Chapter 8

APPLICATIONS: BIOTECHNOLOGY, MEDICINE, AND HEALTHCARE

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8.1 VISION

Nanotechnology is beginning to allow scientists, engineers, and physicians to work at the cellular and molecular levels to produce major benefits to life sciences and healthcare. In the next century, the emerging field of nanotechnology will lead to new biotechnology-based industries and novel approaches in medicine.

8.2 CURRENT SCIENTIFIC AND TECHNOLOGICAL ADVANCES

Major advances in the last several years in scanning probe and scanning optical analytical methods permit viewing the vital chemical processes and microscopic structures in biological systems with unprecedented resolution. These new analytical probes reveal a detailed picture of the microscopic structure of living cells and a view of chemical processes at the molecular scale. The atomic force microscope, for example, can locate and measure the extraordinarily small forces associated with receptor-ligand binding on cell surfaces. Microscopic electrical probes can detect a living cell's exchange of ions with its environment or the propagation of electrical signals in nerves. New high-resolution optical instruments, combined with chemically selective light-emitting fluorescent probes, can follow in detail the chemical processes on the surface of and inside a living cell. This analytical capability allows observation of the biochemical processes and interactions of cells in living systems.

Cells contain exquisite naturally occurring "molecular motors." One of many examples of these naturally occurring nanomachines is F1-ATPase, which is part of the large, membrane-embedded complex that synthesizes ATP within mitochondria (Figure 8.1). This structure, only about 10 nm in size, is a robust, fully functional rotating motor that is powered by natural biochemical processes. In 1998 the Amersham Pharmacia Biotech and Science Prize was awarded to Hiroyuki Noji, a young Japanese scientist who demonstrated the function of this molecular motor by attaching a long actin filament to the rotating part of the motor and observing the rotation in an optical microscope. The detailed understanding of the structure and function of this motor protein and other macromolecular assemblies essential for life is an area of growing scientific importance.

During the last few years, scientists have developed the technology for rapidly mapping the genetic information in DNA and RNA molecules, including detection of mutations and measurement of expression levels. This technology uses DNA microchip arrays that adapt some of the lithographic patterning technologies of the integrated circuit industry.

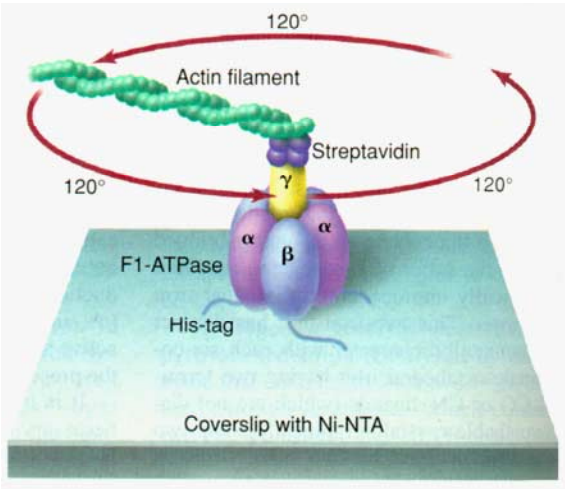


Figure 8.1. The molecular motor protein F1-ATPase. Illustrated here is an experiment reported in *Science*, in which an actin filament is attached to a motor protein to provide load to and allow visualization of the motor rotation (reprinted with permission from Noji 1998, ©1998 American Association for the Advancement of Science).

This is now a commercial technology and is finding its way into biotechnology research and industrial utilization. Work on new types of chemical arrays should expand this approach of parallel biological information processing to analysis of proteins and other biomolecules. Miniaturization of allied analytical processes such as electrophoresis will lead to increases in throughput and reduced cost for other important methods of analysis such as DNA sequencing and fingerprinting. For example, new research (Turner et al. 1998) is aimed at replacing the tedious, slow, and expensive process of DNA sequencing in slab gels with miniaturized integrated microfabricated analytical systems (Figure 8.2).

