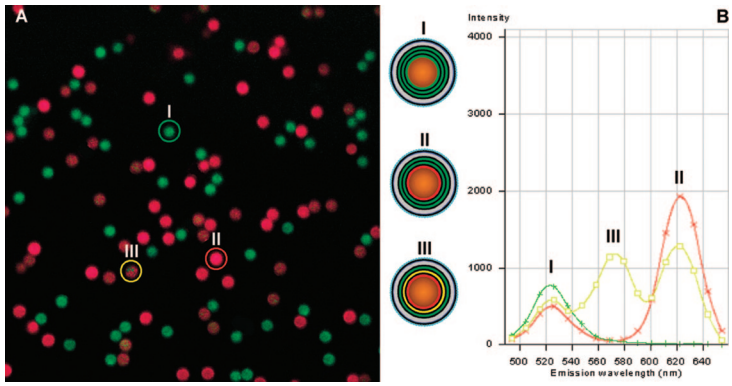


Figure 3. (A) Confocal image of three sets of microspheres encoded with (I) three layers of green QDs, (II) one layer of red QDs and two layers of green QDs, and (III) one layer of red QDs, one layer of yellow QDs, and one layer of green QDs and (B) the emission spectrum of one (circled) microsphere from each set. Color of the circle corresponds to line color of the emission spectra in panel B.

which the QDs occupy a densely packed dark layer sandwiched between the paramagnetic core and the outer shell of SiNPs. The mean thickness of this layer is 38.2 nm. In the images shown, it was assembled from five layers of green QDs having a mean diameter of about 5 nm and six layers of PEI. This suggests that each layer of the PEI contributes a mean thickness of around 2 nm. Microspheres coated with an outer shell of SiNPs and provided with a protein recognition surface were uniform in appearance and highly fluorescent when interrogated with labeled antibodies. When stored at 4 °C in darkness, they retained their activity in multiplexed assays for at least 6 months.

Multiplexed Detection with Encoded Microspheres. Figure 3 shows confocal images of three sets of microspheres and the emission spectra of one microsphere from each set. Each set of microspheres was coated with a different antigenic surface: microspheres encoded with green QDs were functionalized with ovalbumin (OVA), microspheres encoded with green and red QDs were functionalized with bovine serum albumin (BSA), and microspheres encoded with green, red, and yellow QDs were functionalized with human serum albumin (HSA).

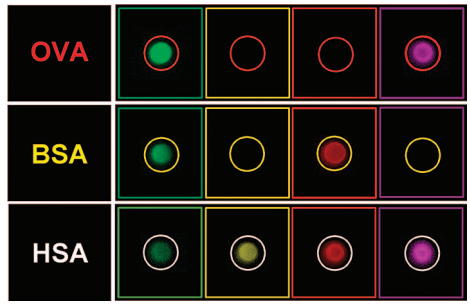
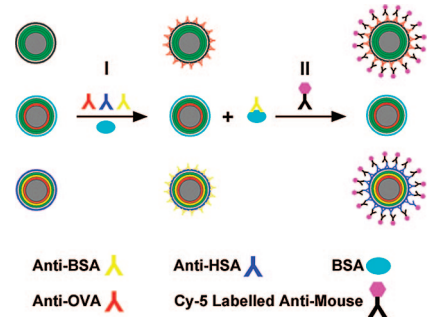


Figure 4. Table showing decoded images of one individual microsphere from each set after it has participated in the multiplexed assay shown in Scheme 3; microspheres specific for BSA are not visible when imaged through the Cy-5 window on the extreme right of the table. The original micrographs from which these images were extracted, and micrographs showing the results of other multiplexed immunoassays can be found in the Supporting Information.

