GOING TO THE SYNCHROTRON

The older I get, the more I dislike traveling. The hassle of airplanes is especially distasteful. I tend to ache from sitting all cramped up in small seats, and I get upset with the people directly in front of me who thoughtlessly put their seats into full recline. And I miss my own bed, and my own bathroom, and the other creature comforts common to one's home.

So ordinarily I'll go to great lengths to avoid travel, even though, as a professional biochemist, I do a lot of it. There is an exception: whenever the occasion presents itself, I'm ready--even eager--to go off to a synchrotron. For me, these trips compare to a mountaineer having a chance to climb Everest. They are expeditions in the traditional sense, brimming with challenge, adventure and risk. The adventure is mainly intellectual (although there is a bit of the old Hemingway definition of discomfort seen from a distance). Many years ago my high school Latin teacher would regale us with the promise that "There is no thrill like the intellectual thrill." Although our adolescent physiologies made us incredulous, I know now that she was right

"What is this synchrotron you're always running off to?" My wife asked me that question one evening while we were having dinner. I had just announced that I'd be flying off to "Brookhaven" in about two months, and the question caught me up short. It's not an easy one to answer without a lot of technical background. The short answer is that synchrotrons are large devices of the kind that used to be called atom-smashers; they're related to the old-fashioned cyclotrons. Many of them are at large Government facilities that have the technology for accelerating subatomic particles like electrons or positrons. Under appropriate conditions these whirling particles can generate very powerful X-rays, which in turn can be used to find the detailed structures of important biochemicals.

But this dictionary-type answer doesn't explain the fascination. Why should an aging biochemist fall under the magnetic spell of whirling electrons? Other men my age take up golf or become serious watchers of sports on television. And they are willing to go off with their wives on cruises to Alaska or motor trips to the wine country. Why was the only travel that sparked my interest going to the synchrotron?

Some of it is personal. A series of unexpected events occurred late in my career that were more than a little rejuvenating. One was that a depressed economy in California led to my being "bought off" from my professor's job at the University of California, which in turn gave me the time and freedom to pursue my research full time. It was during the early 1990's that the University of California sought to solve its perennial financial woes by undertaking a "buy-out" plan, whereby they could move senior professors to the Retirement Fund and use the savings to hire young people at the bottom. The first of these Very Early Retirement Plans (VERIP) was offered in 1991. The initial inducement was to sweeten the formula used to calculate retirement benefits, which involve one's age, time of service, and best-pay years. Any faculty 55 years old or older were eligible for the plan. In truth, the sweetener wasn't very significant, and only the most overburdened with teaching went for it. About two years later, the offer was made again, with a slightly better set of conditions. A few more bit. It was a little like playing blackjack. How high would they go?

Then, early in 1994, the University came back with what they said would be their final offer. As a further lure, our local administrators in San Diego assured those of us who had research groups that we could keep our laboratory space and still take graduate students, so long as we could gather in the usual external grants to pay the bills

The sweetened pot looked pretty good to me and many of my colleagues, but we hesitated on one count: we weren't ready for the "e"-word. The e-word

conjures up the image of a doddering old chap who appears once a week to check his mail, and none of my kind could suffer that. To ease the hobble of "emeritus," the Administration made up a new job title for us, Research Professor, and with that it was a done deal.

I'm sure there are some readers who will not take kindly to professors being so eager to give up teaching and committee meetings in exchange for doing their research, but there is ample precedent for people serving institutions well but eventually needing relief. I am reminded of the philosopher George Santayana, who was a revered professor at Harvard back at the beginning of the 20th century. When he was not quite 50 years old, he came into a small inheritance, and even though he was famous for his wonderful classroom lectures, he decided to give up teaching. In fact, the story goes that he couldn't even wait until the end of the school year. He was lecturing in a classroom on Harvard Yard early in May, and, looking out at the buds on the trees and the blooming forsythia, he is alleged to have said: "Excuse me, gentlemen, I have a date with spring." And with that he walked out. On the day the University offered what turned out to be the final buy-out, I came home and reported to my wife that we had come into a "small inheritance."

