DEPARTMENT OF BIOCHEMISTRY NEWSLETTER

Kansas State University January 1995

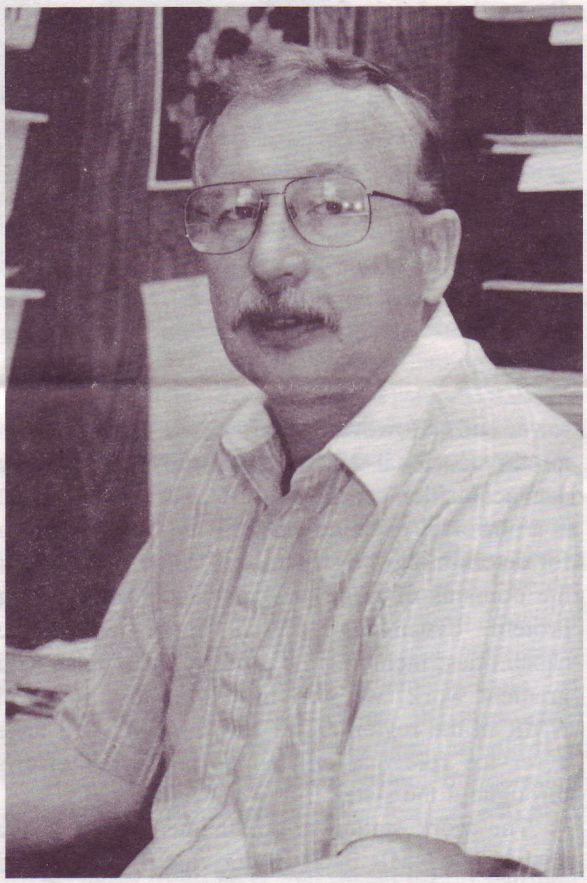
NEWS FROM THE DEPARTMENT HEAD

Tom Roche

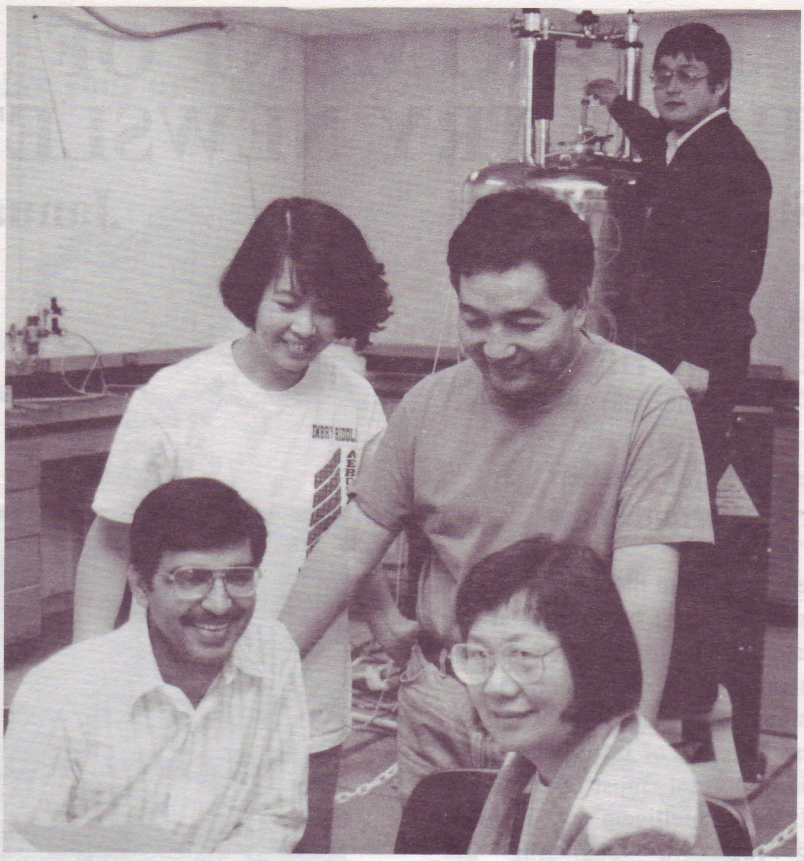
NMR Facility and Protein Structure Determinations

