

PROTEINS DO VITAL WORK THAT KEEPS US ALIVE, not to mention the work that allows us to philosophize, explore the universe, and manipulate molecules. Yet there is no telling how many proteins a human being harbors. And each is a mystery: Out of a near-infinite number of ways to fold, a protein “chooses” one particular pathway and arrives at one particular “destination.” Using nuclear magnetic resonance technology, Angela Gronenborn has studied this dynamic process in a streptococcal protein called GB1, which sometimes folds into mutant or amyloid forms. Her folding landscapes illustrate the energy state of the protein as it folds. A smooth landscape shows a protein that efficiently reaches a low energy state. A rough landscape shows an unstable process with myriad opportunities to fold into an alternate form. Understanding this process can provide a window into diseases like Alzheimer’s, in which fibroid amyloid proteins entangle in the brain as plaque.

LIFE BEYOND VISIBLE LIGHT

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DARK ARTS

Visible light streams outward from the Sun in endless undulating waves clocking 300,000 kilometers per second through the vacuum of space. Eight minutes into that journey, the earth gets in the way. At the University of Pittsburgh's Biomedical Science Tower 3 (BST3), sunlight filters through the great glass windows facing Fifth Avenue. It refracts, reflects, and otherwise rattles around the open stairwells, hallways, labs, and offices. By virtue of a long, open well on the ground floor, a surprising amount of natural light finds its way 25 feet below street level, where it warms the hearts of structural biologists like Angela Gronenborn.

Down here, computer workstations overlook a concrete slab holding six enormous superconducting magnets. These magnets are the ponderous tools of Gronenborn's trade—they have relegated her to basements at the National Institute for Medical Research in London, the National Institutes of Health (NIH), and now Pitt. When architects asked Gronenborn, who heads Pitt's new Department of Structural Biology, if she wanted anything special in her BST3 workspace, she answered, a bit wistfully, "Sunlight." They delivered.

Gronenborn is trim and affable with fine blond hair that goes straight to her jawline and stops. Her pronunciation of consonants reveals her German birth, and her vowels disclose her years spent in the UK. She is an expert in nuclear magnetic resonance spectroscopy (NMR), meaning she uses magnetic fields to create images of important biological molecules, right down to the atoms. As cellular proteins fold (or misfold, as they do in many diseases), Gronenborn can actually watch and understand the process. Each of the magnets she uses is essentially an upright MRI machine in a vat big enough for treading water.

A wavelength of natural light that finds its way to Gronenborn's workstation measures anywhere from 300 to 8,000 Angstroms (30 to 800 nanometers)—small enough that it repeats 100 times or more as it travels the width of a human hair. With modern microscopes, you can see objects and living things that approach this size. However, anything smaller than the wavelength of visible light—viruses, DNA, certain proteins, and organelles—is in a world of darkness. This is the world plied by structural biologists.

