CMLS, Cell. Mol. Life Sci. 61 (2004) 1843–1849
1420-682X/04/151843-07
DOI 10.1007/s00018-004-4036-9


**CMLS** Cellular and Molecular Life Sciences

# Visions & Reflections

# Nanoscale science: a big step towards the Holy Grail of single molecule biochemistry and molecular biology

**R. C. Doty**[a,b,c,*], **D. G. Fernig**[a,b] **and R. Lévy**[a,b,c]

[a] Centre for Nanoscale Science, University of Liverpool, Liverpool L69 72B (United Kingdom)
[b] School of Biological Sciences, Life Sciences Building, Crown Street, University of Liverpool, Liverpool L69 7ZB (United Kingdom), Fax: +44 151 795 4406, e-mail: rcdoty@liv.ac.uk
[c] Department of Chemistry, University of Liverpool, Liverpool L69 7ZB (United Kingdom)

Received 26 January 2004; received after revision 14 April 2004; accepted 23 April 2004

**Abstract.** Light scattering from metal nanoparticles and fluorescence from quantum dots offer distinct advantages over traditional fluorophores when it comes to detection of single molecules in living cells.

**Key words.** Gold nanoparticles; silver nanoparticles; quantum dots; single molecule; single particle; nanobiotechnology.

## Introduction

The sequencing of the genomes of many organisms, including metazoans, has altered the focus of cellular and molecular biology to what is variously known as 'the function deficit', 'post genome science' or 'functional genomics'. Sequenced genomes give us a wonderfully solid foundation for the study of molecular function, but do not shed much light directly onto the molecular and cellular function of molecules whose synthesis is template driven (RNA, proteins). Moreover, sequenced genomes provide little information regarding the function of molecules whose synthesis is not template driven, complex secondary gene products, so lipids, glycolipids, polysaccharides, collectively termed the metabolome or more specifically the lipome and the glycome. These molecules occupy a chemical information space (equivalent to a sequence space or sequence diversity) similar or larger than that of the genome. Their complexity, particularly that of the glycome, co-evolved with metazoans and reflects the increased communication needs of these organisms, which has been met to an extent by use of an interpretative assembly system. Thus, in terms of the function deficit we ignore these molecules at our peril, yet their study is difficult, restricted to biochemical analyses and, for complex polysaccharides where chemical synthesis is not currently possible, purification from natural sources is necessary.

At the heart of the function of biological macromolecules lies their association with other macromolecules to form complexes that are interdependently dynamic in time, composition and cellular location. Thus, to overcome the function deficit we must aim to elucidate the parameters that govern the expression and subcellular location of such dynamic complexes. The analytical methods from molecular cell biology and biophysics that are available, e.g. green fluorescent protein (GFP), epitope tags and monoclonal antibodies, are extremely powerful, particularly when coupled to the fluorescence imaging of single living cells. However, these methods have limitations. For example, the titration of components of molecular complexes that inevitably accompanies overexpression of a tagged species in cells alters all aspects of the dynamics of such complexes. Whereas extremely careful experimentation can exploit such titration to deepen our under-

* Corresponding author.

standing of the phenomenon under investigation [1], more often than not the consequence of this approach is confusion. Other major drawbacks of current approaches include (i) they measure the behaviour of an ensemble and so only the average behaviour of a molecular population is measured, while it is clear that often individual members of a population of molecules at different cellular locations perform different functions; and (ii) their parallelism or level of 'plex' is low. Some consequences are that it is difficult to follow more than two or three molecular species at a time and important associations, which are 'minor' in terms of absolute numbers of participating molecules, but functionally important, will be overlooked.

Tags that are detectable at the single molecule level would solve many of the problems outlined above and so represent a Holy Grail for molecular life scientists intent on providing solutions to the function problem. Molecules carrying such tags would be used at a cellular concentration equivalent to just a few molecules per cell, so removing the problem of titration. Biophysics would study the properties of multiple individual components in individual complexes in single cells. Glycomics and lipomics would finally have an equivalent of polymerase chain reaction (PCR). Although recent progress in fluorescence signal detection has made possible the detection of conventional fluorophores in living cells at the single molecule level [2], these fluorophores photobleach relatively quickly, so there is a need for new tags that allow easier and more photostable detection.

Two products of nanoscale science, semiconductor nanocrystals [3] and metallic nanoparticles [4], have been suggested as the route to this Holy Grail of single molecule detection [5], with a high degree of multiplexing [6]. In the remainder of this review, we examine the synthesis and properties of these materials and evaluate their current performance in the emerging field of nanobiotechnology [7]. Finally, we critically evaluate whether such materials are indeed the Holy Grail they are purported to be.

