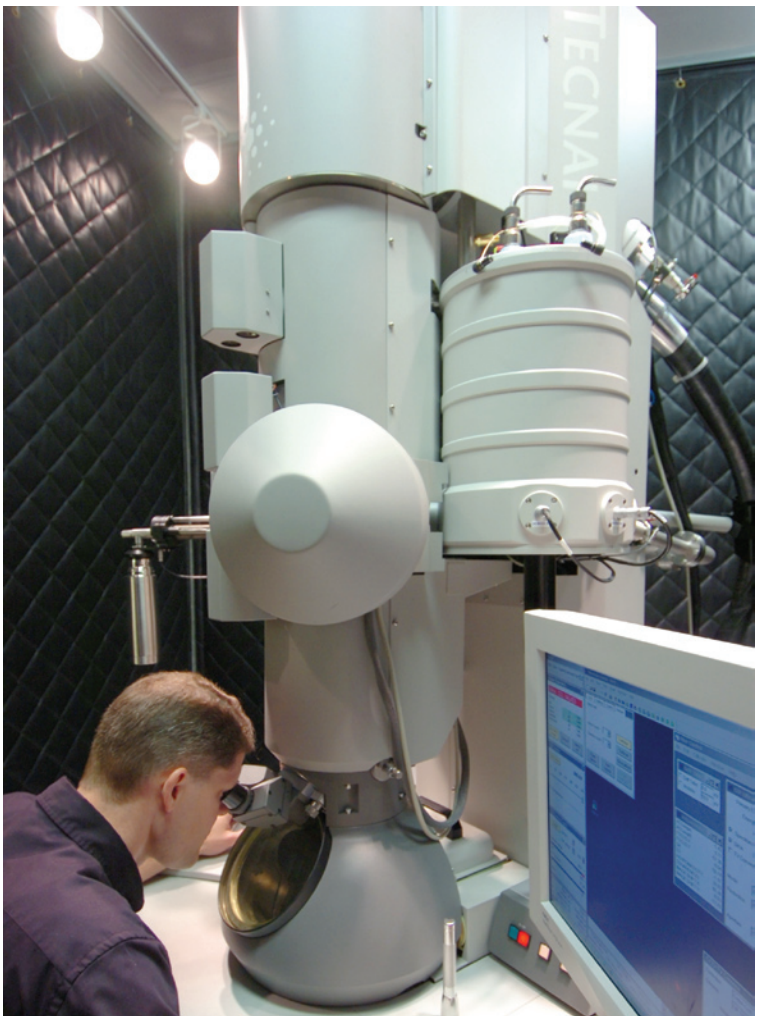
Cellular CAT Scans

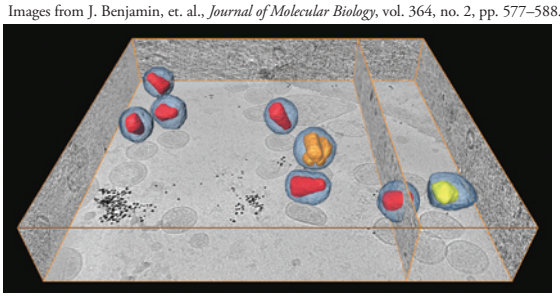
by Douglas L. Smith

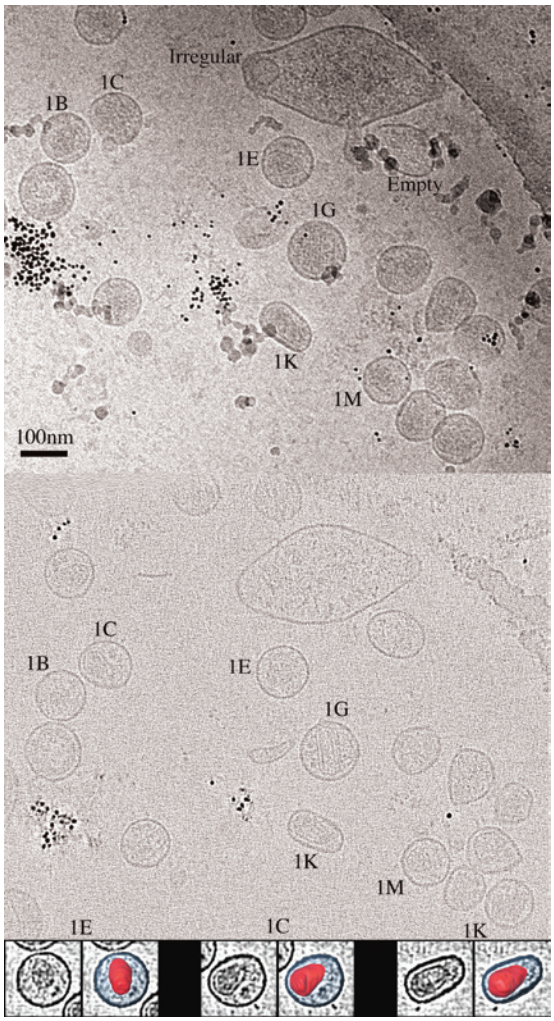


Jensen and the lab's 300-kilo-electron-volt (keV) transmission electron microscope, a liquid-helium-cooled Polara G2. The eyepiece is used for rough positioning of the sample in the vacuum chamber; the actual images will appear on the monitor to his right. The quilted black panels in the background completely surround the apparatus and are part of the airflow-management system.

