

Drug Discovery and Biotechnology Trends

Nanobiotechnology: Giant Successes from Atom-Size Devices



Devices with nanometer-scale features bring much higher resolution to basic research and provide new diagnostic capabilities for medicine. From infinitesimal forces to molecule-size drops of fluids, research scientists in academia and industry keep finding new ways to apply nanotechnology to biological challenges. **BY MIKE MAY AND GARY HEEBNER**

In the last half of the 20th century, electronics shrunk from room-size computers to laptops. In the next century, a similar transition could take biotechnology to the submicron range, which is now known as nanobiotechnology. Nonetheless, this field goes beyond a simple reduction in size. According to Uzi Landman, regents' and institute professor at **Georgia Institute of Technology**, "When you get below a certain length scale, a system behaves differently. You lose the ability to predict that behavior by extrapolating large to small." Consequently, nanobiotechnology generates an entirely new world.

This field attracts attention for a variety of reasons. For one thing, says Landman, "Nanobiotechnology can increase our awareness and understanding of nature." He adds, "This is a lofty goal, and this separates us from cows. We want to better understand nature." In addition, scientists can look at nanobiotechnology from a pragmatic perspective. Landman says that scientists can also use nanobiotechnology to translate knowledge into something that improves lives, such as curing a disease. Both of these goals—basic research and applications—require a new mindset and new tools. "You need to get rid of existing paradigms," says Landman, "and think in a different way." But that thinking also goes along with the demand for new tools. "You don't talk about injecting a drug in

a cell without being able to produce a droplet that is much smaller than the cell itself," Landman says.

Landman understands how ideas can turn into applications. In the cover story for the 18 August 2000 issue of *Science*, Landman and Michael Moseler, now at the **Fraunhofer Institute** in Freiburg, Germany, simulated nanojets with diameters up to 10 nanometers. Before carrying out the computations, Landman and Moseler wondered if such small jets could even exist, but the numbers showed that they could. Now, a team of Georgia Tech engineers builds submicron nozzles. "In a year and a half or so," says Landman, "we will inject biological material through cell membranes." He adds, "We started from a novel concept, and today we can produce nanojets and, shortly, see them in electron micrographs, as well as record their momenta and energy deposited to a target through deflection measurements and infrared imaging when the jets impact on atomic force microscope cantilevers." This project and others show the promise of nanobiotechnology, and even philanthropists see the potential. An any-

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mous donor gave Georgia Tech \$36 million to support nanotechnology, and the state of Georgia added \$45 million. That makes \$81 million of faith in the value of making things small. Other institutions also see vast potential in this field (see “Centers for Nanobiotechnology”).

IMAGING ON THE ATOMIC SCALE

As Landman mentioned, he and his colleagues use electron microscopy to see their submicron jets. Many other scientists in the field of nanobiotechnology also need ways to see what they build, even when the objects dip far below the resolution of light microscopy, which is about 200 nanometers (or 0.2 microns). To see smaller features, scientists turn to electron microscopy or atomic force microscopy.

Electron microscopy provides a resolution down to 0.1 nanometers, and it comes in two forms: transmission electron microscopy (TEM), in which electrons pass through an ultrathin section of material, and scanning electron microscopy (SEM), which provides a three-dimensional view of a specimen's surface. Various companies—including **Jeol**, **Hitachi**, and **Carl Zeiss Nano Technology Systems**—produce electron microscopes for life science research.

Dirk Stenkamp, president of Carl Zeiss Nano Technology Systems, and his colleagues keep adding new features to electron microscopy. With TEM, for example, Zeiss developed in-column energy filters, which increase the contrast by an order of magnitude. That allows life science researchers to image biological samples in their natural form without the need for heavy metal staining. Even more interesting, Stenkamp and his colleagues developed a variable-pressure technology for SEM so that it can image hydrated material, which is impossible with conventional electron microscopy. Stenkamp says, “You can look at unmodified biological tissue, and you can perform experiments over time, such as observing chemical reactions at very high resolution.”

Scientists at Zeiss also developed a new form of electron microscopy called Cross Beam, which is a combination of SEM and focused ion beam structuring with electron-beam live imaging. The ion beam consists of gallium atoms, which can cut into the sample due to their higher mass compared to electrons. Simultaneously, the electron beam provides a high resolution live image of the subsurface structures laid open by the ion beam. Stenkamp calls this “a challenging new technology that we developed, for example, to analyze the interface layers between semiconductor material and human nerve cells for neurobiology implants.” Zeiss tools are being used by researchers working on retinal replacements made of a combination of silicon technology and living cells.