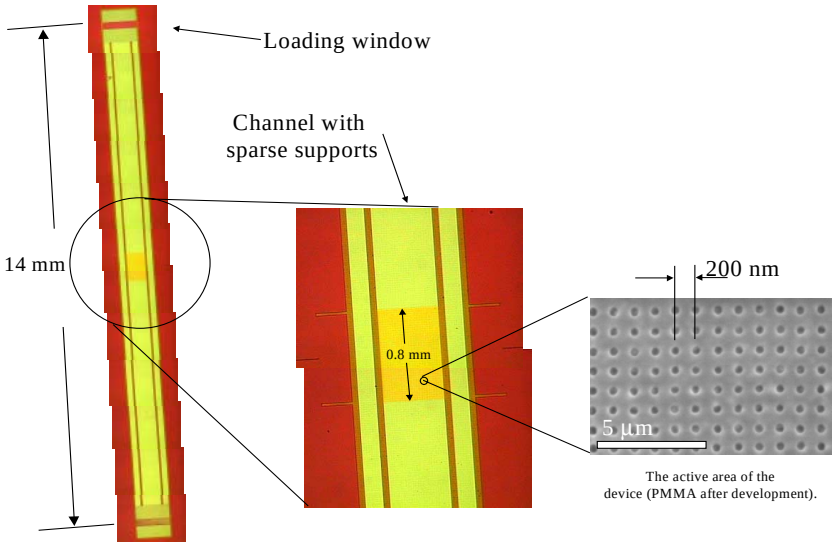


Figure 8.2. Photomosaic of a DNA separation chip. The image is pieced together from twelve optical micrographs. The inset shows a small region 0.8 mm long containing dense pillars that act as a molecular sieve to separate DNA molecules according to size. Conventional gel electrophoresis works essentially the same way, and for this reason these nanofabricated structures are called "artificial gels." This technology, while far from commercialization, has the potential to revolutionize DNA separation techniques by providing an inexpensive, durable, and reproducible medium for DNA electrophoresis (courtesy S.W. Turner, Cornell Univ.).

Using biological systems as a model, scientists are attempting to build ever more complex systems that are capable of self-assembly. As the sizes of components become smaller and manipulation of these components becomes impracticably slow, the need for self-assembling systems is rising. Complex biological systems provide models from which to design components that can come together in only one way to form the desired three-dimensional nanoarchitectural system. Similarly, scientists are using strategies learned from biological systems to design new materials. Spider silk is one of the strongest materials known. Its molecular structure is being used to design better composite polymer systems of increasing strength and utility.

Nanoparticles considerably smaller than one micron in diameter have been used in revolutionary ways to deliver drugs and genes into cells. The particles can be combined with chemical compounds that are ordinarily insoluble and difficult for cells to internalize. The derivatized particles can then be introduced into the bloodstream with little possibility of clogging the capillaries and other small blood vessels, as in the case of insoluble powders. The efficacy and speed of drug action in the human body can thereby be dramatically enhanced. In similar ways, nanoparticles carrying DNA fragments can be used to incorporate specific genes into target cells (Figure 8.3).

Figure 8.3. Pictured here is the "Gene Gun," a system that uses nanoparticles to deliver genetic material to transfect plant and animal cells. In this system, submicron gold particles coated with DNA are accelerated with a supersonic expansion of helium gas. The particles leave the front of the device at high velocity and penetrate the cell membrane and nuclear membrane, thus delivering the genetic material to the nucleus (courtesy Bio-Rad Laboratories).

The ability of DNA to undergo highly controlled and hierarchical assembly makes it ideal for applications in nanobiotechnology. For example, DNA has been used to design lattices that readily assemble themselves into predictable, two-dimensional patterns. These arrays are composed of rigid DNA tiles, about 60 nm^2, formed by antiparallel strands of DNA linked together by a double-crossover motif analogous to the crossovers that occur in meiosis. The precise pattern and periodicity of the tiles can be modified by altering DNA sequence, allowing the formation of specific lattices with programmable structures and features at a nanometer scale. This approach has the potential to lead to the use of designed DNA crystals as scaffolds for the crystallization of macromolecules, as materials for use as catalysts, as molecular sieves, or as scaffolds for the assembly of

molecular electronic components or biochips in DNA-based computers. Similarly, biological-molecule-based scaffolding could take advantage of the unique structural characteristics of RNA molecules, of polypeptide chains, or of the highly specific interactions that occur between DNA and proteins or between RNA and proteins.

Devices that are currently in use to control the interactions of DNA on surfaces can have broader applications for controlling nanoassembly. These devices use electric fields to control the movement of particles toward or away from microscopic sites on the device surface. Charged biological molecules (DNA, RNA, protein) and analytes, cells, and other nanoscale or microscale charged particles can be precisely organized.

