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The use of nanocrystals in biological detection

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In the coming decade, the ability to sense and detect the state of biological systems and living organisms optically, electrically and magnetically will be radically transformed by developments in materials physics and chemistry. The emerging ability to control the patterns of matter on the nanometer length scale can be expected to lead to entirely new types of biological sensors. These new systems will be capable of sensing at the single-molecule level in living cells, and capable of parallel integration for detection of multiple signals, enabling a diversity of simultaneous experiments, as well as better crosschecks and controls.

There are many points of intersection between nanoscience and nanotechnology and the biological sciences. Indeed, the elementary functional units of biological systems—enzymes, motors, membranes, nucleic acids, *etc.*—all comprise complex nanoscale components. In this article, I focus on new means of sensing that arise when inorganic materials, mainly semiconductors and metals, are patterned on the nanoscale. The fact that solid-state materials, metals, semiconductors and magnets, from which we make everyday macroscopic optical and electrical sensors, can now be made on the size scale of individual biological macromolecules will have great impact.

Comparably important advances in the preparation of polymers, dendrimers[1], and other artificial organic nanostructures, as well as topics at the intersection between scanning probe techniques[2] and microfluidics[3] with biotechnology, are not covered here. Although biological macromolecules can be used to great effect to influence the growth of artificial nanoscale materials, this article focuses solely on the uses of inorganic nanostructures in biological detection[4,5]. For a careful consideration of the impact of nanomaterials on human health and the environment, the reader is referred elsewhere[6].

Quantum wells, wires and dots

Many of the developments in artificial inorganic nanostructures are based on a few fundamental concepts in condensed matter physics. In 1973, Leo Esaki was awarded the Nobel Prize in Physics for the development of novel semiconductor quantum devices, in which the tunneling of electrons could be systematically controlled, and for his early espousal of the concept of the 'artificial solid'[7,8]. The electronic and optical properties of a semiconductor arise primarily through the quantum mechanical scattering of the valence electrons by the atomic cores. In the first artificial solids, semiconductor atoms of differing composition were laid

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down sequentially in layers only a few atoms thick, so that electrons were forced to move through an artificial potential, scattering now in a way that the scientist could design. In such 'quantum wells,' it is possible to systematically control the electronic energy level spacings by adjusting the length scale over which the potential varies, compared to the electron wavelength. In such systems, for example, the wavelength of light emission can be directly controlled.

The early experiments in such 'quantum-confined' systems (**Fig. 1**) were extended from layers of atoms in sheets (quantum wells) to lines of atoms ('quantum wires'), and ultimately to 'quantum dots'[9], small three-dimensional groupings of atoms (perhaps a few hundred or as many as 10,000) in which the electron motion is 'confined' by potential barriers in all three dimensions.

In a quantum dot, often called an artificial atom, there are discrete electronic energy levels, much as in an atom or molecule, but in this case, the spacing of the electronic energy levels can be very precisely chosen by the experimenter through variation of the size. Such quantum dots are a fascinating subject for investigation in the physics laboratory (quantum dots even have a periodic table, which has many similarities to the real periodic table, but also its own distinct characteristics[10]). The development of ways to produce colloidal quantum dots in solution led to an explosive growth in research on these materials, because now the new concepts of artificial solid could find use in a much wider range of applications[11].

This was followed closely by the realization that colloidal quantum dots are the size of a typical protein, and that thus it should be possible to introduce colloidal quantum dots into cells. In 1998, both my group[12] and that of Nie[13] reported the first use of colloidal quantum dots for biological labeling and suggested that the photochemical stability and the ability to tune broad wavelength of the quantum dots may make these materials extremely useful for biolabeling.

