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NCRR Reporter

Summer 2006

Critical Resources for Your Research

U.S. Department of Health and Human Services

Solving Chemical Structures To Treat Disease

Washington University's Kristen Mondy and Kevin Yarasheski work together using mass spectrometry in clinical studies of HIV infection

FROM THE DIRECTOR

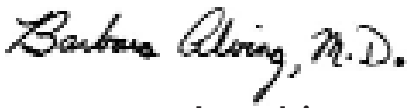
Critical Biomedical Technologies Lead to Improved Human Health

Advances in technology drive innovation and rapid progress in all stages of biomedical research—from basic discovery to clinical investigations. NCRR funds more than 50 specialized Biomedical Technology (BT) Resource Centers across the country, primarily at major academic institutions and health centers.

The BT Resource Centers are hubs for multidisciplinary and interdisciplinary research, linking together collaborators nationally and internationally. To support a range of research pursuits, the centers focus on five technology areas: information technology; optical/spectroscopic technology; imaging technology; technology for structural biology; and technology for systems biology. These diverse tools and expertise have enabled countless groundbreaking discoveries in biomedical science. Many have evolved from utilization for basic research into a broad spectrum of techniques for early detection and diagnosis of diseases.

The cover story in this issue of the *NCRR Reporter* illustrates how the technology known as mass spectrometry—long a workhorse in the analytical chemistry lab—has been developed and enhanced by BT Resource Center scientists to become a critical tool for addressing complex health-related problems, including more accurate diagnosis of amyloid diseases and better understanding of muscle deterioration in AIDS patients.

These examples tell only part of the story. Across the nation, researchers depend on BT Resource Centers for a wide variety of clinical and translational studies. For instance, synchrotron X-ray technologies are providing clues to treating multidrug-resistant infections; imaging technologies are revealing motor learning deficits in autistic children; and laser microbeam technologies are enabling cancer detection and better understanding of cardiovascular disease, neurologic conditions, and metabolic syndrome. The list could continue, but the message is clear—advances in biomedical technology enable researchers to explore answers to a multitude of questions that affect human health.



Barbara Alving, M.D.
Acting Director, NCRR

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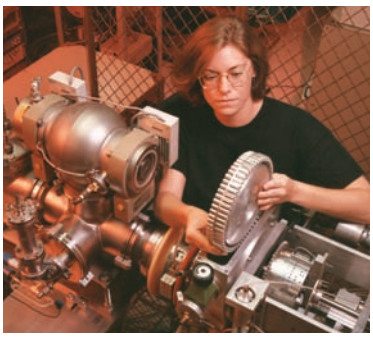

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NCRR Reporter

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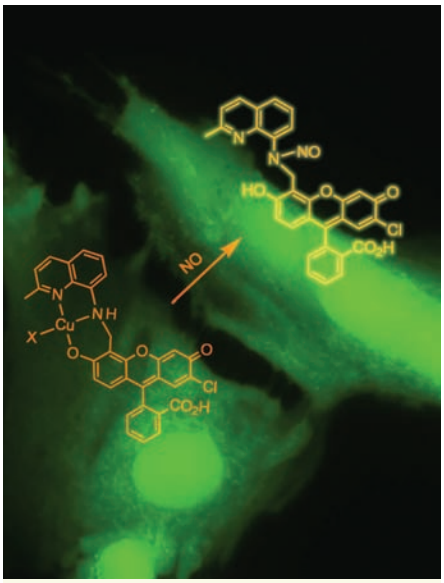
On the Cover: Advanced technologies developed at NCRR-funded resource centers allow scientists to study the molecular underpinnings of human disorders. Working with clinical researcher Kristen Mondy (left) at Washington University in St. Louis, Kevin Yarasheski uses state-of-the-art mass spectrometry techniques to analyze the molecules associated with muscle degeneration in HIV-infected patients.
PHOTO BY PETER NEWCOMB

QUICK TAKES

New tools and updates from NCRR resources



Cancer cells glow green after a new copper-containing sensor (chemical formula, lower left) reacts with NO to produce a fluorescing molecule (upper right).

The Glow of NO in Living Cells

A new fluorescent sensor, developed at the Massachusetts Institute of Technology, will help scientists to scrutinize the elusive and versatile molecule nitric oxide (NO) in living cells. The tiny NO molecule—consisting of just a nitrogen and an oxygen atom—performs surprisingly diverse functions, acting as a neurotransmitter, an antibacterial agent, a maintainer of bone mass, and much more. The molecule has proved difficult to study because it is usually short-lived before reacting with other molecules.

With funding from the National Science Foundation, and an NMR spectrometer purchased through an NCRR Shared Instrumentation Grant, the scientists created and tested a unique copper-containing sensor that readily crosses the cell membrane and glows brightly in the presence of even low concentrations of NO. The probe, described in the July issue of *Nature Chemical Biology*, will greatly aid investigations of how NO functions in cancer cells, neurons, and other cells. ■

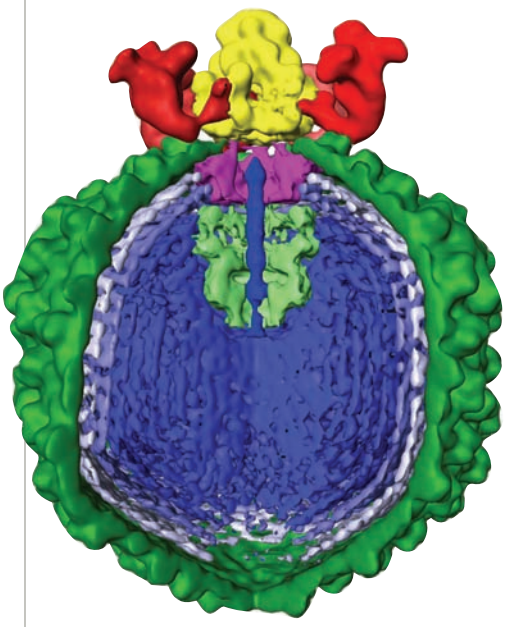
AIDS Vaccine Moves to Clinical Trials

A new multiprotein AIDS vaccine will be evaluated in humans for the first time, after its remarkable success in protecting monkeys from the disease. In studies conducted at the NCRR-funded Yerkes National Primate Research Center, the vaccine protected 96 percent of monkeys from developing AIDS for more than three years, providing better and longer protection than any other AIDS vaccine candidate to date. The vaccine has been under development since 1997 by Yerkes researcher Harriet Robinson and her colleagues at the National Institute of Allergy and Infectious Diseases and the Centers for Disease Control and Prevention. It was licensed for commercial use by GeoVax in 2004.

