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Environmental Molecular Sciences Laboratory

William R. Wiley EMSL Environmental Molecular Sciences Laboratory

EMSL Assisting Purdue Professor with Unraveling a Signal Transduction Mystery

Professor Sandra Rossie decided to spend her six-month sabbatical at EMSL after hearing PNNL's Dr. Richard Smith describe the facility's state-of-the-art mass spectrometry capabilities at a workshop on neuroproteomics.

Rossie—an associate professor of biochemistry at Purdue University—is facing a most challenging biological puzzle: defining the physiological substrates affected by protein phosphatase 5 (PP5) and how this enzyme's activity is regulated.

Reversible phosphorylation—adding and removing phosphate groups on proteins—is a central mechanism by which signals such as hormones control cellular processes. Phosphatases such as PP5, together with protein kinases, are responsible for this process.

A key challenge for Rossie and others studying hormone and neurotransmitter signaling is to identify the substrates for specific kinases and phosphatases. Identifying these substrates can reveal exactly what job or cellular processes these enzymes are controlling.

"When PP5 was discovered several years ago," says Rossie, "No one knew what its job in the cell was." Subsequent literature and research provided clues to PP5's cellular job—involvement in controlling cell growth and proliferation, and a possible role in cellular response to stress hormones and catastrophic events such as generation of large amounts of reactive oxygen species. Now, the question focuses on the substrates of PP5.

"It is really a team effort. You have to have this kind of communication if you are going to get good data."

"As a biochemist, one looks for substrates—the cellular proteins attacked by PP5—because they can reveal the signaling pathways in which PP5 participates and how it controls processes such as cell growth or response to stress," says Rossie. "So basically, I'm on a hunt for the substrates for this enzyme. There are only two substrates that have been identified so far and there are likely to be many more."

According to Rossie, to answer this question, you have to ask the cell where this enzyme is located, what phosphoproteins it attacks, and under what circumstances it happens. Enter proteomics.

Proteomics may be defined as direct characterization of the proteins present in an organism, tissue, or cell

(see ***Signal Transduction***, page 4)

Purdue professor Sandra Rossie (center) studies PP5 with help from **EMSL's 9.4 Tesla, 160 mm bore FTICR mass spectrometer** and postdocs Robert Maxwell (left) and Feng Yang (seated) of the Biological Separations & Mass Spectrometry group.

Proposals for EMSL's 900-MHZ NMR spectrometer are expected to be accepted by Fall 2004.

900-MHz NMR User Access Coming Soon

User Access to EMSL's 900-MHz NMR Spectrometer Just Months Away

Thanks to the perseverance of staff from EMSL's High-Field Magnetic Resonance Facility, users are just months away from being able to access the world's largest, highest-performance nuclear magnetic resonance (NMR) spectrometer.

"We are now testing various samples that are appropriate for its capabilities," says David Hoyt, technical lead of the facility where the 900-MHz NMR spectrometer is housed. "We are currently bringing in specific projects that are of either large size or of a complexity that will require the additional resolution and sensitivity that the high field can provide."

User cases "road tested" thus far include peptide lipid complexes and protein-protein complexes, ranging from a molecular size of 25 kilodaltons to 65 kilodaltons. A large part of the testing involves fine-tuning and developing the instrument's critical solid-state probes.

"We've got three probes that are in the queue—MAS, or magic angle spinning probes for solid samples," says Hoyt.

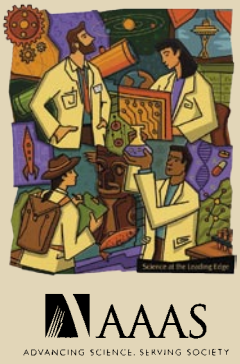
With the capabilities provided by these MAS probes, Hoyt expects to be able to accept biological solids projects requiring the use of the highest field. Such probes will allow users to not only study the typical nuclei—proton, carbon, and nitrogen—found in biosolids, but also will provide some capability to study nuclei that are rare. In addition, one of the probes will allow users to obtain orientation data from protein structures relative to solid surfaces.