Another important factor was that the work in my laboratory had taken a good turn. For many years, I had been making sporadic efforts to crystallize fragments of a very large protein called fibrinogen. Fibrinogen is a molecule that circulates in blood and which is transformed into a polymeric material called fibrin, the principal component of blood clots. Blood clots can be matters of life and death, but they are also intrinsically interesting, and I had long been fascinated by the chemical magic of their formation. By the beginning of the 1990's, with the sand beginning to run out of life's hourglass, I had undertaken a more sustained effort to determine certain molecular details of clot structure, and, together with a beginning graduate student and with the help of another person working with one of my faculty colleagues, began a final push Almost miraculously, crystals of a big piece of

fibrinogen were obtained. Getting crystals of a protein is an artful business. Some proteins are almost as easy to crystallize as is common salt or sugar. Many others, especially large gangly ones, have never been crystallized, despite the efforts of hordes of investigators. If a protein can be crystallized, there's a good chance that its three-dimensional structure can be determined in exquisite detail by X-ray crystallography. A crystal contains vast numbers of molecules, all arrayed in a three dimensional repeating pattern, and, as I will try to show, ingenious methods have been developed to use X-rays to probe their structures.

X-ray crystallography itself actually had its beginnings not long after the serindipitous discovery of X-rays by Wilhelm Conrad Roentgen more than a century ago. Almost everybody has some familiarity with X-rays and knows that they penetrate different materials to different extents. If you put an arm in their path and a film behind, the bones absorb more of the radiation than the muscles and skin, and the silhouette of the bone shows up on the film as being less exposed. One of the first experiments Roentgen did was to make a photograph of his wife's hand just that way. Not only did the bones show up clearly, but also her wedding ring.

What isn't awfully clear to the average person is where these X-rays come from and why they're so penetrating. In Roentgen's case, he was actually studying events that go on inside of an electric discharge tube, a cathode ray tube of the kind that many years later would evolve into the television tube. He noticed that a nearby detector screen, not a part of his experiment, would glow when he applied voltage to the tube. Unexpectedly, something was emerging from the tube as a result of the electrical events going on inside it. Roentgen realized that some kind of radiation that could go through glass was responsible, and he called it X-radiation, or "X-rays."

It was a sensational discovery that captured the public's fancy as well as having a tremendous impact on the field of medicine. We now know that what was

happening was that high velocity electrons, which is what cathode rays are, were slamming into the metallic anode and arousing the resident electrons. In the ensuing excitement and momentary relaxations, radiation was being emitted, consistent with the principle of the conservation of energy.

X-rays are really a form of light--what the physicist calls electromagnetic radiation. Different forms of light are defined by their wavelengths. For example, visible light, the kind we can see and the limits of which are described by the colors of the rainbow, has wavelengths of a sort that allow it to be absorbed by receptors in our eyes. Light with slightly longer wavelengths is called infra-red ("below the red"); microwave and radio energy are kinds of light with even longer wavelengths. On the shorter side, we're all familiar with ultraviolet light ("beyond the purple"); X-rays and gamma radiation have very short wavelengths. As is apparent from this description, the shorter the wavelength, the more energetic--and potentially dangerous--is the light. This is because the speed of light is constant, and the shorter the wavelength, the more beats per second it vibrates. Radio waves cover distances of the order of centimeters or meters between beats; X-rays, on the other hand, are very short and are measured in a term called angstroms (named after the nineteenth century Swedish scientist, Knut Angstrom). One angstrom is about four-billionths of an inch. Fortuitously, bond lengths between atoms are of the same general lengths as X-ray wavelengths, and as such, crystalline materials can diffract them. Within 20 years of the discovery of X-rays, they were used to determine the distances between layers of ions in simple salt crystals. Most of these salts were composed of only a few different atoms.

It's much more difficult to determine the structure of a crystalline protein, however, and it wasn't until 1958 that the first complete three-dimensional analysis was reported, that of a muscle protein called myoglobin. Myoglobin is made up of approximately 1,200 non-hydrogen atoms (hydrogen atoms, the smallest of all atoms, don't have sufficient mass to deflect X-rays). Until then, the largest structure to have been determined was vitamin B12, a molecule

which has 93 (non-hydrogen) atoms. There are numerous technical problems associated with the process, and enormous computational demands. The calculating difficulties have been eased considerably by the development of high-speed computers.

But the first hurdle remains getting good crystals. Usually this is best accomplished by beginning with a homogeneous solution of the target protein. My longtime research assistant Marcia Riley is expert at purifying fibrinogen from outdated blood plasma (which we buy from the San Diego Blood Bank). The next job is to tear the molecule apart into manageable pieces by carefully digesting it with specific enzymes. The pieces must then be separated from each other to yield the necessary homogeneous preparations.