The new vaccine uses DNA to prime the immune response and a genetically modified "pox"-type virus to boost the immune response. Both vaccine components express noninfectious virus-like particles. Phase I clinical trials, conducted through the NIH-sponsored HIV Vaccine Trials Network, began in April 2006 at the University of Alabama at Birmingham, the University of Maryland, and St. Louis University. If successful, the vaccine would face at least four more years of clinical testing. ■

Viewing the Virus

Even in a powerful cryo-electron microscope, the crispness and resolution of an image may depend not only on specific hardware, but also on the software used. By embedding new algorithms in their image-processing software, scientists at the NCRR-supported National Center for Macromolecular Imaging (NCMI) at Baylor College of Medicine are peering deeper into viruses. With the enhanced software, researchers can now see tiny virus features like "tail spikes," with which the virus anchors to the host cell before injecting its viral genome. Studying structures like these can reveal the molecular mechanisms by which a viral pathogen infects a cell.



This 3-D image, showing a cross section of a virus that infects bacteria, was created with the upgraded EMAN software.

The publicly accessible software called EMAN, developed by NCMI scientists, reconstructs the detailed 3-D topology of the molecular components of a virus based on tens of thousands of 2-D electron microscopy images. The upgraded version, EMAN2, is expected to be released in late 2006 and can be downloaded from http://ncmi.bcm.edu/ncmi. ■

IMAGE COURTESY OF CHRISTY TINBERG AND MI HEE LIM, LIPPARD LAB, MIT (ABOVE)
IMAGE COURTESY OF THE NATIONAL CENTER FOR MACROMOLECULAR IMAGING (RIGHT)

Solving Chemical Structures To Treat Disease

Mass spectrometry addresses complex problems in human health.

BY LAURA BONETTA

Many people are familiar with the ravages of Alzheimer's disease: It takes away memories, changes personalities, and leaves many individuals unable to think and act rationally. But less well known is the fact that Alzheimer's disease is one of several disorders caused by harmful deposits of amyloid proteins in various organs and tissues. Depending on the protein involved and where it accumulates in the body, these so-called "amyloid diseases" may produce shortness of breath, weight loss, difficulty swallowing, an irregular heartbeat, or an array of other symptoms that may vary from patient to patient or overlap with other conditions.

This confusing constellation of symptoms can make it difficult for physicians to distinguish among the many types of amyloid diseases and select appropriate therapies. To aid these complex diagnoses, clinicians at the Amyloid Treatment and Research Program at Boston University School of Medicine recruited a powerful ally—an increasingly sophisticated technology known as mass spectrometry.

"Mass spectrometers used to be very difficult to use, often hidden away in the basement of the chemistry department. But they have become routine instruments for biomedical research," says Douglas Sheeley, health scientist administrator for NCRR's Division of Biomedical Technology. Across the nation, NCRR supports five Biomedical Technology (BT) Resource Centers charged with developing techniques and instrumentation for mass spectrometry and making these available to the scientific community through collaborations and training. (See "To Gain Access" on page 8.) "These centers are creating new tools and applying them not only to basic research but also to the clinic," says Sheeley.

Today, mass spectrometry applications range from finding disease-causing mutations in proteins to identifying differences in how patients absorb and metabolize drugs. Mass spectrometers allow scientists to sift through thousands of different molecules in a complex biological soup according to their different chemical compositions, creating a "spectrum" of their masses. The instruments also can select and disassemble specific molecules to reveal more about their makeup. (See box on page 6.)

At Boston University School of Medicine, Catherine Costello and her colleagues use the technique to reveal the identity and structure of amyloid proteins in patients' samples. "Patients are referred to the program from all parts of the country," says Costello, director of the university's Mass Spectrometry Resource for Biology and Medicine, funded by NCRR. More than 30 years ago, as a postdoctoral trainee working at a BT Resource Center at the Massachusetts Institute of Technology, Costello helped to pioneer the clinical application of mass spectrometry by using it to analyze toxic or abnormal substances in the blood and urine of patients who arrived at the emergency rooms of Boston-

Physician Kristen Mondy meets with O. Dan Smith, a volunteer participant in clinical research studies now under way at Washington University in St. Louis. The studies use mass spectrometry to examine the metabolism of bone, muscle, and other tissues.

> The team is identifying thousands of proteins expressed in muscle cells from HIV-infected and uninfected volunteers.

area hospitals. (For more information, see the *NCRR Reporter,* Spring 2002, pages 16-19.)

TARGETED DIAGNOSES FOR AMYLOID DISEASES

Today, Costello's work with amyloid disease primarily involves two types. The first, primary amyloidosis, begins in the bone marrow, where infection-fighting immune cells are produced. Primary amyloidosis arises when high levels of abnormal immune cells generate fragments of antibodies that cannot be efficiently broken down and recycled within the body. Instead, these antibody fragments build up in the bloodstream and clump together, eventually forming amyloid deposits in the heart, kidneys, tongue, nerves, and intestines. This deadly disease is sometimes treated with chemotherapy or bone marrow transplants to limit further production of abnormal antibodies. The second type of amyloid disease, familial amyloid polyneuropathy, starts with deposits of transthyretin, a protein manufactured in the liver. An inherited mutation in the transthyretin gene can change the protein's structure to a sticky form that collects in nerve and muscle cells, producing numbness, weakness, and digestive problems. The only corrective treatment is a liver transplant, which allows the healthy transplanted liver to produce the normal protein.

PHOTO BY PETER NEWCOMB

For patients who come to Boston Medical Center's Amyloid Treatment and Research Program, accurate diagnosis is aided by sophisticated mass spectrometry methods that are usually used for basic research. Blood and urine specimens collected from patients are sent to a laboratory that extracts the rogue proteins from the samples. The proteins' molecular weights are determined by mass spectrometry. Proteins that are abnormal are then broken down into smaller pieces, called peptides, and analyzed by the mass spectrometer, which identifies the portions that differ from their normal counterparts. For example, a change in one amino acid would also change the mass of a particular peptide. Identified differences are then further examined using a more sophisticated tandem mass spectrometer, to pinpoint the exact amino acid change. In cases of inherited disease, like familial amyloid polyneuropathy, and other diseases in which the normal protein or gene sequence is known, the DNA sequence is also determined whenever possible, and the DNA-predicted sequences are compared with the mass spectral data obtained for the expressed protein. "With this inherited disease, an accurate diagnosis might place the patient high up on the list for a liver transplant," says Costello.

Costello and her colleagues have identified hundreds of structural variations in amyloid proteins. Some are substitutions in the sequence of amino acids, and others are modifications that occur after a protein has been manufactured. In addition to helping to make a diagnosis, both types of abnormalities may provide clues to how the different types of amyloid diseases develop.

For example, by collaborating with clinicians and several research groups at Boston University, Costello has been trying to identify structural features and additional tissue components that can make proteins more likely to clump together and form deposits. "We want to find correlations that tell us what impact a change in the protein structure has for the clinical progression of disease," she explains. In addition, Costello and her colleagues use mass spectrometry in combination with atomic-force microscopy to observe interactions of the amyloid proteins with other molecules in the characteristic deposits that may help explain the mechanisms underlying these diseases.

Mass Spectrometry Primer

Mass spectrometers come in all shapes and sizes and have differing capabilities. But every instrument contains three fundamental parts: the ionization source, the analyzer, and the detector.