8.3 GOALS FOR THE NEXT 5-10 YEARS: BARRIERS AND SOLUTIONS

The advances noted above and others involving nanofabrication and nanosynthesis are enabling significant new opportunities for scientific research and commercial applications.

The integration and miniaturization of fluid control, or fluidics, with photonics and electronics is a trend that will lead to a paradigm change in chemical synthesis and analysis. Industries that have not previously been considered high-tech will be transformed by nanofabrication technology in the twenty-first century.

Given the inherent nanoscale of receptors, pores, and other functional components of living cells, the detailed monitoring and analysis of these components will be made possible by the development of a new class of nanoscale probes. Nanotechnology will improve the sensitivity and integration of analytical methods to yield a more coherent evaluation of life processes. The ability to manipulate cells and integrate them with complex inorganic devices and probes will permit scientists to perform a new class of experiments and ask new questions about basic cell functions. For example, integrated cellular systems grown in culture could replace and thus spare animals used for testing drugs and hazardous materials.

Nanoscale sensors. Integrated nanoscale sensors could monitor the condition of a living organism, the environment, or components of the nutrient supply, sampling a range of conditions with a high degree of sensitivity. With arrays of ultraminiaturized sensors that sample a range of chemicals or conditions, the confidence level and specificity of detection would be much greater than is now possible with separate macroscopic sensors. As has been seen with electronic integrated circuits, as the level of device integration increases and the volume of production grows, the costs of highly complex units decreases. One can project that in the next century highly sophisticated, small, and inexpensive sensors employing nanotechnology will be available and used routinely in many parts of our lives.

Nanomachines. To date, development of miniaturized devices is based mostly on nonbiological principles. An example of an autonomous miniaturized controlled-released implantable device (a solid-state silicon microchip) for drug delivery applications is illustrated in Figure 8.4. The microchip can release a single or multiple chemical substance(s) on demand. In addition to drug delivery, this technology may also find use in such areas as diagnostics, analytical chemistry, and others.

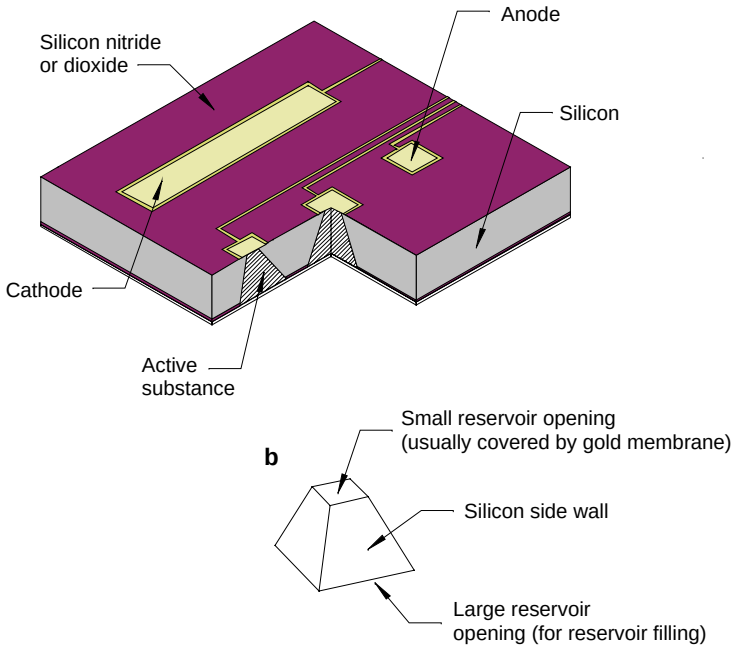


Figure 8.4. Prototype of a microchip device for drug delivery (reprinted by permission from *Nature*, Santini et al. 1999, ©1999, Macmillan Magazines Ltd.).

As integrated nanofabricated systems decrease in size, the ability to retain desired functions will become more difficult. As has been noted, nature has solved many of these same engineering problems and has produced functional molecular motors and many other subcellular functional machines. Further research should allow scientists to integrate these natural systems with inorganic devices and create hybrid systems and a new class of nanomechanical devices. Nanomachines powered by chemically fueled molecular motors could be coupled to devices with integrated valves, pumps, and sensors that can react to changes in the body and the environment. One can imagine, for instance, miniaturized, self-powered machines that sense and identify oil or chemical pollutants in soils and map their distribution and concentration, or medical implants that sense and dispense drugs or hormones in response to body changes.