"We expect to learn some pretty unique things about metal or metallic systems that aren't traditionally looked at from use of these solid-state probes," says Hoyt. "We're really 'ramping up' development of these probes to take advantage of what this instrument should be able to do."

Hoyt anticipates that the 900-MHz NMR spectrometer will be ready for full-time proposal acceptance by Fall 2004.

"We're running it through its paces right now," says Hoyt. "There are some very complicated systems that we have not been able to study as well at a lower field of which we expect to do better at a higher field—we're proving that to ourselves right now through this testing."

Proposals for using the 900-MHz NMR spectrometer can be submitted through EMSL's User System (*http://sos.emsl.pnl.gov:2080/EUS*).



EMSL User Workshop Held at AAAS Meeting in Seattle

Dr. Julia White, Technical Lead for EMSL User Services & Outreach, organized and presented an informational workshop featuring EMSL's proteomics mass spectrometry capabilities at this year's American Association for the Advancement of Science Meeting in Seattle.

The AAAS meeting provided a unique opportunity to interact with researchers performing cutting edge work in biology who were curious about the utility of proteomics and the applicability of that approach to their problems. Two potential collaborations were discussed, one involving work developing vaccinations for Malaria by identifying the proteins in the parasite that are exported and interact with the cells in the host.

The speakers included four PNNL scientists who presented overviews about EMSL facilities, equipment, and specific research examples:

- **Proteomics User Facilities: An Opportunity for Collaboration**
  Dr. George Michaels, Program Director – *Bioinformatics, Computational Biology & Biological Science Facility Development; Biological Sciences Division*
- **Applications of AMT-Based Proteomics**
  Dr. Mary Lipton, Senior Research Scientist – *Biological Separations & Mass Spectrometry; Biological Sciences Division*
- **Managing Large Datasets**
  Gordon Anderson, Technical Lead – *Instrument Development Lab; EMSL*
- **High-Throughput Proteomics at EMSL**
  Dr. Harold Udseth, Technical Lead – *High-Performance Mass Spectrometry Facility; EMSL*

EMSL June Meetings

In June 2004, EMSL staff will be hosting several major events. The first—**EMSL 2004**, the EMSL Users Meeting—will be held **June 14-16**. This year's meeting, "Bridging Technologies in Structural Biology," will feature discussion of current, cutting edge topics in such areas as high-throughput structural biology: nuclear magnetic resonance (NMR) and X-ray crystallography; small-angle neutron scattering; the pathway to the future; and research by EMSL users. A hands on tutorial will be provided on EMSL's virtual NMR capability.

In addition, EMSL Director Bill Rogers will lead discussion about the new EMSL user model, the EMSL user proposal system, the new Collaborative Access Teams, and the new Scientific Grand Challenges.

The **User Advisory Committee Meeting**, to be held at EMSL on **June 15 -16**, allows EMSL 2004 to include a "meet the User Advisory Committee" session that will provide the attending users with an opportunity to relay input about EMSL from a user perspective. In addition to these two concurrent meetings, EMSL's **Science Advisory Committee** will be holding its annual meeting at EMSL on **June 14**.

For more information, see the EMSL 2004 website at *http://www.emsl.pnl.gov/new/emsl2004* or contact EMSL User Services (509-376-2553 or userservices@emsl.pnl.gov).

EMSL 2004, Systems Biology, AVS, and Clay Mineral Societies Meetings on Summer Schedule

June 7 – 8
Northwest Symposium for Systems Biology

EMSL will be the location for the second annual interdisciplinary symposium as it presents the most recent advances in Systems Biology by scientists and researchers who are pioneering 21st century biology. Participants include leading multidisciplinary investigators who are applying novel computational and technical approaches to understanding complex biological systems. Featured topics on the importance of spatial and dynamic regulation of cell function through imaging and modelling include network dynamics, visualization of signaling, spatial regulation, and quantitative proteomics applied to cellular function.