A traditional ionization source turns sample molecules into a gas; gives them an electrical charge, converting them to ions; and pushes them into the mass analyzer. With modern ion sources, samples that cannot be readily turned into a gas are turned into ions and admitted into a gaseous state. Examples of these ionization methods are electrospray ionization and matrix-assisted laser desorption ionization (MALDI), the invention of which led to the 2002 Nobel Prize in Chemistry.

Analyzers operate on a variety of physical principles, but all separate ions according to the ratio of each ion's mass to its charge. After the ions are separated, they can be selected for further manipulation or detected directly. From this information, it is possible to determine the mass of each ion and its relative abundance, and to deduce the elemental composition of the original substance from its overall mass. Further steps allow details of the structures to be determined.

Original methods of ionization were so harsh that they destroyed large molecules such as proteins. But the new, soft-ionization techniques, including MALDI and electrospray ionization, have made studies of larger molecules possible. For more information, visit the American Society for Mass Spectrometry at www.asms.org.

FIGURING OUT MUSCLE WASTING

In another part of the country, scientists at Washington University in St. Louis (WUSTL) are using mass spectrometry to piece together a different medical puzzle—the metabolic syndromes and muscle wasting associated with HIV/AIDS. Today's powerful antiviral drugs have reduced susceptibility to the severe weight loss that often accompanied AIDS in the past. But even when patients are able to maintain their weight, the news is not all good.

Clinical researchers have learned that many HIV-infected patients gain weight in the form of fat, usually deposited around the waist, while fat in their limbs wastes away. In addition, these patients may have elevated levels of blood glucose, triglycerides, and cholesterol—similar to patients who have type 2 diabetes and the cardiometabolic syndrome. "There are many hypotheses for what causes these metabolic changes," says Kevin E. Yarasheski, associate professor of medicine at WUSTL and assistant direc-

PHOTO BY KALMAN ZABARSKY, BOSTON UNIVERSITY (OPPOSITE)

Accurate diagnosis of amyloid disease is aided by mass spectrometry methods usually used for basic research.

tor of the NCRR-supported Resource for Biomedical and Bio-Organic Mass Spectrometry. "We remain uncertain about the fundamental trigger—whether antiviral drugs, HIV infection, genetics, or lifestyle factors."

For the past two decades, Yarasheski has used an isotope ratio mass spectrometer, obtained through an NCRR Shared Instrumentation Grant (SIG), to monitor how body proteins are built up and broken down. (For more information about SIG funding, see the *NCRR Reporter*, Fall 2004, pages 8-9.) The instrument measures the amount of carbon-12, the carbon isotope normally found in the body, relative to carbon-13, a heavier isotope that Yarasheski uses to label different substances like glucose or amino acids, which our bodies use as fuel or raw material for building muscle. The carbon-13-labeled substances are administered to patients. Urine, blood, or tissue samples are then collected by the clinical staff at the NCRR-supported General Clinical Research Center (GCRC) at WUSTL. The amount of carbon-13 in these samples is used to calculate, for example, how fast an amino acid is oxidized, or used to make muscle proteins.

The mass spectrometry resource at WUSTL, which has facilities in both the chemistry department and the medical school, is the only NCRR-supported center with this kind of capability. "This center is among the pioneers for using stable-isotope analysis for biomedical studies. Its applications here date back to the 1970s," says John Turk, co-director of the resource center, along with R. Reid Townsend and Michael E. Gross.

Accurate diagnosis of amyloidosis nearly a decade ago helped to save the life of Connie Hanson (left), whose health remains relatively stable in 2006. Hematologist Vaishali Sanchorawala (right) assisted with Hanson's therapy, and her diagnosis depended on an extensive workup at the NCRR-supported mass spectrometry resource in Boston.

Yarasheski's metabolism research eventually led him to the study of HIV-infected patients. "I entered the HIV field in 1994 because I wanted to understand protein metabolism in AIDS wasting. My group continues to use isotope tracers and mass spectrometry to understand the metabolic disorders associated with HIV, as well as diabetes and other wasting disorders," he says.

Through his isotope tracing experiments, Yarasheski found that HIV-infected people incorporate circulating amino acids into muscle proteins at a slower pace than do healthy individuals. He concluded that HIV muscle wasting is caused by a defect—as yet unknown—in the pathways that regulate amino acid metabolism.

Determining the nature of this defect has given impetus to an ambitious project involving Michael Gross and colleagues in both the chemistry and medicine arms of the mass spectrometry resource. For the past 30 years, Gross has been developing more powerful instruments and techniques for mass spectrometry, most recently employing a new Fourier transform mass spectrometer obtained through an NCRR High-End Instrumentation Grant. (For more information on these grants, see the *NCRR Reporter*, Spring 2006, pages 10-11.) "It is a cutting-edge mass spectrometer that can deal with complex mixtures of molecules," he explains. Gross and Townsend are creating new analytical methods employing this top-of-the-line instrument to follow up on Yarasheski's findings, using the muscle proteome as a paradigm for development of proteomics technologies.

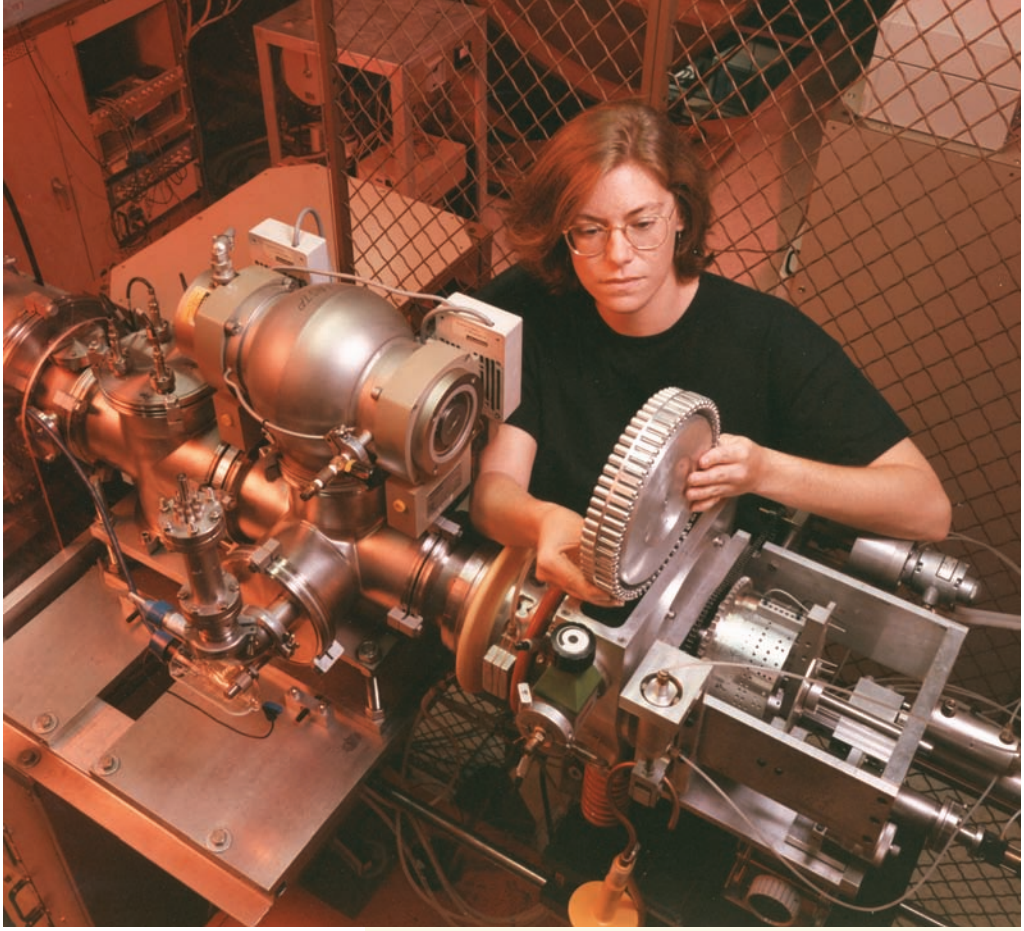
The team is identifying thousands of proteins expressed in muscle cells from HIV-infected and uninfected volunteers. The researchers will determine if these protein expression patterns differ and play a unique role in HIV-metabolic syndromes.