Nanoparticles. Current bioengineered, non-viral gene vectors that are used to introduce new genes into cells are far from perfect. Ideally, DNA nanoparticles with controlled composition, size, polydispersity, shape, morphology, stability, encapsulation capability, and targetability will result in new technologies with improved *in vivo* transfection efficiency. Such nanotechnology will likely have a significant impact on realizing the potential of genetic engineering techniques in agriculture, manufacturing, and environmental applications, as well as in medicine.

Drug development. Technology is dramatically accelerating the discovery of new drug compounds. Continuing advances in nanotechnology will lead to innovative synthetic routes, new processing strategies, and more economical manufacturing. The same or similar processes that have led to the phenomenal increases in computational speed of microprocessors and the increasing density of computer memory will similarly revolutionize the speed with which new compounds are screened for therapeutic potential as new drugs. The pharmaceutical industry projects nearly a tenfold increase in the number of drug compounds that will be evaluated in 2000 compared to 1998, with only a

modest miniaturization of technology. If the trend is similar to that of microelectronics, the rate could grow exponentially. Arrays of nanodrops, each a mere nanoliter in volume, but holding a small cell culture sample, are being used to place hundreds of thousands of cell culture assays on a laboratory desktop, revolutionizing the speed with which new pharmaceuticals can be screened for activity. The time required for new drugs to reach patients could thus be reduced, saving human lives.

Drug delivery. Drug and gene delivery will continue to impact significantly on the practice of medicine. Nanotechnology as applied to drug delivery systems will undoubtedly dramatically improve the therapeutic potential of many water-insoluble and unstable drugs. Microsensors interfaced to a nanoscale drug delivery system could dispense precise amounts of drugs for optimum functionality and minimum toxicity. However, significant challenges still remain in synthesis and processing of drug-carrier nanoparticles at the industrial scale. Nanotechnology may also help reach the hitherto elusive goal of active drug targeting to selected cells within the body. Nanotechnology that can further reduce the size and reproducibly attach targeting ligands to the drug-loaded nanoparticles may help localize the drug to the desired tissues in the body. These nanoparticles may also be valuable tools for molecular and cell biologists to study fundamental cellular processes such as receptor-mediated endocytosis and intracellular trafficking.

Interfaces between biological and other materials. In the repair of the human body with prosthetics or artificial replacement parts, mechanical attachment to the body, or alternatively, rejection by the body, occurs at biological interfaces. The nanoscale chemical and topographical details of the implanted materials determine the reaction of the body. If we can gain sufficient understanding and control of these biological reactions to surface nanostructure, we may be able to control the rejection of artificial implants. Similarly, it may be possible to surround implanted tissue with a nanofabricated barrier that would thwart the rejection mechanisms of the host, allowing wider utilization of donated organs. Ultimately, better materials and understanding of their interaction with the body may lead to implants that the body will not only accept, but that will actually become integrated into the body. Nanofabrication and nanosynthesis give us powerful new tools to address these important medical issues for which a great deal of research is still necessary.

Various bio-inspired ideas are discussed in other chapters (e.g., Chapter 4, on synthesis, and Chapter 6, on nanodevices).

8.4 SCIENTIFIC AND TECHNOLOGICAL INFRASTRUCTURE

The infrastructure needs for nanobiology are similar to those for other fields: multiuser facilities to provide access to specialized technologies, funding mechanisms and organization structures that encourage and support multidisciplinary teams and are responsive to rapid technological change, and training of a new generation of scientists and engineers who are prepared to maximally exploit this new knowledge.

The teaming of physical scientists, engineers, biologists, and health professionals will be required for research and development efforts. The universities should be supported with

grants for training new undergraduate, graduate, and postdoctoral students in these interdisciplinary areas.

