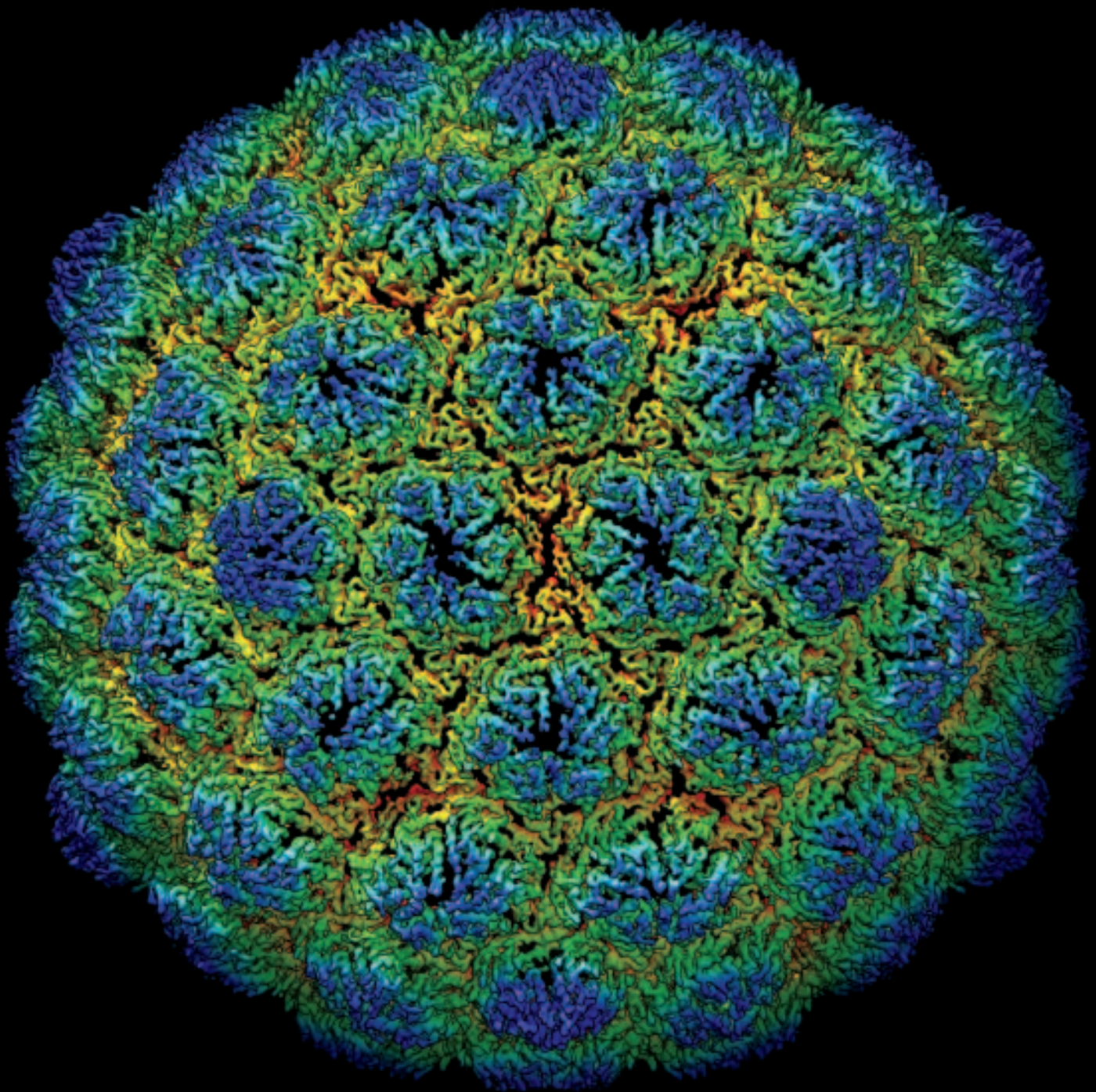


PEERING TOWARD ATOMIC RESOLUTION

BY JASON BITTEL

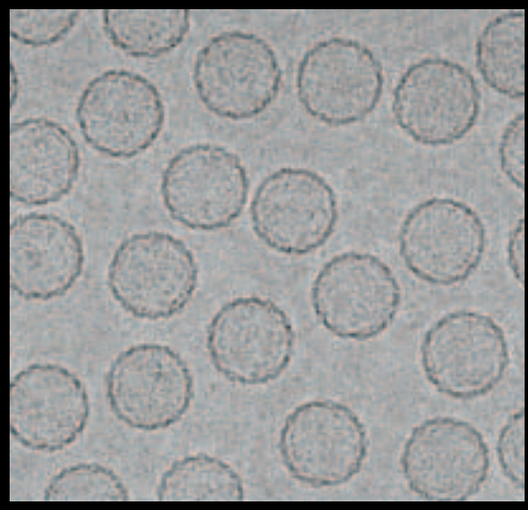
INTO THE VIRUS

James Conway is a PhD associate professor in the Department of Structural Biology at the University of Pittsburgh School of Medicine, and his is a world of the mostly invisible.

Conway specializes in cryo-EM, or cryo-electron microscopy (so-called because specimens are examined at liquid nitrogen temperature, which preserves their structure in the vacuum of an electron microscope). For a decade, he's been training his lab's three microscopes at the protein shells (capsids) of viruses.

