

▶ 0°



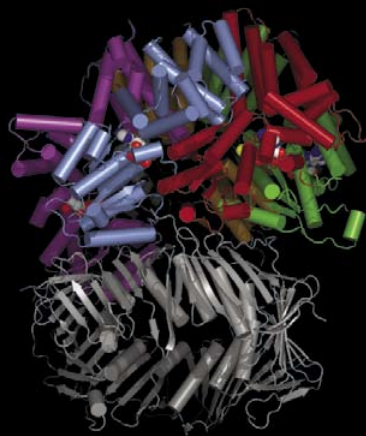
▶ 21°



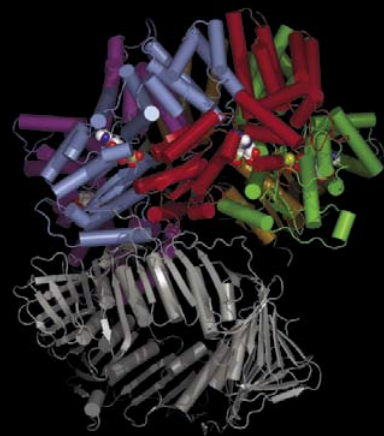
▶ 42°



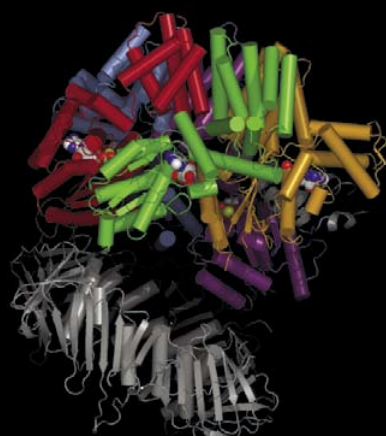
▶ 127°



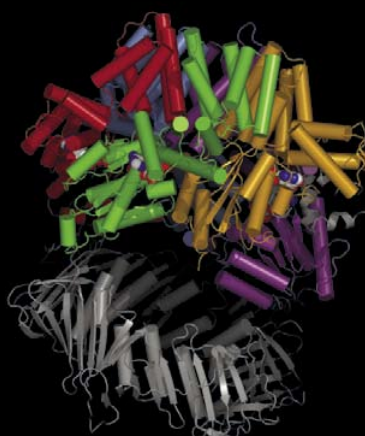
▶ 148°



▶ 169°



▶ 254°



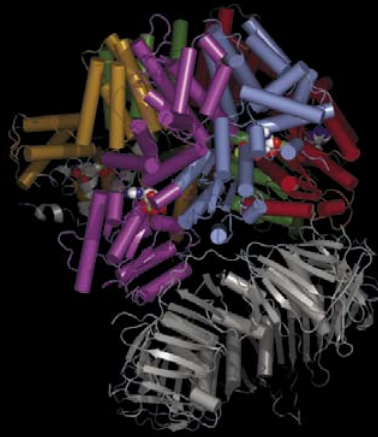
▶ 275°



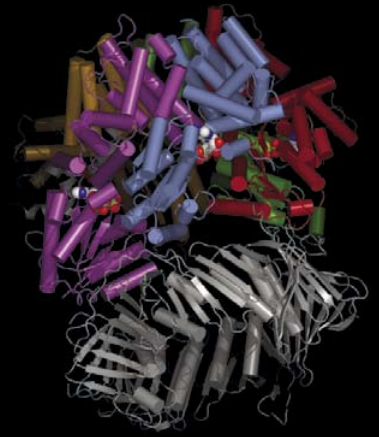
▶ 296°



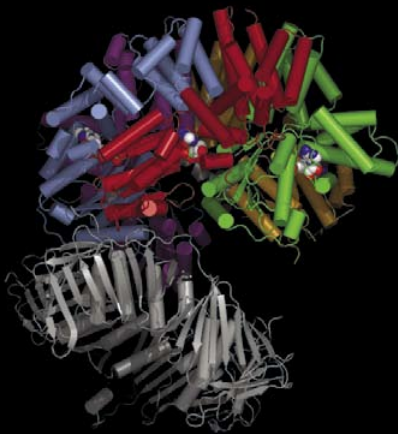
▶ 64°



▶ 85°



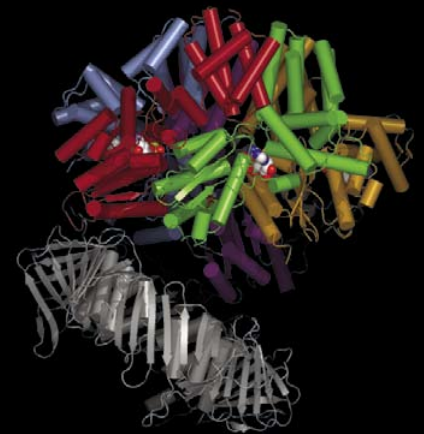
▶ 106°



▶ 191°



▶ 212°

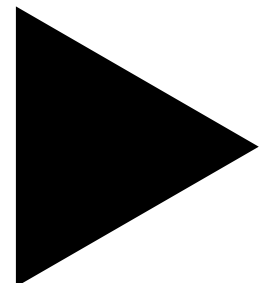


▶ 233°

## VIEWING VITAL STRUCTURES

RESEARCHERS ANGLE FOR BETTER 3-D STRUCTURES OF THE MOLECULAR MACHINES THAT PRODUCE OUR PROTEINS, REPAIR OUR DNA, DEFEND US AGAINST MICROBES, AND, IN EFFECT, CONTROL OUR HEALTH. **BY MAYA PINES**

PHOTO ILLUSTRATIONS BY GREGORY BOWMAN  
THE PHOTO ILLUSTRATION SEQUENCE DEPICTS A 360° ROTATION OF THE CLAMP LOADER ASSEMBLY (SEE PAGE 29). DEGREES OF ROTATION ARE APPROXIMATED.



▶ 318°



▶ 339°



▶ 360°

“This virus is really sneaky,” says Karolin Luger, a newly minted HHMI investigator at Colorado State University, describing the agent that causes Kaposi’s sarcoma, a cancer of the connective tissue below the skin. With the help of x-ray crystallography, a technique that enables scientists to deduce the positions of individual atoms in a structure, Luger and her collaborator Kenneth Kaye of Harvard Medical School have just discovered the devious way in which the Kaposi virus spreads: It piggybacks onto segments of chromatin, a protein- and DNA-containing structure inside the cell’s nucleus, forcing the cell to produce more viral genes every time it copies its own DNA.

Luger made this discovery while analyzing the shape of a nucleosome, the basic repeating unit of chromatin, to which a fragment of the Kaposi virus was attached. “The structure shows that the nucleosome can act as a docking platform for a virus,” she says. “This is a new role for it—and potentially this interaction could be prevented with antiviral drugs.”

Now she hopes to tackle a much bigger problem: discovering the shape of chromatin itself. “Our genetic information, which is stored in DNA, is not read linearly but packed into these highly convoluted and organized structures, and a lot of cancer comes from the wrong readout of genes,” she says. “To discover how this happens, we need to understand the structures involved.”

