PROFILE

Fascinated by Folding

Lila Gierasch uses biochemical tools to understand how linear chains of amino acids turn into complex three-dimensional structures.

BY ANNA AZVOLINSKY

In 1970, as a first-year biophysics graduate student at Harvard University, Lila Gierasch rode a bus through Cambridge every day to travel between the medical school and the main campus. On the bus one day she overheard someone say the word "collagen." Gierasch, now a professor of biochemistry, molecular biology, and chemistry at the University of Massachusetts Amherst, had studied collagen as an undergraduate. Her interest piqued, she struck up a conversation on the bus with the woman who had uttered the word: Barbara Brodsky, then a graduate student in Elkan Blout's laboratory in Harvard's Department of Biological Chemistry. "I just started chatting with her and she told me about her work on collagen in the Blout lab. She and I have been friends ever since," says Gierasch.

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After the chance encounter, Gierasch went to speak with Blout and made up her mind to do her PhD thesis in his laboratory. In the spring, Blout set up an individualized study class for Gierasch and two postdocs in his lab to discuss papers and review articles. "That literature review was fantastic for me. The two postdocs were the same kind of eager minds. They also wanted to learn everything they could about biophysical chemistry. As a first-year graduate student, it was quite a treat."

Gierasch's undergraduate advisor at Mount Holyoke College in Massachusetts had cautioned her that Harvard would most probably be a more intimidating environment than what she was used to. "I think this would have likely been the case except that I had the good fortune of having Blout as an advisor. In his lab, I was both supported and well-nurtured in the way that I had been accustomed to. There were few barriers that I encountered in the lab. It was like a family. He didn't take many graduate students. I was only one of two graduate students and we were treated the same and had the same benefits as the postdocs," says Gierasch.

Blout had come from Polaroid, where he had worked on both the developing of color film and the chemistry of instant photographs, including polymer coatings for the Polaroid films. "When you were in his office, he would get a phone call from the head of an Israeli or Russian institute while you're trying to tell him about your daily research problems." His lab studied how amino acid sequences dictate the conformations and structural architecture of proteins. As a graduate student, Gierasch used nuclear magnetic resonance (NMR) and other biophysical methods to study how amino acid sequence determines the local structure of beta turns, a common type of peptide motif found in many proteins, using six-amino-acid cyclic peptides as a model. She has since spent her career figuring out how simple strings of amino acids turn into elaborately arranged proteins that do the bulk of the work within cells.

Here, Gierasch talks about how the challenges of protein folding have played out across her career, starting from her days as an undergraduate; a job offer at a car repair shop during graduate school; and the gratification that comes with having your students achieve their goals.

GIERASCH GETS GOING

Earning her keep. Gierasch grew up in a family of five, first in the Boston suburb of Wayland, Massachusetts, and then in the western part of the state. Her mother was a teacher and her father was a civil engineer who enlisted his two daughters and son to help with DIY building projects. "There was never an idle moment in our household. I knew how to pour concrete before most kids learned more basic everyday things. When we built a shed, that shed would outlast any commercial one. They were fantastic experiences, such that as my life progressed I didn't have any hesitation taking on pretty demanding projects like fixing cars or chairing a chemistry department."

Multiple talents. In high school, Gierasch enjoyed and excelled in math and science classes. "I had good science teachers who encouraged my innate curiosity," she says. Gierasch's interest in teaching unexpectedly began during a geometry class. "I remember the teacher asking me to explain concepts to the entire class. That experience kindled my love for teaching," she says. Gierasch also had many interests outside of school. She and her older brother Peter, now an emeritus professor of astronomy at Cornell University, went hiking, built radios and telescopes, worked on cars together, and rode horses, which led to a lifelong interest in horseback riding.

PONCE ROS, ACTIONFOTO

LILA M. GIERASCH

University Distinguished Professor, Department of Biochemistry & Molecular Biology and Department of Chemistry
University of Massachusetts Amherst
Editor-in-Chief, *Journal of Biological Chemistry*

Greatest Hits

- Using cyclic peptides as models, identified rules to predict conformations of specific protein motifs (β turns)
- Demonstrated the biophysical interaction of protein signal sequences with the cellular lipid membrane during protein export, helping to establish the technique of using peptides to analyze functional interactions among proteins
- Elucidated molecular chaperone and co-chaperone regions that interact with each other to selectively bind unfolded protein substrates
- Used kinetic techniques to understand the molecular interactions important to assemble a cavity-containing protein
- Uncovered how various conformations of the molecular chaperone Hsp70 allow it to undergo nucleotide-modulated binding to a range of different substrates