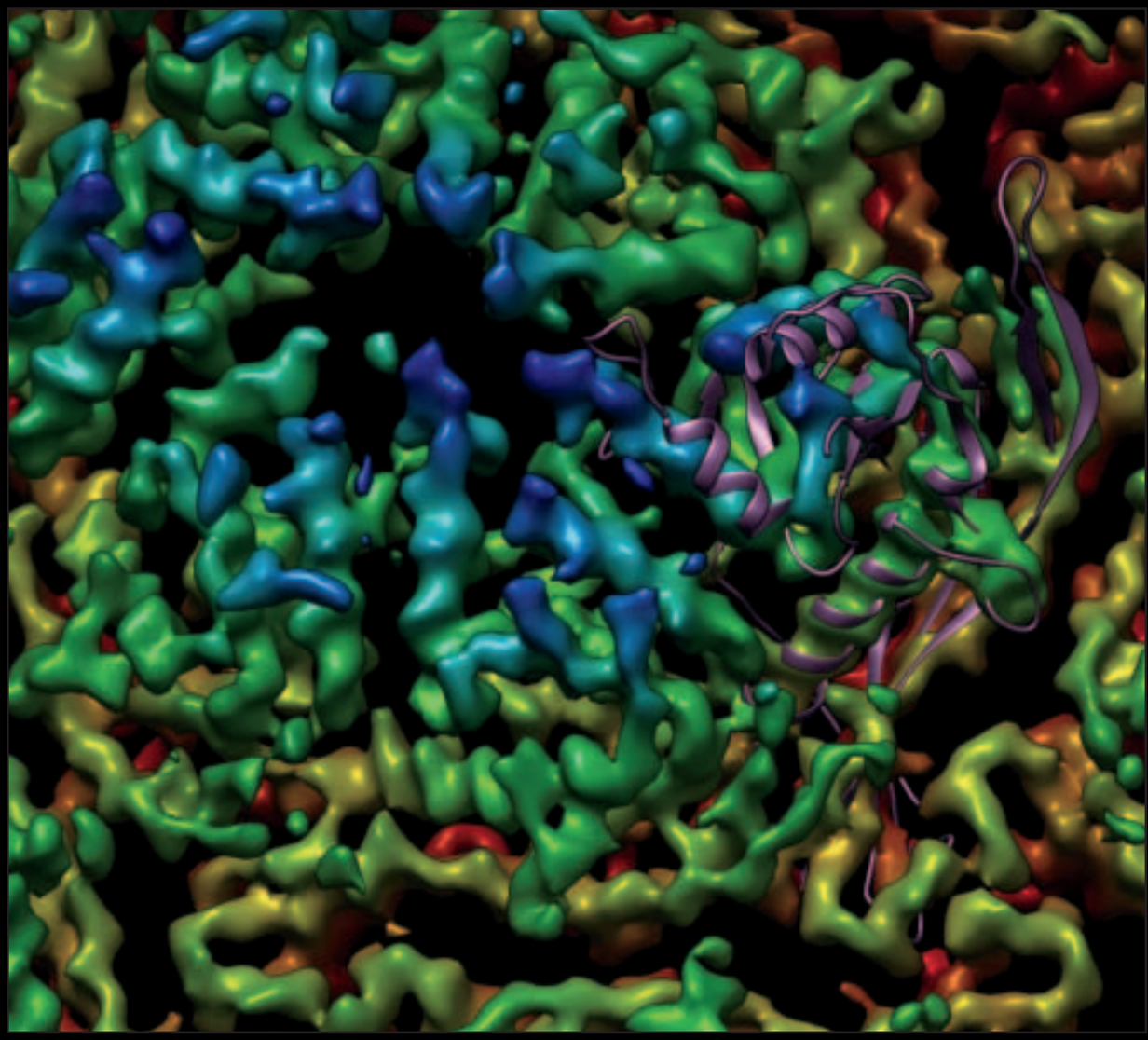
Since the first virus was discovered back in 1898 we've been trying to develop better ways to get a good look at the parasites. In the 1930s, the first electron microscopes (which use a beam of electrons to create an image instead of visible light) brought us closer than ever before. Breakthroughs in mathematics in the '60s allowed us to take the images from those advanced microscopes and translate them into three-dimensional structures.