Luger’s goal illustrates a new trend in structural biology: focusing on the shapes of ever more intricate “molecular machines,” groups of molecules that self-assemble to do key jobs in living cells. Until about 15 years ago, scientists were happy to determine the structure of a single protein at resolution high enough so they could see the position of each of its atoms. Then, spurred by more efficient methods of growing crystals, better computers, and the more intense x-rays produced by a new generation of synchrotrons, they began to solve the atomic structures of single proteins bound to single receptors. Now they want more. They want to see the 3-D structures of the powerful molecular machines that produce our proteins, repair our DNA, defend us against microbes and, in effect, control our health.

These complex functional units consist of perhaps five to a dozen different proteins or nucleic acids that have come together for specific purposes. At times several different molecular machines unite to form even larger functional units.

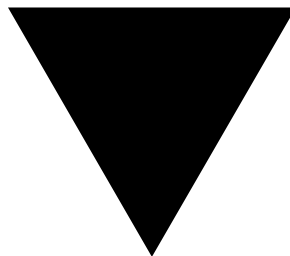
“We’re now able to visualize molecular assemblies of such complexity that I would never have predicted they could be crystallized,” says John Kuriyan, an HHMI investigator at the University of California, Berkeley. Kuriyan’s lab recently solved the intricate structure of a “clamp loader assembly,” a cluster of proteins that positions the machines that replicate DNA. It is difficult enough to grow a well-ordered crystal—the essential first step in x-ray crystallography—when dealing with just one or two components, he points out (see sidebar, “First, Grow a Crystal”). But, in 2000, Thomas A. Steitz, an HHMI investigator at Yale University, Peter B. Moore of Yale, and their colleagues solved the atomic structure of a complicated molecular machine—the large subunit of the

ribosome, the cell’s protein-building factory—at high resolution. This relatively enormous machine contained 3,000 nucleotides of RNA as well as 31 different proteins.

