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SPECIAL EDITION: NATIONAL SECURITY

William R. Wiley
EMSL NEWS
Environmental Molecular Sciences Laboratory

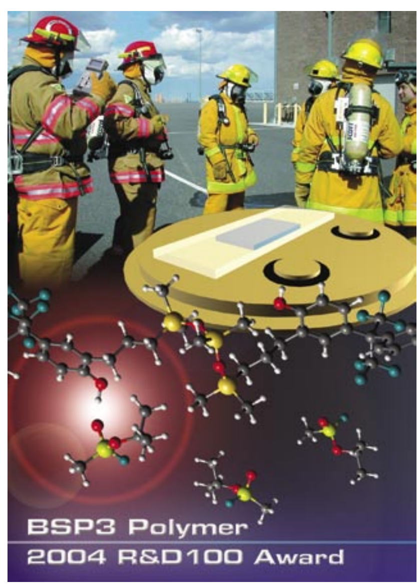
An Award-Winning Chemical Vapor Sensor

Technology provides increased sensitivity to detect airborne chemical agents

Many toxic nerve agents—such as the deadly odorless and colorless sarin—could be used in terrorist acts against populated areas, such as densely packed mass transit systems or crowded office buildings. To mitigate the possibility of chemical warfare-based terrorism within our nation, the U.S. Department of Energy's National Nuclear Security Administration (NNSA) has provided support for the development of real-time detectors that can detect chemical agents in the air quickly and accurately, especially those at low concentrations.

For the past 12 years, Jay Grate has led development of chemical vapor sensors and arrays, using in part sensor development and test equipment housed in Grate's EMSL laboratory—along with EMSL's clean-room facilities, materials synthesis equipment, and surface science characterization equipment. Detection capabilities are created and optimized by coating the surfaces of surface acoustic wave sensors with novel carbosiloxane polymers developed by Grate and his collaborators. These polymers are then able to collect and concentrate vapor molecules from the airborne gas onto the sensor surface through reversible sorption.

One such polymer, the BSP3 Polymer, was designed and synthesized for use in sensors in portable and fixed array-based chemical detectors. Each sensor in the array is coated with a different polymer so that the responses of the array provide a "fingerprint" of a given analyte vapor. When chemical vapors are detected via the sensor, the pattern of responses from the array is processed automatically by pattern recognition methods to allow the capability to recognize and distinguish one compound from another.



Chemical vapor sensor research and development has been ongoing at the Pacific Northwest National Laboratory and in EMSL. Developers of a higher-sensitivity sensor—the BSP3 Polymer—received a prestigious R&D 100 Award in 2004.

Sensors coated with BSP3 Polymer were found to be four times more sensitive than prior similar technologies, and in some systems enabled faster detection to lower concentrations of vapor than was previously possible. In 2004, the BSP3 Polymer garnered a prestigious R&D 100 Award. In addition, Grate and his collaborators have been awarded three patents for their chemical vapor sensor development efforts, with additional patents pending, and have published more than 20 peer-reviewed journal articles during the last several years.

Besides funding from NNSA, much of the sensor development and test equipment housed at EMSL was procured by Grate using a combination of internal funding and funding from EMSL's construction budget. This equipment has been used by EMSL users to evaluate sensor materials, preconcentrators, and sensor technologies.

Dr. Allison Campbell

FROM THE DIRECTOR:

Research Partnerships Addressing National Challenges

This edition of EMSL News is the first in a series that will highlight collaborative research forged between EMSL and the PNNL research directorates to develop **new capabilities in science areas of national importance**—including national security, energy, environmental remediation and more—that EMSL can also **provide to the broader user community**. Within this issue you will see a sampling of the important work we are doing in the national security arena, the impact this work is having, and insights on future research directions at EMSL and how the broader scientific user community can participate.

EMSL is a national scientific user facility dedicated to providing integrated experimental and computational resources to the scientific community in an open, accessible environment. I believe our combination of world-class minds, methods and capabilities uniquely positions EMSL to deliver answers to the questions that matter most to the scientists, the science community and the nation. Access to EMSL and the instrumentation and staff expertise discussed in this newsletter is available through a user proposal process located on EMSL's website (www.emsl.pnl.gov).