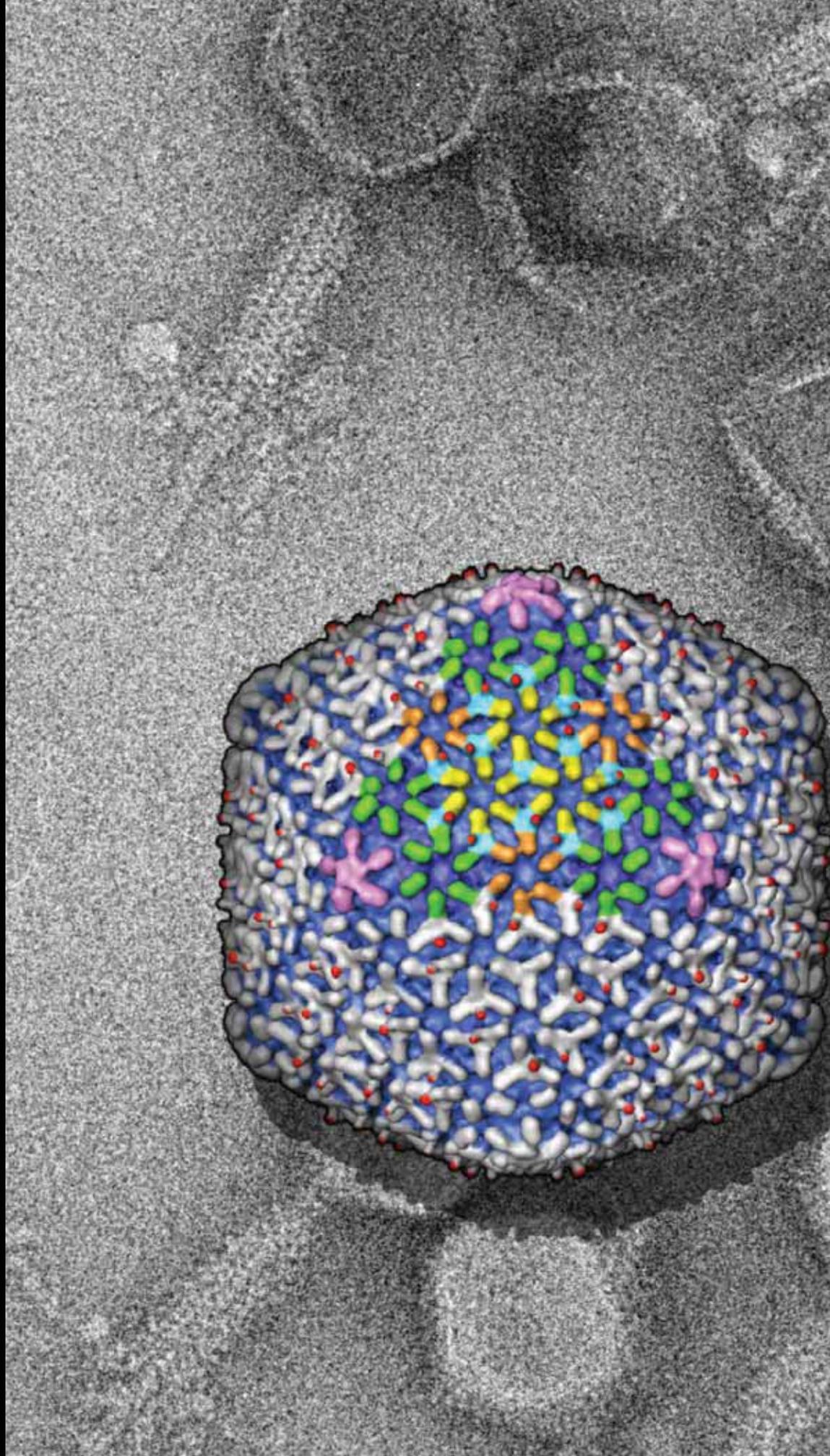


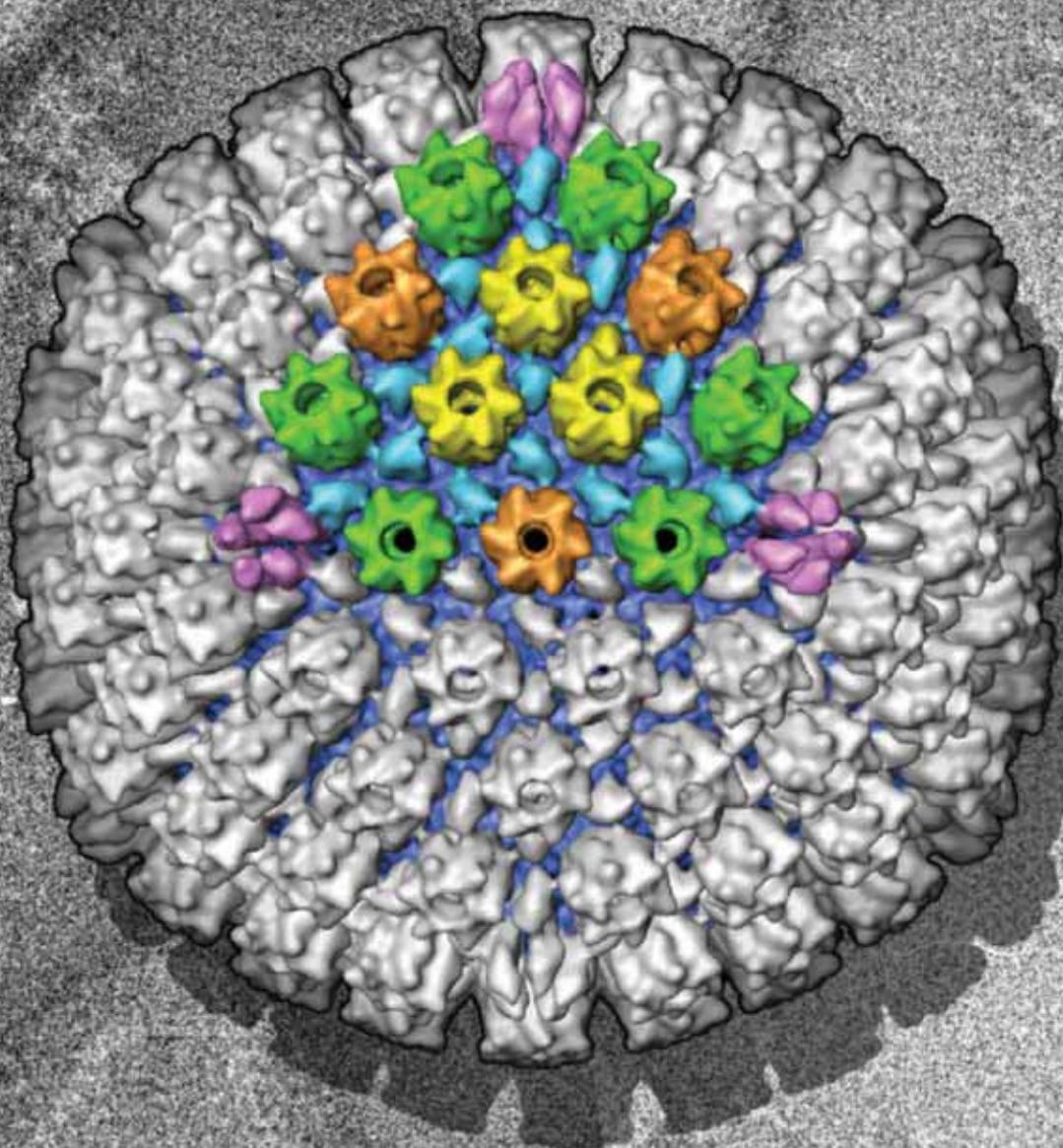
Walk down the corridor away from Gronenborn's magnets, and you'll cross onto another concrete slab. The microscopes here are so sensitive that vibrations originating elsewhere in the building can ruin attempts to capture images of samples. This is where associate professor James Conway practices what he calls (in a New Zealand accent) a "black art."