Auspicious beginning. In 1966, Gierasch entered Mount Holyoke College in South Hadley, Massachusetts, as a "townie," she says, commuting from home. She had wanted to attend Holyoke since she was a small child, both because her mother had gone there and because it seemed "cool." "It was a fantastic place for me. It's a place that encourages women to do whatever they choose and fosters a sense of possibility." During her freshman year, encouraged by her older brother, then a graduate student, and his wife, a tech in Matthew Meselson's Harvard laboratory, Gierasch knocked on the doors of Harvard biochemistry professors, hoping to score a summer research job. Alwin Pappenheimer, who studied bacterial toxins, invited her to chat after everyone else in the Biological Labs building had politely turned her down. "He was this twinkly-eyed, wonderful guy who said that he would come up with a project for me," says Gierasch. She worked in his lab for two summers during college; he later told her that her mention of Mount Holyoke had sparked fond memories of driving back and forth to visit his then girlfriend, a Holyoke student. "So it was part dumb luck that he gave me the opportunity, but he was a major influence in my graduate school decision and scientific interests," says Gierasch. "When I look back, I realize I was adventurous and opportunistic enough to get these chances and make the most of them. I feel very lucky."

Protein-folding roots. While at Harvard for the summer, Gierasch saw a poster advertising a biophysics conference to be held at MIT. Attending the conference helped her decide to study biophysics in graduate school. At Mount Holyoke, she did research with chemist Edwin Weaver that kindled her interest in protein folding. In his lab, she purified collagen from rat tails and studied its ability to refold under different conditions, analyzing the structures using electron microscopy (EM). "Collagen ought to be a simple protein to refold because of its repeated structure, but it's complicated by the fact that it has a triple-helix structure and crosslinks along the three different chains that must wrap around one another," Gierasch says.

GIERASCH GATHERS MOMENTUM

Moonlighting as a mechanic. While in graduate school, Gierasch maintained her hobbies, including working on cars. She found two MG1100s and decided to make one functional car out of parts from both. "There is nothing like putting together a car to figure out how one works," she says. Gierasch disassem-bled the transmission of one car so enthusiastically that the little springs inside flew all over and she needed help getting them back together. She showed up at the local transmission shop with a box of parts, much to the surprise of the MG1100 expert there. "I kept visiting the local shop to buy parts from deeper inside the engine, and by the time I got to the gaskets for the flywheel, they offered me a job."

Rocky road. Gierasch had thought since college that she would pursue a career teaching undergraduates—she wanted to offer the same enabling educational experiences to others that she'd enjoyed. Early in her fourth year in Blout's lab, she saw an ad for a tenure-track chemistry position at Amherst College. Gierasch jumped at the opportunity, becoming an assistant professor of chemistry in 1974 when she was only 24 years old. "Blout appropriately told me to think hard about the decision. Now, whenever I get asked for career advice, I always say, 'Do a postdoc,'" says Gierasch. "I was a pup and had no idea what I was getting into. I survived and landed on my feet, but it was the rockiest period in my life." Gierasch had to juggle completing her thesis, setting up a new laboratory, and teaching. She was among the first six female professors hired by the college, which was transitioning to coeducation, and the only woman on the faculty in math or science. "It was not easy. I looked young and had trouble garnering the respect of the still mostly male students. I left my office door open and was friendly, but was told by my more senior colleagues that I smiled too much. It didn't matter to them that I got an NIH grant and an NSF instrumentation grant," she says. Still, Gierasch enjoyed engaging with the talented undergraduates who joined her lab, and her work on cyclic peptides thrived with undergraduate coauthors.

Fold and secrete. In 1977, Gierasch spent a year-long sabbati-cal at the Université Louis Pasteur de Strasbourg in Paris, working in the lab of Jean-Marie Lehn, an organic chemist who would go on to win the Nobel Prize in chemistry in 1987. A self-proclaimed Francophile, Gierasch says she enjoyed the immersion in French language and culture and in carrying out research on how organic ligands could be designed to bind to anions.

When she returned to the U.S., Gierasch moved her labo-ratory from Amherst to the University of Delaware. She began to shift her research to designing models that mimic protein structural features closer to those found in native biological sys-tems. One focus was the "zip codes" directing protein localiza-tion—amino-terminal signal sequences used by cells to target proteins for secretion. She launched this work in her lab after meeting Tom Silhavy, who was exploring the functions of sig-nal sequences using bacterial genetics. "We would get our two labs together and spend a whole day hashing out how to solve a problem using complementary techniques," she says. Gierasch's work showed, first in collaboration with William DeGrado, that interactions between the protein signaling sequences, generally found on the N-terminus, and the cellular lipid membrane are important for protein secretion in bacteria; and, later, that the signaling peptides can change conformation to induce protein export from the cell.

