WINTER 03

Lens

A New Way of Looking at **Science**

Molecular fingerprints

The search for individualized medicine.

A PUBLICATION OF VANDERBILT UNIVERSITY MEDICAL CENTER

Lens –
A New Way of Looking
at **Science**

WINTER 2003

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The voyage of discovery consists not in seeking new landscapes, but in having new eyes.

– MARCEL PROUST

About the cover: Need help deciphering the fingerprint 'code?' Please turn to the back inside cover.

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ANNE RAYNER POLLO

Lee Limbird, Ph.D.
Associate Vice Chancellor for Research
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Dr. Limbird is professor and former chair of the Department of Pharmacology at Vanderbilt, and a guiding force behind *Lens*.

Capturing light. Providing focus. Altering perspective. That is the intent of our new publication, *Lens*. We are trying to give our readers – scientists and those who watch science alike – an appreciation of the revolution that is occurring in our understanding of health and disease.

Much like the maturation of our children, scientific discovery occurs in two interrelated ways – an incremental, step-by-step grasp of new insights intertwined with fundamental changes and paradigm shifts in our appreciation of life and its underlying processes.

We hope you will come to appreciate through our *Lens* that discoveries that could improve our health are not just about biology and medicine. They are both accelerated and detoured by the motivation and peculiar perspective of investigators. They are catapulted by technological advances. For example, if the promissory notes of genome-wide science are fulfilled, a therapeutic revolution will result, and we will begin to treat underlying diseases rather than simply the symptoms of diseases. Society's definition of health and disease will, of course, influence the direction that this discovery process takes.

One goal of *Lens* is to whet the appetite for a greater understanding of biomedical research for those who have not had the opportunity for formal scientific training. Yet another is to provide a synthesis of the different perspectives on the same topic for experts and the lay public. Hopefully, you will come to appreciate through our *Lens* that discoveries that affect the quality of human life are part of the fabric of our society. They are about public policy, economics, and the balance of these issues in a world that is threatened by more than disease.

It is our hope that *Lens* will provide enjoyable yet thought-provoking reading for a broad audience – from participants in the discovery enterprise to its benefactors, the public at large.

"We are trying to give our readers – scientists and those who watch science alike – an appreciation of the revolution that is occurring in our understanding of health and disease."

– LEE LIMBIRD, PH.D.

"It is appropriate that the magazine's inaugural issue should focus on proteomics, for the science of proteins – many experts believe – will be one of the most important fields influencing 21st Century medicine."

– BILL SNYDER

Imagine yourself driving in the country in a convertible, watching the grassy fields roll by, feeling the wind in your face. Much of what you're experiencing – including the ability to grasp the steering wheel and hear the music on the radio – is due to the actions and interactions of microscopic protein molecules in your nerves, your muscles, your eyes and ears.

Now imagine the interior of your body: the antibodies (also proteins) that fight infection, the complex symphony of protein messages that tell your tissues to grow or not to grow, the signals transmitted by proteins across synaptic junctions in your brain that enable you to think.

Imagine if something goes wrong with some of your proteins, and your body's immune system mistakes your own tissues for bacterial invaders, or because of poorly translated instructions some cells begin to grow out of control, or the signaling across the synapse is disrupted. You may develop an illness like multiple sclerosis or cancer or depression.

Just as the development of the microscope in the 17th Century enabled people to see what was previously un-seeable, today we're going through a similar perceptual revolution. Our modern medicine currently can address only the symptoms or consequences of many illnesses, ranging from dementia to diabetes. But now, through advances in genomics and computer science, we are beginning to appreciate the pervasive role that proteins play in maintaining health or causing disease.

Thanks to technologies like mass spectrometry, protein "chips" and bioinformatics, we are now able to examine nature through a new lens, one that may enable us for the first time to understand the root causes of many diseases, to stop them more successfully than we ever have before, and ultimately to prevent them from occurring in the first place.

This lens is not the private property of doctors or scientists. It is available to all who would look through it.

That is why we at Vanderbilt University Medical Center have produced a new publication accessible to the general public. In this and future issues, we will examine the frontiers of biological science and medical research, and how the new knowledge gained from these inquiries may affect our lives.

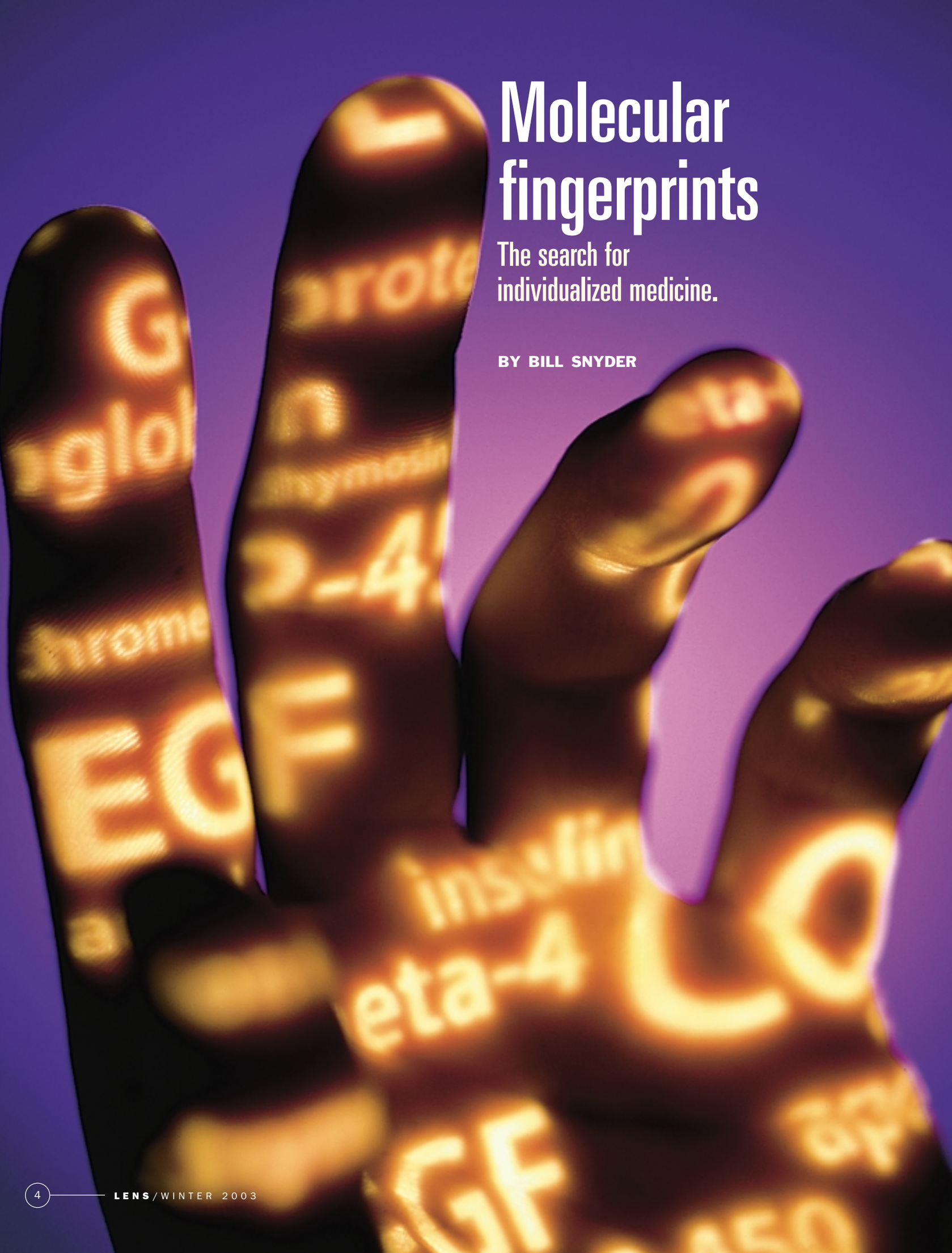
It is appropriate that the magazine's inaugural issue should focus on proteomics, for the science of proteins – many experts believe – will be one of the most important fields influencing 21st Century medicine. Richard Caprioli, director of the Mass Spectrometry Research Center at Vanderbilt and a member of this magazine's editorial board, puts it this way: "We're on the threshold of the journey to cure disease."

As we marvel at the advances that are coming our way, we should not forget the scientists of centuries past who first opened up new worlds of wonder beyond the reach of our eyes. "If I have seen further," said Sir Isaac Newton, "it is by standing on the shoulders of giants."

DEAN DIXON

Bill Snyder
Editor, *Lens* Magazine

For more than 20 years, Snyder covered health care and medical research for daily newspapers, including *The Tennessean* and *Nashville Banner* in Nashville.



Molecular fingerprints

The search for individualized medicine.

BY BILL SNYDER

Proteomics – the science of proteins – is opening up a new world of discovery and understanding of diseases as diverse as cancer and dementia. Using a variety of rapidly developing technologies, and knowledge gleaned, in part, from the successful effort to sequence the human genome, researchers the world over are developing new drugs and diagnostic tests based on proteins that are key to health as well as disease.

One of those methods, called "molecular fingerprinting," attempts to identify patterns of proteins in the blood and tissues that can be used to detect diseases like cancer much earlier and monitor therapy much better than is now possible. This is the story of some of the research – and the essential involvement of a patient – that are helping to make that hope a reality.

Two months ago, Art Haag joined a small, but rapidly growing number of cancer patients who are helping to bring some of the first scientific fruits of the 21st Century into the practice of medicine.

Those fruits include proteomics (pronounced "pro-tee-OHM-ics") – a growing understanding of the power of proteins to determine health and disease. These are not the proteins found in steak or peanut butter. They're already in your body, making up the walls of your cells and tissues, transmitting electrical signals in your brain, and carrying out a host of other genetic instructions that are essential for life.

When they go wrong – perhaps because they're mutated by a genetic error or overproduced – proteins can cause a wide range of problems, from heart attacks to Alzheimer's disease.

Thanks to the technological revolutions of the past quarter century that enabled scientists to sequence the human genome and put vast amounts of computing power on a chip, the previously unseen world of proteins is being revealed. That knowledge is fanning the flames of a new revolution – one in which diseases may be diagnosed earlier, treated much more effectively and ultimately stopped in their tracks.

One of the first clinical applications of proteomics is the early diagnosis of cancer.

Scientists around the world are trying to define "molecular fingerprints" of various cancers – unique patterns of proteins that may signal the presence of tiny tumors not yet detectable by X-rays. The information could help doctors make an earlier diagnosis and determine in advance which treatment will be most effective. It also may lead to the development of new drugs that can – like precision bombs – knock out various steps in the cascade of events leading to malignancy, and without harming normal tissue.

But first, researchers need the help of people like Art Haag, a retired Indiana farmer and land improvement contractor. This summer the former heavy smoker and colon cancer survivor got some bad news – a suspicious spot had appeared on his left lung in an X-ray taken during a check-up. Follow-up tests were inconclusive, so Haag was referred to the Vanderbilt-Ingram Cancer Center.

Haag admits he was scared. "I thought cancer of the lung was just a death threat right there," he says.

In early September, a piece of Haag's lung the size of a strawberry was surgically removed and found to be cancerous. But then something unusual was done to the tumor: it was frozen, sliced, applied to a metal plate and shot with a laser beam in a machine called a mass spectrometer. In a matter of minutes, the machine spit out a spectrum – wavy lines on a graph – a crude picture of the predominant proteins present in his cancer cells.

ANNE RAYNER POLLO

Art Haag, with his wife Wanda, volunteered for the molecular fingerprinting study at Vanderbilt University Medical Center last fall. After his lung tumor was surgically removed (see next page), a mass spectrometer was used to study the proteins it contained. Researchers are looking for patterns of proteins unique to cancer that could help them diagnose it early.

Researchers don't yet know the identity of all of the proteins. Nor do they understand what the spectrum of proteins suggests. But they're learning, thanks to the cooperation of dozens of patients like Haag. In the past year, more than 100 pieces of lung tissue donated by patients have been analyzed this way at Vanderbilt, as part of a "molecular fingerprinting" study led by Dr. David Carbone.

"We're trying to determine if we can identify patterns at the time of diagnosis that can predict how a tumor's going to behave," says Carbone, Ingram Professor of Cancer Research and professor of Medicine at Vanderbilt. "If you knew this tumor was going to respond to chemotherapy, then you might consider giving adjuvant chemotherapy after surgery."

Similar studies of cancerous breast, prostate and rectal tissue are being conducted at Vanderbilt. They join dozens of studies underway around the world. There is some early evidence that such studies can have powerful predictive value.

A complex world

Just what is this new science of proteins? How did we get here, and how far and how fast can we go?

The importance of proteins as the basic building blocks of life has been appreciated for more than 150 years (see "The Power of Proteins," page 8), but until recently, the characterization of these fascinating molecules was a slow and arduous process. A scientist could spend a career trying to isolate, identify and understand a single protein – out of the hundreds of thousands that make up the human "proteome."