Whether you are a practicing biochemist or just a student of the field, you must be amazed at the revolution in understanding biological systems that is underway. The popular literature emphasizes the key role of understanding our genetic make-up, i.e. DNA. While storage of information in a duplicatable form is important, most of the work is being done by all those intelligent and dynamic proteins including roles in facilitating the duplication and retrieval of the information stored in DNA (albeit with the help and intervention of RNA). The amazing capabilities of proteins arise, of course, from their complex structure and dynamic properties and this is what the protein biochemist wants to understand. I bet all alumni of this Department appreciate the importance of proteins. Great advances in molecular biology and biophysical procedures have opened up ever more opportunities for taking this to a precise molecular understanding. Kansas State University has not lagged in adopting the now routine tools of molecular biology but we are just entering the complementary door of exploring the three-dimensional structure of proteins and other macromolecules.

In the last Newsletter, I described development-in-progress (support of an NSF-EPSCoR grant and University sources) for adding a High Field NMR facility. This has now happened and allows us to pursue one of the two biophysical paths for determining protein structure. By design we chose NMR over X-ray diffraction because we had the in-house expertise and several candidate problems and also because the approach is directly served by and integrates well with molecular biology approaches. Dr. Om Prakash joined the Department as Director of the High Field Protein NMR facility in August. Our new 500 MHz NMR



was installed in November along with a powerful Silicon Graphics work station with an optical disk storage system ($800,000 in equipment). Full testing of the essential NMR procedures was completed in April and the instrument was dedicated and brought on line for everyone's use. As many of you are undoubtedly aware, field strength is very important for separating the multitude of NMR signals from the large number of atoms (particularly protons) in a biological macromolecule. 500 MHz places us in this high field range but at what has been considered the



lower end. However, with the new capabilities in multidimensional NMR and the ever expanding data acquisition schemes, our 500 MHz instrument is a powerful machine that provides adequate data for determining the structure of small proteins (or the domains of large proteins) and for analyzing protein dynamics. With these acquisition capabilities, including the capacity to do pulse field gradient work, we now have the most powerful NMR in the region. The NMR facility will have a service role that will support research efforts throughout the state.

The work station is continuously used in the analysis of NMR data, in deriving 3-dimensional structures from those results, and data storage. It also serves the Department by upgrading our capabilities in presenting and understanding the structures of macromolecules that can be retrieved from data bases. Not only the vast store of nucleotide and amino acid sequences but the rapidly increasing library of the three-dimensional structures of proteins can be readily accessed. With the software acquired, structures can be manipulated and altered; for instance, the effect of amino acid substitutions can be approximated by energy minimization techniques. Learning all the NMR procedures and preparing adequate quantities of a candidate protein is not trivial. However, molecular biology approaches often allow expression of high quantities of a candidate protein, and allow production of proteins with stable isotopes incorporated; this greatly expands the capacity of NMR studies to provide information that can be used to obtain the structure of a protein. Come see our new facility in the basement of the Chemistry/Biochemistry building.

FACULTY NEWS

LAURA ANDERSSON

This year the student researchers in my laboratory are all undergraduates. Amy Wilson, Sheri Bylkas, and Anna Johnson are working on "Structural and Functional Analysis of Biological Heme Systems." One project involves exploration of the role of His in the O_2-binding pocket of human myoglobin, comparing properties of seven engineered recombinant mutants. Another project focuses on how protein structure controls substrate specificity for the enzyme cytochrome P_{450}. We have P_{450} enzyme from 5 different sources, and will soon have engineered active-site mutants to examine also. Our biophysical approach enables us to "see" the active-site and directly monitor effects of structural changes on function.

LARRY DAVIS

My laboratory continues to work with nifH mutants. Some are really turning out to be exciting -- they give evidence of a *ras* type binding mode for ATP. Nitrogenase may be an ur-enzyme for nucleotide controlled processes. Another research area that takes a lot of my time is the role of plants in bioremediation. Several graduate students from Chemical Engineering and Chemistry work on that with me. In Phil Nordin's old hood we have a plant chamber that keeps producing interesting results. (We still have to write the papers though.)

