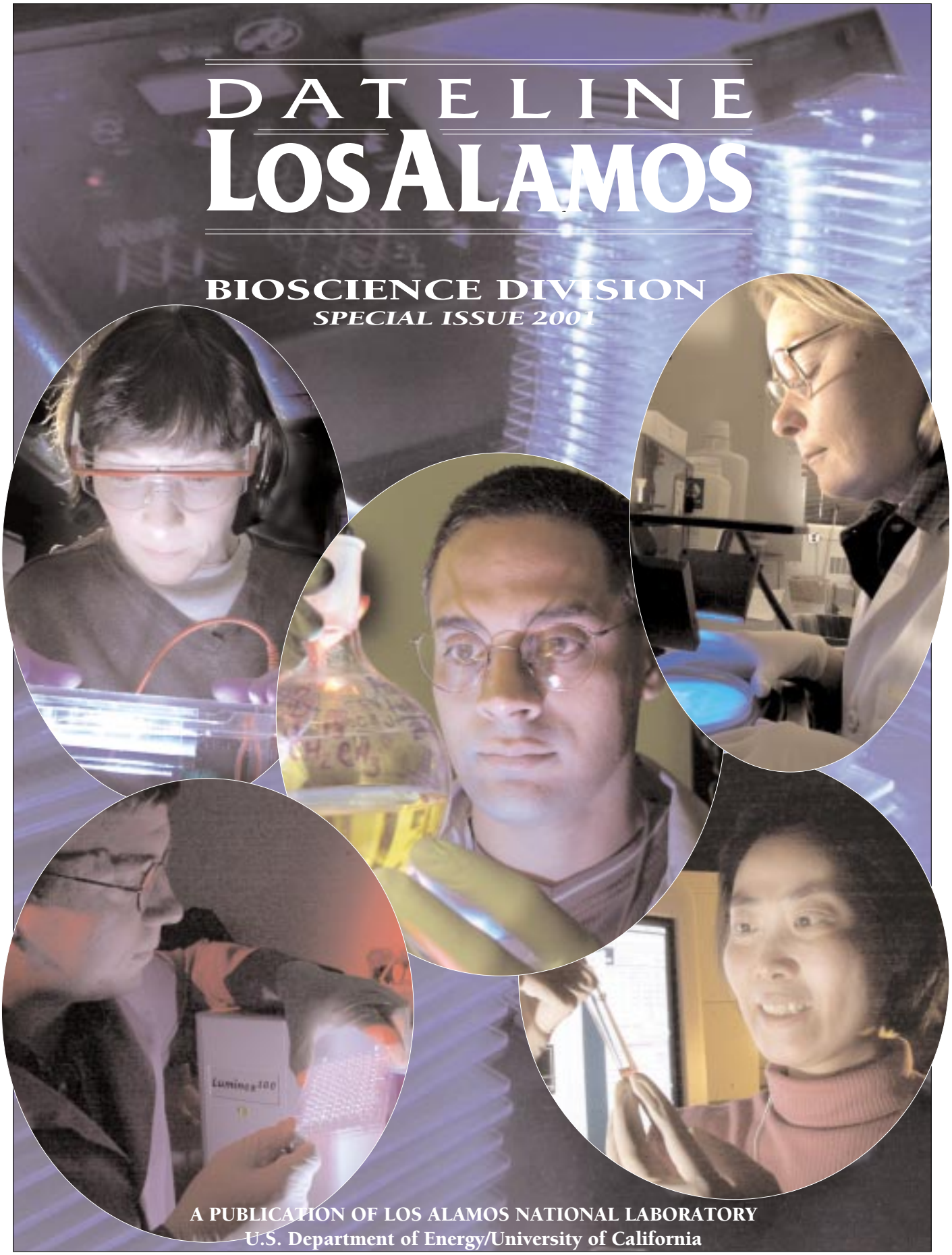
DATELINE LOS ALAMOS

BIOSCIENCE DIVISION

SPECIAL ISSUE 2001



A PUBLICATION OF LOS ALAMOS NATIONAL LABORATORY
U.S. Department of Energy/University of California

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INTRODUCTION

In 1999, Los Alamos National Laboratory created a new division dedicated to biosciences. The formation of Bioscience Division marked the beginning of an exciting era for the Laboratory.

Bioscience involves the convergence of biological, chemical, physical and computational sciences that serve as the tools to begin to unlock some of the secrets of the molecular machines and networks that operate on a cellular level.

This special issue of *Dateline: Los Alamos* outlines the compelling research the Laboratory is doing in this field. The important knowledge gained in this new scientific frontier will provide benefits to national security, public health and the environment.

On the cover are of some of the Bioscience Division scientists featured in this issue: Clockwise from the top left are Cheryl Kuske, Yvonne Rogers, Yulin Shou and John Nolan. In the center photograph is Marc Alvarez.

DATELINE LOS ALAMOS

A MONTHLY PUBLICATION OF THE PUBLIC AFFAIRS OFFICE OF LOS ALAMOS NATIONAL LABORATORY

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SIX STRATEGIC THRUST AREAS IN BIOSCIENCE DIVISION

Biothreat Reduction is concerned with threats to public health that arise from new naturally evolving or genetically engineered bacteria or viruses that cause infectious disease. Research areas are focused on the early detection and identification of bioagents, the understanding of the mechanisms of pathogenesis and the development of bioinformatics tools that disseminate new knowledge rapidly.

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Functional Genomics is the systems approach to understanding the functions of human genes in the context of the whole cell and the entire organism. Initial goals include studies into gene expression and control and development of experimental and computational methods for high-throughput protein analyses.

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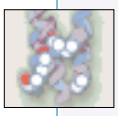

Structural Genomics is focused on the discovery of the 3-D structures of nearly all protein molecules in nature with the goals of providing a foundation for a fundamental understanding of biology at the molecular level. Initially proteins from a single organism, *M. tuberculosis*, will be targeted for structure determination and analysis.

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Natural and Engineered Biochemical Diversity will explore the metabolic and chemical processes that exist in the natural world to enable the synthesis of new molecular approaches. This effort brings together multiple disciplines to develop new technologies for a variety of applications in bioremediation, industrial applications and improved plants.

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Biomedical Technology brings together biologists, chemists, physicists and engineers to develop new technologies for medical diagnostics, health monitoring and biothreat detection.

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Complex Biosystems Modeling is concerned with the management and analysis of information and the use of this data to build empirical models of biological systems.

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The biosciences constitute an era of great revolution and bring together elements of many disciplines. Los Alamos has brought together genomics, chemistry, computational science and microbiology to address the challenging problems of the next century.

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MOLECULES OF LIFE

THE PAST AND FUTURE OF THE HUMAN GENOME PROJECT

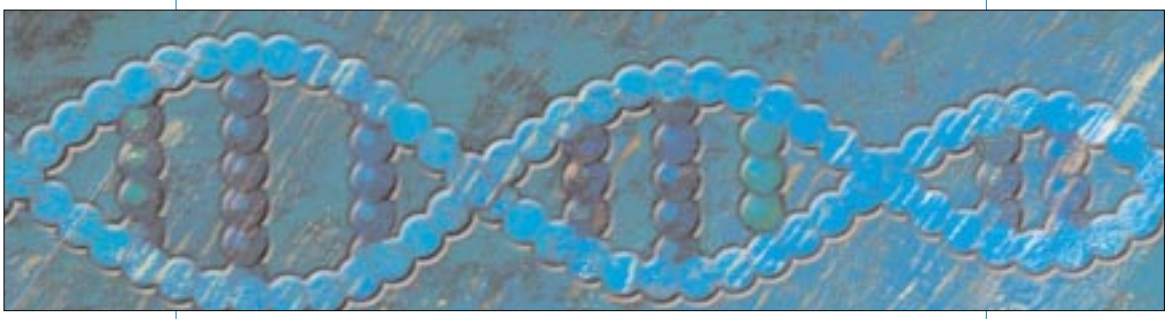
Double Helix with a Twist

Los Alamos National Laboratory has a major role in the U.S. Human Genome Project, a joint Department of Energy/National Institutes of Health effort to identify all the genes in human DNA and determine the sequences of the chemical base pairs comprising the genome.

Rarely has one project held such promise. Knowledge about the effects of DNA variations among individuals may help researchers better diagnose, treat and possibly eradicate the more than 4,000 genetically inherited human disorders.

Los Alamos became interested in genetics early in the Lab's history. The Lab established health research units because radiation was known to cause cell injury and genetic mutation. Researchers conducted early biological research on whole animals to understand better the physiologic and genetic consequences of radiation exposure and to set rational dose limitations for workers. In the 1960s, as this knowledge base expanded, studies became increasingly sophisticated and included investigations at the cellular and subcellular levels.

By the 1970s, Lab scientists were recognized world leaders in the area of cell biology, especially in the development of instruments that could rapidly measure the volume of each cell in a large cell population. Researchers further refined those instruments, called flow cytometers, to detect fluorescence emitted by stained cells. These instruments later were used to analyze chromosomes as well as whole cells.



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In the 1980s, the flow-cytometry resources at Los Alamos combined with newly developed recombinant-DNA technology to construct a series of DNA libraries that represented the DNA in each of the human chromosomes. These libraries were used with other laboratories throughout the world; they provided a means of rapidly mapping the locations of human genes. It was also during this period that a group of Lab researchers created a national repository to store DNA sequence information. The repository was called GenBank and is now maintained by the NIH.

The Los Alamos Center for Human Genome Studies — established in 1988 — better organized the effort to map and sequence the human genome and make these data available to the research community. The Lab's program became part of the international Human Genome Project in 1990.

One of the Lab's biggest accomplishments in genome research was the construction of the first high-resolution physical map of human chromosome 16. Genes on chromosome 16 include those associated with leukemia, breast cancer, prostate cancer, Batten disease, hemoglobin disorders and a type of kidney disease.

In 1996 Los Alamos joined forces with Lawrence Livermore and Lawrence Berkeley national laboratories to form the Department of Energy's Joint Genome Institute (JGI), combining the genome centers of each lab into one virtual organization. The JGI is part of a consortium of five genome institutes that worked together to sequence the entire human genome.

In June 2000, the announcement was made that the human genome had been sequenced. Scientists and historians have compared unraveling the three billion or so base pairs to science and engineering accomplishments such as landing on the moon and splitting the atom.

The sequence of the human genome is a draft sequence; gaps in the sequence remain, but researchers are confident that the draft will develop into a medical revolution.

All remaining gaps in the human genome sequence need to be filled. The Los Alamos Genome Center will close gaps in the chromosome 16 sequence during the coming year. And still other mysteries need to be uncovered, such as the first complete set of RNA molecules produced by the genome or the structural determination of the complement of proteins encoded by the genome. Other milestones include eventually determining which genes are turned on and in what order and how gene products interact with each other to determine how bio-molecular networks function. The Genome Center will evolve from the role it has been playing in mapping and sequencing to playing a role in the determination of how genomes use the information they encode to produce the molecules necessary to sustain life.

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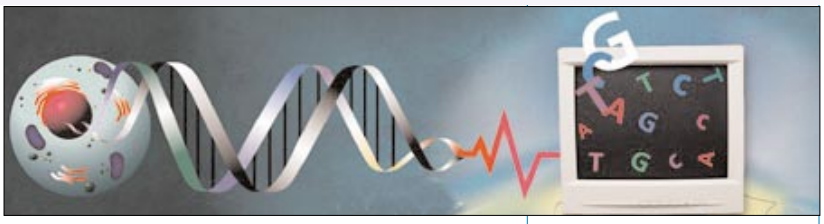
As the mysteries of the human genome are unraveled, new knowledge will yield better pharmaceuticals, forensics tools, predictors of disease susceptibility and clues about our genetic past.



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MOLECULES OF LIFE

GENES TO PROTEINS

As researchers around the world completed sequencing the human genome, scientists and researchers at Los Alamos National Laboratory are setting their sights on a next logical step: understanding the function and complex interactions of the products of these genomes.

An individual's genome contains a set of genes: DNA sequences that code for the individual protein molecules that are essential to build a healthy functioning cell. That genome also contains sequences of DNA that aid in regulating how the genes are "expressed," that is, what happens when a particular gene is turned on so that it results in the transcription of the genetic code, and the subsequent production of its protein. Functional genomics aims to understand how genes are regulated and how an entire suite of gene products, RNA and proteins, act in concert to achieve function.

"If we can understand how a cell works at the level of all its proteins, we will be in a much, much better position to understand how to address the problems that arise from malfunctions in cells, resulting, for example, from inherited disease or infection," according to Los Alamos researcher Norman Doggett.

"We need this to achieve the next advances in medicine and biology," he said.