Good crystals have their molecules arranged in near-perfect three-dimensional lattices. The more perfect the arrangement, the better X-rays will be diffracted and the more information can be gleaned. The term used to describe this quality is resolution. As in ordinary photography, high resolution provides a better image. There is a fundamental difference between the imaging obtained by diffraction compared with ordinary photography, however. As it happens, physicists long ago realized that light can be affected by external objects in three ways: for one, it may be absorbed (as occurred in Roentgen's picture of his wife's hand), or, for another, it may be refracted or bent (the crooked pencil sticking out of a glass of water and what occurs when light passes through a magnifying glass or telescope or microscope). In a conventional microscope, the light scattered by an object is recombined by a glass lens that bends the rays to a focus at a specific point. In an electron microscope, the scattered electrons are recombined and focused by powerful magnets. But there are no lenses or magnets that can focus X-rays. Mathematics and the computer are sometimes described as the substitute for a lens.

Light may also be reflected (as occurs in the case of a mirror); diffraction is a lot like reflection, the rays being reflected by the individual layers of molecules in the crystal. The wavelength is very important here, and

in fact the best diffraction occurs when all the light rays have the same wavelength. When they bounce back from the layers in the crystal, some of the waves will have their crests in phase with others and will be reinforced. Others will be way out of phase and will cancel each other.

As for the arrangement of the molecules in the crystals, I like the two-dimensional analogy of soldiers on a parade field. The goal is to find out what an individual soldier looks like; his or her image. Remember, this is a homogenous assembly and all of the soldiers are look-alikes; we want to find out how tall they are, how wide, the shape of the hats, etc. In order for the analysis to work, the soldiers must be in some kind of repetitive formation; if they're just standing around randomly, the method won't work. The more perfectly they arrange themselves in some formation--to the point where we can look down a rank or column and not see one offending elbow--the better the resolution and the better the final image. There is a little more to it; each soldier is in virtual contact with her neighbors. For example, each might have her left arm extended out to the side and her right arm extended forward, the finger tips in each case just touching the neighbor. The contacts can dictate the nature of the formation. For example, if the right arm is moved leftwards, the soldiers in the rank in front will be shifted leftwards, also. It works the same way in the crystal; the intermolecular contacts direct the overall pattern. As mentioned earlier, there is a huge number of molecules in a crystal, typically more than a trillion in a protein crystal the size of the period at the end of this sentence. It's easy for them to get out of line and blur the diffraction. The diffraction itself depends on the X-rays being reflected from successive layers of molecules and only those rays re-emerging which have their waves in exact register, which is to say, in phase.

I love to look at crystals. I have an old, low-power microscope that magnifies things about fifty-fold. The crystals are grown in small drops of solution sitting on tiny platforms in plastic trays covered with transparent tape which can be cut open to get at the crystals. The trays, which are about

four by five inches and perhaps three-quarter of an inch tall, are divided into 24 chambers with platforms in their centers. Each drop can be surrounded, moat style, with a larger volume of a solution of a somewhat different composition and the two liquids allowed to come into a kind of equilibrium by local evaporation. The trick is to get the protein, which is initially dissolved in the little drop, to gradually come out of solution. It takes any where from a few days to months to grow protein crystals. Different proteins crystallize into different shapes, and a given protein may yield different crystal forms depending on the exact solution conditions. Some crystals are like little glass plates, others like diamonds, some like small pieces of translucent "two by fours" (the lumber kind). Often the first growth crystals aren't good enough for X-ray diffraction studies, but they can be used to seed subsequent batches, and, by a number of artful manipulations of the conditions, bigger, better, smoother crystals can be encouraged. Sometimes the crystals are clumped together and need to be separated. When it comes time for the X-ray beam, it's very important that it be a single crystal. If two stuck-together crystals were used in the beam, the equivalent of a double-exposure would result. The clumps can be broken up in various ways, but I like to use a single paint brush bristle. Like many of the operations in this business, it requires a certain amount of practice. Coordinating the bristle-tip while staring into the binocular eyepiece is just a little like rubbing your stomach with one hand while tapping your head with the other.

Sunday morning is my favorite time for examining crystals. With no one around, it's easier to get all your faculties focused on the job. The rows and columns of the sectored dishes are numbered and lettered. As I examine each drop, I record in my notebook what I see. Sometimes nothing at all has happened, and the drop has remained clear. Other times the protein has simply precipitated into a large white tangle. Often the first indication that useful crystals might be gotten are clusters of needles that look like little sea urchins. The reward comes when there are smooth-faced prisms. Now I write: Plate DD141 (8-3-98). B4. Good crystals. May be ready for the synchrotron.