CHARLIE HEDGCOTH

Efforts to understand lysine tRNAs continue under the guidance of Ph.D. student Dimitri Tamalis with characterization of various genes for lysine isoaccepting tRNAs. Ph.D. student Ping Wei is exploring the relationship between mitochondrial gene expression and cytoplasmic male sterility in wheat. At a considerably slower pace, I am searching for tissue specific promoters with which to construct artificial male sterile wheat and someone to fill a postdoc opening.

MIKE KANOST

In my laboratory we are continuing to study proteins that are present in insect hemolymph. We are working on serine proteinases, serine proteinase inhibitors from the serpin superfamily, and a bacteria-induced protein from the immunoglobulin superfamily. We are also studying insect hemocytes to begin to understand how they recognize and respond to pathogens and parasites. Three of my graduate students will graduate this semester: Haobo Jiang, Yang Wang, and Lei Zhao.

KARL KRAMER

I continue to work with many students and collaborators on insect cuticle and gut physiology and metabolism, and on experimental insect control proteins. This past year we completed studies on the properties of two high molecular weight catechol-containing glycoproteins that probably become cross-linked during insect cuticle sclerotization; the structure and distribution of several catecholamine glucosides that serve as storage precursors for cuticle tanning agents; and the metabolism and transport of catecholamines in wild type and pale body colored strains of the medfly. We also bioassayed many proteins for anti-insect activity, and identified an enzyme, some enzyme inhibitors, and some carbohydrate binding proteins that retard insect growth and development. We engineered an insect-chitinase full-length cDNA into a baculovirus and demonstrated that the recombinant, chitinase-producing virus kills insects more rapidly than does the wild-type virus. Because of this work, we submitted a patent disclosure application for the use of recombinant insect chitinase as a biocide. Working with the bacterial insecticide *Bacillus thuringiensis* (Bt), we found that gut proteinases present in some strains of the Indianmeal moth that are resistant to Bt do not activate the insecticidal protoxin as efficiently as enzymes from the wild-type strain, and proposed that altered protoxin processing is a potential mechanism of insect resistance to Bt.

On the home front, all is well except for my hairline, which is too mobile. My wife Virginia is teaching Spanish at Manhattan High School, son Karl is a senior at MHS and is also taking a Spanish class at KSU, and daughter Kristina is an eighth grader at the Manhattan Middle School. As part of her 4-H activities, Kristina is busy fostering a yellow labrador retriever puppy to become a service dog for a handicapped individual. Next year three family members should graduate, one from high school, one from middle school, and one from Kansas Specialty Dog Services.

R. KRISHNAMOORTHI

Grace is doing post-doctoral work in NMR of biomolecules at Washington University, St. Louis. Sylvia, after getting a Master's Degree, went with her husband to Pakistan to serve as a volunteer. Gong continues to take care of the laboratory and contribute to the various projects in progress. Thanks to the NIH support, Drs. Mengli Cai and Jianjua Liu joined the laboratory in the early part of 1994. They have been putting our new NMR instrument to constant use! Recently, I received an NIH Research Career Development Award. Mengli and Gong have accomplished the three-dimensional solution structure determination of CMTI-V. Liu has obtained exciting results with C-13 relaxation measurements on CMTI-III and CMTI-III*. On a personal note, my family and I had the pleasure of a 4-month visit from my 70-year-old mother from India.

DEL MUELLER

I'm still teaching most of Biochemistry II Laboratory and Physical Biochemistry, like always, but several years ago started teaching IOB too. I'm also still doing research on Rubisco. The Rubisco work has taken an exciting turn. I took a sabbatical with Jake Schaefer at Washington University, St. Louis, in the spring of '92 to apply some of his group's new solid-state NMR methods to measure distances between phosphorus nuclei of a bound inhibitor and the activator carbon on Rubisco. (Yes, we visited the Arch too and I took a picture of it that's hanging on my office wall.) After getting some problems "ironed-out," we got very good and precise data. Currently, we have produced uniformly ^{15}N-labeled Rubisco and are beginning distance measurements (up to 6 Å) between active site nitrogens and other labeled groups under several conditions to accurately assess the suspected conformational flexibility of the active site. These should be really fun times!