A cell isn't merely a bag of enzymes sloshing around in a thick soup of cytoplasm. According to Assistant Professor of Biology Grant Jensen, it's more like a multistory factory—a set of interwoven production lines complete with conveyor belts, forklifts, and steel I-beams to hold up the roof. Or, if you prefer, the world's most elaborate Rube Goldberg contraption. The cell's cogs and camshafts, springs and motors, girders and sheet metal (or, in the Rube Goldberg case, gloved hands on sticks, precariously balanced bathtubs, and spring-loaded mallets) are protein molecules. Protein machines conduct the cell's metabolic business; protein motors make muscles contract, amoebas crawl, and paramecia swim. When a cell is preparing to divide, protein diazo machines make a duplicate set of the genetic blueprints, and then protein winches and cables pull the two copies to opposite ends of the cell. Shells of interlocking proteins armor-plate viruses, protein trusswork gives cells their shape, and protein stickers on the protein girders tell the cell which end is front. Jensen's research group wants to photograph each rod, flywheel, and bearing and work out its mechanical interactions with its fellows, in terms as solid as a cast titanium sprocket. As Jensen puts it, "Ultimately, of course, we want to understand how things work at an atomic level—a proton goes here and it causes this atom to move over there, which causes that atom to move over here, and the sum of it all is that the cell swims, or eats, or reproduces itself."

The Jensen lab works in an emerging field called electron cryotomography. Says Jensen, "We're doing a mixture of technology development and basic biological research: trying to answer fundamental cellular questions that are really only answerable by this new technology. How do bacteria maintain their shape? How do they establish polarity? How do they segregate their DNA? How do they cinch off in the middle and divide? And how do they divide asymmetrically, so





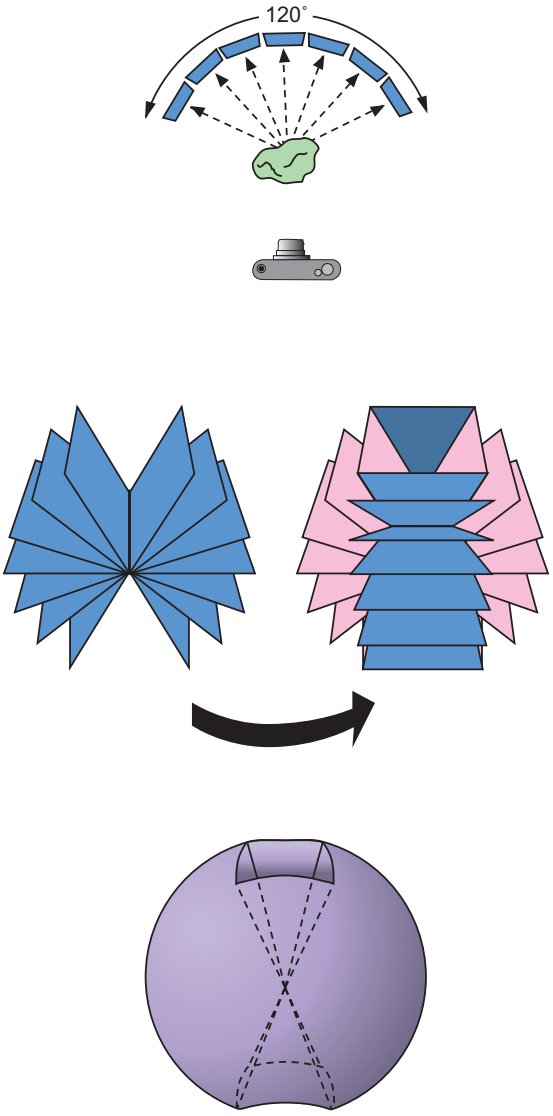
Above: A 3-D rendering of part of a water droplet containing plunge-frozen HIV viruses. The shadows on the aquarium floor, as it were, are viruses above or below the slice. Right, top: One of the raw electron-microscope images on which the rendering was based. The viruses, some of which have been labeled 1B, 1C, and so on, are floating amid other cellular guck. The small black objects that look like buckshot are 10-nanometer-diameter gold spheres used to align the images. Right, center: A slice through the cleaned-up 3-D reconstruction, taken at the same tilt angle as the raw image. Material above and below the image plane is no longer visible. Right, bottom: Three individual virus particles after further processing, rendered in two and three dimensions.

that the 'baby' buds off and swims away, while the 'adult' stays where it is? It's like building a newer and greater telescope—you build a bigger telescope to see deeper into space. We're building a better microscope to see deeper into the smallest cells."

The transmission electron microscope has been around since the 1930s. As the name implies, it uses a beam of electrons rather than a beam of light to look at the very, *very* small. For something to be visible, it must be larger than the wavelength of the radiation you're shining on it. Visible light has wavelengths between 400 to 700 or so nanometers (nm), or billionths of a meter, but individual atoms are 0.1–0.2 nm in diameter, and your average protein molecule runs two to five nanometers in size. The high-energy electrons in a high-end electron microscope have a wavelength of about 0.002 nm. "We accelerate electrons to about 80 percent of the speed of light, so they're really moving, and then we fire them through the sample," says Jensen. When the electrons hit an atom in the sample, they scatter. The scattered electrons interfere with the ones that continue to fly straight and true, and some of each get refocused into a so-called phase-contrast image by a set of electromagnetic coils that act as lenses. In the resulting picture, atoms or regions containing densely packed atoms show up as dark spots. Much of what we know of cellular structure comes from electron microscopy; the key new features that Jensen exploits are the "cryo" and the "tomo."

Like a stop-motion animator, the "cryo" portion freezes living cells in the act of whatever they're doing. Each experiment begins with a droplet of microbe-laden water wicked by surface tension over a set of 2,000-nm-, or two-micron-, diameter holes in a sheet of carbon atoms only 100 nanometers thick. This carbon film, the equivalent of a glass slide in an optical microscope, in turn fits into a three-millimeter-diameter opening in a copper "grid" that acts as the microscope's stage. Ice is less dense than water, which makes life on Earth possible—otherwise the oceans would have frozen solid in the very first Ice Age—but it makes life difficult for electron microscopists. Says Jensen, "When you freeze water gradually, it expands, bursting some cells and distorting the rest and ruining the experiment. But in the late 1980s, people discovered that you could plunge the grid into liquid ethane at about 80 kelvins and freeze the water so fast that the molecules can't bounce around and move apart to form the hydrogen bonds required to make crystalline ice." This amorphous ice, with its molecules caught in random orientations, occupies the same volume as the liquid water it froze from.