Although each set of microspheres in this example could have been encoded with only one color of QD, the ability to encode with multiple colors is a key requirement for higher levels of multiplexing. Equal amounts of microspheres from each set were mixed to form a suspension array,[27–29] which was then used to screen for three different types of serum albumin. The reagent-limited format of these assays was similar to multianalyte screens for pathogens except that these are based on microspheres of different size and gold nanoparticles.[30] The assays were carried out in two stages. In the first stage, the suspension array was incubated with the sample and antibodies specific for the albumins, and in the second stage, the suspension array was incubated with fluorescent Cy-5-labeled antibodies specific for the antialbumin antibodies. The progress of a multiplexed immunoassay performed on a sample that contained BSA is shown Scheme 3. In the first stage, the suspension array was mixed with the sample and antibodies specific for BSA, HSA, and OVA; antibodies specific for HSA and OVA bound to microspheres functionalized with HSA and OVA, but the antibodies specific for BSA did not bind to microspheres functionalized with BSA. In the second stage, the suspension array was mixed with Cy-5-labeled anti-mouse antibodies; the labeled antibodies bound to microspheres specific for HSA and OVA but did not bind to microspheres specific for BSA. Images of individual microspheres that have participated in a multiplexed immunoassay performed on a sample that contained BSA are shown in Figure 4. Microspheres encoded with green (OVA) and red, yellow, and green (HSA) are fluorescent when viewed through the Cy-5 filter, but microspheres encoded with red and green (BSA) are not visible through this filter. We also used the suspension array to perform multiplexed immunoassays on



Scheme 3. Multiplexed immunoassay for serum proteins. In step I, the suspension array is incubated with a sample containing one serum protein (BSA) and antibodies specific for three different serum proteins (BSA, HSA, and OVA); no anti-BSA antibodies bind to microspheres encoded with red and green QDs because they are bound to BSA in the solution. In step II, the suspension array is incubated with fluorescent antibodies that are specific for all the antibodies used in step I, but they do not bind to microspheres encoded with green and red QDs because these microspheres did not bind anti-BSA antibodies in step I.

samples that contained (A) no added albumins, (B) HSA, (C) OVA, and (D) both BSA and OVA. The results of all these immunoassays are shown in the Supporting Information.

CONCLUSIONS

We have developed a simple method for preparing microspheres that are provided with a spectral code matched to a specific recognition surface and demonstrated their use in multiplexed detection. In this method, TOPO-capped QDs are assembled between intervening layers of high molecular weigh polyamines in an aprotic solvent. The method is less complicated than those based on water-soluble QDs, which require more QDs and more layers of intervening polymers to achieve the same result. Because the amount of QDs assembled depends only on the surface area of the microspheres and because surface does not become more disperse during the assembly process, it allows microspheres to be furnished with resolvable spectral codes. These codes were matched to a specific recognition surface by overcoating the QDs with an outer shell of SiNPs functionalized with proteins. The SiNPs conferred stability on the microspheres in the presence of detergents and blocking proteins and provided a foundation for the covalent attachment of antigens. Because the shell was optically transparent, it had no effect on the excitation or emission properties of the QDs, and because it was chemically similar to glass, it could be activated by traditional silane chemistry. In order to functionalize the shell with a biological recognition surface, a reaction scheme that prevented irreversible aggregation of the microspheres was developed. The method for assembling QDs should also be useful for preparing photoluminescent thin films for other applications in optoelectronics and sensing.

METHODS

Materials. Paramagnetic polystyrene microspheres functionalized with epoxy groups were from Dynal, Wirral, U.K.; they had a mean diameter of 4.5 μm (CV ~ 5%) and were supplied at a concentration of ~4×10^8 microspheres/mL (30 mg mL^{-1}). TOPO-coated CdSe/ZnS core–shell QDs in toluene were from Evident Technologies, Inc., Troy, NY. Silica nanoparticles (Ludox TM-40; SiNPs) were the kind gift of W.R. Grace & Co, Columbia, MA. Poly(sodium 4-styrenesulfonate) (PSS; MW 70 kDa), low molecular weight branched poly(ethylene imine) (low MW PEI; MW 25 kDa), aminopropyl triethoxysilane (APTES), glycidylpropyl trimethoxysilane, *o*-xylene (anhydrous), and *N*,*N*-diisopropylethylamine (biotech grade) were from Aldrich. Branched poly(ethylene imine) (PEI; MW 750 kDa), bovine serum albumin (BSA), human serum albumin (HSA), albumin from chicken egg white (OVA), 2-iminothiolane (Traut's reagent; HCl salt), succinimidyldithiopropionate NHS (SPDP), dithiothreitol (DTT), monoclonal anti-bovine serum albumin (anti-BSA; mouse ascites fluid), monoclonal anti-human serum albumin (anti-HSA; mouse ascites fluid), monoclonal anti-chicken albumin (anti-OVA; mouse ascites fluid), and gelatin (from cold water fish skin) were from Sigma. Cy-5-labeled monoclonal anti-mouse IgG (raised against whole molecule) antibodies were from AbCam Ltd., Cambridge, U.K. Other reagents were of Analar (or equivalent grade) or higher, and all solutions were prepared in HPLC grade water from Aldrich.