Then came the fruits of the genomic and computer revolutions – methods for cloning genetic material (the "DNA") and mass producing large quantities of it; automation and miniaturization; and the ability to create and sift through huge "libraries" of data on genes and proteins.

The recent sequencing of the 35,000 or so genes that make up the human genome has created a vast pool of information from which scientists hope to fish out ways to prevent dementia, cure cancer, and perhaps even eliminate ancient afflictions of the developing world.

For example:

– At the University of Virginia, Donald Hunt and his colleagues have identified peptides – fragments of proteins – that trigger the body's immune system to kill melanoma (skin cancer) cells. This could lead to the development of an effective vaccine against the disease.

– Researchers at Stanford University have created a microarray or glass slide containing thousands of molecules that can bind to antibodies in the blood. It's being tested as a way to improve the diagnosis of autoimmune diseases like rheumatoid arthritis, in which the body's immune system attacks its own tissues.

– Scientists at Johns Hopkins University have developed a new blood test for malaria using mass spectrometry, a way of measuring and identifying proteins. The method could lead to improvements in early diagnosis and treatment of the disease, which kills more than a million people in equatorial countries every year.

– An "electronic taste chip" has been developed at the University of Texas at Austin that mimics the ability of the human taste bud to rapidly detect proteins and other chemicals in environmental samples. One possible application: detection of biological or chemical weapons.

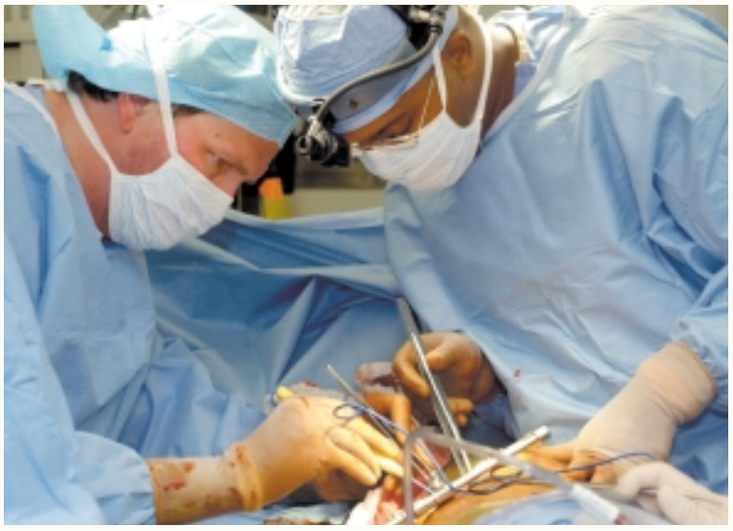
As tantalizing as these examples are, few scientists predict that the discovery and understanding of important proteins

– continued on page 9

The search for telltale proteins

1

DANA JOHNSON



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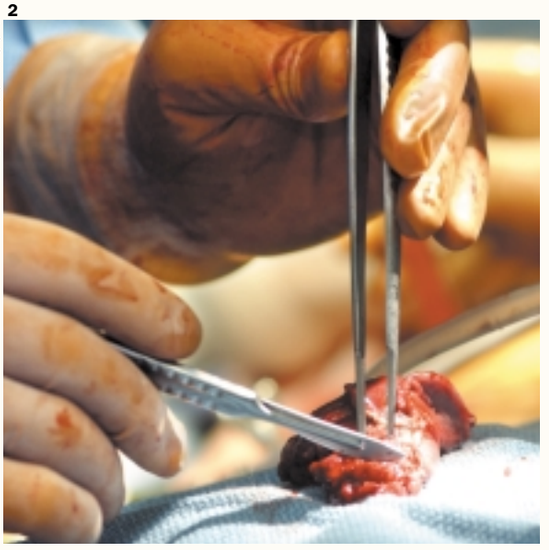

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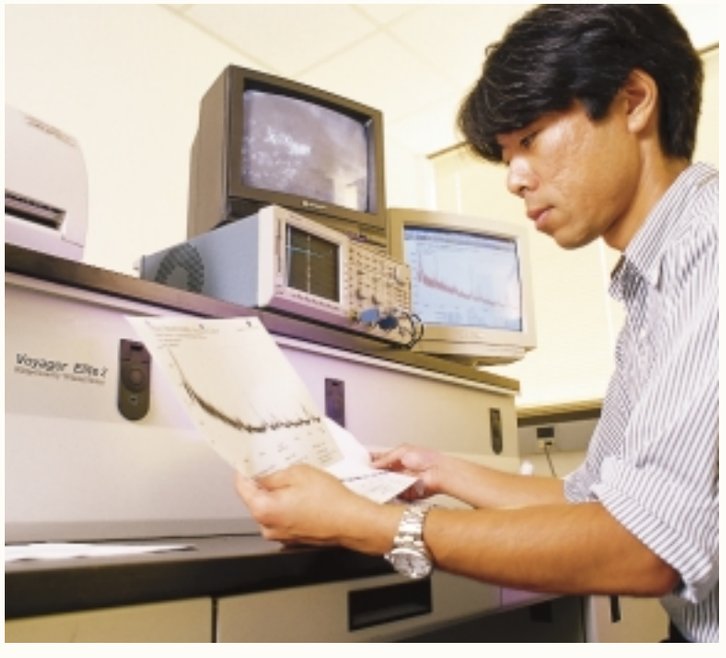


1.
John Roberts, M.D., left, chief of general thoracic surgery, operates on Art Haag with the help of surgery resident John Stewart, M.D.

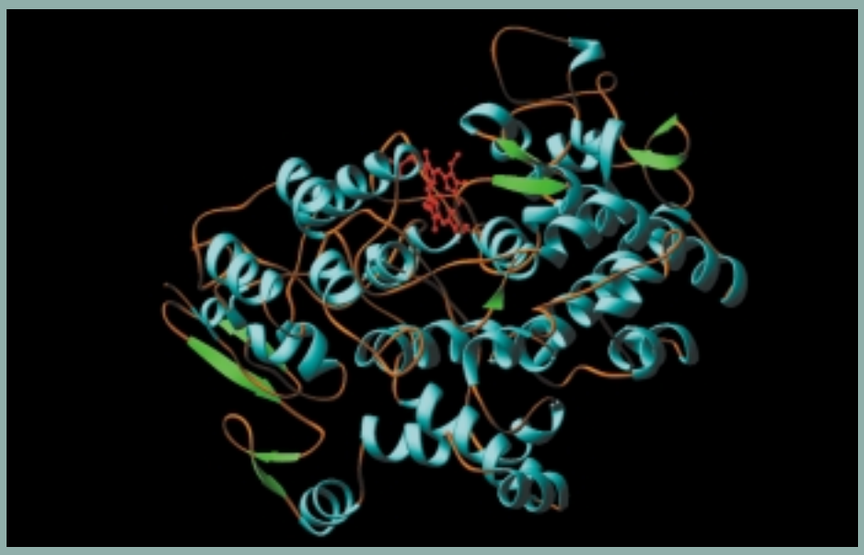
2.
The tumor, removed from Haag's left lung, is about an inch long.

3.
Adriana Gonzalez, M.D., examines a lung tumor in the surgical pathology lab.

4.
Slices of a tumor are prepared for a mass spectrometry study.

5.
Kiyoshi Yanagisawa, M.D., Ph.D., reads the mass spectrum of proteins in a tumor.

THE POWER OF PROTEINS – A BRIEF HISTORY



This ribbon diagram shows the three-dimensional structure of the cyclooxygenase or COX-2 enzyme, a protein that plays an important role in inflammation, pain and tumor growth.

Courtesy Larry Marnett, Ph.D.

The term "protein" goes back to 1838, when Swedish chemist Jöns Berzelius coined it from the Greek proteios (primary) to emphasize the importance of this group of molecules as the primary building blocks of life.

By the turn of the 20th Century, most of the 20 common amino acids that form the protein "backbone" had been discovered. Scientists also had identified certain proteins, called enzymes, that could catalyze chemical reactions, and others, called antibodies, that could stimulate the body's immune response to foreign "antigens."

A groundbreaking discovery in 1922 demonstrated the unique power of proteins. That year, Canadian researchers used a purified extract of insulin, which they had isolated from the pancreas, to save the life of a 14-year-old diabetic boy. Within a year, the manufactured protein became available worldwide. For the first time in history, there was an effective treatment for diabetes.

In the early 1970s, a succession of key developments in genetics and immunology opened the door to protein therapeutics. The ability to transplant genes between different species led to the development and marketing of the first genetically engineered drug, human insulin, in 1982, followed by human growth hormone in 1985, and the first genetically engineered vaccine, to fight hepatitis B, in 1986.

Nine years earlier, in 1975, British researchers Georges Köhler and César Milstein figured out a way to fuse antibody-producing cells from immunized mice with antibody-secreting mouse cells derived from a type of cancer called myeloma. The result was a "hybridoma," a line of hybrid cells that could be grown indefinitely and, when injected into mice, could produce large amounts of "monoclonal" antibodies, mass-produced to recognize a specific molecular target.

Genetic engineering techniques were used to "humanize" the mouse antibodies so they were less likely to be rejected by the body's immune system. Since 1986, the U.S. Food and Drug Administration has approved 11 monoclonal antibodies, primarily to prevent rejection of transplanted organs and combat cancer. Herceptin, approved in 1998, is a monoclonal antibody used in the treatment of breast cancer.

Technological advances and the urgency of the AIDS epidemic led to a new field in the early 1990s – the design of drugs based on the three-dimensional structure of target proteins. The first drugs to come out of this drug-design pipeline were the protease inhibitors, which block an enzyme used by the AIDS virus to make infectious copies of itself.

In combination with other drugs, protease inhibitors can reduce the AIDS virus to undetectable levels in the blood, and they have substantially increased survival rates.

The identification, about a decade ago, of the two cyclooxygenase (COX) enzymes is another example of the power of proteins. The enzymes produce prostaglandins, fatty-acid molecules that exert a wide range of effects, from wound healing to inflammation to blood clot formation to promoting cancer growth.

Prostaglandin production by one of the enzymes, called COX-1, protects the stomach lining, whereas activation in other tissues of a related enzyme, COX-2, can lead to inflammation, pain and tumor growth.

This finding led to the development and the marketing of the blockbuster arthritis drugs Celebrex and Vioxx, which specifically inhibit the COX-2 enzyme without affecting the activity of COX-1. Their ability to discriminate between the two COX enzymes means they can relieve pain and inflammation without causing stomach upset and ulcers, a problem with other non-steroidal, anti-inflammatory drugs that block both enzymes. Celebrex and Vioxx also are being tested at Vanderbilt and elsewhere for their potential to prevent colorectal cancer.

This is only the beginning, says Larry Marnett, Mary Geddes Stahlman Professor of Cancer Research at Vanderbilt. Marnett and his colleagues have been studying the three-dimensional structure of the COX enzymes with an eye to developing new and more effective drugs to inhibit them. "The building 'tsunami' of information about the structure and function of proteins is going to have a major impact on drug design," he predicts.

LARRY MARNETT, Ph.D.
Director, Vanderbilt Institute of Chemical Biology

Proteins are constantly in motion. Some proteins come on the scene just long enough to do their jobs, and then – in the blink of an eye – they're gone.

will be as straightforward as the decade-long effort it took to read our genetic script.

While human beings apparently have fewer genes than it takes to make a rice plant, there are hundreds of thousands of different human proteins, perhaps more than a million.

The diversity and complexity of proteins is absolutely mind-boggling.

Insulin, which carries sugar into the cells for fuel, is made up of two polypeptide chains, one with 30 amino acids and the other with 21. Hemoglobin, the oxygen-carrying protein in red blood cells, is a complex three-dimensional molecule with four chains, each more than 140 amino acids long. The epidermal growth factor receptor, a target for some of the new cancer drugs, is a single-chain protein with nearly 1,200 amino acids (see "One Protein's Story," page 22).

Proteins also have many different jobs. They form the elastic and resilient framework of muscles, nerves and other body tissues. They carry signals within and between cells, and – in the case of antibodies – sound the alarm when a germ invades. Many of them are enzymes, catalyzing chemical reactions. Still others help "read" the genetic code so it can be "translated" into more proteins.

Scientists used to think that for every gene there was only one protein. They now know that while genes determine the sequence of amino acids that make up the protein backbone, the genetic instructions can be translated and implemented in more than one way.

Once produced, proteins can be modified – by the addition of a phosphate or sugar molecule, for example – in ways that change their shape and function. And while our double-helical string of genes is relatively static and unchanging, proteins are constantly in motion. Some proteins come on the scene just long enough to do their jobs, and then – in a blink of an eye – they're gone.

Different parts of the body have different populations of proteins. They change, too, in response to environmental influences, like the digestion of a piece of cherry pie – or even the time of day. "Your proteins won't be the same tonight as they are now," says Richard Caprioli, director of the Mass Spectrometry Research Center at Vanderbilt.