The "tomo" part is best known from the medical profession. Says Jensen, "In a CAT scan, Computerized Axial Tomography, they take X-ray pictures of your head through a range of angles and put them together in a computer to get a 3-D model of your skull, your brain, your eyeballs, et cetera. And then you can take a slice through that model



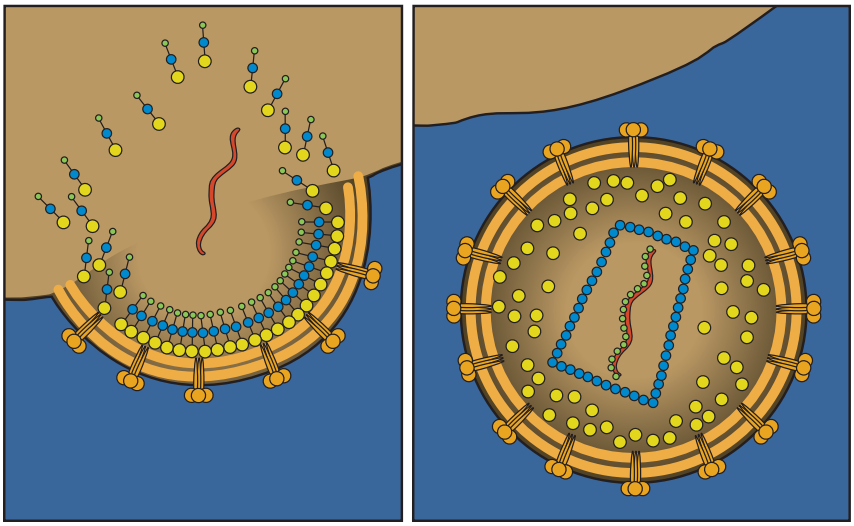
Doing tomography along one arc gives a set of pictures (blue) that don't show the subject from all angles, leading to distortions in the 3-D reconstruction in the wedge where data is missing. Rotating the sample 90 degrees and repeating the scan gives a second set of pictures (pink) that doesn't provide complete coverage either, but fusing the two sets (purple sphere) covers most points of view.

anywhere you want, like cutting through a block of cheese, and see what's in that cross section. We're doing the same thing with bacteria." The microscope automatically tilts the sample grid through about 120 degrees as the series of electron pictures is taken. But, as shown at left, this leaves a wedge of data missing around the north and south poles, as it were, of the 3-D reconstruction. So Jensen's lab bought a prototype device that, at the end of the scan, turns the grid by 90 degrees—without having to remove it from the high-vacuum sample chamber—to do a second scan perpendicular to the first. "Our microscope is exceptional," he says, "because it's the first one that allows us to routinely record a tilt series one way, and then rotate frozen samples and record a second tilt series the other way. This makes the missing wedge turn into a missing pyramid."

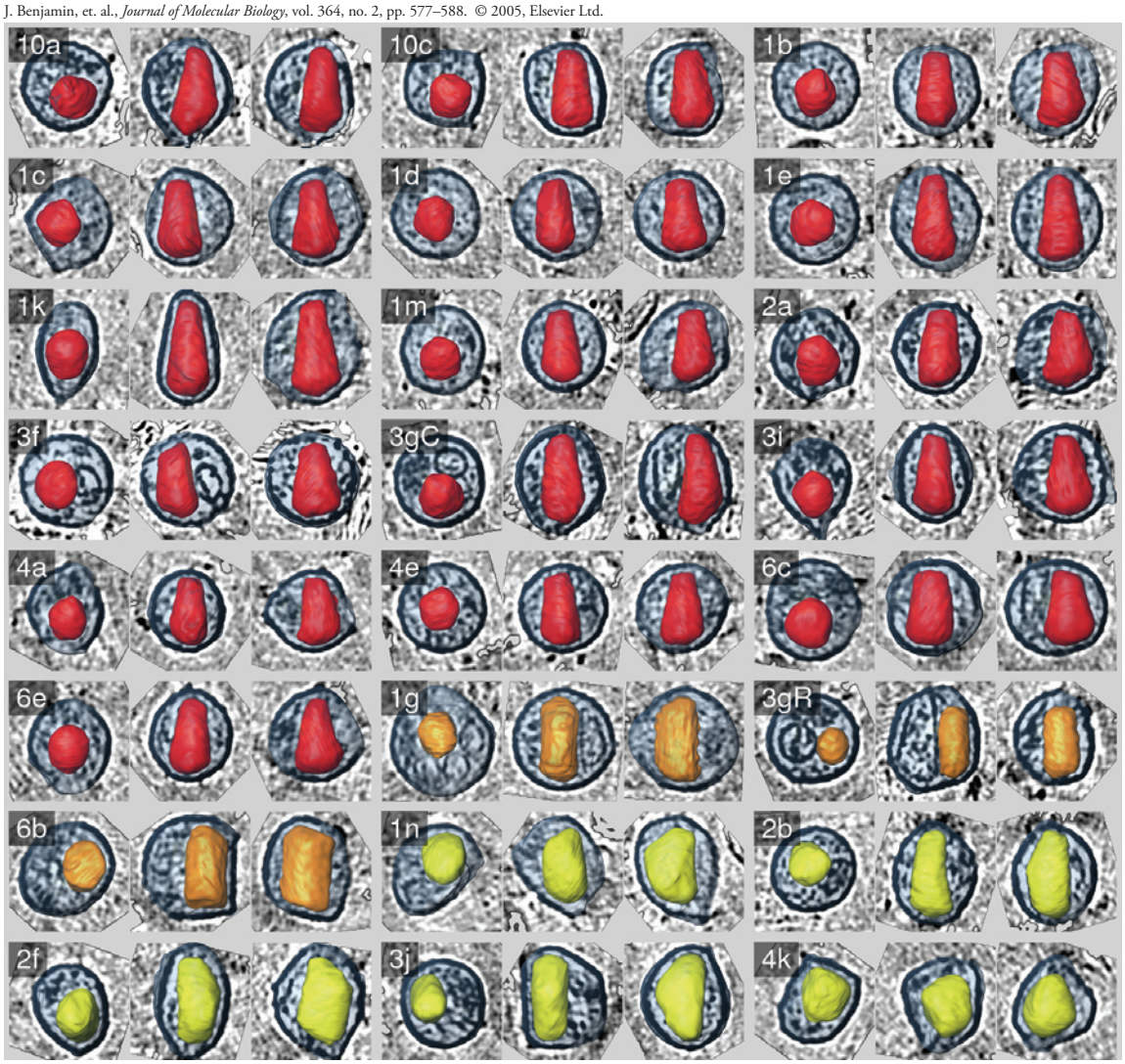
As befits Caltech, it's the world's most automated electron microscope. The scans are recorded on a CCD camera, and a computer using software written by grad student Christian Suloway and a number of collaborators elsewhere interprets the images and tells the microscope where to take the next set of pictures. So once the samples are loaded, six grids at a time, says Jensen, "it operates around the clock, taking tens of gigabytes of data a day without any user intervention. That's kind of cool—a multimillion-dollar, state-of-the-art microscope taking pictures of bacteria all night long for us."