We hope you find EMSL News interesting, informative and a useful resource for information on research activities here in EMSL. *– Allison*

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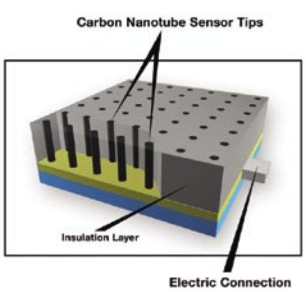
Ongoing Nanotube Research Results in Miniscule Biosensors



Carbon nanotube—10,000 times smaller than a human hair—is regarded as one of the materials of choice for the next generation of biosensors because of its strong electrocatalytic activity and capability to minimize fouling of sensor surfaces.

For the past three years, researchers at PNNL have been studying these minuscule materials for biosensor applications as funded through PNNL's Laboratory-Directed Research and Development program. The research paid off in 2004, when PNNL researcher and EMSL user Yuehe Lin claimed the first successful lab tests of a glucose biosensor and then an organophosphate sensor developed using carbon nanotubes.

Organophosphate compounds are used in pesticides and as raw materials for nerve agents. To construct the sensor, Lin chemically fused carbon nanotubes to an acetycholinesterase enzyme, which served as a biocatalyst. A 2- by 4-millimeter sensor surface was then peppered with carbon-nanotube bits and their fused enzymes. Enzyme activity has been found to dampen in the presence of organophosphate. Acting as electrodes, the nanotubes sensed the enzyme inhibition as a muted signal and passed that information to an off-the-shelf electrochemical detector, which showed an instant reading of organophosphate at traces of as little as 5 parts per billion.

Much of the research conducted by Lin and his coworkers has benefited using the state-of-the-art, high-speed/high-sensitivity electrochemical equipment located in EMSL's Advanced Electrochemical Facility.

Continued development of biosensors constructed of carbon nanotube material promises to be instrumental in detecting chemical signatures that can lead to early warning of chemical warfare, the quick screening of chemical warfare agents for border and transportation security, the rapid identification of chemical warfare agents for first responders, and forensic analysis.

* *Much of the technical information in this article taken from the Spring/Summer 2004 issue of the PNNL magazine* Breakthroughs.

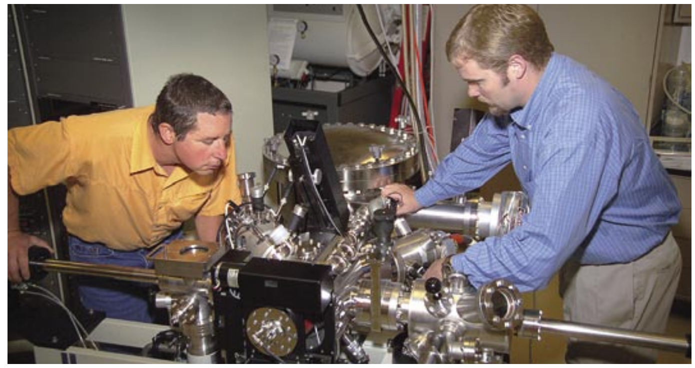
Mass Spectrometry Scratches Surface of Bioforensics

If an envelope delivered through a mail-handling facility contained a white, powdery substance, scientific experts would be able to analyze the envelope's contents and quickly determine the type of bacterial spores in their presence—such as anthrax—down to the individual strain.

However, even now, the same experts could not readily tell you how the spores were grown, where they were grown, or how they got into that envelope in the first place. With the relatively new and emerging field of bioforensics, researchers at EMSL and PNNL are working at the behest of the Homeland Security Initiative, a PNNL Laboratory-Directed Research and Development initiative, to solve the mystery these questions pose—specifically, under what conditions the spores were grown.

"Bioforensics is a fairly new field to which researchers are still becoming acquainted," said EMSL researcher Dan Gaspar, who is helping to lead the project with users John Cliff, Karen Wahl, and Dave Wunschel of PNNL's National Security Directorate; Kris Jarman of PNNL's Computational and Information Sciences Directorate; Nancy Valentine of PNNL's Environmental Technology Directorate; and Steve Golledge of the University of Oregon. "We're leveraging more than a year of experience in bioforensics and are making concrete progress in this arena."

