WAKE FOREST UNIVERSITY BAPTIST MEDICAL CENTER

INSIGHTS: RESEARCH, EDUCATION, MEDICINE

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visions

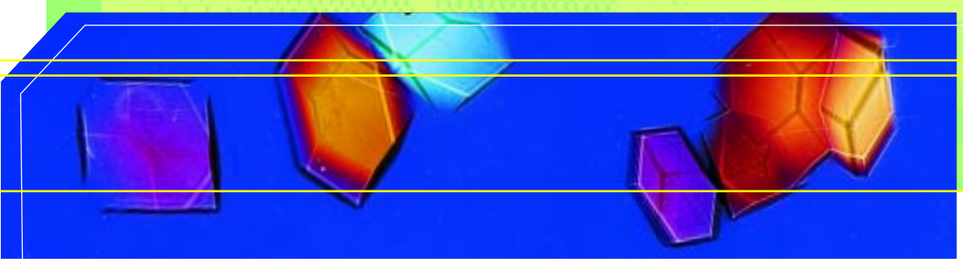
ON THE EDGE:
STRUCTURAL BIOLOGY RESEARCH

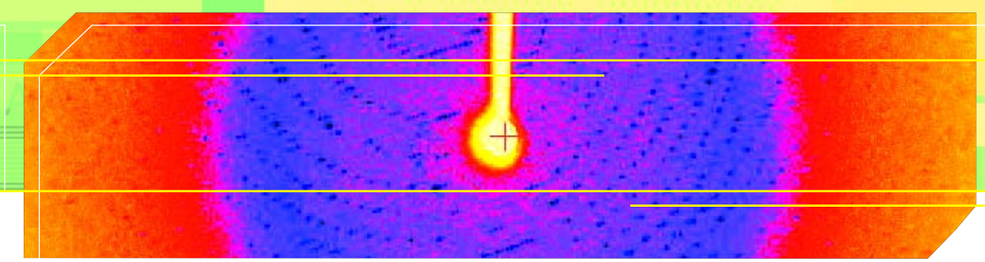
ALUMNI EDITION

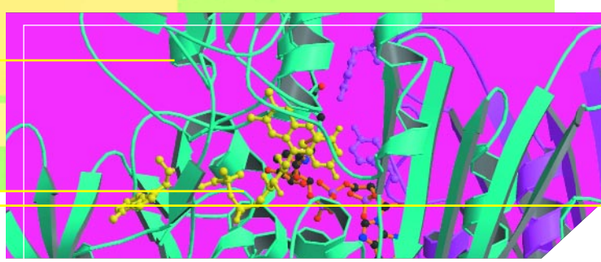
By Michael Massoglia

THE SHAPE of Things to Come: ON THE EDGE OF STRUCTURAL BIOLOGY RESEARCH

WITH THE LATEST X-RAY CRYSTALLOGRAPHY EQUIPMENT AND A DYNAMIC, COLLABORATIVE RESEARCH TEAM NOW IN PLACE, WAKE FOREST IS EMERGING AS A LEADER IN THE FIELD OF STRUCTURAL BIOLOGY





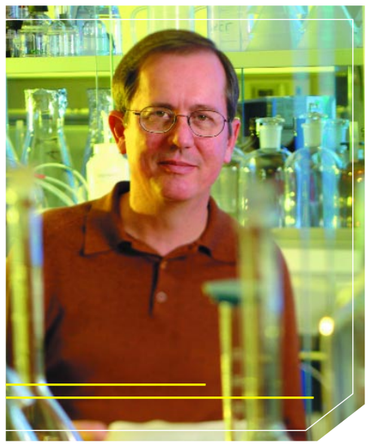


Crystals of the flavoprotein NADH peroxidase (left); X-ray diffraction pattern of an NADH Oxidase crystal (middle); portion of the Coenzyme A Disulfide Reductase crystal structure (right).

FIFTY YEARS AGO, JAMES WATSON and Francis Crick reported the double helical structure of DNA. Almost as an afterthought, they wrote in *Nature*, "it has not escaped our notice" that the proposed structure could offer a mechanism for genetic material to duplicate itself. It does, of course, and the past half-century has seen dramatic advances in molecular biology and genetics culminating, in the public imagination, perhaps, with the sequencing of the human genome. In reality, that feat was only a beginning. Still remaining are fundamental questions of structure and function at the molecular level: How does the DNA in our genes work to make the proteins in our cells, and how do these proteins interact with one another?