Today, a trinity of technologies has evolved to the point that Conway and his colleagues are coming close to mapping the very atoms that make up viruses and other infinitesimal bits of matter.



Bacteriophages are viruses that prey upon bacteria. (Their name comes from the Greek word *phagein*, which means "to eat" or "to devour.") It may seem counterintuitive, but Conway's cryo-EM microscopes can actually see smaller particles, like phage D3 pictured above and to the left, more clearly than larger ones. "The quality of the images is the same," explains Conway; it's just that larger capsids are more difficult to work on. The gray image shown above is indicative of what Conway actually might see—what cryo-EM produces. The colorful images presented on the opposite page and elsewhere in this feature are modified with the freely available molecular graphics software, UCSF Chimera. From the preparation of grids to the angle of the image, there are a thousand things that can go wrong with each shot. That's why three-dimensional models are best created after Conway has taken, on average, tens to hundreds of thousands of images.

PHAGE D3 IMAGES (PP. 30-33) COURTESY: JAMES CONWAY, DEPARTMENT OF STRUCTURAL BIOLOGY. ROBERT DUDA, ROGER HENDRIX, DEPARTMENT OF BIOLOGICAL SCIENCES.



Perhaps the most important among these technologies is the field emission gun—a powerful tool that creates a focused beam of electrons. In the 1990s, the introduction of the field emission gun transformed cryo-EM by drastically improving the signal-to-noise ratio and spatial resolution of specimens. It also allowed electron microscopes to achieve resolutions down to 10 angstroms (Å) and below. For reference, scientists consider 3 Å to be truly atomic—or at the level that lets us perceive individual atoms. Conway's lab recently managed to see bacteriophages (viruses that infect bacteria) at about 4 Å.

"It's incredibly exciting that we're now heading toward atomic resolution with the new technologies," he says. "I can get a sample, put it in the microscope, and by this evening or tomorrow, I may be the first person to see what a particular virus looks like."

Automation has also propelled the field forward. One of the electron microscopes in Conway's lab can take up to 2,800 images a day, a boon when you consider that tens of thousands of images are required to stitch together a three-dimensional model like the colorful images on these pages.