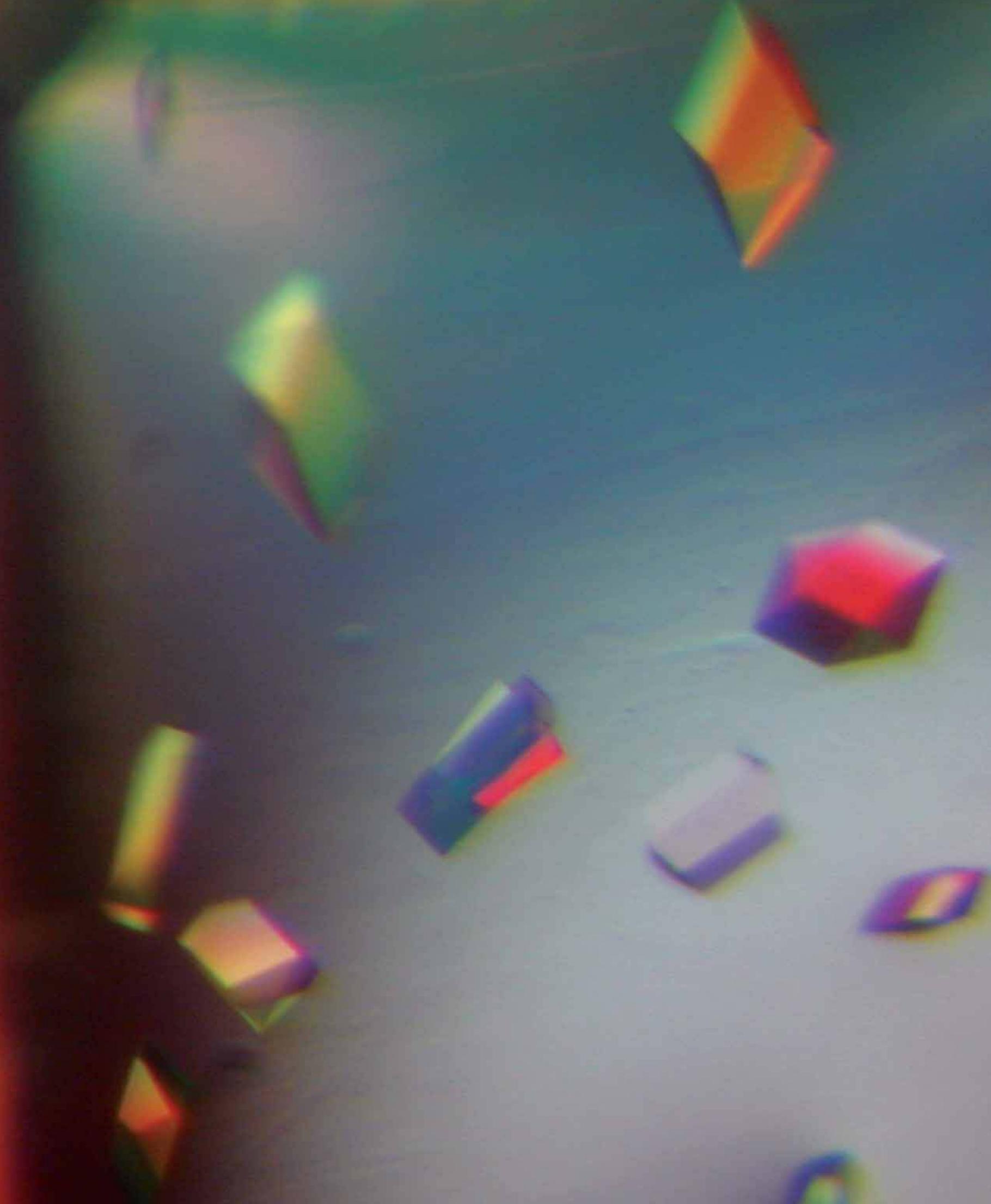
The art, in a nutshell: Place your sample—a solution containing viruses, for example—onto a copper grid. Use blotter paper to soak up the excess. (Herein lies some alchemy—the proper length of time to apply the paper is a guess, but timing is critical to image quality because it determines the thickness of the ice surrounding the sample.) Plunge the grid into a bath of liquid ethane, which rapidly freezes the sample. Now it's ready for the cryoelectron microscope, which fires a beam of electrons with a wavelength measuring a fraction of an Angstrom.