Researchers must have tools to build answers to those questions. In an unusual cross-campus collaboration between the Medical School and Reynolda campuses, Wake Forest University has created a structural biology program and housed it in refurbished state-of-the-art laboratory space in the Bowman Gray Technical Center of R.J. Reynolds Tobacco Co.

A useful metaphor for this effort might be found in the process of protein crystallization that is central to much of the research going on there. That's because coaxing a protein to crystallize — like finally realizing the idea of a research facility more than a dozen years in the making — can be a daunting investment of time, patience, energy and creativity with no guarantee that even the best attempt will bring the desired crystals into being. And if it does, if a million billion protein molecules suspended in solution finally array themselves into an ordered lattice suitable for further, more detailed study — like the shipping cartons, crates and construction pallets strewn about the floor opened and arranged to equip 9,000 square feet of wet lab space, X-ray diffraction laboratory, biomolecular resource facility, computing and graphics stations, academic offices and conference rooms — that's only the first step in what can be a long journey of research and discovery into the structure and function of just what makes us tick.



The new structural biology facility enhances teamwork, says Dr. Mark Lively.

"BEFORE YOU CAN UNDERSTAND how a machine works, you must have a notion about how it's made, how it's shaped," said MARK O. LIVELY III, PH.D., professor of biochemistry and director of Molecular Genetics. "That's what structural biology is about: defining the shapes of the molecules, which are the machines that make the cells work. The functional aspects of a cell depend entirely on how the proteins work. And you can't understand how the proteins work unless you know what their shape is."

The fundamental relationship in biology between structure and function is an idea first put forth by Aristotle more than 2,500 years ago. Today, the principle is no different, only the questions modern technology allows researchers to explore:

Can we use knowledge of the protein structure to design a drug that disrupts the activity of HIV protease, an enzyme essential to replicating the virus that causes AIDS?

How can we use a molecular understanding of DNA metabolism to design a drug that keeps cancer cells from resisting chemotherapy?

Is it possible to individualize drugs that work differently in different people?

Are there ways to turn off genes that cause heart attacks and turn on ones that cut the risk?

What would it take to keep cells from dying naturally of old age?

Although the development of HIV protease inhibitors is a concrete example of where such inquiries can lead, Lively advises against unrealistic expectations of what Wake Forest might achieve; structural discoveries will not move quickly into the clinic. "It's very basic research," he said. "You can't readily take any given experiment that's going to be done in this laboratory today and say this is absolutely going to become a clinical treatment for disease X in two weeks or six months or five years. It's not at that level, for sure."

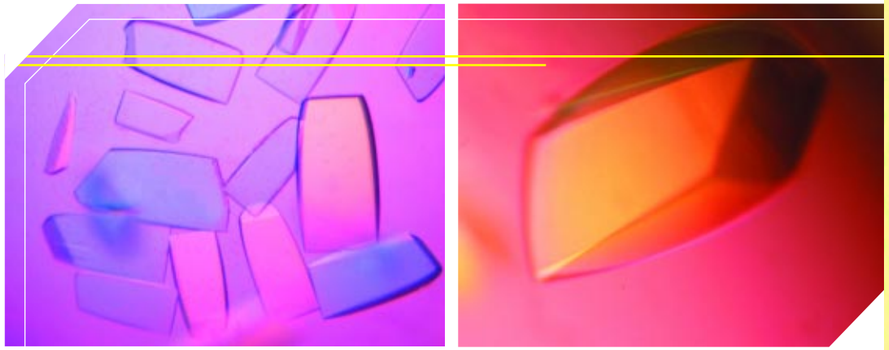
Proteins have shape and structure — including folds, contours, twists, turns, coils, loops, whirls, whorls and other topographical features defined by a protein's polypeptide "backbone" in space, its posture partly a function of how its own atoms attract or repel one another. Researchers studying protein structure search for "active sites" where protein-to-substrate interactions take place. In theory, once they know where and how an interaction takes place, they can experiment to find ways of gumming up the works. In simple terms, this is how HIV protease inhibitors keep people from developing AIDS; in broader terms, this is the kind

of investigation Wake Forest has equipped itself to pursue. With 30,000 to 40,000 human genes coding for the construction of what might ultimately be more than a million proteins, and with chains of hundreds of amino acids often required to link into a single protein via complex biochemical reactions that are catalyzed by other proteins, there is no shortage of important questions for researchers to explore.