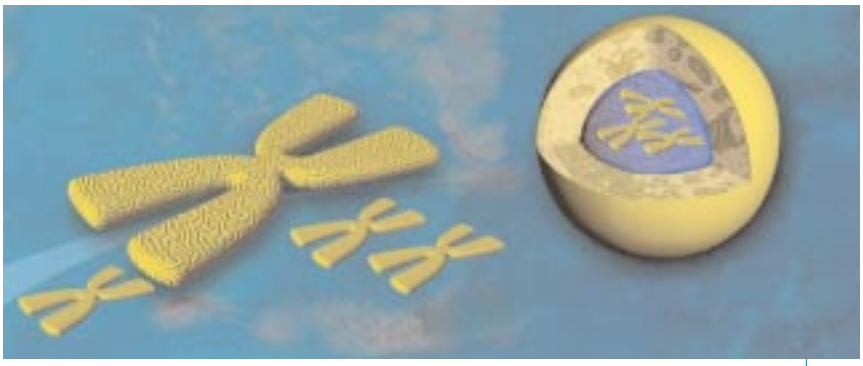
Functional genomics will help to determine or assign function to each gene or gene product of the genome. "It's understanding the purpose of every gene in the genome."

Up to this point, researchers have decoded the characters that make up the genes — researchers for example know there are about 30,000 to 35,000 genes in the genome. "But that still doesn't tell us what those genes do," said Doggett.

One approach, global gene expression analysis, uses a fluorescently tagged DNA copy of a cell's messenger RNA, or ribonucleic acid, to bind to a collection of genes that are spotted on a glass slide.

Using specially treated microscope slides, researchers can place about 40,000 DNA spots corresponding to different genes into an area that is smaller than a postage stamp. The fluorescently labeled DNA copy of messenger RNA binds to its matching genes to let researchers know which genes are active in a given cell type.

Each individual's DNA heritage is contained in 22 pairs of chromosomes —plus one sex-determining pair — for a total of 46 chromosomes. Only about 5 percent of the total DNA pairs contains genes.



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In this global gene expression study, scientists can determine which genes are transcribed into RNA and which in turn are translated into protein.

"We want to know for each gene which tissue or cell type it is expressed in — in which tissue or cell type it is turned on, thereby making an active protein." said Doggett.

Los Alamos has created a database and supporting computational software to address global gene expression data that researchers around the world are using.

Another method of functional genomics that researchers are actively engaged in is being pioneered at Los Alamos: phage display. Phage is a virus that infects bacterial cells; bacteria phage is used as a tool for producing antibodies. Antibodies are important tools for functional genomics analysis because they help researchers understand the function of proteins in a cell. Researchers also use the phage-display approach to isolate and identify proteins.

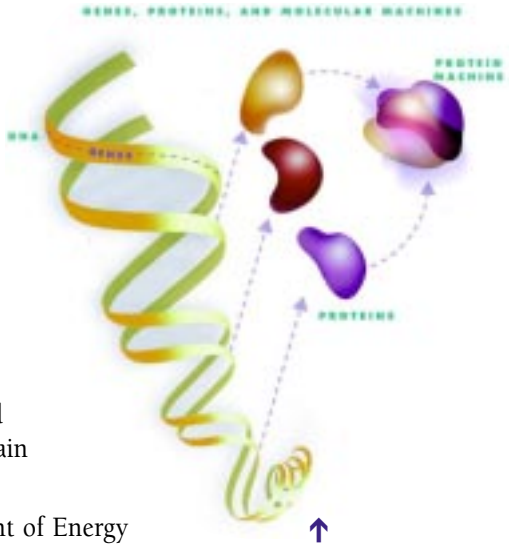
Yet another approach, called mass spectrometry, uses a mass spectrometer that allows researchers to take an unknown protein and determine enough of its amino acid composition to identify what protein it is and what gene it came from.

This approach is particularly useful for determining how cells respond to external stimuli such as low dose radiation or exposure to pathogenic agents.

"If I want to study how a cell responds to the influenza virus, this is a very important approach ... we will see how a cell responds at the protein level," said Doggett.

Researchers using this approach also can see how proteins bind to other proteins, what are their biochemical pathways, which again is important in determining how genes function.

In addition to work being done at Los Alamos, the Department of Energy recently announced a new initiative that builds upon existing genome research. The "Genomes to Life" initiative will develop genome-scale high-throughput and computational approaches for how genes function.



↑ Information is stored in genes and other DNA sequences. Genes contain recipes for the proteins. Proteins act alone or in complexes to perform all cellular functions.

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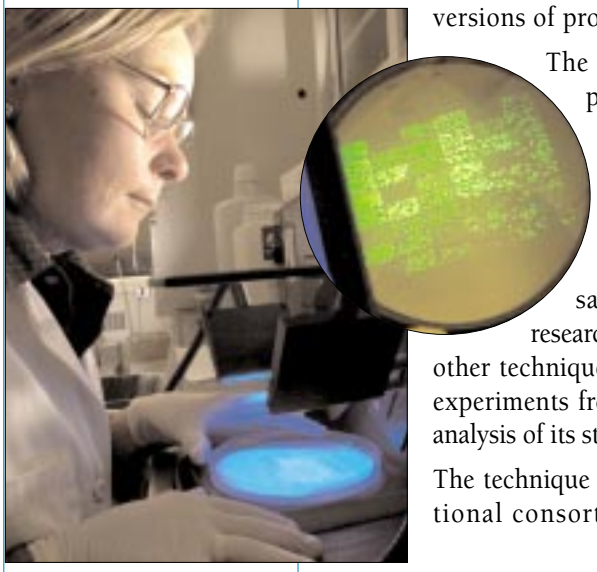
MOLECULES OF LIFE

SHAPING THE FUTURE

Yvonne Rogers of Los Alamos' Bioscience Division screens clones of tuberculosis proteins using a green fluorescent protein-folding reporter. The Los Alamos-developed technique allows her rapidly to determine if the proteins have organized themselves into the proper shapes so they will be useful in further experiments to study their structure. The inset shows the different degrees of fluorescence in cell colonies.



Proteins are the biological workhorses that make life possible. They provide structure, synthesize complicated chemicals, control the ability to move, help transmit neural impulses and perform countless other biological demands. Their ability to function properly is intimately tied to their structure — a complex arrangement of twists, loops, spirals and folds. Understanding this molecular origami is crucial in developing a fundamental understanding of molecular biology, designing disease-fighting drugs and repairing malfunctioning proteins.

One problem in studying protein structure has been an inability to rapidly synthesize large quantities of proteins for investigation. Los Alamos National Laboratory researchers have developed a technique called a "folding reporter assay" to address this problem. "You need large quantities of proteins, but they need help in folding (organizing themselves into the proper shape) when produced in large quantities," said Geoffrey Waldo of Los Alamos' Bioscience Division, developer of the technique. "Our assay lets us evolve new versions of proteins that fold properly."

The method involves fusing a green fluorescent protein, or GFP, with a target protein. The GFP fluoresces only in the presence of successful folding, showing which colonies of cells are the most valuable for propagating. The shape and function of the target protein does not have to be known to use the technique, says Waldo. In recent months, Los Alamos researchers have used the folding reporter assay and other techniques for the first time to complete a full set of experiments from initial engineering of a protein to a full analysis of its structure.

The technique is a key element for the work of an international consortium conducting research to analyze the

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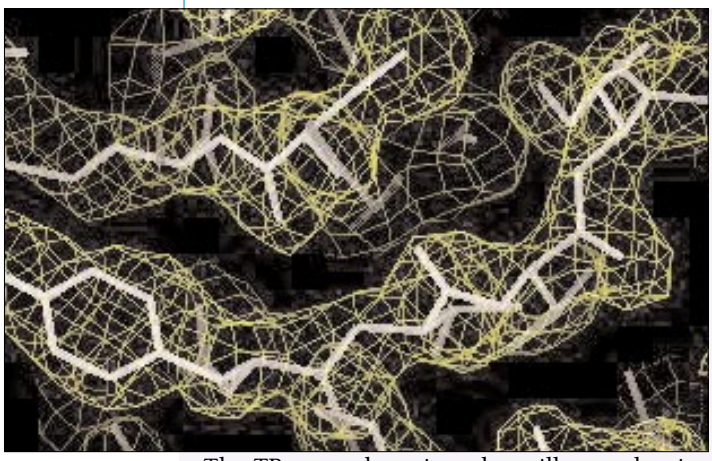

↑ SOLVE is an automated tool for producing electron-density maps of protein molecules based on X-ray diffraction data. The tool is Web-based and is used at more than 300 universities and institutions worldwide.

structures of some 400 proteins of the bacterium that causes tuberculosis, the world's No. 1 infectious disease, as declared by the World Health Organization to be a global health emergency. The Los Alamos-led collaboration, which involves approximately 40 institutions in move than 10 countries, received a five-year, $28.5 million grant last fall from the National Institutes of Health for the project.

The TB research project also will use other innovative technologies developed at Los Alamos, according to project leader Tom Terwilliger of the Bioscience Division. One of them is SOLVE, a computer program that creates 3-D pictures of protein molecules faster than any other method. SOLVE, which produces electron-density maps based on X-ray diffraction data, is used at more than 300 universities and research institutions around the world.

"By combining the technologies and experimental capabilities at Los Alamos with those of the entire TB structural genomics consortium, we are in a great position to make enormous progress in determining protein structures from TB," Terwilliger said. Information developed during the project will be placed on the World Wide Web for use by other researchers and companies in efforts to develop more effective drugs and treatments for the disease.

Other strengths Los Alamos brings to studying the molecular frontier include powerful computational resources, large research facilities and a multidisciplinary research environment that includes experimental and theoretical capabilities. In addition to the understanding of basic biology and its consequences for human health, the Laboratory is applying its molecular research capabilities to national and international problems related to the environment and mitigating the grave threat of biological terrorism.

Support for structural genomics research at Los Alamos comes from the Department of Energy's Office of Biological and Environmental Research, the NIH and the internal Laboratory-Directed Research and Development program.

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MOLECULES OF LIFE

THE CHEMISTRY OF LIFE'S BUILDING BLOCKS

Life's molecules are made up from chemical building blocks that can be synthesized in a laboratory. The ability to synthesize these molecular components is extremely important in the quest for understanding the structures and functions of the biological macromolecules, DNA, RNA and proteins. While X-ray crystallography allows researchers to identify protein structures that form crystals, many proteins are difficult to grow into crystals.

For these "difficult" proteins, a different technique called nuclear magnetic resonance spectroscopy, or NMR, is commonly used to study their structure. To use NMR, though, the specific atoms in the proteins must first be "labeled" or "tagged" to make the identifiers visible.

The tagging process is necessary because all proteins, which are composed of amino acids, are made up of carbon and nitrogen atoms. Unfortunately, regular carbon and nitrogen atoms are invisible to the NMR. To get around this, Los Alamos scientists Ryszard Michalczyk, Jurgen Schmidt, Rudy Martinez, Clifford Unkefer and Pete Silks are using synthetic chemistry to imitate nature to recreate the building blocks of proteins — amino acids. As the scientists synthesize the amino acids, they change them slightly by replacing the regular carbon and nitrogen with special, stable isotopes of the elements that have one additional neutron. For instance, instead of regular carbon-12, they will insert in its place carbon-13. The NMR is able to "see" these stable isotopes.

The group also has developed similar chemistry for the building blocks of DNA and RNA nucleotides. The team studies protein and DNA molecules labeled with these stable isotopes using NMR methods.

Jurgen Schmidt of the Lab's Bioscience Division creates molecules labeled with stable isotopes that can be observed and studied using nuclear magnetic resonance, or NMR, spectroscopy methods. ↓

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One of the big challenges has been to find ways to streamline the methods of synthesizing new molecules so that it can be done using the smallest amounts possible of stable isotopes. This is important because the stable isotopes, such as carbon-13 or nitrogen-15, are very expensive.

In addition to researching the structure of proteins and DNA, the group also is working on developing synthetic, biomimetic (nature-imitating) compounds that could ultimately be used in types of "antisense" therapy. For instance, the group is studying the possibility of creating DNA analogs (molecules that mimic DNA structure). These analogs could bind to defective genes — those that are causing a particular disease —and provide a "marker" for disease detection.

The research being conducted now is a long way from medicinal or diagnostic applications, but it demonstrates some of the exciting possibilities that may lie ahead. The National Institutes of Health Stable Isotope Resource (more information can be found in "Stable Isotope Research Resource: Structural Biology Research Depends on Supply of Labeled Building Blocks") has been critical to the success of Los Alamos' synthetic chemistry efforts, serving as one of the major funding sources for critical research and development work.

Also on the cutting edge of molecular synthesis is the research being done by Martinez. He is collaborating with Los Alamos computer scientists and theoretical physicists to create compounds that are used in quantum computing research — a whole new type of computing in which computations could be done in a tiny molecule, as opposed to today's central processing unit. The speed of quantum computers one day might be marshalled to model the most complex systems we know — a living organism or perhaps even an ecosystem.