Recently, this area of investigation has developed significantly. Colloidal quantum dots are robust and very stable light emitters and they can be broadly tuned simply through size variation. In the past two years, we have seen the development of a wide range of methods for bio-conjugating colloidal quantum dots[14–18] in diverse areas of application: cell labeling[19], cell tracking[20], *in vivo* imaging[21], DNA detection[22,23] and multiplexed beads[24]. It has been demonstrated that colloidal quantum dots can have a significantly larger linear absorption cross section for excitation compared with phycoerithrin[25], and orders of magnitude larger cross section for two-photon excitation compared with conventional organic chromophores[26]. Colloidal quantum dots with a wide range of bio-conjugation and with high quantum yields are now available commercially, so that it is no longer necessary for each experimenter to grow their own (which takes quite a bit of practice) or to become lost in the myriad discussions concerning the best way to render colloidal dots water soluble and bio-compatible. The range of
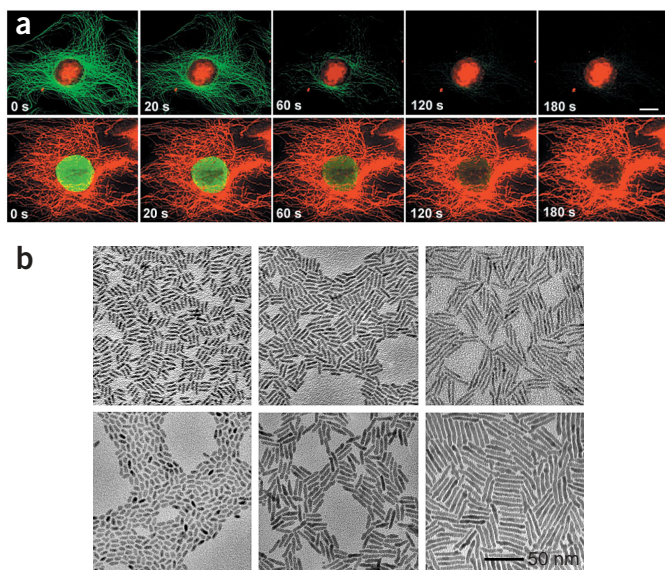
Figure 1 Quantum confinement in semiconductors and new biological labels. (**a**) Cell labeling with quantum dots and illustration of quantum dot photostability, compared with the dye Alexa 488. In the upper panels, the nucleus is stained red with quantum dots and the actin fibers are stained green with the dye. In the lower panel, the labeling is reversed. (Reprinted from ref. 19.) (**b**) Transmission electron micrographs of quantum rods—a new nanostructure that may have uses as a biological label with polarized emission, reduced blinking and faster radiative rates than dots. The time course is the bleaching of the dye fluorescence as a function of laser irradiation time. (Reprinted by permission of the American Chemical Society from ref. 97.)

Figure 2 Photonic band gap materials for biological sensing. (**a**) A colloidal crystal impregnated with a polymer hydrogel diffracts light. The hydrogel specifically adsorbs glucose, swelling the gel and changing the colloidal crystal diffraction. (Reprinted by permission of the American Chemical Society from ref. 36.) (**b**) Light emission from a polymer bead microcavity with a single colloidal quantum dot attached and calculation of the electric field in the vicinity of the structure. Such structures represent a new wave of emerging materials that combine quantum confined structures with photonic structures. (Reprinted by permission of the American Chemical Society from ref. 94.)

biological experiments that these materials are employed in is growing rapidly, and this is one of the first commercial applications of modern nanotechnology.

Photonic crystals

In quantum dots, the potential in which electrons move is controlled so as to make a three-dimensional 'box.' In the 1980s, Eli Yablonovitch first proposed that by analogy with the control of the density of electron states in semiconductor quantum wells, wires, and dots, it should be possible to also control the density of photon states by creating a medium with artificially designed regions of varying index of refraction. Yablonovitch[27] has called these materials "photonic crystals," or semiconductors for light. The goal in research into photonic crystals is to control the patterns of materials on a length scale comparable with the wavelength of light, in one, two, and three dimensions, thus creating materials with designed optical characteristics. Here, nature is way ahead of us because such variations already result in the beautiful colorings of butterfly wings[28] and are a common feature of opals. If scientists could artificially control photonic crystals at will, it would be useful for much more than making beautiful colors. Consider that for an electronically excited atom or molecule to radiate, there must be a state for the outgoing photon. Indeed, the Fermi's golden rule expression for the quantum mechanical radiative rate of a molecule is directly proportional to the photon density of states of the medium surrounding the atom or molecule. By embedding a molecule in a photonic crystal, it is possible to control the rates and directions in which molecules emit light[29]; this is complementary to systems where electrons are confined, and in which the energy of the emission can be controlled.

