INVESTIGATING THE MINUTE MOLECULES IN BIG BIOLOGY

BY ALLA KATSNELSON

GREAT EXPECTATIONS

Structural biology, a research endeavor that examines the smallest possible scale of biological life, demands some of the most imposing tools in life science. Amble down the aluminum spiral staircase to the basement of the University of Pittsburgh's Biomedical Science Tower 3 and you'll find a massive chamber with sunshine-yellow walls, a concrete floor, and shiny chrome ladders on casters flanking tall white canisters of various sizes. This is the Department of Structural Biology's fleet of nuclear magnetic resonance (NMR) spectrometers. Drop a small tube of your chosen protein into an opening that leads down into the heart of one of the spectrometers, and the magnetic fields it generates—thousands of times stronger than the Earth's—allow researchers to construct three-dimensional images of your or any other macromolecule, down to the last atom. Down a short corridor, three cryogenically cooled electron microscopes detect cellular and subcellular architecture. In a ground floor suite, X-ray generators powered

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Pitt structural biologist Angela Gronenborn, right, has assembled a fleet of nuclear magnetic resonance spectrometers and other tools for three-dimensional protein analysis. Michael Delk, left, manages the lab.


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Angela Gronenborn surveys her sandbox—the research space she assembled to fuel the team-based discoveries of Pitt structural biologists.

by a central computer yield high-resolution atomic structures of crystallized proteins in a technique known as X-ray crystallography. In nearby labs, researchers can produce milligrams of pure protein—the functional equivalent to buckets, on the microscale—for any of these studies.

This is the space that Angela Gronenborn, PhD, calls her sandbox—the space she built to give Pitt structural biologists a top-notch place to play. A relatively young field at the junction of chemistry, physics, and molecular biology, structural biology endeavors to determine how the 20 amino acids encoded in the genome take shape to form the proteins that power all biological processes. Gronenborn is a world leader in the effort. Recruited to Pitt in 2004 to build the Department of Structural Biology and serve as its founding chair, she arrived with a clear vision: Acquire the best instrumentation available and assemble researchers with diverse skills to use it and the compatibility to collaborate. "I'm a strong believer in what I would call 'team science,'" says Gronenborn, an NMR expert and a member of the National Academy of Sciences, who holds the UPMC-Rosalind Franklin Chair in Structural Biology. "To understand big biological systems, you have to combine different methodologies and perspectives, and then integrate it all to get the final picture."

The approach is paying off. Under Gronenborn's leadership, the department has achieved acclaim worldwide and launched a small but growing graduate program. She and her colleagues also established a National Institutes of Health–funded center for structural research on the human immunodeficiency virus (HIV)—one of five such programs nationwide. Last May, *Nature* published the team's landmark description of the structure of the virus' capsid, or protein coat, pegged to an unprecedented resolution. Perhaps just as important as Gronenborn's role in that singular discovery is her role in creating an environment that allows investigators to pursue great science, surrounded by all of the tools they need to do what scientists do: follow their noses. "That approach has been so good to me," she says.

Gronenborn likes to say she's been lucky in science. Born in Cologne, Germany, she attended an all-girls boarding school. As graduation neared, the principal and her father dissuaded her from becoming a mathematician. She chose physics and chemistry instead. After completing her undergraduate degree at the University of Cologne in 1975, she stayed on for her PhD in physical chemistry. NMR, a technology used to measure the physical properties of molecules to create atomic-scale images of them, had captured her imagination. NMR was already widely used in physics and chemistry, but Gronenborn envisioned a different application.

Her brother Bruno was a PhD student at the same university, and she would often join him in his lab, where he was studying how proteins regulate gene expression by binding to DNA. The field of molecular biology was in its infancy. Although the structure of DNA had been described by British scientists James Watson, Francis Crick, and Rosalind Franklin in 1953, researchers knew little about how genes were transcribed into proteins, and the ability to sequence DNA was on the cusp of invention. "I found the whole area absolutely fascinating," Gronenborn says. When she finished her PhD in 1978, she was determined to use NMR spectroscopy to solve the structures of protein complexes—particularly protein interactions with DNA—in order to study how genes are switched on and off.