Solutions. The following solutions were used: PBS, 15 mM sodium phosphate, pH 7.4, 0.15 M NaCl; PBS-Tween, PBS containing 0.05% (v/v) Tween-20; antibody diluent, PBS-Tween containing 1 mg mL^{-1} gelatin; blocking solution, PBS containing 10 mg mL^{-1} gelatin; antibody solution I, antibody diluent containing anti-BSA, anti-HSA, and anti-OVA in which the antibodies were diluted to final IgG concentrations of ~5 μg mL^{-1}; antibody solution II, antibody diluent containing Cy-5-labeled anti-mouse at a final IgG concentration of 10 μg mL^{-1}; bicarbonate solution, 0.1 M $NaHCO_3$, pH 8.6.

Equipment. Magnetic precipitation of Dynal microspheres was carried out using an MPC-S sample concentrator (Dynal), and (slow-tilt) rotation of microspheres was carried out on a MX2 sample mixer (Dynal). UV/vis spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer LS 50B luminescence spectrometer. Epifluorescence images of encoded microspheres were acquired with a Leica DMBL fluorescence microscope equipped with a SPOT 2 camera system (SPOT Diagnostic Instruments, inc, Sterling Heights, MI) and custom filters matched to the emission peaks of Cy-5 and the QDs. Cy-5 was excited at 660 nm, and the QDs were excited at 400 nm. The image acquisition time was 250 ms. All images were acquired with a 100× oil-immersion objective lens at a total magnification of 1000×. Confocal images were acquired with a Zeiss LSM 510 laser scanning confocal microscope equipped with a Zeiss META detector. Samples for transmission electron microscopy (TEM) were prepared by adding a drop of the microsphere suspension in methanol to Agar 100 epoxy resin (Agar Scientific) and allowing it to polymerize overnight at 60 °C. Seventy nanometer sections of the polymerized resin were cut with an ultramicrotome and viewed with a Tecnal Spirit transmission electron microscope at an accelerating voltage of 100 kV. Samples for scanning electron microscopy (SEM) were prepared by placing a drop of the microsphere suspension on a standard aluminum SEM stub and allowing it to dry in air. The sample was then sputter-coated with 60% gold/palladium (Polaron E5100) and imaged with a Philips XL30 SEM at an accelerating voltage of 20 kV. Zeta potential measurements were made with a Zeta Plus zeta potential analyzer supplied by Brookhaven Instruments Ltd., Reddich, U.K.

Polymer-Coated Microspheres. One molar NaCl containing 100 mg mL^{-1} HMW PEI was mixed 1:1 (v/v) with a vortexed suspension containing 6 mg of washed epoxy microspheres in 500 μL of water and rotated overnight at room temperature. At the end of this time, the microspheres were washed with (1) 4 × 1 mL of 0.5 M NaCl, (2) 4 × 1 mL of 0.1 M sodium borate solution, (3) 4 × 1 mL of 0.1 M sodium acetate, pH 4.5, and (4) 6 × 1 mL of 0.5 M NaCl. The microspheres were then resuspended in water and stored at 4 °C.