S. MUTHUKRISHNAN

My laboratory is focusing on the isolation, characterization, and expression of defense genes in plants. Recently we have generated transgenic rice plants constitutively expressing a rice chitinase. These plants appear to be more resistant to fungal infection. In collaboration with Dr. Karl Kramer, we have introduced an insect chitinase cDNA into tobacco plants. Tobacco plants that express the insect chitinase are more resistant to feeding damage by insects than control plants. These results point to possible use of these defense genes in protecting plants from fungal and insect pests.

OM PRAKASH

I joined the Department of Biochemistry as director of the newly established high field protein NMR facility during last year. Previously I had worked at CIBA-GEIGY Pharmaceuticals Company, New Jersey and the University of Arizona. At KSU my prime responsibility is directing the NMR laboratory for macromolecules and assisting area researchers with NMR investigation of macromolecules, especially proteins. This laboratory will also support the grant application of life scientists at KSU, KU and WSU. Besides running multi-dimensional and other fancy NMR experiments, my research interests are structure-function studies, structural characterization and molecular recognition using NMR and NMR-structure based drug designing.

GERALD REECK

In the broadest terms, the work in my laboratory is on the structure and function of proteins -- particularly enzymes and inhibitors -- and on the structural basis for strong and selective interactions between proteins. In all cases, encoding DNAs are crucial to our experimental approach, as is expression of those "genes" in *E. coli* and the creation of mutant proteins by mutagenesis of their cloned DNAs. A new and potentially exciting twist to our work is an active collaboration with X-ray crystallographers on the three-dimensional structures of our proteins.

TOM ROCHE

My laboratory is continuing to have fun investigating the function and regulation of the pyruvate dehydrogenase complex (PDC). We have evidence that the kinase component walks around the surface of the complex by a "hand-over-hand" mechanism. We have firmly established a signal translation (yes, translation, not transduction) mechanism whereby PDC activity is throttled down to lead to conservation of body carbohydrate reserves. My former students will recognize that this sound like results that are founded in earlier work. While our research has gone well in recent years, funding has been every more difficult to obtain. We have just learned, however, that we will receive a four year NIH grant starting in April. Shengjian Liu obtained his Ph.D. last year and is now doing postdoctoral work with

Arthur Kornberg at Stanford. Sundari Ravidran and Weli Li also obtained M.S. degrees since our last newsletter. Sundari is working in my laboratory still and Weili is working for Merck in New Jersey. Gary Radke has moved into our Biotechnogy Core Facility. Since they both did some work in my laboratory and since some former students will remember them at a smaller size, I will mention that Tonya Roche is finishing an M.S. in Biomedical Engineering this semester at Boston University and Eric Roche is in his first year of graduate school at MIT in the Department of Biology.

DOLORES TAKEMOTO

This year my laboratory has been very busy with the arrival of a new postdoctoral fellow from Russia, Dr. Alexander Yakhin. Karen Gonzalez expects to finish her Ph.D. this year on the role of protein kinase C in galactosemia. A past student, Joan Cunnick, is now an Assistant Professor at Iowa State. This plus two children have kept her very busy. Dan Morrison, also a past student is now a research associate at the University of Illinois in Chicago. He has continued his work in the retina field. Brenda Oppert is now a research scientist at the Grain Marketing Research Laboratory here in Manhattan, where she has been very successful in her work.

Several undergraduates have left to fulfill their career goals, including Christian Twamley who is now in optometry school in Texas and Jennifer Adams who is now a student in K-State's College of Veterinary Medicine. My laboratory now has a new graduate student, Dong Mei Wang, who is an optometrist from China. She will continue to work on diabetic retinopathy.