"As it happens in science," said Leslie B. Poole, Ph.D., associate professor of biochemistry, "we get more detail and more detail and more questions."

Even before the human genome was sequenced in 2001, the National Institutes of Health announced its Protein Structure Initiative. The goal is to determine 10,000 unique protein structures in coordinated fashion over 10 years. Researchers supported by grants from the National Institute of General Medical Sciences — including many within Wake Forest's structural biology program — may apply for supplemental awards to facilitate detailed functional studies of particular proteins for which structures have been obtained.

"STRUCTURAL BIOLOGY has just caught fire," said AL CLAIBORNE, PH.D., professor of biochemistry, who leads the program with Lively.



Single crystals of the antioxidant enzymes methionine sulfoxide reductase, which help reverse redox damage.

They worked for years to convince the administration such a program would be essential to the future of medical research and graduate education. "We were missing out on this all of that time," Claiborne said. "Now we are on the same ball field as the Dukes and the Chapel Hills and the Seattles. This is going to bring us much closer to unknown discoveries than we've been previously. It will allow people to look at this Medical Center, or Wake Forest University Health Sciences or Wake Forest University, for that matter, in a context that says, yes, we in fact are considered to be part of that relatively elite group of basic scientists."

When advocates for a program in structural biology pressed forward with funding requests in the 1990s, they lacked one important ingredient to make their case: a researcher trained in X-ray diffraction, a fundamental tool of structural analysis. In this experimental method, a finely tuned beam of X-rays bombards a purified protein crystal, and a computer records where the X-rays go. The electrons that give a protein its structure will obey certain laws of physics and diffract or reflect X-rays into patterns. Changing the angle at which X-rays strike the sample will change the diffraction pattern in measurable ways. By repeating this approach and collecting data in systematic fashion, a researcher can deduce protein structure by calculating backward from where the X-rays landed to how the electrons were arranged when the X-rays struck them.

Although the N.C. Biotechnology Center did approve funding during this period for core equipment still being used to analyze enzyme function, Claiborne said, it was not enough: "We just couldn't go to the next step in structural biology because every time, without question, we'd run into this chicken-and-egg problem. You want to ask for $250,000 for establishing an X-ray diffraction facility, but where is your X-ray crystallographer? On the other hand, if you don't have the facility, you can't get the X-ray crystallographer."

Enter Conn Mallett, Ph.D., now assistant professor of biochemistry. In 1997 he was a third-year graduate student in Claiborne's lab when Claiborne and Poole visited crystallographers in Japan. Through an introduction, Claiborne met a renowned crystallographer at the Institute for Protein Research at Osaka University who agreed to collaborate with them on crystal studies from Wake Forest — if they would send a grad student to Japan. In the spring of 1998, Mallett left Winston-Salem for his final year and a two-year post-doctoral fellowship.

"Part of it was probably adventure," Mallett said, explaining why he went to Japan to learn X-ray crystallography. "Part of it was wanting to see a project through, because the project I had been working on in Al's laboratory really needed a crystallographic and a structural component. The only way to do that

(cont. p. 8)

By Mark Wright

PROTEIN CRYSTALLOGRAPHY SHEDS LIGHT ON AGING, INFECTION AND DISEASE PROCESS

IT SHOULD COME as no surprise that a chemical phenomenon that scientists now think underlies the human aging process is a version of the same phenomenon that causes burning, rusting, weathering of wood, and the more subtle deriving of energy from respiration and photosynthesis.

It is the same chemistry that gives you household cleaning fluids; it is used to purify the metal for your jewelry, and it will eventually destroy it.

"It" is the oxidation-reduction reaction, or "redox" in scientific parlance.

In its simplest form, oxidation occurs when an element or molecule takes on an oxygen atom, forming different molecules. Simultaneously another molecule is giving up an oxygen, or is being reduced.