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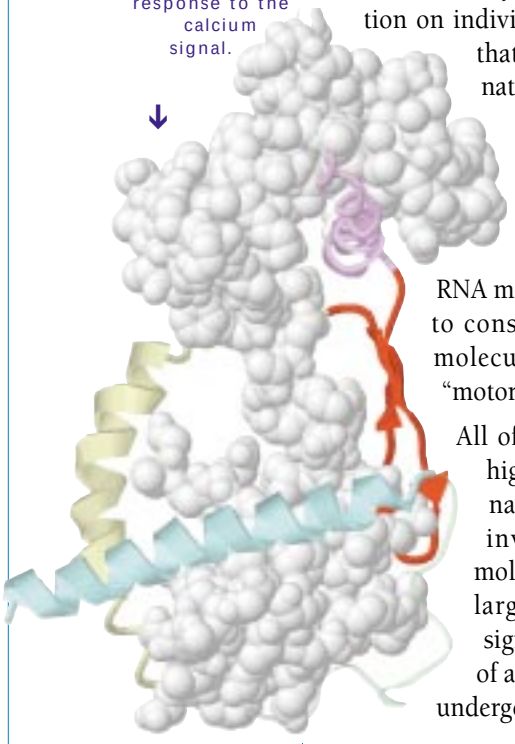
MOLECULES OF LIFE

MOLECULAR MACHINES AND NETWORKING

We began the 20th century with very little knowledge of the molecules of life. For the first 50 years, researchers focused largely on trying to understand molecules' make up, wondering how molecules were able to do such mysterious things as pass on hereditary information.

In 1953, James Watson and Francis Crick, 1962 Nobel laureates, discovered the double helical structure of DNA and deduced the chemistry of how the subunits, the nucleotides, on each intertwined strand interacted. This discovery led to the understanding of the genetic code. In that same year the first crystal structure of a protein was solved, the oxygen storing muscle protein myoglobin, and researchers gained insights into how proteins achieved the chemistry of life.

For the next 50 years researchers gained a wealth of detailed information on individual biological molecules and came to understand that the biological function is achieved by the coordinated functions of many proteins. Indeed most of life's processes are carried out by what can be called "molecular machines" that are made of dozens of proteins that act in concert to carry out multiple functions such as read the genetic code from the genome and transcribe it by creating the messenger RNA molecule; read the messenger RNA containing the code to construct a protein and synthesize it; repair a DNA molecule damaged by radiation or chemicals; or form a "motor" that can create force and movement as in muscle.



Muscles contract when calcium ions activate a molecular switch. Shown below are two intertwined protein molecules, troponin C and troponin I. The red structure modifies the 3-D shape of the complex in response to the calcium signal.

All of these complex processes must be performed in a highly coordinated and regulated manner. This coordination is achieved through signaling networks that involve multiple proteins as well as small signal molecules. The language of these signaling networks is largely conformational — the proteins respond to signals in the cells such as a change in concentration of a small signal molecule by binding that molecule and undergoing a conformational change. This change allows it

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to interact with the next protein in the signaling network, thus transmitting the message. And in this fashion the signal can be passed along through many partners. It also can be amplified.

A major challenge for the 21st century is to unlock the secrets of molecular machines and networks. At Los Alamos, researchers are approaching this challenge by studying "second messenger" signaling. A second messenger is a small signal molecule, in some cases a simple ion like a calcium ion that is released inside a cell when a "first messenger" signal is received at the surface of the cell. A well-known first messenger signal is the binding of a hormone, like adrenaline, to a cell surface receptor in response to a fright – this message must be translated into the molecular action needed for a response, such as fleeing. To achieve flight, the brain must signal the cells in our bodies to make energy, so they can then turn on the molecular machines, our muscles, that will help us escape.

Capturing the interactions in the signaling networks and machines that keep us healthy and functioning is a great challenge. Protein complexes are often dynamic and transitory and are not well suited for study by a single technique that must trap the complex in a particular physical state. Los Alamos researchers have approached the problem by using hybrid experimental data and computational modeling. The experimental data came from many different sources, such as crystallography and nuclear magnetic resonance for the structures of individual protein components; fluorescence and cross-linking data for distances between components; neutron scattering for the shapes and position of components; and mutagenesis data for the proximity of different amino acids on different components combine and with computational methods develop models that best fit all of the known data.

In this manner, researchers have developed a model for the molecular switch that controls muscle contraction that now can be further tested and refined.

Muscle is made of assemblies of protein molecules that form thick and thin filaments. The filaments sliding past each other via the action of "cross-bridges" between the filaments give rise to contraction and movement. The cross-bridge interactions are controlled by calcium-ion signals that affect molecular switches sitting on the thin filaments. The Los Alamos model for these molecular switches shows them to be made from two intertwined protein molecules, called troponin C and troponin I. When troponin C sees a calcium-ion signal, it undergoes a conformational change that causes it to "grab" part of the troponin I molecule that, in the absence of the calcium-ion signal, binds to the thin filament protein actin. This action releases troponin I's inhibitory effect on the cross-bridge formation and triggers the contraction.

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DATELINE: LOS ALAMOS

NEW TECHNOLOGIES FOR HEALTH

'SNP'ING' AWAY AT HUMAN HEALTH ISSUES

In the summer of 2000, scientists around the world cheered as the effort to unravel the mystery of the human genome reached a milestone — a completed draft of the human genome sequence. The sequence is a set of instructions that determines individual characteristics ranging from the cosmetic, such as hair and eye color, to the medically important, such as susceptibility to disease and response to treatments.

The challenge for scientists is that although they have sequenced one human genome, there are approximately six billion people in the world, all with different physical characteristics and genes. In a comparison of two random people, 99.9 percent of their genetic sequence is identical. Individual differences, then, are defined by this distinctive tenth of a percent of the genetic sequence.

The study and use of information about human genetic variation are becoming increasingly important now that the genome is sequenced. Individual genetic differences are very important because by understanding the differences, scientists and physicians will be able to identify genes associated with diseases, develop new drugs to fight disease and design better treatments for individual patients. Several pharmaceutical companies promote the idea of "personalized medicine," resulting from the integration of genomics and drug discovery and design. This has led to a new field known as "pharmacogenomics."

The most common type of genetic variation is single nucleotide polymorphisms, or SNPs. In the human genome, there are millions of SNPs.

Among other things, locating SNPs will ultimately help physicians prescribe the most effective treatments for their patients. Every year approximately two million people have adverse reactions to drugs, resulting in nearly 100,000 deaths. Many of these deaths could be avoided if doctors knew more about the person's genetic background. Specifically, physicians would be able to treat patients more effectively if they had an understanding of the genetic variations that affect drug metabolism. With this information, the physician could prescribe a treatment that would be maximally effective and less likely to produce an adverse reaction in that specific patient.

Researchers at Los Alamos National Laboratory have developed a novel way to search genetic sequences for targeted subsets of the many millions of SNPs. Researchers Scott White, John Nolan and Hong Cai have collaborated to create a method of analyzing SNPs by putting synthetic DNA probes on tiny plastic microspheres for the purpose of studying them with a fluorescent measurement instrument called a flow cytometer. The benefit of the Los Alamos system is that it can analyze a very large number of samples quickly, inexpensively and relatively easily, in comparison to other methods of analysis.

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Bioscience Division researcher John Nolan examines a microplate with multiple wells. Each well contains a microarray that is composed of multi-colored microspheres used for high-throughput analysis of individual genetic variation. Rapid diagnostics that can identify single nucleotide polymorphisms (SNPs) will play a major role in the future of human health.

One of the first groups of SNPs that the team studied identified a genetic marker for beryllium disease, an illness that can affect nuclear and industrial workers who handle this metal. Thanks to the development of the new SNP analysis method, Los Alamos researchers have developed a test to identify specific individuals who are at risk for development of the disease. Those workers who are most susceptible can then be advised of their increased risk, and overall safety of the workforce can be improved.

Beyond beryllium disease, the researchers are addressing the role of genetic variation in susceptibility to cancer and response to drugs. They also are working to develop a better understanding of human history and population structure. In addition, these researchers are applying this technology to the study, detection and identification of human pathogens, including the influenza virus and pathogenic bacteria such as *E. coli* and other food-borne pathogens.

The Los Alamos team has filed patent applications on its process and is working closely with a commercial partner to bring its SNP analysis method to the marketplace. The team also has had interest from pharmaceutical companies, clinical diagnostic laboratories and others. This new technology is being implemented in Los Alamos' Bioscience Division facility for the large-scale analysis of genetic variation. The facility will enable Laboratory researchers and academic collaborators to access this powerful new tool to address critical issues that include human health and disease.

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DATELINE: LOS ALAMOS

NEW TECHNOLOGIES FOR HEALTH

UNMASKING THE MYSTERIES OF CHRONIC BERYLLIUM DISEASE: NEW TESTS IDENTIFY SENSITIVITY, GENETIC SUSCEPTIBILITY

Beryllium is a unique lightweight metal used in nuclear weapons and, in the commercial sector, for telescope mirrors, golf clubs and a variety of other applications. While solid beryllium and beryllium alloys are safe, fine particulate beryllium is hazardous if inhaled.

In certain individuals, breathing microscopic beryllium particles can lead to Chronic Beryllium Disease (CBD), sometimes called berylliosis. CBD is a long-duration, allergic-type lung response that can make the sufferer abnormally weak and is sometimes fatal.

Research into beryllium health effects in the Bioscience Division at Los Alamos National Laboratory center on identifying worker sensitivity and increased risk caused by genetic factors. Only a small percentage of people exposed to beryllium become sensitized to it, meaning they experience an immune-system reaction to exposure. In addition, it appears that not everyone who is sensitized develops CBD.

A team led by Bioscience Division researcher Babs Marrone has devised an improved Lymphocyte Proliferation Test, or LPT, a blood test that can identify sensitized individuals. The researchers also have found genetic markers that indicate increased susceptibility.

Bioscience Division technician Yulin Shou holds a tube of human lymphocytes that has been prepared for analysis by using a flow cytometry-based test for beryllium sensitivity called Immuno-LPT. The flow cytometry results are displayed on the computer screen behind Shou.

The new test, called the Immuno-LPT, takes advantage of the fact that both sensitization and CBD are immune-system responses. Using flow cytometry, a laser-based, cell-analysis technique developed at Los Alamos, researchers can detect a proliferation of a specific white blood cell, or lymphocyte, known as a

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CD4+ cell, that forms in response to beryllium. Results suggest that because CD4+ cell proliferation in people sensitized to beryllium matches the response in people who have CBD, the Immuno-LPT may be quite accurate in predicting the development of CBD.

Because some individuals develop sensitivity and disease when exposed to only miniscule amounts of beryllium, while others with high levels of exposure never get sensitized or develop CBD, scientists wondered if there could be a genetic risk factor.

"Our research has shown that the majority of individuals with CBD, or with a CD4+ response to beryllium, have rare variations of a gene on chromosome 6 containing what is known as a 'Glu69' marker," said Marrone. "We looked closely at the variations around the marker, and we found other contributing genetic factors that help us pinpoint those who are at increased risk."

Because these genetic differences are inherited and not caused by beryllium exposure, researchers could use the genetic markers to identify individuals with greater susceptibility to develop beryllium disease.

All genetic-marker information must be kept confidential, said Marrone. And taking the test must be the decision of the workers — with their informed consent. Given that, the Lab would like to offer the genetic-marker test to more than 3,000 current and former Lab employees who either worked with beryllium or may have had incidental exposure.

Ethical and legal issues must be carefully considered in any genetic testing program, either at the Lab or in industry, according to Marrone. "We want to get the best possible information to the workers so that they can then make informed decisions about their work situation," she said. "At the same time, we must ensure that information from genetic testing does not lead to any kind of discrimination."

Marrone and her colleagues continue to work with industrial hygienists, physicians, environmental scientists, chemists and health physicists to understand better how beryllium damages the immune system, with the ultimate goal of a cure for beryllium disease. Experts in legal and ethical issues also seek to integrate new information about genetic markers into beryllium medical surveillance practices.