NMR was a nascent method, and few researchers at the time believed it could be extended to molecules as large as proteins, but Gronenborn was undeterred. She headed off to London for a postdoc with James Feeney at the Medical Research Council's National Institute for Medical Research in Mill Hill, where Feeney's lab was just beginning to explore the use of NMR in proteins. The group was studying how drugs bind to an enzyme called dihydrofolate reductase, and Gronenborn set out to reveal how ligand and drug binding affect the enzyme. Structural biologists had already solved the structure of a few dozen proteins by X-ray crystallography, but the work provided just a freeze-frame image. To determine the effects of ligands on the enzyme would require repeatedly crystallizing the ligand in complex with the enzyme—a daunting task. NMR offered an easier approach. By measuring the spectral peaks of dihydrofolate reductase in solution, Gronenborn made her first foundational observation: The protein could exist in different conformations. "That showed us that there were a lot of dynamics going on in proteins—they were not all standing still," she says. "It also showed that NMR can give information that is not available by other techniques, such as crystallography."

"[The National Institutes of Health] said, 'You can work on whatever you want, if you occasionally also look at an HIV protein,'" Gronenborn says. "Ever since, I've been working on HIV or HIV-related proteins."

Gronenborn wasn't standing still, either. London was a refreshing change after Germany's highly hierarchical university system. "I was given the freedom to follow my own curiosity, and I was daring enough to do things that were difficult but very interesting," she says. She met Marius Clore, a student at the institute, and the two of them tackled the topic that had originally captivated her. They spent long hours in the lab, fumbling with early protocols for synthesizing DNA and then running the samples, thrilled with the yield of NMR spectra that had never been seen before. In 1981, Gronenborn was awarded a permanent position at the institute; her reputation in the field was gaining traction. One day in the lab, she got a surprising phone call: On the line was crystallographer Max Perutz, who had shared the 1962 Nobel Prize in Chemistry for determining the structure

of hemoglobin. Now he wanted her to work some NMR magic on a project with which he was struggling. A multiyear collaboration was born. "She was one of the very few people in those days who had the rare combination of biochemical skills and NMR expertise—not to mention her exceptional drive—necessary to develop the fledgling area of biological NMR," Feeney says.

In her six years at Mill Hill, Gronenborn published more than 50 papers. In 1984, she and Clore, by then married, accepted a joint post at the Max Planck Institute of Biochemistry in Munich. There they headed the biological NMR group and continued refining their use of NMR to study protein structure and function. Klaus Schulten, a computational biophysicist now at the University of Illinois at Urbana-Champaign, met her there when he was on the faculty at the Technical University of Munich. "When she came back to Germany," he says, "she was already a very established scientist and she had a very clear and interesting goal."

"Angela has this amazing intuition for how to approach things that look risky, yet are very promising questions from the standpoint of general biological insight," says biophysicist Tatyana Polenova.

In 1988, the National Institutes of Health came knocking with an urgent problem. Researchers had already identified HIV as the virus responsible for a deadly new epidemic ravaging gay men and intravenous drug users, and the agency had launched a clinical trial of a vaccine, but little was known about its modus operandi. Not a single structure for the virus or its associated proteins had been determined. The agency recruited Gronenborn and Clore to Bethesda, Md., where Gronenborn became chief of the structural biology section at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). "They said, 'You can work on whatever you want, if you occasionally also look at an HIV protein,'" Gronenborn says. "Ever since, I've been working on HIV or HIV-related proteins."

Gronenborn and Clore set up shop in the basement, where Adriaan Bax, PhD, a biophysicist and NMR spectroscopist, had just installed a powerful new magnet for the HIV work. NMR uses very strong magnets. Place any molecule—a protein, say—into a magnet, and the nuclei of every atom in the protein interact with the magnetic field. Interject radiofrequency pulses, and the communication among the atoms—known as the exchange of magnetization—can serve as a sort of ruler, allowing researchers to calculate distances and angles among the nuclei and ultimately build a model of the protein. Gronenborn's and Clore's expertise lay in expressing and labeling proteins with NMR-active isotopes and developing algorithms to determine their three-dimensional structure. Bax, meanwhile, was a brilliant choreographer, coaxing the spectrometer to deliver sequences of pulses that allow the nuclei to converse. "I would go to Ad and say, 'Oh, can you do this type of experiment?'" Gronenborn recalls. "And he would always say, 'Ah, it's impossible.' And then, a few hours later, he would say, 'Oh, let me try this … .' And he would try it, and it would work!"

