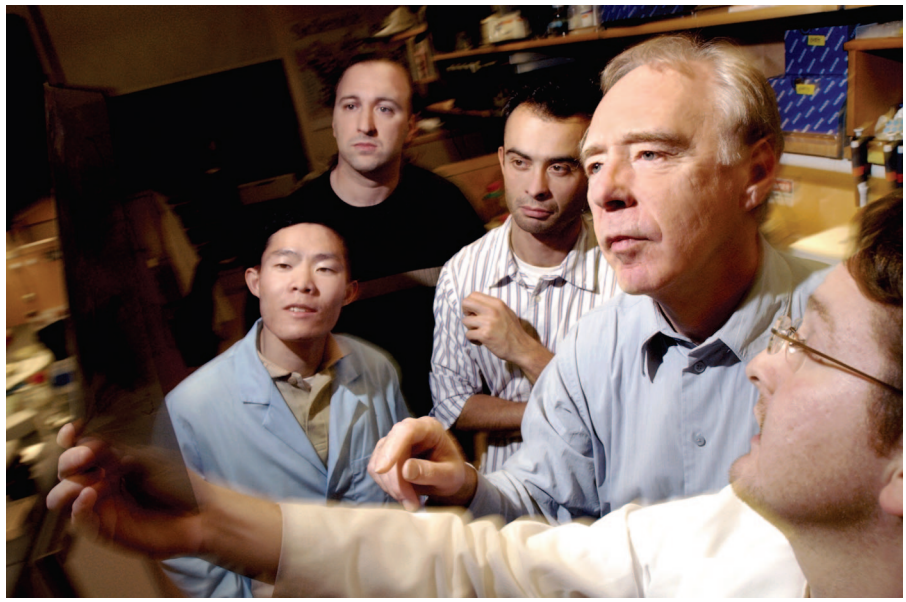
Profile of Robert M. Stroud

For a cell, membranes are life-enabling demarcations and barriers that must be crossed, and many specialized proteins and channels play crucial roles in facilitating transport across membranes. One such transmembrane protein, Ire1, is responsible for putting the brakes on protein folding run amok. Determining how Ire1 actually senses and responds to a backlog of unfolded proteins in the endoplasmic reticulum (ER) has been a focus of structural biologist Robert M. Stroud. Elected to the National Academy of Sciences in 2003, Stroud's joint Inaugural Article (1), published in a previous issue of PNAS with colleague and fellow Academy member Peter Walter, is yet another example of how understanding protein structure leads to an understanding of function.



Robert M. Stroud, second from right, with (from left) graduate student Joe Ho, postdoctoral researcher Franz Gruswitz, senior scientist Pascal Egea, and graduate student Ian Harwood (holding film).

From Infinite to Infinitesimal

Currently a professor in the Department of Biochemistry and Biophysics at the University of California, San Francisco, Stroud was born in Stockport, England, in 1942. Growing up, he was intrigued by the stars. "I set up the telescope in the garden on a clear night and wondered at the enormity of the universe," he says. His father, an engineer, shared his love of science and mathematics with his son. Stroud recalls, "He would build an electric motor from parts he made on a lathe and by hand, in front of my eyes, and it would work!" Inspired, Stroud designed electronic devices, amateur radio transmitters, a pH meter, tape recorders, and amplifiers, all in a garden shed that his father provided.

Despite his interests, Stroud recalls that, "I was not much of a student until I was about 13 years old, when I encountered a rather Germanic math teacher, Charles Oefner, and, later, Brian Gaskell, at Cheadle Hulme School (Stockport, England). They were inspirational," he says. Oefner would read aloud test scores. "There was no grade point inflation. Rarely did anyone score over 65%," recounts Stroud. Then one day Oefner announced, "Stroud, 100%." "From that point on, I realized how much I loved the logic of mathematics and calculus," he says.

Stroud entered Cambridge University (Cambridge, England) on scholarship in 1961 to study physics and mathematics. At Cambridge, he encountered the science of crystallography for the first time. The infinitesimal nature of matter fascinated Stroud just as much as the infinite nature of the universe had in his childhood garden. "If you got to the finest level of matter, things turned into discrete little things, atoms. It still strikes me as the most incredible thing in the universe," he says. At Cambridge, Stroud had an inspirational teacher in crystallographer Michael Bown. "It resonated with me that you could see atoms and that the way they were put together had to do with the properties of matter," he recalls.

Upon graduating in 1964, Stroud planned to continue at Cambridge for a Ph.D. in physics. During the summer after graduation, he traveled with the water polo team in Europe with plans to return to the laboratory. About a week before the start of classes, Stroud realized, "It wasn't really so much physics that enchanted me, but more that molecular structure could lead to an understanding of biological function." Stroud began to inquire about places where he could work on biological structures and chose J. D. Bernal's laboratory at Birkbeck College (London).