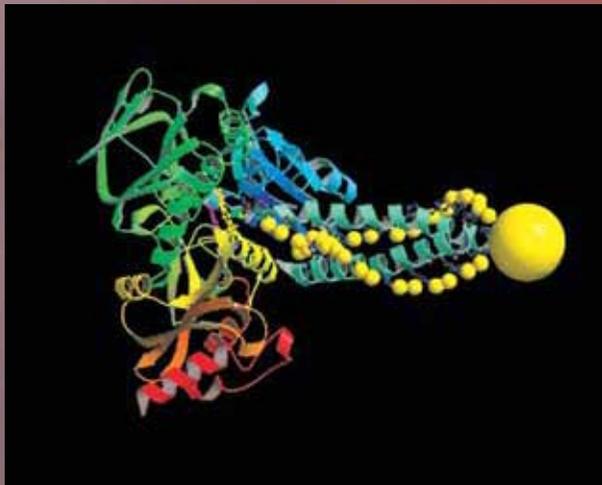
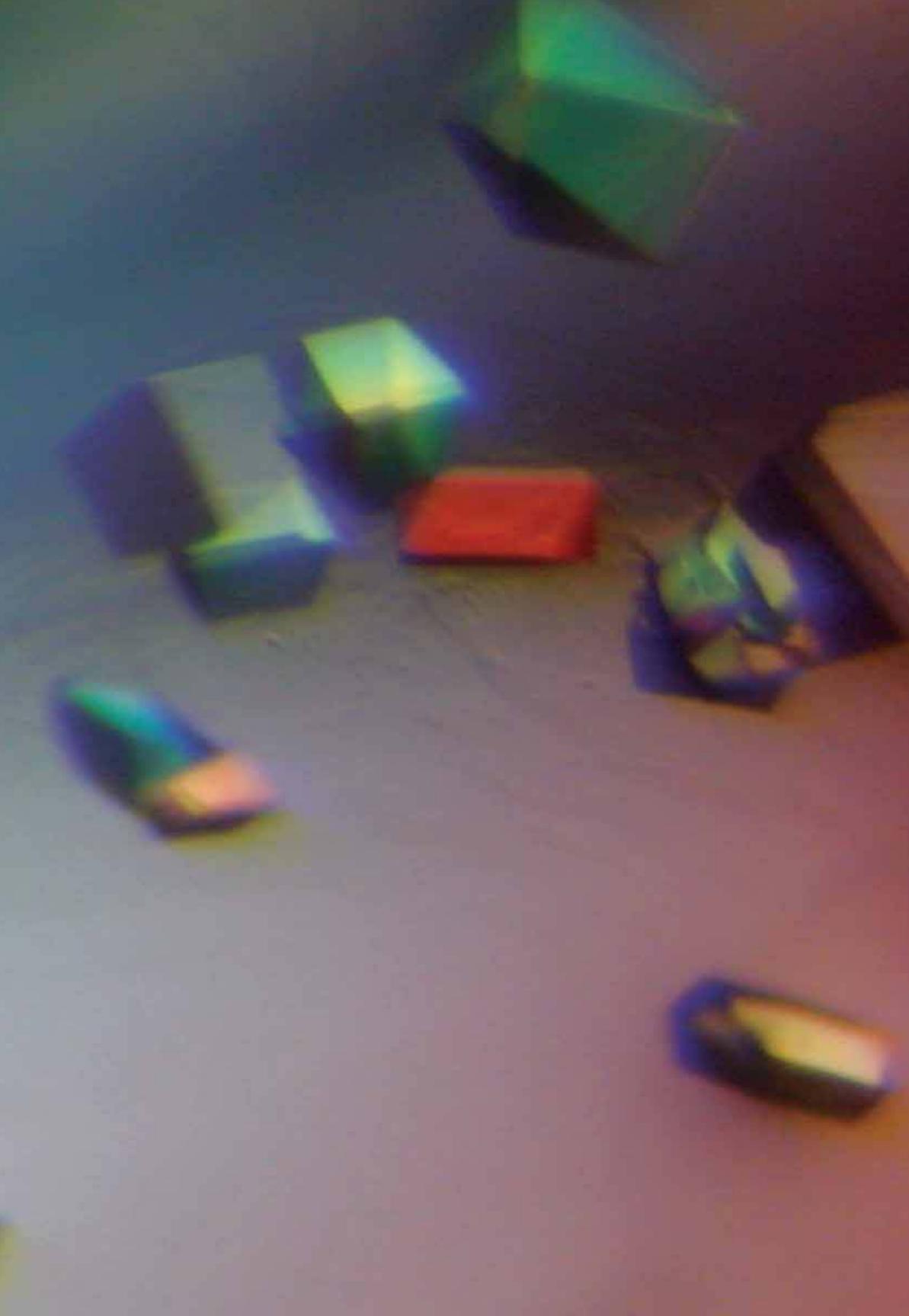
Conway uses cryoelectron microscopy to produce detailed structural images of viruses and other large protein complexes. With colleagues at Pitt, the NIH, and Scripps Research Institute, he has unraveled some of the secrets of virus life cycles. And he's advancing this black art. In 1997 he succeeded in improving resolution from 17Angstroms down to 9—the difference between seeing a red line on a baseball as it whizzes past and noticing individual stitches in the leather as you hold it in your hand. At Pitt, he aims to break the 5Angstrom barrier.