Our factory tour begins by looking at proteins that act as modular steel scaffolding. The mature HIV virus has a spherical skin, a disguise fashioned from the cell membrane of its former host, which encloses a conical shell of protein molecules that contains a wad of protein that swaddles the genetic information, in this case RNA. Says Jensen, "The whole thing can be thought of as a capsule that packages this infective RNA and then sneaks it into a fresh cell, where it causes the cell to build a thousand more copies of itself. Then they all bubble

In an HIV infection, the hijacked cell (brown) produces HIV RNA (red) and a protein called Gag (the linked yellow, blue, and green spheres). The Gag proteins migrate to the cell membrane (orange), sticking to its underside and making it blister up. Gag then gets cut into three smaller proteins—matrix (yellow), capsid (blue), and nucleocapsid (green)—inside the maturing virus.



J. Benjamin, et. al., *Journal of Molecular Biology*, vol. 364, no. 2, pp. 577–588. © 2005, Elsevier Ltd.



A rogue's gallery of 23 HIV viruses, each rendered in three mutually perpendicular views. The capsids are color-coded by shape: conical (red), cylindrical (orange), and other (yellow). To further baffle structural microbiologists, images 3gC and 3gR are actually of the same particle, which contains two capsids and thus a double dose of RNA—something that happens, for unknown reasons, fairly frequently.

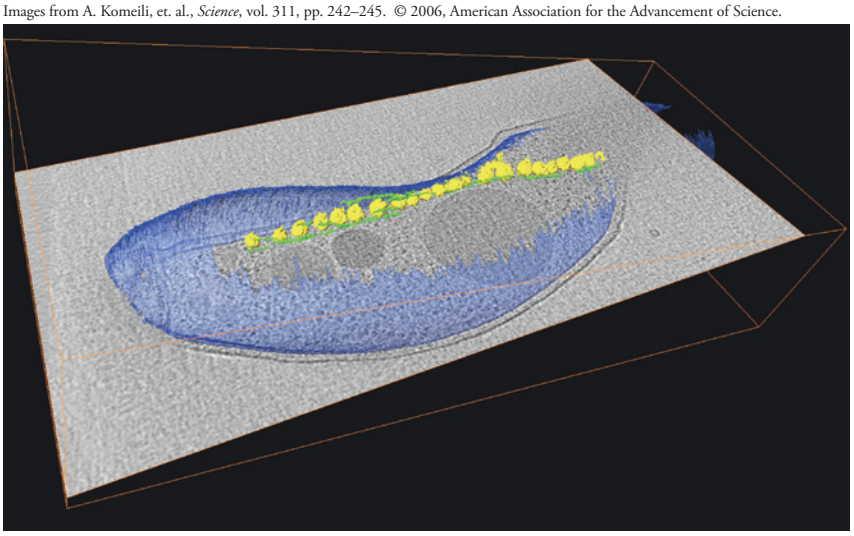
out from the cell surface to go infect more cells."

