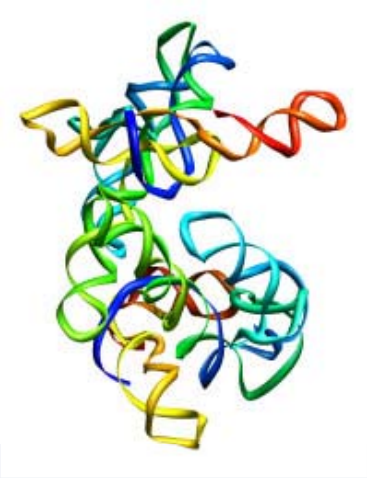
OF WALTZING PROTEINS AND WHISPERING RNA

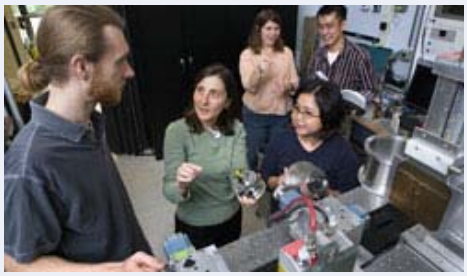


Lois Pollack seeks answers to questions at the forefront of molecular biology

By Sourish Basu

Lois Pollack loves to build tools. One of her favorites is a paper-thin purple square of silicon, less than an inch across, with channels thinner than a human hair. "This is one of the earlier mixers I built," says the associate professor of applied and engineering physics. Looking at it, it's hard to believe that this "mixer"—two microscopic channels etched cross-ways—in the late 1990s became a new window on how proteins danced their tangled dance during the first few moments of folding.

Proteins, which account for about a fifth of our weight, are polymeric chains of up to 20 different amino acids. Each protein is hundreds to tens of thousands of amino acids long and performs a very specific function in our body; hemoglobin in our blood carries oxygen, and myosin in our muscles gets us moving. To perform such specific functions, proteins have to fold up in specific three-dimensional structures and sometimes toggle between different ones. A protein called VIVID, for example, found in a bread mold, changes its conformation when exposed to light to keep the fungus' Circadian rhythms in sync with the sun.



Graduate student Steve Meisburger (left), postdoctoral associate Suzette Pabit (right), and Associate Professor Lois Pollack (center) discuss beamline components in the CHESS G-1 station. In the background, graduate students Jessica Lamb and Lee Li look on.

A protein—forged as a linear chain—must find its correct structure from several billion possible folded forms. Counting on a protein to do that regularly is like expecting a mile-long yarn to knot up in precisely the same tangled ball every time—yet the protein usually succeeds. "Somehow the instruction as to how a protein is going to fold up is chemically written into the pattern of amino acids," says Pollack, but it's a writing that we haven't learned to read yet. How a protein acquires its biologically active structure, and how it responds to stimuli—such as VIVID to light—have therefore been open questions in biology for several decades.

Proteins and their structural polka are half of Pollack's research; interacting RNA and DNA strands make up the rest. What they have in common are the tools she builds to study them. "I love to find a problem that really needs to be solved, and ask 'Why has nobody been able to solve this before, and what's been limiting progress?'" she explains. "And then I do my best to build a tool to solve that problem. But it doesn't always work." No wonder, once you look at some of those problems. How does the flu virus fool our immune system every year? What controls our circadian rhythm? How do our cells decide when to make certain proteins?

Today, we know these to be questions at the forefront of molecular biology. Hard to believe, then, that Pollack slipped down this path perchance while flipping through a magazine, waiting to collect data for her real research, more than a decade ago.

Early Years

Pollack received her Ph.D. from the Massachusetts Institute of Technology, working with Thomas Greytak. In 1989, she came to Cornell to work with Physics Professor Robert Richardson as a post-doctoral associate. A low temperature physicist by training, she started studying nuclear spin ordering in 1995.

Her experiments with Richardson involved extremely long spin relaxation times; each run would take several hours, even days, to finish. During one such run, Pollack picked up an issue of *Physics News* and flipped to an article on protein folding. Within half a page, she had found her calling.

In 1995, the "energy landscape" picture of protein folding was just beginning to emerge. Each possible structure of a protein, the picture says, has a specific energy. Just as a ball will roll downhill to the point of lowest energy, a protein switches through many possible structures until it finds its lowest energy structure, which is also its biologically active structure.