The proliferation of the ubiquitous term 'nano' can be traced back to the mid-1990s, when research groups on the East and West Coasts of the United States synthesized quantum-confined, spherical cadmium selenide (CdSe) nanocrystals, with control of both size and size distribution [8, 9] and researchers in Liverpool synthesized small (2–8 nm), spherical gold (Au) nanocrystals with moderate control of size and size distribution [10]. Both of these new syntheses resulted in nanocrystals stabilized by alkane chains and soluble only in nonpolar organic solvents. While the as-synthesized nanocrystals are not water soluble and, hence, virtually useless for biological applications, incredible progress has been made in the last decade to remedy this situation.

## Quantum dots

Semiconductor nanocrystals, also called quantum dots, are fluorescent particles. Their usefulness derives from the ability to tune the wavelength of emitted light by using particles of different diameters. This property, known as quantum confinement, refers to the fact that the diameter of semiconductor nanocrystals is smaller than the equilibrium separation distance of the electron-hole pair (exciton) formed in the bulk material upon promotion of an electron from the valence band to the conduction band. The smaller the diameter of the nanocrystal, the more confined the exciton is, resulting in a larger gap between the valence and conduction bands and, hence, a blue shift in the emitted light. By changing the composition of the nanocrystal, in addition to its diameter, the wavelength of emitted light can be varied from the ultraviolet, through the visible and into the infrared (IR) region of the electromagnetic spectrum. While the spectrum of emitted light is very narrow [<30 nm full width at half maximum (FWHM)], the absorption spectrum is very broad. This allows a single light source to excite all of the semiconductor nanocrystals, regardless of their emission wavelength. In an effort to increase the quantum efficiency of light emission, an epitaxial layer of another semiconducting material was added to the nanocrystals to create a core/shell structure. Initially the shell material was chosen to have a higher band gap than the core, confining the electron-hole pair to the core and separating it from the non-radiative traps and defects on the surface of the nanocrystal [11–13]. This allowed quantum yields to approach 100% while still retaining the same emission spectra as the original nanocrystal. Recently, a similar approach was employed to confine the electron to the core, while confining the hole to the shell, and vice versa [14]. The emission spectra resulting from this situation is considerably red shifted compared to the original nanocrystal, opening up even more of the electromagnetic spectrum for a particular core material. Although the quantum yield peaks around 50%, these particular core/shell nanocrystals show the most promise in photovoltaic applications where efficient charge separation is crucial.

## Metallic nanoparticles

While metallic nanocrystals do not exhibit the same efficient fluorescence characteristic of semiconductor nanocrystals, they do still possess a distinctive optical property, known as surface plasmon resonance, which is effectively due to the collective oscillation of free electrons. For the two most-studied metal nanocrystals, Ag and Au, the wavelength at which this occurs is in the vicinity of 400 and 500 nm, respectively, and is a function of the nanocrystal size and composition, the molecules

attached to the surface of the nanocrystal and the surrounding medium. Most of the current biological applications of metal nanocrystals, rely on their light-scattering properties, mainly because scattered light can be detected thousands of times more sensitively than transmitted light [15, 16]. A solution of nanocrystals will scatter an incident beam of monochromatic light at that very same wavelength, but when illuminated with an incident beam of white light, the wavelength at which the scattered light is a maximum is a function of particle size, shape and composition. Through a combination of Ag and Au nanocrystals of different sizes, one can view scattered light of every colour in the visible light spectrum. The particle size dependence of the wavelength of scattered light was predicted by Mie using classical electromagnetic theory [17, 18]. In the Rayleigh limit ($R < \lambda/20$, where R is the radius of the nanocrystal and $\lambda$ is the wavelength of incident light) the wavelength of scattered light remains unchanged, while the intensity increases as $R^6$. For nanocrystals outside the Rayleigh limit, an increase in particle size broadens the scattering spectrum and shifts it to longer wavelengths. In general, the maximum intensity of light scattered from a Ag nanocrystal is at least 10 times that of a Au nanocrystal of the same size, and the spectral shifts with regard to particle size are more pronounced. The scattering spectra are also affected by the refractive index of the medium containing the nanocrystals. Increasing the refractive index increases the intensity of scattered light and shifts it to longer wavelengths. The intensity of scattered light for Au nanocrystals is enhanced by an increase in the medium refractive index more so than Ag, but the spectra of Ag shift more than those of Au [15]. These effects are important experimentally because raising the refractive index of the medium increases the signal-to-noise ratio of a particular measurement by both increasing the scattering from the metal nanocrystals and decreasing the background scattering from dielectric materials, such as microscope slides, cuvettes and so on. Light scattering from metal nanocrystals cannot be quenched, and the nanocrystals do not degrade under optical excitation like many molecular fluorophores, permitting time-dependent studies. In fact, the scattered light intensity of a 60-nm-diameter Au nanocrystal is approximately equivalent to the fluorescent light intensity of 270,000 fluorescein molecules [15]. This allows the detection of a single particle using a simple optical microscope in dark-field mode. The main drawback of this detection strategy is the background scattering from the biological sample and from defects in microscope slides. This background cannot be removed by an emission filter in an experiment where the illumination and detection have to be at the same wavelength, but, as mentioned above, refractive index matching can be effective. To ensure that the detected light comes from a metallic particle, more sophisticated instrumentation is needed in which the scattered light is analysed using a spectrometer coupled to the microscope or a tunable filter to recognize the characteristic scattering spectrum of the metallic nanoparticles. A novel method based on light absorption (photothermal detection) ist not subject to background scattering.