Proteins aren't lone wolves, either. They act and react as part of intricate networks and pathways that transmit signals to and from the DNA, across the cell membrane and through the blood to distant parts of the body. Thus, proteins have been called "molecular machines," at work in cellular "factories."

Just as proteins carry out many of the functions necessary for life, they also are at the root of many diseases. A form of diabetes, for example, results from an inadequate supply of insulin.

A change in a single amino acid in the oxygen-transporting hemoglobin molecule alters the protein's three-dimensional shape, and results in sickle-cell anemia. Red blood cells with the abnormal protein are misshapen (sickle-shaped), break apart and block small blood vessels, causing pain and low blood count (anemia).

Too much protein also can be a problem. Elevated levels of the receptor for epidermal growth factor, for example, has been linked to a variety of cancers.

Molecular fingerprints

This connection between proteins and cancer, in particular, is driving a new growth industry in proteomics. Much of the effort is aimed at developing new drugs targeting specific proteins. But the search for new diagnostics is equally intense. That's because some diseases, including some forms of cancer, typically escape notice until they are in an advanced, hard-to-treat stage.

Currently cancer is diagnosed through a variety of techniques, both non-invasive (ultrasound, X-rays, CAT scans, etc.) and invasive (primarily surgery). There are a few blood tests for cancer, which detect cancer-related antigens (proteins that bind with antibodies), but these are far from definitive.

For example, prostate cancer is associated with increased levels of prostate-specific antigen (PSA), but PSA levels also rise in response to exercise, infection and certain medications. Levels of cancer antigen 125, a screening tool for ovarian cancer, are abnormally high only about half the time in early disease.

Challenges ahead

There are limits to the new science of proteins.

For example, researchers often don't speak the same scientific "language," making it difficult for them to share information or collaborate. To solve that problem, the international Human Proteome Organization is developing standards for the reporting of experimental data.

Pharmaceutical companies also may have to shift their focus from the search for the next blockbuster drug to the development of combinations of drugs, tailored for different segments of the patient population.

That's easier said than done. Currently it can take 15 years and more than $800 million to bring a drug to market – in part because of rigorous testing requirements and the high number of potential drugs that never make it through the testing process.

If drug companies could tell in advance which patients – because of their genetic make-up – are most likely to respond to a new medication and are unlikely to experience serious side effects, "you could reduce the number of people you have to study … and you can greatly reduce your cost," says Alastair Wood, professor of Medicine and Pharmacology at Vanderbilt and an expert on pharmacogenomics. But, he adds, "no one's worked out how to do that yet."

Gene- and protein-based technologies may speed drug development, thereby lowering the cost. But intellectual property issues and a regulatory structure designed to approve one drug at a time could stifle efforts to develop "cocktails" of different drugs – made by different companies – that attack a disease at several points simultaneously, experts say.

"One of the things that's really inhibiting progress is that the FDA (U.S. Food and Drug Administration) has never dealt with approval of multi-use agents for an indication like this," says Raymond DuBois, professor of Medicine and Cancer Biology at Vanderbilt who helped discover the role of the COX-2 enzyme in colon cancer.

"I think it's going to be hard to get an individual pharmaceutical company to fund these combination trials using drugs from different companies together," DuBois says. "One possibility would be for the National Cancer Institute to step up to the plate and put some trials together to look at this."

Five years ago, researchers at the U.S. Food and Drug Administration and National Cancer Institute joined forces in an attempt to improve early detection of cancer, determine in advance which treatments are likely to be most successful in individual patients and, ultimately, to develop new "targeted" therapies that effectively stop cancer growth without harming normal tissue.

"Certainly cancer is underpinned by genomic disorders but functionally it's a proteomic disease," says Emanuel Petricoin, the project's lead FDA researcher. "Effectively it's the rewiring and miswiring of the protein circuits, the signal pathways, that cause cells to grow and not die."

Lance Liotta and his colleagues at the National Cancer Institute already had developed a microscope technique for teasing apart normal cells from their cancerous neighbors with the help of a laser. The cancer cells were then split open, and their contents poured onto a "protein chip," a glass slide lined with "bait" molecules to which the cellular proteins stuck.

Extraneous material was washed away, and the slides were put into a mass spectrometer. As a first step, the slide was zapped with a laser, giving the proteins an electrical charge and spinning them off into a vacuum chamber toward an oppositely charged electrode. Their molecular weight was determined by the time it took them to get there.

The mass spectrometer then spit out a spectrum showing the molecular weights of all of the proteins and protein fragments. Dozens of experiments revealed that different cancer cells had unique spectra, or patterns, of proteins and thus potentially could be identified by their "molecular fingerprints."

Using these techniques, the researchers are analyzing biopsies of cancerous tissue removed from patients before and after treatment to see if they can predict, from the protein patterns, which patients are likely to respond to specific drugs. The hope is that doctors one day will be able to "tailor" treatments to individual patients, while avoiding – in advance – therapies that won't work.

The researchers also began testing the serum, the clear liquid part of the blood from cancer patients, to see if they could detect the characteristic protein patterns without actually removing any cancerous tissue.

Last February, they reported their first success. With the help of pattern-recognition computer software developed by scientists at Correlogic Systems in Bethesda, Md., the government researchers found unique patterns of five proteins in serum that could detect ovarian cancer with near-100 percent accuracy.

"Ovarian cancer is ripe for using this technique because it is usually diagnosed too late, when it's almost untreatable, whereas if you catch it early enough, surgery alone can cure this disease most of the time," Petricoin says.

Using a similar technique, researchers led by Bao-Ling Adam and George Wright at Eastern Virginia Medical School in Norfolk have identified a pattern of nine serum proteins that accurately distinguish between prostate cancer, benign prostate hyperplasia (non-cancerous enlargement of the prostate gland), and healthy tissue.

The technique was considerably more specific than the PSA test, which has a high rate of false positive results, the researchers reported last summer. If validated by further study, "this approach would have immediate and substantial benefit in reducing the number of unnecessary biopsies," they concluded.

While there is growing evidence that tumor cells can be distinguished from normal cells by their protein fingerprints, "at this point that's probably more of an assumption than absolutely proven," cautions Roy Jensen, associate professor of Pathology and Cancer Biology at Vanderbilt.

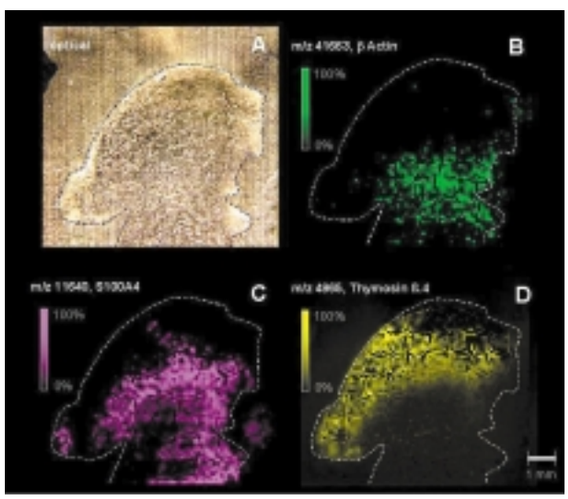
Jensen is working with Richard Caprioli and his colleagues, who have developed another mass spectrometry technique, called "imaging mass spectrometry," that can actually "look" inside the tumor itself.

Using a laser and high-speed electronics and computers, they modified standard spectrometers so they could generate digital pictures, showing the distribution of individual proteins in cells and tissues. In April 2001 the researchers reported finding high levels of thymosin beta-4, a protein that may be a harbinger of malignant growth, in the outermost, proliferating edge of a human brain tumor that had been implanted in a mouse.

These "molecular photographs" may help improve the diagnosis and treatment of cancer. By pinpointing the precise location in the tissue where high levels of a protein are spurring cancerous growth, the technique could improve the accuracy of cancer surgery.

Repeat studies also can help determine how quickly tumors are growing, and whether they are responding to drug treatment, Caprioli says. This is a "molecular insight that can be directly applied to patient care," he says.

Petricoin acknowledges criticism that he and his colleagues have not identified the proteins they're picking up in the



Imaging mass spectrometry of a human brain tumor that had been grown in a mouse reveals a high concentration of a number of specific proteins in the fast-growing edge of the tumor, including thymosin beta-4 (d). This suggests that these proteins may be important in tumor spread. Illustration by Richard Caprioli, Ph.D., and his colleagues and previously published in *Nature Medicine*.

"What this is going to take is all aspects of humanity, if you will, working together. It's going to take the physicists, it's going to take the mathematicians, computer scientists, biologists, chemists – you can go down the line. We as humans have to network before we can understand protein networks."

Richard Caprioli, Ph.D.
Stanley Cohen Professor of Biochemistry

DEAN DIXON

blood. That will be crucial, other scientists say, for understanding the role the proteins may play in cancer growth, and how to develop new drugs to stop them. That's true, agrees Petricoin, but patients need this technology now.

"We're failing in the war against cancer," he told a group of researchers at Vanderbilt in September. "For patients who have ovarian cancer … who have seen their mothers die of cancer, they don't care what the underlying identity (of the protein) is. We have to test this hypothesis in the clinic. (But) we could use that pattern as a diagnostic today."

Discovery science

"I don't think they (the 'omic' strategies) will provide the answers to all of life's mysteries," responds Walter Chazin, Chancellor's Professor of Cancer Research and director of the Center for Structural Biology at Vanderbilt. "Nothing significant has been achieved yet … from using proteomics. It's really in the technical development stage. The more we learn, the more complex it becomes.

"However, the one thing I do believe in is the value of these initiatives (to develop) high throughput technologies," Chazin says. That's the ability to accelerate the research process and generate huge amounts of data essentially overnight.

The new technologies, in fact, are opening up a new way to conduct research (see "Discovery Science," page 16).

The traditional approach, exemplified by Stanley Cohen's Nobel Prize-winning work at Vanderbilt four decades ago, is to begin with a biological observation. For example, Cohen noticed that an extract of mouse salivary glands causes newborn mice to open up their eyes earlier than normal.

That observation led to the discovery of epidermal growth factor (EGF) and, later, to the identification of the receptor through which it acts. Hypotheses were formulated to explain the phenomena that were observed, and experiments were designed to test them.

Meanwhile, certain cancers were found to have abnormally high levels of the EGF receptor. Technological advances enabled scientists to determine the three-dimensional structure of the protein, and to synthesize molecules that can block its activity. Cohen's body of work, beginning with an incidental observation, helped lead to a new field of cancer research and drug development.

The tried-and-true hypothesis approach hasn't been abandoned. But increasingly, scientists are first mining the genome and now the proteome for all the data they can. Rather than studying a car by kicking the tires, they're taking the entire vehicle apart – including the engine – then fiddling with each part to see what it does.

"It's like Magellan," says Andrew Link, Ingram Assistant Professor of Cancer Research at Vanderbilt who has developed a new method for studying complex mixtures of proteins. "You send your boat out. You don't know what you're going to find."

Pie in the sky

Disappointing results in recent clinical trials have tempered optimism about the ability of some of the early – and highly touted – "targeted" therapies to stop disease in its protein tracks.

In August, the pharmaceutical giant AstraZeneca reported that adding Iressa, a drug that blocks the EGF receptor, to a standard cancer drug did not significantly improve survival in a large-scale study of patients with advanced non-small cell lung cancer, the most common form of the disease.

The two drugs may have antagonized each other's effects, however. When given as a single agent, the response of tumors to Iressa "is still felt to be remarkable," says Carbone, who directs a federally funded Specialized Program of Research

DEAN DIXON

"Different patients may respond to different cocktails, so the more you know about the biology of cancer, the more likely you are to be able to develop intelligent therapies."

David Carbone, M.D., Ph.D.
Professor of Medicine, Ingram Professor of Cancer Research

Excellence in lung cancer at Vanderbilt. The drug, which has been given to more than 18,000 people worldwide, is at least two to three times better in treating recurrences of lung cancer "than anything else out there, as well as being convenient and safe to take," he adds.

Simply blocking the EGF receptor may not be enough to stop the cascade of events that leads to cancer, however. "You give 100 patients Iressa, 15 percent will respond, and there's no way for you to know right now which one," Carbone says. "My belief is that cancer is more complicated than that … It might well be 50 genes that you're going to have to look at simultaneously."

The ultimate treatment may come in the form of "cocktails" of several drugs that target different genes and protein pathways, depending on the unique characteristics of the patient's cancer.

"Different patients may respond to different cocktails, so the more you know about the biology of cancer, the more likely you are to be able to develop intelligent therapies," Carbone says.

That's why Carbone is carefully analyzing and cataloging the patterns of proteins found in the lung tumors removed from patients like Art Haag.

The patients will be followed and, over time, the researchers will see if there are unique patterns that correlate with specific outcomes, such as a recurrence of the cancer in the brain, response to chemotherapy, or poor survival. Eventually, they hope to be able to predict the course of a patient's disease shortly after they remove his or her tumor, and plan treatment accordingly.

"If we know this tumor will have a propensity to go to the brain, we can give prophylactic cranial irradiation," Carbone says. "If you knew this tumor was going to respond to chemotherapy, then you might consider giving adjuvant chemotherapy after surgery."