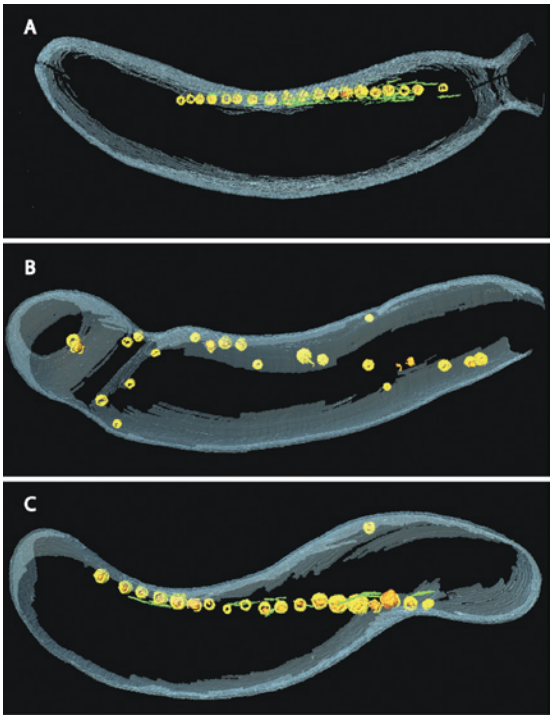
The RNA also forces the infected cell to manufacture a protein called Gag, which is shaped like a wedge of pie. Gag's rounded piecrust binds to the host cell's membrane, and the sides of the slice, where the filling oozes out, stick to the sides of other wedges. So as the wedges lock into place, the membrane starts to curve, forming a bud called the Gag lattice. Once the lattice has grown to become a complete sphere, the bud detaches. The virus finally matures after an enzyme called protease snips the Gag protein into three smaller proteins. (In fact, our best anti-HIV drugs are protease inhibitors, which keep the Gag intact and render the virus noninfective.) The outermost chunk of the Gag protein, the piecrust, having served its purpose, becomes a protein called "matrix" that lets go of the cell membrane and drifts around within the virus. The middle part of the wedge becomes a smaller protein called "capsid" that forms the inner cone containing the RNA, and the little piece at the tip becomes a protein called "nucleocapsid" that coats the RNA.

All this is well known, but teasing out how the individual proteins arrange themselves to form these shells has been confounding because each HIV particle is unique. For one thing, "each virus has a different number of Gag units, so the virus size varies," says Jensen. This alone throws a monkey wrench in the crystallography, and the traditional methods of staining and fixing viruses for microscopy often destroy the Gag structure, so that the workings of the assembly process remain largely hypothetical. But Jensen's grad student Jordan Benjamin and postdoc Elizabeth Wright, collaborating with Wesley Sundquist's group at the University of Utah, have successfully taken pictures of the virus in the immature and mature states and are beginning to discover how the Gags fit together. In the process, they've found that some of the anatomical features that other people had reported were merely artifacts of their sample-preparation methods. The next step will be to try to figure out what interplay of forces locks these three proteins into their proper places, and perhaps—many years from now—figure out some way to stop them.

Let's now consider proteins as I-beams. Protein chains called cytoskeletal filaments give cells their shapes. But Jensen's group has found that there are a *lot* of these filaments. "It's kind of like the first X-rays of humans, when we saw the whole skeletal structure at once. Long before that, we knew about bones from dissections, but X-rays allowed them to be seen in their living context. Similarly,

Images from A. Komeili, et. al., *Science*, vol. 311, pp. 242–245.





Above: A partial 3-D reconstruction of *M. magneticum*. The inner cell membrane is blue, the magnetosomes are yellow, and the filaments are green. Right: In a normal cell (A), the magnetosomes line up along the filaments. In a mutant (B) that does not make the MamK protein, there are no filaments, and the magnetosomes scatter around the cell's periphery. But if you turn the *MamK* gene back on (C), the filaments appear, and the magnetosomes regroup as best they can.

you can get a lot of important information by taking cells apart, but we've taken pictures of intact bacteria and seen more protein filaments than were expected—in their native arrangements."

Most recently, postdoc Zhuo Li, geobiology postdoc Arash Komeili (now an assistant professor of microbiology at UC Berkeley), Professor of Geobiology and Professor of Biology Dianne Newman, and Jensen collaborated on studies of *Magnetospirillum magneticum*. Like other so-called magnetotactic bacteria, *M. magneticum* has little structures called magnetosomes. Each magnetosome is a sack filled with a single crystal of magnetite (Fe_3O_4); when properly aligned, the magnetosomes act as tiny compass needles to help the bacterium orient itself. They tend to be arranged in chains, and in *M. magneticum* they all lie in a line running the length of the cell. Electron cryotomography's close-ups revealed that a protein filament runs like a girder down one side of the magnetosomes and presumably holds them in place. Komeili's fluorescence-labeling studies suggest that the filaments consist of a protein called MamK, and, indeed, in mutants he made that lacked MamK, the magnetosomes were scattered like errant marbles.

Surprisingly, the magnetosomes aren't sealed bubbles within the cell, but are, in fact, pouches—"invaginations" is the technical term—of the cell's inner membrane. This means that magnetosomes are open to the periplasm between the cell's inner and outer membranes, which may help explain how they get filled with magnetite. The dissolved iron destined to become magnetite can probably diffuse across the cell's leaky outer membrane pretty easily, and earlier studies had suggested that the magnetite's precursor, a mineral called ferrihydrite, precipitated out in the periplasm. This solid mineral would then somehow have to get through the cell's inner membrane and, if the magnetosomes had actually been free-floating within the cell, the magnetosome membrane. But this way, a ferrihydrite particle could slowly make its way through the pouch's neck into

the magnetosome, where further chemical reactions would turn it into the magnetite crystal.

Structural members are important, although their job is kind of dull. But proteins with moving parts—now, *that's* cool! Some bacteria propel themselves by long, thin filaments called flagella ("flagellum" is Latin for "little whip") that thrash about and provide thrust. A bacterium known as *Treponema primitia* has two flagella, one on each end, that lie along the bacterium's tubular body between the inner and outer cell membranes. Says Jensen, "The motors that spin bacterial flagella are the quintessential molecular machines—nanoengines that turn an axle. Other people have taken the engine apart and named the pieces, but we don't know how the whole thing fits together. And when the engine is taken apart, some pieces are lost. But because Gavin Murphy, one of my graduate students, is taking pictures of whole cells, we get images of the complete engine, *intact*. If he thawed the cells out, they'd swim away."