JOHN TOMICH

My group is positioning itself to bridge the disciplines of molecular biology and medicinal chemistry. We are currently involved in the design and synthesis of novel branched peptides. These chemically synthesized biomolecules are patterned after native biological proteins such a bound ionic channels and receptors. We have been developing the tools to assemble and characterize their structures. The biophysical properties and biological activities of these molecules are measured at other institutions through collaborative arrangements. My group has recently begun synthesizing unusual amino acids with novel chemical properties. Recently these synthetic amino acids are incorporated into chemically synthesized peptides or proteins and their properties analyzed . To date we have synthesized three new amino acids. My group consists of two senior research associates, two graduate students, two technicians and five undergraduate students.

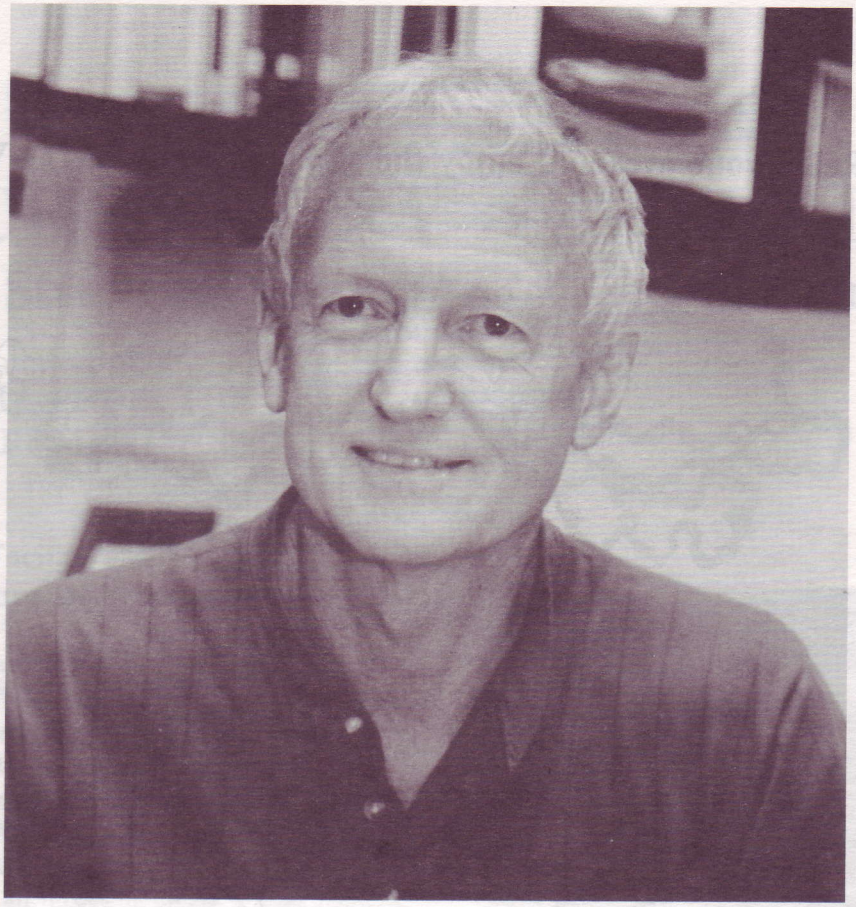
XUEMIN WANG

The research in my laboratory centers on the control and cellular significance of phospholipid turnover. Jim Dyer, a Ph.D student in my group, has made significant contributions to the identification and characterization of isoforms of phospholipase D in plants. He is a recipient of the 1994 Biochemistry Department Outstanding Graduate Research Award. Some of his work has been published in two recent articles (Arch. Biochem. Biophys. 1993, 306:486; Plant Physiol. 94, 105:715). Liwen Xu, another Ph.D student, has finished sequencing of a Ricinus phospholipase D and determined its intracellular location. Some of her results have been published recently in J. Biol. Chem (1994, 269:20312). Dr. Stephen Ryu has been a postdoctorate with me for almost two years working on the role of phospholipases in plant stress response. Stephen and I both received travel awards from Northern American Plant Lipid Group this summer for presenting our data at the 11th International Meeting on Plant Lipid held in Paris, France. Suqing Zheng has recently joined in my group as a visiting scholar from China, and we are looking forward to a fruitful collaboration with her.

