

Freeze Frame Cryo-EM is a way to view protein structures—now at atomic resolution—as they do their thing in the biological world.

FOR AN ELECTRON MICROSCOPE, WHICH NEEDS A VACUUM to clear the air, water is the enemy. “It contaminates the sample, makes the vacuum dirty, and essentially destroys the experiment,” says Nikolaus Grigorieff, an HHMI investigator at Brandeis University. “Of course, in biology, water is the one substance that is present everywhere.”

The solution is to flash-freeze the sample and use the electron microscope, one of the most sensitive tools in science, to look at proteins or other biological structures trapped in a frozen matrix. In electron cryomicroscopy, or cryo-EM, researchers freeze a sample in liquid ethane so quickly that the watery environment becomes a glassy, vitreous solid before it can spoil the experiment or crystallize into regular ice. The living structure is preserved whole, suspended in time.

The technique has recently begun to achieve the degree of sensitivity that makes it useful for structural biologists who want to resolve molecular structures at the atomic level. Increasingly, cryo-EM is seen as a natural complement to—and sometimes a substitute for—gold-standard methods such as x-ray crystallography for studying proteins, nucleic acids, and the complexes they form inside cells.

Grigorieff is a specialist in the field of cryo-EM. Working with fellow HHMI investigator Stephen Harrison of Harvard Medical School and Children’s Hospital, Boston, he recently reported a structural map of an outer-coat protein locked in place on an intact rotavirus particle (see Upfront, “Piecing Together Rotavirus’s Unique Approach”). The picture was not as detailed as Harrison’s images of the protein captured by x-ray crystallography, but it allowed the scientists to visualize the protein’s placement on the intact particle and infer its role in rotavirus infection.

For almost any technique in structural biology, the trick is to achieve high resolution without losing the fuller picture of how a structure looks and behaves inside a cell. That’s a big challenge for x-ray crystallographers, who must isolate, purify, and coax living structures into an artificial crystalline lattice to study them. For one thing, crystallization is not always possible, especially for large structures made of many interlocking molecules. In addition, the crystallized form captures only one state of the structure: cellular

parts are made to move, and locking them into a static position gives only one part of the story. Other structural biology techniques are limited in that they require dehydration, dyes, or harsh fixatives that can disrupt fragile chemical bonds.

Developed in the 1970s, cryo-EM has the advantage of being able to preserve molecular structures in multiple states and hence to give a more complete picture of a macromolecular machine in action.

