



PRESS RELEASE

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HWI, U of R Scientists Make Breakthrough In Studies of Human Proteins Involved in Disease *Dr. Michael Malkowski and Dr. Elizabeth Grayhack's Work Published in Prestigious Scientific Journal*

Dr. Michael G. Malkowski, Hauptman-Woodward research scientist, in collaboration with University of Rochester yeast geneticist and research associate professor of biochemistry and biophysics Dr. Elizabeth Grayhack have made a remarkable discovery which has now been published in the prestigious Proceedings of the National Academy of Sciences.

Drs. Malkowski and Grayhack are collaborators on a project which has resulted in a way to more effectively and efficiently study the structure of human proteins involved in diseases.

They have solved a critical structural biology problem by utilizing the combined knowledge and skill set of yeast geneticists and x-ray crystallographers.

In the world of x-ray crystallography, a target protein is selected for study, the protein is produced in an organism (typically bacteria), it is then purified and crystallized. X-rays are then shot at the crystallized protein and the resulting diffraction pattern is solved to determine the three-dimensional structure of the protein. This information is used in rational drug design and to understand the root causes of disease.

It is a lengthy process and it all centers around production of large amounts of highly pure protein. Up until now many human proteins that are targets in drug development were not readily accessible because they could not be produced at high levels in *E. coli*. Many human proteins are insoluble in the bacterial host. While the yeast host is able to deal with the complexities of these proteins, it was unable to incorporate a heavy atom that is necessary for solving the crystal structure – and in fact, when that heavy atom was introduced it was fatal for the yeast.

But that dilemma has been solved.

Dr. Grayhack, along with research associate Erin Quartley, and the other members of her lab have developed a strain of yeast that allows scientists to incorporate a heavy atom into proteins grown in yeast.

“The need for a eukaryotic (yeast or human) host in which to produce proteins for x-ray crystallography is absolutely clear. Both we and other scientists have found that up to 70% of foreign proteins are insoluble in *E. coli*, and thus cannot be purified,” Grayhack said. “However, in order to use yeast as a source of proteins, we needed to figure out why selenomethionine (the source of the heavy atom in the crystal) could not be efficiently incorporated into proteins in yeast. We inferred that the cause of selenomethionine toxicity lay in a particular chemical conversion of selenomethionine and that genetic manipulation of the biochemical pathways to prevent this reaction would solve the problem.”

“While we were able to express these proteins, it was very difficult to solve the structures because the yeast had been unable to incorporate the heavy atom derivative into the protein. The heavy atom is used to solve the protein’s structure,” Malkowski said. “Dr Grayhack and her team have figured out how to manipulate the yeast genetics to make this work.”

“We have been able to work as the other half of this equation because as crystallographers we have shown this method works and can be used by the whole structural biology community,” Malkowski said. “We have used this yeast method, grown the crystals and solved a structure. This work is a breakthrough.”

What Reviewers Have Said About This Publication

“This is an excellent manuscript that reports an important advance for the production of selenomethionine-substituted proteins expressed in a eukaryotic (yeast) host. These findings promise significant impact for the field of protein structure determination by x-ray crystallography and hence of the myriad connected disciplines.”

“This is an excellent, clearly-written manuscript that reports an exciting result, namely, the construction of a yeast strain that when used as a host allows efficient labeling of recombinant proteins with selenomethionine. The breakthrough reported in this communication will provide an important alternative means of producing eukaryotic proteins for structural studies. As such, it should be received with great interest.”

“The authors have made a clever guess that the reason why selenomethionine incorporation into proteins in yeast is very difficult is due to conversion of SeMet to Se AdoMet. They then construct strains of yeast deficient in the two conversion enzymes and find a strain that can grow in SeMet and incorporate it into protein effectively.”

About Grayhack

Grayhack received her Ph.D in Biochemistry from Cornell University in Ithaca, NY and did postdoctoral work with Dr. Ira Herskowitz at the University of California at San Francisco. Grayhack works together with Eric Phizicky at the University of Rochester. Together with Stanley Fields at the University of Washington, they developed the biochemical genomics approach in which genomic collections of affinity tagged proteins are used to rapidly link biochemical activities to genes. The MORF library, their most recent genomic collection, was made in collaboration with Mark Dumont at Rochester and Mike Snyder at Yale. Analysis of protein expression from 5,573 MORF strains provided a critical tool for development of yeast as a source of proteins for x-ray crystallography and jump started their efforts with the Center for High-Throughput Structural Biology.

About Malkowski

Malkowski received his Ph.D. in Biochemistry from Wayne State University in Detroit, Michigan and his bachelor's degree in Biochemistry from the University of Detroit in Detroit, Michigan. He resides in Williamsville, New York. In addition to the research discussed above, the Malkowski laboratory also deals with the structural characterization and functional analysis of other enzymes involved in lipid metabolism. Malkowski is also the Project Manager and a co-PI for the Center for High-Throughput Structural Biology (CHTSB), housed at HWI, where he is involved in the development of tools for high-throughput characterization of membrane proteins. The CHTSB is one of six specialized research centers established nationally through the Protein Structure Initiative within the National Institute of General Medical Sciences at the NIH.

About HWI

With more than 50 years of exceptional scientific research, HWI is an independent, non-profit facility specializing in the area of fundamental biomedical research known as structural biology. Our team of more than 75 staff members is committed to improving human health by studying the causes of diseases, as well as potential therapies, at their basic molecular level. We are located in the heart of the Buffalo Niagara Medical Campus in downtown Buffalo, New York, in a new state-of-the-art structural biology research center at 700 Ellicott Street. For more information, visit HWI's website at www.hwi.buffalo.edu or call 716-898-8600.

About the University of Rochester

The University of Rochester, founded in 1850, is one of the nation's leading private universities. With just over 4,500 undergraduates, Rochester is one of the smallest and most collegiate in character among the top research universities.