Pierre Massion, assistant professor of Medicine at Vanderbilt, is working with Carbone on a related study – testing tissues removed from the lungs of smokers who do not have cancer, to see if they can predict which smokers are likely to develop cancer in the future.

"It's like Magellan. You send your boat out. You don't know what you're going to find." - Andrew Link, Ph.D.

Jensen says he hopes to do the same thing with breast cancer.

"The ideal thing would be that these early (breast) lesions, before they develop invasive cancer, would secrete a protein into the blood that we could detect with a simple blood test," he says. "Further down the road, if we truly understand these molecular pathways that are giving rise to these lesions, we could design specific drugs that would target these proteins and the woman wouldn't even have to undergo a surgical procedure."

In lung cancer, too, "the whole paradigm has shifted," Carbone adds. "Ten years ago, we had a couple of drugs, they weren't very effective and most people weren't receiving them. Now we have a huge number of . . . interesting, molecularly targeted therapeutics, and some of them are showing real clinical responses.

"I think a new era in lung cancer has started."

"I didn't realize they'd gotten that far," agrees Haag, whose Vanderbilt surgeon, John Roberts, successfully removed all detectable traces of the cancer. But he also gives himself credit for going in for regular checkups after his previous bout with colon cancer. "The six month to a year check-up is worth a million dollars," he says. **LENS**

ANNE RAYNER POLLO

Laser beams are used to eject proteins from the thin slices of tumor for further study, as suggested by this photo-illustration of a mass spectrometry study. The goal is better understanding of how cancer develops and, ultimately, better ways to diagnose, treat and prevent the disease.

DOES PROTEOMICS NEED A "BIG GOVERNMENT" APPROACH?

The field of proteomics is burgeoning, thanks in large part to a generous flow of research funding – both public and private. But some observers wonder whether faster progress could be achieved with a concerted government effort – a "big science" program like the Human Genome Project (see "The Future of Proteomics," page 26).

A big-government approach is warranted, some argue, because of the current limits of technology and knowledge. For example, computer-enhanced techniques including high-performance liquid chromatography and mass spectrometry are allowing researchers to reach conclusions even with the tiniest amounts of protein (see "Mining for Proteins," page 14). But there is still no protein equivalent of the polymerase chain reaction, developed in the early 1980s, which allows almost limitless mass-production of genetic material for study.

Another reason for attempting a "Man on the Moon" approach to proteomics is the urgency of medical problems that are waiting to be addressed.

"Our ability to improve on human biology I would argue is one of the most important possible goals of the 21st Century," says Daniel Perry, executive director of the non-profit Alliance for Aging Research, which advocates for more research dollars on diseases that affect older people.

In the next 30 years, "a wave of chronic disease and disability," including a quadrupling of the number of people with Alzheimer's disease, will sweep over the country as the population ages, predicts Perry, a former member of the federal Task Force on Aging Research and former advisor to the White House Conference on Aging.

"If we can fine-tune the human biology at the level of genes and proteins and growth factors, and use those insights to extend healthy years of life and reduce to a bare minimum the years and months or weeks spent in a dependent state at the far end of life, that would be an enormous social accomplishment," he says. "I think it's a goal and a vision that could very well be served by national leadership in the public sector."

A big government approach isn't universally embraced. Some scientists worry that serendipity – the chance discovery that dramatically shifts thinking about a biological problem – would be squelched by a bureaucracy which determines the research agenda in advance.

Other researchers believe that the diversity and complexity of the protein world simply doesn't lend itself to a "Human Proteome Project."

"There is no human proteome as far as I'm concerned," says Emanuel Petricoin, co-director of a clinical proteomics program operated jointly by the U.S. Food and Drug Administration and the National Cancer Institute.

"The proteome is constantly changing and fluctuating in the context of the person, what that person is exposed to, the time of the day, the underlying disease process, so I don't think we're going to decipher a human proteome set," Petricoin says.

"I think what we're going to try to understand is the proteins that are changing as a consequence of the disease. We're going to use those proteins as therapeutics, biomarkers and tools to basically drive the clinical decision. That's where the money is in the pharmaceutical industry."

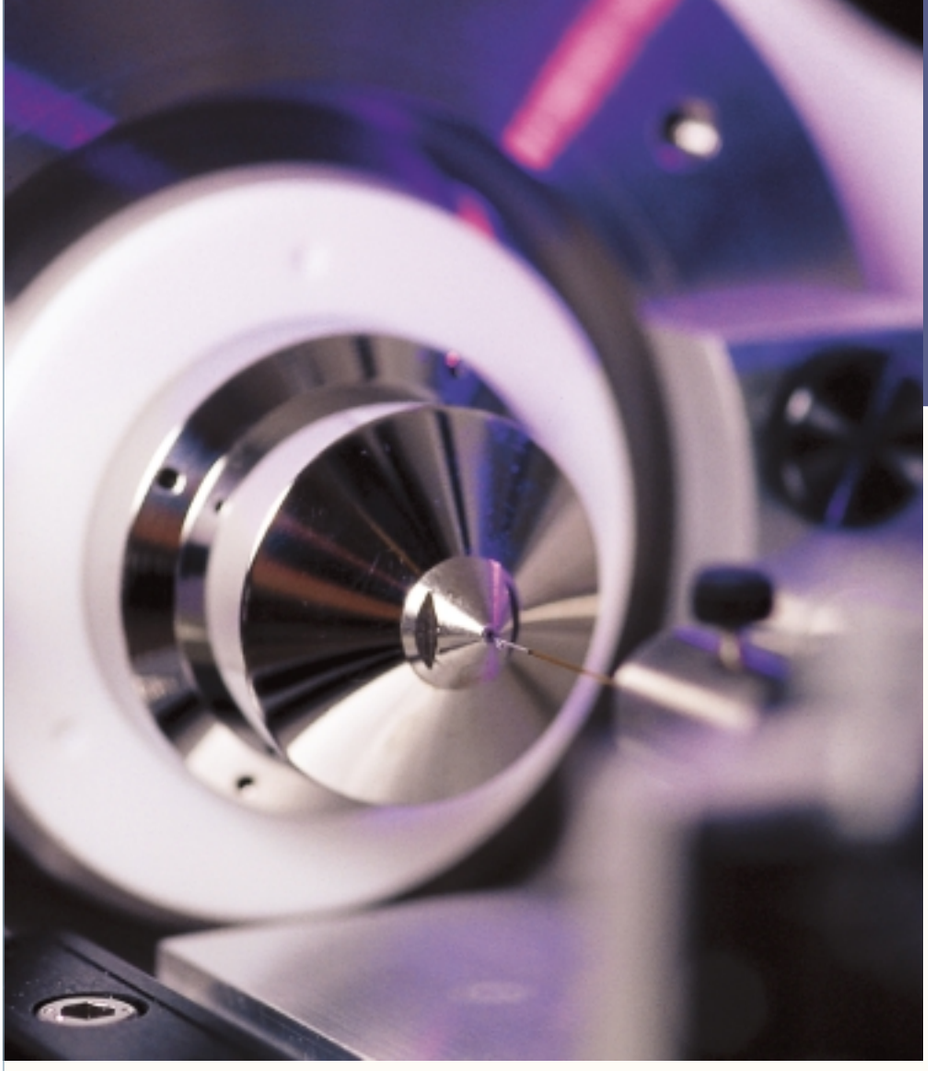
While proteomics may lead to significant improvements in the diagnosis and treatment of disease, those applications will be expensive, at least initially, and health care consumers will be required to pay an increasing proportion of the cost, predicts ethicist Daniel Callahan, director of international programs for the Hastings Center in Garrison, N.Y.

The rich will be able to afford the higher price tag, but the poor will not. "A two-tier health care system is the inevitable result, and one where the gap between the tiers gradually increases," Callahan writes in the June 17, 2002 issue of the *Journal of Molecular Biology*.

Callahan urges a different approach, one that places more emphasis on the socioeconomic determinants of health and disease prevention. "The principal test for biomedical progress would be its impact on population health rather than individual health," he writes. "It would not aspire to conquer each and every disease, but only those that shortened life or harmed its quality to some significant degree."

After all, he argues, "the main determinants of population health are not research progress and improved health care (except perhaps for the elderly). They are instead the socioeconomic conditions under which people live."

In the United States, fully 50 percent of all deaths can be ultimately traced to behavioral causes, such as smoking, obesity and lack of exercise. "The best predictor of a healthy life," Callahan concludes, "is education, followed closely by economic security."



ANNE RAYNER POLLO

Mining

Proteomics is a technology-driven field. The photo at left, for example, shows proteins separated by high-performance liquid chromatography being sprayed into a tandem mass spectrometer, which will further separate them according to their "mass-to-charge" ratios. Here is a sampler of other important technologies:

Two-dimensional gel electrophoresis

This technique separates proteins across a gel, first according to their charge (isoelectric point) and then by their size (molecular weight). After separation the gels are stained, revealing dark spots where the proteins have concentrated. Spots of interest can be extracted from the gel and analyzed to identify the proteins.

In recent years several advances have improved the accuracy and sensitivity of 2-D gels. More reliable analytical methods, including robotic machines, have replaced hand-processed gels. By tagging proteins from two separate tissue samples with different fluorescent markers, researchers now can compare them on the same gel.

"This allows us to quantify the increase or decrease of a given protein," in different stages of cancer, for example, says David Friedman, director of the proteomics laboratory in the Vanderbilt Mass Spectrometry Research Center. "It is very powerful at imaging thousands of proteins at a given time, yet it is biased toward more abundant proteins in the cell," Friedman says.

Mass spectrometry

Mass spectrometry is becoming an increasingly valuable and versatile technique for identifying proteins, studying interactions between proteins, and mapping the location of proteins within tissue.

Ordinarily, proteins must be "cleaved," or broken into manageable pieces called peptides, before they can be "weighed" in the mass spectrometer. The peptides are "ionized," or given an electrical charge, so they can be propelled by an electric or magnetic field through a vacuum toward the instrument's detector.

From the motion of the peptides, the spectrometer calculates their "mass-to-charge" ratio, which is related to their molecular weight, and generates a spectrum of the relative amounts (or intensity) of all the peptides that make up the original protein. The resulting peak-and-valley pattern of the mass spectrum is the protein's unique "fingerprint."

Techniques developed in the 1980s have greatly improved the ability of mass spectrometry to analyze large, intact proteins and complex protein mixtures.

Electrospray ionization, developed by John Fenn and his colleagues at Yale University, is a method of squirting a protein solution through an intense electrical field in order to create a fine spray of charged droplets. The liquid evaporates, sending relatively large proteins into the mass spectrometer for analysis.

Another method, called matrix-assisted laser desorption/ionization or "MALDI," was developed independently by researchers in Germany and Japan. Protein or tissue samples are mixed with a matrix, a material that can absorb laser energy, and applied to a target probe or plate. When struck by a laser, the matrix is partially vaporized, and electrically charged proteins are jettisoned into the mass spectrometer.

"It's like hitting a golf ball out of a sand trap," explains Vanderbilt mass spectrometry expert Richard Caprioli. "You hit the sand around it."

For their contributions to mass spectrometry, Fenn and Japanese researcher Koichi Tanaka were awarded the Nobel Prize in Chemistry last fall. They shared the award with Kurt Wüthrich (see "Determining Protein Structure," below).

Detecting protein interactions

In the late 1980s at the State University of New York at Stony Brook, Stanley Fields and Ok-kyu Song pioneered a technique for studying protein interactions called the "yeast two-hybrid system." They broke apart a yeast protein that binds to and turns on a gene essential for cell growth. One half was attached to a known protein, or "bait," and the other half was attached to the "prey," the protein being studied. If the bait and prey interact, the two halves of the original protein will be brought back together, the gene will be turned on, and yeast cells will grow.

for proteins

Techniques for isolating and identifying proteins

The technique does not pick up all of the interactions that occur in nature, but it has helped researchers construct "maps" of protein-protein interactions in organisms like the *H. pylori* bacterium, the major cause of ulcers. This information may lead to new ways to treat the infection.

Protein microarrays or "chips" provide another way to study interactions. The chips contain a variety of molecules that can bind specific proteins. Bound proteins, in turn, are detected through fluorescence labeling and other tagging techniques. This information is helping scientists understand how the cell operates at the molecular level, and how tiny changes in protein function can lead to disease.

Determining protein structure

X-ray crystallography uses X-rays to determine the three-dimensional structure of the crystallized form of proteins and other important biological molecules. The technique has led to the development of drugs that specifically bind to and inhibit disease-related enzymes, including one essential for production of the human immunodeficiency virus, which causes AIDS. It can take days, weeks or even years to crystallize proteins in order to study them. "But the payoff for all of this effort is well worth it," says Walter Chazin, director of Vanderbilt's Center for Structural Biology. "X-ray crystallography, as the core technique of structural biology and structural genomics, is providing a paradigm shift in the ability of scientists to understand, control and design new biomedical activities."

