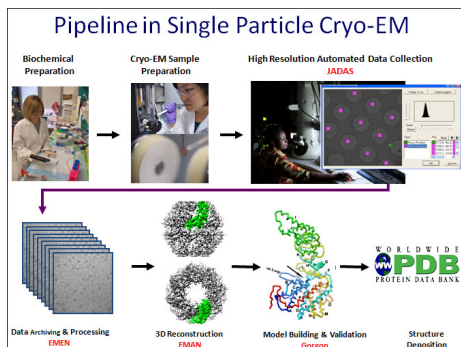
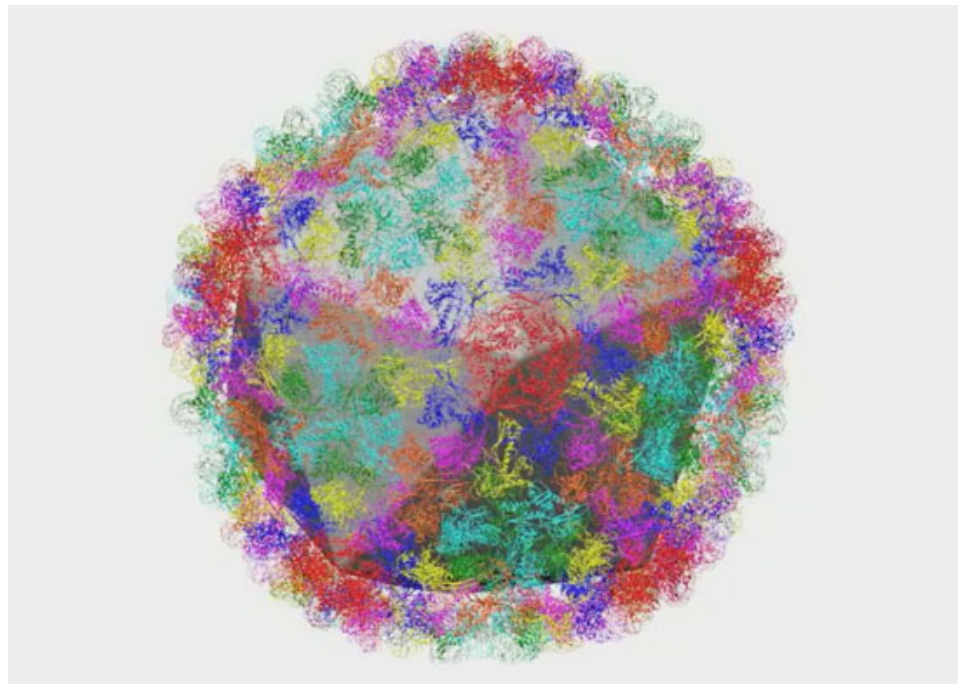


Sharpening Electron Imaging

TACC supercomputers help researchers at the National Center for Macromolecular Imaging define virus structures

The electron microscopes that Wah Chiu oversees can image a virus down to its individual protein side chains. Yet, questions remain regarding how to convert these images into useful information to aid physicians and scientists in the understanding and diagnosis of disease?

Chiu is the founder and director of the National Center for Macromolecular Imaging (NCMI) — a National Institutes of Health-funded center that provides cryo electron microscope (Cryo-EM) resources to the scientific and biomedical community, as well as the Center for Protein Folding Machinery. He is a leading developer of the computational methods through which Cryo-EM imaging becomes useful for a wide spectrum of applications.



Experimental procedure in single particle cryo-EM starting from biochemically purifying the protein complex made up of many protein components, freeze preserving the protein complex in a matrix of thin layer of vitreous ice, imaging the frozen, hydrated protein complex maintained at liquid nitrogen in a 300 kV electron microscope, classifying and aligning the 2D images of the protein complex in random orientations by image processing software, reconstructing the 3D image with tens of thousands of aligned images, modeling the individual protein components of the 3D density map of the protein complex, and depositing the map and associated models to EMDb databank and PDB. Click image to see larger version.

Chiu is exploring these issues with the goal of understanding enough about the architectural organization of the proteins that make up viruses to develop new

the dynamics of a bacteriophage procapsid, using a 3.8 angstrom cryo-EM map and model. therapeutic strategies by which infections can be limited.

To appreciate the problem, it is necessary to consider how cryo-EM microscopes work. Electron microscopes image specimens by shooting electrons at samples (rather than photons as in optical microscopes). These microscopes then capture the diffracting electrons to form a two-dimensional image, which contains structural details of molecules on a sub-nanometer scale. The technology also allows for the observation of frozen, hydrated biological specimens that have not been stained or chemically fixed in any way, showing them in their native solution environment.

Visualizing images from a single electron microscope sample is trivial. But to reconstruct data from 50,000 or 100,000 particle images to determine the three-dimensional location of atoms, and then to combine that data into a simplified visual model that humans can interpret, well, that requires advanced computers of the highest order.

“How do we extract useful three-dimensional information from multiple two-dimensional images, and then put them together in a coherent manner?” Chiu said. “This is exactly where the computing comes in.”

Chiu has been working at the interface of microscopes and advanced computers since early in his career, both with the National Science Foundation and National Institutes of Health. He saw early on that computers would be essential for interpreting microscopic images at near atomic resolution.