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NEW TECHNOLOGIES FOR HEALTH

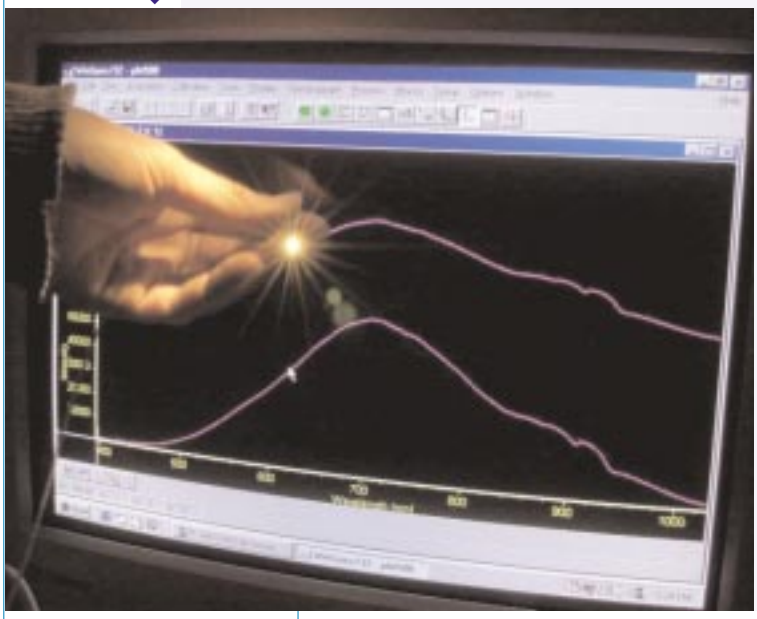
OPTICAL BIOPSY STUDIED AS BREAST CANCER TREATMENT AID

Breast cancer is the leading cause of cancer-related death in women aged 40-59 and was expected to total more than 45,000 deaths in the United States last year, according to the American Cancer Society. A Los Alamos National Laboratory-developed technology, the Optical Biopsy System (OBS), may aid in not only the diagnosis of breast cancer, but the success of the surgical treatment as well.

An OBS is a real-time probe based on white-light interaction with tissue, which can be used either through an endoscope or a biopsy needle. The system consists of small optical fibers that shine tiny bursts of light onto tissue and then collect the scattered light traveling through the tissue. A computer then analyzes the scattered light.

OBS technology works because cancerous tissue scatters and absorbs light differently than normal tissue. A computer uses artificial intelligence and pattern recognition codes to analyze the scattered light spectra and discern the spectra of normal tissue from diseased tissue.

"Breast cancer is very complicated because breast tissue consists of a wide variety of tissue types," said Irving Bigio, formerly with the Laboratory's Bioscience Division and now at Boston University. "Types of cancer tissues from the breast vary greatly, as do types of normal tissue, so it's a difficult process doing diagnostics." Bigio and Paul Ripley, a post doc at the Laboratory from the United Kingdom, are currently working with doctors at the University College of London Medical School on the OBS clinical breast cancer studies.



As a researcher moves the optical biopsy probe over an area, a computer analyzes the scattered light, as shown on the screen, to determine if cancerous tissues are present.

So far, data from the clinical studies being conducted by the UK collaborators are promising. The OBS data agree with findings from standard pathology more than 80 percent of the time, based on two parameters: sensitivity and specificity. Sensitivity

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refers to the instrument's ability to find cancer when there is cancer, and specificity refers to the instrument's ability to discern cancerous tissue from healthy tissue.

"We're very encouraged by these results, although preliminary," said Bigio. "The fact that we're getting this level of agreement with results from pathology means that the OBS has excellent potential for aiding doctors during breast cancer surgery."

In another aspect of the research, Bioscience Division's Judith Mourant is studying the properties of tissue that affect light scattering and make systems like OBS predictors of cancerous tissue. Light scatters from an object depending on its composition and shape. Consequently, one might expect that the scattering of light from tissue would change when the form and structure of the tissue changes. Mourant has had a National Institutes of Health-funded program since 1996 to study how changes in cell structure that accompany carcinogenesis affect light scattering. "One of our first tasks was to determine what structures in the cells scatter light. We now have strong evidence that light is scattered by small, internal cellular structures," Mourant said.

The light scattering from these small, internal cellular structures is particularly important when the light illumination point and the scattered-light detection point are located close to each other on the surface of the tissue. Further, Mourant has studied the light-scattering properties of tumor-causing cells grown in a 3-D culture. She is now using this information to develop more sensitive methods to noninvasively measure properties of tissue related to cancer-causing cells.

Mourant now is developing fiber optic probes that deliver and detect polarized light, because this provides additional information than garnered with traditional methods.

For general information on the Optical Biopsy System:

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DATELINE: LOS ALAMOS

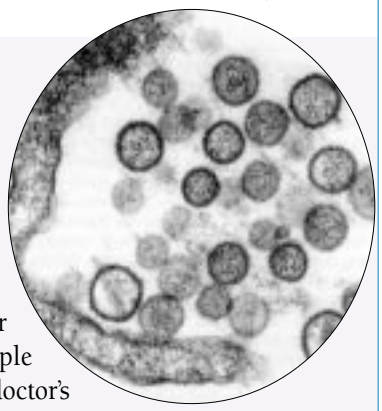
ENSURING OUR SECURITY AGAINST BIOLOGICAL THREATS

EARLY DETECTION FOR PROTECTION

Being able to rapidly detect biological agents is among the most difficult and yet urgent tasks facing the nation. Whether the threat is from a natural outbreak or a terrorist's release of threat agents, medical treatment cannot effectively begin without first identifying the bioagent. At the same time, effective understanding and response to a biological threat requires rapid communication across the health-care system.

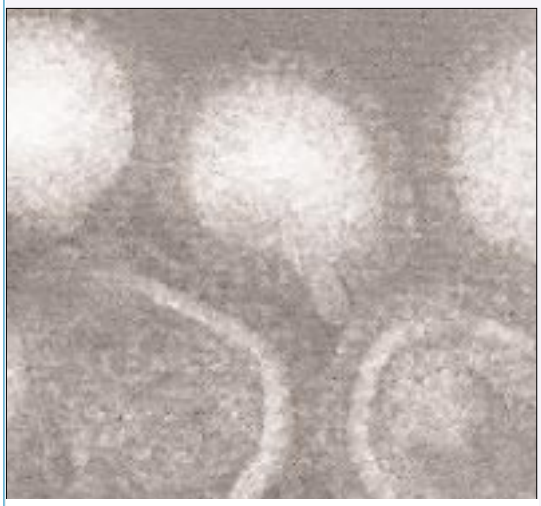
Two new Los Alamos National Laboratory projects, both internally funded by the Laboratory-Directed Research and Development program, seek to address these identification and communication challenges, one from the approach of a specific detection tool, and the other from a broader analysis of the system and the varied tools that could speed a national response.

Below is a microscopic view of the hantavirus.



Early Identification of Influenza and Hantavirus

One of the new projects is based on the detection of signature proteins that decorate the surface of the influenza virus particle. In collaboration with University of New Mexico Medical School, Lab researchers are developing a compact sensor system using thin films that mimic cell membranes and waveguide-based optical detection. This small hand-held device will be simple to use and capable of detecting influenza early after infection. The project's objective is a simple inexpensive device that could be used in a doctor's office to guide treatment or in the field to provide information on the spread of the infection.



Above is a micrograph of a flu virus magnified 150,000 times.

The initial effort for this detector is focused on influenza and hantavirus. Influenza is one of the leading causes of death in the United States, and health experts are predicting a likely occurrence of a virulent new influenza strain striking in the near future, similar to the 1918 pandemic that killed more than 20 million people worldwide. Hantavirus, which is common in many South American countries, has now been found in more than 25 states, and its hemorrhagic fever kills approximately 45 percent of its victims. In many cases, at rural clinics patients with flu-like symptoms are sent home with a diagnosis of influenza, only to return in shock and quickly succumb to the infection before proper treatment can be initiated. With early diagnosis, patient survival rates improve significantly because they quickly can be referred to advanced care facilities for appropriate treatment.

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Infection from many biothreat agents (e.g., *Bacillus anthracis* that causes anthrax) also produce flu-like symptoms. Accordingly, in the event of a bioterrorist attack, one of the problems facing first responders and health-care workers is to be able to distinguish bioagent infection from influenza. Without the disease's early identification, the patient would likely be sent home only to die days later when the infection moved beyond the treatable stage. A robust hand-held device that could quickly screen potential victims to distinguish between influenza and infection or exposure to a biothreat agent is critical for triage and will help save lives.

Early Warning — a Prognostic Epidemiology Network

The need for rapid identification combined with the ability to swiftly notify other members of the health-care community forms the basis of a second Los Alamos effort, that of an integrated early warning system for influenza.

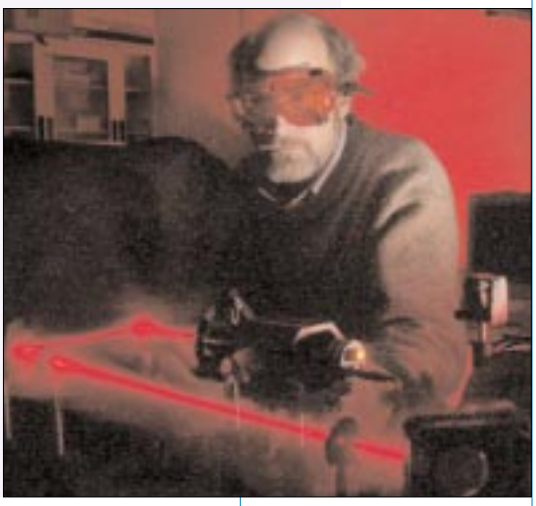
The researchers are working to combine the skills of chemistry, biology, space science and systems analysis to anticipate the course of an influenza outbreak and speedily mitigate or eliminate its impact. Talent in genomics, cell biology, sensor development, modeling and simulation, statistics and advanced systems engineering will all come into play for this project, as will relationships with the Centers for Disease Control and Prevention, the UNM School of Medicine and the New Mexico Department of Health. Once perfected in the influenza application, the components will then be adapted to a range of other natural and terrorist-derived threats.

Such a program requires a multi-level approach, an approach that Los Alamos is an acknowledged leader. Among the components of the system will be the following:

- new data sources for influenza strain identification,
- new computer models for predicting disease spread and evolution and
- a nationwide network for communicating key information and planning a response.

The first part of this effort, early identification of influenza strains, will leverage the work already described, namely the development of a small hand-held device capable of detecting influenza early after infection. Further development will identify genetic markers that are associated with virulence of the virus and incorporate those into models of influenza evolution and transmission. This research can provide guidance as to which measures are most effective in containing an impending outbreak.

Andy Shreve, of the Lab's Bioscience Division, is adjusting a laser-based spectroscopic instrument that characterizes thin molecular films used in biosensor devices. The ultrathin films are created using self-assembly techniques and are designed to mimic aspects of the structure of natural biological systems.



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In part two, detection sensor research will be expanded to identify and detect toxins, viruses and DNA strands at vanishingly small concentrations. The sensor described previously uses a fluorescence-based recognition system. Another promising effort already afoot, tags nucleotide recognition elements to allow identification of viral RNA sequences. Yet a third could be effective as well, that of using "molecular beacons" that each detect a different target, marking single nucleotide variations in different virus strains.

Part three goes beyond the challenge of seeing what an infected patient is battling. In this phase, the objective is to determine what the patient has been exposed to, even before symptoms are evident. Chemical changes within the epithelial lining of the lungs appear to provide useful information in this area. If patients can be identified before they are either sick or infectious, simple public-health steps such as isolating and breaking the expanding cycle of infection before it begins possibly can stop an impending epidemic.

The final phase of this aggressive program is called a Multi-Level Heterogenous Data Fusion. It addresses the crippling isolation of the very health-care facilities that find and treat the earliest victims of an outbreak or biological attack. Early detection of influenza-like illnesses is critical to the nation's ability to detect and respond to a biological attack. Yet current medical reporting techniques involve a health-care worker writing a case analysis some hours or days after a patient is seen. While the potential exists to gather extensive data from the scattered health-care facilities, until these facilities are networked and reporting information in a more timely, structured fashion, information is too little, too late.

On the local scale, health-care providers immediately need to recognize unusual events and take appropriate action. Regionally or nationally, the local information must be transmitted to the decision-makers quickly enough that mitigating measures can be put into place.

Existing prototype systems at Los Alamos can be tested to determine if they are of use in filling these information gaps. Current Los Alamos projects such as the Biological Aerosol Sentry Information System and a medical surveillance system, called Rapid Syndrome Validation Project, (see "Rapid Syndrome Validation Project") both demonstrate the Laboratory's experience with networked sensor and information systems and the ability to work with industry to provide open standards for combining clinic information systems. Research that uses tools such as Mobile Software Agents that can perform detailed pattern searching on data that reduces an impenetrable mass of information to the key items can tell users whether or not a virulent outbreak of the flu is at hand.

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ENSURING OUR SECURITY AGAINST BIOLOGICAL THREATS

RAPID SYNDROME VALIDATION PROJECT

Los Alamos National Laboratory is collaborating on a new tool that will provide public health officials with an early warning and response system for threats to public health.