DANA JOHNSON

Another way to determine protein structure is to analyze the way the nuclei of the atoms that make up the molecule "resonate" under the influence of radio waves in ultra-high magnetic fields, a technique called nuclear magnetic resonance spectroscopy.

In the 1980s, Swiss biophysics professor Kurt Wüthrich worked out a method for interpreting the NMR spectra of proteins in solution. Since then, he and his colleagues have determined the NMR structure of more than 50 nucleic acids and proteins, including prion proteins, which are thought to cause mad cow disease.

Bioinformatics

Bioinformatics is the collection, organization and analysis of large amounts of biological information using networks of computers and databases. Vast, on-line "libraries" of confirmed protein sequences and the mass spectra "fingerprints" for thousands of proteins, predicted from the genes that encode them, have greatly improved the ability of researchers to identify proteins.

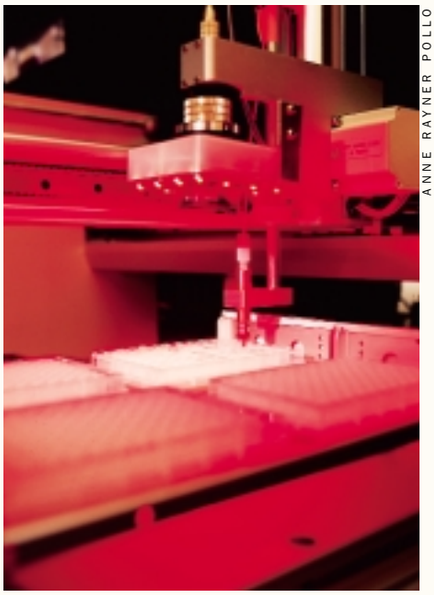
For example, John Yates, Jimmy Eng and their colleagues at the University of Washington in Seattle developed a computer program, called SEQUEST, that helps researchers search databases rapidly for a possible "match" to the protein they've discovered.

Andrew Link, a former post-doctoral fellow in Yates' lab who now is Ingram Assistant Professor of Cancer Research at Vanderbilt, uses SEQUEST in a technique he developed, called "direct analysis of large protein complexes" (DALPC).

"X-ray crystallography, as the core technique of structural biology and structural genomics, is providing a paradigm shift in the ability of scientists to understand, control and design new biomedical activities."

Walter Chazin, Ph.D.
Director of Vanderbilt's Center for Structural Biology

ANNE RAYNER POLLO



A robotic machine punches protein-containing "gel plugs" out of a two-dimensional gel, based on coordinates determined by laser imaging. The gel plugs will be processed so that the proteins within them can be studied further. This and other technologies have greatly increased the speed and capabilities of proteomic research.

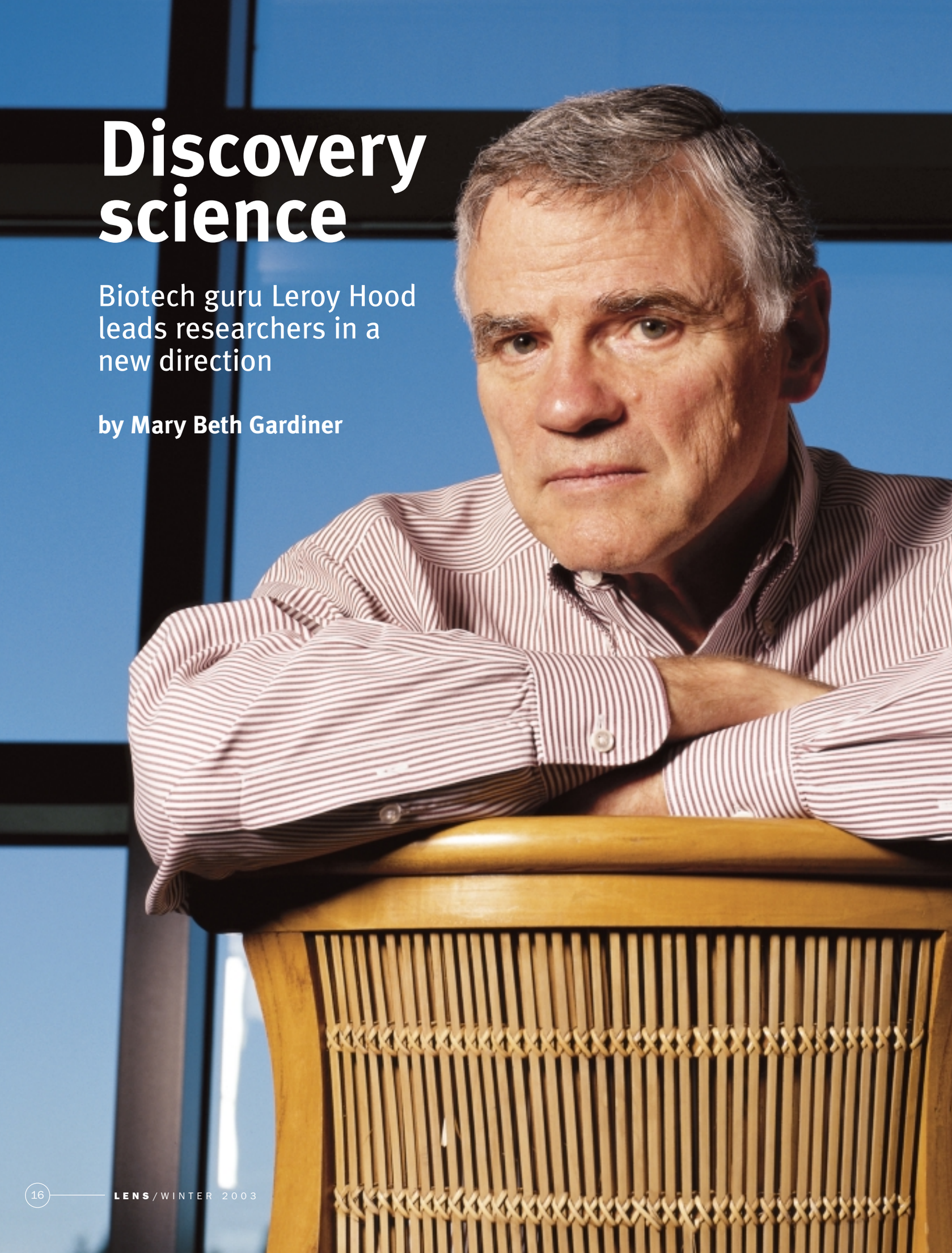
Proteins are fragmented and the resulting peptides are separated by multi-dimensional liquid chromatography. This is a method for separating tiny amounts of peptides according to a variety of physical attributes, such as size or charge, as they travel through flexible plastic columns no bigger than the size of a human hair.

The peptides are analyzed by mass spectrometry, then fragmented to even smaller pieces and analyzed again to get information about their amino-acid sequences (a technique called "tandem" mass spectrometry). SEQUEST is then used to help identify them, thereby avoiding the need to purify each protein individually. DALPC "gives us the ability to look at complexes of hundreds of proteins at a time, and figure out what they are in a single experiment," Link says. **LENS**

Discovery science

Biotech guru Leroy Hood leads researchers in a new direction

by Mary Beth Gardiner



BRIAN SMALE

For his honeymoon, Leroy Hood planned to take his new wife on a 110-mile trek across five mountains. Eager to please, Valerie Logan strapped on her new backpack, a wedding gift from a friend, and fell in step beside him. By the end of the second day, they hadn't reached the lake where they intended to camp before night began to fall. With only craggy ground as far as the eye could see, Hood made his decision: they would turn off-trail and head uphill, through the woods, to get to the campsite faster. They bushwhacked up the side of the mountain, struggling over rocks and past grasping branches as the darkness deepened.

Considering the events of the lifetime that has unfolded since that honeymoon trip, the decision made by the 24-year-old newlywed on the trail that summer night is vintage Lee Hood. The tenacity of this physician-scientist, called by some the father of biotechnology, is evident in a career marked by ground-breaking innovations in biological instrumentation and analysis, and by his bellwether efforts to shift responsibility for scientific discovery from individuals to cross-disciplinary, interactive groups.

Along the way he has directed a number of large and productive research labs; helped to found 11 companies, Applied Biosystems and Amgen being two of his earlier efforts; and patented a dozen inventions. Hood has co-authored more than 500 scientific articles and textbooks, and won numerous honors – among them the Lasker Award and Kyoto Prize, known, respectively, as the American and Japanese equivalents of the Nobel Prize.

His motivations often misunderstood, Hood's maverick tendencies have won him both praise and criticism. His detractors see him as solely a technologist, one who has too closely aligned himself with the business world. His colleagues know him as a visionary, but also a realist – a practical man with a practical purpose.

Hood says the inspiration behind his fervor is simple. "I'm interested in making things better for the world."

The hum of Hood's ideas and values has reached a crescendo in the Institute for Systems Biology. This innovative research center, established three years ago in Seattle, Wash., is dedicated to the integration of biology, computation, and technology as a means of furthering the fundamental revolution that is taking place in medicine, the move toward predictive and preventive medicine.

Within 10 to 15 years, Hood forecasts, physicians will be able to predict a person's predisposition for disease based on his or her genetic makeup. And not long after that, they will understand the biological networks within which the defective genes reside, and be able to block the harmful effects.

"The idea is that all the elements of a biological system can be defined and put into a database," he says. "Sequencing the human genome and placing the sequence in a database is pure discovery science …, which raises the possibility of globally analyzing the behavior of all human genes in, for instance, normal cells versus cancer cells."

Discovery science, Hood says, stands in contrast to hypothesis-driven science – the tried and true approach that has prevailed for years, where a hypothesis is formulated and experiments carried out to test that hypothesis.

Though his proposed interdisciplinary, systems approach to biological discovery is revolutionary, traditional hypothesis-driven methods have not been abandoned. Rather, they are embedded in the new approach, which advocates figuring out what happens normally within a system – a signaling pathway in a cell, for example – then disrupting that system repeatedly, making note of what changes occur. If you know how the system can break down, Hood says, you should be able to figure out how to fix it.

Supported by both private and public funding, the non-profit institute currently has a staff of 180 employees – about half the ultimate goal – and is engaged in 10 industrial and three academic partnerships. The nine faculty members run the gamut from astrophysicist to immunologist to mathematician to protein chemist to computer scientist, all learning how to speak each other's language and apply their tools to understanding biology's complexity.

"At first blush, they all think biology's easy, and that they all have the ways to help us out," says Hood. "But in most instances, they are useful only in direct proportion to how well they understand biology.

"Biology is infinitely complicated, so what you have to do is use hypotheses to shed light on global studies of very selected aspects of complexity. Then you can start to sort out what it's about."

- Leroy Hood, M.D., Ph.D.

BRIAN SMALE

"These people can develop techniques and computational tools, but the development has to be driven by biological frontiers. There are all sorts of fancy tools that let you measure things, but who cares if the things aren't relevant? You have to figure out how to measure important things rather than measure anything."

Hood's unique take on how technology supports biological discovery is not always well appreciated, especially in academic circles, says George Lake, a founding faculty member at the institute and astrophysicist who is applying his expertise to the study of biological networks and the role of retroviruses in evolution.

"If you're a technologist at a university, that's a tough position to be in," Lake says. "There are very few people with the deep scientific insight that (Hood) has got who so embrace technology."

Nature as teacher

Hood developed a deep connection to the natural world in his youth. Born in 1938 in Missoula, Montana, a town split by a rapidly flowing river and lying at the convergence of two forested mountain ranges, he began roaming the woods before the age of six, and camping by himself shortly thereafter.

Summers spent at his grandfather's ranch in the Beartooth Mountains of southwestern Montana shaped his character and his destiny. Hood was the son his grandfather had never had, and from the man, Hood says, he learned "the power of love, commitment and friendship." On the ranch he learned to ride horses, tend the animals, and climb the rugged mountains, further bolstering his confidence and independence.

Because his high school was small, Hood was able to explore a ragbag of activities, from music to acting to debate to sports. Football's camaraderie and rough-and-tumble held particular appeal for him.

Another highlight was the chance meeting of Valerie Logan, the woman who would one day become his wife. They lived in neighboring towns, and met at a state speech, debate, and drama meet in the spring of their junior year. They would date, off and on, in a cross-country courtship that lasted seven years.

Hood excelled in mathematics and science. During his junior and senior years, he took courses in his grandfather's geology camp, a summer program attended by students and faculty members from a number of Ivy League universities, and was a field assistant for several geology mapping projects in the nearby mountains. One of these projects was the basis of his Westinghouse Science Talent award, the first ever from Montana.

By his senior year, his science teacher, Cliff Olson, asked him to take over the biology classes, which he did, gleaning his lesson plans from issues of *Scientific American.*

"I enjoyed getting the sophomores excited about biology," Hood says, "and more importantly, I learned enough to begin to see the enormous potential of biology in the future. I remember being fascinated by one article on the structure

of DNA, discovered just three years earlier in 1953. I came away convinced I wanted to go into biology."