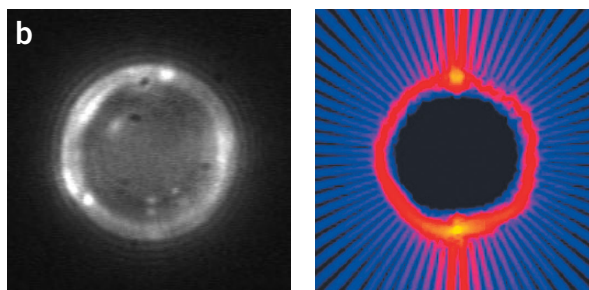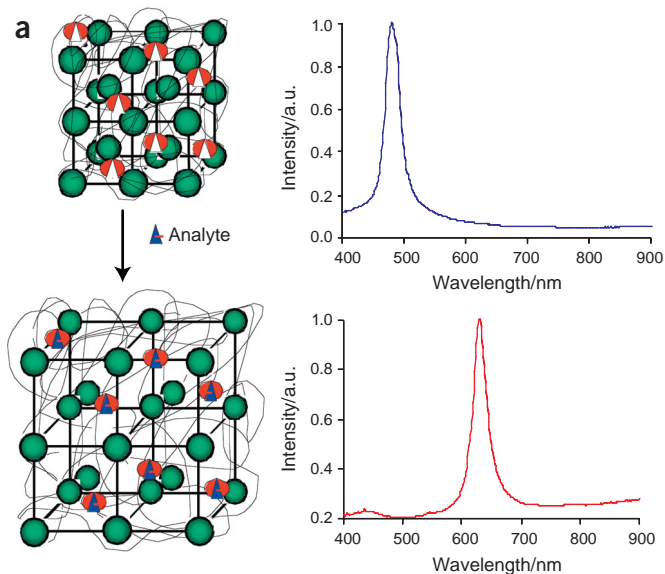Many photonic band-gap materials can now be prepared by a variety of means, some of which are potentially compatible with the incorporation of biological molecules[30–33] (a true three-dimensional photonic gap crystal remains elusive and the subject of a great hunt). Nonetheless, the existing materials already show promise in biological detection.

Consider photonic crystals consisting of an array of silica or polymer beads of a few hundred nanometer in size. The voids are large enough to directly incorporate a variety of biological macromolecules. Furthermore, a binding event within these macromolecules can produce a change in the spacing of the beads or in the index of refraction of the surrounding medium. The Asher group[34–36] has demonstrated the use of this scheme for detection of lead ions and carbohydrates in blood (**Fig. 2**).