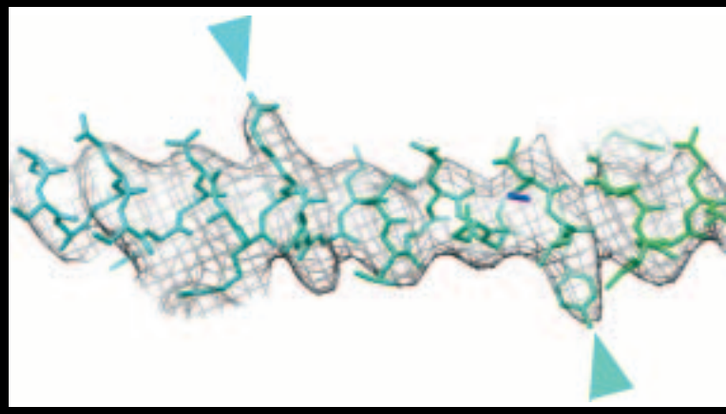
Most recently, a new type of camera has allowed the microscope to collect electrons directly rather than having to convert electrons into photons much like an old television set would.

Getting to the near-atomic isn't cheap. The new camera system costs close to half a million dollars. (The one in Conway's lab is currently on loan.)

Of course, seeing the virus clearly is only part of the objective.

"Understanding how the virus assembles, its architecture, how the virus interacts with its environment and host receptors and antibodies—that gives you the basis on which to start designing something useful as a therapy," says Conway.

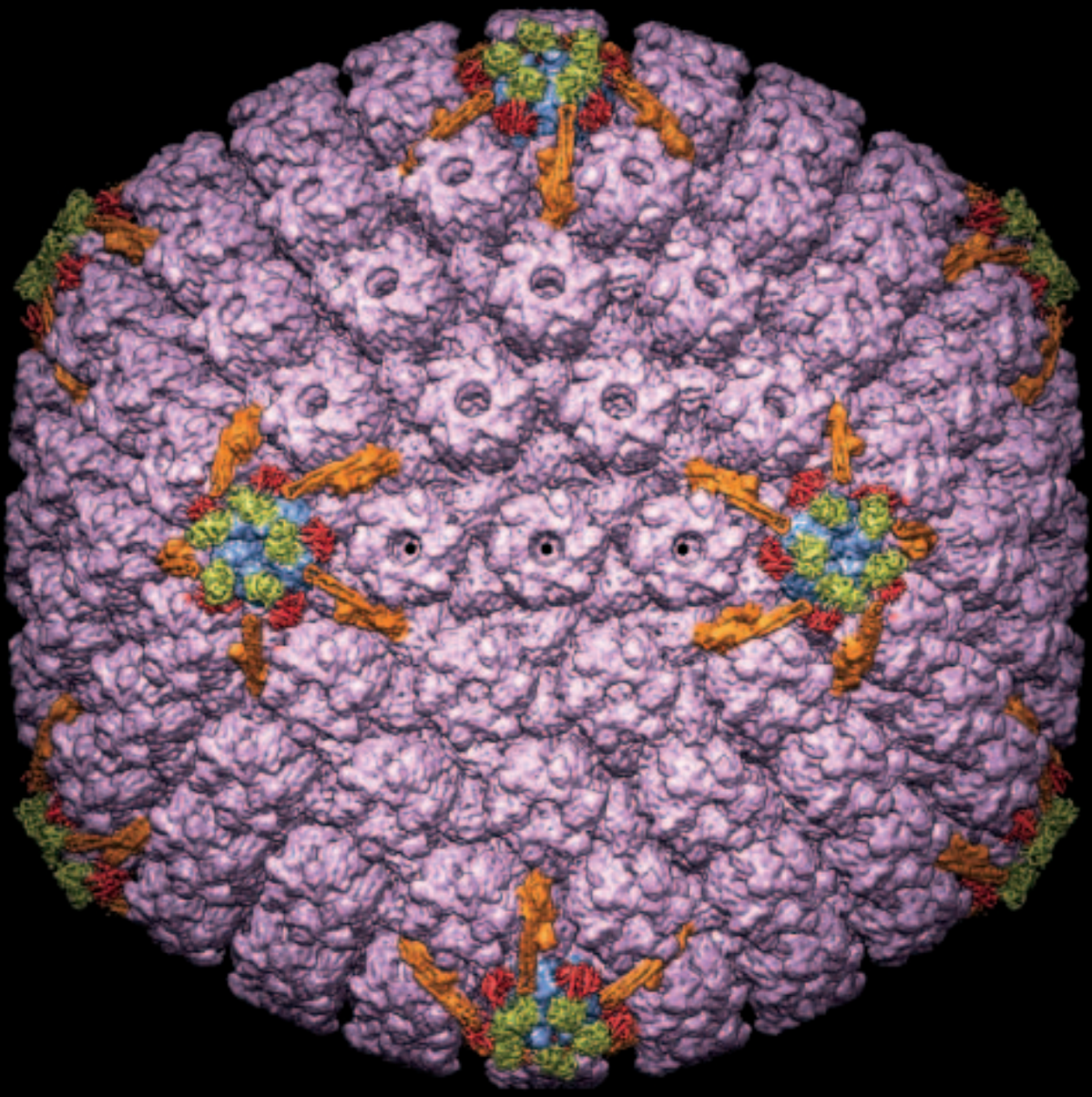
"But until you know how the pieces fit together, you can't even really start." ■