Hood had been leaning heavily toward a liberal arts college in Minnesota. But Olson convinced him to attend his alma mater, the California Institute of Technology (Caltech), because of its elite faculty and students and its outstanding research reputation. After graduation in 1960, Hood went on to medical school at Johns Hopkins University in Baltimore, Md., to get more background in human biology.

"I found it staggering how little we really understood about human biology," he says. "I remember asking a pediatric intern, a resident, and then a visiting professor, in that order, what caused diarrhea. Each could list organisms and diseases that did so, but could not speak to the pathophysiological mechanisms. It was a descriptive view of biology that was quite different from what I was used to."

Having developed a keen interest in immunology, Hood returned to Caltech to work with biology professor William Dreyer on theories of antibody diversity. Their work advanced the idea – radical at the time – that antibody chains were encoded by two distinct genes, which became physically rearranged during maturation of antibody-producing immune cells. The theory helped explain the tremendous adaptability of the body's immune system to a wide range of infectious assaults.

"By my second year of graduate school, I was giving lectures at universities and national meetings," Hood says. "The general reaction of the scientific community to the two gene/one polypeptide hypothesis was skepticism and even reprobation. I realized for the first time how threatening new ideas are to many scientists."

Dreyer's mentorship was pivotal, inspiring conceptual thinking and creativity in his protégé, and providing the two pieces of advice that have colored all of Hood's subsequent scientific efforts: One, always practice biology at the leading edge, and two, if you really want to change the field, invent new technologies to push the frontiers of biological knowledge.

Hood and Logan married while he was in graduate school. After earning a Ph.D. in biochemistry, Hood served a three-year stint in the Public Health Service, pursuing his research on antibody diversity at the Immunology Branch of the National Cancer Institute. But by far his most memorable experiences during those years, he says, were the births of his son, Eran, and his daughter, Marqui.

A new view of technology

In 1970, Hood returned to Caltech again, this time as a member of the faculty, his mentor's imperatives ringing as loudly in his ears as ever. "I went to Caltech with a commitment to split my time between biology and technology," he says. "The idea was that biology should drive the choice of the technology developed, and that the technology should lift barriers to the deciphering of important biological information."

"The general reaction of the scientific community to the two gene/one polypeptide hypothesis was skepticism and even reprobation. I realized for the first time how threatening new ideas are to many scientists."

- Leroy Hood, M.D., Ph.D.

Over the next 20 years, Hood's lab would develop four instruments – a protein sequencer and synthesizer, as well as a DNA sequencer and synthesizer – that allowed scientists to move readily from a protein sequence to its gene sequence and vice versa, and allowed for the synthesis of genes and fragments of proteins.

This suite of four instruments formed the technological foundation of modern molecular biology, opening the door for many new and powerful strategies, including the polymerase chain reaction (PCR). The lab's work on the automation of DNA sequencing contributed directly to the planning and realization of the Human Genome Project.

The discoveries that were enabled by the protein sequencer in the early 1980s led to a number of key disease-related breakthroughs, including the treatment of chronic anemia with erythropoietin and the implication of prion proteins in mad cow disease and its human variant, Creutzfeldt-Jakob disease.

Hood's own research was expedited by the technologies developed in his lab, which allowed him to move from an analysis of antibody proteins to an analysis of antibody genes. The combined efforts of Hood's lab and the laboratories of Susumu Tonegawa and Philip Leder established that the two gene/one protein theory was correct, that there were many antibody genes in our DNA, and that the genes were capable of undergoing adaptive mutation to further increase antibody diversity.

In recognition of these efforts, the three scientists were awarded the prestigious Albert Lasker Award for Basic Medical Research in 1987. That year Tonegawa was the sole recipient of the Nobel Prize. Hood admits he was disappointed not to be selected to share the award. "On the other hand, you do science for the fun of doing science, you don't do it to win prizes," he says.

Meanwhile, Hood was getting his first taste of the blossoming biotech industry. Nineteen companies turned down his proposal to commercialize the gas-liquid phase protein sequencer that he and Michael Hunkapiller, a senior research fellow in his lab, developed in the late 1970s, even though it was 100 times more sensitive than current equipment, and capable of sequencing important yet relative scarce proteins like the prion.

A venture capitalist from San Francisco, Bill Bowes, had heard that Hood was shopping his instrument unsuccessfully, and offered up $2 million to start a company to market the sequencer. The result was Applied Biosystems, today part of Applera Corp. and a world-leader in molecular instrumentation.

In the spring of 1985, Lee Hood was one of a dozen scientists who gathered in Santa Cruz, Calif. to discuss whether sequencing the human genome was a good idea. It was the first meeting ever held on the Human Genome Project.

Hood recognized that a new approach to science would come from having this comprehensive cache of biological information. This radical new opportunity, he realized, held enormous promise for human health. With the new tools, it would be possible to identify defective genes, the first step toward understanding their roles in human disease and how to overcome their dysfunction.

His lab at Caltech already had developed a unique cross-disciplinary culture of biologists and technologists, but Hood wanted to go further. So in 1987, he applied for and received funding from the National Science Foundation to transform his lab into a "Science and Technology Center."

During the next five years, the center cut the first turf in the field of proteomics, and helped pioneer the technology for putting oligonucleotides, single-stranded pieces of DNA, on "chips." DNA chips have greatly accelerated genetic research.

Shaking the Etch-A-Sketch comes at a price, however. When Hood proposed a new Division of Molecular Biotechnology,

the Biology Department – which would have been its administrative home – balked at the technological direction his lab was taking.

Convinced at this point that his cross-disciplinary vision of science was essential to biological discovery, Hood moved his lab to the University of Washington School of Medicine in 1992. With $12 million of support from Microsoft founder and Seattle native Bill Gates, Hood established the Molecular Biotechnology Department.

The new department thrived, deepening and expanding the efforts of the transferred Science and Technology Center. It spawned two of the 16 Genome Centers that worked on the Human Genome Project, and gave rise to a multitude of industrial collaborations. The center also developed ink-jet printer technology for making DNA chips, and a fluorescence-activated cell sorter of unprecedented power.

Body as system

A concept had been marinating in Hood's mind since the early 1990s – systems biology, the study of the interaction of all the elements in a system, be that a cell or an organism, rather than studying one gene or one protein at a time. Hood's lab moved increasingly toward a systems approach in its research efforts, using global technologies to study prostate cancer as well as bone marrow stem cells and autoimmune disease.

In 1996, Hood proposed the creation of an Institute for Systems Biology at the University of Washington, to be modeled after the successful Whitehead Institute for Biomedical Research in Cambridge, Mass. Three years of discussion were not enough to clear the path of administrative obstacles, however, so in December 1999, Hood resigned to co-found an independent institute with faculty colleagues Alan Aderem and Ruedi Aebersold.

Originally housed near the University of Washington campus, the Institute for Systems Biology now occupies a new building, not far away, on the shore of Lake Union. The design of the three-story facility consciously reflects the mission.

The open floor plan encourages flow from one work group to the next – cubicles of biologists adjoin those of physicists, mathematicians, computer scientists, engineers, and chemists. Labs are quietly efficient, the din from banks of instrumentation sequestered in windowed rooms across from rows of streamlined workbenches. Walls are painted with the colors of nature – sunflower, olive, burnt sienna, deep lilac – and pools of sunlight fall into nooks furnished with overstuffed chairs.

Lee Hood blends into the background here, though his schedule on any given day is anything but inconspicuous. One recent morning, Hood addressed a delegation of Taiwanese scientists, ministers, and business leaders on cooperative biotechnology ventures. Afterward, he gave a tour of the facility to U.S. Congressman Adam Smith.

As they walked, the two discussed the best way to identify an agent used in a bioterrorist attack and why intellectual property rights are important to the development of drugs. By noon, Hood was across town, addressing the Democratic Leadership Council on the ethical and social consequences of the biotechnology revolution.

Some research efforts, especially the use of embryonic stem cells, have led to calls for greater government regulation over what scientists can do in the laboratory. Hood agrees that the research should be monitored; where he disagrees is at what point. "Our responsibility is not to block the creative discovery process, but to carefully shepherd and control the applications of the technologies for the good of society," he says. "Technology is going to present society with enormous opportunities and enormous challenges.

"We must educate ourselves so that we can participate in the social decision-making process, balancing these opportunities and challenges," Hood adds. "Only if we think rationally and analytically, and are at least informed about the basic facts of what biology and biotechnology is about, can we think effectively about these social and ethical issues."

Hood is inexhaustible and stays perpetually warmed up and ready to speak on any topic, his gears seemingly stuck in drive. A fit man with an intense gaze, only his graying hair betrays his 64 years. His days often start early with a solitary run, a time when he says he does his best thinking. Younger colleagues marvel at his stamina, finding their own energy flagging under the duress of building the infrastructure of the center while maintaining their research.

Though he's much more likely to pick up the sports page than the business section of the newspaper, Hood understands deeply how industry can serve his vision. The long-term plan, he says, is to launch small start-up companies that can develop the discoveries of institute scientists. Venture capitalists already have committed funding

Navigating Montana's rocky terrain as a child spawned in Hood an enduring love for mountaineering that has since carried him across countless peaks, including the Sierra Nevada Mountains in California (left) and the Swiss Alps (right).
Photos courtesy Leroy Hood, M.D., Ph.D.

"One of the most important roles of a mentor is to create unbounded environments where people can move in any direction they want, and to give them a problem that is unbounded and will challenge them. Then, just let them do it."

- Leroy Hood, M.D., Ph.D.

BRIAN SMALE

for a new building he calls the Accelerator, where the companies will be housed while they get a foothold.

"The business is just a means to an end for Lee," says his colleague, George Lake, "a way to get the technology matured to the point that it benefits science. If people in medicine or science have something to do with a company, that is instantly a conflict of interest, something dirty. But the fact of the matter is, if you have something that will actually improve people's well-being, if you don't get financial backing it will take years longer to develop, and whatever benefit it has is lost to those people who suffered for those years."

Hood is quick to give credit to the myriad talented scientists who have populated his large and busy labs over the years, and who are primarily responsible for the technological and methodological innovations.

"Students will often come up with solutions to problems that the mentor would neither have the time nor, in some cases, the specialized skills to solve," he says. "One of the most important roles of a mentor is to create unbounded environments where people can move in any direction they want, and to give them a problem that is unbounded and will challenge them. Then, just let them do it."

"His lab works very well for people who are independent and are able to find their own resources for learning," says Jared Roach, a physician and research scientist at the Institute. "That said, Lee is really there for you when you need him."

"He's very open to people having done different things with their life – it's one of his endearing qualities," adds Lee Rowen, a senior research scientist at the Institute who has doctoral degrees in biochemistry and philosophy.

Since his days in medical school, when he volunteered to teach science to inner-city high school students, Hood also has been committed to K-12 science education. He and his wife currently are involved in an effort to revise the way science is taught in Seattle public schools.

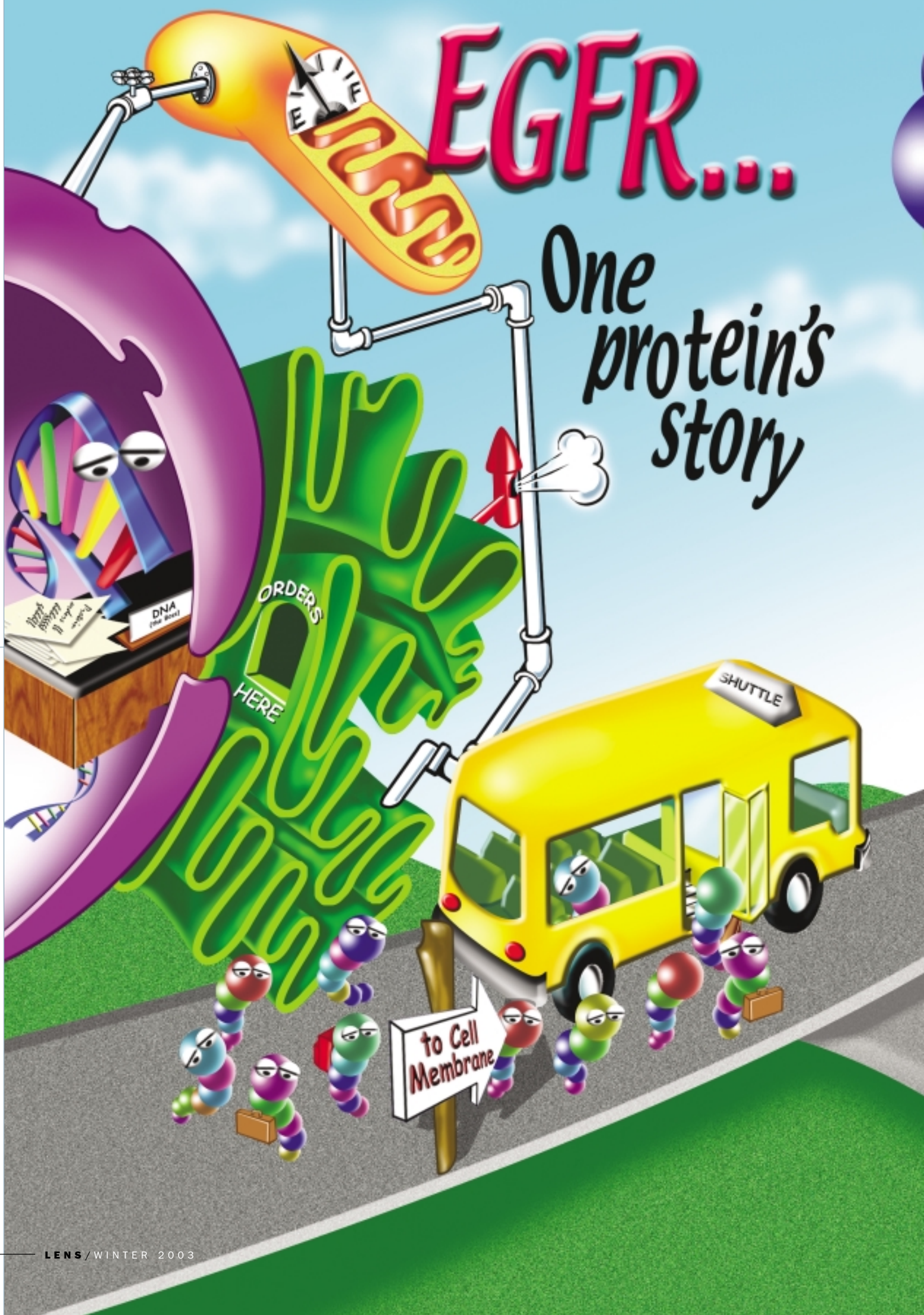
The goal: to lift science education from the doldrums of rote memorization to a more active, inquiry-based method, one that sparks interest in young minds and instills a long-lasting habit of questioning. The program, supported by a National Science Foundation grant, includes summer workshops for teachers. "You just have to go and see kids getting turned on by hands-on, inquiry-based thinking to realize that this is the way you really want to teach things," Hood says.