Metallic nanoparticles

In the luminescent materials we have discussed thus far, the optical response is due to the excitation of single electron-hole pairs. Some of the most promising avenues for enhanced optical detection schemes arise through the use of noble metal nanocrystals. In a metallic nanoparticle, incident light can couple to the plasmon excitation of the metal, which involves the light-induced motion of all the valence electrons[37].
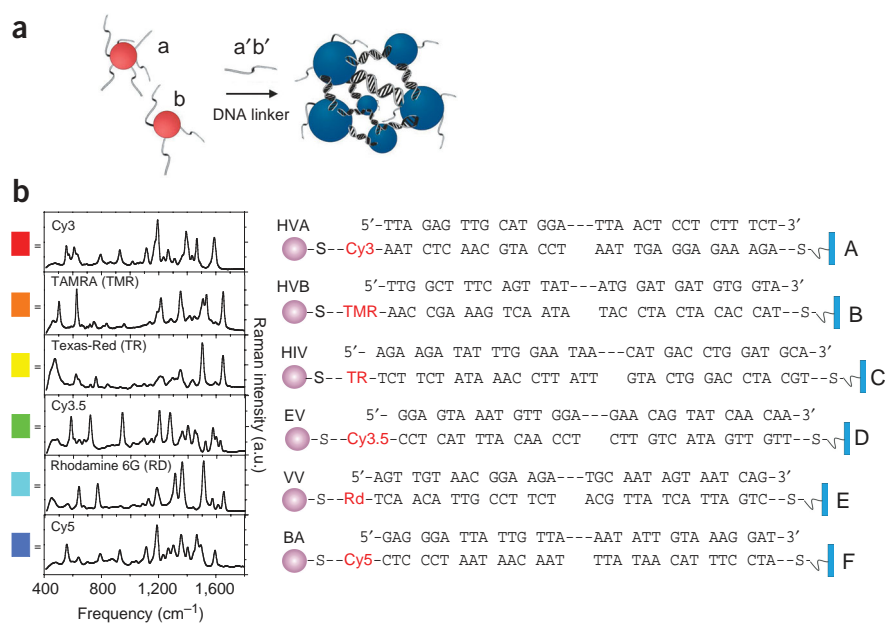
Figure 3 Noble metal nanocrystal based biosensors. (**a**) The DNA-induced aggregation of gold nanocrystals leads to a shift in the plasmon resonance. This has been developed as a sensitive probe for oligonucleotides. (Reprinted by permission of the American Association for the Advancement of Science from ref. 48.) (**b**) Surface-enhanced Raman effect for gold nanocrystals designed to create a large number of specific biological labels. Dye molecules attached to specific oligonucleotides can be detected by their characteristic Raman spectra; these Raman spectra are detectable because of the large enhancement of the radiation field in the vicinity of the metal nanocrystals. (Reprinted by permission of the American Association for the Advancement of Science from ref. 50.)

Thus, the cross section for elastic light scattering from a 50-nm gold nanocrystal can be a million-fold larger than the cross section for absorption or emission of electromagnetic radiation from any molecule or even quantum dot chromophore. Although these objects are somewhat large for use inside cells, they nonetheless provide a powerful and evolving toolkit for biological detection[38,39]. For example, it has been shown that the plasmon resonance is strongly dependent on shape and size[40–43], so that it is possible to make a wide range of light scatterers that can be detected at different wavelengths. Such nanoparticles are readily bio-conjugated and are commercially available. Using specific organic molecules[44] or DNA[45–47], it appears possible to make designed, discrete aggregates of nanoparticles, in which the spectra will depend sensitively on the particle arrangement, providing a rich system for detection.

The electromagnetic field in the near-field region around a metallic nanoparticle is greatly enhanced, providing important new mechanisms for detection. In the most famous example, when many gold nanoparticles are located nearby each other, their plasmon resonances couple to each other via the near field, shifting the plasmon resonance to higher energy. Mirkin and colleagues[48] showed how this change in the optical response from isolated to aggregated metallic nanocrystals can be used to sensitively detect nucleic acids, using gold nanocrystals coated with a high density of oligonucleotides on the surface (**Fig. 3a**). Interestingly, one practical difficulty of working with metallic nanocrystals—overcoming their tendency to aggregate under conditions of high ionic strength—is removed by the addition of a dense layer of oligonucleotides. Such detection schemes have progressed rapidly and are now under development commercially.

It is extremely desirable to be able to optically detect a 'fingerprint' spectrum, but ordinarily this is only possible with vibrational (infrared and Raman) or magnetic resonance spectroscopies, and none of these has sensitivity anywhere close to what is possible with luminescence. The large field enhancement in the vicinity of gold nanocrystals is well known to lead to the surface enhanced Raman scattering (SERS) effect, and developments in this area may well change the picture[49]. Mirkin and colleagues[50] have shown that it is possible to detect a wide range of biological macromolecules through binding events involving gold nanocrystals that have been coated with specific molecules that offer a distinct Raman signature (**Fig. 3b**). Although the SERS effect has been known for some time to provide an enhancement of as great as 10^5 in Raman cross section for molecules on a rough gold surface, it was not until the advent of single molecule studies that it was discovered that in fact only a very small number of molecules on the surface (one in 10^5) actually provide for the enhanced signal[51,52]. These few molecules are now thought to be located at special sites in the gap between two nearly touching gold nanocrystals[53].