The threat to public health from infectious diseases is increasing. New, evolving and emerging diseases have appeared in unexpected locations with increasing regularity as society becomes more mobile. Diseases such as HIV, dengue fever and hantavirus present unique and significant challenges to the public health infrastructure, both in recognizing their presence and dealing with their effects. The familiar flu virus takes a significant toll every year, causing thousands of deaths, mostly among the aged or the young.

The threat of bioterrorism also has increased. A rogue group could introduce virulent biological pathogens into a population with potentially catastrophic results, if we do not have the tools to detect and respond. Most flu epidemics are recognized only after they happen, and therefore, appropriate medical attention is often too late.

Physicians and public health officials realize that information systems to recognize and respond to public-health threats need to be better. Officials need tools to recognize an outbreak, make quick diagnoses, disseminate information throughout the health-care community and allocate the resources to deal with such threats.

A new project will put important new tools in health-care providers' and emergency planners' hands. The Rapid Syndrome Validation Project is a collaboration of several institutions including Los Alamos and Sandia national laboratories, the University of New Mexico Emergency Medicine Department and the New Mexico Department of Health Office of Epidemiology.

Al Zelicoff, senior scientist in the Center for National Security and Arms Control at Sandia, conceived the project.

"Its primary objective is to provide early warning and response to emerging health threats," Zelicoff said.

RSVP is a computer-based system that operates at the nexus of public health and bioterrorism. Los Alamos scientist Dave Forslund provided the software package that resulted in a portable system that can run on any computer platform anywhere in the world that has access to a Web browser. The portability along with the low cost of running the program through a Web browser makes the technology ultimately available to anyone, anywhere.

Input computers can be set up with touch screens to make the data input faster, which would help emergency room personnel. Health-care providers enter a patient's symptoms on a highly secure, encrypted Internet transmission. After the new syndrome is entered, the output screen shows a map, organized by zip code, of all similar syndromes.

The project is being piloted on six syndromes: flu-like illness, fever with skin findings, fever and altered mental status, acute bloody diarrhea, hepatitis and acute respiratory distress.

Physician judgment determines the syndrome that best represents the patient's condition and is partially captured by the syndrome selections. The reporting system assists the physician with appropriate clinical and epidemiological data. If the syndrome matches a reportable disease, an alert will be provided to the physician and automatically sent to the Department of Health. The reportable-disease database provides the DOH with data on potential outbreaks and other public health risks.

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RSVP may benefit another Los Alamos project called EpiSIMS, a disease-outbreak computer model primarily designed for tracking respiratory illnesses. EpiSIMS currently is being developed to model the spread of influenza through a population and is a derivative of the TRANSIMS model, which predicts the flow of traffic. By coupling the infectivity rates of an influenza virus with the flow of people and their contacts with each other, the goal is that EpiSIMS will develop into a powerful model for projecting the scope and impact of an influenza outbreak. EpiSIMS would be a tool to benefit public health planners and disaster response officials in responding to public health emergencies of either natural or man-made origin.

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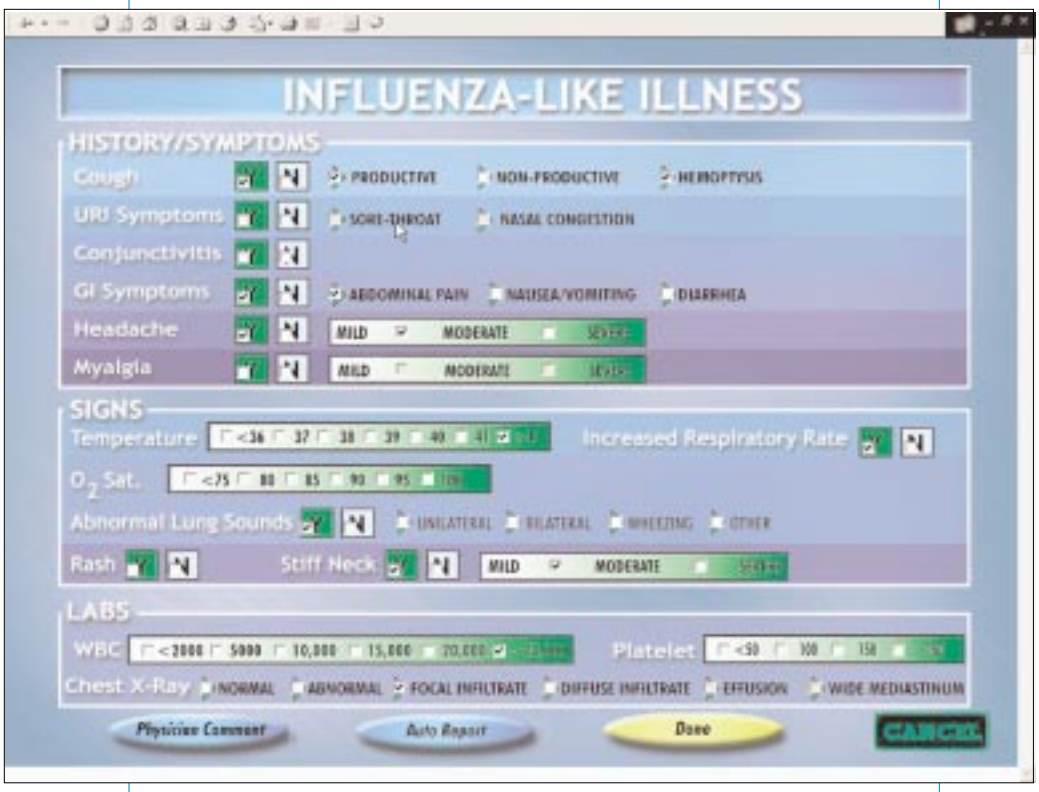

Influenza-like illness is an important clue to alert health-care providers of an emerging outbreak, such as a new virulent flu strain, hantavirus or the release of a bioagent by terrorists. Virtually all infectious disease caused by bioagents that might be used by terrorists initially look like a flu-like illness, for example, anthrax. A surveillance system could monitor unusual numbers of flu-like illness and give health-care workers time to develop intervention strategies.

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ENSURING OUR SECURITY AGAINST BIOLOGICAL THREATS

UNRAVELING ANTHRAX

Los Alamos National Laboratory's Bioscience Division researchers have developed technologies that can uniquely identify the origins of biological organisms based on information in the DNA.The technique, known as Amplified Fragment Length Polymorphism (AFLP), is used to create a library of genetic profiles for hundreds of different *Bacillus anthracis* strains, the organisms that cause anthrax in livestock and humans. Specific DNA fragments from the AFLP profile are then used to design a new set of fragments, known as polymerase-chain-reaction, or PCR, primers, that can specifically detect these fragments in complex samples.

↑ Bacillus anthracis

Based on the analysis of tissue samples, Los Alamos researchers proved that the victims of the 1979 anthrax outbreak in the former Soviet Union were infected with at least four different strains of *B. anthracis*. This provided definitive evidence that the deaths were not caused by a natural infection. It was later revealed that the deaths had been caused by the accidental release of *B. anthracis* spores from a Soviet military biological research facility suspected by western intelligence experts of producing large quantities of spores. More recently, Los Alamos DNA analysis of samples from Iraq in the aftermath of the Gulf War was directly linked to Iraq's disclosure of an offensive biological warfare program that included the use of *B. anthracis*.

A team of Los Alamos researchers, including Paul Jackson, Karen Hill and Larry Ticknor, uses AFLP markers as genetic characters to determine the relationships among bacterial isolates. AFLP DNA fragment libraries have been developed for a large number of *Bacillus* species and a smaller number of pathogens. Researchers use the libraries to analyze medical, veterinary, forensic and environmental samples to determine their microbial content. The goal of the project is to generate an AFLP profile from a sample

B. anthracis bacteria form spores when exposed to oxygen. The spore-form can survive for decades when buried in dirt, thus accounting for anthrax outbreaks in cattle when an old site is unwittingly exposed through grazing.

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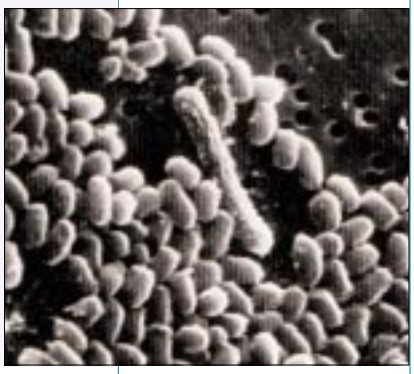

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containing unknown microbes, compare it electronically to all the archived profiles, and thereby determine its phylogeny, and possibly, its exact identity and geographic origin.

The technology recently was used to properly classify a misidentified pathogen that severely infected the wound of a French peace-keeping soldier wounded in Bosnia. The microbe isolated from the infection was misidentified as a *B. thuringiensis* strain commonly used as a biopesticide. This caused considerable public concern about the use of this species for insect control. Los Alamos analyses showed that the pathogen actually was related closely to *B. anthracis*, explaining why the soldier suffered a serious infection, and was only remotely related to the *B. thuringiensis* strains used as biopesticides.

B. anthracis, discovered in the 1870s, was the first organism shown to cause a particular disease. It causes anthrax in animals, mostly cattle, horses, goats, sheep and in humans. Cutaneous anthrax in humans occurs most frequently on the hands and forearms of persons working with infected livestock or products from these animals. It results in sores that develop coal-black scabs. The term anthrax comes from the Greek word for coal.

Cheryl Kuske, a technical staff member in the Lab's Bioscience Division, is interested in understanding the relationships between the pathogenic *B. anthracis* bacterium and non-pathogenic close relatives of *B. anthracis* that are naturally present and widespread in the environment. She has been comparing sets of genes from the pathogen with DNA from several different nonpathogenic *Bacillus* species to identify genes that are unique to the pathogen. These genes have potential for use in rapid, DNA-based detection strategies for *B. anthracis* in environmental samples for use in forensic analysis.

Another Bioscience Division project addresses the host-pathogen interface. Michael Altherr and Tom Brettin's work will create novel databases and integrated computational tools to investigate the interaction between pathogens and human cells, including *B. anthracis*. Several key technologies have emerged through this project that allow the analysis of the entire transcriptional and translational components of a cell. The knowledge gained from these studies may someday help lessen the consequences of exposure to the pathogens and aid in the development of vaccines.

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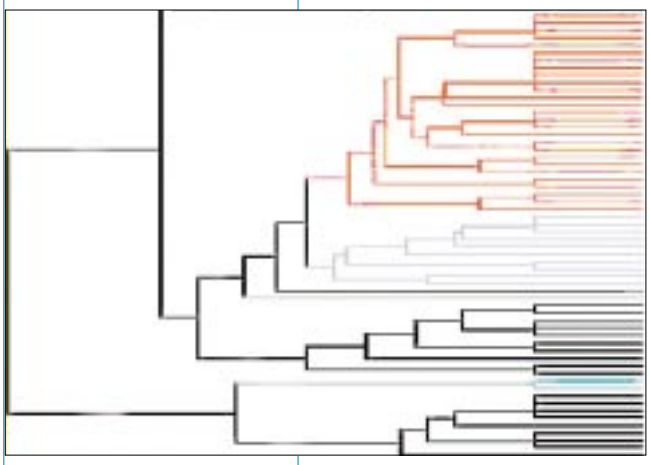
ENSURING OUR SECURITY AGAINST BIOLOGICAL THREATS

USING PATHOGEN SEQUENCE DATA

As scientists delve into the vast quantity of biological data currently being produced, the problems of handling such a treasure trove of information are daunting. New tools and techniques for managing, storing, analyzing, mining and visualizing this information are the focus of much attention in the scientific community, especially when the data can have a bearing on public health and even emergency response.



↑ Each bacterium carries its own DNA fingerprint, enabling a researcher to classify different species and strains within a species. Each species generates a "family tree" based on its genetic similarities. This phylogenetic tree illustrates how bacteria are related to each other. The group in red represents pathogenic organisms and includes *B anthracis*.

Los Alamos National Laboratory is trying to increase the knowledge of specific biological agents that terrorists might use. Researchers are working to develop biological and computational tools to assist in identifying the biological "signatures" that will allow rapid identification of these agents.

The Los Alamos effort provides bioinformatics support to the researchers of the U.S. Department of Energy's Chemical and Biological National Security Program. Los Alamos researchers are studying *Yersinia pestis,* the bacteria responsible for plague, and *Bacillus anthracis*, the source of anthrax, and are beginning to study other threat agents. In collaboration with others, this work may lead to better methods of treatment and prevention of major illnesses. In addition, this work is likely to lead to better methods of performing forensic and epidemiological analysis of biological incidents, determining whether an outbreak is natural or engineered and providing a possible identity to perpetrators.