What makes protein folding a hard nut to crack is that it's impossible to see a protein fold. Typical protein molecules—between a few and several hundred nanometers long—too small to be seen with light microscopes. Instead, scientists look at how a protein scatters X-rays to paint a picture of its complex contortions. A change in pH initiates the dance. The first steps begin in just milliseconds, so the pH must be changed very rapidly. Otherwise the pH-change's effects will show up in the painting. The scattered X-rays must be measured just as quickly. When Pollack first turned her attention to the problem, the pH change took longer than the folding. "Proteins fold in the blink of an eye," says Pollack. "They were too fast to be detected by the tools you could buy, and those were the tools that everybody was using."

Pollack had an idea for building a much better tool: a micro-flow mixer—a tiny square wafer of silicon with channels etched out in the center—that could change pH 1,000 times faster and allow rapid X-ray measurements. She came up with the design in collaboration with Robert Austin of Princeton University and Sol Gruner, director of Cornell's High Energy Synchrotron Source and Austin's former colleague at Princeton. Luckily for Pollack, she was near one of the few synchrotron sources in the United States.

Professor Lois Pollack and students in the in the CHESS G-1 station.

X-rays produced inside the synchrotron come out in an extremely narrow beam. Focusing this beam on her mixer, Pollack produced snapshots of the first milliseconds of folding, offering a peek into the part of a protein's life that had been invisible. "In fact, it was the only experiment I've ever done in my life that worked the first time," she fondly reminisces. "The first time I did that experiment, I took data that was almost immediately publishable and really astonishing."

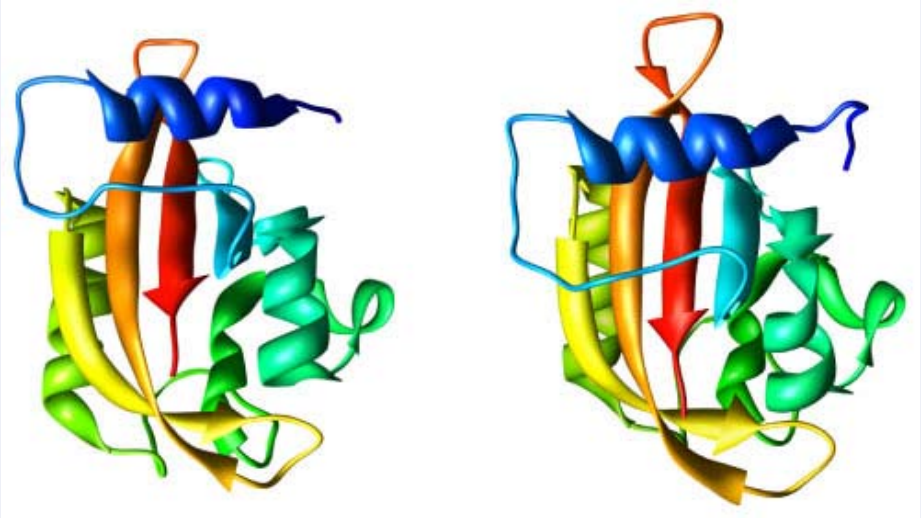
Protein Functions

For many proteins, how they perform their functions is as poorly understood as how they fold. Armed with her tool that could take time-resolved X-ray snapshots of proteins, Pollack, with Microbiology and Immunology Associate Professor Gary Whittaker, Chemistry and Chemical Biology Associate Professor Brian Crane, and Applied and Engineering Physics Professor Watt Webb, has been studying the functional stage of some proteins.

Hemagglutinin (HA)—the 'H' in the infamous bird flu-virus H5N1—a so-called viral fusion protein, is a trickster. When a flu virus invades, "the cell realizes that it's a foreign body, and feeds it to the cell's internal digester, a compartment called the endosome," explains Pollack's graduate student Jessica Lamb. The endosome acidifies the virus in an attempt to digest it. Unlike most proteins, however, HA is triggered by acidity to attain its active structure. It fuses the virus with the endosome boundary and the viral genetic material tumbles out inside the host cell.

More importantly, says Lamb, "Hemagglutinin is the thing that evolves to evade our immune system every year," requiring a new flu vaccine every season. Scientists are baffled how, despite its annual mutation into a structurally different protein, HA still retains its functionality. Nor do they understand why HA is triggered by acidity. With her toolkit, Pollack hopes to see HA at work, literally, and demystify this unique protein.