Above, from a close-up of a phage D3 protein helix, you can see how an atomic model (sticks) fit into the cryo-EM density (chickenwire). The shell of phage D3 is made up of 540 copies of the major capsid protein, which is, in turn, created by 20 different kinds of amino acids stringing together. Each of those amino acids has distinct side-chains, which give them their specific chemical and structural character. The way that proteins interact, and eventually assemble as building blocks or enzymes, is wholly dependent on the nature of those side-chains. Being able to "resolve" such blueprints of structure tells scientists a lot about function. Conway's team has resolved D3's side-chains.

The panel on the opposite page illustrates how far the field of cryo-EM has come. Just a few years ago, this same image would have looked like a bunch of "blobs and sausages" (that's the technical term, says Conway). Instead, the proteins here appear more like corkscrews—right-handed corkscrews, to be specific. Conway explains, "For the first time, we're really seeing secondary structure, not just a blob of the right length or a smooth tube of the right length. We're seeing an actual chiral helix." (In other words, he is able to see the direction of the spiral.) The purple ribbon above is a protein crystal structure identified from bacteriophage HK97 (so named because it was identified in Hong Kong), which infects *E. coli*. Notice the way it fits almost perfectly onto the helices of phage D3. "We're seeing that we have a tremendous correspondence between this virus and the other virus," says Conway.

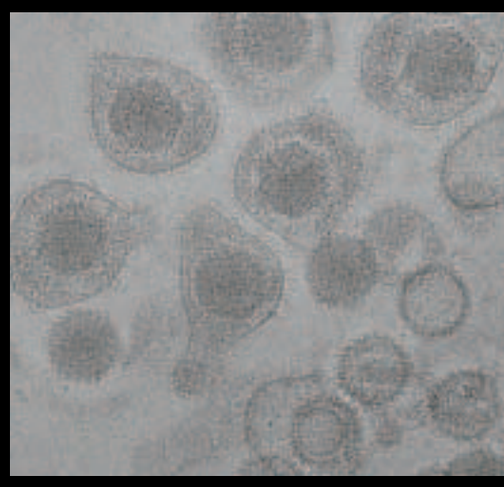
PURPLE "RIBBON" IMAGE OF BACTERIOPHAGE HK97: RETRIEVED FROM PROTEIN DATA BANK (ENTRY 3E8K)