“When I first heard Steitz describe this work ... I felt much as I did when humans first stepped on the moon,” Kuriyan recalls. It was the largest molecular-machine structure that had ever been solved in such detail. Around the same time, the smaller subunit of the ribosome was also visualized, and this year the total ribosome structure—which shows how the ribosome produces new chains of protein, one amino acid at a time—was solved at reasonably high resolution.

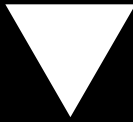
## BOLD COLLABORATIONS

The conquest of the large ribosome subunit emboldened scientists to tackle other molecular machines that in the past had seemed forbiddingly large. Their efforts have had some early and potentially useful results, such as leading biologists to learn exactly how certain classes of antibiotics kill bacteria and why certain bacteria are resistant to drugs. In order to prevent bacteria from producing new proteins, drugs actually target the bacteria’s ribosomes. Recent studies in Steitz’s lab and elsewhere have identified the specific crevices of bacterial ribosomes into which particular antibiotics fit. This discovery could lead



## FIRST, GROW A CRYSTAL

► **The first commandment of x-ray crystallography**—grow a crystal of the molecule you’re interested in so you can peer at its atomic structure—can be a scientist’s biggest stumbling block. ► **A crystal is a form of perfection**, in which all the atoms of each molecule are arranged in precise order and their pattern is repeated regularly in three dimensions. X-rays beamed at such crystals are then diffracted in regular patterns, which scientists can use to figure out the positions of the atoms that make up the molecule. But obtaining such crystals used to require enormous luck. It is still particularly difficult when dealing with large, complex, and flexible molecules. And in some cases it may be impossible, as Karolin Luger knows in connection with chromatin, the subject of her next experiments. “Chromatin’s structure is not regular enough to produce a good crystal,” she says. She realizes she will have to use different tools, such as an analytical ultracentrifuge or an atomic-force microscope. ► **Recently, scientists** have made several improvements in their methods of growing crystals. For example, it is now possible to test in a few minutes whether a particular protein is likely to crystallize in certain conditions, saving hours of trial and error. Instead of depending on just one set of conditions to make things crystallize, scientists can rapidly set up about 1,000 different conditions for growing crystals with the aid of new robotic dispensers that operate on the level of a nanoliter (one-billionth of a liter). All this can be done with just one milligram of protein. Then the results can be read with an automated microscope. According to David Agard, “Such methods are completely changing how we do crystallography.”



## CLAMP LOADER ASSEMBLY

THE INTRICATE STRUCTURE OF A CLAMP LOADER ASSEMBLY, A CLUSTER OF PROTEINS THAT HELPS POSITION DNA DURING REPLICATION, WAS RECENTLY SOLVED IN THE LABORATORY OF HHMI INVESTIGATOR JOHN KURIYAN.

Each of the five clamp loader subunits contributes a helical bundle to form a cylindrical structure called the collar domain. This tight association appears to be primarily responsible for keeping the five subunits together throughout the clamp loading cycle.

A nucleotide molecule with a triphosphate tail is trapped at each interface between nucleotide binding modules. As a result of subunit movements and DNA binding, the nucleotide tail is hydrolyzed from adenosine triphosphate (ATP) to adenosine diphosphate (ADP), which in turn stimulates the clamp loader to release the DNA sliding clamp.

Five-Subunit Clamp Loader Complex

The peanut-shaped nucleotide binding module for each clamp loader subunit packs in an organization determined by the type of bound nucleotide and the presence of the DNA sliding clamp.

DNA Sliding Clamp

The circular, central pore in the clamp is large enough to allow easy passage of double-stranded DNA. After forming a closed ring around the DNA target, the clamp becomes topologically linked to the double helix, and can slide freely along the DNA duplex.



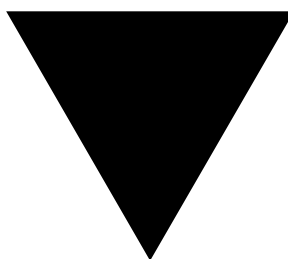
to drugs that fit more tightly, are more effective at low doses, and have fewer side effects.