One of the keys to investigating growth media lies with a surface analysis technique known as time-of-flight secondary ion mass spectrometry (ToF-SIMS), which the researchers are currently using to study elemental metal signatures in bacteria in order to gather clues about the media in which they were grown.



A time-of-flight secondary ion mass spectrometer, such as the one shown here at EMSL with EMSL researcher Dan Gaspar (right) and PNNL researcher John Cliff (left), is instrumental to the emerging field of bioforensics.

ToF-SIMS involves the bombardment of a surface with finely focused primary ions (in the researchers' case, oxygen and gold clusters), effectively damaging the surface and causing electrons and positive and negative ions to "shoot" from the surface. Ions of one polarity or the other are then collected and analyzed using time-of-flight mass spectrometry to obtain signature data such as which metals are present in the sample. Statistical models are then generated to differentiate the signatures due to different media.

The researchers began this effort in 2004 by evaluating four different growth media to demonstrate that the technique could be used to distinguish the medium in which a particular spore type was grown. The method is now being extended to other questions related to the actual composition of the media. A paper on the subject—one of the first to Gaspar's knowledge—is currently in press with *Applied and Environmental Microbiology*.

Chemical Compounds "Fingerprinted" by Infrared Spectral Library

When the World Trade Center Twin Towers collapsed and burned on September 11, 2001, the smoke was thought to contain several toxic gases such as phosgene due to burnt Freons. To facilitate hazardous materials response in the chaotic aftermath, infrared remote sensing systems and a vapor-phase infrared library called Northwest Infrared were used to identify the specific gases emitted to the atmosphere.

Six years in the making at EMSL and PNNL, Northwest Infrared was funded by the National Security Directorate and the U.S. Department of Energy's National Nuclear Security Administration's Office of Nonproliferation Research and Engineering as a sound method for identifying potentially harmful gases that can infiltrate the atmosphere.

Northwest Infrared provides high-resolution "fingerprints" of chemicals.

The library provides high-resolution, quantitative "fingerprints" of more than 400 chemical compounds obtained by Fourier transform infrared (FTIR) spectrometers in EMSL. While the library has generally been used by academia and industry to study

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›› *Fingerprinted*, cont'd from page 3:

atmospheric pollution and smokestack emissions, the technology possesses great potential application to national security, where it can be used to identify precursors to deadly nerve agents and facilitate hazardous materials response. A handful of Northwest Infrared's more-than 200 users currently registered to use the system represent the Department of Homeland Security.

To assemble the library, researchers first used FTIR spectrometry to study the light that is absorbed by certain vapor-phase chemicals. When infrared light shines on the chemical compounds, the compounds vibrate as the light is absorbed. Each compound is unique and will only vibrate at certain characteristic frequencies, providing a spectrum containing the distinctive fingerprint for that particular chemical compound. The spectrum is then placed in the library, where registered users can identify their unknown spectra by comparing them against those contained in the library.

"There are maybe only two other databases in the world—and they are much smaller—that come close to the quality of our database," said PNNL researcher and EMSL user Steve Sharpe, who was instrumental in development of the library. "This is certainly the highest quality data one can get publicly."

Much of the instrumentation in Sharpe's EMSL lab was procured to support this research—including a Bruker 120HR FTIR spectrometer that plays a key role. Much of the instrumentation is commercially available; however, it has been modified to support development and growth of the library.

The research not only addresses the gas phase, but also is developing a similar database for liquids. While the latter represents a client-only endeavor, the gas-phase portion (nwir.pnl.gov) is publicly available, with unlimited access following a $200 one-time access fee, which is funneled into website maintenance.

Hallmark Proteins of Plague Bacterium Come to Light

Yersinia pestis, the causative agent of plague, is considered by the Centers for Disease Control (CDC) as a high-priority organism for study because of its potential use for bioterrorism. While the infamous bubonic plague is commonly transmitted through the bite of an infected flea, pneumonic plague is transmitted when a person inhales *Y. pestis*.