Although pushing crystals around with a paint-brush bristle takes practice and some manual dexterity, getting a crystal the size of a period out of a drop and into a suitable glass tube for the X-ray analysis is even harder. A finely drawn out glass capillary tube can be used. The tube is broken off so that its small end opening is just a little larger than the longest dimension of the target crystal. The other end of the capillary is stuck into a little plastic adaptor which in turn is attached to a length of latex tubing. A plastic fitting is attached to the other end of the tubing for a mouthpiece. All in all, it amounts to a kind of flexible straw with a very small opening at the other end. The goal is to slurp up a crystal into the capillary--but not too far up--all this while peering into the binocular scope. It's a hand-eye-mouth coordination that must be mastered by practice. Once into the capillary, the crystal, suspended in fluid, must be transferred into another glass tube sealed at one end called the mounting capillary; it's the one the crystal will sit in while the X-rays are bombarding it. At this point the surrounding fluid must be carefully removed so that the crystal won't slip during the exposures. It's also useful to get the crystal positioned in a way that its best face is oriented as an easy target for the beam at the start, even though the whole system will have to be moved around later. After the crystal is set, a drop of solvent is added nearby--but not touching the crystal--to keep the space moist; the crystal mustn't dry out. Then we seal up the open end of the capillary tube with beeswax, and it's ready for the beam

All of this takes practice. It can be a frustrating business. A prize crystal gets sucked up in a turbulent froth, only to disappear because of an errant exhalation that takes on typhoon proportions in this microscopic world. I think it's an easier business to master when you're young. Initially, I learned most of these techniques from one of my graduate students, Stephen Everse, who in turn had learned them from a graduate student working for one of my faculty colleagues. Joe Kraut is a real crystallographer and a very old friend. Many years ago the plan had been that, should our group ever get

crystals, he would be the person to do the structure. Actually, it was a member of his group who managed the first small crystals of our fragmented fibrinogen material. But Joe also took the Very Early Retirement Incentive Plan (VERIP) and made the switch to Research Professor, and he had his own agenda that didn't include working on fibrinogen. Instead, he generously gave us a key to his facility and let us learn by doing. It was a great experience, learning all the things one usually learns as a graduate student or a postdoc in a new field. Now I have a research assistant (Leela Veerapandian) who sets up the crystals on a routine basis; her dexterity is impressive. Leela prefers to use one of her own hairs attached to a little nyon handle as her prod. I will never match Stephen or Leela in speed, but slow or not I find great satisfaction when I manage a well mounted crystal on my own.

After a crystal is mounted in the capillary, it should be tested as soon as possible. Although some protein crystals evidently endure for long periods, ours seem to hold up in the mounting tubes for only a few days . When we first began, we would hike over to Professor Kraut's lab in another building on campus where he had an X-ray generator. The capillary containing the crystal would be inserted into a positioning device called a goniometer, which can be adjusted to orient the capillary and crystal so that when the beam is turned on it will hit the crystal just right. The next job is to get some kind of picture of the X-rays scattered by the crystal. In fact, there are many different systems for catching the diffracted X-rays, the simplest of which is ordinary photographic film. The X-rays blacken the (developed) film with a pattern of spots that is reciprocally related to the way the molecules are arrayed in the crystal. The secret to the molecule's structure is hidden in how dark each of the spots becomes. All our early work was done with film, and it was a good way to learn. After we were more experienced, Joe Kraut let us use a more sophisticated apparatus called an area detector, which can catch a bunch of diffracted X-rays electronically and store them in a computer directly. One can take many different exposures this way relatively quickly, although, as we shall see, not nearly as quickly as one can at a synchrotron. In either case,

a motorized device can be activated that will twist the capillary in space in a specified way so that the various faces of the crystal are serially exposed frame by frame. Typically, it may take a hundred exposures or more to get complete coverage of a crystal, what is called a "complete data set." Each of those exposures will have hundreds of diffracted rays ("spots"), so in the end one is evaluating tens of thousands of "reflections," which is a term used by crystallographers for the spots made by diffracted rays.

During 1994 and 1995, we used Kraut's area detector to collect data on hundreds of different crystals of a large piece of human fibrinogen called "fragment D." Fibrinogen is one of those large, gangly proteins that no one could crystallize. Its general structure had long been known to be extended and bolo-like on the basis of electron microscope pictures. Our strategy was to sever connections that hold together the better defined terminal parts, and it was one of these terminal parts-- the fragment D--that we were able to crystallize. Tens of thousands of past biochemical experiments by countless researchers had provided a host of other details, including the fact that fibrinogen is actually a Siamese twin, two identical halves being joined at a central point. In the crudest sense, each half of a fibrinogen molecule consists of two cabbage-shaped globules attached to a cluster of long rhubarb stalks. The leafy ends of the rhubarb stalks are attached in the middle. Fragment D is the equivalent of two cabbages and the severed stump of a rhubarb stalk cluster.