According to Stroud, Bernal's group at Birkbeck was famous for crystallography. Rosalind Franklin had studied there, also under Bernal. "I realized partway through graduate school that I was sitting at the desk that had been Rosalind Franklin's desk. I had enormous respect for her work," he says. Stroud also met other important figures during his time there, including James Watson and Max Perutz. "It was very, very exciting to have met the founders of the field," he says.

For his thesis, Stroud worked on defining small peptide and nucleoside structures. Stroud was one of the first to decode a crystal structure, that of the anticancer agent tubercidin, by using noncentrosymmetric direct methods (2). Noncentrosymmetric direct methods use the inherent mathematical relationships between the diffracted amplitudes as the means to derive the structure of a molecule "without any more knowledge than the observable diffraction patterns themselves," Stroud explains. He wrote his own computer programs, to calculate things like Fourier transforms, in the days of punch paper tapes. "That was back when computers actually made noise," he says.

While working on his Ph.D., Stroud shared an apartment with the editor of *Medical News* (London), the main medical newspaper of the time. Stroud was roped into writing for the publication and translated molecular biology for medical doctors. Reading and digesting the literature brought about a revelation for Stroud. "The atomic nature of matter, the most phenomenally amazing thing in the universe, has a direct impact on human health. If you knew how these proteins worked, you could make better therapeutics," he says.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 18773 in issue 52 of volume 102.

www.pnas.org/cgi/doi/10.1073/pnas.0600255103

Permanent Deferral

Upon finishing his doctorate in London in 1968, Stroud sought to work with a scientist who had left an impression on him several years earlier. "When I was a graduate student in 1965, I had the unbelievable thrill of hearing David Phillips as he unveiled the structure of lysozyme," he says. The talk was "enchanting," he recalls. In 1968, Stroud wrote to Philips inquiring about a position at Oxford University (Oxford), where Phillips had relocated. Philips offered Stroud a post as lecturer but explained that it would not be available until the following year and suggested that Stroud do a postdoctoral stint, something that was not as common then. "I thought it would be a good time to explore the U.S.A.," says Stroud. He wrote to Richard Dickerson at the California Institute of Technology (Pasadena, CA). "Caltech was the pioneering crystallography school in the U.S.," he recalls. Before that time, Linus Pauling, whom Stroud greatly admired, was the Chair of the Chemistry Department. "He originated the notion that you could understand proteins by understanding the amino acids and the peptide bonds that joined them together. I was lucky enough later on to have his brilliant grandson, Sasha Kamb, join me in the lab. I have a piece of Linus Pauling's beta pleated sheet model on my wall, given to me by Sasha as a gift, which I highly prize," says Stroud.

At the end of his postdoctoral tenure, Stroud enjoyed Caltech so much that he wrote Phillips to ask for a year-long deferment of the Oxford lectureship in order to continue at Caltech as an instructor. At the end of that year, as an instructor with a laboratory and a few students of his own, he was "still having fun," he says, and was granted another deferment. Eventually, in 1973, Stroud was hired as an assistant professor at Caltech. "Regretfully, I missed the Oxford school created by David Phillips that I so much admire," he says.

Moving up the Coast

In 1976, Stroud received an offer to help start a structural biology program at the University of California, San Francisco. The school was small at the time. "I had only just become aware of its increasing stature," he says. William J. Rutter, whom Stroud calls a visionary, had started a program aimed at understanding biological processes in disease. Stroud began to build a graduate program in 1977 and helped recruit, among others, colleagues Robert Fletterick and David Agard, a former student of Stroud's from Caltech. "We really nucleated a graduate program that focused on the science of biophysics" and emphasized good teaching, Stroud says.

At the University of California, San Francisco, Stroud continued his work on understanding cellular processes at the level of atomic structure. He took advantage of a local model organism, *Torpedo californica*, to understand how the nervous system can signal so rapidly. *T. californica* is an electric ray that lives in the waters of the Pacific Ocean, just off the coast of northern California. In 1977, Stroud published his first paper on the acetylcholine receptor in *T. californica* with Michael Ross (3). By 1979, Stroud and Ross had published the first three-dimensional structure of the acetylcholine receptor (4). "It is something I am extremely proud of. It was one of the first uses of density modification to determine structure and grew out of my earlier structures determined by direct methods," says Stroud. Density modification uses the knowledge that structures always have positive scattering density and finite size as a constraint in refinement of structures. Today, other derivatives of this principal are now used in almost all structure determinations.

"The atomic nature of matter, the most phenomenally amazing thing in the universe, has a direct impact on human health."