The culmination of a lifetime's work, the Institute for Systems Biology is Hood's meridian hour, his greatest chance to make a contribution to society. Through the systems approach to biological discovery, he aims to expedite the move toward personalized medicine. And by grassroots efforts to transform K-12 science education, he hopes to produce a citizenry better able to question and understand the momentous changes ahead.

Asked whether retirement is on the horizon, Hood first says no, then hedges.

"Yes, in the sense that I see myself doing different kinds of things," he says. "I think I will always stay scientifically and intellectually involved, but in a less demanding way that would give Valerie and me more time to have fun, to do some things in the outdoors while we're still able. Because there will be a time when we'll be more fragile."

Lee Hood fragile? Not a chance. **LENS**


EGFR...
One protein's story
DNA
ORDERS
HERE
SHUTTLE
to Cell Membrane

We invite you to take a journey into a busy cell, to learn about proteins – how they're made and what they do. We've selected the EGF receptor, a protein involved in signaling pathways that regulate cell growth, as our guide.

by Leigh MacMillan

One protein's story

EGF (epidermal growth factor) and its receptor are special to Vanderbilt University. Stanley Cohen, now an emeritus professor of Biochemistry, was awarded the Nobel Prize in 1986 for his discovery of EGF and its role in cell growth. Cohen and colleagues were the first to characterize the EGF receptor and describe its fundamental features.

EGF and its receptor are now known to be members of a large family of proteins, all involved together in the complex regulation of cell growth. These family members are targets for drugs designed to block the abnormal growth characteristics of cancer cells.

We all start out the same way – being pieced together like so many beaded necklaces – according to the plans. And yet, even though we come off the same assembly lines, we are not at all the same.

The plans instruct some of us to act like two-by-fours, supporting the walls. They direct others of us to act as couriers, or janitors, or assembly line workers. Others still get sent out, to work in the larger world.

Our world is a single cell. We are proteins.

You can think of our world, the cell, as a sort of factory – a very tiny factory, and only one among the millions that make up the human body. It is a place busy with manufacturing. We are the factory's products and its staff. So really, we manufacture ourselves. But not without the plans.

Here's how it works. The plans – the DNA blueprints – are stored in a central office, the nucleus, where they are cared for, you might even say coddled, by proteins. The proteins in the nucleus fancy themselves as having the most important jobs. They rush in

to patch tiny tears in the blueprints. Or they copy parts of the plans to send to the assembly lines for the production of new proteins. Or they coat the DNA and keep it safe during storage. They do keep things humming in the central office, but still I'd rather have my job – at the factory wall.

I am a receptor, specifically an EGF receptor. I spend my time at the cell surface, part of me poking out of the cell, part poking in. Kind of like the doorman who greets Dorothy and her friends at the Emerald City with his head and upper body sticking out the door, the rest safely inside. I will tell you about what I do at the cell surface, but first I want to give you a little background on how I got here.

Like the rest of the proteins in the cell, I was put together on one of the assembly lines. Earlier, I referred to us proteins as being like beaded necklaces. Here's why. We are made of chemical

"beads" called amino acids. Twenty different kinds of amino acid beads are strung together in a particular order for each protein, based on a copy of the plans. So the blueprints might say something like: purple, green, green, blue, red, yellow, purple, yellow, blue…, really the DNA spells out the order of the amino acids, not colors of beads, but you get the idea. The assembly line proteins read out the order, pick out the right amino acids, and put them together in long protein strings.

The order of the beads, then, makes each of us unique. The order determines what we do and the shapes that we take. At the end of the manufacturing process, we don't end up with our amino acids all in a tidy straight line. Instead, we coil and twist as we are made, with the help of folding proteins, until we look like hopelessly tangled necklaces. Scientists call this our structure. Though it may look like a globby mess, the bumps and dips in our structures are carefully constructed for the interactions we have with other proteins and molecules inside and outside the cell.

After we come off the assembly line, we load into a series of shuttles for transport

to our job sites in the cell. En route, we can receive some additional decorations – flourishes added to some of our beads – like you might put on a scarf, or a hat. Our decorations are things like sugars and fatty acid chains; they outfit us for our jobs. Because of varying accessories, even two EGF receptors might end up looking a little bit different from each other. We proteins, you see, are as individual as are you human beings.

So I made it out to the cell surface, along with a shuttle full of other proteins bound for the same destination. As I told you, I am a receptor, which means my job is to receive incoming messages. Well, only certain arriving messages. I am specialized to respond to epidermal growth factor (EGF) and a handful of other signals that are similar to EGF.

EGF – itself a protein, by the way – is one of the many messenger molecules that continuously bombard the outside of the cell. A few of these signal molecules can pass directly through the membrane, but most must interact with a receptor to have their message sent on. Hundreds of different receptors, as well as other kinds of proteins, stud the cell surface. I am not

the lone receiver of EGF signals – other EGF receptors and our family members join me in the task. Our family members actually work as pairs to send messages inside the cell.

When EGF sticks to us and we team up, we pass the signal along to a host of proteins inside the cell. These courier proteins are waiting nearby, just inside the walls, ready to take the message from us and speed off, transmitting it to parts deep inside the factory.

The courier proteins know that a pair of us has received a message because we perform a chemical reaction, called phosphorylation, on ourselves. We take a kind of sticker – a phosphate group – from a molecule called ATP and stick it onto some of our amino acid beads. These phosphate stickers are signals for the courier proteins to interact with us so we can tell them the message. They then share the information with still other proteins, and eventually the message can make it all the way to the central office, the nucleus, where the DNA is stored.

The messages that make their way to the nucleus affect which plans are copied for the assembly of new proteins. This is how messages from outside the cell alter the types of proteins being manufactured inside the cell.

Signals from my receptor family will send the cell into a growth mode; the cell will duplicate its DNA and split itself into two cells. With continued growth signals, these two cells can split again, making four, and so on. It doesn't take long to have a bunch of new cells. This is good if you're trying to make a new organ, or replace damaged tissue. It's bad if the cells are dividing for no healthy reason, like tumor cells do.

I'm sorry to say that my family members and I participate in processes that can turn normal cells into tumor cells. It happens when things go awry – the plans get changed – and the assembly lines churn out way too many of us. Or we're made a little differently so that we're able to send signals all the time, not just in response to EGF.

In situations like these, there are new drugs that aim to keep us in check. Some of these medicines work by jamming up the place where EGF sticks to us. Some, like a new one called Iressa, bind our hands, in a sense, making us unable to put the phosphate "stickers" on ourselves or on other proteins. These drugs may help, but tumor cells are a tricky lot; they're good at figuring out ways to get around such roadblocks.

As for me, I'm nearing the end of my shift. I've put in a good day's work here at the factory wall, and I'm spent. The cell will replace me with a brand new EGF receptor. And I will board a shuttle bound for the lysosome – our cellular recycling center. There, I will be dismantled so that my amino acid beads can be reused to manufacture some other protein. I'm hoping they don't end up in one of those central office types! **LENS**

Among the scientists, business leaders and government officials who led the successful effort to sequence the human genome were two officials of Applera Corp., the parent company of the Applied Biosystems Group and the Celera Genomics Group.

Michael W. Hunkapiller, Ph.D., Applera's senior vice president and president of its Applied Biosystems Group, pioneered development of the automated machines that made it possible to unravel the human genetic code. Tony L. White, Applera's chairman and CEO, was a guiding force behind the creation of Celera Genomics and its race with the publicly funded Human Genome Project.

Recently White and Hunkapiller shared their thoughts about the potential of proteomics to improve human health, the importance of industry-academia collaboration, and the challenges that lie ahead.

The future of proteomics.

An interview with Tony White and Michael Hunkapiller.

BY BILL SNYDER

TONY WHITE
APPLERA'S CHAIRMAN AND CEO

Lens: What are the next advances that are about to happen? What will the market and medicine look like in 10-15 years?

Hunkapiller: I think a lot of the drugs that are coming on the market now have come about because of a more thorough use of tools that allow in-depth analysis of the biology underlying the choice of targets and the choice of molecules that interact with those targets. I think we will see drugs coming to market at a more rapid pace.

What is clear is that when people are given drugs now, there tends to be a mix of beneficial effects for some people and harmful effects for others, and no effect at all for a third group of the population. One thing that will happen as we get a better understanding of all the biochemical pathways that may be impacted by a particular therapeutic compound, we can begin to do drug development in a way that maximizes the positive and minimizes the negative effects of some of those compounds. We will be able to target exactly the right disease-causing agent with the right drug as opposed to more of a hit and miss approach, which is what has somewhat characterized past efforts.

Understanding the underlying biology also can help us understand the preventive measures to take against disease. Most of the diseases that have a genetic underpinning also have a big interaction with the environment that can trigger those diseases. If we understand the underlying biology, we can begin to understand how to deal with preventive measures as well as the therapeutic ones.

Lens: Should there be a "big science" project to move the field along?

White: I certainly believe that with the exception of a situation where a research program might require a very large, centralized capital expenditure or capability, a decentralized research program is going to be better for everybody – one like we have today, where the government provides grant money to the individual researcher at the laboratories, rather than bringing the money into one big centralized center. I'm not sure that would work very well.

These research programs tend to have a boss and whatever that boss thinks is the right science, is the only science that gets done, as opposed to under the grant mentality, where there are a lot of ideas coming up from different places. I think you get better science. You get more diversity and you get more for your money.

Lens: What challenges must be overcome to achieve the fruits of proteomics?

Hunkapiller: A lot of the technology efforts up until now have been to design systems that allow people to study an individual out of an entire population. The human genome project was to do that essentially.

One of the challenges now is to develop tools that go beyond just looking at the overall blueprint. Scientists now want to look at the individual differences among large populations of individuals to help identify changes that might lead to disease or to identify why some individuals remain healthy. The tools that are required to look at those differences across a large number of individuals are pretty substantial, as opposed to tools used to decipher the basic blueprint of life.

The main issue is reducing the cost of the technologies to a level that is affordable to perform these studies on a large number of people. The technologies that will allow large-scale studies are available, but they are currently somewhat cost prohibitive. I think the same issue applies to the proteomics industry.

On top of that, there is the challenge of making the technology cost effective to do a complex study of both protein structure and function in a broad survey sense, which has not been done yet. It requires development on the one hand, and invention on the other. Science and technology have a good way of playing off of each other, and coming up with solutions to these kinds of challenges.

Lens: If Congress, in an attempt to reduce the cost of pharmaceuticals, makes it easier for generic drugs to get to market, would this have an impact on drug discovery?