Atomic force microscopy (AFM) takes scientists in for an even closer look with resolution in the range of 0.01 to 10 nanometers. “AFM works like a phonograph,” says Mike Merion, vice president of metrology at **Veeco Instruments**. “A sharp point drags over a surface, and the instrument records how much the tip moves.” AFM crosses a specimen line by line to create a topographical image. In addition, AFM can measure material properties. For example, applying a voltage from the cantilever tip enables the

measurement of impedance across a specimen's surface. Merion adds, “In the past, it took \$200,000 or more and a Ph.D. to do AFM; now an AFM can cost less than \$100,000 and requires only a few days of training.”

In so-called scanning probe microscopy, the tip attaches to a single biological molecule. By moving the tip up and down, it records forces. Merion says, “You can measure the force to unwind a protein's alpha helix or β -pleated sheet. It's really amazing, and you can do it one molecule at a time.” This can reveal the thermodynamics of a protein and how, for instance, it might behave as a drug or drug target.

IMAGING INTERACTIONS

Some of today's devices—especially microarrays and microfluidic technologies—help scientists image molecular interactions. These processes can reveal the steps in signal transduction, how a drug attaches to a target, and so on. Seeing molecules coming together, though, requires some way to label them. Tags or probes small enough to work within these tiny devices and even within living cells are always in demand, and companies including **Molecular Probes** and **Quantum Dot Corporation** are continually creating ever-more-sensitive probes.

Andy Watson, vice president of Quantum Dot, says, “Quantum dots are tiny pieces of semiconductor material that light up brightly. We attach them to biological molecules so that they can report when those molecules bind to something.” These quantum dots start with a core that is just a few nanometers across and end up with a diameter of 10 to 15 nanometers, depending on the size of the attached biological molecule. Moreover, a quantum dot lights up brightly, and Watson's company currently offers quantum dots in seven colors, which allow the simultaneous tracking of several events. “In cancer,” Watson says, “you can look for a combination of biomarkers in single cells.” Quantum Dot already offers these probes with a variety of antibodies attached. This company also offers conjugation kits so that a customer can add their own antibodies to the quantum-dot core.

“This new visualization method,” says Watson, “allows researchers or drug-discovery scientists to be more accurate and definitive in describing basic processes and the mechanisms behind disease.” As an example, in the 17 October 2003 issue of *Science*, a team of investigators from Paris reported the use of quantum dots to track individual glycine receptors in living cells. Watson says, “These scientists watched how single proteins move around in a living environment and interact. It let these scientists ascertain a whole new set of rules for receptor dynamics and how receptors diffuse and dock.”

The interest in single molecules attracts many companies. For example, Stefan Seeger, founder of **Molecular Machines & Industries**, says, “We

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have developed a fluorescence technique that can detect a single molecule recognition event.” Specifically, this technique can record virtually any receptor-ligand binding, and measures the fluorescence in a tiny volume—just 10^{-18} liters. This technology uses glass-bottom microtitre plates and the LB8 Platereader from Molecular Machines & Industries. Seeger says, “The main field of application is for clinical diagnosis and drug discovery.”

MOVING MICROSCOPIC VOLUMES

In nanobiotechnology, developing and using atom-size devices also demands the control of tiny volumes of liquid, such as samples that must be moved precisely. Products for handling these small volumes range from conventional tools, like pipettors and 96-well plate fillers, to combinations of sophisticated robotic and microfluidic devices. **Eppendorf, Gilson, and Hamilton Company** created much of the technology used today for liquid handling.

Luke Roenneburg, product manager for Gilson, says, “Miniaturizing assays requires ways to handle miniaturized volumes of liquids.” In fact, liquid handling comes in right at the start. “The liquid handling is vital because it is the delivery function for a chip, plate, or slide regardless of the assay or application,” says Kary Staples, director of marketing at Gilson. “It is the initial step and one of the most important components to ensure success for any nanoassay.”

Gilson provides a variety of automated liquid handlers. The Constellation 1200, for example, automatically dispenses precise volumes—ranging from 5 nanoliters to 1 microliter—and uses advanced robotics to dispense the liquid in the proper *x-y* position. Roenneburg says, “The Constellation is a large-bed robot that processes high-density array plates or chips. It can even handle viscous fluids.”

In addition to handling small volumes of liquid, some technologies focus on keeping fluids flowing in the right places and proper volumes. Various companies—including **Agilent** and **Nanostream**—are working to develop so-called microfluidic applications with high-pressure liquid chromatography (HPL). For example, Nanostream recently introduced its Veloce system, which is a micro parallel liquid chromatography system. In describing this technology, Surekha Vajjhala, director of marketing at Nanostream, says, “We took established analytical technology and put it on a platform that lets customers analyze more samples simultaneously.” The system uses a 24-column Brio cartridge that provides simultaneous separations and real-time ultraviolet detection.