In its early days, protein NMR used mainly proton spectra. Unfortunately, all but the smallest proteins had too many overlapping peaks for scientists to resolve. Gronenborn and her colleagues realized that expanding the spectra from two dimensions to three and four could alleviate this problem. As Clore and Gronenborn analogized in 1991, imagine a macromolecule as a large encyclopedia. Arranged in a single line of text, the letters would crowd into an unreadable jumble. Expanded to two dimensions—a page—the overlap would remain too strong to discern more than a few letters here or there. In three dimensions, spaced out into a book, many of the words would be distinct. But only in four dimensions, as a set of tomes, would the entries be fully readable. They combined this methodology with a new way of labeling proteins—on nitrogen and carbon nuclei, as well as the conventional protons—to untangle the crowded spectra and correctly assign individual peaks to every atom. Their technique has since become routine. "This was a huge breakthrough," says Tatyana Polenova, a University of Delaware biophysicist and a member of the Pittsburgh Center for HIV Protein Interactions. "Without this seminal work from her lab, we would not be able, today, to solve structures of large proteins."

The group's first major achievement with these methods was solving the structure of interleukin-1 beta, a protein with 153 amino acids. Today, a protein that size is relatively easy for a structural biologist to wrangle, but at the time, its size posed a formidable challenge. Other important structures followed: several cytokines and chemokines, a host of HIV-related proteins. The group also solved the structures of several protein-nucleic acid complexes. "Those were things that very early on I had wanted to do but were impossible to do," Gronenborn says. "So now that we had the technology, we could do it, and that was very gratifying."

On paper, Gronenborn, Clore, and Bax each had their own research groups, but in practice they collaborated closely; the 30 or so postdocs among them shared a study area, equipment, and resources. "There was tremendous synergy," says Bax. "Basically, everybody was there 7 days a week, 12 hours a day." When they weren't working, he adds, they would be out drinking together or mingling at the frequent parties that Gronenborn organized. Known for their fantastic food and wine, the gatherings were a particular draw for cash-strapped postdocs.

Jun Qin, now a professor of molecular medicine at the Cleveland Clinic, still draws on the collegiality that he experienced as a postdoc in Gronenborn's lab. Many of his cohort of trainees remain in touch two decades later. "We still exchange ideas and talk about grants and papers," he says. Like Gronenborn, Qin came to structural biology from a chemistry background, and he was impressed to find Gronenborn herself teaching him how to grow bacterial

cultures and prepare proteins. "Of course, I made some mistakes," he says, recalling how, early on, he tried labeling a 6-liter batch of cell culture with isotope-labeled glucose. He dumped a few thousand dollars' worth of the pricey sugar solution into a container and was horrified to find that the cell culture didn't grow. "I thought it was the end of the world," Qin says. "But she just said, 'Okay, sit down and calmly think about what you did and what happened.'" (Happily, together they found the cause of the problem, and his second attempt at labeling worked.) "I learned all the state-of-the-art technology in her lab," Qin says, "and I also learned how to do science—how to address important questions."

Gronenborn could have continued on at NIH indefinitely, but she began to feel the tug to leave a legacy larger than her own body of research. She had recently parted ways with Clore when Arthur S. Levine, senior vice chancellor for the health sciences and John and Gertrude Petersen Dean of the School of Medicine, reached out to lure her to Pitt in 2004. Medical schools rarely have structural biology departments, but Levine believed that structural, computational, and developmental biology together form the bedrock of contemporary biomedical research. He set out to erect a research building in which structural biology research would serve as a literal and metaphorical foundation. Gronenborn, for her part, was drawn to the challenge of building a new venture. She knew her vision carried a big price tag—more than $10 million for the NMR instrumentation alone—and was impressed by Levine's support. "People pay lip service to the need to understand things at the atomic and molecular level, but to really do it as a big enterprise takes a lot of money and commitment," she says. "And the institution here has been committed—that is what attracted me."

