Profile of William A. Eaton

Biophysicist William Eaton, who was elected to the National Academy of Sciences in 2006, probably would not be where he is today—chief of the Laboratory of Chemical Physics at the National Institutes of Health (NIH, Bethesda)—if it were not for the Vietnam War. He had completed his medical degree at the University of Pennsylvania (Philadelphia) and was finishing the research for his doctorate in the fall of 1967 when a draft notice arrived in his mail.

"I didn't even think about taking an internship after graduating from medical school," Eaton said, "because the dean had told me that without an internship I would be able to work toward a Ph.D. and would not be drafted."

The government did not see it that way. After he received a draft notice, Eaton rushed to the draft board to tell them that they had made a mistake. He was a scientist, not a physician, he said. His plea fell on deaf ears. "I was asked whether I had a medical degree and when I said, 'Yes,' I was told, 'Well, *Doctor* Eaton, you're drafted.'"

This pronouncement motivated Eaton to do some rapid research. "I learned that I could satisfy my military obligation by becoming a medical officer in the Public Health Service and do basic research at the National Institutes of Health," he said. "So that's how I got here."

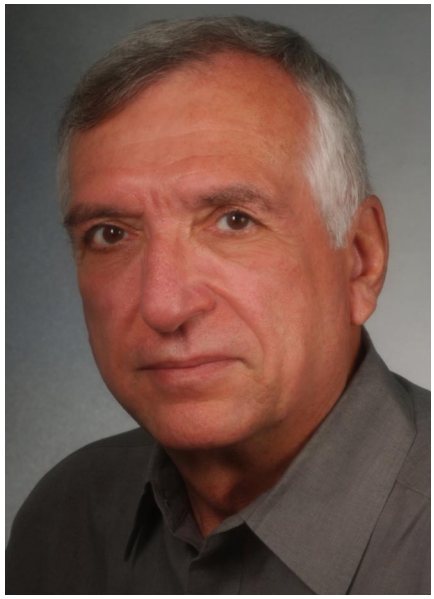
During his wide-ranging career, Eaton has made important discoveries on cooperative interactions in proteins and the molecular basis of sickle cell anemia. More recently, his work has turned to understanding how proteins fold, and he has pioneered the development and application of fast kinetic methods to work on this challenging topic.

In his inaugural article (1), published in the December 2, 2008 issue of PNAS, Eaton and colleagues examine the folding of a very small protein, the villin subdomain, from 3 different theoretical perspectives.

Tinker, Tailor, Scientist, Spy

Eaton has made his name as a researcher, but when he started college, his plan was to become a practicing physician. He took a pre-med track at the University of Pennsylvania, majoring in chemistry. But he decided to take a break before starting medical school and went to West Berlin in 1959 as the first Willy Brandt exchange student between the University of Pennsylvania and the Free University of Berlin.

Eaton had a remarkable year. "It was a great experience," he said. "Berlin was the center of world politics at the time. It was



William Eaton

one of the best decisions I ever made. I was a pretty naïve kid, and essentially grew up in Berlin. I didn't spend much time at the university. Instead, like many students in those days, I spent most of my time discussing all manner of things in cafés or bars. I could sit in the best seat in the main opera house on Unter den Linden for less than a dollar. I saw 35 operas that year."

Eaton also played a role in the Cold War that might have been written by John le Carré. He roomed in Berlin with a high school friend, Martin Rice, who had become a Russian language expert in the Army Security Agency. Rice's job was to listen in on radio conversations among the Soviet troops surrounding Berlin.

During a visit to the Soviet embassy in East Berlin to explore the possibility of a visa to the USSR, Eaton met the "Cultural Secretary," who turned out to be a high-level KGB agent. As a result Eaton and Rice became entangled in an American counterintelligence operation. "It was exciting, but I was frankly very scared," Eaton said.

The next year he realized how much danger he had been in. The Soviets arrested his successor in the student exchange program, Marvin Makinen, while he was traveling in the USSR, and sentenced him to 2 years in prison and 6 years in a corrective labor camp on espionage charges.