Redox gives new meaning to the word "ubiquitous." It is, quite literally, everywhere — from the interaction of solar radiation with the Earth itself to the molecules of the cells of your body.

You are a virtual hotbed of redox reactions, and the science of structural biology is advancing our understanding of the significance of these processes.

Todd Lowther peered through a microscope at the structural biology program's newly renovated lab at RJR's Bowman Gray Technical Center. He was looking at protein crystals that were grown in the lab, the product of an exacting isolation and purification process.

Even to the untrained eye, the tiny crystals are breathtakingly beautiful — triangles and quadrangles and explosions of sculpted rods, all smaller than half a millimeter, and each representing a miniature piece of the puzzle of life.

The crystal is the key to unlocking the structure of the protein molecule. Crystallization holds the molecules still so that the researchers can aim a single-electron X-ray beam at them. The electrons refract off the electrons of a molecule and create a pattern of dots on an X-ray film or digital camera chip. The pattern can then be converted to a density map that is the same general shape as the molecule. From that the scientists can determine which amino acids make up the protein and how they are arranged.

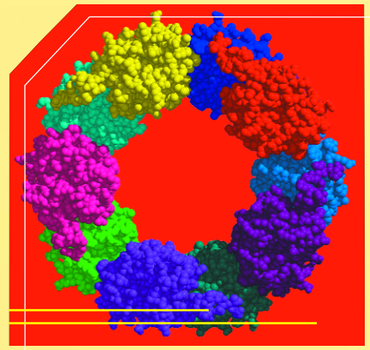
Methionine, one of the 20 amino acids and one thought to be important in the aging process, is a focus of Lowther's research. Other scientists elsewhere are also studying methionine, but Lowther is the only one using X-ray crystallography.

In doing so, he can clearly see the redox reaction that takes place when methionine encounters hydrogen peroxide (H_2O_2). Because of its sulfur content, the amino acid is oxidized to form methionine sulfoxide. "This is damage," Lowther explained. "By adding that one oxygen it can alter the ability of the protein to function properly. It could lose its ability to interact with another protein, or with DNA, or even RNA (ribonucleic acid, which is involved in protein production). Some proteins will actually lose their enzymatic activity."

Although the body is able to reverse some of the damage with methionine sulfoxide reductase (MSR), he said, "as you get older you have more and more and more of this oxidation, and our bodies are not able to keep up."

What if you had a little help with your MSR production? Fruit flies that were given the gene that expresses MSR lived 70 percent longer than average. On the other hand, mice who had their MSR genes "knocked out" had a 40 percent shorter life and an increase in neurological disorders.

This suggests that MSR might play a key role in holding off Parkinson's disease, Alzheimer's and other forms of dementia.



Proteins can be doughnut-shaped, like this model of redox protein alkyl hydroperoxide reductase (AhpC).

Although some redox reactions are irreversible and part of a natural process, such as aging, others are imminently reversible. Structural analysis by Leslie Poole, a senior member of the structural biology team, has shown these reactions to be part of the body's vital "signaling" mechanism between and within cells.

The redox-dependent functions of a bacterium that causes hospital-acquired infections are an area being explored by Al Claiborne, a co-director of the structural biology program. The bacterium depends upon a particular enzyme and co-enzyme to defend itself against hydrogen peroxide, a powerful oxidant that is toxic to bacteria.

"Knowledge of the structure of the enzyme could lead to the development of something that would inhibit its function," Claiborne said, "and that might lead to a new antibiotic to help solve the problem of hospital-acquired infections."

Poole, who has worked with Claiborne since she was a graduate student, is a firm believer in the value of sharing scientific information, and she is particularly excited about the new location for the structural biology program.

"This space is enhancing the potential for collaboration."

was to solve the structure of the enzyme. I wanted to do that myself."

While Mallett was training in Japan, a program in structural biology was budding at the medical school. First came the computational power to crunch the data and solve the structures of crystals taken outside for study; later would come an in-house diffraction lab. When Mallett was ready to return to the United States, the school was ready to buy the equipment — if he joined the faculty to run it. "The position was there," he said, "and that's part of what led me back — and wanting to see a project through again, wanting to continue the growth of crystallography and structural biology here at Wake Forest."