Research Support/Education
(more news from the Department Head)

During the past two years, the faculty have succeeded in attracting all time highs in extramural support ($1.2 million and $1.4 million). This was the product of a huge effort -- last year the total requests in applications sent out by the Department was over $ll million, and Biochemistry faculty were on multi-investigator grants from other Departments totalling several million in requests. Some of the most productive programs submitted grant applications that received very favorable reviews but were still not funded. This is happening all across the country and is seriously damaging the continuity of outstanding research programs. (Having just been in Japan, I can assure you that the same is not happening there.) The expanded capabilities derived principally from molecular biology and biophysical approaches in solving biochemistry questions have opened many doors. This has spawned development of many significant and productive research efforts throughout the country; however, the available funding has only risen slightly. Review panels are forced to make priority judgements that are based on secondary considerations. Thus, U. S. tax payers (and research foundation donors) can be assured that solid research is being conducted with the small portion of taxes that are supporting this research. However, there is tremendous inefficiency due to the need to expend a huge amount of time in search of research dollars; due to stop/go funding that leads to loss of continuity including loss of experienced research assistants; and due to the demise of research programs that have been continuously successful and built up irreplaceable expertise through years of experience. We are happy to report that acquisition of the NMR has already favorably affected acquisition of research funds (see paragraph by Dr. Krishnamoorthi) and we anticipate this will be a long-term (but slowly developing) trend.

How does all of the above relate to our capacity to effectively educate graduate and undergraduate students? In answering this, I will not dwell on the well known concept that classroom education is intertwined with the knowledge and insight of the instructor, and that an active researcher can convey unique insights that instill a special value and dynamic in any class. Instead, I want to briefly emphasize the direct consequences of the functioning of an effective research enterprise on educating future biochemists. The expanded research capabilities of the Department and the success in attracting extramural funds translate into students carrying out novel research. The undergraduate researcher (particularly but not exclusively biochemistry majors) are significant participants in research projects throughout the Department. This endeavor is often considered by those students to be among the most valuable experiences in their undergraduate education; furthermore, it regularly leads to authorship on publications and can have a longstanding influence on future pursuits. As you know, the research experience is at the core of educating graduate students, and their capacity to complete meaningful thesis projects depends on extramural support and the equipment that is available. Just as the transition has been made to expecting most graduate students to employ molecular biology techniques, it will become a general expectation that students increasingly use and manipulate data bases and become familiar with thinking in an interactive way about 3-D structures of proteins. The Department looks forward to many graduate degrees that utilize NMR as a primary tool. If the trend towards ever increasing difficulty in obtaining extramural funding continues, not just important research results will be lost, but KSU and other institutions engaged in graduate and undergraduate education will simply be able to provide less meaningful education with long-term consequences for the creative and competitive capabilities of this country.