PDP Functionalized Albumins. SPDP (0.2 mg) in 100 μL of DMF was added dropwise to a stirred 10 mg of albumin (BSA, OVA, or HSA) in 1 mL of bicarbonate solution. After being stirred for 1 h, the PDP-albumin (PDP = pyridyldithiopropionyl) was purified by gel exclusion chromatography on Sephadex G25. The PDP concentration was determined at 343 nm after reduction with DTT ($\epsilon = 8.08 \times 10^3$ M^{-1} cm^{-1}), and the protein concentration was determined from the absorbance at 280 nm (1% = 0.71) corrected for PDP. The molar ratio of PDP to albumin in the purified products was ~2:1.

Assembly of Quantum Dots on Paramagnetic Microspheres. The following protocol describes how microspheres were encoded with the first two layers of QDs; additional layers were added by repeating steps 2 and 3: (1) 20 μL (60 μg) of polymer-coated paramagnetic microspheres were magnetically precipitated and washed with 4 × 1 mL of HPLC grade water (this had a pH of

6.5) and 4 × 1 mL of methanol and then rotated in 1 mL of chloroform containing an excess of TOPO-capped QDs for 1 h; (2) the microspheres were magnetically precipitated and washed with 1 mL of chloroform, 4 × 1 mL of methanol, and 1 mL of water and then rotated for 1 h in 1 mL of a pH 8.0 solution containing 1 mg mL^{-1} PEI and 0.5 M NaCl; (3) the microspheres were washed with 4 × 1 mL of HPLC grade water and 4 × 1 mL of methanol and then rotated in 1 mL of chloroform containing an excess of TOPO-capped QDs for 1 h.

Assembly of Silica NPs on Paramagnetic Microspheres. (1) Encoded microspheres (60 μg) with outer layers of PEI/PSS/PEI were rotated for 1 h in HPLC grade water containing an excess of SiNPs; the pH of this solution was 9.5. (2) The microspheres were magnetically precipitated and washed with 4 × 1 mL of water and then rotated for 1 h in 1 mL of a pH 8.0 solution containing 1 mg mL^{-1} low MW PEI and 0.5 M NaCl. Step 1 was repeated three times, and step 2 was repeated twice giving microspheres with outer layers of SiNP/PEI/SiNP/PEI/SiNP.

Encoded Paramagnetic Microspheres Functionalized with an Antigenic Protein Surface. SiNP-capped encoded microspheres (60 μg) were washed with 4 × 1 mL of ethanol and then rotated overnight in 1 mL of a mixture of ethanol/H_2O/APTES (95:3:2 v/v). At the end of this time, the microspheres were washed with 4 × 1 mL of ethanol and resuspended in 0.5 mL of bicarbonate solution. The microspheres were then added to 0.5 mL of bicarbonate solution containing 1 mg of Traut's reagent and rotated for 30 min. At the end of this time, the microspheres were washed with 4 × 1 mL of bicarbonate solution and resuspended in 1 mL of PBS containing of 0.5 mg of PDP-albumin. After rotating overnight the microspheres were washed with 4 × 1 mL of PBS and rotated in 1 mL of blocking solution for 1 h. At the end of this time, the microspheres were washed with 4 × 1 mL of PBS and stored in darkness at 4 °C.

Multiplexed Immunoassays with a Suspension Array of Encoded Microspheres. Sample solutions were prepared by dissolving one or more of the albumins (BSA, HSA, or OVA) in antibody solution I, such that the final concentrations of the albumins were 10 μg mL^{-1}. Suspension arrays were prepared by mixing three sets of encoded microspheres. Set 1 was encoded with green QDs and functionalized with OVA. Set 2 was encoded with green and red QDs and functionalized with BSA. Set 3 was encoded with green, red, and yellow QDs and functionalized with HSA. Equal amounts of microspheres from each set in antibody diluent were mixed and diluted 1:1 with the sample solution. After being rotated for 15 min, the microspheres were magnetically precipitated and washed with 1 × 1 mL of PBS-Tween. They were then resuspended in antibody solution II and rotated for a further 15 min. At the end of this time, the microspheres were washed with 1 × 1 mL of PBS-Tween and imaged with the epifluorescence microscope.

Acknowledgment. This research was partly funded from the U.K. MoD Science Innovation and Technology Budget through the Electronic Systems Research Domain. Electron microscopy was carried out with technical support form Cornelia Muncke, Department of Physiology, Liverpool University.