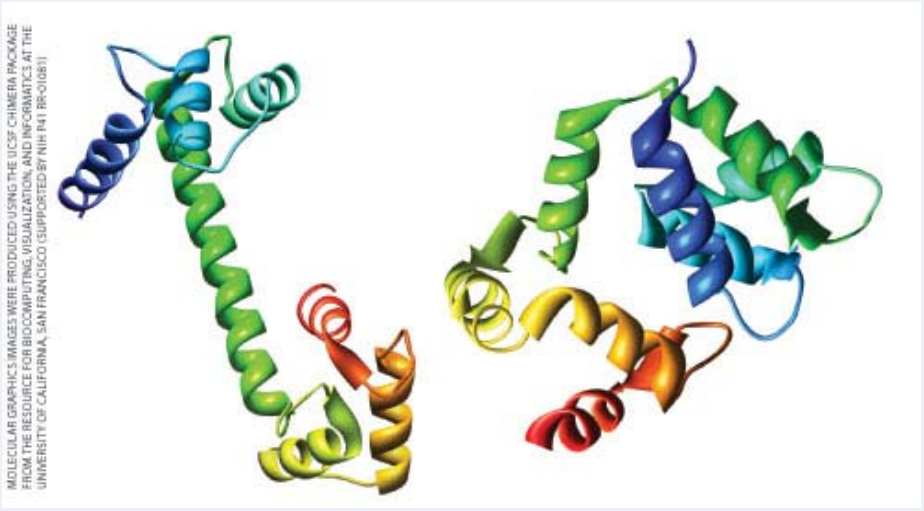
Proteins like HA catalyze structural changes, such as the fusion of the viral and endosome membranes. Others are molecular motors, such as myosin in our muscles and dynein in bacterial flagella. Yet other proteins, such as VIVID and phototropins in plants, respond to external stimuli such as light. The response of these proteins is very often a structural change, easily detectable by the suite of tools Pollack has at her disposal. Lately, she has trained those tools on two proteins that respond to stimuli.



The group measures the structural change that the fungal protein VIVID (2PD7.pdb, left) undergoes when it senses light (2PDR.pdb, right), which keeps a mold's biological clock in sync with the sun.

VIVID, a protein found in bread mold, controls the mold's circadian rhythm, the same physiological rhythm that makes us sleepy at night. The Pollack group, in collaboration with the Crane group, observed that two VIVID molecules merge when hit with blue light, starting a cascade of reactions that ultimately makes the mold's biological clock tick. Although VIVID itself is found in only a few organisms, it "is one of a larger family of proteins that is found in everything" including mammals, says Lamb. These proteins don't always control circadian rhythm, she clarifies. For example, a very similar protein NPH1 in *Arabidopsis* is responsible for the tendency of plants to grow towards light, or phototropism.

Calmodulin, on the other hand, senses calcium ions. "The body works a lot like a circuit, and ions carry the currents in the circuit," Pollack explains. The flow of calcium ions across the cell membrane plays an important role in muscle contractions. Calmodulin senses these ions and converts their concentration into chemical signals, which trigger motor proteins in charge of muscle functions. Such proteins that convert or "transduce" one kind of signal into another are fairly common in biology. Understanding how VIVID and calmodulin work, therefore, will provide insight into two of biology's most important processes; photo-detection (how organisms respond to light), and signal transduction.



Pollack's group studies the dance that proteins like calmodulin (CALcium MODULated proteIN) perform when they change from being bound with calcium (1CLL.pdb, left) to not being bound with calcium (1QX5.pdb, right).

While protein functions kept her fascinated, Pollack realized quite early that studying them one after another had the danger of becoming monotonous. "It occurred to me that I could spend my entire life studying different proteins," she says. As a physicist, she craved diversity in the problems she tackled. In 2002, armed with her microsecond imaging tools, the tool-builder looked around for another problem that needed solving. "And then I found RNA folding," she remembers.

RNA: The New Frontier

Up until the late '70s, the central dogma of biology was quite clear: proteins did all the work and were made by translating the genetic code in messenger RNA (mRNA). The mRNA, in turn, fetched the code from a DNA library—and that was about all the RNA did.