The structural biology program builds naturally on the expertise in the application of X-ray and imaging technologies long demonstrated within the Division of Radiologic Sciences and elsewhere. NMR spectroscopy, for example, plays a major role in structural analysis; it can generate a dynamic, time-averaged picture of a protein. But despite the enormous effort, diffraction studies only yield pictures of a protein frozen in time. What happened along the biochemical way that made it fall into just the right shape? How long did it take for the protein or enzyme to fold into the shape that will facilitate the redox interaction that enables hemoglobin to deliver oxygen from the lungs?

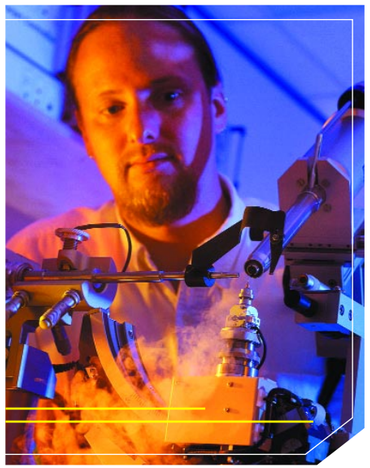
"WHAT I THINK IS REALLY INTERESTING is understanding how proteins move," said JACQUE FETROW, PH.D., Reynolds Professor of Computational Biophysics at Wake Forest University and part of the structural biology program. "You look at a protein structure and it looks like this static object, but it's really not. You've got pieces of it moving this fast," she said, vigorously waving her hand, "you've got pieces of it sort of swaying in the breeze. There are all kinds of different motions on all kinds of different time scales.

"You're talking about a vast range of time scales, from the really fast to the really slow – from picoseconds and nanoseconds to milliseconds and seconds."

Put another way, that would be like the difference in motion between the blink of an eye and plate tectonics.

Fetrow received her joint appointment between the Physics and Computer Science departments on the Reynolda campus earlier this year after GeneFormatics, Inc., a structural informatics company that she helped to found as chief scientific officer, morphed into a drug discovery company and was merged out of existence.

Although she envisioned returning to academe after working in the commercial sector, Fetrow wasn't looking for her next appointment when she learned of the proposal involving researchers from the two campuses. So she explored her options.



Conn Mallett aligns a CryroJet for an X-ray diffraction experiment.

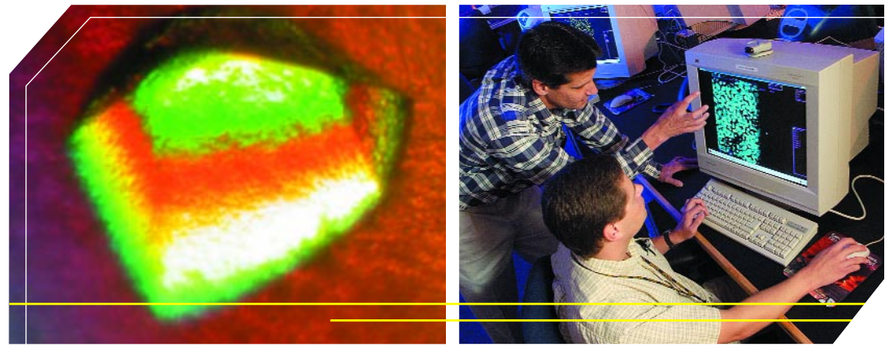
"When I heard that the university was starting the structural biology program, that was really added impetus because a lot of the data that I use — including information about protein motions on a functional site — come from structural databases," Fetrow said from her office on the Reynolda campus. "When I do my research on the computational side and think that I might have something interesting worth further exploring, having the structural biology facility that I can go to and colleagues I can go to and say, hey, this is really interesting, can we test it? — having that at the university was really important."

Like Fetrow, Tom Hollis, Ph.D., and Todd Lowther, Ph.D., assistant professors of biochemistry, were recruited to Winston-Salem before the structural biology facility had fully crystallized. In the fall of 2001 they were X-ray crystallographers finishing post-doctoral fellowships — Hollis at Harvard Medical School, Lowther at the University of Oregon — and both were deciding where they would continue their research. By then, however, Mallett was running X-ray diffraction studies from his crowded lab on the second floor of the Hanes Building. Quickly named to the search committee, he could relate to the candidates as an enthusiastic peer who shared a vision of what the new program could mean for Wake Forest and for themselves.