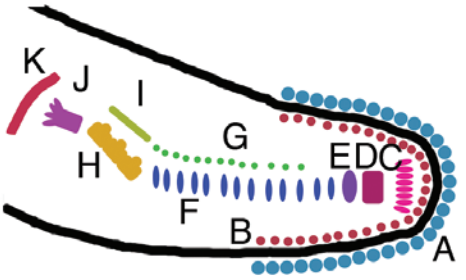
The motors make the flagella spin like a pair of worm gears, which causes the bacterium to spin as well. Like a drywall screw into a 2 x 4, the cell torques itself into the medium ahead of it. "When you're that small, water is like cold tar," Jensen notes.

The engine straddles the inner cell membrane. Embedded in the membrane is a ring-like component called the stator, and nested inside the stator is the moving part, the rotor. "Protons flow through the stator," Jensen explains, "which causes the stator to spin the rotor. It's kind of like a playground merry-go-round, where you have people all around pulling on it to make it spin." The rotor is attached to a rod that leads to the flagellum and causes it to turn. The rod passes through another part, called the P ring. P stands for peptidoglycan, says Jensen, "and the P ring is like a bushing. It greases the rod as it spins through the peptidoglycan layer, which is like the chicken-wire frame that gives the cell its shape." And below the motor, in the cell's cytoplasm, lies a component called the C ring. "Now there's another cool part to this," Jensen says zestfully. "The C ring acts like a transmission. It receives signals from the cell through proteins that dock on its underside and cause the whole motor to either rotate clockwise or counterclockwise. So it's like a forward gear and a reverse gear." It's thought that the C ring and the rotor spin together, as a unit, but that level of detail remains to be seen—as does the actual gear-shifting mechanism.

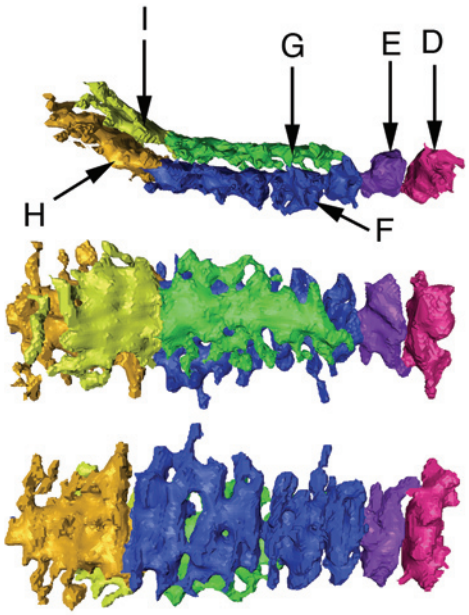
How *T. primitia* gets around also isn't clear, but it's generally assumed that the motors make the flagella spin like a pair of worm gears, which causes the bacterium to spin as well. Like a drywall screw into a 2 x 4, the cell torques itself into the medium ahead of it. "When you're that small, water is like cold tar," Jensen notes. There's a lot of resistance that bigger organisms don't experience. *T. primitia* lives in the guts of termites, swimming through "bits of wood, lots of juice, and thousands of other bugs." The work was done in collaboration with Assistant Professor of Environmental Microbiology Jared Leadbetter, who studies the amazingly elaborate, cellulose-digesting ecosystem that inhabits the termite belly.

But there's more than one way to propel a bacterium. *Mycoplasma pneumoniae*, which causes some types of pneumonia, has a "foot" called the attachment organelle that allows it to stick to surfaces and crawl around inside your lungs. How this works is a mystery, but grad student Greg Henderson's work, in which, says Jensen, "we labeled all the parts and named them, with incredible creativity, A through K" allows for some educated guesses.

In the schematic view on the opposite page, the blue dots labeled A are proteins that coat the outside of the foot, and presumably enable it to stick to the mucus-coated landscape of your lungs. Inside the foot, giving it its shape, are two rodlike



Right: A schematic of the key proteins in *M. pneumoniae*'s attachment organelle.



Below, right: Three 3-D views of the two rod-like structures that act as the motor.

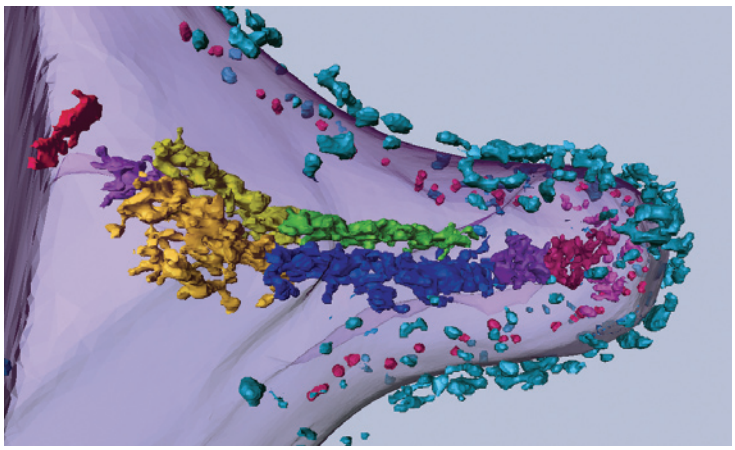
structures—components F and H form the larger, thicker rod, and G and I the shorter, slimmer one—that abut a "terminal button" (C, D, and E) over which the cell membrane is stretched. The base of the rod, where the foot joins the cell body, is attached to a shallow bowl (K) by something that resembles a waiter's hand, fingers splayed, supporting a tray. Since G and F are not solid entities but sets of disconnected segments separated by sizable gaps, says Jensen, "the idea is that the rod contracts and then all of a sudden kicks out. And as it kicks, it's more likely that the front goes forward than that the back goes backwards." Like a paddle against the water, the bowl in the back would meet a lot of resistance as it pushed against the cell's cytoplasm. So the terminal button in the front moves instead, thrusting part of the cell membrane, and the sticky As, ahead. "And then the motor contracts again and kicks, and the cell just rolls forward like the treads on a tank." Once the foot crawls past, the theory goes, the As detach from the lung's surface and diffuse toward the front of the foot again, wading in the cell membrane like an angler waist-deep in a river.