8.5 R&D INVESTMENT AND IMPLEMENTATION STRATEGIES

- Fund basic science and technology development needed for future biotechnology, health, and national security (biowarfare, nanobiodevices, and survivability) needs. This must include basic research in the cell and molecular biology of the many naturally occurring nanomachines within cells.
- Fund efforts to train clinicians in the use of the emerging technologies and their integration into medical instruction.
- Promote funding in proposals with rapid turnaround times for exploratory, agile response to developing opportunities uncovered by advances in nanotechnology.
- Encourage interdisciplinary cooperation of academic, industrial, and Federal laboratories.
- Support coordinated research by teams that represent the required diversity of disciplines, at sufficient magnitude to make rapid progress.

8.6 PRIORITIES AND CONCLUSIONS

- Exploratory research should be encouraged and new ideas promoted aggressively in the area of nanobiotechnology.
- A systematic investigation should be undertaken of natural structures with intrinsic patterns at the nanoscale, as well as in use of the identified nanoscale patterns for new materials and devices.
- Interaction of biomolecules with inert materials is an area of special interest both for medical application and for understanding the role of environment on the origin and evolution of life on Earth.
- It is important to support universities in interdisciplinary training of undergraduate and graduate students at the intersection of biological, physical, and engineering sciences.

8.7 EXAMPLES OF CURRENT ACHIEVEMENTS AND PARADIGM SHIFTS

8.7.1 Special Attributes of Biological Systems

Contact person: L. Jelinski, Louisiana State University

Biological molecules and systems have a number of attributes that make them highly suitable for nanotechnology applications. For example, proteins fold into precisely defined three-dimensional shapes, and nucleic acids assemble according to well-understood rules (Figure 8.5). The ribbon diagram of the oxygen-binding protein myoglobin, found in muscle cells, is illustrated in the lower portion of the figure, a diagram constructed from atomic coordinates provided by the Protein Data Bank. Antibodies are highly specific in recognizing and binding their ligands, and biological assemblies such as molecular motors can perform transport operations. Because of these

and other favorable properties, biomolecules, biophysics, and biology are themes that run through all of the topics of this report (Jelinski 1999).

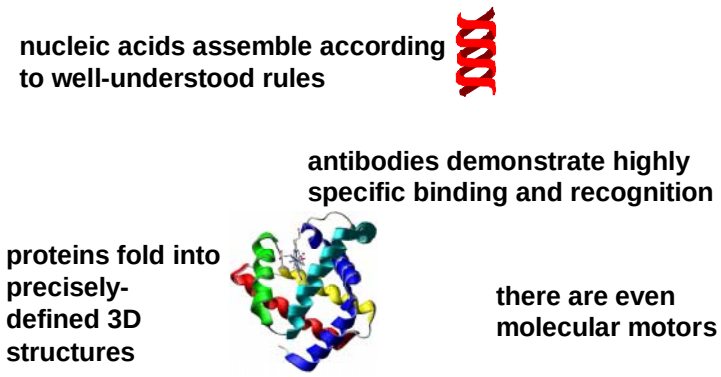


Figure 8.5. Examples of biological systems (courtesy L. Jelinski; lower diagram courtesy L. Pollack, Cornell University).

8.7.2 Nanoscience and Nanotechnology in Tissue Engineering

Contact person: D.J. Odde, University of Minnesota

Between the typical size of an animal cell, ~10 μm, and that of a protein molecule, ~5 nm, is where nanotechnology advances can effect better understanding and control of living cells. Achieving greater control of cell behavior will likely facilitate efforts in the emerging area of tissue engineering. Tissue engineering is directed toward using cells and their molecules in artificial constructs to compensate for lost or impaired body functions. Commercial ventures are currently spending ~$500 million/year in research, development, production, and marketing. In 1998 the first two tissue-engineered products came on the market after Food and Drug Administration approval (Lysaght 1998). These first two products are both engineered skin equivalents, although many more tissues are at various stages in development and clinical trials. Undoubtedly, a vast array of new nanotechnologies could potentially facilitate future tissue engineering efforts, both in basic and applied research. Four procedures are highlighted here as examples of applications of nanoscience and nanotechnology to tissue engineering.