For additional information or on-line registration, see the Northwest Symposium for Systems Biology website at *http://www.pnl.gov/northwestsymposium* or contact Marla Seguin (marla.seguin@pnl.gov).

June 15 – 18
Pacific Northwest Chapter of the American Vacuum Society Meeting

EMSL will host the 26th Annual Symposium on Applied Surface Analysis (Surface Analysis '04), to be held in conjunction with the 15th Annual Symposium of the Pacific Northwest Chapter of the American Vacuum Society. This year's topics include, but are not limited to, surface science; state-of-the-art methods of characterization; and application of surface science to material systems including semiconductors, oxides, minerals, ceramics, metals, polymers composites, and biomaterials.

For detailed information about the program or registration see the Surface Analysis '04 website at *http://www.emsl.pnl.gov/new/pnwavs2004* or contact Theva Thevuthasan (theva@pnl.gov) or Don Baer (don.baer@pnl.gov).

June 19 - 24
Clay Mineral Society Meeting

This year's theme, "Gorges, Clays, and Coulees," reflects the dramatic landscapes carved in the basalt bedrock of the region by the floodwaters of glacial Lake Missoula during the last Ice Age. This 41st international conference, held at the Red Lion Inn in Richland, Washington, will bring together leading clay scientists and engineers for a pre-meeting workshop focusing on methods for investigating microbial-mineral interactions, along with tours of EMSL; technical symposia (covering topics ranging from the chemistry of spectroscopy of clays to the impacts of microorganisms on clay reactivity); theme sessions; and regional field trips. For detailed information about the conference or to register, see the CMS2004 website at *http://www.pnl.gov/cms*. Contacts: Jim Amonette (jim.amonette@pnl.gov) and Paul Gassman (pl.gassman@pnl.gov).

Signal Transduction (continued from page 4)

under a given set of physiological or environmental conditions. Proteomics provides the ability to directly examine proteins and changes in proteins such as phosphorylation that occur during hormonal stimulation. Rossie's approach is to compare phosphoproteins from cells that have different levels of PP5 activity. This approach poses two challenges: each cell contains thousands of proteins, and the signaling proteins that PP5 may attack are rare.

"If you go looking for substrates in this way, you are looking for a small number of needles in a very large haystack," says Rossie. "You have to be able to isolate the phosphoproteins and identify them. This requires analyzing a large number of samples with very high sensitivity."

Thus, Rossie turned to EMSL. "EMSL provides access to resources that would be impossible for most individual investigators to build or obtain on their own." These resources include high-sensitivity, high-throughput mass spectrometers along with the scientific expertise in proteomics, which is what this research project requires.

"EMSL is driven to develop the technology to process many samples at a very high rate with high sensitivity. This permits one to ask questions about all the proteins in a cell and how they have changed in response to a signal such as a hormone," says Rossie.

In addition to EMSL's state-of-the-art instrumentation, Rossie is receiving help from EMSL's collaborative research teams with expertise in sample processing, mass spectrometry, and bioinformatics to run these experiments.

"Me—I want to know what the biological pathways of PP5 are," says Rossie. "It's not possible for me to be an expert in all of these areas."

One important part of this collaboration with EMSL, according to Rossie, is good communication.

"These teams cooperate with each other to a very high degree, and this helps them get the most information possible from a biological sample," says Rossie. "It is really a team effort. You have to have this kind of communication if you are going to get good data."

No doubt the EMSL experience will help Rossie find success in her quest.

"EMSL is a very impressive operation with a tremendously stimulating scientific environment," says Rossie. "There are a lot of very talented people here who can help a biologist like myself do proteomics at the most sophisticated level."

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The William R. Wiley Environmental Molecular Sciences Laboratory (EMSL) is a Department of Energy national scientific user facility located at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. The EMSL is operated by PNNL for the DOE Office of Biological and Environmental Research.

For additional details about the capabilities and research being performed at EMSL, please visit our web site at http://www.emsl.pnl.gov or call us at 509-376-2553.