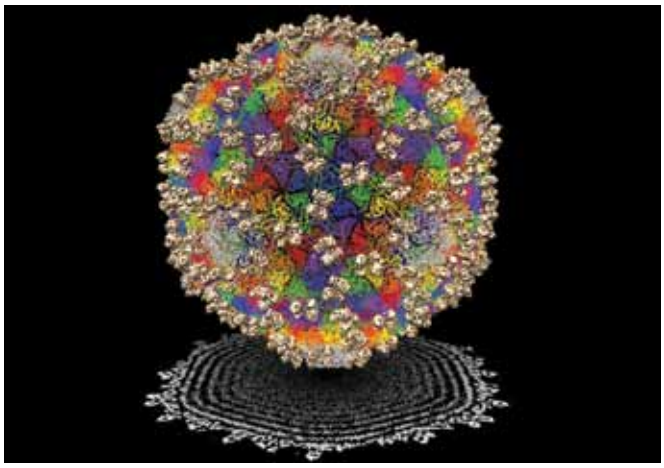
Peering More Deeply into Proteins

A new protocol developed at the National Center for Macromolecular Imaging (NCMI) by Director Wah Chiu and his colleagues provides greater insight into protein structures, which might lead to the design of better drug interventions, ultimately helping to improve human health. Proteins are not only dynamic; they often form large complexes of multiple molecules, which are difficult — and sometimes impossible — to see using the conventional techniques that probe molecular structures at the atomic level, such as X-ray crystallography or nuclear magnetic resonance. Supported in part by NCRR, NCMI focuses on specimens that cannot currently be studied by these techniques. The center works on developing three-dimensional images and atomic models of complex molecular machines that can guide the design of drugs and vaccines for a variety of diseases.

NCMI uses the technique of single-particle electron cryomicroscopy (cryo-EM), in which large molecules are rapidly frozen to preserve them in their natural state, yielding snapshots of the dynamic processes these molecules might undergo. However, unlike X-ray crystallography, which can provide atomic resolution with the aid of protein crystals, cryo-EM has been unable to achieve such resolution.

Chiu and his colleagues are changing that. They have developed a new, five-step computational protocol to obtain a higher-resolution structural model from electron images of molecular machines without making crystals. First, with the image data recorded in a 300 keV electron cryomicroscope, they use their home-built software, EMAN, to combine tens of thousands of two-dimensional images of molecules in random orientations and generate a three-dimensional volume density map. Then, they use an application called SSEHunter to find regions of the cryo-EM map that might correspond to structural elements like alpha-helices or beta-sheets. Once they identify these elements, Chiu and colleagues use a shape-analysis technique called skeletonization to determine how the elements are connected. They next look at the primary amino acid sequence of the protein to predict how the amino acid sequence relates to these structural elements. Once they make these assignments, they can accurately reconstruct the topology of the entire protein.



■ The National Center for Macromolecular Imaging has used its new electron cryomicroscopy protocol to reconstruct a model of the epsilon 15 virus, which infects salmonella. This model also shows the virus' DNA inside the capsid, the shell of proteins that protect the DNA.

"We use all these pieces of information to pin down where the protein begins, how it winds through space and where it ends," Chiu said.

Using this new protocol, Chiu and his colleagues have built a three-dimensional picture of the epsilon 15 virus shell, which is composed of hundreds of proteins, at near-atomic resolution. They also have validated the protocol by using it to look at another molecular machine (GroEL) for which the X-ray crystallographic structure is known.

NCMI has teamed up with researchers from various institutions, including Purdue University; Stanford University; the University of California, San Francisco; Massachusetts Institute of Technology; and the University of Washington, to further refine the new protocol. This refinement will allow NCMI to reconstruct models of protein complexes at even higher resolution and accuracy.

Chiu's protocol can offer new insights into how proteins and viruses work and, ultimately, into ways to improve health. Epsilon 15 is a virus that infects salmonella. Many people across the United States have recently suffered from salmonella poisoning related to peanut products.