Perhaps it will be possible to prepare biological sensors consisting of a biological macromolecule with specific affinity, and located in the gap between two 50-nm gold nanocrystals. Such a system would be extremely specific and sensitive. Halas and colleagues[54,55] have shown that it is possible to alternately pattern metal and dielectric materials radially in shells, providing a high degree of control over plasmon resonances and the Raman scattering process[54] and providing an important tool for biological detection[55].

Detection systems

Molecular events can be sensed and detected in biology using three main formats: optical detection, electrical detection, and magnetic detection.

Optical detection. Optical detection remains the most widely used mechanism for detecting biological binding events and for imaging in biological systems. In the future, the goal will surely be to enable single molecule detection *in vivo,* despite the large background present in a living system. Combinations of quantum-confined systems, plasmon excitations in metal nanoparticles, and manipulation of the local fields in their environment, with control over the photon density of states, could in fact yield such systems in the coming years.

Electrical detection. Even though optical techniques continue to evolve, the fact is that electrical detection remains extremely desirable. Electrical systems can be miniaturized and integrated into systems, offering many advantages over optical detection schemes. Here, nanotechnology has a great deal to offer.

Pseudo-one dimensional nanostructures, such as semiconductor nanowires[56,57] and carbon nanotubes[58] offer the greatest chance yet for creating robust, sensitive, and selective electrical detectors of biological binding events. Current flow in any 'one-dimensional' system is extremely sensitive to minor perturbations, and in nanowires and nanotubes, the current flows extremely close to the surface. Biological macromolecules bound to the surface of a nanowire and undergoing a binding event with conformational change or change of charge state, may thus perturb the current flow in the nanowire. Thus, it is possible in principle that these materials will form the basis of new electrical biosensing systems, and important strides in this direction have been made.
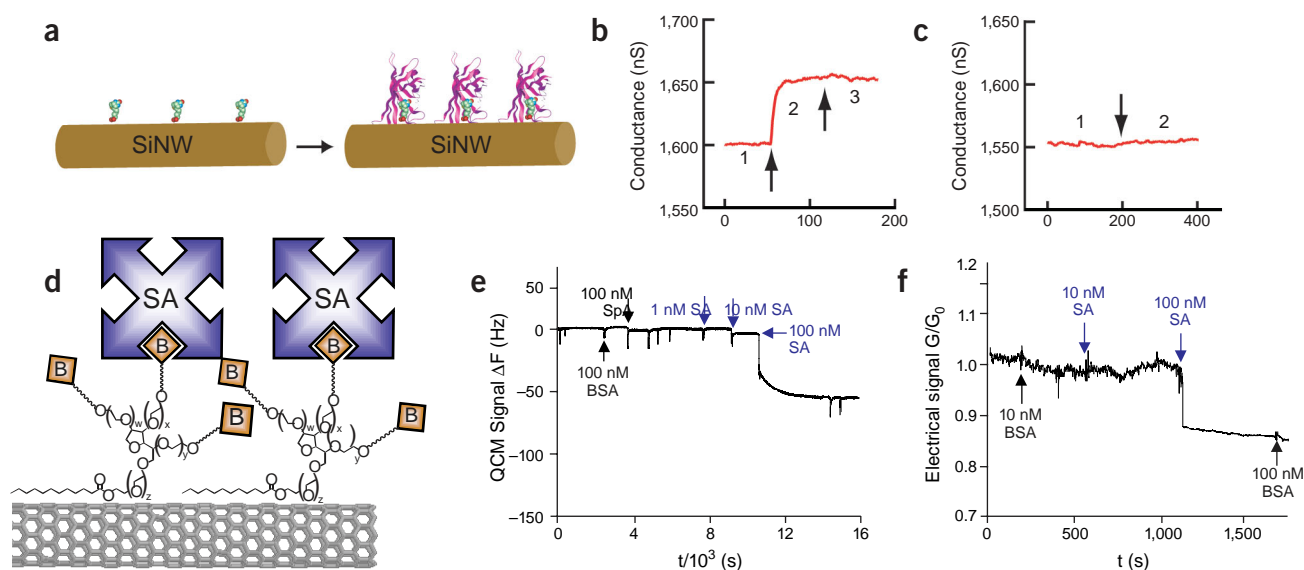
Figure 4 Nanowire- and nanotube-based electrical biosensors. (**a**) Scheme showing silicon nanowires functionalized with biotin. (**b**) On exposure to streptavidin, the nanowires show changes in conductivity. Plot of conductance versus time for a biotin-modified SiNW, where region 1 corresponds to buffer solution, region 2 corresponds to the addition of 250 nM streptavidin, and region 3 corresponds to pure buffer solution. (**c**) A nanowire that is not functionalized with biotin shows no response. Conductance versus time for an unmodified SiNW; regions 1 and 2 are the same as in **b**. (**a–c**, Reprinted by permission of the American Association for the Advancement of Science from ref. 59.) (**d**) Scheme showing nanotubes functionalized with biotin shows similar changes. (**e,f**) Quartz-based microbalance signal (**e**) and electrical signal of nanotubes after addition of different concentrations of streptavidin (**f**). (**d–f**, Reprinted by permission of the National Academy of Sciences, USA, from ref. 98.)