Getting the crystal structure of fragment D was a more challenging problem than I had realized and would have proved difficult even for an experienced crystallographer. In spite of our determined efforts, a structure was not emerging, which is to say, the diffraction data we had collected were not up to the task. By May, 1995, Stephen Everse had been a graduate student for almost six years and had certainly accomplished more than enough for a Ph.D. Accordingly, we published a preliminary paper on our results and he was awarded his degree. The paper also served as a public notice that we were working on

the fragment D structure--a kind of claim stake--although I felt certain that most people in the fibrinogen community already knew what we were doing. In this regard, we had long known that there was another group working on an even bigger fragment--called fragment X. Fragment X amounts to two fragments D connected to each other by another, smaller, fragment called E. We had been in touch with the other group and, in response to their requests, had provided them with our methods and general protocols. They had been stuck for a very long time, unable to obtain a detailed structure for reasons I'll explain momentarily, and it didn't seem to me that we had anything to fear by sharing our methodology.

Not surprisingly, Stephen wanted to stay in our lab and continue the work as a postdoc. To our good fortune, we were joined by another postdoc from England, Glen Spraggon, who had just completed his Ph.D. in Oxford (where they actually call the degree D.Phil.). Glen was trained in protein crystallography and was more experienced in many of its aspects than were we. One of the first things he said to us after reviewing the data we had collected so far was, "We've got to get to a synchrotron."

As I noted earlier, synchrotrons were originally the balliwicks of physicists who were smashing sub-atomic particles into each other. The incidental radiation that was being given off was to them only a nuisancesome by-product.

Then, in 1971, some European scientists wrote an article in the journal *Nature* in which they showed that the powerful synchrotron radiation could be harnessed for use in X-ray diffraction studies. The beams available were more than a hundred times brighter than the conventional X-rays being used, and the potential for improvement was enormous. It was a seminal paper, and in the interval since its appearance the method has gone from one that was of necessity parasitic on the experiments of physicists to the situation today

where there are synchrotrons whose principal use is macromolecular crystallography.

I don't know exactly how many synchrotrons there are in the world; probably there are only a dozen with facilities for protein crystallography. I know of five in the United States, a few in Europe and one in Japan. Most of the ones in this country are sponsored by the Department of Energy; they include facilities at the Brookhaven National Laboratory, the Argonne National Laboratory, the Stanford Linear Accelerator and the Cornell High Energy Synchrotron Source. A new synchrotron has recently begun operation at the Lawrence Berkeley Laboratory Even before Glen's arrival, I had put in an application for time at the Stanford synchrotron, which is the one nearest to San Diego. These applications are judged by a committee of experienced crystallographers who rank the applications according to importance and demonstrated need for the very powerful synchrotron radiation. In fact, my priority score was not high enough to make the list for beam time. My guess is the committee probably thought the project was important enough, but they could justifiably wonder about our lack of experience. Undaunted, I put in another application, and also one to the synchrotron at the Brookhaven National Laboratory in New York. Fortuitously, Glen was corresponding by email with his former D.Phil. adviser back in Oxford and mentioned the problem to him. As it happened, the adviser was very keen to have Glen come back to Oxford for a few weeks to finish some papers they were trying to publish. Perhaps if I could provide the airfare for Glen and Stephen to make the trip, he would be willing to give up one day of his own time at the synchrotron in Daresbury, England.

This was an agonizing decision for me. One day is not much time. Lots of things could go wrong. Would the crystals survive the long trip? Furthermore, our money situation was always tight in that we had only a single modest N.I.H. grant to cover every aspect of our program. But synchrotron radiation is so much more powerful than what we had available on our campus in San Diego that any data collected were certain to be better. Even more compelling, the

wavelength of synchrotron radiation can be adjusted ("tuned"), and our particular need was for a special kind of data (called "anomalous") collected on crystals of protein that were decorated with gold atoms. Gold and other heavy metals scatter X-rays much more than do the lighter atoms found in proteins, and one can use the metal scattering as a kind of sign-post to chart the other lighter atoms. Gold scattering is best measured at certain wavelengths that can be selected at the synchrotron but not on small conventional X-ray generators. The two young men were naturally eager to try this, and after tossing and turning for a night or two, I agreed. And so in March, 1996, they took off for England. We stayed in daily email contact. I was nervous, even more so after "our day" got postponed. But in fact Glen and Stephen eventually were able to get some of our crystals on to the beam, and they came home with magnetic tapes containing the data.