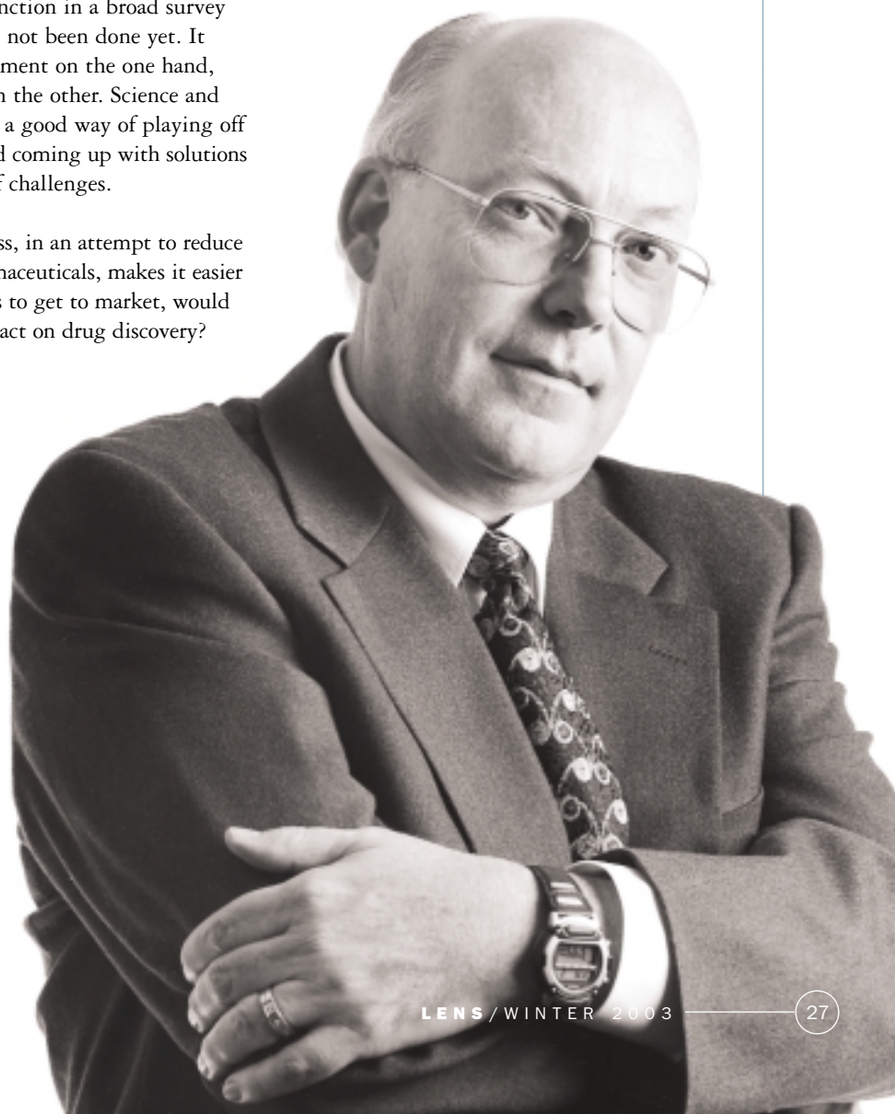
White: If you consider a complete cessation of all research and development an impact, I would agree.

I don't see why a right-minded public company would spend anything on developing a drug if they weren't going to be able to have patent protection around it. It costs a billion dollars or so to develop a drug. Most companies are not inclined to do that so that somebody can steal it from them.

As a matter of principle, patent protection in pharmaceutics is just as important as it is in any other commercial endeavor.

Lens: What are the barriers beyond the science – financial, political, and social?

Hunkapiller: I would certainly say that the stem cell issue and the ethics surrounding that could easily become one of the bigger obstacles to understanding how some of these biological systems work and, more importantly, figuring out ways of translating that knowledge into practical benefits. It is one of the areas in which the potential for using a lot of this information in a pretty dramatic way to impact people's health would seem to be pretty astounding, and I personally would hate to see the politics of that override the benefit of it.



MICHAEL W. HUNKAPILLER, PH.D.
APPLERA'S SENIOR VICE PRESIDENT

"One of the challenges now is to develop tools that go beyond just looking at the overall blueprint. Scientists now want to look at the individual differences among large populations of individuals to help identify changes that might lead to disease or to identify why some individuals remain healthy. The tools that are required to look at those differences across a large number of individuals are pretty substantial, as opposed to tools used to decipher the basic blueprint of life."

Michael W. Hunkapiller, Ph.D.

White: I guess I'd put it another way. I think the stem cell debate was a very instructive one, in that you took a scientific initiative, and it was kidnapped by people with a different agenda. I'm not favoring either side. I'm simply saying that people with other social agendas try to insert themselves into science in fairly ignorant ways.

Cloning is another one. Leave it up to the people who want to alarm society, and who want to take their own agenda and insert it into the scientific debate, absent an educated public they'll get away with it.

People need to be better informed about this science before they start talking. Unfortunately, that is not the way politicians typically work. They usually start talking and somewhere along the way they find out what they're saying.

Hunkapiller: I'd add a second area where I think the complication of some of this knowledge has been put into a debate in which people with different agendas have captured the media attention as well, and that's in the whole area of genetically modified food.

To some degree, one can understand the concern. I think that a lot of the companies that started to use the technology went about it not in an inappropriate manner, but they may have chosen the wrong first targets. The big seed companies that were also big herbicide companies tended to promote the use of the technology in a way that would foster their interest as opposed to highlighting the benefit for the public.

We can argue about whether we need corn that is resistant (to insects), but if we go to third-world countries where nutrition is a key to survival, having crops there that provide vitamins the population could not get otherwise is a pretty big deal. And people there, I think, would laugh at the arguments that go on in the U.S. and some countries in Europe.

I think it is a disservice to the public at large to lump the less than stellar use of science with the cases where it is obviously beneficial. And I think that sometimes the media misses the distinction between those two things.

Lens: Is collaboration between academia and industry important for the development of the field of proteomics and for drug development, and, if so, why?

White: It's valuable. I think the key is that everybody has to know what they want to get out of the relationship, and if the relationship can be structured so both parties can accomplish what they want to accomplish, it's a good thing.

Hunkapiller: It's a tradeoff. The academic world has a mission to create new knowledge and make that available for other people to build upon. Companies have an imperative to increase value for their shareholders. Certainly where those missions are in concert, there is synergy between the two, and an opportunity for fruitful collaborations, such as when basic discoveries are developed into practical applications to help people.

Clearly one has to be mindful of the fact that there occasionally are conflicts in determining what are the goals. Mechanisms have to be in place to minimize those conflicts. But the university system is a source of a lot of basic knowledge that is applied towards practical applications. Congress recognized that a long time ago, by fostering within the biological area programs that push universities to get some of their basic discoveries into the commercial world.

Lens: There is growing concern that industry affiliation represents a potential financial conflict of interest for university-based researchers, one that threatens to jeopardize the public trust in their integrity and honesty. What do you do to avoid conflicts of interest, and ensure that industry-academic collaborations succeed?

Hunkapiller: We tend to do collaborations with universities during what I would call the pre-competitive development or the basic discovery stage in the technology field. We try to make sure through our relationships with academia that we have access to developing technologies. We do not tend to push for exclusive access because exclusivity, I think, can tilt over frequently into problems with the relationship.

It becomes trickier when one is dealing with later-stage development issues, and that can become a bigger challenge publicly in some of the drug development efforts. One just has to be very careful to make sure there is full disclosure, as to who pays for development costs and who reaps the benefits of those developments.

Lens: How can your company balance the need to keep stockholders happy with the need to do good science?

White: I don't see the conflict. We have to do good science to stay in business, so I think the best thing we can do is make to sure we have informed shareholders. We spend lot of time communicating with them, trying to make sure they understand our strategy and our plans and the basis for our scientific endeavors. We spend a lot of money, a quarter of a billion dollars a year, on research and development. That is the nature of our business. If we didn't do it and we didn't do it very well, we wouldn't last.

We chose to be in field that is technology intensive, and so we have a risk profile that is different than if we were developing the next generation farm tractor. **LENS**

glossary

PROTEOMICS — A NEW LANGUAGE

The term "proteome" was coined by Australian researchers in the mid-1990s to describe all the PROTEins expressed by the genOME, the complete set of genetic material necessary for life. Proteomics, then, is the study of the expression, function and interaction of proteins in health and disease.

Here's a guide to proteins we included in our cover illustration:

ApoE – apolipoprotein E, a protein involved in the transport of cholesterol and other fatty molecules. One form of this protein is a major risk factor for Alzheimer's disease.

CD4 – a protein on white blood cells that binds to HIV, the virus that causes AIDS.

Cytochrome P-450 – a class of enzymes that play important roles in the metabolism of drugs and toxins in the liver.

COX – cyclooxygenase, an enzyme that exists in at least two forms, and which exerts a wide variety of effects in different tissues through the production of locally acting hormones called prostaglandins (See page 8)

EGFR – epidermal growth factor receptor, a protein that plays a role in signaling cell growth, including cancer growth. (See page 22).

G proteins – known for their ability to bind guanine nucleotides. They transmit signals from a large class of proteins called G protein-coupled receptors (GPCRs). Together, G proteins and GPCRs play a role in a wide range of physiological functions, including the regulation of blood pressure, the production of glucose by the liver, and the transmission of signals between nerve cells in the brain.

Ion channel – proteins that allow ions, or electrically charged molecules like sodium, calcium or chloride to pass through cell membranes. Ion channels generate and orchestrate the complex language of electrical signals essential to the function of muscles, lungs, heart, brain and other vital organs.

Kinase – a family of enzymes that attach phosphate groups to specific amino acids in proteins. Tyrosine kinases, for example, attach phosphate groups to tyrosine, a key event in many "signaling pathways," including blood sugar regulation by insulin and cell growth triggered by epidermal growth factor.

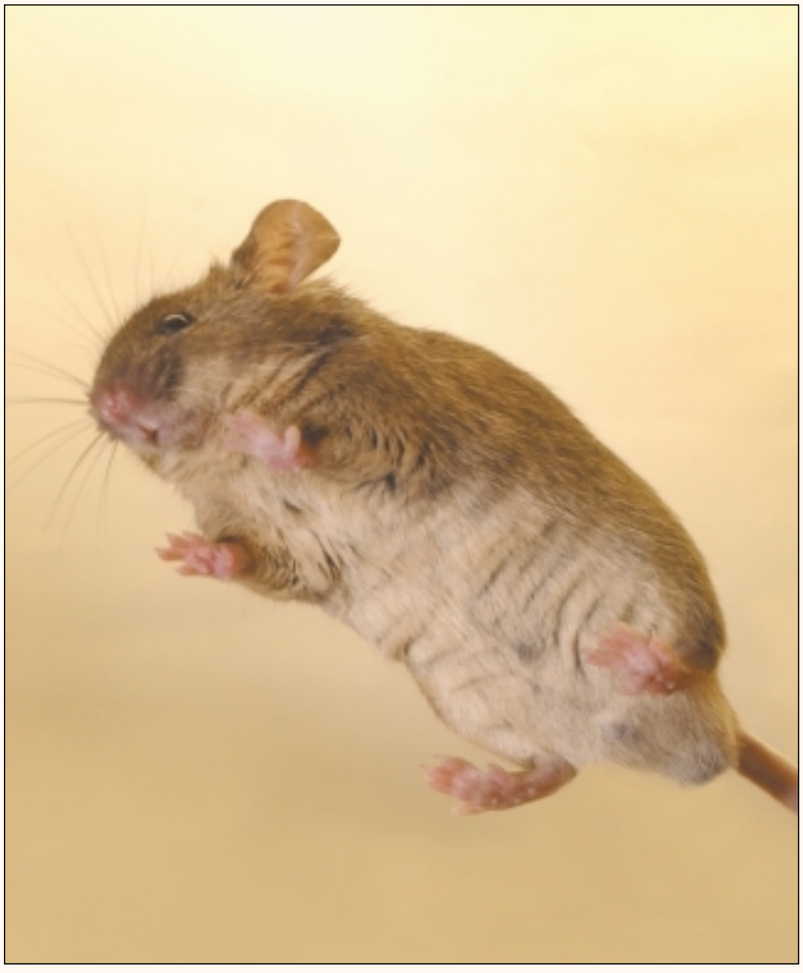
Nuclear pore protein – groups of proteins that allow molecules to pass in and out of the nucleus of the cell.

PAI-1 – plasminogen activator inhibitor, a protein that can block natural clot-busting enzymes in the body, including tissue-type plasminogen activator (t-PA). High levels of PAI-1 are therefore a risk factor for cardiovascular disease.

PSA – prostate-specific antigen, a protein marker found in the blood that is associated with prostate cancer (See page 9).

TGF-beta – transforming growth factor beta, a protein first identified in cancer cells that suppresses cell growth.

Mice play a key role in improving understanding of many human diseases, including diabetes.



ANNE RAYNER POLLO

IN THE NEXT ISSUE:

The holy grail of diabetes
Researchers throughout the world are trying to turn stem cells into transplantable, insulin-producing beta cells. A progress report.

The second time around
Oscar Crofford helped establish the value of strict glucose control. Today he has a new life – on the farm.

The perils of modern life
Fatty foods and inactivity are fueling an epidemic of diabetes in children and minorities. What can be done about it?

Lens

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