The Department of Homeland Security (DHS) is funding joint research between PNNL and Lawrence Livermore National Laboratory (LLNL) to characterize the proteomes of *Y. pestis* and the highly infectious microbe that causes tularemia, *Francisella tularensis*, another microbe of high priority to the CDC. Future studies may also focus on *Bacillus anthracis*, the bacterium responsible for anthrax.

PNNL scientist Kim Hixson uses a ThermoFinnigan LTQ mass spectrometer, one of many instruments in EMSL's High-Performance Mass Spectrometry Facility used by Mary Lipton's research team to identify peptides from potential bioterror organisms.

EMSL user Mary Lipton (PNNL) has already completed proteome analyses of the three pathogenic *Yersinia* species: *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*. Lipton plans to complete six more proteomes by year end with collaborators Kim Hixson, an EMSL scientist, and Luther Lindler, a pathogen microbiologist at the Walter Reed Army Institute of Research, who will provide the biomass samples. Recent *Yersinia* samples were provided by Sandra McCutchen-Maloney of LLNL.

The research began in 2003 and was originally funded by the U.S. Department of Energy's Chemical and Biological Nonproliferation Program to better understand *Y. pestis*' pathogenicity—a focus on what proteins make the organism infectious and their mechanisms. When the DHS was formed, however, the scope changed to biomarker discovery and validation to identify the proteins that indicate exposure to disease.

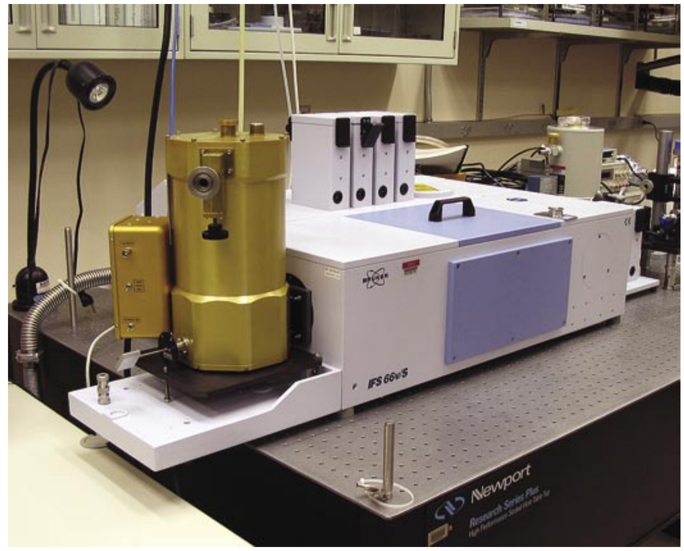
The process involves comparison of spectrometric analyses of enzymatically digested protein samples to an accurate mass and time (AMT) tag marker database. The AMT tag process helps researchers identify protein biomarkers in an organism by correlating raw measurements from liquid chromatographic separations and diverse mass spectrometers.

The research uses EMSL's full suite of LTQ and Fourier transform ion cyclotron resonance (FTICR) mass spectrometers, including the 11.5- and 9.4-tesla FTICR mass spectrometers for ultra-high-resolution sensitivity.

"EMSL houses all of the instruments and the proteomic infrastructure that we need—it's like one-stop shopping. And there's a lot of support," said Lipton.

Library Developed as Quick, Reliable Bacteria Identification Tool

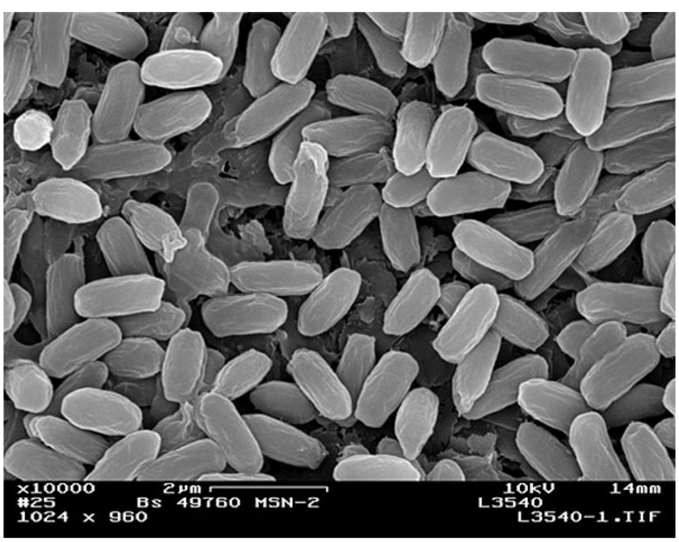
In October 2001, fear struck the nation when a white, powdery substance began appearing randomly in pieces of mail. A handful of those who inadvertently inhaled the substance perished after what they believed was a cold and sore throat quickly worsened to severe breathing problems and shock. The substance was identified as the spore-forming bacterium *Bacillus anthracis*, which causes the acute infectious disease anthrax.