BRINGING PERSONALIZED MEDICINE TO PATIENTS

The mass spectrometer Yarasheski uses for his studies on metabolism is a distant cousin of an instrument that has long been used for analyzing geological samples. First developed in the mid-1970s, the accelerator mass spectrometer (AMS) is an exceptionally sensitive instrument for measuring trace amounts of radioactive isotopes in very small samples. In the late 1980s, scientists at the Lawrence Livermore National Laboratory in Livermore, California, were among the first to apply AMS to bio-

medical research by looking at how small doses of carcinogens affected the DNA of mice.

Today, Kenneth Turteltaub, principal investigator of Livermore's NCRR-funded National Resource of Biomedical Accelerator Mass Spectrometry, uses the technique to trace how the human body processes small amounts of toxins or nutrients, in amounts an individual might normally ingest from food. Volunteers swallow a substance labeled with a harmless amount of radioactive isotopes, such as carbon-14 or hydrogen-3. AMS is then used to measure the radioactive isotopes in blood, urine, or tissue samples collected over several hours to several days. The results show the quantities and the rates at which the substance is absorbed or excreted.



Scientists at Lawrence Livermore National Laboratory in California use accelerator mass spectrometry to discover how the human body metabolizes trace amounts of nutrients, toxins, and other compounds.

Scientists recently used this approach to develop a test for detecting differences in the way people absorb vitamin B12, needed for the production of red blood cells. Severe problems in absorbing this vitamin can lead to anemia or birth defects. "Using this kind of test would allow physicians to tailor the amount of B12 that should be administered to an individual," says Turteltaub. The same principles would apply to determining the ideal dose of a drug to give to an individual patient, a concept sometimes referred to as "individualized medicine."

AMS also may have applications to the drug development pipeline, Turteltaub says. Traditionally, drug development protocols have depended on studies of animal models to predict how people might metabolize drugs. But the comparisons are not always apt. An alternative approach might involve giving harmless trace amounts of a labeled drug to human volunteers and then tracing its absorption and metabolism using AMS.

MASS SPECTROMETRY'S BROADER APPLICATIONS

Beyond the five NCRR-supported mass spectrometry research resource centers, the technique also is being employed at several NCRR-supported centers for proteomics and glycomics. (For more information, see the *NCRR Reporter*, Spring 2004, pages 5-7.) These centers integrate high-throughput analytical techniques to look for changes caused by disease in the thousands of proteins or carbohydrates in complex samples. Studies focus on breast cancer, infectious agents, and cellular events that underlie human health. "In order for these techniques to be employed for clinical research, the analyses must be very precise and robust," says NCRR's Douglas Sheeley. "Proteomics isn't there yet, but today's advanced mass spectrometers can meet these challenges and will play an increasingly important role as clinical studies become more complex."

TO GAIN ACCESS: NCRR supports five Biomedical Technology Resource Centers that develop new tools and applications and offer diverse types of mass spectrometry services and training, free of charge, to qualified scientists. Web sites and Principal Investigators (PIs) for these resource centers are listed below.

- National Bio-Organic Biomedical Mass Spectrometry Resource, University of California, San Francisco. PI: A. L. Burlingame. http://donatello.ucsf.edu.
- Mass Spectrometry Resource for Biology and Medicine. Boston University School of Medicine. PI: Catherine Costello. www.bumc.bu.edu/msr.
- National Resource for Biomedical Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, California. PI: Kenneth W. Turteltaub. www.llnl.gov/bioams.
- National Resource for Mass Spectrometric Analysis of Biological Macromolecules, The Rockefeller University, New York. PI: Brian T. Chait. http://prowl.rockefeller.edu.
- Resource for Biomedical and Bio-Organic Mass Spectrometry, Washington University, St. Louis. PIs: Michael L. Gross and John Turk. http://wunmr.wustl.edu/~msf.

PHOTO COURTESY OF LAWRENCE LIVERMORE NATIONAL LABORATORY

Nanoparticles Aid Ultrasound Imaging

Imagine a human-made sphere so small that you could wrap dozens of them around a red blood cell. Picture particles so tiny they could slip through pores in blood vessels and lodge themselves into a tumor. Researchers at Ohio State University are studying such nanospheres for their ability to enhance ultrasound images and possibly aid in the clinical diagnosis of cancer.

Thomas Rosol and Jun Liu examined the imaging properties of spheres about 100 nanometers in diameter after injecting them into the tail veins of mice. The nanospheres, delivered in a watery suspension, traveled throughout the circulatory system and accumulated in the liver, where they were collected by bacteria-destroying cells. Once in the liver, the nanospheres enhanced tissue reflectivity when examined through ultrasound. Changes in liver reflectivity could be seen 15 minutes after injection and lasted for up to 1.5 hours.

Thomas Rosol and Jun Liu use nanoparticles to enhance the ultrasound imaging of liver tissue in mice. The team hopes to use nanoparticles to detect small cancer lesions.