First, scanning probe microscopy can be used to elucidate the nanometer-scale structure of protein filaments (Hameroff et al. 1990). These filaments include both intracellular and extracellular structures that are linked together via transmembrane receptors to provide the mechanical continuity that holds tissues together. Second, optical forces in the form of laser-tweezers can be used to measure motor protein motions on the nanometer scale (Svoboda et al. 1993). Understanding how molecular motors work will help us to better understand the fundamental contractile and propulsive properties of tissues. Third, biomaterials can be fabricated that have nanometer-scale features representing the imprinted features of specific proteins (Shi et al. 1999). Such imprinted surfaces could potentially provide highly stable, biospecific surfaces for the long-term maintenance of an engineered tissue equivalent. Fourth, nano/micro particles, including living animal cells, bacteria, and colloidal gold (100 nm), can be optically guided and deposited in arbitrarily defined three-dimensional arrays, a process called “laser-guided direct-writing.” As shown in Figure 8.6, individual spinal cord cells can be confined and guided along a laser beam axis to generate a steady stream of particles. By combining

various cell types and biomaterials, arbitrary three-dimensionally patterned cell constructs can potentially be assembled to more closely mimic the architecture and structure of native organs (Odde and Renn 1998; Renn et al. 1999).

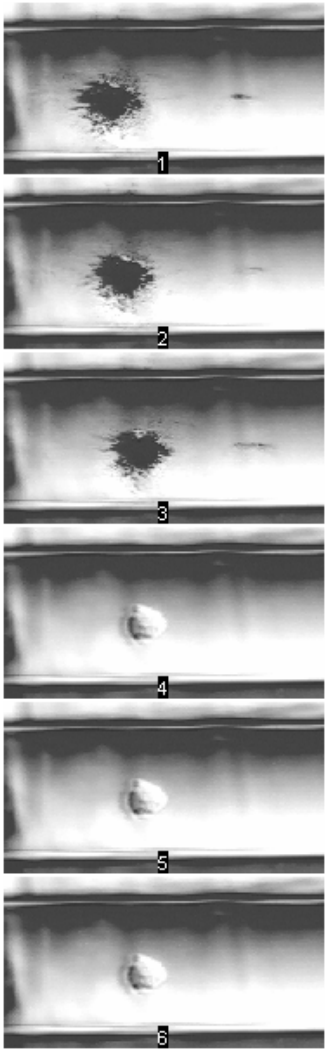


Figure 8.6. Laser-guided transport of an individual spinal cord cell inside a hollow optical fiber. The laser light comes from the left and imparts a propulsive force on the cell. The laser beam can be directed onto a surface and cells deposited into arbitrary patterns. Subcellular particles (~100-500 nm) are also guided (cell diameter, 9 μm; time interval between frames, 300 msec).

8.7.3 Biodetection

Contact Person: J. Murday, Naval Research Laboratory

Nanotechnology promises revolutionary advances in military capability. For instance, the confluence of biology, chemistry, and physics at the nanometer scale is enabling significant advances in military sensors for biological and chemical warfare agents. Civilian disaster response teams and commercial medicine will benefit as well. We cannot afford to respond to a nerve gas attack, such as the 1995 Aum Shinrikyo incident, by carrying a canary as a sensor (Figure 8.7). Defense research and development programs are pursuing many sensor options; two related technologies are nearing fruition and will have medical applications as well.

Figure 8.7. Canary sensor (courtesy Sankei Shimbun).

One is a colorimetric sensor that can selectively detect biological agent DNA; it is in commercial development with successful tests (Figure 8.8) against anthrax and tuberculosis (Mirkin 1999). Compared to present technology, the sensor is simpler, less expensive (by about a factor of 10), and more selective—it can differentiate one nucleotide mismatch in a sequence of 24, where 17 constitutes a statistically unique identification.

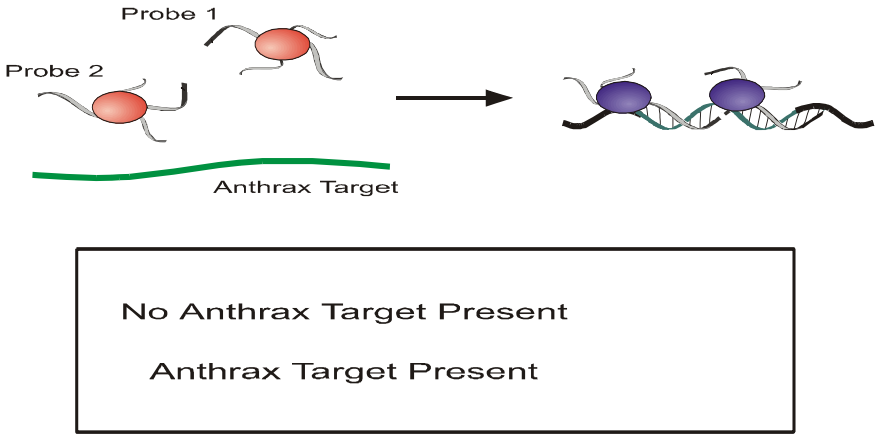


Figure 8.8. Anthrax detection: when the anthrax target is present, pairs of nanoparticles assemble together via the DNA filaments and change the color of the respective suspension (courtesy C. Mirkin, Northwestern University).