M. pneumoniae may also be a so-called minimal cell. A mere 700 or so genes suffice to build and run this little bug, which is pretty amazing. It also leads to the hope that we could identify each of the 700-odd proteins those genes produce, determine their 3-D structures by X-ray crystallography, and figure out how they all work and fit together. A group led by Sung-hou Kim at UC Berkeley is working on the structures and has gotten well over half of them.

And when you are working on structures, sometimes it does help to look at things in isolation—single-particle analysis, as it's called. Electron cryotomography can do that, too, when the particles are large enough. Among the right-sized particles are complexes where several different proteins form loose associations to perform some task—enzyme A takes a molecule, tweaks it a bit by cutting a bond here or adding an oxygen atom there, and hands it off to enzyme B, and so on, until you wind up with a molecule of, say, fat. In other words, protein complexes are the machines in the cell's assembly lines.

So instead of freezing whole cells, you freeze solutions of the enzymes in the process of doing their thing. What results are pictures of small, blurry blobs, but, says Jensen, "electron cryotomography is the highest-resolution technique currently available to image individual, unique protein

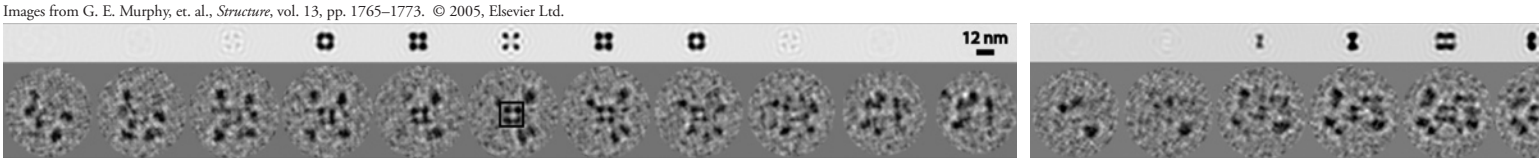
Below: A 3-D reconstruction of the entire foot, coded in the same colors as above right.

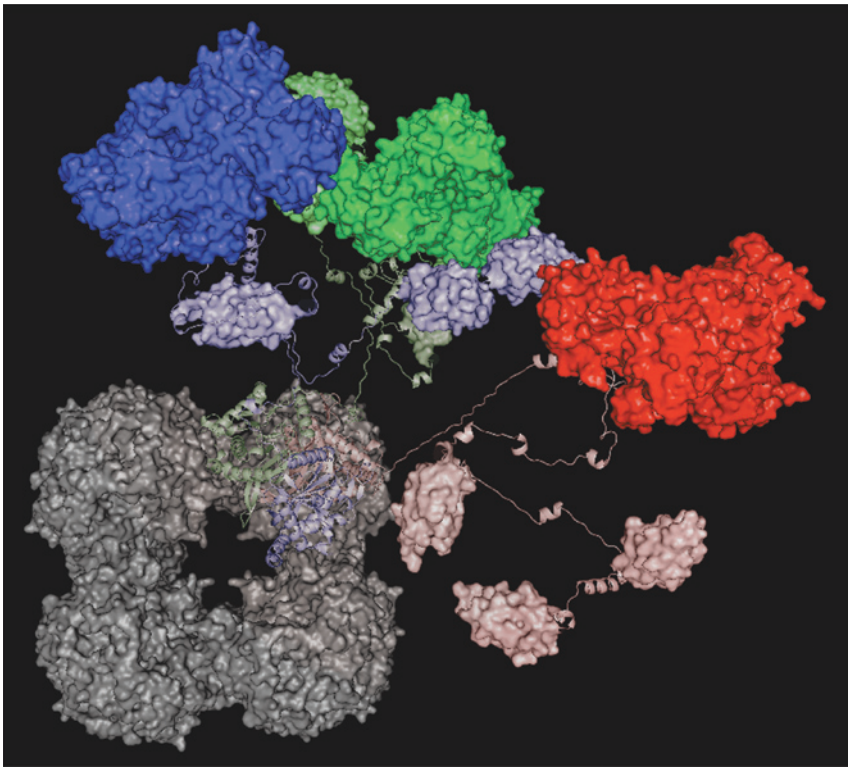


Images from G. P. Henderson, et. al., *Molecular Microbiology*, vol. 60, no. 2, pp. 376–385.

Below: Each of these three strips is a set of slices, taken 2.5 nanometers apart, through an *E. coli* pyruvate dehydrogenase complex. The bottom part is the raw image. The top part shows just the core crystal of enzyme number two. These three complexes were chosen because they happened to be oriented along the crystal's 4-, 2-, and 3-fold axes of symmetry, respectively.

Images from G. E. Murphy, et. al., *Structure*, vol. 13, pp. 1765–1773. © 2005, Elsevier Ltd.



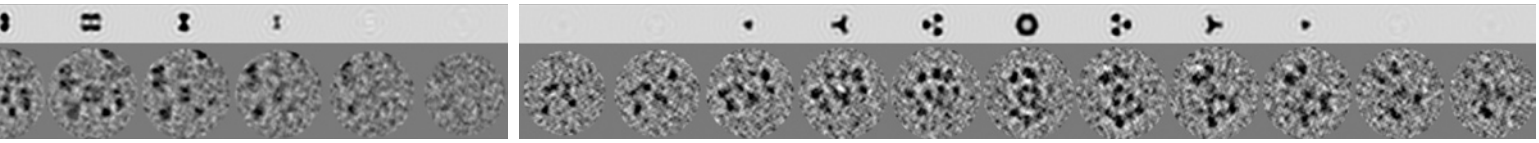


A 3-D model of a core holding three enzyme balloons. The hollow gray square is the central crystal of enzyme number two, with the three specific enzyme molecules holding the balloon strings rendered in light blue, light green, and pink. The corresponding blue, green, and red balloons are all dimers (two molecules bound together) of enzyme number three.

complexes—the other techniques require averaging lots of copies of identical complexes under special conditions." Grad student Murphy has been examining the pyruvate dehydrogenase metabolic pathway, which consists of three enzymes that together catalyze five reactions that burn a simple carbohydrate (called pyruvate, oddly enough) to fuel the cell.