## Practicalities of nanoparticles: first steps

While fluorescence from semiconductor nanocrystals ranging from the ultraviolet (UV) to the near-IR is best achieved in particles less than 10 nm in diameter, sizes easily obtained by syntheses in organic solvents, metal nanocrystals need to be greater than 30 or 40 nm in diameter in order to take advantage of their light-scattering properties. This is too large to be synthesized in organic solvents, but an aqueous synthesis utilizing sodium citrate as both reducing agent and electrostatic stabilizer is capable of producing Au nanocrystals between 10 and 150 nm in diameter [18]. For reproducible, quantitative results, nanocrystals need to be monodisperse in both size and shape. Unfortunately, the citrate-reduction synthesis gives highly polydisperse results when trying to synthesize nanocrystals greater than ~30 nm in diameter. Recent work using 12-nm citrate-stabilized Au nanocrystals as seeds for the growth of larger nanocrystals has made possible the synthesis of monodisperse, spherical nanocrystals, with diameters between 30 and 110 nm, and monodisperse, ellipsoidal nanorods of similar size [19].

Although substantial advances have been made in the synthesis of large, water-soluble Au nanocrystals, the same cannot be said for Ag nanocrystals. Ag oxidizes much more readily than Au, and the larger van der Waals attraction between Ag spheres compared to Au spheres reduces the effectiveness of electrostatic repulsion as a method of stabilization. Thus far, the solution to this problem has been to deposit Au around the Ag nanocrystal, much like the seeded growth syntheses mentioned previously, in order to reduce the oxidation of the Ag surface. In monolayer and bilayer quantities, it has been shown that this does not affect the absorption or light-scattering properties of the original Ag core [20].

Synthesis of semiconductor nanocrystals occurs almost exclusively in organic solvents because they allow precise control of the nanocrystal size, shape and size distribution. The remaining challenge is to transfer these nanocrystals into an aqueous environment. The two most common techniques to bring about this phase transfer are ligand exchange and silanization.

Solubilization via ligand exchange requires displacement of the hydrophobic molecule attached to the surface of the nanocrystal by a hydrophilic one, usually a carboxylic acid-terminated molecule [21, 22]. The efficiency of this technique is dependent upon the relative bond strengths

between the two molecules and the nanocrystal surface, and usually requires a large excess of the hydrophilic molecule in solution during the ligand exchange. Unfortunately, this requirement precludes the direct attachment of some biological molecules that are not available or are prohibitively expensive in such large quantities, many peptides and proteins, for example.

Another approach to making nanocrystals water soluble is to encapsulate them in a layer of silica/siloxane via a surface silanization procedure [23, 24]. In subsequent cross-linking reactions, charged molecules can be incorporated into the shell to provide electrostatic stabilization to the water-soluble nanocrystals, or long-chained, hydrophilic molecules, such as polyethylene glycol, can be used to provide steric stabilization. The resulting silica shell has exposed amino, mercapto and phosphate groups that can be used to further functionalize the nanocrystal surface. While the silica-encapsulated nanocrystals are very stable and do not aggregate in aqueous solution, gradual decomposition of the silica layer eventually leads to nanocrystal precipitation and limits the length of time they can be left in water.