Come If You Like

In the fall of 1960, Eaton returned from Berlin to enter medical school at the University of Pennsylvania. But apart from a biochemistry class, he was not enthusiastic about his medical school courses.

"I was generally a pretty unhappy guy as a medical student, except each summer when I got involved in a research project," he said. "One of the great things about Penn was they encouraged us to do lab research in the hope that some of us would become full-time biomedical researchers."

During his first summer project, Eaton worked on muscle biochemistry. For his second summer, he traveled to the Medical Research Council Laboratory of Molecular Biology in Cambridge, U.K. with his newlywed, Gertrude McBride, to work on protein biosynthesis in the laboratory of Sydney Brenner, one of the founders of molecular biology.

"I heard Sydney give a lecture in Atlantic City on the genetic code, which was simply spellbinding," said Eaton. "I wrote a letter asking if I could come to work with him. His response was characteristically Sydney, a thin aerogram that read:

'Dear Bill,
Come if you like.
Sydney'."

The 6 principal investigators in the Cambridge laboratory at that time were Brenner, the head of the laboratory Max Perutz, Hugh Huxley, Fred Sanger, Francis Crick, and John Kendrew.

"It was quite a group," Eaton said. "They won 6 Nobel prizes. Listening to Sydney and Francis create modern biology at coffee, lunch, and afternoon tea in the canteen was a great experience. The summer convinced me that I wanted to do research full time as a career."

Infectious Enthusiasm

After his return from England, Eaton joined the University of Pennsylvania's new combined MD/Ph.D. program. As one of its first students, however, Eaton ran into some political turbulence between the clinicians and the basic scientists.

"A professor of hematology didn't like the idea that I was going to take 12 weeks of elective time in my senior year to do lab research," Eaton said. "So he had me sent off to a community hospital in West Philadelphia to sew up knife wounds in the emergency room and hold retractors for surgeons in the operating room."

After completing medical school, Eaton began his Ph.D. research with a year doing protein purification, potentiometry, and calorimetry in the laboratory of Philip

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 18655 in issue 48 of volume 105.

Eaton with his daughter Helen at her graduation from Juilliard.

George in the chemistry department at Penn. He then transferred to the spectroscopy laboratory of Robin Hochstrasser. His newfound interest in spectroscopy stemmed from social sessions with 3 young faculty, who adopted him as an honorary fourth member of their beer-drinking group. One was a classical experimental physical chemist, another was a statistical mechanician, and the third was Hochstrasser, a spectroscopist.

"Each believed his area to be very much superior to the other two," Eaton said. "A wonderful part of my graduate education was listening to these 3 guys argue about the relative merits of thermodynamics, statistical mechanics, and quantum mechanics."

One evening in a bar, Eaton told Hochstrasser that he had a beautiful, large crystal of a protein, cytochrome *c*, which had a conformationally sensitive absorption band at 695 nm. Hochstrasser insisted that they had to study this crystal immediately.

"After drinking quite a few beers, we went over to his laboratory at about midnight," Eaton recalled. Hochstrasser mounted the crystal on a microscope stage, turned the microscope on its side, and, using the microscope lamp as a source, focused polarized light passing through the crystal into a large spectrograph.

After developing the photographic plate, Hochstrasser announced that the 695-nm band was polarized perpendicular to the plane of the heme and that it was an extraordinarily important result.

"I knew essentially nothing about molecular spectroscopy at this point, so I really didn't know what Robin was talking about," Eaton said. "I was simply bowled over by his excitement and decided this was the kind of science for me. What was a genuine interest in research when I started as a full-time student in Robin's lab turned into a deep passion, in large part due to Robin's brilliance, extraordinary creativity, and infectious enthusiasm."

In January 1968, immediately after completing his doctorate in Hochstrasser's laboratory on single-crystal optical spectroscopy of heme proteins, Eaton began research at the NIH as a Public Health Service (PHS) medical officer.

"We came up with a very simple and powerful idea."