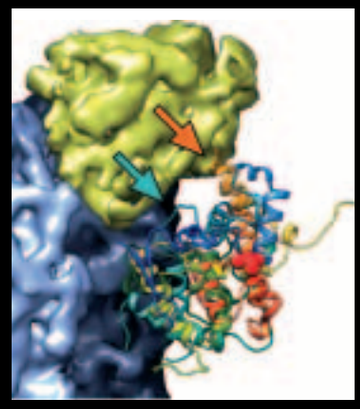
Behold the complex brilliance of a herpesvirus capsid (above and right). When people use the word "herpes" they are usually referring to HSV-1 or HSV-2, but a large family of viruses actually comprise *Herpesviridae*. Eight types are known to infect humans, including varicella zoster virus (the cause of chickenpox and shingles), Epstein-Barr virus (think, mononucleosis), and Kaposi's sarcoma–associated herpesvirus (the cancer-causing KSHV is known for its opportunistic infections in AIDS patients and was discovered by Pitt's Yuan Chang and Patrick Moore). According to the Centers for Disease Control and Prevention, about 90 percent of adults have been exposed to one kind of herpes or another. This makes it an extremely interesting virus for research, says Conway.

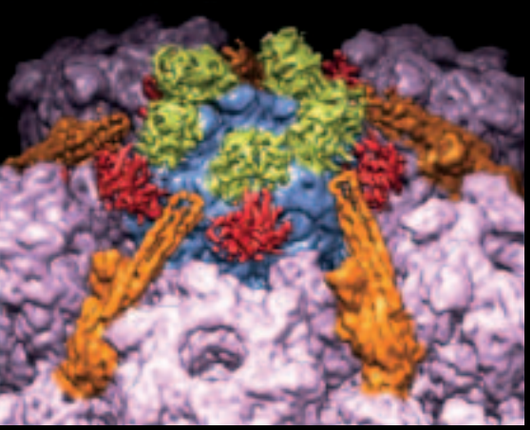
HERPESVIRUS CAPSID IMAGES COURTESY: JAMES CONWAY, ALEXANDER MAKHOV, ALEXIS HUET, DEPARTMENT OF STRUCTURAL BIOLOGY. FRED HOMA, JAMIE HUFFMAN, DEPARTMENT OF MICROBIOLOGY AND MOLECULAR GENETICS.

PHAGE 121Q (SHAPE OF MAGNIFYING GLASS, OPPOSITE PAGE) IMAGES COURTESY: JAMES CONWAY, ALEXIS HUET, DEPARTMENT OF STRUCTURAL BIOLOGY. ROBERT DUDA, ROGER HENDRIX, DEPARTMENT OF BIOLOGICAL SCIENCES.