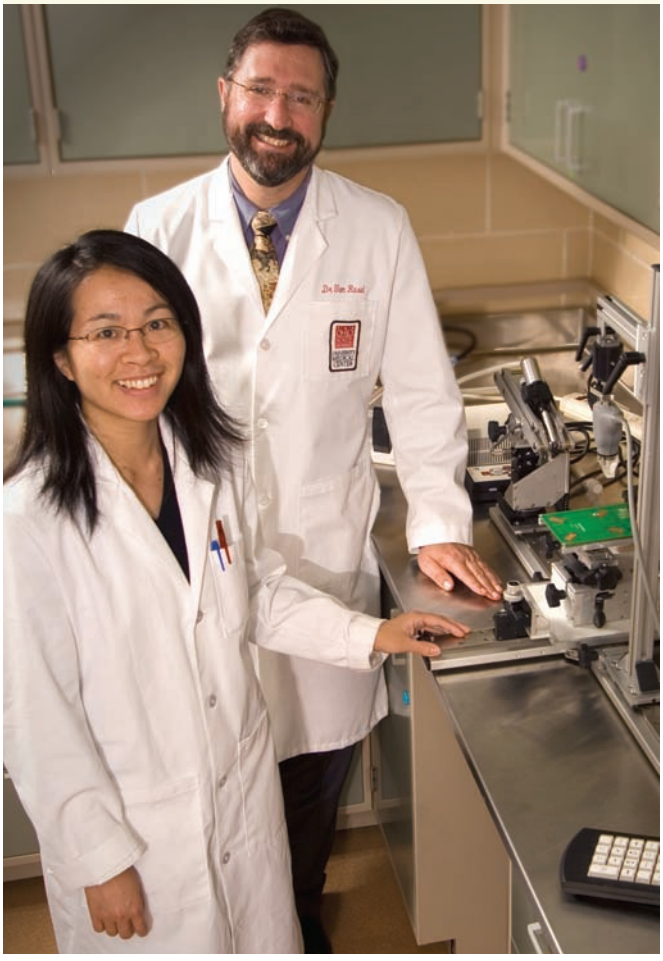


PHOTO BY J. R. HARVEY, COLLEGE OF VETERINARY MEDICINE, OHIO STATE UNIVERSITY

Rosol and Liu found that brightness of the liver tissue was directly correlated to the concentration of nanospheres delivered. Their data suggest that nanospheres can indeed be used as tiny imaging agents.

Nanospheres are ideally sized for clinical use because bigger particles, those larger than a micron, are quickly recognized and cleared by the body. Nanospheres not only temporarily evade recognition—thus providing extended time for imaging—but also are small enough to fit through the pores of a tumor's leaky blood vessels and reach its cells.

In future studies, Rosol and Liu plan to attach the nanospheres to antibodies that bind to tumor cells. A large concentration of these nanosphere-labeled antibodies, lodged in a small tumor lesion, could then be detected via ultrasound. "Traditionally, ultrasound cannot see very small lesions. So the goal is to use the nanospheres to detect these lesions before the tumor starts to get too big," says Rosol.

Rosol and Liu noted no adverse effects of the nanospheres up to two weeks post-injection. "These particles are so small that they are taken up by the macrophages and can be eliminated through the lungs or the gastrointestinal system. Nonetheless, we eventually want to use biodegradable nanoparticles," says Rosol.

Rosol's research was supported through NCRR's Midcareer Investigator Award (K26) in Mouse Pathobiology Research, which he has leveraged to obtain additional funding. "By using preliminary data from the grant, we were successful in obtaining a Shared Instrumentation Grant from NCRR to purchase a Micro CT scanner," he says. The K26 award allows established pathobiologists, like Rosol, to spend up to 50 percent of their time performing research and mentoring the next generation of mouse pathobiologists. (*Phys. Med. Biol.* 51:2179-2189, 2006)

—AL STAROPOLI

NCRR RESOURCES: The Midcareer Investigator Award (K26) in Mouse Pathobiology Research provides support for pathobiologists within 15 years of completing their specialty training. Grantees act as mentors for beginning investigators, thereby increasing the pool of researchers who can conduct mouse pathology studies. This award provides up to five years of support. For more information on this and other funding opportunities in comparative medicine, visit www.ncrr.nih.gov/compmed/cm_rcdtf.asp.

Additional support for this research comes from the Susan G. Komen Breast Cancer Foundation, the National Cancer Institute, and the National Science Foundation.

Developing Essential Research Tools

Grants create shared resources for preclinical research.

Steven Britton has spent 10 years of his life breeding some of the fittest rats on earth. These rats can literally outrun others in their species. He also breeds their exact opposite—rats so unfit they are prone to disease. The latter are used as models for studying complex disorders such as hypertension, diabetes, and coronary heart disease.

Development of these specialized rats is funded by a Resource-Related Research Grant (R24), awarded by NCRR. These five-year grants support projects that develop resources for the biomedical research community. R24s have been used by grantees to develop immunological reagents, gene chips, computer models, and a wide variety of specialized animals—all of which are eventually made accessible to investigators. "Once you have a proven model, researchers should not have to spend their time reinventing the wheel," says Raymond O'Neill, a health scientist administrator with NCRR's Division of Comparative Medicine. "This is advantageous because it saves time and money."

Genetic analysis of unfit rats, seen here on a treadmill, may reveal markers for predisposition to cardiovascular disease. With NCRR funding, these specially bred rats are now available to investigators worldwide.

Britton, a professor of molecular and integrative physiology at the University of Michigan in Ann Arbor, began using rats to study hypertension about a decade ago. To study this complex disorder, he needed a rat with characteristics that closely resembled the disease. Rat models available in the mid-1990s were developed by selective breeding based solely on the trait of high blood pressure. But these single-trait models were not complex enough to mimic the multifaceted features of human hypertension.

So in 1996, Britton and his colleague Lauren Koch embarked on a quest to develop a better rat model, one that closely mirrored the complex disease he wanted to study. They began with 168 rats, running each on a treadmill to determine its level of aerobic running capacity. Britton and Koch then bred the fittest with the fittest and the least fit with the least fit, and did likewise with their offspring. By the 15th generation, they had created two groups of rats, one that was highly fit and another that was highly unfit.

In the end, the fittest rats could, on average, outdistance the unfit rats by a factor of 6. The unfit rats not only were slower but also showed a marked predisposition to disease. This was expected, as Britton worked on the supposition that low aerobic capacity is a strong predictor of disease and, eventually, mortality. Britton believes that genetic analysis of the unfit rats will reveal markers for predisposition to complex diseases, such as cardiovascular disease and diabetes.

In 2003, while still developing his rat model, Britton received an R24 grant from NCRR. "The R24 absolutely made the difference between doing everything on a small scale and working on a larger scale with numerous laboratories performing experiments that could not be done in one lab," he says.

Since then, Britton and Koch have selectively bred hundreds of rats that have been distributed to laboratories worldwide. "The rats are in high demand, even without advertising. Requests result mainly from meetings and publications," says Britton. Today, the rats are provided to investigators free of charge. "If we didn't have the R24, developing these rat models would be a limiting factor in research," says Britton.

Keith Reimann received an R24 grant to develop research tools for a completely different disease: AIDS. In 1997, Reimann and Joern Schmitz began producing reagents, composed of recombinant antibodies, that could be administered to nonhuman primates to study the AIDS viruses. Their reagents have

PHOTO BY MARTIN VIOLET, UNIVERSITY OF MICHIGAN IN ANN ARBOR

been useful in evaluating candidate AIDS vaccines and understanding AIDS pathogenesis. Reimann leads the NIH Nonhuman Primate Reagent Resource, housed at Beth Israel Deaconess Medical Center in Boston.

To understand a complex immune system response to a virus like AIDS, Reimann administers reagents to disarm specific parts of the nonhuman primate immune system, such as particular subsets of T cells. In doing this, he can determine what role these cells play in defending the body against a viral attack. The use of reagents is, in essence, a complicated game of isolating variables in order to understand the immune system's multifaceted disease-fighting mechanisms.