Refining Resolution

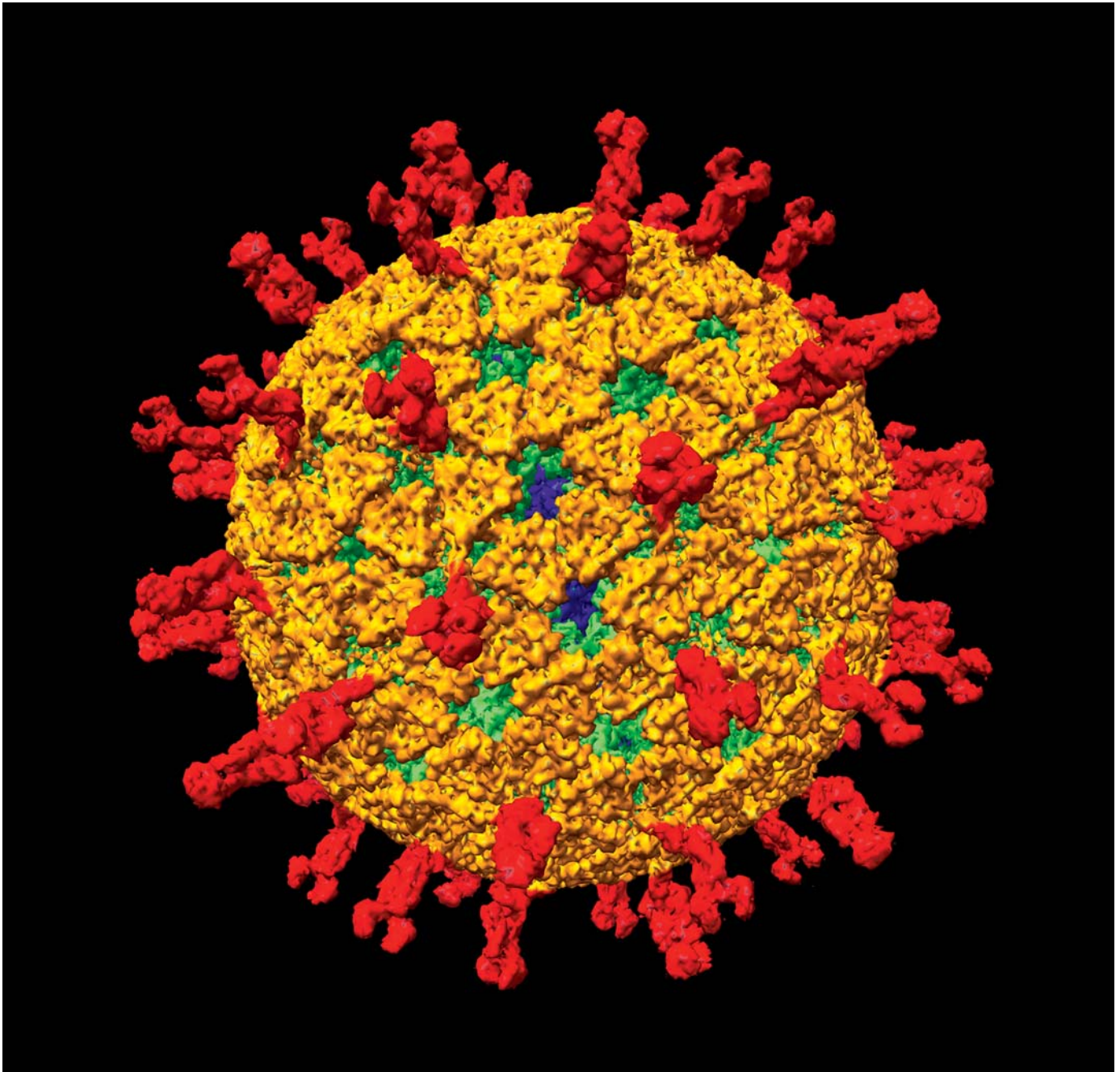
It’s not easy to prepare a sample in this way, but the bigger challenge for the cryo-EM community, says Grigorieff, has been getting to an atomic-scale resolution of one to five angstroms (the average atom is roughly two angstroms).

“For a long time, people were getting around 20 angstroms resolution, where they might be able to distinguish different proteins but not different amino acids within the proteins,” he says. “Then it was six angstroms or so, which still isn’t good enough to show atomic-level detail.”

Grigorieff is intent on pushing that limit. In the rotavirus study, his group achieved a resolution of about four angstroms.

There are two primary ways to get three-dimensional structures from cryo-EM images. Grigorieff uses an approach called single particle reconstruction, taking two-dimensional images of thousands of copies of the virus particle and then averaging them with computer algorithms into a three-dimensional picture. In the other approach, called computed tomography cryo-EM, a single molecular structure is photographed in a series of pictures from many angles to arrive at a similar three-dimensional average, much like the CT-scan technology used by physicians. In both cases, the raw images appear fuzzy, so the averaging step is key.

“Basically, you have a bunch of very snowy images, and then you stack them up on top of each other and out comes a pattern,” says Grigorieff. “When we do enough of these—100,000 or even a million—then we are suddenly able to see much more detail in the underlying pattern.” Or rather, computers see the pattern and interpret it, creating a color-mapped model that shows the orientation of molecules and the ways they interact with each other.



This three-dimensional cryo-EM image of a rotavirus enables researchers to infer the role of two proteins on the virus's temporary, outermost coat. The VP4 spike protein (red), which helps the virus puncture the membranes of target cells, is held in check by the VP7 protein (gold) until the virus is primed for infection. In green is a third protein, VP6, found in the middle coat.

Grigorieff spends much of his time creating and refining those algorithms, which must be powerful enough to analyze all those thousands of pictures—and flexible enough to interpret a variety of structures. It helps, Grigorieff says, to work with a highly symmetrical particle like rotavirus, which has the same symmetry as the 20-faced solid called the icosahedron. “The symmetry makes the task of averaging one million molecules much easier,” he explains.

“The rotavirus work showed that we can get sufficient resolution to build atomic models,” he says. “Because we now have proof of

concept ... we have more confidence that we will eventually be able to get similar resolution with more difficult samples.” Eventually, Grigorieff would like to tackle cellular behemoths such as the spliceosome, a structure made of 150 or so proteins that edits messenger RNA into a more readable form. ■

—SARAH GOFORTH



WEB EXTRA: Visit the online *Bulletin* to learn more about Grigorieff's cryo-EM images of rotavirus. www.hhmi.org/bulletin/nov2009