The first job in analyzing X-ray data is to sort out all the spots and evaluate their intensities on a high speed computer, operations referred to as "indexing and scaling." There is a marvelous computer software package called "Denzo" put together by Zbyszck Otwinowski (a well known name in crystallography circles) that facilitates matters greatly. The programs can quickly determine in which crystal system the molecules are arrayed, which is like finding the particular formation the soldiers on the parade field have adopted. It's the first piece of information needed to find out what the soldiers themselves look like. This process is followed by numerous quality checks and much evaluation, followed by analyses that generate three-dimensional maps. It was about a month before we were able to see the first "pictures," or electron density maps, but they were better than we had dared to hope for. Indeed, we were absolutely giddy when tubes of electron density appeared that were obviously the rhubarb stalks The stalks are really a cluster of long alpha-helices wound around each other. It was now clear to us that sooner or later we would get a good structure, although we knew it would probably take another year and much more data. Even with the limited data we had, however, we could begin building a model. Model-building in a computer is

delightfully tedious. The goal is to construct balls and sticks--atoms and bonds--into the clouds of electron density shown in the three-dimensional maps.

The first job is to trace the path of the polypeptide chains that constitute the protein's backbone. Almost everybody knows--or should know--that proteins are long chains of amino acids. The order--or sequence--of the amino acids determines how a chain folds up in three dimensions. That's what the crystallographer is trying to determine. After a chain is pretty much set in place, then the orientation of each of the amino acid side-chains needs to be found. The positions of the atoms are kept track of in the computer by a long list of values for x, y and z. These are "the coordinates" that can be sent to anyone who wants to reconstruct the model. They are the equivalent of latitude and longitude in a two-dimensional map. Imagine that you wanted to describe the geography of the United States strictly by a list of latitudes and longitudes of its cities. You might start with a list of only five big cities, say, New York, Chicago, Seattle, Los Angeles and Miami. This would constitute a low-resolution description of the country, but as the list was extended to include more and more cities, the "model" would gradually fill out. By the time the hundred largest cities were included, a graph of the numbers would be a good representation of the whole country. If, on the basis of other information, it was also known that the major cities were connected by highways, that network could be included in the model also, just as the protein crystallographer knows the order of amino acids in the polypeptide chain. So it is that a computer rendering of the long list of three-dimensional coordinates for the atoms in a protein reproduces the model calculated from the original data.

Remarkable computer programs have been developed to help in the process. The one we were using is called "O;" it was developed by a Welshman named Alwyn Jones who has spent most of his career in Sweden. It is really a suite of programs, each designed for a specific task. For example, there is a subroutine called "BONES" with which one can examine an electron density map and "skeletonize" the clouds so that a framework map of the highest density is

revealed. Ideally, the skeleton should correspond to the polypeptide backbone, at least as a first approximation.

We had divided the model building among us so that Glen and Stephen each took a cabbage, and I concentrated on the rhubarb stalks, which as I mentioned are really entwined alpha helices, called a "coiled coil." We each had our own computer, so we could work along side each other all day every day. Not surprisingly, Stephen and Glen were much better at the work than I; they could also work longer without interruption (the VERIP hadn't relieved me of all my responsibilities). In the end, Glen and Stephen would have to help finish up the rhubarb stalks, too.

Our euphoria was short-lived. In April, I received a brief email message from a friend at another university who had seen a posting on the internet that a high resolution structure of a portion of the fragment we were working on had been solved by another group. They had succeeded in crystallizing one of the cabbages. We were stunned. Somehow it hadn't occurred to us that we had immediate competition other than the group working on the larger fragment X. The fragment X project had been stalled for a long time because they couldn't obtain suitable metal-decorated material. They knew that if we succeeded in obtaining a structure, the solution could be applied directly to their data and the much larger structure of fragment X quickly gotten, even without metals. The reason is, if one knows a significant part of the soldier's structure, the rest can be gotten even without heavy metal decorating. Similarly, if we knew the structure of the smaller fragment, we'd be able to get our own structure directly. This appealed to Glen Spraggon, who, having worked on the project for less than a year had less of an emotional investment than the rest of us. To me, however, this would be like climbing almost all the way up a mountain and then having a competitor throw you a rope down from the top. So I wasn't disappointed at all when, for reasons of their own, it turned out that the other group decided not to make their data public for almost a year from the time we

first heard the news. It meant that we were able to solve our own structure by ourselves.

