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Science Made Possible

A Night-and-Day Difference

"Grand Challenge" demonstrates team-based science approach; provides new systems-level understanding of microbes important for biofuels and carbon sequestration.

In late 2004, when Dr. Himadri Pakrasi walked into the crowded EMSL Auditorium, he recognized only one scientist he knew. In the hours that followed, discussions circled around a single challenge: designing a multi-disciplinary, multi-institutional effort to understand the ways unique cyanobacteria harness energy from sunlight by day and generate their own fertilizer at night. The ultimate goal? To make discoveries that support engineering of such microbes for energy and environmental purposes.

Team Science The nearly 30-member Membrane Biology Grand Challenge team included multi-disciplinary experts from 6 university laboratories and 10 national laboratory groups. **Inset** Principal Investigator Dr. Himadri Pakrasi of Washington University in St. Louis.

The day was the original scoping meeting for the Membrane Biology Grand Challenge, which Pakrasi had learned about through an advertisement in *Science*. His team at Washington University in St. Louis (WUSTL) had submitted a proposal, and months later, he arrived to begin his work as Principal Investigator. The new research effort—to form large, integrated teams around key scientific questions—was initiated by EMSL, the Environmental Molecular Sciences Laboratory, a DOE national scientific user facility located at Pacific Northwest National Laboratory.

When Pakrasi left the auditorium that day, he had the feeling he had created vital relationships with several collaborating scientists. He was right. Fast forward through a half-dozen years—and past a small mountain of systems biology discoveries, achievements, spin-off products, and new projects—to the moment in May 2011 when Pakrasi revisited EMSL for the team's final wrap-up meeting. This time, he was essentially attending a family reunion.

"These are good friends," he said. "Many of them, like Dave Koppenaal [EMSL's chief technology officer and Co-PI of the Grand Challenge] and Steve Wiley [EMSL's lead biologist], are colleagues I pick up the phone and call to share research ideas. And they do the same."

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Getting Past "Alpha"

While relationships are valuable, the Grand Challenge's family-like team arose from a purely technical need. Exploring the complexities of the cyanobacterium called for true collaboration between experts from several fields.

"Early on, the team recognized how to function as a family. Each member understood the unique contributions of the others," Pakrasi remembered. "This is crucially important, especially in systems biology." This represented a hurdle many teams aren't able to overcome because of an ingrained focus on individual results.

"As scientists and technologists, we are traditionally trained to establish ourselves as 'alpha' operators," Pakrasi continued. "But this simply doesn't work when a team is trying to achieve a systems-level understanding."

What did work was to set up open channels for the sharing of both technical data and research ideas. Using data-sharing portals and meetings of all kinds—formal and informal, virtual and in-person—leaders enabled team members at all levels to keep their daily work firmly within the context of the broader team.

"After the first year or so, it became self-driven at the grassroots level," Pakrasi said. "From graduate students to PI's, everyone felt free to connect and collaborate with anyone else, and a mutual respect was fostered."

Exploring the complexities of the cyanobacterium called for true collaboration between experts from several fields.

Koppenaal added, "And the cool thing was: people started really supporting one another and pushing other team members to do better science. We began to speak one another's languages, across the different disciplines involved. A dataset one group thought was most interesting sometimes wasn't what the group across the country needed to move the project forward. So we educated each other."

Through these collaborations, a more complete picture of a complex biological system began to come into focus.

A Bug's Life

That original meeting laid the groundwork for the team and its work. Several scientists in the room who normally assume an "alpha" role were deciding the project's main objectives and how best to achieve them. Discussion turned to a unique feature of *Cyanothece* 51142, a particular cyanobacterial strain. The marine microbe shares a phenomenon with more complex organisms: circadian rhythms. Just as humans experience "jet lag," *Cyanothece* responds drastically to day-night cycles. Specifically, it separates nitrogen fixation and photosynthesis temporally in each cell—switching its molecular machinery twice daily to accommodate this separation. The presence or absence of light, in part, triggers a cascade of gene expression, protein translation, intercellular signaling, and shifts in membrane organization that lead to the functional changeover. The scientists in the room had established that cyanobacteria do this; they just didn't know the underlying mechanisms.

"Steve Wiley and I just looked at each other as if to say, 'Isn't this the grand challenge, then?'" Pakrasi remembers.

They recognized that an integrated, systems-level understanding of these processes could hold the keys to how *Cyanothece* might be put to work as a viable large-scale component of carbon sequestration or renewable energy production. But in the near term, these future innovations would require unprecedented discoveries of the overall system's energy production and cell cycling processes. During the core years of the project, from 2005 to 2008, this was the goal.

> The Membrane Biology Grand Challenge team generated the most comprehensive functional and quantitative analysis for any photosynthetic organism to date.