"There was a lot of competition among young physicians to land a position at NIH because of the war, and I was very lucky to obtain one with Elliot Charney, an excellent spectroscopist and incredibly nice man," Eaton said, "To our amusement we PHS officers were called 'yellow berets' by the officers from the naval hospital across Rockville Pike."

Eaton was quickly given a tenured position in the newly founded Laboratory of Chemical Physics, based on his work on the molecular spectroscopy of hemes and nucleic acid bases. Marvin Makinen, who was released by the Soviets in a prisoner exchange after 2 years and had returned to the University of Pennsylvania for his medical degree, also came to the NIH as a PHS officer and coauthored one of Eaton's first papers on hemoglobin (2). Makinen is now a professor of biochemistry at the University of Chicago (Chicago, IL).

Sickle Cell Biophysics

Soon after arriving at NIH, Eaton began work on sickle cell disease. "There was a lot of interest in sickle cell disease at that time, and I thought this was an area where I could take advantage of my training in both science and medicine to make a contribution," he said.

Working with James Hofrichter, who has been a close collaborator ever since, Eaton used the microspectrophotometer he had built for crystal studies to measure the polarized absorption spectra of the bundles of aligned fibers that formed in sickled red cells. In analyzing the results, Eaton and Hofrichter discovered that a structure of the fiber proposed by John Finch and Max Perutz based on electron microscope images could not be correct.

Eaton wrote a letter to Perutz, who invited him to come to a meeting at the United Kingdom's Royal Society in January 1973. "Max paid all of my travel and living expenses to hear me talk about his mistake," Eaton recalled.

The optical study aroused Eaton's interest in understanding how sickle hemoglobin aggregates into fibers. The fibers form in red blood cells when hemoglobin is deoxygenated in the tissues. They stiffen and distort the cells, which can cause them to become stuck in the capillaries and block the circulation. The result is chronic organ damage and extremely painful episodes known as "sickle cell crises."

Together with Hofrichter and Philip Ross, an expert in calorimetry, Eaton began to study the thermodynamics and kinetics of hemoglobin fiber formation. They made the remarkable discovery that fiber formation is preceded by a marked delay period that depends inversely on the 30th power of the initial sickle hemoglobin concentration, by far the highest concentration dependence ever observed for the kinetics of any system (3). With Frank Ferrone, Eaton and Hofrichter developed a novel double nucleation mechanism that explained both the existence of a delay period and the high concentration dependence (4).

They were surprised when Ferrone first made measurements on very small solution volumes by using a newly developed laser technique and found that the delay times were not reproducible.

"We were initially puzzled and were quite worried," Eaton said, "but it quickly dawned on us that we were observing random fluctuations from nucleating single fiber molecules and were quite excited when we realized that it was a natural prediction of the mechanism."

In addition to sickle hemoglobin concentration, Eaton and Hofrichter found

that the delay time is supersensitive to several other solution conditions, including temperature, pH, the fractional saturation with oxygen, and the presence of normal adult or fetal hemoglobin.

"We came up with a very simple and powerful idea—that disease severity is determined by whether the delay time is longer or shorter than the transit time through the narrow capillaries of the tissues," Eaton said.

The Italian Connection

While subsequently working with Andrea Mozzarelli, who was visiting from the University of Parma in Italy, Eaton and Hofrichter showed that the delay time for the vast majority of cells is sufficiently long that they escape the capillaries into larger vessels before fibers begin to form. A small fraction of cells, mainly those with the highest sickle hemoglobin concentration, have a short enough delay time to sickle while still in the capillaries, which enhances the possibility of becoming stuck. The delay makes the disease survivable and explains why the heterozygous version of the condition, called "sickle trait," produces no disease. Sickle trait cells contain 40% sickle hemoglobin and 60% normal adult hemoglobin, and the delay times are so long that they rarely sickle in vivo.

"To treat sickle cell disease, we don't have to completely inhibit fiber formation," Eaton said. "All we have to do is increase the delay time to allow more cells to escape the capillaries before fibers begin to form. This can be accomplished with a small dilution of the sickle hemoglobin. This is exactly how hydroxyurea works [5]."