Acquiring enough protein to study provided a challenge. Stroud describes the *T. californica* ray as essentially having a large biological battery on its back. When triggered, it delivers a 45-V, 120-A shock generated by rows of cells. Each of these cells has numerous acetylcholine receptors, a key to obtaining enough protein to study. Stroud followed the acetylcholine receptor work by analyzing the structure of toxins known to form similar transmembrane channels, including colicin Ia from *Escherichia coli* (5). The problem of getting enough protein for mechanism and structure determination of membrane proteins still occupies Stroud today. "We'd like to express and study human membrane proteins," he says, but acquisition of such proteins is a rate-limiting step. "I would like to help determine the ability to make human membrane proteins in functional and biochemically active forms," he says, so that the field can study not only the structure and mechanism but also can use these proteins for drug development. He thinks it would be especially useful for neuropsychopharmaceutical development.

Using, and developing, techniques of structural biology, Stroud had determined the nature of the acetylcholine receptor, a protein that is key to the neuromuscular junction and action of anesthetic agents. Stroud says that the most dramatic recent evolution in structural biology has been the use of synchotron x-ray radiation. The ability to tune specific wavelengths of x-rays that hit the crystal and then diffract offers greater resolution and efficiency. Stroud relates that as early as the mid-1970s at Caltech, he recognized that synchotron radiation could be key and worked on mirrors to focus the x-rays (6, 7).

Crossing Membranes

Stroud's interest in transmembrane proteins, the subject of his PNAS Inaugural Article (1), began with his work in acetylcholine receptors. "Transmembrane channels and signaling have long fascinated me. There would be no life without membranes," he says. In 2000, Daxiong Fu, Larry Miercke, and Stroud's group determined the first atomic structure of an aquaporin, for the *E. coli* glycerol facilitator, GlpF (8). The group has since determined the structure of three more aquaporins: the *E. coli* protein aquaporin Z, AqpZ (9); the eye lens channel, AQP0 (10); and, most recently, an archaebacterial channel that conducts hydrogen sulfide, AqpM (11). In 2004, Stroud's work on ammonia channels was featured on the cover of *Science* magazine (12). "It was the first structural understanding at the molecular level of how ammonia channels work. It's the highest resolution of any transmembrane channel currently in the literature," he says. The article was recognized by *Chemical & Engineering News* as one of five outstanding achievements of 2004 in the field of chemistry.

The year 2004 brought recognition of Stroud's work with University of California, San Francisco, collaborator and joint Inaugural Article author, Peter Walter (1). Walter had defined the mechanisms of chaperoning membrane proteins into their correct folded form and targeting them into membranes, a path that Stroud says fascinates him and gets to the heart of his own interests in mechanism and structure of membrane proteins. After more than 12 years working and publishing together, in 2004, Stroud and Walter, along with col-

leagues Pascal Egea and Shu Ou Shan, published the structure and mechanism showing precisely how the signal recognition particle (SRP) targets membrane proteins to synthesis in the ER membrane (13).

Says Stroud of Walter, "Peter is a visionary biologist whose insights are constantly challenging. As things progressed, Peter discovered the important system that provides a response to the cellular stress of overproduction of proteins that then do not fold correctly." Walter identified the transmembrane receptor that detects these unfolded proteins, called Ire1 (14). "Ire1 detects proteins being made too fast without caution," explains Stroud. Walter, with Joel J. Credle and Feroz R. Papa, expressed, mutated, assayed, and crystallized Ire1, while Janet S. Finer-Moore solved its structural analysis. "Together we were able to show how sensing of unfolded proteins may bring together multiple receptors on the luminal side," says Stroud.

In contrast to former mechanistic proposals, Ire1 appears to itself recognize incompletely folded proteins by using a groove on a portion of the protein that brings neighbors together to elongate the binding groove. The proximity of receptors in the ER lumen then translates into a signaling complex in the cytoplasm. This action is the signal that starts a cascade that induces many changes in gene induction and makes hundreds of changes in metabolism to synthesize more ER membranes and more chaperones (1).

Toward Improved Drug Design

Stroud continues to be oriented toward understanding biological molecules. "My entire focus has been biological and chemical from the beginning of my Ph.D. work," he says. In addition to finding ways to make biologically active membrane proteins and to determine function, Stroud wants to use the knowledge of protein structure of drug targets to improve drug design. He sees drug design as the "ultimate challenge for calibrating how well we know what we are talking about when we happily discuss molecular interactions," he says. Three pharmaceutical companies have invited him to be a founding advisor.

Stroud also wants to explore how proteins act together to make cellular machines that make drug-like molecules. He gives the example of erythromycin synthesis (15, 16). "As we determine the structural basis for making erythromycin, we will also be able to proscribe how to alter these protein specificities so that they make different molecules," he says.

In addition to his election to the Academy, Stroud is a Fellow of the New York Academy of Sciences, the Royal Society of Medicine, and a former Alfred P. Sloan Foundation Fellow. Despite all of these honors, Stroud remains focused on those who work with him. "None of this would have been possible for me without the company of the many postdoctoral scholars, associates, and graduate students. I owe them lifetime gratitude. The greatest thrill of all is to see how their careers have evolved and taken these fields forward and made them their own," he says.

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