The simplest organisms just let the enzymes in this pathway slosh around in the cytoplasm and wait for lucky collisions to happen in the right order. Sophisticated cells like ours prefer to pack many copies of these enzymes into a near-crystalline machine—a regular icosahedron containing more than 60 copies of each of the three enzymes. "We just pass the substrate from here to there to there to there, and we're done," says Jensen. "It's an efficient pipeline." There are large numbers of metabolic pathways in every cell, and the more sophisticated the cell, the more these pathways tend to get streamlined into such tightly organized structures.

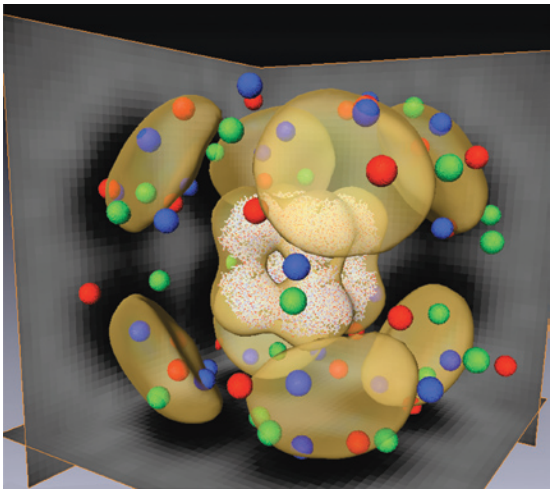
The bacterium *E. coli*, which turns out to lie midway along that scale of sophistication, keeps 24 copies of enzyme number two in a cubelike core, around which many copies of enzymes number one and three drift. Murphy discovered that enzymes one and three are actually tethered to enzyme number two, rather like a child holding a helium-filled balloon on a string in each hand. (The analogy is not quite exact, as the strings are regions of enzyme number two that seek out and attach themselves to handy copies of enzymes one and three.) But picture a classroom of 24 such kids at their desks, their balloons bobbing in the breeze from an open window, and you begin to see why the complete structure had proven unsolvable. But, says Jensen, "Electron cryotomography gave us both the quaternary structure, which means how all the proteins are arranged with respect to each other, and the conformational heterogeneity, which is how free they are to move around."



Combining the 3-D info from single-particle microscopy with all the atomic structures already in protein databases, says Jensen, leads to "another feat that has not been possible before, which is to make a complete map of a whole cell. We see each individual protein molecule, and we can identify some of them by their shapes. So we can actually map out where they are in 3-D, and that's really novel." While the little gray blobs in any individual slice all look pretty much alike to the naked eye, they're quite distinctive to the computer, which reassembles the slices into 3-D images. Imagine a basket full of fresh vegetables: if you took one slice through the basket, a radish and a carrot might show up as two equal-sized circles; but stack several slices together, and their identities are soon revealed.

The vast majority of the cell's proteins are just diffusing through the cytoplasm, but some are bolted to other molecules. And of course the structural members, the girders and I-beams, don't move. Says Jensen, "We'll be able to identify all the big or specifically arranged components, and since we already know how to model the diffusion of smaller proteins, we'll have a nearly complete description of the cell. A lot of what happens in cells depends on where things are. This is the best way to see how all the parts are positioned with respect to each other."

Below: The average structure of 305 pyruvate complexes. The individual atoms in the central crystal of enzyme number two can be seen, while all the copies of enzymes number one and three drift around within the eight brown, jelly-donut-shaped objects surrounding the core. The red, blue, and green spheres mark the specific locations of proteins number one or three in three of the 305 individual pyruvate complexes examined.



It takes a lot of computational horsepower to reassemble all those vegetables from their slices—even in a small cell!—and then rotate and examine them from all possible angles, superimposing them on the collection of reference shapes until a match is found. The Jensen lab is one of the chief users of the Caltech Structural Biology Supercomputer, a 280-unit cluster of dual-processor IBM Power PCs that, when bought in 2005 on a grant from the Parsons Foundation, was one of the Top 500 supercomputers in the world. "We're biologists," says Jensen, "but we use a lot more computers than pipettes."

Jensen is at Caltech thanks to the Biological Sciences Initiative, which also built the Broad Center for the Biological Sciences that houses him. When the initiative was launched, says Jensen, "the faculty got together and asked themselves, 'What are the most exciting new fields we'd like to get into?' One of the areas they identified was cryo EM, and so they started a faculty search that went on for about six years. In the meantime, Caltech built Broad, complete with specially designed rooms for state-of-the-art electron microscopes." The basement facility has two microscope rooms, each with a four-foot-thick, vibrationally isolated concrete mounting slab and a custom-designed air-handling system that bathes the microscope in a stable, laminar flow of air held at a carefully controlled temperature. (If the temperature changes more than a few tenths of a degree, the resistance of the coils of wire that act as electromagnetic lenses changes, and the microscope's focus is thrown off.) The Moore Foundation funded the microscopes—the 300-keV one that is the lab's workhorse and a 120-keV model used for preliminary studies. "A lot of people deserve credit for this. The biology division's vision created this opportunity, and by the time I finished my postdoc, it was an unbeatable offer. *Unbeatable.* They already had the building. They already had the rooms ready, and funds allocated to buy the world's best microscopes. I would have had to be crazy not to come." □