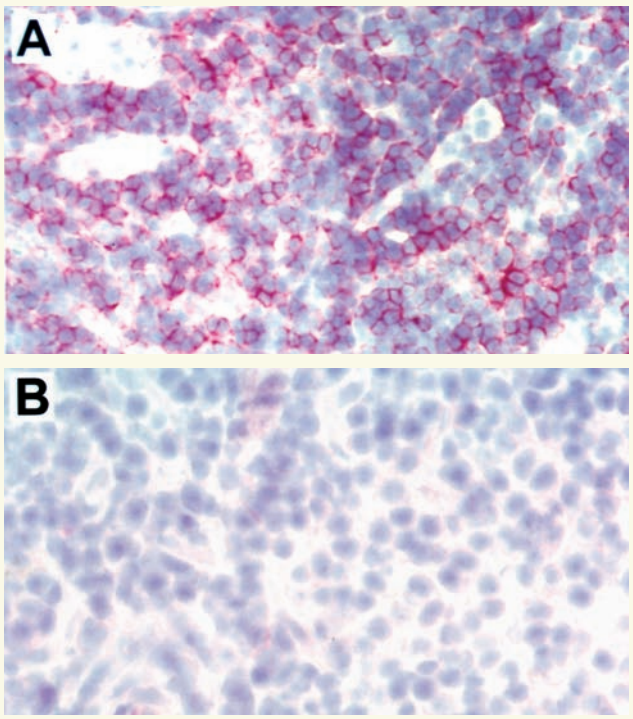
Reimann first produced recombinant antibodies for his own AIDS studies because reagents for use in nonhuman primates were not available through commercial sources. This is still the case today. "If you pick up a commercial reagent catalog, you'll see an extensive selection of reagents for rats and mice, because there is a huge market for those," Reimann says. "However, there is little economic incentive for the private sector to develop reagents specifically for nonhuman primates."

When other researchers learned of Reimann's effective reagents, requests began to trickle in. By sharing his reagents, Reimann hoped to save others the time and effort of producing reagents from scratch, thereby accelerating their research. "It's a tall order to produce the quality and quantity of reagents needed for administration to nonhuman primates," says Reimann. The reagents worked so well that soon he was swamped with requests.

"I went to an NIH official and said 'I'm getting overwhelmed with providing these reagents to other investigators. What do you think we should do to help meet these needs?' " In 1999, Reimann learned of NCRR's new R24 awards, and a year later he received his first grant. "Whether it's a new antibody developed in our lab, or one obtained by agreement from a pharmaceutical company, the R24 has allowed me to produce and distribute validated reagents to other investigators," he says.

Last year alone, Reimann provided reagents for use in nonhuman primate studies to more than 40 investigators funded by eight NIH Institutes and Centers. The reagents also have been distributed internationally. Reagents are distributed either free of charge or at the cost of production.

Reimann's reagents also are used to study infectious diseases other than AIDS. Over the last five years, the Nonhuman Primate Reagent Resource has provided reagents for the study of Ebola, hepatitis B, tuberculosis, malaria, measles, and even the flu. Aside from studying complex diseases, the reagents also are used for basic research in understanding fundamental phenomena of the immune system, such as the regulation of T cells.



■ *Lymph node tissues before (A) and after (B) a reagent has depleted red-stained immune cells. Investigators can now use these reagents to study immune responses in nonhuman primates.*

Investigators designing complex, multiyear studies can depend on standardized reagents provided by Reimann, because they have been successfully used and tested by the scientific community. "If these reagents were not available, some types of studies would not be done," says NCRR's O'Neill. "Research related to AIDS vaccines could be slowed down tremendously."

In 2004 Reimann's work was supplemented by additional funding from the National Institute of Allergy and Infectious Diseases, which has expanded the scope of the resource to develop new reagents. With a multiyear renewal of his R24 from NCRR, Reimann hopes to develop new reagents for future studies. "The R24 is really designed to serve other investigators, so they can optimize use of their resources," he says.

—AL STAROPOLI

APPLY FOR FUNDING: NCRR's Resource-Related Research Grants (R24s) are designed to encourage investigators to develop or improve resources that can be shared with the biomedical community as a whole. Resources are defined as animal models, cell cultures, or computer/mathematical models that have the potential of becoming well used by other researchers. Applicants for these grants, which offer up to five years of support, must demonstrate a need for the resource in the biomedical research community.

For information about R24 and other investigator-initiated grants from NCRR's Division of Comparative Medicine, visit www.ncrr.nih.gov/compmed/cm_rpg.asp.

PHOTO COURTESY OF NIH NONHUMAN PRIMATE REAGENT RESOURCE

Peptide Delivers Poison to Cancer Cells

Cargo-carrying molecule injects cells with therapeutic payloads.

BY ELIZABETH TRACEY

The word "flip" may bring to mind a sizzling pancake, flipped fresh from the griddle, or perhaps a gymnast's graceful, inverted dismount from a balance beam. These elegant maneuvers move an object from one unique environment to another. Researchers Yana Reshetnyak, Donald Engelman, Oleg Andreev, and colleagues co-opted the word, and its concept—but changed the spelling to pHLIP—to identify a new chemical tool that can "flip" a drug or other molecule from the outside to the inside of a diseased cell. In their most recent studies, the scientists showed that pHLIP can deliver a deadly mushroom-derived poison, known as phalloidin, across the cell membrane and into cancerous cells.

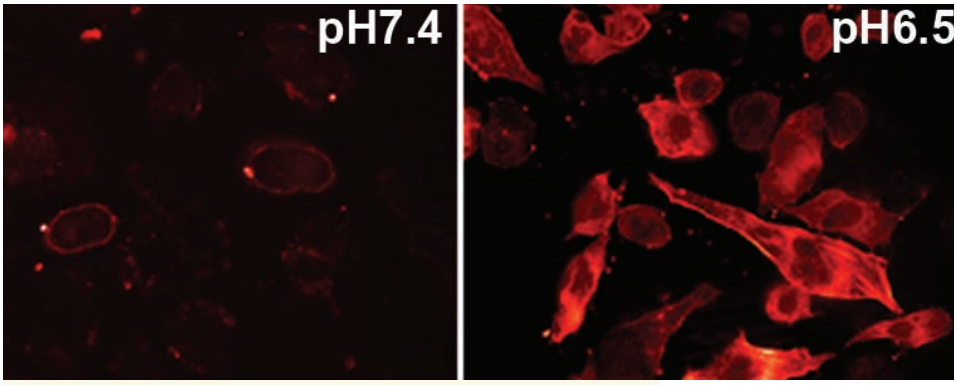
"pHLIP stands for pH(low) insertion peptide, which describes the action of the peptide when it's in an acidic environment," says Engelman, professor of biophysics and biochemistry at Yale University in New Haven, Connecticut. "In acidic conditions, the peptide inserts itself into the cell membrane, and it has the capability to transport a cargo across the membrane." This type of cargo-maneuvering peptide may eventually prove useful as a therapy, because many human disorders—including cancer and stroke—are associated with acidic extracellular environments, which have a low pH.

pHLIP is related to a helical polypeptide first identified in Engelman's lab nearly a decade ago. The polypeptide was derived from the C segment of a protein known as bacteriorhodopsin. In contrast to other lipid-favoring helices from the same source, the polypeptide identified by Engelman and colleagues was soluble in a watery environment. But studies of this molecule stalled until Reshetnyak, as a postdoctoral fellow, and Andreev, as a visiting scientist in Engelman's lab, resurrected interest in this unusual peptide, subsequently characterizing it and more completely describing its behavior. Now assistant professors of physics at the University of Rhode Island in Kingston, Reshetnyak and Andreev today rely on NCRR funding that created the Rhode Island Network for Molecular Toxicology, which supports the development of research facilities and the mentoring of junior investigators. (See box.)