EMSL's FTIR spectrometer is an instrumental tool for developing the bacterial spore library.

During the past three years, the Department of Homeland Security and the U.S. Department of Energy's National Nuclear Security Administration (NNSA) have funded researchers at PNNL and EMSL to develop a tool to allow for quick, reliable identification of such potentially deadly bacteria in the event of biological warfare. The result is a library of spectra—or "spectral signatures"—of bacterial spores that were grown in PNNL laboratories and analyzed using Fourier transform infrared (FTIR) spectroscopy at EMSL. Similar in theory and protocol to the Northwest Infrared database (see accompanying article), the library provides a set of reference data so that spectra of unknown bacterial spores can be quickly matched to a library signature and identified.

To develop and grow the library, PNNL microbiologist and EMSL user Nancy Valentine first cultured and grew several *Bacillus* species in her PNNL laboratory. Her collaborator, PNNL researcher Tim Johnson, along with several researchers from EMSL and PNNL's research directorates, then used EMSL's FTIR spectroscopy laboratory resources to distinguish between the various *Bacillus* types (e.g., the relatively innocuous *Bacillus subtilis* versus the harmful *Bacillus anthracis*) as well as between *Bacillus* and other non-biological materials (e.g., *Bacillus anthracis* versus talcum powder). To ensure the greatest data reliability, the researchers reproduced the data to make sure the same type of *Bacillus* cultured on different days and in various conditions generated the same results—as subtle, yet reproducible, differences in the spectra can help distinguish one *Bacillus* from another.



Spores of *Bacillus subtilis 49760*, as it is cultured in preparation for inclusion in the bacterial spore library. EMSL capabilities were used to analyze these bacterial spores and obtain spectra to populate the bacterial spore library.

Now that the researchers can easily distinguish among *Bacillus* species and possibly even strains, and as the library grows, they are moving towards the emerging field of bioforensics—in other words, not only identifying various *Bacillus*, but determining the source of *Bacillus* culturing. Johnson, Valentine, and their collaborators have obtained preliminary and encouraging results using surrogate *Bacillus*; however, further research in this area will require use of a combination of analytical techniques and associated experts to attempt to determine the sources of *Bacillus*. Some of these methods include FTIR spectroscopy, matrix-assisted laser desorption ionization, secondary ion mass spectrometry, liquid chromatography, and gas chromatography-mass spectrometry. Many of these techniques, along with the associated scientific expertise, are available at EMSL and PNNL.

Besides funding from the Department of Homeland Security and NNSA, this research is supported by PNNL's Laboratory-Directed Research and Development program.

BEADS *Breeds Successful Automated Sample Preparation System*

A few years ago, "bioterrorism" was a mostly unfamiliar term; today, it is a household word. Bioterrorism threatens the components of our environment—the air we breathe, the water we drink, the food we eat, and the soil we sow. When suspected of being tainted with biothreats such as bacteria or chemical toxins, samples of these molecularly complex environmental resources must be obtained and analyzed without delay to mitigate the potential impact.

Currently funded by the Department of Homeland Security, the Biodetection Enabling Analyte Delivery System (BEADS) is an automated, mobile sample preparation and analyte detection system developed by researchers at PNNL. The system can be used to analyze trace amounts of pathogens and toxins in complex samples on location. BEADS' modular design means it can be tailored to a remarkable range of applications, which—along with its portability—makes it an exceptional solution to monitoring the environment.