The team also is developing various computational tools to assist in this project. They have developed interactive visualization tools; repeat-analysis tools; and a body of practices and procedures for data exchange that allow researchers to perform analyses more efficiently and exchange data more readily. The project also contributes to the

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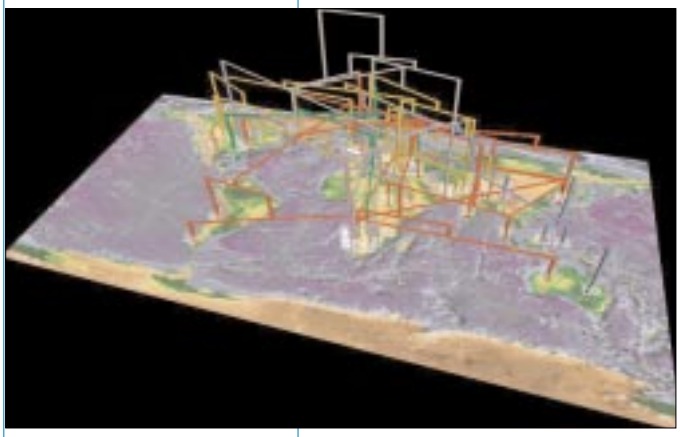

This computer graphic is a 3D simulation of the family tree of *B. anthracis* strains indicated on the previous page. Individual strains are located geographically on a world map according to their origin.

challenge of improving the integration and interoperability of large software systems dealing with large amounts of data. The team's work is accessible to researchers across the field via the Web page (http://www.cbnp.lanl.gov/) that also links various other efforts in this area.

The bioinformatics work is distinctly a team effort. The members work closely with the best researchers who are investigating the organisms of particular interest to them, regardless of affiliation. The team is working with researchers at Lawrence Livermore National Laboratory on bacterial threat agents, with the U.S. Army Medical Research Institute of Infectious Diseases and the University of Alabama on pox viruses. This work also is coordinated closely with Los Alamos work on sexually transmitted diseases being done for the National Institutes of Health. The NIH work is being done in collaboration with world experts on herpes, papilloma and chlamydia viruses and others.

Said team member Murray Wolinsky of the Lab's Bioscience Division, "Because of the large need for tools and the limited funds available, we have to avoid 'reinventing the wheel' and use existing tools whenever feasible. For all of these reasons, we stay abreast of the work being done here and elsewhere and focus our efforts on unmet current and future needs."

Such coordination helps ensure that the Lab project uses best practices in sequence annotation and develops tools needed by the community in general.

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UNDERSTANDING & PROTECTING OUR ENVIRONMENT

MICROBIAL DIVERSITY

They have been called the foundation of the biosphere, invisible yet essential. And now researchers know bacteria are unimaginably abundant but just don't know exactly who they are.

Bacteria are microscopic, single-cell organisms that are everywhere in the environment, including in the soil, air, in and on our bodies and in extreme environments like hot springs. They can be good or bad: Some make us sick, others help us digest food, still others are used for antibiotics. But researchers in Los Alamos National Laboratory's Bioscience Division have discovered that they are not as simple as once believed, and that there are more different types of them than anyone ever imagined.

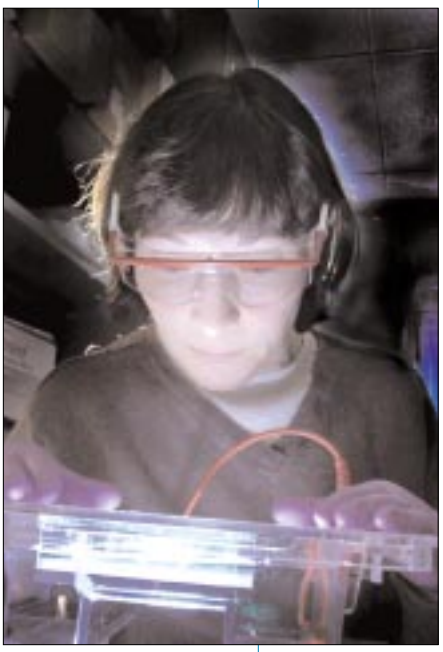
After 150 years of studying bacteria colonies in petri dishes, scientists have finally acquired an arsenal of molecular, DNA-based techniques that allow them to observe the organisms in their natural environments.

"What can be cultured is only a very small fraction of what's out there," said Cheryl Kuske, a scientist working on the project. "Microbial organisms are much more diverse than we ever imagined. We are just beginning to understand how vast that diversity might be."

The Department of Energy has an interest in identifying these previously unknown bacteria for a number of reasons. Bacteria are critical for decomposing and recycling nutrients at a global scale. They also are a valuable resource of novel metabolic abilities useful for pharmaceuticals and industrial processes. There also is a need to survey the natural microorganisms in the environment to be able to detect pathogens for forensic applications.

"We need to know which bacteria are naturally present in our environment to be able to specifically detect outsiders," Kuske said. "It's kind of like 'Where's Waldo?' — trying to detect pathogens in a diverse environment where the background may have traits that are similar to

Cheryl Kuske of the Lab's Bioscience Division looks through a gel electrophoresis unit used to detect and separate, by size, DNA fragments.



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the detection target. We've been tasked to survey a number of different environments, everything from natural soils to city air, and look at how variable the background bacteria might be and how they fluctuate."

The procedure for identifying these "new" bacteria involves extracting and sequencing one gene from all the bacteria in an environmental sample. The bacterial genome is about a tenth the size of the human genome. The 16S gene is the marker gene that all bacteria have, and with the use of molecular biology techniques, scientists can sample all representatives of that one gene and analyze them. In this way, researchers have been able to assign relationships to bacteria, essentially constructing family trees.

"A number of people have conducted studies of individual copies of this gene, developing 16S gene libraries," Kuske said. "We analyzed many of these libraries and together we've compiled an enormous tree of the bacteria we've found. In all of our studies, we haven't seen the same thing twice. We think there are probably millions of different organisms."

Kuske and her team use different scientific methods to answer questions about the bacteria, depending on what scale they are studying. In a single cell, they want to understand how the bacterium's DNA controls cell functions. In studying entire communities of microbes, the questions include who are the community members and what their functions are in the ecosystem.

Some of the other questions Kuske and her team would like to answer include how complex are these communities and how are they changing? Because of the difficulty in studying so many organisms, they have developed community fingerprinting techniques, essentially looking at the problem at a much lower resolution. This molecular fingerprint analysis can be used to monitor bacterial complexity, the relative abundance and dynamics of these microscopic communities at a landscape level in the real world.

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DATELINE: LOS ALAMOS

UNDERSTANDING & PROTECTING OUR ENVIRONMENT

BUILDING A BETTER CATALYST FOR BIOREMEDIATION

There are only a few ways to handle toxic waste. Dump it, put it in a landfill, move it someplace else or change the contaminant into something less hazardous. Dealing with toxic waste is a major problem that is beginning to be addressed in an innovative way: using bacterial enzymes, catalytic proteins produced by living cells, to transform the waste.

Biotransformation of one substance into another using bacteria isn't something new. It has gone on as long as there has been life. But now, Los Alamos National Laboratory's Bioscience Division is trying to create a toolbox, of sorts, using bacteria to clean up industrial waste. For the national laboratories, that also means a way to clean up legacy waste from operations related to the nuclear weapons complex.

The idea seems simple enough: use an enzyme that will react with the contaminant, called a substrate, and convert it into something benign. The idea is appealing because the process could be used to treat contaminants otherwise too far underground to get at easily and economically.

Jim Brainard and his team are concentrating on organic contaminants. The vast diversity of bacteria raises the prospect for the creation of a catalog of designer enzymes to use on waste. The process turns organics into more bacteria or something else such as carbon dioxide. Work on bioremediation of inorganic contaminants such as toxic metals, actinides and other man-made compounds also is in the early stages at the Lab.

"I think you can find an enzyme that will degrade almost any chemical in the environment," said Brainard.

The team uses a combination of rational and evolutionary design approaches. With the rational approach, functional properties and structural features of different enzymes are compared and combined. Then, site-specific testing is done to see if results of the "marriage" of the enzymes accomplish desired goals. In the evolutionary design approach, the team makes large libraries of random mutations in proteins then picks out the enzymes that work well with a particular contaminant. The whole idea is to go from a good catalyst to a better catalyst.

So far, Brainard and his team have had success with a few well-known enzymes. Most are not the best catalysts for initiating contaminant transformation. So, some biological engineering has to take place to improve the efficiency of their reactions.

Many enzymes that transform toxic contaminants only work efficiently with high concentrations of contaminants. "Most of the known enzyme systems will not transform contaminants at low enough levels to catalyze transformations down to regulatory limits," Brainard noted.

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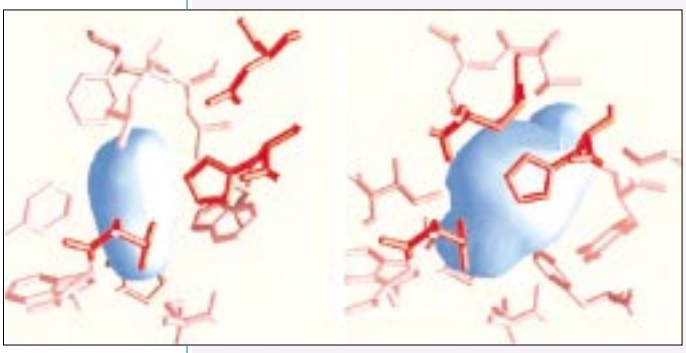
Brainard's colleagues are improving the interaction between an enzyme and contaminant so it will bind with the substrate at low concentrations.

The benign product of an enzyme-contaminant reaction also might stop complete conversion of the contaminant altogether. "Product inhibition and binding of enzyme to substrate are both problems that need to be overcome if we are going to reach regulatory levels and be able to say we have 'cleaned' the site," says Brainard. Lab scientist Cliff Unkefer and his team have engineered enzymes that are no longer product inhibited. The designer enzymes will be carried to the site that need remediation by microbes.



↑ Molecular models shown above illustrate the difference in size of the active site (blue object) for two similar enzymes that degrade the industrial contaminant trichloroethylene. Through molecular models, a new mutant enzyme was designed that enlarges the active site. The mutant enzyme captures the desirable properties of both enzymes in a new form — with a faster reaction rate and decreased product inhibition.

Microbiologist Cheryl Kuske and her team are developing tools to better understand microbes in the environment (*see "Microbial Diversity"*). The majority of bacteria is something about which little is known because there are so many bacteria and most do not grow under laboratory conditions. Using a tree as an analogy, all animals make up just one small branch, plants are another and other microorganisms make up the rest. Microorganisms are the most diverse forms of life and more than 95 percent are still unknown.

Another possible application for a library of "designer" enzymes is in the chemical industry. Biomaterials rather than petrochemicals could be used to develop feed stock. Glycerol for example, derived biologically, could be converted into a feed stock for many biochemicals. That raw material would be used to make polymers and plastics — all from the same enzyme process being developed to deal with organic chemical waste.

"That's a money-maker rather than a cost. There are certainly more opportunities for research support if you have a target that potentially will make a chemical company money as opposed to a cost to clean up a site," Brainard said. "I would claim that research into better bioremediation tools is a national need. I think it's an important thing for us to do."

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DATELINE: LOS ALAMOS

UNDERSTANDING & PROTECTING OUR ENVIRONMENT

METABOLITE DISCOVERY ALLOWS FOR FAST PLANT GROWTH

A project that uses modern biotechnology to produce plants that grow faster, are more robust and contain more protein is ongoing in Los Alamos National Laboratory's Bioscience Division. The project stems from the discovery of a naturally occurring plant metabolite that allows plants to regulate their own nitrogen metabolism rates, resulting in plants that reach peak growth more rapidly because they fix more carbon dioxide.

The success of the project and its inevitable commercialization could have significant impacts for worldwide crop yields, and its beneficiaries could include environmentalists, farmers, industry in the United States as well as people in some developing nations. An added benefit is the increased sequestration of carbon dioxide, potentially lessening the effects of global warming.

Los Alamos scientist Pat Unkefer's team discovered the plant regulatory system that coordinates these functions. "Plants function in a world with abundant carbon, but limited nitrogen," she said. "And they must maintain the proper ratio of

The tobacco plants on the right show enhanced growth when genetically altered to overproduce a metabolite that increases their nitrogen uptake. The control plants are on the left.