For example, in a breakthrough series of experiments, Lieber and colleagues[59] have shown that semiconductor nanowires can be functionalized with biological macromolecules and incorporated into electrical circuits, and that in this configuration, the current that flows in the nanowire is very sensitive to binding events of the macromolecule (**Fig. 4**). The precise sensing mechanism is not fully established. To see one possibility, consider the example of a silicon nanowire coated with a thin layer of silica, and immersed in a buffer. The wire is surrounded by a double layer of ions, and any perturbation to this ionic environment, such as may occur when a protein on the wire surface undergoes a binding event, may alter the field experienced by the wire. Similar experiments may be possible with nanotubes, although in this case care must be taken to separate the complex mixture of metallic and semiconducting nanotubes that are created in the nanotubes generation process. For instance, Zettl and colleagues[60] and Dai and colleagues[61] have shown that carbon nanotubes can be extremely sensitive detectors for a variety of gases. In this case, the transduction mechanism can be more readily understood as involving direct adsorption of the gas on the tube, leading to a change in the electronic structure that can be detected electrically. However, similar efforts to bioconjugate nanotubes and to use them in biological detection schemes are in progress in many laboratories[58,62,63].

Nanomaterials that transport ions rather than electrons may also form extremely interesting artificial electrical biodetectors. Here, physical scientists are learning first from nature, where transport through gated nanopores in membrane proteins are used to sense and transmit signals. Bayley and colleagues[64,65] have beautifully demonstrated the kinetics of transport thorough individual protein pores and have shown how these can used in biosensors. The shape in the nanopores is just the inverse of the nanowire and nanotube described above because the ions now move through a one-dimensional channel, and therefore again, sensitivity and control can be maximized, and of course selectivity can be achieved via the protein. This has

sparked the nanoscience community to further consider ways in which nanopores can be created and used for biological sensing and detection[66]. Perhaps the most well known proposal is to rapidly sequence a single DNA molecule by electrically sensing the base pairs as they pass by electrodes that are embedded around a nanopore in a semiconductor material[67]. Different polynucleotides can be distinguished from each other as they pass through an alpha hemolysin channel[68], and entirely artificial nanopore/detector schemes are under active investigation[69].

Magnetic detection. More complex physical behavior, beyond quantum-confined semiconductor systems (single-electron-like behavior), metals (with collective plasmon excitations), or even ordinary electrical devices, arises in systems with correlated electron behavior, including nanoscale magnetic systems and superconductors. The physics of small magnetic systems was first discussed decades ago[70], and is undergoing a renaissance as it becomes possible to study magnetic phenomena in individual nanoparticles[71] and even individual molecules[72]. Magnetic crystals behave as a single magnetic domain, with all the spins in a crystal coupled together to create a giant magnetic moment. In a very small crystal, and at a high enough temperature, this moment wanders randomly (superparamagnetic); above a critical size, this moment becomes locked in a fixed direction (ferromagnetic). The critical size is about 25 nm for iron oxide, and about 11 nm for cobalt nanocrystals. Magnetic nanocrystals 25 nm in size appear in magnetotactic bacteria, which contain a chain of such particles that acts as a compass[73]. Magnetic nanocrystals are also widely employed in artificial biological detection and separation systems, serving important roles as magnetic resonance contrast enhancement agents[74–76], and as the basis for a wide range of magnetophoresis experiments[77–79].