BEADS' design overcomes the often cumbersome practice of manually preparing a sample for delivery to analyte detectors by quickly and automatically purifying and concentrating the sample in modular steps. The prepared sample is then delivered directly to an integrated detection device. BEADS can use almost all existing bead types such as polymer, glass, or magnetic beads. The surface of the beads is tailored for the application by attaching specific recognition molecules, such as DNA oligonucleotides or antibodies, to enable concentration, purification, and detection of the corresponding bioanalyte.

PNNL scientist Cynthia Bruckner-Lea began work on the technology 10 years ago. Several of the capabilities housed in EMSL's High-Field Magnetic Resonance Facility and Interfacial and Nanoscale Science Facility have been used at one time or another during the decade of BEADS development, including automated fluidic systems and surface analysis equipment, high-resolution x-ray photoelectron spectroscopy equipment, and nuclear magnetic resonance instruments for the characterization of bead surface chemistries and quantum dot reporters used for detection.

"Having all of the necessary instrumentation in one location is unique," said Bruckner-Lea. "The rate of development was also aided by the proximity of chemists, biologists, and engineers within a single facility."

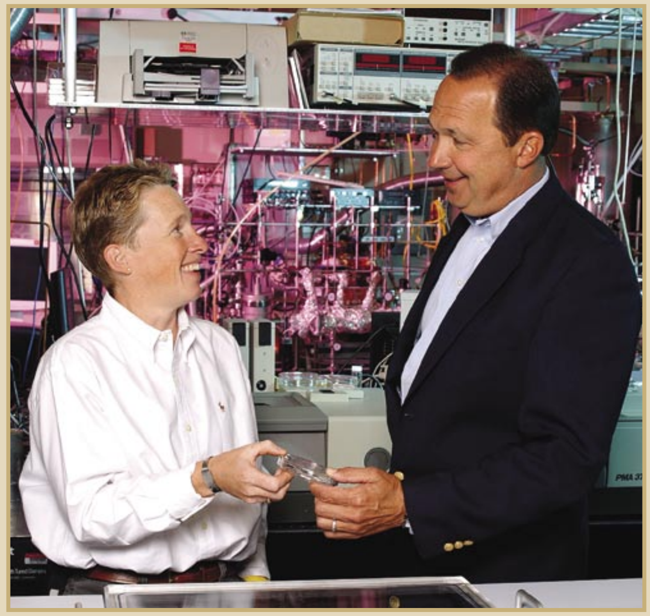
Initially, the development of BEADS involved characterizing the functionalized surfaces required for concentrating and purifying biological analytes and demonstrating detection-limit performance in modules. With the aid of EMSL's Instrument Development Laboratory, all functions have been engineered into one automated system, and now prototypes are being developed for testing for certain applications.

BEADS technology has resulted in more than 10 publications and has earned several patents during the past decade. Besides the support of the Department of Homeland Security, entities that have provided support in the past include the National Aeronautics and Space Administration, the U.S. Navy, the U.S. Department of Energy's Chemical and Biological Nonproliferation Program, and the America Waterworks Association Research Foundation in conjunction with the Environmental Protection Agency.

PNNL researcher Cindy Bruckner-Lea used several of the resources in EMSL to develop BEADS, an automated sample preparation and analyte detection system.

A Question and Answer Session with Allison Campbell *and* Mike Kluse

PNNL's National Security Directorate (NSD) boasts one of the fastest growing areas of research at the Laboratory, with more than $384 million in business volume projected for Fiscal Year 2006. Several key NSD research projects critical to the defense, nonproliferation, intelligence, and homeland security needs of the nation are, or in the past have been, conducted using the unique capabilities housed in EMSL. Mike Kluse, PNNL Associate Laboratory Director for National Security, and Allison Campbell, EMSL Director, discussed some of the connections between NSD research and EMSL.



How has EMSL supported the national security work that has been ongoing at PNNL?

Campbell—EMSL has a robust user program in place that allows many of our users whose research focuses on national security to easily access the facility's state-of-the-art instrumentation and expert staff necessary to drive their work. In fact, we provide several of these researchers with permanent office and laboratory space to facilitate their work not only to support research in national security areas, but also to support EMSL's mission to create an integrated environment for discovery and technological innovation in pursuit of the nation's most difficult and critical scientific challenges.