Some biologists have been using the ribosome's structure as a jumping-off point to examine what happens to newly born protein chains when they emerge from the ribosome. Good health depends on proteins getting where they're supposed to go, but no one knew precisely how new protein chains make their way across various cell membranes—or how some of these chains become lodged inside the membranes. In 2004, cell biologist Tom A. Rapoport and structural biologist Stephen Harrison, both HHMI investigators at Harvard Medical School, led a team that solved the atomic structure of a surprisingly narrow membrane channel that new proteins must squeeze through, as through a birth canal. Docking next to this channel, the ribosome extrudes limp nascent protein chains right into the channel opening and pushes

them in. Once on the other side of the membrane, the chain folds into its active shape and gets to work.

The channel turned out to have a very tricky structure—it is shaped in part like a clamshell that opens and shuts, allowing a number of proteins to cross the membrane while holding back millions of others. It also directs some proteins sideways, to positions inside the membrane.

“The system is extremely ancient,” Rapoport explains. Every bacterial or mammalian cell that has an outer membrane or some internal compartments must be able to transport proteins across



## A BEAM GLOWS IN BERKELEY

► **A billion times brighter** than the sun and traveling at the speed of light, a thin beam of x-rays streams out of the ringlike Advanced Light Source (ALS) synchrotron in Berkeley, California. After hitting a small crystal, it bounces off, producing patterns that enable scientists to decipher the crystal's atomic structure.

► **Researchers who want to discover** the 3-D shape of a biological molecule—and have obtained a crystal of it—need the help of x-rays so intense they can be produced only by synchrotrons. In these large facilities, electrons that travel at nearly the speed of light are forced off their normal straight paths and into a circular route by magnets. At each bend, the electrons emit beams of light ranging from bright ultraviolet to x-rays. ► **Two years ago**, HHMI opened two “dedicated” beamlines at this synchrotron facility—with the cooperation of the U.S. Department of Energy, which operates it—to enlist these x-rays for regular use by structural biologists. ► **“It’s been super-successful,”** says David A. Agard, an HHMI investigator at the University of California, San Francisco, who notes that much of his own work on the shapes of large molecules that play important roles in protein folding would have been impossible without ALS’s new beamlines. At the Rockefeller University, Roderick MacKinnon, who won a Nobel prize in 2003 for solving the structure of a potassium channel, used the ALS to gain important clues about the channel’s shape. John Kuriyan and scores of other researchers, both in and out of HHMI, also sing the praises of the ALS. ► **Despite its prodigious power**, the ALS is not the most brilliant—or expensive—x-ray source in the United States. The Advanced Photon Source (APS) of the Argonne National Laboratory in Argonne, Illinois, outside Chicago, holds that distinction. To solve the structure of the ribosome’s major subunit, for instance, Thomas A. Steitz and his colleagues used the APS to build on their earlier results with the Brookhaven National Lab’s Synchrotron Light Source on Long Island, where HHMI installed its first dedicated beamline a decade ago.

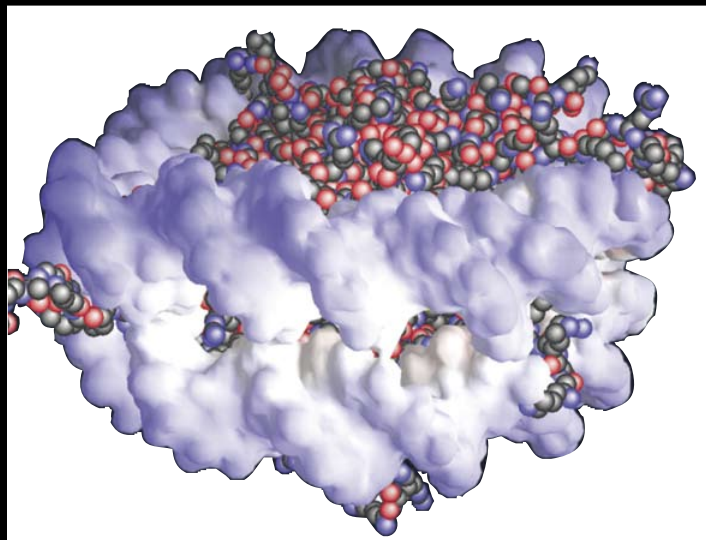
► **The beauty of the ALS**, scientists agree, is its power combined with its ease of operation. In addition, says Douglas Rees, “it is moving toward complete automation.” This should make it possible for biochemists and others who are not crystallographers to solve structures there—and greatly speed up the discovery rate of important molecular shapes.

these membranes to their destination as well as find ways to respond to its environment. Proteins such as insulin need to exit from the cell and travel to other parts of the body. All cells need certain proteins to be embedded in their membranes, to act as receptors for signals from other cells. The membrane channels that conduct such proteins in different species are amazingly similar. If they malfunction—if essential proteins are misdirected, misfolded, or destroyed—a variety of diseases can result.