She was deliberate about faculty recruitment. Rather than assembling researchers based on biological interests, she sought to create a team that was able to innovate methodologically. She began by recruiting people with a wide variety of technical expertise—NMR, crystallography, cryo-electron microscopy (cryo-EM), protein expression—and the ability to collaborate to address big questions.

"It's very rare that all these techniques can come together in one place, and that's what I wanted to do here," Gronenborn says. She also wanted to bring in more than one specialist in each technique. "I've learned over the years that you always need to talk to people who are close to you in methodology," she says.

Gronenborn didn't explicitly plan to include HIV in her own or the department's scope, but in 2006 the NIH requested funding proposals for research centers focused on structural elements of the virus. Because she had worked on the virus at NIDDK, she decided to apply and she invited Peijun Zhang, PhD, a new faculty hire and an expert in cryo-EM, to help write the application. "I said, 'I have no experience with HIV,' but she said we would pull together all the expertise necessary to carry out the project," recalls Zhang. "That's the way she directs the center. She brings the right people together."

One of the HIV experts Gronenborn engaged was Christopher Aiken, a Vanderbilt University virologist. "I'd seen her name," he says, "but she wasn't that central a player in the HIV field." However, he was impressed with her idea to focus the center on early events in the HIV life cycle, a wide-open topic at the time, and was further hooked by the quality of the draft proposal, which—after years in Europe and at the NIH—was Gronenborn's first competitive grant application. "I thought, *Wow, this looks like a winning horse*."

And indeed it was. With the grant awarded in 2007, the center's researchers—based at Pitt as well as other universities in the U.S. and abroad—embarked on solving the structure of the HIV-1 capsid, the protein shell that carries viral DNA into the cell. That knowledge might point to new ways of thwarting the virus before infection takes hold, a workaround to the drug resistance that occurs with current treatments. "The structural biology of the HIV capsid is extremely challenging," explains Zhang. That's because capsids of retroviruses don't form uniform particles. Structural biology methods, on the other hand, are all based on averaging, and they work best when components are uniform.

To get around the problem, Zhang and Gronenborn led the center's efforts to use cryo-EM and NMR to work out an intermediate-resolution structure of an in vitro-assembled capsid. That structure, which *Cell* published in 2009, offered hints on how the capsid forms and dissolves. Other avenues were also pursued. "Angela has this amazing intuition for how to approach things that look risky, yet are very promising questions from the standpoint of general biological insight," says biophysicist Tatyana Polenova. An expert in a type of NMR conducted in condensed phases rather than on molecules dissolved in solution, Polenova joined the center in 2010, when Gronenborn asked whether her technique might work for studying the HIV capsid. She was happy to give it a shot, but noted that it would be a challenge. "Angela said, 'Okay, my philosophy is: We may go down in flames, but let's have fun trying.'"

Meanwhile, Zhang continued to push the HIV-1 capsid structure to high resolution, ultimately combining her findings with computational modeling conducted by Schulten to yield an approach in which individual atoms could be discerned. At 4 million atoms, and 1,300 proteins, the HIV capsid structure is one of the largest ever solved. (See how Zhang and her colleagues solved the puzzle on p. 18.)

As this project moved toward completion, Gronenborn set out on a new quest—one that took her away from the bench. For the past two years, she had held an ongoing discussion with Sandra Mitchell, PhD, chair of Pitt's Department of History and Philosophy of Science, about success in the scientific process. So she used a sabbatical at Berlin's Institute for Advanced Studies to try to quantify the value of an idea that has been central to her work: the power of team science. Using data from granting bodies and publication records, she attempted to track whether increasingly interdisciplinary research teams could show measurably higher levels of success.

The answer to Gronenborn's big question proved more elusive than she expected, though she hopes to return to the problem. Meanwhile, she did learn one thing: If she ever takes another sabbatical, it had better be in a lab. Returning to Pittsburgh this fall, she realized how deprived she had felt. "The initial glimpse of new data that tells you, *hmm, it's not what you expected*," she says, "that's still the most thrilling thing." ■