Hydroxyurea is the only drug known to have some success in treating sickle cell disease. It dilutes the sickle hemoglobin by replacing ≈20% with nonaggregating fetal hemoglobin, but not in all cells.

"With the recent availability of large compound libraries," Eaton said, "it should be possible to rapidly discover drugs that affect all cells by screening for their effect on the delay time."

Eaton has also made important discoveries about normal hemoglobin. "Understanding cooperative oxygen binding by hemoglobin has always been a fascinating subject for me," he said. In the early 1980s Eaton and Hofrichter, together with Eric Henry, a theoretical physicist, began a series of investigations, combining nanosecond-resolved absorption spectroscopy with theoretical modeling, to explain the complex conformational and ligand-binding kinetics of hemoglobin.

Eaton also began a still-ongoing collaboration on hemoglobin cooperativity with frequent trips to Mozzarelli's laboratory in Parma. Their work on oxygen binding to single crystals of hemoglobin confirmed 1 of the major postulates of the allosteric model of Monod, Wyman, and Changeux, that binding without a change in quaternary structure is perfectly noncooperative, settling what was at the time a 20-year-long controversy (6).

Shifting Gears

Eaton made a major shift in his career in 1991 after a chat with theorist Peter Wolynes at a meeting near Moscow on protein physics. "Peter suggested that I start working on the protein folding problem, because he said the field really needed experimental physical chemists," Eaton said.

That conversation was the first of innumerable useful discussions on protein folding with Wolynes, who came to the NIH for a sabbatical year in 1997. "Peter is incredibly smart and his theoretical work has motivated a lot of what I have done in the field," Eaton said.

Eaton's first experiments on protein folding measured the kinetics of intramolecular contact formation triggered by photolysis of the cytochrome *c* complex with carbon monoxide. His optical triggering method increased the time resolution in protein folding experiments by ≈6 orders of magnitude and was the beginning of the so-called "fast folding" field.

These experiments led to the concept of a protein folding "speed limit," which Eaton estimated to be ≈1 μs, >1,000 times faster than the fastest folding protein known at that time (7).

"Our notion of a 'speed limit' had an immediate impact on the field," he said, "and motivated the search to find fast-folding proteins and to engineer them via mutations to fold even faster. We've recently engineered the villin subdomain to fold in 700 ns."

Three Perspectives on Folding

In Eaton's inaugural article (1), he and his colleagues present 3 different perspectives on the folding of the villin subdomain.

"It's the smallest naturally occurring sequence that folds into a globular-like protein, so it has all of the properties of much larger single domain proteins even though it's just 35 residues," he said.

In the article, the researchers analyzed a range of equilibrium and kinetic data with 3 different models: a 3-state chemical kinetics model, a physical kinetics model, and the Ising-like statistical mechanical model that Eaton developed with Victor Muñoz (8).

The first 2 models provide some insights, but yield much less information about the folding mechanism than the Ising-like model, which enumerates ≈100,000 microstates and fits the data with fewer parameters.

"This incredibly simple model is remarkably successful in quantitatively explaining a wide range of equilibrium and kinetic measurements, so it must capture much of the underlying physics," he said.

Eaton pointed out that the next great experimental challenge is to determine the distribution of microscopic pathways that connect the folded and unfolded states.

"This information can be obtained from theoretical models and simulations, but it is not available from ensemble measurements, which only measure average properties," he said. "So a major focus of my lab now centers on making single-molecule fluorescence measurements, with the long-term goal of actually watching individual molecules as they fold and unfold [9]."

As chief of the Laboratory of Chemical Physics at the National Institute of Diabetes and Digestive and Kidney Diseases for the past 23 years, Eaton is extremely proud of building an outstanding group of biophysical scientists.

"I have tried to follow the advice I got from Max Perutz many years ago," he said. "Max told me to 'hire the brightest scientists and let them do what they want.' And we've been able to do that in my institute. The NIH has been a great place to work and I have been fortunate to have had so many terrific postdocs and wonderful colleagues. My friends in academia tell me that I have the best job in science, and I agree completely."

Kaspar Mossman, *Science Writer*

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