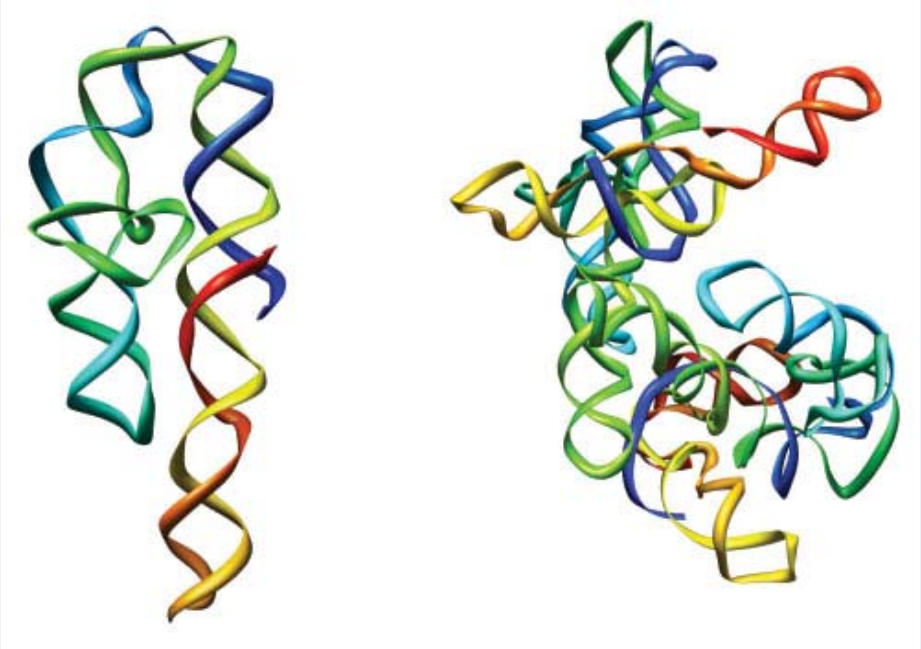
Then in 1978, scientists discovered introns—certain sections of mRNA that excised themselves and spliced the remaining mRNA pieces back together. "An intron, to cut itself out, has to form a specific three-dimensional structure" exactly like a protein, explains Lamb, which at that time went against all established notions of genetics. And it blew everyone away.

Nor was the intron the only "active" RNA; by 2000, scientists had discovered another stunner. The ribosome, the cell's protein-making factory, is itself a complex of RNA and proteins; bacterial ribosome, for example, has three RNA and 50 protein molecules. Electron microscopy revealed that those three RNAs, and not the 50 proteins, catalytically joined amino acids to churn out proteins.

RNAs perform both these functions by acquiring the "correct" structure for the job, like proteins. Since Pollack had already built the tools to study folding proteins, using them to see folding RNA was the natural next step. But their protein-like folding was not the only fascinating aspect of RNAs; as Pollack says, "About every decade there's a revolution in what people know about RNA." Once thought to be mere messengers, RNAs were found to regulate gene expression, inhibit and trigger protein production, and mimic antibodies to fight infections. For Pollack, these

diverse functions were signatures of interacting RNA; if she could decipher how RNA strands talked to each other and to other molecules, she could understand what drove their folding, or how they inhibited protein production—in short, what made them so versatile.

Further, Pollack explains, much of RNA interactions boil down to the force between charges —something physicists have been studying for several centuries. In solution, RNA—and its sidekick DNA—exist as long strands of negatively charged ions. Their electrostatic forces dominate all other forces and reduce the problem of RNA folding into something deceptively simple: how does a line of charge behave in the presence of an ionic background? "It's all about electrostatics. I can solve Coulomb's law to find out how RNAs interact," she explains.



They also study how small pieces of RNA (1GID.pdb, left) acquire their structure within larger folded RNA, like the tetrahymena ribozyme (1X8W.pdb, right).

Since RNA strands always exist in ionic solution, however, the problem is not a simple one. The negatively charged RNA attracts proteins and positive ions that surround it and screen its charge. So any force the RNA exerts on other molecules is always through a veil of screening charges and depends on the exact geometry of this screening cloud.

To "see" this cloud, Pollack resorted to small-angle X-ray scattering of DNA strands (they're electrically similar to RNA, but a lot cheaper). "We've also measured the effective charge on DNA helices using some other techniques," says Pollack, "and it's surprising that nobody has done that before."

With the screening charge distribution mapped out, Pollack's group is figuring out how electrostatic forces galvanize these nucleic acid chains to function as gene regulators and antibodies. In time, they hope to have a crack at their original problem—how RNA folds up to function like a protein.

"Our cells have lots of tiny RNAs, but people thought they were insignificant—and now they're turning out to be really important. So it's a great time to be studying RNA!" exclaims Pollack. While biologists discover newer roles for RNA, she digs into what makes them tick. With her toolbox of micro-flow mixers, X-rays, and fluorescence markers, this tool-maker eavesdrops on RNA strands whispering to each other and spies proteins waltzing from structure to structure, so that we may learn their secrets.

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