Eventually, microfluidic devices will merge with microarrays. For example, **Affymetrix** recently joined forces with **Caliper Life Sciences** to develop hybrid microarray-microfluidic systems. In essence, these systems will move preprocessing steps onto a microarray. Stephen Fodor, chairman and chief executive officer of Affymetrix, says, “Microarrays will eventually include microfluidics and reagents.” He adds, “Microarrays are great because they really fire up people’s imaginations, and there is lots of work that we and others will do to integrate fluid devices.” For example, a microarray could include onboard microfluidics that provide

Centers for Nanobiotechnology

Institutions around the world keep creating new centers for nanobiotechnology. For example, the **National Science Foundation** funded the Center for Biological and Environmental Nanotechnology at **Rice University**. A variety of other U.S. universities—including **Cornell**, the **University of California at Los Angeles**, and the **University of Michigan**—also developed nanobiotechnology centers. Centers in other countries include the Center for NanoScience at the **Ludwig-Maximilians-Universität** in Munich, Germany, and the Center for Science in Nanometer Scale at **Seoul National University** in Korea. For even more information on nanotechnology around the world, visit:

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DNA amplification and labeling. In fact, Affymetrix received a grant from the **U.S. National Institute of Standards and Technology** in the mid 1990s to integrate microfluidics with commercial microarrays. “So we have already made prototypes,” Fodor says.

Carl Zeiss Nano Technology Systems also got in the microfluidics field when it acquired distribution rights to nanoimprint tools from **Molecular Imprints**. With this technology, developers use ultraviolet light—instead of heat—to cure the attachment of a pattern of material on a substrate. Stenkamp of Zeiss says, “This technique lets you combine three-dimensional nano- and micro-size features with just one template, which is hard to do with conventional lithography.” This technique also creates nanometer-size pipes with such smooth edges that fluid flow remains laminar. Stenkamp and his colleagues are developing this technology to make high resolution microarrays for DNA and protein chips. He says, “This will allow much faster DNA and protein sampling in the future.”

Other companies also use nanotechnology. **Nanosys**, for example, is developing ultraminiature nanoarrays for biomolecular analysis. Another company, **Symyx**, develops high throughput screening of thousands of materials for use in various products and biomedical devices.

AN ATOMIC ATTACK ON DISEASE

Microarrays and many other small-scale devices can also be used to develop diagnostic and therapeutic instruments. **C Sixty**, for example, is using fullerenes—small molecules with unprecedented antioxidant properties—in biopharmaceutical applications. **SkyePharma** uses nanotechnology to develop drug-delivery mechanisms; **pSivida Limited** is developing nano-structured porous silicon for applications in human and

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animal health care. In addition, **Immunicon Corporation** uses nanotechnology to attack cancer.

Specifically, Immunicon developed CellTracks, which can separate, isolate, and characterize circulating tumor cells. Leon Terstappen, senior vice president of research and development and chief scientific officer at Immunicon, says, "It is well established that cancer patients die of their metastatic, not primary, cancer. That means that there must be cells traveling through the blood." So Terstappen and his colleagues look for cancerous cells in blood samples. CellTracks relies on magnetic nanoparticles—just 100 nanometers in diameter—that are labeled with antibodies against epithelial cells. "These cells are the basis of all carcinomas," says Terstappen, "and they should not be in the blood. If they are, we assume there is cancer somewhere." After the antibodies attach to targets, the magnetic particles can be collected. "Once you have the cells," Terstappen explains, "you can determine the presence or absence of treatment targets by genotyping and phenotyping." In a recent clinical trial, investigators from Immunicon showed that this technique reveals when treatment is working for metastatic breast cancer, because the circulating cells disappear. So, I have a tool that I can use very early on in the treatment cycle."

In addition to detecting diseased cells, clinical scientists always need new ways to deliver drugs. Consequently, **Flamel Technologies** uses polymer based nanotechnologies for controlled release of drugs. Rémi Meyrueix, scientific director for Flamel, says, "Therapeutic proteins such as interferon-alpha2b, interleukin-2, or insulin have a short half-life after being injected. Consequently, the therapeutic protein concentration in a patient, which is high after injection, drops fast." That means that the drug concentration can be dangerously high at first, and then quickly drops to levels where it fails to work. To balance the delivery of unstable drugs, scientists at Flamel developed the Medusa system, which is a suspension of 30-nanometer particles made of amino acid polymers. These nanoparticles absorb a drug, and then release it over time. In 2003, Flamel licensed Basulin, a long acting human insulin based on Medusa technology, to Bristol-Myers Squibb. Long acting interferon-alpha2b and long acting interleukin-2 are in pre-clinical development at Flamel.

From better imaging to more accurate diagnostics, a wide variety of nanotechnologies will advance life science research and medicine. Reducing the dimensions of devices and the theories behind them will change how scientists interpret results and engineers design instruments.

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