"Bacterial viruses might not normally be viewed as medically relevant," Chiu said. "But understanding the structure of epsilon 15 and how it is destructive to salmonella bacteria could lead to the design of an intervention for salmonella poisoning. That's down the road, but thinking about translational

PHOTO BY MATTHEW BAKER FROM WEN JIANG ET AL., COURTESY OF *NATURE*

potential is natural with technology developed at a Biomedical Technology Research Center."

—FRANCES MCFARLAND HORNE

TO GAIN ACCESS: The National Center for Macromolecular Imaging (NCMI), funded in part by NCRR, is one of 52 NCRR-supported Biomedical Technology Research Centers (BTRCs). Investigators at NCMI collaborate to resolve structures of large, complex molecules. All mature image-processing software is freely available on the Web at http://ncmi.bcm.edu/ncmi/test_software. NCMI is also working with researchers at Washington University in St. Louis to develop a graphical toolkit to help other investigators use the new structure mining protocol. For more information about this and other BTRCs, visit www.ncrr.nih.gov/btrc.

ADDITIONAL READING: Ludtke, S.J., Baker, M.L., Chen, D.H., Song, J.L., Chuang, D.T., Chiu, W. De novo backbone trace of GroEL from single particle electron cryomicroscopy. *Structure* 16:441–448, 2007.

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A New Way to Preserve Fish

Some fish species, like zebrafish and medaka, have become important models for human disease research and developmental biology studies. For 15 years, Terrence Tiersch's laboratory at the Louisiana State University Agricultural Center has been preserving fish species by freezing their sperm to facilitate the sharing of these animals among researchers. With the thousands of mutant and transgenic fish lines created around the world, as well as U.S. Customs restrictions on shipping live animals, freezing fish sperm is an easier and more cost-efficient way to maintain and share the lines. Now, Tiersch's group has received funding from NCRR to standardize the freezing process and expand it to a wider scale.

■ For 15 years, Terrence Tiersch of the Louisiana State University Agricultural Center has been preserving fish species, such as the zebrafish shown here, by freezing their sperm. Currently, Tiersch and colleagues are creating a standardized, high-throughput process that the NCRR-supported Zebrafish International Resource Center and similar facilities can use to maintain fish lines.

PHOTO COURTESY OF TERRENCE R. TIERSCH

Currently, there is no standard process for freezing zebrafish or medaka sperm. Individual investigators find their own way, but these methods are often slow, laborious and inconsistent. "People have homemade recipes and borrow technologies from other animal models," said Michael Chang, program director at NCRR.

Indeed, for the past 10 years, Tiersch's group has been using a nearby commercial facility that freezes bull sperm. The facility allows Tiersch access to its equipment, but he has to use the same process for fish that is used for bulls, which is not ideal. Also, being very small, zebrafish and medaka might yield only three microliters — no more than a tiny drop — of sperm sample at most. And even though zebrafish and medaka are similar in size, they are different species, living in different environments, and their sperm behave differently. That means the conditions and requirements for freezing their sperm also differ.

Tiersch's group and three collaborating laboratories are creating a standardized, high-throughput process that stock and resource centers, such as the NCRR-supported Zebrafish International Resource Center, can use to maintain fish lines. The first phase of this work will go beyond freezing alone. The group will identify all the steps on the path, from the state of the fish before sperm collection, to the coding and databases needed to keep track of specimens, to the final distribution of samples to investigators. This work will generate a "first draft" process, which will be fine-tuned in the second phase in collaboration with the resource centers. The final draft will be a process that allows resource centers, which once needed a day to freeze a small number of sperm specimens, to freeze thousands of samples in a shorter period of time.

"We're not just developing a technique or saying things are feasible," Tiersch said. "We're past that. The small scale is already there. We're coming up with the industrial scale."

—FRANCES MCFARLAND HORNE

TO GAIN ACCESS: The new protocol for freezing zebrafish sperm will be available through resource centers, such as the NCRR-supported Zebrafish International Resource Center (http://zebrafish.org), which stores and maintains more than 1,000 different zebrafish lines. The new protocol will also be available to other resource facilities and research institutions that breed zebrafish and medaka.