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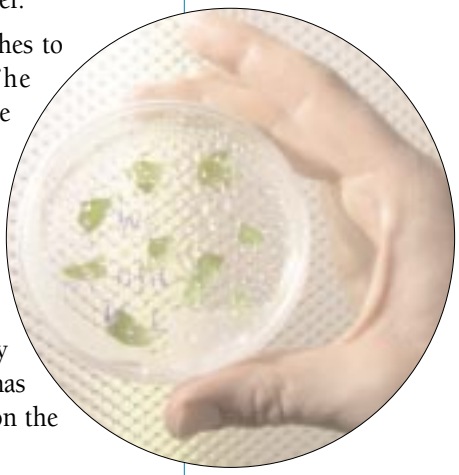
carbon, nitrogen, phosphorus and sulphur. What we've done is to trick the plants into operating as if they have more nitrogen, and consequently they take up more nitrogen, which is good for farmers because they will need less fertilizer." Unkefer points out that the metabolite is neither a pesticide nor a hormone: It is a metabolic trigger.

Unkefer's team pioneered research into several approaches to stimulate the mechanism in a variety of plants. The approaches include topical application of the metabolite and, more recently, bioengineering of tobacco plants to overproduce it. Plants subjected to either of these approaches grow faster, have greater biomass and contain more protein. Plants that have responded to the topical application include corn, oats, alfalfa, soybeans, lettuce, tomatoes, cantaloupe and cotton. The team has developed an efficient and environmentally friendly chemical synthesis for this metabolite; a patent has recently been allowed for this synthesis. Other patents on the technology are pending.



↑ Tobacco leaves are incubated with a special virus containing the gene responsible for enhanced metabolite production.New plants are regenerated using the leaves that contain the specialized enhanced growth mechanism.

A second approach to increasing the amount of the metabolite in plants has been accomplished through a combination of bioengineering and classical plant breeding. The approach has been demonstrated in tobacco, a "lab mouse" for plant researchers. The researchers identified the critical genes, created engineering plants and then bred for the desired traits. Unlike some new technologies, these approaches do not require adding foreign DNA to plants. The plants' normal processes are used to accomplish these beneficial changes.

"An exciting part of this technology is that we can achieve these beneficial changes in a manner that is more friendly to the environment than many previous biotechnology approaches," Unkefer said. "We hope to continue to study this beneficial, 'green' metabolite and to establish industry partnerships to help make these benefits available to the wider community."

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UNDERSTANDING & PROTECTING OUR ENVIRONMENT

THE WHO'S WHO OF SPOTTED OWLS

A unique molecular biology study of endangered Mexican spotted owls nesting in the Jemez Mountains near Los Alamos National Laboratory is being conducted in the Lab's Bioscience Division and has revealed valuable information about levels of genetic diversity present within the owl population. Genetic diversity is one of several aspects of spotted owl biology that Los Alamos scientists hope to better understand with the aid of data gleaned from the field. The study, which also 'will provide valuable information about the birds' habits to wildlife researchers interested in stabilizing endangered species population, will use genetic information derived from a particularly noninvasive technique: The DNA has been acquired from feathers collected from the owls' habitat.

The team has extensive experience studying bird genetics and has developed a method of analyzing the DNA characteristics of the Jemez Mountain owls and of tracking their habits by collecting and analyzing their feathers.

The Mexican spotted owl was listed as federally threatened in 1993. This subspecies of the spotted owl is found in northern Arizona, southeastern Utah and southwestern Colorado, south through New Mexico, west Texas and into Mexico. The Mexican spotted owl generally inhabits mixed conifer, pine-oak and riparian habitat in mountains and canyons.

The U.S. Fish and Wildlife Service's recovery plan for the subspecies requires research on population biology, gene flow and genetic isolation of populations. To gain such information in the past, researchers needed to capture and handle the birds to take blood or tissue samples. By studying feathers the birds have molted in and around their nests, scientists have eliminated concerns of hurting the owls or changing their behavior.

The Los Alamos team has collected more than 1,300 Mexican spotted owl feathers from 19 identified territories in the Jemez Mountains over the past 17 years. They now have a database of feathers: Each one has been photographed and given a number for tracking purposes, then placed in a bag to ensure no cross contamination occurs.

In the lab, the feather DNA is isolated for analysis. The end of a feather is diced up and placed in a solution that ruptures the cells and releases the DNA. After eliminating the proteins and other contaminants, the relatively pure

Genetic variation in spotted owl DNA is illustrated below. Each vertical lane represents one individual owl. The red bands represent standards that are run in each lane for each sample to provide a size reference. The blue bands show the variability between individual owls.

The Mexican spotted owl, above, is molting a feather that was used in DNA research. Scientists want to uncover more information about this threatened subspecies' migration, nesting and mating habits.

form of DNA that remains looks like water. The sample is examined for a specific set of repetitive sequences, usually one to six base pairs repeated over and over. These sets of repeats are known as microsatellites.

The team has constructed a library from a DNA sample of a Mexican spotted owl housed in a rehabilitation center in Albuquerque. The library provides a way to archive a series of DNA fragments that together constitute multiple copies of the entire genome of the spotted owl. The researchers have isolated individual microsatellite clones from the library and have determined their DNA sequence. The sequence data allowed the researchers to build short DNA primers for the microsatellite locations. Together with a method known as the polymerase chain reaction (PCR), these primers enable the researchers to analyze the microsatellite repeats in feather DNA. Differences that are found at the microsatellite locations within feather DNA will be used to create a database of genetic variations for the spotted owl.

When the database is complete, the team will be able to identify every individual bird within the population. Some of the questions that will be answered in the study include how far the birds travel, how long they live, how successful they are at reproducing and whether they are site-specific.

A comparison of DNA from different populations will be useful in making management decisions if the number of one group begins to decline. Restocking decisions could depend on information about genetic similarities between healthy populations and one that is in crisis.

The genetic data also will help researchers assess the long-term effect, if any, that the Cerro Grande Fire will have on the spotted owl populations. After the fire was put out and the smoke cleared, researchers found that 90 percent of Los Alamos County's habitat for the Mexican spotted owl had been lost. Luckily, none of the nests being monitored by the team studying the owl's genetic characteristics were damaged. Nonetheless the fire did reduce the overall area that young owls can occupy as they become reproductively mature and set out to develop their own nesting territories. The ongoing project will help determine if this loss of habitat will result in any significant change in the population's level of genetic diversity.

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LESSONS FROM NATURE FOR NEW MATERIALS

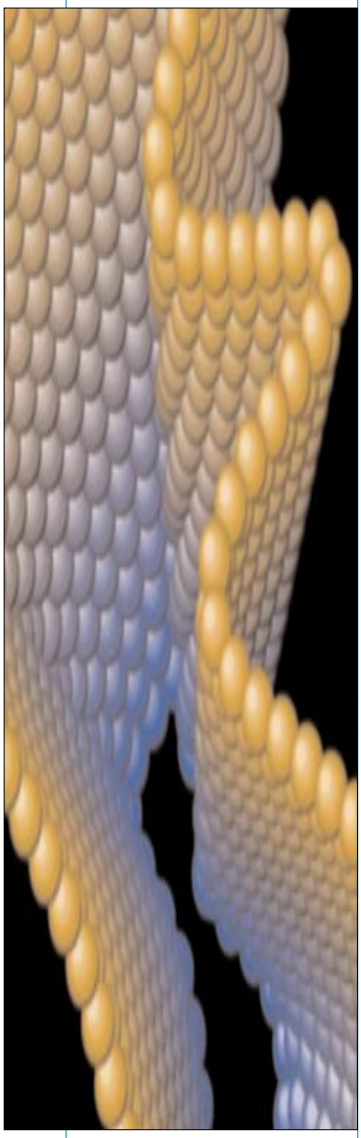
BIOLOGICALLY INSPIRED NANOTECHNOLOGY

Much of today's scientific revolution is taking place at the nanometer scale. There is growing recognition that an ability to design and manipulate materials at the nanoscale will allow scientists to not only improve existing materials, but also develop entirely new classes of intelligent or "smart" materials for everything from miniaturized laboratories and micro-computers to drug delivery systems. To this end, lessons from biology offer revolutionary approaches.

Nanoscience is the design and fabrication of materials from the nanometer-length scale up to create novel and significantly improved devices and materials. In contrast, traditional materials science builds from large-scale objects down. The semiconductor industry, for example, has relied on developing smaller and smaller features in large silicon wafers to fabricate computer chips. In contrast, using a nanoscience approach, one can self-assemble chains of molecules to replace wires on conventional computer chips, and it allows the semiconductor industry to produce revolutionary computer chips that are not only smaller, but also faster and more powerful than anything that exists today.

To put a nanometer-length scale into perspective, one nanometer — one billionth of a meter — is 100,000 times smaller than the width of a human hair. The building blocks in the biological world are nanometer-sized molecules such as proteins and sugars that, when assembled into intermediate length-scaled objects, determine and control biological function.

In addition to developing nanoelectronics, many other features of the biosynthetic process lend themselves to devising nanotechnological materials. To do its work, nature uses highly sophisticated processes, for example, selection; self-organization; and self-assembly to provide an enormous range of "bio"-materials that ultimately form cells, tissues and organs. These materials exhibit remarkable powers of memory, replication, self-healing and self-repair.



↑ This is an artist's rendering of nanometer building blocks.

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In the case of bone, for example, nature has developed a composite ceramic material with overlapping levels of structural hierarchy and functional complexity. Self-assembled bio-organic materials, such as lipids and proteins, form nanoscale templates for inorganic components, guiding the final structure and shape of bone. It is the mixture of two very different materials — inorganic silicates and organic proteins — that gives bone its exceptional strength.

Biological membranes, which encapsulate all cellular machinery, represent another such example. Here, nanoscale organization and complex interactions of its constituents such as lipids and cholesterol, allow them to filter undesirable molecules from entering the cell. Recent advances in materials synthesis and biotechnology have enabled scientists to use these lessons from biology to produce highly ordered nanostructured materials with unique properties.

At Los Alamos National Laboratory, researchers recently have developed nanofilters by mimicking the biosynthesis of bone. These nanofilters are made up of ordinary glass, which has pores and channels that can be adjusted in size from four to 20 nanometers. By controlling the size and chemical properties of the pores, the constituents of complex mixtures can be separated. These nanofilters could be employed, for example, as masks to prevent exposure to biological pathogens such as viruses that can be as small as 30 nanometers in diameter. This work is supported by the Department of Energy's Office of Basic Energy Sciences in a joint project with Sandia National Laboratories and the University of New Mexico.

Los Alamos researchers also have developed miniaturized biosensors that can detect bioagents and markers for disease by mimicking cellular membranes and depositing these membranes onto optical chips. The surface of these membrane-based sensors look like the natural target of a biological agent, receptor molecules that decorate the surface of a cell membrane. By copying nature's functions using nanoscaled materials, the useful properties of sensors can be optimized permitting entirely new approaches to, for example, the early detection of disease. This cross-disciplinary effort spans fundamental science in Los Alamos' Strategic and Supporting Research Directorate and systems engineering in the Threat Reduction Directorate and is supported by the departments of Energy and Defense and by Laboratory-Directed Research and Development funds (see "Early Detection for Protection" for more on LDRD projects).

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LESSONS FROM NATURE FOR NEW MATERIALS

SHINING A LIGHT ON NOVEL POLYMERS

A rapidly growing field of research, recognized by a 2000 Nobel Prize in chemistry, focuses on electrically conductive plastic, once thought to be an impossibility. Plastic, after all, is a well-known insulator. But with certain modifications in its chemical structure, a type of plastic called a conjugated polymer becomes conductive, a property that has been used in recent years to make lighted displays, solar cells and some television screens.

Conjugated polymers are long chains of molecules — some resembling strings of pearls.

At Los Alamos National Laboratory, researchers have discovered an unusual characteristic of conjugated polymers that makes them highly valuable in sensors to detect biological and chemical agents. "This unique property greatly increases a sensor's sensitivity," said Liaohai Chen of Los Alamos' Bioscience Division. "We can detect bioagents or chemical agents rapidly at extremely low levels."

Conjugated polymers are long chains of molecules. They are joined by alternating single and double bonds that can enable the electrons to move along the backbone of the chain. Normally they become conducting when they are "doped" by certain doping molecules. This discovery in the late 1970s earned the aforementioned Nobel Prize in chemistry.

Some conjugated polymers that fluoresce can be treated as a string of glowing "pearls" linked together. The fluorescence of the polymer can be turned off by the presence of molecular "quenchers" attached to the polymer and turned back on when the quenchers are removed.

Chen and Los Alamos colleagues Hsing-Lin Wang, Duncan McBranch and David Whitten discovered in 1999 that one quencher attached to a polymer chain would quench every "pearl" of the entire chain, and that when this single quencher was removed, the whole chain would fluoresce.

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"This was a very surprising result, not only to us, but to the whole polymer community," said Chen. "What's more, the property is reversible, so the polymer can be turned on and off, depending on whether the single quencher is attached or not. This was against all expectations. Even though no current theory or models can explain this observation, the tremendous signal amplification process definitely opens a new avenue for sensor development."