A complementary effort is based on atomic force microscopy with a sandwich immunoassay attaching magnetic beads to a microfabricated cantilever sensitive to small displacements (Figure 8.9; Colton 1999). In the laboratory this technology is already 100 to 1,000 times more sensitive than conventional immunoassays.

Both colorimetric and magnetic bead technologies might be implemented in detector arrays that provide simultaneous identification of multiple pathogens. For instance, GMR memory elements can sense the presence of the magnetic beads (Colton 1999).

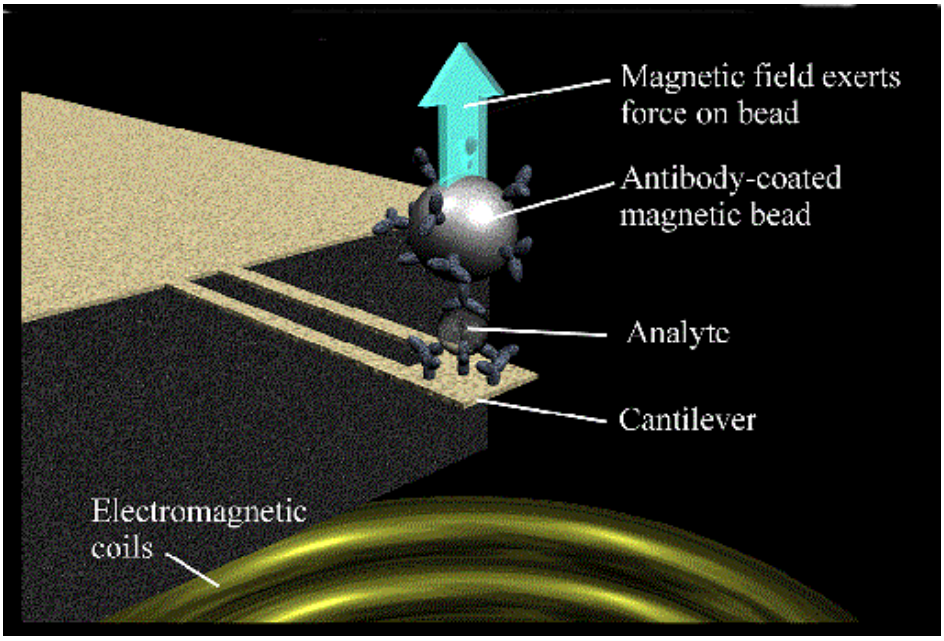
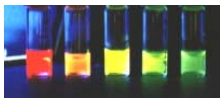


Figure 8.9. Atomic force microscope (AFM) immunoassay (courtesy Naval Research Laboratory; reprinted with permission from Baselt et al. 1996, ©1996 American Vacuum Society).

8.7.4 Semiconductor Nanocrystals as Fluorescent Biological Labels

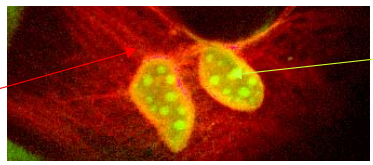
Contact person: P. Alivisatos, University of California, Berkeley

For more than a decade there has been an intensive effort to prepare high-quality nanometer-size colloidal crystals of many common semiconductors. At the onset, this effort had a strong focus on fundamental studies of scaling laws, in this case, quantum confinement of electrons and holes. Over this decade, tremendous advances occurred in both the spectroscopy and the fabrication methods. This yielded a new class of very robust macromolecules with readily tunable emission energy. To the extent that applications of this technology were envisioned at the onset, they were focused in the domain of optoelectronics. Yet quite unexpectedly, it turns out that these colloidal nanocrystals can be used as fluorescent labels for biological tagging experiments. Biological tagging is one of the most widely employed techniques for diagnostics and visualization. As shown in Figure 8.10, it appears as though for many applications, the colloidal nanocrystals are advantageous as labels, when compared to existing organic dyes (Bruchez et al. 1998; Chan and Nie 1998). This has led to rapid commercialization of the new nanotechnology.