The uniformity of citrate-stabilized Au nanocrystals, the ease with which the citrate molecules can be displaced from the Au surface, and the unique optical properties of both individual and aggregated clusters of Au nanocrystals are critical to their successful utilization in biological applications. One area where this came to fruition early was the attachment of thiol-terminated DNA oligonucleotides to Au nanocrystals, in which complementary strands of DNA were used to assemble both dimers and trimers and large, aggregated structures of nanocrystals [25, 26]. The nucleotide-induced aggregation caused a red to blue colour change in the aqueous nanocrystal solution. This aggregation is reversible upon heating the solution above the melting temperature of the DNA. In this way the absorbance of Au nanocrystals is used to detect DNA hybridization [27, 28]. A more solid-state approach to the detection of DNA hybridization is to immobilize a strand of DNA on a substrate and flow a solution of nanocrystals containing the complementary strand of DNA over the substrate [29, 30]. The attachment of nanocrystals on the surface can be detected by light scattering. This technique offers greater sensitivity and can detect DNA in the femtomolar concentration range [29, 31]. The DNA can also be used as a substrate for restriction enzymes [32].

The most promising application of semiconductor nanocrystals is the fluorescent labeling of biological samples. Quantum dots possess numerous properties that make them more attractive than conventional fluorophores, including narrow, tunable and symmetric emission spectra; broad, continuous excitation spectra; and lifetimes long enough to allow the filtering of cell autofluorescence. The biggest obstacle to widespread application of quantum dots as biological labels is tailoring the surface chemistry to reduce non-specific binding and the propensity of these materials to degrade and aggregate in aqueous environments. Three early examples successfully implemented different techniques to fluorescently label cells with CdSe core/shell quantum dots. Alivisatos and co-workers used CdSe/CdS core/shell nanocrystals encapsulated in a silica shell [33]. Small, green-emitting nanocrystals with trimethoxysilylpropyl urea and acetate groups selectively bound to the nucleus of 3T3 mouse fibroblast cells, presumably through the well-known uptake of polyanions by nuclei. Larger, red-emitting nanocrystals with biotin attached to the surface bound to F-actin filaments prelabeled with phalloidin-biotin-streptavidin. The sample was imaged with a wide-field microscope using a mercury lamp as the excitation source in order to view both colours at the same time. Nie and co-workers employed mercaptoacetic acid to make their CdSe/ZnS core/shell nanocrystals water soluble [21]. Proteins were cross-linked to the free carboxyl group via reactive amine groups. For example, the attachment of transferrin allowed receptor-mediated endocytosis of the transferrin ligand to be followed in cultured HeLa cells. In another experiment immunoglobulin G was attached to the quantum dots, which were then specifically aggregated by a secondary antibody in order to show extensive aggregation in the presence of a specific polyclonal antibody. In a comparison with rhodamine 6G, the quantum dots were found to have a spectral width 1/3 that of rhodamine 6G, stability to photobleaching 100 times greater, and fluorescence emission intensities equivalent to about 20 rhodamine molecules. Thompson and co-workers treated CdSe/ZnS core/shell quantum dots with dithiothreitol to form a hydroxyl-terminated surface [34]. Further treatment with 1,1′-carbonyl diimidazole and 5′-aminated oligonucleotides produced highly water soluble nanocrystals with no significant aggregation or loss of oligonucleotide after 2 months. These oligonucleotide-coupled quantum dots were then used in a fluorescence in situ hybridization assay for a Y chromosome-specific sequence in human sperm. The result was highly specific quantum dot binding to cells containing Y chromosomes and a lack of non-specific binding for quantum dots with just a hydroxylated surface and for those coupled to random oligonucleotides.

One potential benefit of using quantum dots as fluorescent labels is the degree of multiplexing they offer, though there is a trade-off in terms of size, since genome-scale degrees of multiplexing require mesoscale particles. Thus, by embedding different-sized quantum dots in micron-sized latex beads at precisely controlled ratios, Nie and co-workers predict a theoretical coding of $10^6$ proteins or nucleic acids by using six different colours and 10 intensity levels [35]. Realistically, they envision detection of five or six different colours and six intensity

levels, giving 10,000–40,000 biological labels. The beauty of their approach is that the CdSe/ZnS core/shell quantum dots do not need to be made hydrophilic. They are embedded into the beads in their as-synthesized, hydrophobic state. The pores are then sealed with a thin polysilane layer to protect the quantum dots from degradation in aqueous conditions and the temperature cycling conditions of DNA hybridization assays. Biological molecules are attached to the latex beads by cross-linking with surface-bound carboxylic acid groups. For more detailed information concerning the biological applications of semiconductor nanocrystals, the reader is directed to the handful of recent reviews devoted specifically to this topic and the references therein [36–39].