"One of the reasons I came here was the commitment from the administration and from the Biochemistry faculty as a whole wanting structural biology to go ahead," Todd Lowther said. "Now it's all really come full circle. We have the state-of-the-art facility in North Carolina, where two years ago, who would have known that that would have happened? The other factor for me is that my area of research is in oxidative stress, which is similar to Al Claiborne's and Leslie Poole's. I had been following their work for many years, and they had seen mine as well. It was a natural fit, just based on our research areas."

Hollis' research, on the other hand, focuses on DNA metabolism and damage-and-repair mechanisms mediated by nucleases that recognize, excise and correct strings of erroneous base pairs — the adenine-thymine, cytosine-guanine bonded-partner nucleotides that carry genetic code. "As soon as I started interviewing," he recalled, "Wake Forest went to the top of the list very quickly because of the enthusiasm of the faculty and the department and the institution for structural biology. It was clear they were committed to building structural biology — particularly crystallography. It didn't take long once I started comparing them with other schools and other institutions to become excited about coming here." Once here he began a collaboration with Fred W. Perrino, Ph.D., associate professor of biochemistry, and it quickly bore fruit: the crystal structures of TREX1 and TREX2, enzymes discovered in Perrino's lab that could impede the potency of chemotherapy.

"It took about three months worth of work to get it done, and we're in the process of publishing that now and also writing a grant on the project," Hollis said. "It reaffirmed and validated my decision to come here." X-ray crystallography, he added, "is a very time-consuming field. It's known for requiring an enormous amount of work to get few papers. I had as a personal goal to get a structure done in the first year. I was able to accomplish that because of the environment, both the commitment by the institution and the enthusiasm of the other faculty members in starting collaborations and being willing to work with the new faculty in just instituting collaborations with us."



Single crystal of the flavoprotein NADH oxidase from *Streptococcus pyogenes* (left). Fred Perrino (standing) and Tom Hollis study a crystal structure in the crystallography computer lab (right).

For Perrino, whose lab remains on the Bowman Gray campus, hooking up with Hollis was the whole idea.

"It wasn't just Tom as a structural biologist," said Perrino, who was also on the search committee, "but that his training is in the area of proteins that interact with DNA. The fact is Tom has an interest in these types of enzymes. That will drive the project forward because he'll be interested in writing the papers, he'll be interested in writing grants. He's going to build his career on structural biology of these types of enzymes." Perrino, in fact, had put off work on the project until Hollis' arrival meant in-house access to the particular combination of skill, training and interest that the project needed.

"I've been down the road where I've collaborated with folks at other institutions," Perrino said. "The small details that become very important in making these types of projects successful are sometimes difficult to communicate when you're collaborating either by e-mail, or by telephone, or by driving to different institutions, or flying. The things that you don't write down on paper can be critical to the success of the project."

The difficulty of long-distance collaboration is a point Claiborne, Lively and others had made for years. It can be more than frustrating to depend on a long-distance collaborator whose timetables, and priorities and procedures are not your own. Claiborne, an enzymologist, was Poole's graduate advisor in the late 1980s when she was studying NADH peroxidase, an enzyme that appeared to catalyze a new class of reactions. This area of inquiry has since exploded, both in Poole's lab and elsewhere, but when the first paper was published out of Wake Forest in 1989, the authors had no way to solve the three-dimensional structure. But they had the protein crystals, and Claiborne wrote to a crystallographer in Freiburg, Germany, whose team had solved similar structures, and he agreed to take on the project with some stipulations.

"What they required us to do was to do all the crystallization here, and then to actually pack up each individual crystal in a Pasteur pipette — a glass pipette with a fine point — actually take the solution in which the crystals were prepared, pick the crystals out under the microscope, shove them into the wide end of the Pasteur pipette, seal the other end with parafilm, and then seal up the toppings," Claiborne recalled.

"So we shipped boxes of these Pasteur pipettes, packed very carefully in cotton and everything else, to Germany."