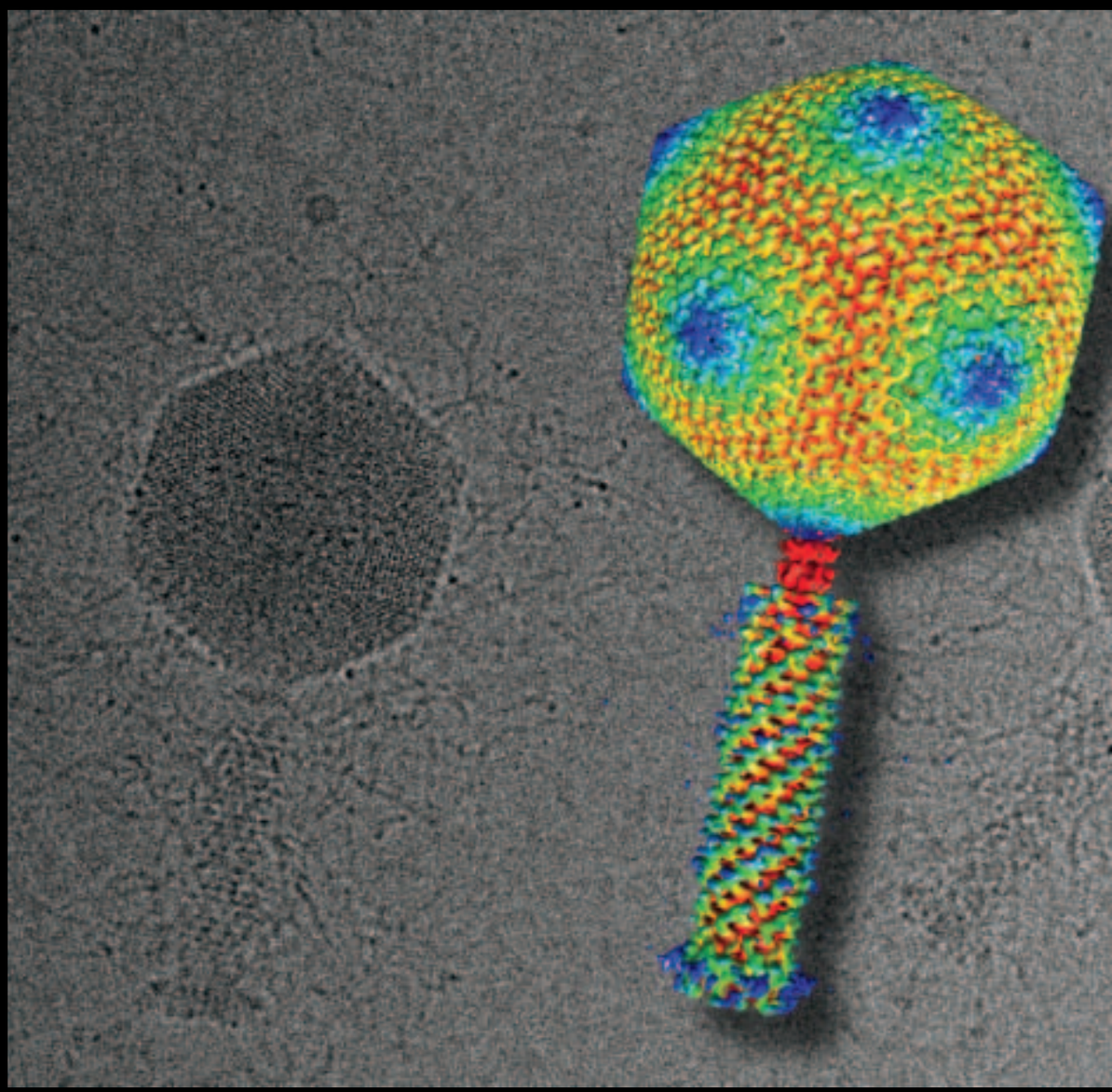


At 6 Å, the image to the right offers a rare glimpse into the way proteins interface on a herpesvirus capsid. Here, the red, burr-shaped protein (UL25) from the starfish-shaped constellation (shown lower left) has been replaced with an atomic structure obtained through X-ray crystallography—a field complementary to cryo-EM requiring proteins or protein fragments that can be crystallized. While this may look like no more than New Year's confetti to most of us, to Conway it represents an opportunity to short-circuit the virus. The arrows point to sections where protein UL25 interacts with protein UL36. Perhaps scientists can develop a drug that mimics UL36 and binds with the confetti strands instead of UL36. Such a drug would have the advantage of being extremely specialized and unlikely to get in the way of any of the other cellular processes on which our bodies depend. With nonspecialized drugs, says Conway, "the medicine might be as bad as the disease—or worse."





Compared to other viruses, the herpesvirus capsid is both massive and multifarious. We're only just beginning to understand how it functions. Learning more about the capsid's surface could yield more effective virus-fighting therapies. For instance, imagine a drug particle coming into contact with the vast wasteland of purple shown to the left. There are lots of places to land, but what should it target? The image above shows a starfish-shaped collection of proteins that we know to be responsible for maintaining the DNA stored inside the capsid. These proteins are crucial to the virus's success—that also means they're ripe for exploitation.



Phage 121Q, before and after: The gray image in the background shows the phage as Conway eyes it using cryo-EM. On the right, Conway has superimposed what that same particle looks like after a modeling makeover. This image shows the full bacteriophage particle, tail and all. When the phage finds a suitable host, it's the tail that actually attaches to the cell and serves as a corridor through which the phage can pump its DNA.