The question of how quantum dots and nanoparticles enter the cell, and whether or not they can be designed to specifically target particular subcellular compartments is as important to their future applicability as how they perform once they get there. Given their size, it is perhaps not surprising that these materials may be placed in the cell interior not only by direct physical methods, but also by methods that use the cellular machinery which is adapted to the transport of proteins from the cell exterior to the cell interior and, once in the latter, to specific subcellular compartments. A great deal of work thus far has focused on the labeling of cells via bonding to membrane-bound receptor proteins [40–42] and examining quantum dot stability upon microinjection into cells [43–45]. Kyriacou and co-workers [46] studied the accumulation of Ag nanoparticles in living bacterial cells, as a result of aztreonam-induced disruption of the cell wall and the concomitant increase in membrane permeability. These particles, however, are quickly extruded from the cell by an efflux pump. Superparamagnetic nanoparticles derivatized with a membrane translocation signal, HIV-Tat peptide, were efficiently internalized into haematopoietic and neural progenitor cells, presumably via adsorptive endocytosis, and were retained for several days [47]. Quantum dots coated with homing peptides and injected into mice were able to specifically bind to various vascular sites depending on which peptide was bound to the quantum dot [48]. The coadsorption of polyethylene glycol was shown to reduce nonspecific uptake into the liver and spleen by 95%. Tkachenko and co-workers [49] conjugated peptides to Au nanoparticles modified with bovine serum albumin in order to achieve both receptor-mediated endocytosis and nuclear targeting of intact HepG2 cells. It was shown that the efficiency of nuclear targeting was greater for nanoparticles coated with two groups of short peptides, one coding for receptor-mediated endocytosis and the other possessing a nuclear localization signal, compared to nanoparticles coated with long peptides that coded for both processes. The movement of the nanoparticles in the cell was followed by video-enhanced colour microscopy and differential interference contrast microscopy, and made possible by the large visible light absorption and scattering cross-sections of the 20 nm Au nanoparticles.

In addition to using nanocrystals to detect biological molecules, biological molecules can be used to self-assemble nanocrystals into patterns that are not otherwise thermodynamically or kinetically favourable. Complementary strands of DNA can be attached to nanocrystals of different size, shape and/or composition. In this way, large nanocrystals can be surrounded by small ones [50], metal nanocrystals can be surrounded by semiconductor ones [51] and so on. The challenge with this technique is to control the degree of nanocrystal ordering. Ideally, one would like to form dimers, trimers and other well-defined structures, such as monolayers and multilayers [52]. However, this requires precise control of the number of DNA molecules attached to the surface of the nanocrystal, and it is not currently possible to generate a solution of nanocrystals with only, and exactly, one (or two, or three) DNA molecules per nanocrystal. In a given solution there is a statistical distribution of desired capping ligands per nanocrystal. The challenge then is to separate the nanocrystals with one oligonucleotide from those with two, three, four and so on, which at the present time has only been consistently achieved by gel electrophoresis [53, 54]. To achieve maximum resolution, the nanocrystals should be size monodisperse and homogeneous in charge, and the DNA molecules should have a mobility similar to that of the nanocrystals so that the attachment of only one or two oligonucleotides has a significant effect on overall mobility through the gel. Besides being time consuming and relatively inefficient, the pore size of the gel presents a serious limitation to the size of nanocrystals that can be separated using this technique.

## The biology-nanoscale science synergy: Holy Grail in sight?

The above pioneering work highlights the potential of nanocrystals. It is clear that the ligand shell is critical for exploitation of the remarkable properties of these materials, since nanoparticles otherwise tend to aggregate and/or oxidize, particularly in aqueous solutions. Current ligand shells (polymers, including nucleic acids, self-assembled layers, e.g. mercaptoundecanoic acid) fall short of the ideal, since they do not meet one or more of the following criteria: (i) protection of the nanocrystal from complex experimental environments, which may range from electrochemical to biological; (ii) provision of the means to couple functional groups specifically at defined stoichiometry; and (iii) a defined thickness such that the geometry of assemblies of nanocrystals can be predicted and the prediction verified against experimental observations. Thus, the field is still some way off from routine

synthesis of monodisperse nanocrystals with an inert ligand shell substituted by active groups (DNA, peptide and so on) at an exact stoichiometry. One consequence is that standard preparative biochemical chromatographic techniques, which are suitable for industrial scale-up, as would be required for mass production of a sensor, cannot be applied to most existing preparations of nanocrystals.