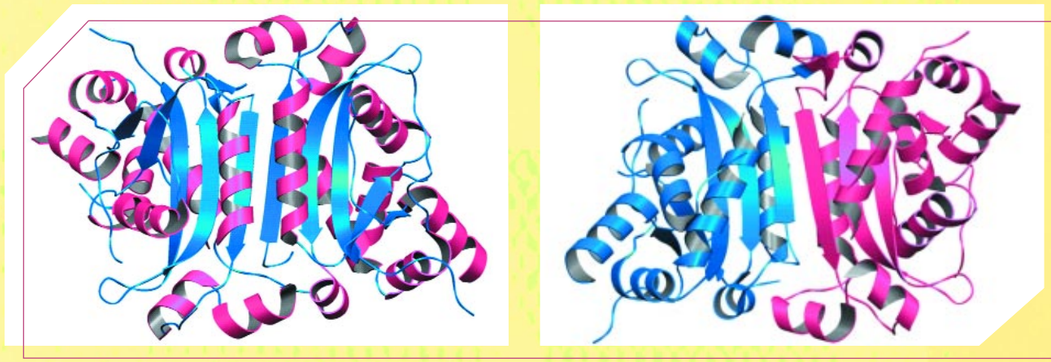
(cont. p. 11)

By Robert Conn

MAKING TREX: DEMYSTIFYING DNA REPAIR

THE NEW STRUCTURAL BIOLOGY program is helping Wake Forest scientists solve the mysteries behind two enzymes that repair DNA.

Fred Perrino, Ph.D., and his colleagues first cloned the genes encoding these DNA repair enzymes, called TREX1 and TREX2, in 1999. "We cloned the genes, but we didn't know what these enzymes looked like or how they might work," he said.



In a collaboration with Dr. Fred Perrino, Dr. Thomas Hollis determined the X-ray crystal structure of the DNA repair protein TREX2, pictured in the ribbon diagrams above.

That waited until they figured out how the structure of the enzyme reacted with DNA, which helped them understand why the enzyme worked.

"That information only came by solving its three-dimensional structure," said Perrino, associate professor of biochemistry. "That's why structural biology becomes a very important component of figuring how these enzymes work."

Perrino discovered TREX1 and TREX2 while trying to determine why an anti-leukemia drug stopped working — or never worked — in some patients. He had theorized that the drug acted by inhibiting the replication of DNA in these leukemia cells, which prevented them from reproducing. He thought DNA repair molecules called exonucleases repaired the DNA in the leukemia cells by removing the leukemia drug, enabling the cells to resume multiplying and the leukemia to get worse.

He said TREX1 and TREX2 were the first exonuclease genes to be identified from human cells. "Its normal function in cells would not be to remove a chemotherapeutic drug. It is likely to function whenever the ends of DNA get messed up during the process of replication, so the wrong building block of DNA gets put on. This enzyme might be responsible for removing it."

In fact, after the whole human genome was published in 2001, TREX1 and TREX2 remained the only two exonuclease genes of this kind to have been discovered. "It looks as if there aren't going to be as many as we thought possible. They may be two of the really key enzymes that perform this function."

Perrino and WFUHS received patents on TREX1 and TREX2 in October.

The team used biochemical engineering techniques to introduce the genes into bacteria, to make larger quantities of the TREX enzymes, which were crystallized. Thomas Hollis, Ph.D., assistant professor of biochemistry, then solved the structure of the enzyme, which has 236 amino acids.

When the team analyzed the resulting structure, they couldn't see amino acids 157 through 168, probably because that portion of the structure was moving. "Three of those amino acids were the type of amino acid — arginine — that would bind to DNA."

They tested that idea in the test tube by swapping another amino acid, alanine, for the arginine, and making a new enzyme. "We demonstrated that it binds to DNA about 100-fold less efficiently. So it clearly is the position where DNA is binding in this molecule," said Perrino.

Now Perrino has a new target in his effort to stop leukemia: attack this weak spot in the TREX enzyme. "That would be a good place to go, because if we can make the enzyme not be able to bind to DNA, then this enzyme is not going to work."

If the repair enzyme is stopped, then the original leukemia drug might be more effective and perhaps stop the leukemia.