SEPARATED AT BIRTH? Scientists have long assumed that viruses plaguing humans don't have much in common with those that affect bacteria. Now they're rethinking that premise. The background image here shows bacteriophages (SPO₁) viewed with electron microscopy. Pitt's James Conway, working with Roger Hendrix and Robert Duda of the Department of Biological Sciences, used cryoelectron microscopy to compare the bacteriophage's outer protein shell (left) with that of a human herpes virus (right). Shell proteins are color coded, showing a shared structural pattern, which had previously been associated exclusively with herpes. This lends support to the theory that these viruses share a common ancestor.









Upstairs, Pitt associate professor Joanne Yeh grows microscopic crystals that glimmer and shine as light refracts from their surfaces. Although the recent recruit from Brown University admires the crystalline beauty of these formations, they are only an intermediate step in figuring out the structure of the protein molecules within.

In 1952, working in a cellar at King's College in London, Rosalind Franklin used x ray crystallography to produce an extraordinary DNA image that she labeled "Photo 51," which, without her knowledge, was shown to James Watson and Francis Crick, giving them clues they needed to decipher the double helix structure. Yeh's work is in Franklin's tradition (in fact, her department head, Gronenborn, holds the Rosalind Franklin endowed chair in structural biology), though x ray crystallography has come a long way from those days. Yeh can reveal the structure of molecules to atomic resolution detail. And analyses that might have taken Franklin a year, Yeh can do in an afternoon with computational advances and 3-D modeling programs. Once her lab prepares a pure sample of protein to crystallize (this form ensures the molecules are stacked in an orderly fashion, like rows of identical parts packed into a warehouse), she fires an x-ray beam at it. By solving the diffraction pattern, Yeh can generate a 3-D model, showing the location and orientation of each and every atom. With this information, pharmacologists can design drugs and bioengineers can make nanomachines that work at the molecular level.

HOW DO YOU BUILD A MACHINE OUT OF A MOLECULE? First learn its exact structure. Joanne Yeh crystallized a pure solution of an enzyme that metabolizes hydrogen peroxide (a marker of inflammation); analyzing patterns of x rays diffracting off the crystals allowed her to then solve the enzyme's structure. **INSET:** With physicists and engineers, Yeh linked the molecule to a gold nanoparticle (large sphere) using a peptide (green ribbon) bound with cobalt (small yellow spheres). When the team immobilized the enzyme assembly onto gold nanoelectrodes, the tiny machine conducted electrical signals in the presence of hydrogen peroxide. This technology may one day be used to create biosensors to diagnose disease states like inflammation long before symptoms become detrimental.

LARGE IMAGE: Crystals of macromolecules.

Gronenborn admits that she could have happily remained at the NIH, where she led a vigorous and successful program. But she says she was thrilled by the opportunity to bring NMR together with x ray crystallography and cryoelectron microscopy. Each is such an expensive enterprise that most institutions have added them piecemeal in scattered departments and buildings. At Pitt, structural biologists are next-door neighbors, each practicing his or her obscure art with the latest generation of equipment. These investigators can interact daily with Pitt's computational biologists, pharmacologists, and clinicians (and, of course, with each other) to bring the finest bits of life out of the darkness and into the light. ■

STRUCTURAL WORKHORSE: When proteins mutate to amyloid forms, bad things happen like type 2 diabetes. To understand the basics of this process, Angela Gronenborn probes a protein called GB1—the “workhorse” of her lab. GB1 sometimes mutates and forms long amyloid fibers, as seen in the gray cryoelectron microscopy images. Using NMR, Angela Gronenborn creates cartoons to decipher the arrangement of the thousands of atoms involved. Red and teal ribbons represent two presentations of the backbone atoms and show how they arrange themselves into a long helical strand; many strands together form an amyloid fiber. Such representations help scientists design drugs to interfere with the amyloid formation process, like the protease inhibitors that are the current standard of care for HIV/AIDS.