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Protein folding inside the cell. In 1988, after eight years at the University of Delaware, Gierasch moved to the University of Texas Southwestern Medical Center in Dallas. There, her work on signal sequences prompted her to ask how proteins fold dur-ing their biosynthesis inside the cell, and led her to join in the discovery and functional characterization of intracellular folding assistants—molecular chaperones—which help proteins progress from linear amino acid sequences to three-dimensional shapes in the complex cellular environment. "The concept of molecular chaperones was just emerging, and our lab was primed, using bio-physical tools, to understand how these proteins recognize their substrates. The structures of the chaperones were not known initially, and it was a fundamental puzzle how these proteins bind many different substrates and yet selectively bind substrates that are unfolded. We wanted to understand the basis of recognition of unfolded substrates and how chaperone interactions facilitate folding in the cell," says Gierasch. In 1992, using NMR, Gierasch's postdoc Samuel Landry showed that the same substrate binds differently to two bacterial chaperone proteins, GroEL and DnaK. Gierasch's lab also characterized the important regions of Gro-EL's co-chaperone, GroES, that modulate GroEL's affinity for its substrates, and in 1996, collaborating with Johann Deisenhofer, solved the crystal structure of GroES.

GIERASCH GAZES AHEAD

Country mouse. In 1994, Gierasch moved again, this time to the University of Massachusetts (UMass) Amherst, where she was recruited to chair the Department of Chemistry. At UMass, Gierasch's lab continued to work on chaperones and also tackled the folding mechanism of beta-sheet proteins that have a cavity in their center to encapsulate a hydrophobic ligand. Because prior models for folding such complicated proteins were confounded by both the beta-sheet character and the hydrophobic collapse of the central cavity, Gierasch's lab decided to tackle the problem head-on. She and then graduate student Patricia Clark followed the folding kinetics of a cavity-containing retinoic acid binding protein and found that amino acid side-chain interactions, rather than stable hydrogen bonding, play a major role in creating the cavity and the overall architecture of the protein.

Still into the fold. After two stints as department head, first for Chemistry and later for Biochemistry & Molecular Biology, Gierasch was ready to dive back into research without the burden of administrative duties. Gierasch's lab tackled the challenges and vulnerability that proteins face when folding in vivo as a result of the cellular environment. Cells deploy a complex network of components, called the protein homeostasis system, to ensure that folding occurs with fidelity and that potentially deleterious mis-folded species are degraded. The protein-folding system within the cell is so complex that Gierasch decided to enlist the help of another protein-folding and mathematical-modeling expert, Evan Powers, to come up with a computational model of protein homeostasis in *E. coli* that they call FoldEco. "We use it to simu-late the fate of a protein as it is synthesized and folds and then do experiments to test if our model encapsulates the process appropriately," Gierasch says.

Recently, her graduate student Karan Hingorani found that an exogenous small molecule acts as a targeted pharmacological chaperone and greatly improves folding of a poorly folded large protein. The results may have implications for designing ther-apeutics for diseases that involve protein misfolding, including Huntington's and Alzheimer's. The lab has also delved into the mechanism of a major class of molecular chaperones, the Hsp70s. In a 2012 *Cell* paper, then postdoc Anastasia Zhuravleva showed how the different conformations of *E. coli* Hsp70, DnaK, enable it to carry out its chaperone functions. Ongoing work in the Gierasch lab is building on this foundation and extending to human chaperones that mediate a wide array of cellular functions.

Gierasch gives back. A year ago, Gierasch assumed the reins as editor-in-chief of the *Journal of Biological Chemistry*, published by the American Society for Biochemistry & Molecular Biology. She is excited about the opportunities ahead for science publishing in general and this venerable journal in particular, and now spends half of her time on leading JBC into the future.

With gratitude. "One of the most gratifying things about my career is the people. It is so fantastic to see my former students, who gave so much during their training, succeed in running their own labs. Those are the proudest moments of my career." She also credits her husband, John Pylant, whom she met when they were both taking dressage lessons from the same riding instructor in Texas, for providing the personal support that makes life enjoyable and doable.

Chance favors the prepared mind. "I feel very fortunate because many times I was in the right place at the right time and was able to capitalize on that. You can make things happen if you keep a positive outlook and pour in your energy and convince people by your passion." ■