Meanwhile, scientists have been pursuing the 3-D structures of several other “large molecular machines that control the birth, growth, and death of proteins,” says Kuriyan. Some researchers are even studying machines as dynamic as the spliceosome, a structure in the cell nucleus that is put together very loosely from different components that keep shifting location as the machine does its work. The spliceosome acts on RNA molecules that are copied from genes; its job is to excise any noncoding intervening sequences (introns) from mRNA and stitch together coding (exon) sequences to make “mature” mRNA that is then translated to proteins encoded by the gene.

At Brandeis University, HHMI investigators Melissa J. Moore and Nikolaus Grigorieff are collaborating in an effort to map the spliceosome’s structure, and the scope of the challenge is clear. The spliceosome must be exceedingly precise while splicing out introns, because a mistake that shifts even one nucleotide in a splice site will throw the entire gene-coding region “out of frame” and produce possibly dangerous mutations. Splicing errors are the basic cause of genetic diseases such as retinitis pigmentosa, some forms of dementia, cystic fibrosis, spinal muscular atrophy, and cancer. To prevent such outcomes in humans, the spliceosome must accurately identify more than 100,000 introns in diverse sequences of RNA.

Using electron microscopy, the scientists obtained a low-resolution structure of the spliceosome that showed several asymmetric sections forming an unusually large number of tunnels and bridges—an intriguing start. They could not use x-ray crystallography for these studies, Grigorieff explains, because



◀ **NUCLEOSOME CORE** SOLVING THE STRUCTURE OF THE NUCLEOSOME—A FUNDAMENTAL CHROMATIN COMPONENT MADE UP OF A DISK OF PROTEINS SURROUNDED BY DNA—WAS A STARTING POINT FOR KAROLIN LUGER. SINCE THAT ACHIEVEMENT, SHE HAS SHIFTED HER FOCUS FROM WHAT THE NUCLEOSOME IS TO WHAT IT DOES, AND HOW THE STRUCTURE CHANGES AS IT INTERACTS WITH OTHER MOLECULES. IN THIS SIDE VIEW OF THE NUCLEOSOME CORE PARTICLE, DNA IS DEPICTED AS A LIGHT BLUE SURFACE; ATOMS OF THE HISTONE OCTAMER ARE REPRESENTED AS SPHERES.

there are relatively few spliceosomes in a cell nucleus—far too few to grow into a crystal—and because “for crystallization, all spliceosomes would have to assume essentially the same shape.” Nevertheless, he says, “We have collected and averaged thousands of images of spliceosomes, which should give us a detailed structure at a higher resolution.”

### BETTER TOOLS FOR THE JOB

Many factors have come together to produce the current crop of new findings in structural biology. “All operations are faster,” says Douglas C. Rees, an HHMI investigator at the California Institute of Technology. “It’s also easier to decipher structures on the basis of data, thanks to computational programs developed by

Axel Brunger [an HHMI investigator at Stanford University] and others.”

In addition, all research on structures has benefited greatly from recent progress in genomics. “Now, when we’re interested in understanding a particular mechanism, we can pull out the proteins that carry out that function from many different genomes,” says Kuriyan. “Sometimes the genes from one organism produce proteins that for some reason are more stable and crystallize better than the human or other genes that you were working on originally.”

Scientists are also learning how large and shifting molecular machines can be caught in the act and crystallized as a whole. “Some of it is just luck,” says Kuriyan. “But some of it is the result of

doing experiments that tease out how the molecules work at a biochemical level. It’s like photographing a tiger at the water’s edge. You need to understand that the tiger comes to the water, know when it comes to water, position yourself by the pool—finally you get that moment when everything is right, and you snap it.”

As these methods improve, researchers will have more opportunities to see for themselves “how a structure talks to you,” as Nobel prize winner Roderick MacKinnon, an HHMI investigator at the Rockefeller University, once described the value of structural biology. Eventually this work will lead to a better understanding of how living cells function and how to repair them when they fail. ■