The main difficulty in designing new ligand shells is our lack of understanding of the organization of the ligand shell itself; interference by nanocrystals with sensitive spectroscopic techniques, e. g. IR, which would normally be used to establish the structure of the ligand shell, being a major contributing factor. The use of flat surfaces as a proxy for nanocrystals, e. g. Au(111), in lieu of Au nanoparticles may advance substantially the use of nanocrystals.

Nanocrystals will only become part of the routine of cell and molecular biology when the above problems are satisfactorily resolved. However, new solutions to these problems are being published on almost a daily basis [55–60]. Moreover, major products based on nanocrystals are finding their way onto the market, e. g. the Qiagen HiLight system (Qiagen, Hilden, Germany), which detects DNA by use of the resonant light scattering of Au and Ag nanocrystals. So the Holy Grail may not be quite in our grasp, but it certainly is in sight, and this exciting and fast moving field will have solved many of its current major problems by the end of the decade. By this time cell and molecular biologists are likely to have nanocrystal-based detection kits, some of which will be single molecule sensors, and the health care system will have simple but very sensitive sensors for common clinical tests.

*Acknowledgments.* We acknowledge the support of the Biotechnology and Biological Sciences Research Council, the Cancer and Polio Research Fund, the European Union and the North West Cancer Research Fund.

1 Nelson G., Paraoan L., Spiller D. G., Wilde G. J., Browne M. A., Djali P. K. et al. (2002) Multi-parameter analysis of the kinetics of NF-kappaB signalling and transcription in single living cells. J. Cell Sci. **115:** 1137–1148
2 Sako Y. and Uyemura T. (2002) Total internal reflection fluorescence microscopy for single-molecule imaging in living cells. Cell Structure Function **27:** 357–365
3 Murray C. B., Kagan C. R. and Bawendi M. G. (2000) Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies. Annu. Rev. Mater. Sci. **30:** 545–610
4 Collier C. P., Vossmeyer T. and Heath J. R. (1998) Nanocrystal superlattices. Annu. Rev. Phys. Chem. **49:** 371–404
5 Schultz S., Smith D. R., Mock J. J. and Schultz D. A. (2000) Single-target molecule detection with nonbleaching multicolor optical immunolabels. Proc. Natl. Acad. Sci. USA **97:** 996–1001
6 Cao Y. C., Jin R. and Mirkin C. A. (2002) Nanoparticles with raman spectroscopic fingerprints for DNA and RNA detection. Science **297:** 1536–1540
7 Niemeyer C. M. (2001) Nanoparticles, proteins and nucleic acids: biotechnology meets materials science. Angew. Chem. Int. Ed. **40:** 4128–4158
8 Murray C. B., Norris D. J. and Bawendi M. G. (1993) Synthesis and characterization of nearly monodisperse CdE (E = S, Se, Te) semiconductor nanocrystallites. J. Am. Chem. Soc. **115:** 8706–8715
9 Alivisatos A. P. (1996) Semiconductor clusters, nanocrystals and quantum dots. Science **271:** 933–937
10 Brust M., Walker M., Bethell D., Schiffrin D. J. and Whyman R. (1994) Synthesis of thiol-derivatised gold nanoparticles in a two-phase liquid-liquid system. J. Chem. Soc. Chem. Commun. 801–802
11 Hines M. A. and Guyot-Sionnest P. (1996) Synthesis and characterization of strongly luminescing ZnS-capped CdSe nanocrystals. J. Phys. Chem. **100:** 468–471
12 Dabbousi B. O., Rodriguez-Viejo J., Mikulec F. V., Heine J. R., Mattoussi H., Ober R. et al. (1997) (CdSe)ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. J. Phys. Chem. B **101**: 9463–9475
13 Peng X., Schlamp M. C., Kadavanich A. V. and Alivisatos A. P. (1997) Epitaxial growth of highly luminescent CdSe/CdS core/shell nanocrystals with photostability and electronic accessibility. J. Am. Chem. Soc. **119:** 7019–7029
14 Kim S., Fisher B., Eisler H.-J. and Bawendi M. (2003) Type-II quantum dots: CdTe/CdSe(core/shell) and CdSe/ZnTe (core/shell) heterostructures. J. Am. Chem. Soc. **125:** 11466–11467
15 Yguerabide J. and Yguerabide E. E. (1998) Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications: I. Theory. Anal. Biochem. **262:** 137–156
16 Yguerabide J. and Yguerabide E. E. (1998) Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications: II. Experimental characterization. Anal. Biochem. **262:** 157–176
17 Mie G. (1908) Contributions to the optics of turbid media, especially colloidal metal solutions. Ann. Phys. **25:** 377–445
18 Frens G. (1973) Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nature Phys. Sci. **241:** 20–22
19 Brown K. R., Walter D. G. and Natan M. J. (2000) Seeding of colloidal Au nanoparticle solutions. 2. Improved control of particle size and shape. Chem. Mater. **12:** 306–313
20 Cao Y., Jin R. and Mirkin C. A. (2001) DNA-modified core-shell Ag/Au nanoparticles. J. Am. Chem. Soc. **123:** 7961–7962
21 Chan W. C. W. and Nie S. (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science **281:** 2016–2018
22 Mattoussi H., Mauro J. M., Goldman E. R., Anderson G. P., Sundar V. C., Mikulec F. V. et al. (2000) Self-assembly of CdSe-ZnS quantum dot bioconjugates using an engineered recombinant protein. J. Am. Chem. Soc. **122:** 12142–12150
23 Gerion D., Pinaud F., Williams S. C., Parak W. J., Zanchet D., Weiss S. et al. (2001) Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots. J. Phys. Chem. B **105:** 8861–8871
24 Schroedter A. and Weller H. (2002) Ligand design and bioconjugation of colloidal gold nanoparticles. Angew. Chem. Int. Ed. **41:** 3218–3221
25 Mirkin C. A., Letsinger R. L., Mucic R. C. and Storhoff J. J. (1996) A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature **382:** 607– 609
26 Alivisatos A. P., Johnsson K. P., Peng X., Wilson T. E., Loweth C. J., Bruchez J. et al. (1996) Organization of 'nanocrystal molecules' using DNA. Nature **382:** 609–611
27 Elghanian R., Storhoff J. J., Mucic R. C., Letsinger R. L. and Mirkin C. A. (1997) Selective colorimetric detection of polynu-