“The imaging capability was improving, the microscopes were getting better, and what I saw was needed was computing,” Chiu said. “That’s what I’ve put a lot of effort into in the last 10 years; to explore new image processing algorithms and to use high performance computers in our research.”

Recently, Chiu has been testing his Cryo-EM analysis methods using the Ranger supercomputer at the Texas Advanced Computing Center.

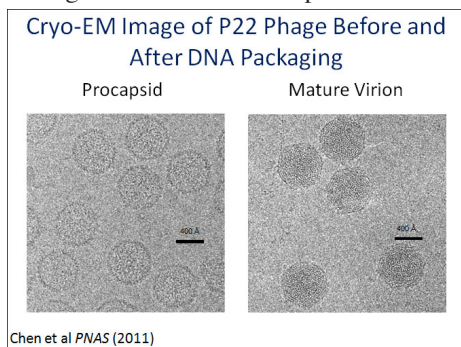
“The three-dimensional density map is extremely difficult for a human being to comprehend, so we need to simplify the representations of the density in terms of lines, curves, and the connectivity from one amino acid to another in a protein molecule,” Chiu said.

The process is iterative, with human insight feeding into and improving the computational models, so biological discoveries can be made.

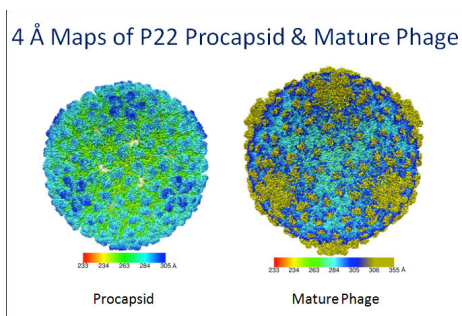
Unlike most applications that use high-performance computing, Chiu’s work is not a simulation.

“We’re doing image processing of an experimental observation,” he explained. “What we’re getting is not an imaginary photograph. It all originates from physical experiments.”

Working with colleagues at Baylor, Chiu resolved precise images of several bacterial and animal viruses to understand how they change shape and package the viral DNA during the virus maturation process.



Cryo-EM images of P22 phage before and after DNA packaging. Extracted from Chen, D. H., M. L. Baker, C. F. Hryc, F. Dimaio, J. Jakana, W. Wu, M. Dougherty, C. Haase-Pettingell, M. F. Schmid, W. Jiang, D. Baker, J. A. King, and W. Chiu. (2011). Structural basis for scaffolding-mediated assembly and maturation of a dsDNA virus. *Proc Natl Acad Sci U S A* 108:1355-60.



3D density maps of P22 procapsid and mature phage using ~20,000 particle images. The resolution of these maps is determined to be ~4 Å. From Chen, D. H., M. L. Baker, C. F. Hryc, F. Dimaio, J. Jakana, W. Wu, M. Dougherty, C. Haase-Pettingell, M. F. Schmid, W. Jiang, D. Baker, J. A. King, and W. Chiu. (2011). Structural basis for scaffolding-mediated assembly and maturation of a dsDNA virus. *Proc Natl Acad Sci U S A* 108:1355-60.

“Viruses are mere proteins and nucleic acids. They start with one protein and build the whole virus with multiple proteins forming a shell,” Chiu explained. “But the shell is not a static object. It can breathe and expand, and that expansion requires a structural rearrangement of the proteins. In our model, we were able to see that rearrangement and explain exactly how the change takes place within one protein and at the interfaces among all its neighboring proteins.”

The resolved models illustrated “before and after” images of the virus, first empty and then filled with viral DNA. To enact the transformation – a virtual ballooning – some amino acids are forced apart while others are moved closer to each other, making way for the negatively charged DNA to enter the virus from one entry and, simultaneously, for the scaffolding proteins to exit from another opening.

This discovery has important ramifications for many infectious diseases, including HIV, herpes, and bacteriophages.

Whereas most applications that run on supercomputers are highly parallel, Chiu’s method is “embarrassingly parallel” requiring many small, independent calculations to create a complete three-dimensional picture of the virus. Fortunately, Ranger and other TACC systems were designed to accommodate serial, high-throughput and other disparate research methods, all of which are required for science to advance.

“A lot of computer facilities are only

interested in the CPUs, but have no interest in real-world applications,” Chiu said. “TACC is interested in taking care of scientists like us, who have real world problems to solve.”

“Dr. Chiu’s research is an excellent example of the sort of work that we aspire to enable here at TACC,” said Michael Gonzales, TACC’s computational biology program director. “By leveraging the advanced computing technologies of our center, his research is providing critical insight into the fundamental physical properties governing viral infections.”

Chiu’s research findings have been published in the *Proceedings of the National Academy of Science* (Jan. and July 2011), *Structure* (May 2011), and the *Journal of Structural Biology* (May 2011).



Wah Chiu, founder and director of the National Center for Macromolecular Imaging (NCMI).

The NCMI assists more than 100 research projects a year, imaging samples relevant to disease and to the basic of understanding of life’s processes. As director of the center and lead developer on a number of community tools that allow for enhanced Cryo-EM images, Chiu is supporting discoveries around the world.

“Scientifically, these tools allows biologists to see things that they couldn’t see before, things that are closer to reality rather than idealized situations,” Chiu said. “Just as physicists study the hydrogen atom, but the periodic table has more than hydrogen, in a similar way, we can study more realistic systems to understand the biological activities, not as an isolated test, but in the cellular environments.”

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