"When the pH becomes more acidic, the polypeptide becomes more hydrophobic and would rather be in a water-free environment, like the cell membrane," says Reshetnyak. "Therefore, the peptide inserts itself into the membrane, which is an energetically more favorable state. Some researchers might find this behavior to be a disadvantage, but I thought it was a great advantage. It creates high selectivity, because the peptide inserts itself only in acidic surroundings."

Reshetnyak investigated how the peptide exists in solution, whether it forms aggregates, and how it releases energy. She found that, in an acidic environment, one end of the peptide moves into the cell, while the other end remains outside. She also observed that the efficiency of the insertion increased as the pH decreased.

To evaluate the peptide's ability to translocate, or carry, other molecules across the cell membrane, Reshetnyak and colleagues



The red-labeled toxin phalloidin is barely visible when bound to the peptide pHLIP outside of cancer cells (left) but becomes prominent when the pH is lowered (right) and pHLIP transports the toxin through cell membranes.

synthesized a new version of the original polypeptide, adding a single cysteine residue to one end—and pHLIP was born. The additional cysteine allows easy attachment of select cargo molecules via a chemical bond that is stable outside the cell but breaks apart inside the cell. When the cargo-carrying end of pHLIP moves through the cellular membrane, the internal environment of the cell cleaves the bond and releases the cargo.

"We assessed the ability of pHLIP to translocate a cargo of dye molecules into cultured cancer cells," Reshetnyak says. "We found that the dye accumulated intracellularly and that the lower the pH, the greater the dye uptake."

Reshetnyak and her colleagues could see that cancer cells were an ideal testing ground for pHLIP, because the environment surrounding cancer cells is known to be acidic. Cancerous cells have an accelerated metabolic rate and rapidly pump protons into the extracellular space. Phalloidin, a toxin from the *Amanita phalloides* mushroom, was chosen to study delivery of a cyclic peptide cargo to cancer cells.

Phalloidin has long been used by researchers to study cell division and the functions of the cytoskeleton, because it inhibits these activities. But the toxin is too soluble in water to get across the cell membrane. With his expertise in cell biology and cancer, Andreev recognized the therapeutic potential of phalloidin in slowing the proliferation of cancer cells, but the cell mem-

INBRE Grants Give a Competitive Edge

Funding from NCRR's IDeA Network of Biomedical Research Excellence (INBRE) Program created the Rhode Island Network for Molecular Toxicology. This support allowed the University of Rhode Island to purchase state-of-the-art instrumentation and create core research facilities for biophysical and biomedical studies—all critical to Yana Reshetnyak's work with the cargo-delivering peptide known as pHLIP. Reshetnyak credits INBRE funding with helping to advance her investigations of pHLIP and to compete successfully for a three-year research grant—awarded last year by the U.S. Department of Defense—to study pHLIP's potential applications to prostate cancer.

Reshetnyak's success represents an important goal of the INBRE Program, which was designed to enhance research opportunities and boost the number of competitive investigators in states, like Rhode Island, that typically receive a lower share of competitive funding from NIH. To broaden the geographic distribution of federal research funding, NIH has designated 23 states and Puerto Rico as being eligible for infrastructure-enhancing grants from NCRR's Institutional Development Award (IDeA) Program. (For more information about the IDeA Program, visit www.ncrr.nih.gov/resinfra/ri_idap.asp.)

The Rhode Island network, one of more than 20 INBRE-supported networks nationwide, seeks to support and develop talented scientists—especially junior investigators like Reshetnyak—and build a productive multisite program for collaborative research in molecular toxicology. The Rhode Island network includes a state-of-the-art core research facility, established in 2002. The facility, the only one of its kind in the state, offers access to advanced instrumentation for proteomics research, cell culturing and imaging, and chemical analysis.

The biomedical studies of Yana Reshetnyak (left) and Oleg Andreev (right) are enhanced by INBRE funding, which provides access to mentoring and advanced instrumentation at the University of Rhode Island.

COURTESY OF YALE UNIVERSITY (TOP),
COURTESY OF UNIVERSITY OF RHODE ISLAND (RIGHT)

brane was a major barrier to its use.

"Those who have worked with phalloidin in the past have used techniques to disrupt the cell membrane, allowing the toxin to enter," says Andreev. "We suspected that attaching phalloidin to pHLIP might permit delivery into living cells, with pHLIP selecting cancer cells because of the acidic environment. Once inside, we expected the toxin to inhibit cell contractility and division."

Tissue culture work demonstrated that the pHLIP is able to deliver phalloidin into cells, and once there, the toxin does inhibit cell division. Over time, precancerous cells form but cannot divide, and ultimately die. "Now we're working with a mouse model of breast cancer using pHLIP and phalloidin," says Reshetnyak. "We've seen that once the mouse is injected with the peptide, it localizes to the tumor because of the acidic environment. The ratio of how much of the peptide is found in the tumor, compared to how much is found elsewhere, is about 5:1," Reshetnyak says.

Another exciting application for pHLIP is the delivery of peptide nucleic acids, or PNAs, into cells. "These molecules are similar to nucleic acids like DNA and RNA, but PNAs have a polypeptide backbone rather than the sugar backbone found in DNA and RNA," Engelman explains. "PNAs are difficult to deliver across the cell membrane, and this is where pHLIP may prove useful." Intracellular delivery of PNAs might eventually allow scientists to inhibit or enhance the activities of specific genes. Once again, the acidic environment outside cancer cells would allow such agents to localize to tumors and insert the PNA cargo. Tissue culture work to date has shown that pHLIP can insert a 12-base PNA into a cell.

Engelman, Reshetnyak, and Andreev predict that pHLIP could be helpful in any disease or condition where extracellular acidic conditions exist. A short list might include atherosclerosis, stroke-damaged tissue, sites of infection or inflammation, or tissue damaged by trauma. Preliminary studies in vivo show that pHLIP can localize to aggregations of cancer cells that are still too small to be seen, suggesting a role for the peptide in the early identification and perhaps treatment of metastatic lesions. Early identification of minute tumors in a screening capacity may be another possibility.

"We feel fortunate that chance has favored the prepared mind," says Engelman. "We still have quite a lot of work to do, and we'll need an industrial partner to develop pHLIP for clinical applications, but our studies have been surprisingly promising to date."