Supporting Information Available: Method for assembly of quantum dots in quartz cells and figures showing UV/vis and fluorescence spectra acquired at intermediate stages in the assembly, TEM and SEM images of intermediate stages in the assembly of quantum dots and silica nanoparticles on microspheres, and fluorescence micrographs showing results of multiplexed immunoassays for albumins. This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1 Matsui, I. Nanoparticles for Electronic Device Applications: A Brief Review. *J. Chem. Eng. Jpn.* **2005**, *38*, 535–546.
2 Alivisatos, A. P.; Gu, W. W.; Larabell, C. Quantum Dots as Cellular Probes. *Annu. Rev. Biomed. Eng.* **2005**, *7*, 55–76.
3 Zhao, J. L.; Bardecker, J. A.; Munro, A. M.; Liu, M. S.; Niu, Y. H.; Ding, I. K.; Luo, J. D.; Chen, B. Q.; Jen, A. K. Y.; Ginger, D. S. Efficient CdSe/CdS Quantum Dot Light-Emitting Diodes using a Thermally Polymerized Hole Transport Layer. *Nano Lett.* **2006**, *6*, 463–467.
4 Nazzal, A. Y.; Qu, L. H.; Peng, X. G.; Xiao, M. Photoactivated CdSe Nanocrystals as Nanosensors for Gases. *Nano Lett.* **2003**, *3*, 819–822.
5 Radtchenko, I. L.; Sukhorukov, G. B.; Gaponik, N.; Kornowski, A.; Rogach, A. L.; Möhwald, H. Core-Shell Structures Formed by the Solvent-Controlled Precipitaion of Luminescent CdTe Nanocrystals on Latex Spheres. *Adv. Mater.* **2001**, *13*, 1684–1687.
6 Pejova, B.; Tanusevski, A.; Grozdanov, I. Chemical Deposition of Semiconducting Cadmium Selenide Quantum Dots in Thin Film Form and Investigation of their Optical and Electrical Properties. *J. Solid State Chem.* **2003**, *172*, 381–388.
7 Dabbousi, B. O.; Murray, C. B.; Rubner, M. F.; Bawendi, M. G. Langmuir-Blodgett Manipulation of Size-Selected CdSe Nanocrystallites. *Chem. Mater.* **1994**, *6*, 216–219.
8 Lowman, G. M.; Nelson, S. L.; Graves, S. A.; Strouse, G. F.; Buratto, S. K. Polyelectrolyte-Quantum Dot Multilayer Films Fabricated by Combined Layer-by-Layer Assembly and Langmuir-Schaefer Deposition. *Langmuir* **2004**, *20*, 2057–2059.
9 Decher, G.; Schlenhoff, J. B. *Multilayer Thin Films*; Wiley-VCH: Weinheim, Germany, 2003.
10 Shavel, A.; Gaponik, N.; Eychmüller, A. The Assembling of Semiconductor Nanocrystals. *Eur. J. Inorg. Chem.* **2005**, 3613–3623.
11 Mamedov, A. A.; Belov, A.; Giersig, M.; Mamedova, N. N.; Kotov, N. A. Nanorainbows: Graded Semiconductor Films From Quantum Dots. *J. Am. Chem. Soc.* **2001**, *123*, 7738–7739.
12 Franzl, T.; Klar, T. A.; Schietinger, S.; Rogach, A.; Feldmann, J. Exciton Recycling in Graded Nanocrystal Structures. *Nano Lett.* **2004**, *4*, 1599–1603.
13 Lin, Y. W.; Tseng, W. L.; Chang, H. T. Using a Layer-by-Layer Assembly Technique to Fabricate Multicolored-Light-Emitting Films of CdSe@CdS and CdTe Quantum Dots. *Adv. Mater.* **2006**, *18*, 1381–1386.
14 Yang, W. J.; Trau, D.; Renneberg, R.; Yu, N. T.; Caruso, F. Layer-by-Layer Construction of Novel Biofunctional Fluorescent Microparticles for Immunoassay Applications. *J. Colloid Interface Sci.* **2001**, *234*, 356–362.
15 Wang, D. Y.; Rogach, A. L.; Caruso, F. Semiconductor Quantum Dot-Labeled Microsphere Bioconjugates by Stepwise Self-Assembly. *Nano Lett.* **2002**, *2*, 857–861.
16 Wang, X. Y.; Ma, Q.; Li, B.; Li, Y. B.; Su, X. G. The Preparation of CdTe Nanoparticles and CdTe Nanoparticle-Labelled Microspheres for Biological Applications. *Luminescence* **2007**, *22*, 1–8.
17 Salgueiriño-Maceira, V.; Correa-Duarte, M. A.; Spasova, M.; Liz-Marzán, L. M.; Farle, M. Composite Silca Spheres with Magnetic and Luminescent Functionalities. *Adv. Funct. Mater.* **2006**, *16*, 509–514.
18 Hong, X.; Li, J.; Wang, M. J.; Xu, J. J.; Guo, W.; Li, J. H.; Bai, Y. B.; Li, T. J. Fabrication of Magnetic Luminescent Nanocomposites by a Layer-by-Layer Self-Assembly Approach. *Chem. Mater.* **2004**, *16*, 4022–4027.
19 Ma, Q.; Wang, X.; Li, Y.; Shi, Y.; Su, X. Multicolor Quantum-Dot-Encoded Microspheres for the Detection of Biomolecules. *Talanta* **2007**, *72*, 1446–1452.
20 Gao, X. H.; Chan, W. C. W.; Nie, S. M. Quantum-Dot Nanocrystals for Ultrasensitive Biological Labelling and Multicolor Optical Encoding. *J. Biomed. Opt.* **2002**, *7*, 532–537.
21 Gaponik, N.; Talapin, D. V.; Rogach, A. L.; Hoppe, K.; Shevchenko, E. V.; Kornowski, A.; Eychmüller, A.; Weller, H. Thiol-Capping of CdTe Nanocrystals: An Alternative to Organometallic Synthetic routes. *J. Phys. Chem. B* **2002**, *106*, 7177–7185.
22 Parak, W. J.; Gerion, D.; Pellegrino, T.; Zanchet, D.; Micheel, C.; Williams, S. C.; Boudreau, R.; Le Gros, M. A.;