Kluse—EMSL has been and will continue to be an important partner for NSD. We excel at delivering solutions to our clients that are based on innovative science, and much of that science comes from use of EMSL resources. For example, some of the "pioneer" NSD projects at EMSL involved development of the Biodetection Enabling Analyte Delivery System—an automated, mobile sample preparation and analyte detection system—and chemical vapor sensors. This research is now being further developed for detecting toxins with funding from the Department of Homeland Security, so these early projects continue to benefit today from the use of EMSL capabilities.

In turn, how is the NSD work benefiting EMSL?

Campbell—Our research relationship with NSD has allowed us to leverage resources to purchase state-of-the-art equipment that can serve the needs of immediate national security clients, as well as a broader base of users in those research areas. For example, use of our instrument development, spectral signature, mass spectrometry, and nanomaterial capabilities is allowing NSD researchers to develop microtechnologies for military applications, support quantum cascade laser technology development, and detect and obtain trace signatures of weapons of mass destruction. This research and development is earning the respect of our NSD clients and continues to enhance the reputation of EMSL as a state-of-the-art national user facility, as well.

What areas of national security are addressed using the instrument and staff resources in EMSL?

Kluse—EMSL supports every aspect of our national security mission: nuclear nonproliferation, homeland security, defense, and intelligence. It is a key resource with capabilities and facilities that cut across our business markets. All of our clients want to be able to identify smaller quantities of threatening substances such as chemical weapons, and to do so more quickly and remotely, and then be able to develop actionable intelligence as a result. The expertise housed in EMSL helps us understand the science of detection and analysis so that we can improve our detection and prediction capabilities.

What are some examples of NSD products and the resources at EMSL used to help develop these products?

Campbell—One excellent example is the ongoing spectral signature research, an area that has provided great benefit to our homeland security and defense clients, especially following 9/11. EMSL's infrared spectral technologies, such as its suite of leading-edge Fourier transform infrared

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›› *Q&A*, continued from page 7:

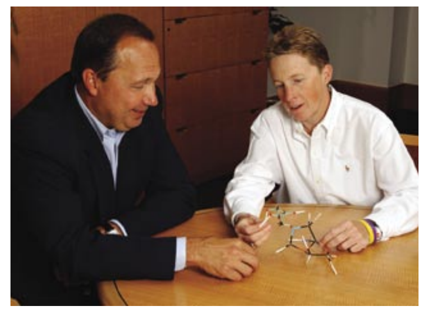
spectroscopy and spectrometry instruments, have been significant to the development of libraries used for quick identification of harmful bacterial spores, such as those that cause anthrax, and gas- and liquid-phase agents that can be used by terrorists for chemical warfare.

Kluse—Another fine example is the use of EMSL's suite of state-of-the-art mass spectrometry instrumentation to support quick identification of deadly "bugs" that can be used in biological warfare, such as *Yersinia pestis*, the causative agent of the plague. Understanding the biomarkers of *Y. pestis* can aid in detection, post-infection treatment, and vaccine development for this lethal pathogen.

What is the future of the connection between PNNL's National Security Directorate and EMSL?

Kluse—Ours is a long-term partnership. In the next year, we expect to begin devising how the National Security Directorate can partner with EMSL to address a significant challenge facing the homeland security community. In fact, I believe we have a unique opportunity to address a biosecurity issue. EMSL staff have expertise in addressing so-called "Grand Challenges," or the big problems facing the scientific community. In the homeland security science and technology complex, biosecurity is of particular interest. We'll be venturing into that arena to identify areas where EMSL and PNNL could fill an important need. I consider EMSL and the fundamental science work conducted in support of the Department of Energy Office of Science to be key resources as we grow our national security business. We will continue to use these capabilities to support our efforts in addressing pressing national and homeland security challenges.

Campbell—The relationship between EMSL and NSD is a shining example of what is possible. Through this work, we have been able to provide critical tools to address critical problems in the national security arena. We give clients and users alike a means to achieve tangible results, and I see that continuing and growing for years to come.



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