I can only guess at the reasons the other group was so secretive. Initially we thought the reason was because they submitted the structure to a structure prediction contest called CASP (Computer Assisted Structure Prediction) held every May at the conference center in Asilomar, California. Theorists are always trying to anticipate experimentalists, and there have been legion attempts to predict protein structures on the basis of the known sequence of amino acids alone. The best of these have only been modestly successful and have only led to crude representations of actual structures, but even crude information is often valuable. Indeed, we ourselves had long before made a prediction of what these cabbages should look like. The contest came and went, and the details of the structure were not revealed; there must have been more to it.

Ironically, the young men were barely back from the synchrotron in England when we received notice that, as a result of a cancellation, we had been awarded some immediate beam time at Stanford. This was good news, because we had plenty of crystals and we still needed much more data to improve our maps. In May, Stephen and Glen made the short trip north to Palo Alto and collected two day's worth of assorted data. Unhappily, I was committed to serve on a review committee in Washington, D.C. that weekend and couldn't go with them. Within days, however, we received notification that we had three day's worth of time at the National Synchrotron Light Source at the Brookhaven National Laboratory. Suddenly, we seemed to be in the catbird seat. By this time, also, Marcia and Leela had purified another more complex fragment we had long had our eyes on. It was the equivalent of fragment D except it was isolated from a fibrin clot instead of from the starting fibrinogen molecule. If we could obtain its structure, we'd learn a lot about how the molecules are packed together to form the clot. Not only were we able to get crystals, but we were able to grow them with some physiologically important peptides bound to

important places in the double-D molecules. We had plenty of material for another synchrotron trip, and this time I was determined to go along myself.

And so late in July, 1996, I set out one Sunday morning on a trip to the East coast. I flew to Chicago and changed planes for MacArthur Airport on Long Island. Stephen and Glen had left San Diego on Saturday in order to get the cheaper airfares that come with a Saturday night stay, a requirement not needed for my Senior Coupons. So while I was flying cross country, they were spending Sunday site-seeing in Manhattan. The plan was that they'd meet me at the airport with a rental car, and in fact they were waiting when I deplaned about 7:30 Sunday evening Stephen and Glen are both very big guys, and because I am as skinny as I am old, it was I who slithered into the small back seat of a two-door Tercel sub-compact, the cheapest rental available.

We drove east on the Long Island Expressway, turning off at Exit 68. Instead of heading north on the William Floyd Parkway towards the BNL, we headed south to the Windmill Diner for some supper. The young men had burgers and fries; I had a bluefish sandwich and a Sam Adams. Glen also had a beer, but Stephen doesn't drink alcohol. The caffeine in soft drinks appeals to him, however.

It was dark when we drove up to the lab. Stephen slowed down at the gate and the guard, recognizing the little rental car, waved us through. Apart from the guard, the place seemed completed deserted. We proceeded to the housing office where a lone person stays on duty until midnight. I got my dorm key and paid the $14.50 per night fee (it's gone up a dollar recently) Our rooms were in the men's dormitory called Cavendish, named for Henry Cavendish, the discoverer of hydrogen. The plan was to meet at the car at 7:00 AM and go to breakfast at the cafeteria, after which we'd appear at the administrative wing of the synchrotron in order to take the mandatory safety class as soon as possible after the 8:00 AM opening.

My room was small and spartan but clean. It had a cot, a small writing desk and a tiny refrigerator, noisy enough that I had to unplug it. I opened the window to let in the cool night air and unpacked my bag. Then I went on a simple exploration to find the various facilities. I wanted to call my wife to let her know of my safe arrival, but the one pay telephone was being used. There was a group of young men watching the Olympics on a television in a lounge area, so I joined them. After a while, I went back to the phone, but the same guy was still gassing away. I gave him the cranky old man treatment, and he begrudgingly hung up. I made the call and then went off to find the bathroom nearest to my room; I shaved, brushed my teeth, and took one of the eight sleeping pills I allow myself each year on occasions when I change time zones and need to be well rested at the start of the next day.

The Brookhaven National Laboratory, which has a permanent staff of 3,000, is located sixty-five miles east of New York City on a sprawling 5,265-acre site almost dead center on Long Island. The former site of Camp Upton, an Army training camp during both World War I and II, it still has a military look, its 250 mostly modest buildings being widely spread out, and the two-story wooden dorms having that familiar barracks-style. All told, there are two dormitories for men, one for women, and one that accommodates both men and women, for the use of the 4,000 visitors a year who like us come to use the remarkable resources. There is also a guest house, which apparently costs quite a bit more than the dorms. The nearest communities are five or more miles away, although there are a number of facilities right on site, including a fire house and a gas station. The setting is bucolic, and deer sightings are common.