cleotides based on the distance-dependent optical properties of gold nanoparticles. Science **277:** 1078–1081
28 Storhoff J. J., Lazarides A. A., Mucic R. C., Mirkin C. A., Letsinger R. L. and Schatz G. C. (2000) What controls the optical properties of DNA-linked gold nanoparticle assemblies? J. Am. Chem. Soc. **122:** 4640–4650
29 Taton T. A., Mirkin C. A. and Letsinger R. L. (2000) Scanometric DNA array detection with nanoparticle probes. Science **289:** 1757–1760
30 Taton T. A., Lu G. and Mirkin C. A. (2001) Two-color labeling of oligonucleotide arrays via size-selective scattering of nanoparticle probes. J. Am. Chem. Soc. **123:** 5164–5165
31 Park S.-J., Taton T. A. and Mirkin C. A. (2002) Array-based electrical detection of DNA with nanoparticle probes. Science **295:** 1503–1506
32 Kanaras A. G., Wang Z., Bates A. D., Cosstick R. and Brust M. (2003) Towards multistep nanostructure synthesis: Programmed enzymatic self-assembly of DNA/gold systems. Angew. Chem. Int. Ed. **42:** 191–194
33 Bruchez J., Marcel, Moronne M., Gin P., Weiss S. and Alivisatos A. P. (1998) Semiconductor nanocrystals as fluorescent biological labels. Science **281:** 2013–2016
34 Pathak S., Choi S.-K., Arnheim N. and Thompson M. E. (2001) Hydroxylated quantum dots as luminescent probes for in situ hybridization. J. Am. Chem. Soc. **123:** 4103–4104
35 Han M., Gao X., Su J. Z. and Nie S. (2001) Quantum-dot tagged microbeads for multiplexed optical coding of biomolecules. Nat. Biotechnol. **19:** 631–635
36 Michalet X., Pinaud F., Lacoste T. D., Dahan M., Bruchez M. P., Alivisatos A. P. et al. (2001) Properties of fluorescent semiconductor nanocrystlas and their application to biological labeling. Single Mol. **2:** 261–276
37 Parak W. J., Gerion D., Pellegrino T., Zanchet D., Micheel C., Williams S. C. et al. (2003) Biological applications of colloidal nanocrystals. Nanotechnology **14:** R15–R27
38 Chan W. C. W., Maxwell D. J., Gao X., Bailey R. E., Han M. and Nie S. (2002) Luminescent quantum dots for multiplexed biological detection and imaging. Curr. Opin. Biotechnol. **13:** 40–46
39 Gao X. and Nie S. (2003) Molecular profiling of single cells and tissue specimens with quantum dots. Trends Biotech. **21:** 371–373
40 Winter J. O., Liu T. Y., Korgel B. A. and Schmidt C. E. (2001) Recognition molecule directed interfacing between semiconductor quantum dots and nerve cells. Adv. Mater. **13:** 1673–1677
41 Jaiswal J. K., Mattoussi H., Mauro J. M. and Simon S. M. (2003) Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nat. Biotechnol. **21:** 47–51
42 Rosenthal S. J., Tomlinson I., Adkins E. M., Schroeter S., Adams S., Swafford L. et al. (2002) Targeting cell surface receptors with ligand-conjugated nanocrystals. J. Am. Chem. Soc. **124:** 4586–4594
43 Kim S., Lim Y. T., Soltesz E. G., De Grand A. M., Lee J., Nakayama A. et al. (2004) Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat. Biotechnol. **22:** 93–97