Larabell, C. A.; Alivisatos, A. P. Biological Applications of Colloidal Nanocrystals. *Nanotechnology* **2003**, *14*, R15–R27.
23 Nann, T. Phase-Transfer of CdSe@ZnS Quantum Dots using Amphiphilic Hyperbranched Polyethylenimine. *Chem. Commun.* **2005**, 1735–1736.
24 Gómez, D. E.; Pastoriza-Santos, I.; Mulvaney, P. Tunable Whispering Gallery Quantum-Dot-Doped Mode Emission from Microspheres. *Small* **2005**, *1*, 238–241.
25 Hermanson, T. *Biconjugate Techniques*; Academic Press: New York, 1996, pp 57–60.
26 Carlsson, J.; Drevin, H.; Axén, R. Protein Thiolation and Reversible Protein-Protein Conjugation — N-Succinimidyl 3-(2-Pyridyldithio)Propionate, A New Heterobifunctional Reagent. *Biochem. J.* **1978**, *173*, 723–737.
27 Nolan, J. P.; Sklar, L. A. Suspension Array Technology: Evolution of the Flat Paradigm. *Trends Biotechnol.* **2002**, *20*, 9–12.
28 Finkel, N. H.; Lou, X. H.; Wang, C. Y.; He, L. Barcoding the Microworld. *Anal. Chem.* **2004**, *76*, 353A–359A.
29 Wilson, R.; Cossins, A. R.; Spiller, D. G. Encoded Microcarriers for High-Throughput Multiplexed Detection. *Angew. Chem., Int. Ed.* **2006**, *45*, 6104–6117.
30 Benecky, M. J.; Post, D. R.; Schmitt, S. M.; Kochar, M. S. Detection of Hepatitis B Surface Antigen in Whole Blood by Coupled Particle Light Scattering (Copalis^{TM}). *Clin. Chem.* **1997**, *43*, 1764–1770.