The ability for action on a single molecule to affect an entire polymeric chain makes a biological or chemical sensor extremely sensitive. All it takes is for one element in a quenched chain to recognize the target and pull the quencher away for the entire chain to light up, sending a luminescent or electronic signal that can be easily read. In principle, such a sensor can be hundreds or thousands of times more sensitive than sensors using conventional fluorescence mechanisms.

Thus far, conjugated polymer-based biosensors have successfully detected very low concentrations of toxins and viruses. Los Alamos researchers are trying to optimize a sensing system by further increasing the sensitivity and verifying which quenchers and other elements work best with specific bioagents or toxins. Along with researchers elsewhere, they also are trying to understand why the polymeric chain behaves the way it does. The mechanism for the multiplier effect remains mysterious.

The Los Alamos work is supported by the Department of Energy's Office of Biological and Environmental Research.

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DATELINE: LOS ALAMOS

USER FACILITIES FOR THE NATION

STABLE ISOTOPE RESEARCH RESOURCE: STRUCTURAL BIOLOGY RESEARCH DEPENDS ON SUPPLY OF LABELED BUILDING BLOCKS

The ability to apply research techniques to important problems in biology and medicine depends on the availability of isotopically labeled compounds.

Within the last 30 years, the Los Alamos National Laboratory's Stable Isotope Research Program has evolved into a "multifaceted" program that serves biomedical researchers around the world.

Researchers have created more efficient routes for synthesizing stable isotope labeled compounds, which are distributed to accredited researchers. Many scientific studies make use of such isotopes as carbon-13 and nitrogen-15, benefiting the biochemical, chemical, clinical and pharmacological realms.

Molecules are tagged with stable isotopes so they can be traced spectroscopically using various methods that include Nuclear Magnetic Resonance, Electron Spin Resonance, vibrational and mass spectroscopy, said SIR Director Clifford Unkefer. Such techniques have potential for studying the structure and function of DNA, RNA and proteins. Spectrometry is the measurement of energy spectrums emitted or absorbed by a substance.

The Laboratory's SIR program is supported by the National Institutes of Health, the world's foremost medical research center and the federal focal point for medical research in the United States.

SIR develops methods for site-specific labeling of amino acids and nucleotides, the components of large molecules such as proteins and DNA. In addition, the organization develops methods for producing labeled proteins and nucleic acids for structural studies.

Such medical treatments to arise from collaborative research between Los Alamos and other labs include a breath test used to diagnose ulcers. Scientists also have figured out how a natural product, such as penicillin, is synthesized by nature.

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Marc Alvarez of the Lab's Bioscience Division is working on the synthesis of an isotopically labeled compound for its use in the quantum computing field.

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USER FACILITIES FOR THE NATION

PROTEIN CRYSTALLOGRAPHY RESOURCE AT NEUTRON RESEARCH CENTER FOR IMAGING PROTEINS

Thanks to a $4.8 million capital commitment from the U.S. Department of Energy, Los Alamos researchers have completed a state-of-the-art neutron diffraction station at Los Alamos' Neutron Scattering Center, part of the Los Alamos Neutron Science Center, known as LANSCE. The new station went on line in December 2000.

The neutron diffraction station may provide critical data for pharmaceutical companies to develop new designer drugs to combat debilitating diseases. It also may allow genetic researchers to understand better the events that lead to activation of "genetic switches" that result in deformities or maladies. Genetic switches might also fight off illnesses or trigger immune responses.

Understanding the structure of proteins and polymers is also key to understanding the messages encoded in human DNA.

At the facility, researchers place a sample of a material in the path of a beam of neutrons. Neutrons in the beam scatter when they interact with the atoms in the sample. A detector behind the sample records how the neutrons were scattered and renders a two-dimensional, black-and-white stippled image — a crude mandala of sorts. This diffraction pattern is manipulated and then analyzed by a computer to reveal the 3-D structure of the sample. The computer analysis accurately portrays the relative distances between the atoms that make up the structure, the lengths and angles of the bonds between atoms in the structure and the position and location of each hydrogen atom in the structure.

The neutron-diffraction-imaging process is a little like making and interpreting an X-ray image. In fact, structural biologists use X-ray diffraction to make images of organic molecules. But unlike X-ray diffraction, which is "blind" to hydrogen, neutron diffraction yields accurate information about hydrogen atoms contained in a structure. Being able to see and understand hydrogen atoms in a polymer, like DNA, or in a protein segment is key to understanding how the protein or polymer functions. Hydrogen is key to many internal and external reactions of proteins with other biological chemicals. Hydrogen atoms act like tiny switches to activate a chemical or to keep it in "standby" mode.

Because ordinary hydrogen can be substituted by deuterium — a stable isotope of hydrogen that has an extra neutron in its nucleus — and because the neutron scattering data can distinguish between hydrogen and deuterium atoms, scientists will be able to get a better idea of which hydrogen atoms are actually switches and which are simply chemical place-holders in a molecule.

Moreover, because neutron diffraction can see hydrogen, researchers will be able to better understand the role that water (two hydrogen atoms bound to an oxygen atom) plays in proteins and polymers. In some cases, water molecules are simply a structural building block. In others, water arranges itself into a funnel configuration that allows other chemicals to move from one area to another. Understanding the role of water in biological chemicals is a key field of study for some Los Alamos researchers.

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USER FACILITIES FOR THE NATION

NATIONAL FLOW CYTOMETRY RESOURCE CENTER

For more than 30 years, scientists and researchers from Los Alamos National Laboratory and academia have been using flow cytometry to analyze, characterize and sort thousands of biological cells, chromosomes or molecules in minutes. Los Alamos has been at the forefront of flow cytometry technology development from the beginning.

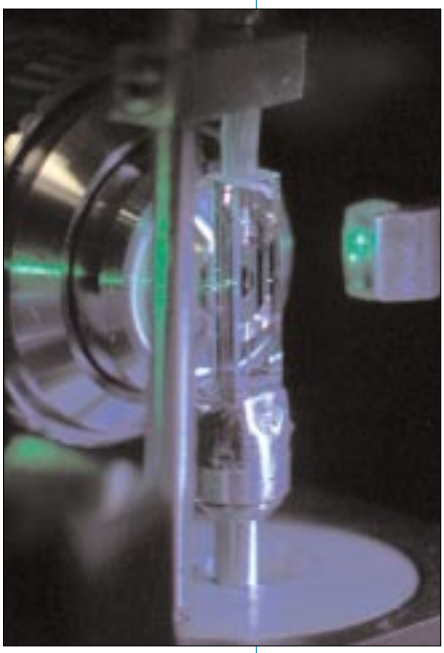
In July 2001, Los Alamos begins the 20th year of its National Flow Cytometry Resource (NFCR), a research and user facility. Over the years, Los Alamos researchers have made advancements in the technology that now allows researchers, among other things, to identify DNA fingerprints of bacteria, including biological threat agents, and develop tests to identify people sensitive to chronic beryllium disease.

More recently, Los Alamos researchers have developed a new flow cytometer that measures the sizes of DNA fragments 100 times faster and is 200,000 times more sensitive than conventional techniques. And newer cytometers are small enough to fit on a researcher's desk.

Flow cytometers use lasers, lenses, computers and other high-tech equipment. Researchers use fluorescent "markers" to determine the size, shape and function of hundreds of thousands of cells in a span of minutes. Researchers can then separate "tagged" particles, such as abnormal cells, for further analysis. This cutting-edge work is helping doctors and researchers worldwide better understand how cells function normally — and malfunction in diseases and disorders such as leukemia, cancer and AIDS.

The National Flow Cytometry Resource is recognized as a leading research resource in flow cytometry by the National Institutes of Health's National Center for Research

In this flow cytometer, DNA fragments pass through the clear glass tube in front of the microscope, where the fluorescent tags become excited by the light emitted by a solid state laser. The tags emit photons, which are recorded by a detector.



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Resources (NCRR). The Los Alamos center is a NCRR-funded Biomedical Technology resource in flow cytometry.

Los Alamos' newest flow cytometer allows researchers to measure the sizes of individual fragments of DNA. This Los Alamos-developed flow cytometer can determine the size distribution of DNA fragments with 98 percent or better accuracy in less than seven minutes from a prepared sample, regardless of the length of the fragments. Less than two-trillionths of a gram of DNA is required to perform the analysis. Compared with conventional techniques such as pulsed-field gel electrophoresis, researchers can determine sizes with 90 percent accuracy, but pulsed-field gel electrophoresis requires relatively large amounts of DNA — roughly one-millionth of a gram — and 14 to 24 hours to obtain a fingerprint from a prepared sample.

"The new flow cytometer also has the potential to identify specific strains of bacterial species," noted Los Alamos researcher and co-Resource Director James Jett.

This is crucial in epidemic tracing and forensics. It also works for responding to a bio-agent attack where quick identification of the specific strain of an organism aids in tracing its origin.

An earlier version of a Los Alamos-built flow cytometer received a 1997 R&D 100 Award from the Illinois-based R&D Magazine as one of the 100 most significant products, materials or processes with commercial promise for that year.

Researchers are developing a smaller, portable version of the tool and are seeking an industrial partner to manufacture it. A patent has been granted.

With the recent opening of Los Alamos' new state-of-the-art Beryllium Technology Facility, the new test for chronic beryllium disease will help Los Alamos screen employees and potential new employees who will work in the facility in support of Los Alamos' national security missions.

Other NFCR activities include collaborating with approximately 25 university and medical school investigators; providing instrument access to investigators; and conducting a course in flow cytometry at Los Alamos every other year, which attracts an international audience of about 50. The next course is scheduled for June 2001.

This funding also facilitates a unique relationship that began 11 years ago between Los Alamos and the University of New Mexico. UNM pathology professor, Larry Sklar, conducts research for the NFCR while also serving with Jett as its co-director.

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CONCLUSION

The Bioscience Division at Los Alamos National Laboratory provides the nation with forefront science and technology development that improves public health, protects our environment and enhances national security. In pursuing this mission we draw on a diversity of scientific disciplines and the talents of around 300 researchers and technical specialists in safety, security, facilities, administration, financial operations, human resource management and communications.

↑ Jill Trewhella, Director of Bioscience Division, received her doctorate in Inorganic Chemistry from the University of Sydney, Australia, in 1980. She also holds a master's degree in physics. Trewhella joined Los Alamos National Laboratory in 1984 to begin a biological neutron scattering program as part of a joint appointment with Life Sciences and Physics divisions. During her tenure at Los Alamos, she has held multiple positions and was selected as Lab Fellow in 1995. She pursues research on the molecular basis for signaling and regulating of protein functions in cells.

The members of the Bioscience Division came to Los Alamos from near and far, from local communities and from all over the world, with rich experiences and heritage. And in the short time we have been a community, we have implemented innovative leadership and management systems that seek to foster broad community involvement in the development and ownership of our vision and how we plan to achieve it.

Bioscience Division was "born" Oct. 1, 1999. Its creation was inspired by the recognition that one of the most exciting frontiers for science and technology in the 21st century lies in seeking to understand and use biological complexity to improve the human condition.

The completion of the Human Genome Sequence, an achievement that Los Alamos played a key role in, has heralded a new era of genomic-scale biology that challenges us to develop a "systems" understanding of biology in which we discover how literally thousands of biological molecules operate together to achieve healthy function.

The quest for this understanding has already opened up enormous potential for health technologies and biotechnology applications. And in our quest for understanding biology

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in a predictive way, the Bioscience Division brings physicists, chemists and computational scientists together with biologists to probe specific biological systems and how they function at levels of detail that are unprecedented. This understanding enables us to more efficiently engineer enzymes or organisms for modified function to be used in applications such as bioremediation and carbon management; synthesization of new molecules that mimic biological functions to create novel materials for new applications; understanding the molecular details of protein malfunctions that interfere with healthy living; and designing detectors and sensors for identification and early warning of biological threats or disease.

This issue of Dateline showcases just a small sampling of our accomplishments and aspirations. We hope it will transmit to you the excitement and commitment to excellence, innovation and public service that is at that heart of our community.

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BIOSCIENCE DIVISION SPECIAL ISSUE 2001

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BRIEFLY

FROM DIRECTOR JOHN BROWNE

I am pleased to introduce this special issue of Dateline: Los Alamos that highlights the compelling research that Los Alamos is conducting in the biosciences.

Los Alamos National Laboratory became interested in health-related research early in the Laboratory's history because radiation was known to cause cell injury and genetic mutation. Early biological studies were devoted to improving our understanding of the physiological and genetic effects of radiation exposure and to setting appropriate dose limitations for workers. As this knowledge base expanded, Los Alamos' research and development work became increasingly sophisticated and included investigations at the cellular and subcellular levels, leading to today's atomic-level studies.

The results of research on this new scientific frontier will benefit national security, public health and environmental protection.

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