44 Larson D. R., Zipfel W. R., Williams R. M., Clark S. W., Bruchez M. P., Wise F. W. et al. (2003) Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science **300:** 1434–1436
45 Dubertret B., Skourides P., Norris D. J., Noireaux V., Brivanlou A. H. and Libchaber A. (2002) In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science **298:** 1759–1762
46 Kyriacou S. V., Brownlow W. J. and Xu X.-H. N. (2004) Using nanoparticle optics assay for direct observation of the function of antimicrobial agents in single live bacterial cells. Biochemistry **43:** 140–147
47 Lewin M., Carlesso N., Tung C.-H., Tang X.-W., Cory D., Scadden D. T. et al. (2000) Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat. Biotechnol. **18:** 410–414
48 Akerman M. E., Chan W. C. W., Laakkonen P., Bhatia S. N. and Ruoslahti E. (2002) Nanocrystal targeting in vivo. Proc. Natl. Acad. Sci. USA **99:** 12617–12621
49 Tkachenko A. G., Xie H., Coleman D., Glomm W., Ryan J., Anderson M. F. et al. (2003) Multifunctional gold nanoparticle-peptide complexes for nuclear targeting. J. Am. Chem. Soc. **125:** 4700–4701
50 Mucic R. C., Storhoff J. J., Mirkin C. A. and Letsinger R. L. (1998) DNA-directed synthesis of binary nanoparticle network materials. J. Am. Chem. Soc. **120:** 12674–12675
51 Mitchell G. P., Mirkin C. A. and Letsinger R. L. (1999) Programmed assembly of DNA functionalized quantum dots. J. Am. Chem. Soc. **121:** 8122–8123
52 Loweth C. J., Caldwell W. B., Peng X., Alivisatos A. P. and Schultz P. G. (1999) DNA-based assembly of gold nanocrystals. Angew. Chem. Int. Ed. **38:** 1808–1812
53 Zanchet D., Micheel C. M., Parak W. J., Gerion D. and Alivisatos A. P. (2001) Electrophoretic isolation of discrete Au nanocrystal/DNA conjugates. Nano Lett. **1:** 32–35
54 Zanchet D., Micheel C. M., Parak W. J., Gerion D., Williams S. C. and Alivisatos A. P. (2002) Electrophoretic and structural studies of DNA-directed Au nanoparticle groupings. J. Phys. Chem. B **106:** 11758–11763
55 Xie H., Tkachenko A. G., Glomm W. R., Ryan J. A., Brennaman M. K., Papanikolas J. M. et al. (2003) Critical flocculation concentrations, binding isotherms and ligand exchange properties of peptide-modified gold nanoparticles studied by uv-visible, fluorescence and time-correlated single photon counting spectroscopies. Anal. Chem. **75:** 5797–5805
56 Ryadnov M. G., Ceyhan B., Niemeyer C. M. and Woolfson D. N. (2003) 'Belt and braces': a peptide-based linker system of de novo design. J. Am. Chem. Soc. **125:** 9388–9394
57 Chakrabarti R. and Klibanov A. M. (2003) Nanocrystals modified with peptide nucleic acids (PNAS) for selective self-assembly and DNA detection. J. Am. Chem. Soc. **125:** 12531–12540
58 Wu X., Liu H., Liu J., Haley K. N., Treadway J. A., Larson J. P. et al. (2003) Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat. Biotechnol. **21:** 41–46
59 Penn S. G., He L. and Natan M. J. (2003) Nanoparticles for bioanalysis. Curr. Opin. Chem. Bio. **7:** 609–615
60 Alivisatos P. (2004) The use of nanocrystals in biological detection. Nat. Biotechnol. **22:** 47–52