The research described in this article is supported in part by NCRR, the National Institute of General Medical Sciences, and the U.S. Department of Defense.

ADDITIONAL READING: Reshetnyak, Y. K., Andreev, O. A., Lehnert, U., and Engelman, D. M. Translocation of molecules into cells by pH-dependent insertion of a transmembrane helix. *Proc Natl Acad Sci USA* 103:6460-6465, 2006.

NEWS FROM NCRR

People, Awards, Grants, and New Developments

Workshops Plot Future Directions

NCRR recently sponsored three workshops for the biomedical community. The workshops convened experts to discuss new genetic tools for rhesus macaques, network connectivity for biomedical research, and the inclusion of support for clinical and translational research in the nationwide health information network.

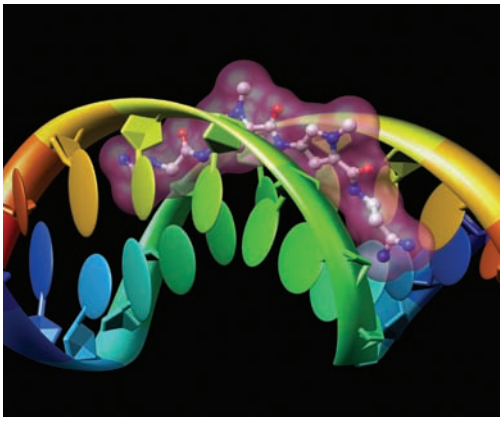
The first workshop, "Genetic Tools for Optimizing the Use of Rhesus Macaques for Translational Research," was held April 19-20 on the NIH campus. Participants summarized the characteristics and current uses of existing rhesus-specific genetics tools, such as physical and genetic maps, databases, microarrays, and the rhesus genome sequence. Experts then discussed the application of genetic tools and unmet needs in specific research areas, including transplantation, aging, cardiovascular disease, AIDS research, emerging infectious diseases and neurobiology. Participants delineated future needs and recommended the specific genetic tools that should be developed. Further refinement and development of genetic tools for the rhesus macaque should enhance the use of this critical animal model for translational research.

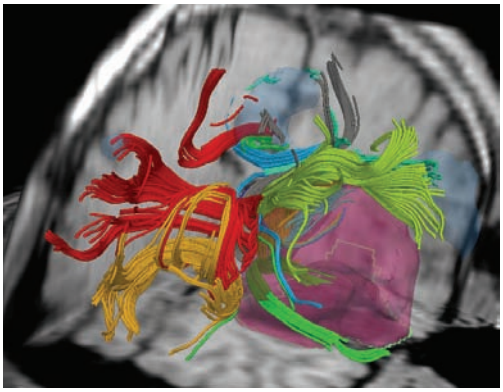
A second workshop, "Supporting Connectivity for Biomedical Research," was held on April 24 in Arlington, Virginia. Networking experts, biomedical researchers, and representatives from government and private industry identified and discussed key challenges to improving network connectivity and utilization across a broad spectrum of users, including those with access to cutting-edge networks and those who have little or no connectivity. The charge to the group was to examine best practices for implementing collaborative research networks and to identify key needs and priorities for cyberinfrastructure development during the next several years. The workshop encouraged efforts to strengthen and build new partnerships among funding agencies, academic organizations, and the private sector to better coordinate, expand, and optimize investments in network infrastructure. The workshop also sought to leverage intersections between biomedical research and

health care to broaden community participation in health research and facilitate development of clinical and translational research networks. The workshop was co-sponsored by NCRR and the U.S. Army Medical Research and Materiel Command.

On May 9, investigators, researchers, physicians, industry representatives, and policy analysts convened in Chevy Chase, Maryland, for the workshop "Ensuring the Inclusion of Clinical Research in the Nationwide Health Information Network (NHIN)." The meeting aimed to create a plan to incorporate support for clinical and translational research as part of the emerging nationwide health information exchanges that build on the increasing adoption of electronic health record systems. Participants discussed the ability of the NHIN to support various types of clinical research, including clinical trials of new drugs. One of the specific uses discussed was more efficient and inclusive discovery of potential participants in clinical studies and trials. The meeting was jointly sponsored by NCRR, the Agency for Healthcare Research and Quality (AHRQ), and the nonprofit organization FasterCures.

For more information on these and other NCRR-supported workshops, visit www.esi-bethesda.com/ncrrworkshops/workshops.htm. ■



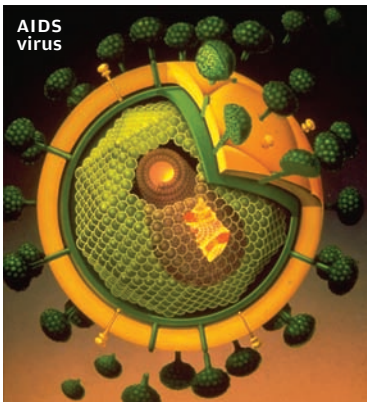


Biotechnology for Tomorrow's Clinic

Principal investigators and staff from Biomedical Technology (BT) Resource Centers around the country convened on June 19-20 for the meeting "Creating Biotechnology for Tomorrow's Clinic," held in Bethesda, Maryland. Topics discussed included medical devices, therapies, and diagnostics; use of computation in modeling and visualization; proteomics and glycomics; and image-guided therapy. The meeting, co-sponsored by NCRR and the National Institute of Biomedical Imaging and Bioengineering, allowed participants to present scientific achievements, foster alliances among centers, and support emerging technologies that can advance biomedical research.

Technologies developed at NCRR-funded centers include a versatile molecular graphics software known as Chimera (top) and a computational technique that models the changing shapes of brain structures during surgery (bottom).

NCRR's Division of Biomedical Technology funds more than 50 specialized BT Resource Centers, which support the discovery, development, and dissemination of powerful leading-edge technologies that have broad applications to the study of biology and medicine. Over the years, the Resource Centers have been responsible for the development of several critical technologies, including magnetic resonance imaging, multi-photon microscopy, and use of synchrotron X-rays for structural biology. The BT Resource Centers focus primarily on the development of core technologies but also are involved in training, dissemination, and collaborations. For more information about the NCRR-supported centers, visit www.ncrr.nih.gov/ncrr prog/btdir/btdirectory.asp. ■



Upcoming AIDS Symposium

The 24th Annual Symposium on Nonhuman Primate Models for AIDS will be held on October 4-7, 2006, in Atlanta, Georgia. Participants will discuss the latest AIDS-related findings in primate virology, immunology, pathogenesis, vaccines, therapeutics, and genetics. The meeting will be hosted by the Yerkes National Primate Research Center (NPRC) at Emory University. Funding is provided by NCRR and the National Institute of Allergy and Infectious Diseases.

For more information, visit www.yerkes.emory.edu/NHPM2006. The Yerkes NPRC is one of eight National Primate Research Centers supported by NCRR. Additional information about NCRR's primate center program can be found at www.ncrr.nih.gov/compmed/cm_nprc.asp. ■

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