Faculty in Focus: Charles Hedgcoth

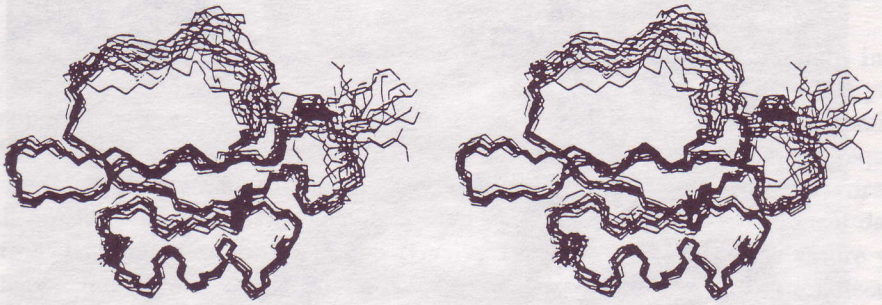
Tom Roche

Finally, as in past newsletters, I want to end by briefly commenting on one faculty member. This year's victim is Professor Charles (Charlie) Hedgcoth. Charlie is the senior full professor in the Department. He has just finished a five year stint as Chairman of Graduate Biochemistry Group. This is one of those tasks (especially graduate student recruiting) that involves a lot of time and effort but receives very little credit. As usual Charlie's research program is active and diverse. He just received a two year USDA grant for $150,000. This involves studies on a specific mitochondrial gene in wheat that is altered in male-sterile wheat. Understanding how male sterility is introduced could afford new opportunities for control in wheat breading. He has had industry support to develop an approach to controlling male sterility. Indeed, Charlie has performed much of the experimental work on this project. He continues to have interests in unique functions of Lys t-RNA in mammalian cells and the gliadin storage proteins of wheat. Charlie is recognized as one of the best teachers in the Department. He always obtains outstanding student evaluations (often the best among 6 faculty who teach the Biochemistry I/II series). For several years he has taught Biochemistry and Society; he has had an outstanding student response in this course. The University (without extra resources, of course) is trying to introduce more general education courses. Charlie's Biochemistry and Society presentations should be the model. Not surprisingly, Charlie was one of the first members of the Department to receive a Stamey Teaching Award. He continues to attract graduate students from all over campus to his Nucleic Acids course and has also taught General Biochemistry in recent summers.

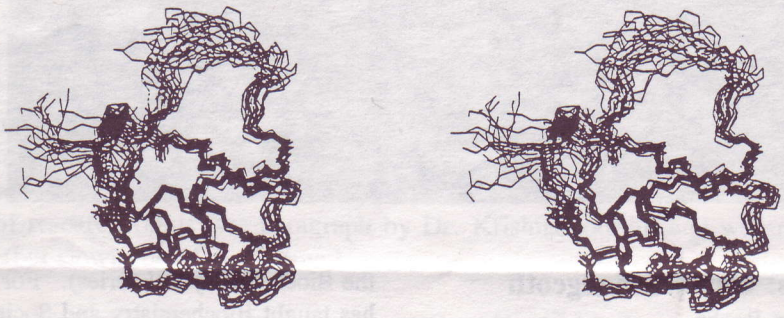
Yes, Charlie is still very active playing, coaching, and refereeing soccer and he jogs almost daily. A lot of younger faculty wish they were in such good shape. Finally, inside and outside the Department, Charlie's council is always respected. He has just stepped down from his third year of service on the Graduate Council. Not long ago he served on a committee for hiring an interim dean. He is on a very large number of graduate student advisory committees. Whenever we are preparing a policy document, Charlie functions as our in-house lawyer. Charlie may be the most senior member of the Department but none of us would want to contest him in a two mile race or lose his invaluable contributions.

Relax your eyes and enjoy the stereoviews of CMTI-V !

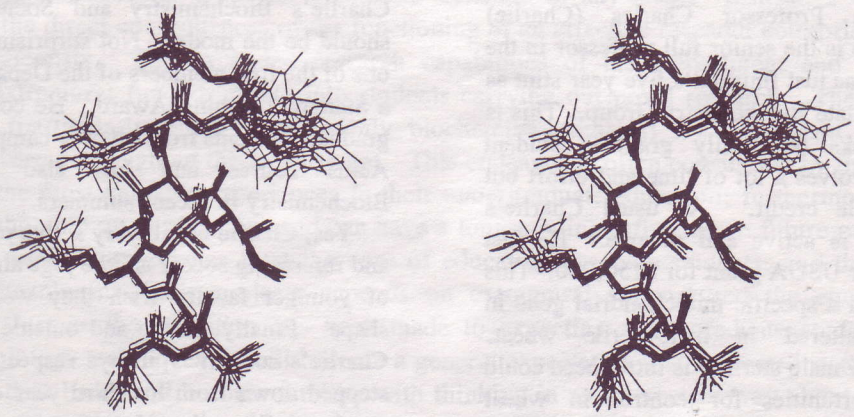
A



B



C



A & B: Stereoviews of the best-fit superposition of 22 NMR-derived structures. The main chain atoms (N, C_α and C_{CO}), Trp9 side-chain atoms and the Cys3-Cys48 disulfide bridge are shown.

C: Stereoview of α-helix from Asn28 (top) to Ser18 (bottom)

(Cai, Gong, Kao and Krishnamoorthi, to be published)