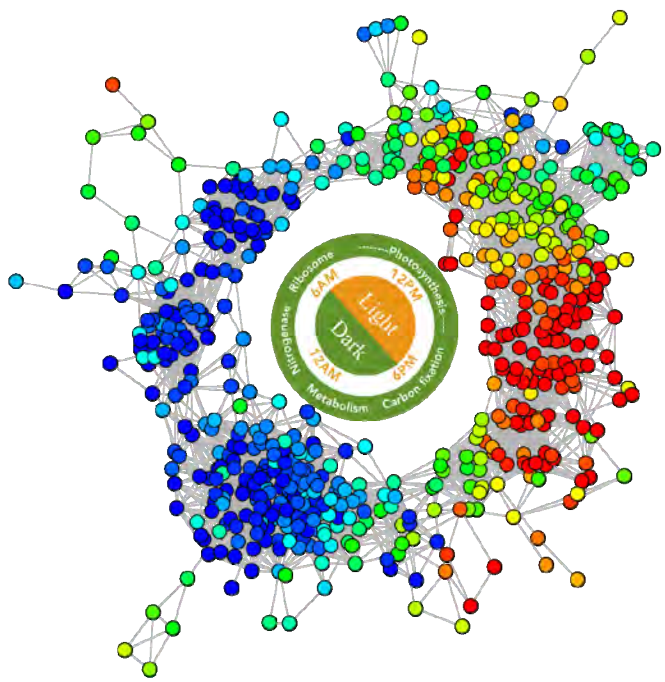
Game-Changing Outcomes

The team went to work, with members hailing from WUSTL, PNNL, EMSL, Purdue University, St. Louis University, the Donald Danforth Plant Science Center, and Shanghai Institute of Plant Physiology and Ecology. EMSL's extensive mass spectrometry and supercomputing capabilities were used to track *Cyanothece* protein responses to real-world conditions, helping the team generate the most comprehensive functional and quantitative analysis for any photosynthetic microbe to date. In doing so, they established *Cyanothece* as a new model organism in photosynthetic microbiology, creating a springboard for applications such as biofuels and carbon sequestration.

Members of the team published widely, stimulating new thinking in the research communities involved. They gave more than 30 professional talks and produced more than 30 scientific publications, including three in *Proceedings of the National Academy of Sciences*.

Resources for Research

Beyond results, the Grand Challenge team produced novel tools to accelerate research in a growing field. Perhaps most importantly, extensive new proteomic and metabolomics databases for *Cyanothece* have enabled exciting follow-on studies. The structures of several proteins were revealed, providing insights into functions within the organisms' energy-generating and carbon/nitrogen-fixing processes. In addition, the team established pure *Cyanothece* cultures and acquired genome information for seven *Cyanothece* species—from both marine and freshwater environments. Because the culturing process required better research hardware, the team used EMSL's in-house capabilities to design, fabricate, and



A Transcriptomic Wreath Network In this representation of a Cyanothece transcriptome (species ATCC 51146) under 12 hour light/dark cycles, each circle is a gene, colors indicate levels of expression (red is highest), and lines between genes indicate similar patterns of expression. The biological processes labeled arrange themselves on the wreath according to the environmental conditions and internal clock cues they require. This data visualization is one of many that allowed Grand Challenge scientists to derive detailed insights into a complex system in action.

deploy new photobioreactor systems.

The project required new software tools as well—the development of which was one of the project's original goals. Grand Challenge experiments resulted in new solutions for data integration across laboratories, and tools for managing, browsing, and interrogating cyanobacteria datasets, all of which are now available to the broader scientific community. Users can analyze cyanobacteria interactions of membrane proteins and membrane systems, energy production mechanisms, and the central "clock" apparatus that drives the back-and-forth switch between nitrogen fixation and photosynthetic energy production.

In addition to new knowledge, hardware, and software, team members developed novel procedures to cryo-freeze live *Cyanothece* cells such that inherent structural relationships of membranes, internal storage granules, and other organelles are preserved for observation. This allows new insights into photosynthetic membrane continuities and granule organization, using 3-D tomographic techniques to reconstruct entire volumes of the cell. For portions of the ultrastructure reconstruction, EMSL scientist Alice Dohnalkova won a Diatome U.S. First Place Award in August 2007.

A Grand Legacy

According to Pakrasi, the breadth and depth of these accomplishments are a result of tackling the problem with a highly functional, multi-disciplinary team. Moving forward, scientists interested in cyanobacteria are armed with the wide-ranging fruits of the team's labor. And in the quest for new clean energy and emissions control technologies, this makes a night-and-day difference.

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In one example that builds upon the knowledge base provided by the MBGC, a December 2010 *Nature Communications* paper by Pakrasi's Washington University team describes the largest volumes of hydrogen production ever recorded in natural cyanobacteria under normal atmospheric conditions. This result is promising for economically scalable microbial biofuels: while pursuing genetic modifications to increase feedstock production, the approach relies upon natural conditions and fertilizers, reducing costs.

Grand Challenge leaders also emphasize the effort's broader legacy—confirmation of a highly integrated team science approach.

"While it isn't a scientific result, we think of the team concept and how it was implemented as the overarching success of the Grand Challenge," said Koppenaal. "The scientific community is certainly putting the technical outcomes to work. But in new EMSL research campaigns, we're also transferring the concept of team science to new problems, and refining it for even greater impact."

In addition to Pakrasi and Koppenaal, key contributors to the Grand Challenge team include Louis A. Sherman of Purdue University, Rajeev Aurora of St. Louis University, Tom Smith of the Donald Danforth Plant Science Center, and Teruo Ogawa of the Shanghai Institute of Plant Physiology & Ecology, and many others from the institutions involved.

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Research: DOE Office of Science, Office of Biological and Environmental Research

Scientific User Facility: Environmental Molecular Science Laboratory

Selected Publications

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Contact

Himadri Pakrasi, Principal Investigator:
pakrasi@biology2.wustl.edu

David Koppenaal, Co-Principal Investigator:
david.koppenaal@pnnl.gov

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