Band gap vs. size in CdSe nanocrystals
10 year study of scaling laws and synthesis

Unexpected applications in biological labeling
Example: Two-color stain of mouse fibroblast cell



- Significant advantages over conventional dyes:
- Reduced photobleaching
- Multi-color labeling, parallel screening
- Infrared labels, blood diagnostics
- Molecular size nanocrystals are bio-compatible, with many other possible applications

Figure 8.10. Semiconductor nanocrystals as fluorescent biological labels (reprinted with permission from Bruchez et al. 1998, ©1998 American Association for the Advancement of Science).

8.7.5 Nanofabrication of DNA "Chips"

Contact persons: M. Sussman, University of Wisconsin; P. Brown, Stanford University

DNA detector arrays that today operate in the micron size range provide the potential to do thousands of experiments simultaneously with very small amounts of material. Figure 8.11 is an image of a chip with 6,400 microdots, each containing a small amount of a different gene in the yeast genome and capable of determining how active that gene is in yeast. Yeast cells were grown under various conditions; the amount of red or yellow light represents the level of RNA produced from the DNA in that gene, under those conditions. Similar experiments using this or related technologies can now be performed with tens or hundreds of thousands of human genes. By comparing the pattern of gene expression of normal tissue with cancerous tissues, scientists can discover which few genes are being activated or inhibited during a specific disease. This information is critical to both the scientific and clinical communities in helping to discover new drugs that inhibit cancer-causing genes. The important point is that these technologies allow physiological changes in yeast or humans to be characterized, molecule by molecule, in just a few hours. Five years ago, an experiment like this would have taken dozens of scientists months to complete.

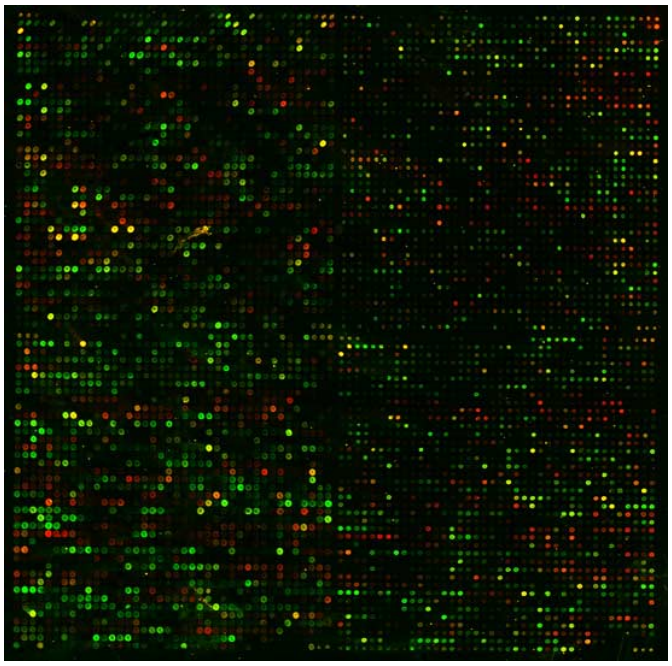


Figure 8.11. The full yeast genome on a chip (Brown 1999).

This technology therefore represents a paradigm shift in the way biologists do research, providing a means for using the vast amounts of information being revealed by the Human Genome Project. Some scientists have likened this to 150 years ago, when the periodic table for the chemical elements was discovered, ushering in a century of breakthroughs in chemistry. By analogy, the human and plant genome projects may organize all biological information in a way that may usher in a century of basic and applied research in the manipulation of life. Despite the power of the new technology, coupled with genome sequences, it is still in its nascent forms and is largely limited in its sensitivity, selectivity, and requirement for expert operators. Nanotechnology has the potential to do the following:

- Further reduce the size of the assays, allowing larger numbers of genes to be studied in each experiment
- Increase their sensitivity, for example, through better detection methods
- Result in wider application of these systems in hospitals, clinics, or perhaps even as real-time sensors within the body, for example, by enabling new ways to integrate sequential steps in lab procedures into ultraminiaturized lab-on-a-chip devices that are less subject to operator error

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