Superconductors already play an important role in biomedicine, as they form the basis for the magnets used in magnetic resonance imag-

ing. Will such materials also play an important role on the nanoscale? Two trends are very encouraging in this respect. The first is the emergence of new detection schemes based on magnetic nanoparticles and superconducting quantum interference device (SQUID) magnetometers. In a SQUID, matter must be patterned to create regions where two halves of a superconducting loop are separated by insulating gaps. The resulting device is very sensitive to magnetic fields, and SQUIDs can detect the change when ferromagnetic nanoparticles stop rotating freely in solution, owing to a biological binding event[80]. The detection limits may eventually be pushed down to the single molecule level[81]. The exquisite sensitivity that arises in a system with quantum interference is more clearly brought home by another stunning development: the remarkable feat of magnetic resonance imaging using microtesla fields (the Earth's magnetic field is 100 times greater) rather than a large external magnet. This new advance is only possible due to the control of correlated electron behavior and quantum effects in the SQUID, and also points out that beyond the question of detecting biological macromolecules, imaging at the nanoscale remains a major goal, and advances in nanomaterials are sure to drive this further. (A trend in this area is the development of scanning probe techniques[82], including the proposed scanned probe magnetic resonance imaging[83], which are not reviewed here.)

Perspectives

There is much to be done with respect to materials development for nanocrystals. For example, one negative feature of colloidal quantum dots is the fact that they 'blink' or emit light intermittently when excited with high intensity[84]. This arises not only as a by-product of multiple-charge nonradiative inelastic scattering (which is enhanced in quantum dots where the charge carriers are tightly confined and the overlap of charges is large[85]), but also from the difficulties of growing a thick surrounding shell of high band-gap material to fully 'confine' the photo-generated charges. It is important to note that embedded dots, grown by molecular beam epitaxy, do not show this feature[86], and therefore it is surely possible to make colloidal quantum dots that do not blink. It is very likely that this may occur through the study of colloidal quantum rods[87], which in addition to showing highly polarized light emission[88], may well also show reduced blinking effects owing to their greater volume.

Another key feature of interest is the radiative rate. Quantum dots emit with a lifetime of a few tens of nanoseconds, which is very good for gated detection to suppress background from biological systems[89], but definitely limiting for applications in which it is necessary to cycle the chromophore from excited to ground state rapidly in a short period of time (e.g., in flow cytometry). Rods are likely to have highly enhanced radiative rates compared with dots (A.L. Efros, Naval Research Laboratory, Washington DC, USA, personal communication).

Furthermore, it may be possible to control the radiative rates of dots by embedding them in an environment that enhances the local electromagnetic field in their vicinity[90]. In any case, in the next few years, it is likely that a quantum-confined system for biological detection with a sub-nanosecond radiative rate will be developed.

As this article has outlined, both the electron density of states and the photon density of states can be used to alter fundamental materials properties. In the next decade, these two areas of research will merge. Physical scientists are increasingly looking into ways to simultaneously control the electronic energy levels and the photon density of states[91,92]. There have been studies recently of single quantum dots embedded in microscopic cavities that can control the allowed modes of light emission[93,94]. Much of this research is directed more towards sophisticated techniques of manipulating information optically[95]; however, if such

systems can be further developed and coupled to biological macromolecules, they may provide narrow band, efficient, directional light emission, coupled with highly specific and very high gain detection of binding events.

Concurrent with advances in the nanomaterials themselves, progress is rapid particularly in electrical and magnetical systems used for their detection in biosensors. The case of superconductivity is only one in which we see that the ability to control a quantum system can yield entirely new ways of detecting and imaging. Current research on highly correlated electron systems and on controlled quantum systems, such as solid state qubits[96], may seem very remote from new biotechnologies today, but will surely form the basis of future technologies, much as quantum confined systems emerged as a biotechnology over the past decade.

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