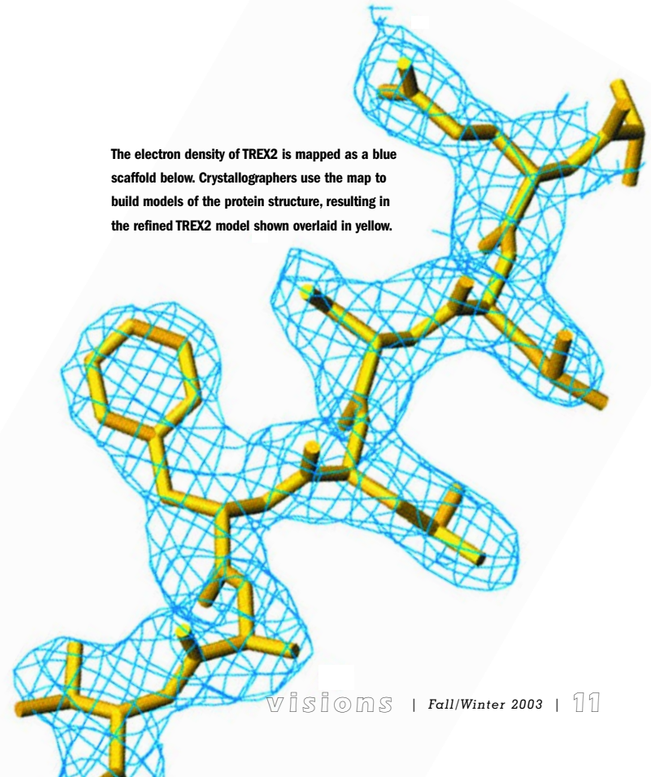
Now researchers from either campus can purify and crystallize proteins on one side of a long hallway in the Bowman Gray Technical Center, then walk their prepared samples to the other side for X-ray diffraction studies. From there it's just a stroll down the hall to the biomolecular computing and graphics facilities, where researchers can crunch their data and manipulate 3-D models at dual monitors and a theoretical peak performance of 1 billion floating-point operations per second.

The facility was established in industrial laboratory space leased from R.J. Reynolds Tobacco Co. Based on the details outlined in a five-year plan developed and shepherded by Lively and Claiborne, medical school administrators approved the overhaul and upfit of the vacant space. Through spring and early summer, vise-grip pliers, socket wrenches, tape measures, crowbars, power saws, drills and hammers were the tools of choice that made ready the space for the DNA sequencer, DNA synthesizer, fermentors, incubators, sterile cell culture hood, centrifuges, high-pressure cell homogenizer, fast-protein liquid chromatography system, stopped-flow spectrofluorimeter, refrigerators, stereo microscopes, anaerobic glove box, liquid nitrogen cryojet, rotating anode generators — and all of the more generic tools of the biochemist's trade.

Besides Claiborne, Fetrow, Hollis, Lively, Lowther, Mallett, Perrino and Poole, researchers associated with the new program include Derek Parsonage, Ph.D., assistant professor of biochemistry, who is on site, Roy Hantgan, Ph.D., associate professor of biochemistry, Dave Horita, Ph.D., assistant professor of biochemistry, and Susan Hutson, Ph.D., professor of biochemistry, all from the Bowman Gray Campus; and Bernie Brown, Ph.D., assistant professor of chemistry, and Fred Salsbury, Ph.D., assistant professor of physics, both from the Reynolda campus. They, their students and researchers from departments and centers across the medical school now have access to what Claiborne calls "an academic think-tank for structural biology" that will enhance interdisciplinary collaboration and the potential for future discovery.

Drs. Al Claiborne, Leslie Poole, Todd Lowther and Derek Parsonage (l-r) build upon each other's research.

"IT TAKES FACILITIES LIKE THIS to train the biologists and do the research because of the skill set you need," MARK LIVELY said. "You need to be a molecular biologist; you need to be a protein chemist, you need to understand protein structure and function, purification properties; you need to be a physicist, because this is very much a physicist's method – X-ray beams, diffraction, the whole issue of something called the phase problem of how you take the diffraction data and back-calculate to the actual structure; you have to be a computer scientist. It's gotten to the point where no one individual is fully capable of doing everything that absolutely needs to be done. We have the team here."



The electron density of TREX2 is mapped as a blue scaffold below. Crystallographers use the map to build models of the protein structure, resulting in the refined TREX2 model shown overlaid in yellow.