The real centerpieces of the laboratory aren't awfully noticeable, being mostly underground. One prominent structure is a now decommissioned high-flux reactor with a huge silver-colored dome. Not long ago it was hounded into inactivity by an irate public when it was found to be the source of a small tritium leak. Other large structures include a particle collider, a very big synchrotron, and a smaller synchrotron used for the X-ray source we were going

to be using. The BNL is the home of a very large number of research endeavors, ranging from very pure physics to applied genetics, and the activities of the smaller synchrotron, which are officially administered by the National Synchrotron Light Source, or NSLS, comprise only one modest segment. The synchrotron itself is embodied in a large circular structure, the main floor of which is one story below ground level. Windowless labs and offices and other facilities are situated around the periphery. The center section houses the synchrotron itself, a circular tunnel about 70 yards in diameter through which electrons are accelerated. The radiation emitted by these energetic particles is tapped off on tangential beam lines at 30 stations around the circle; the beams are modulated on their passage into secure bunkers by a number of devices: wigglers and undalators that enhance the brilliance, collimators and filters that select certain wavelengths, and focusing devices that target the beam with great precision. Many of the stations have "splitters," so the number of beam lines is actually greater. Only seven of the beam lines are used for macromolecular crystallography (mostly proteins but occasionally RNA); the others are used in a variety of physical experiments, including tests on the nature of materials, surface chemistry, and many other things I don't know anything about. Of the seven used for protein and RNA work, two are leased to the Howard Hughes Medical Institute, whose pampered investigators never have to want for anything. The remainder are funded by a grant from the Division of Research Resources of the National Institutes of Health. As such, academic groups whose applications pass muster don't have to pay for the use of the facility. Industry-sponsored workers do pay a fee.

The matter of safety is taken very seriously at synchrotrons. The most critical rule is never to risk a person being exposed to the beam; the beam is so intense that even a few seconds exposure would mean instant serious injury if not death. There is an elaborate interlock system between the area where the experimenters sit by their computers and monitors and the pill-box hutches where the samples are positioned in line with shutters on the beamlines. There are time locks and keys that have to be turned and removed, and buttons to be

pushed and emergency horns and colored lights. Beyond that, everyone wears a badge for monitoring radiation exposure, even though the beam is well shielded with lead and steel and radioactivity per se is not a real threat.

In the morning, I met Stephen at the car. Glen had still been asleep when Stephen rapped on his door, so we had to wait a bit. Then we drove over to the cafeteria. It's an excellent facility with a wide selection of good foods. The dining area has floor to ceiling windows that look out among beautiful pine trees. It would have been nice to have a leisurely breakfast and enjoy the setting, but in fact we ate hurriedly, cleared our dishes and headed over to the synchrotron about five blocks away. There were already a few groups ahead of us filling out forms when we got there, but the three women running the office were efficient, and in short order we were watching a safety video, taking a written test on various aspects, and sitting for individual photographs that would be affixed to our admittance badges. We were also issued radiation badges that would be carefully monitored. About ten o'clock, one of us having swiped his newly issued badge through the strip reader at the entrance to the experimental floor, we crossed over into another world The low hum of heavy machinery and air conditioning and the fluorescent lighting made it just a little eerie. We knew that only a short distance in front of us on the other side of a concrete wall a billion electron-volt stream of electrons was racing around. Our assigned beamline was X12C We had entered opposite X1, so we turned left and walked clockwise not quite half way around the circle.

Bob Sweet is in charge of beamline X12C, and he was waiting when we arrived. He had been checking things over from the group before us, who had departed about 8:00 AM. Bob is a well known crystallographer and the co-editor of a very important set of books on protein crystallography. He gave us a quick run-down on what equipment was available and what to do if problems arose. He telephoned John Skinner, the person in charge of the computers, and John came over to set up our computer account and give us a password. During all of this rapid instruction, I kept nodding my head as if I understood

everything that was whistling past, but really I was counting on Stephen and Glen to find out the vitals. Stephen is a wizard at computing, and I was confident he would explain the directory system and related software to me later. I was just a little overwhelmed at the amount of complicated instrumentation that was jammed into the little alley way in front of the hutch where the beam came in. There was a bank of four high speed computers with big consoles lined up on a low counter; three or four battered office chairs on wheels haphazardly filled much of the alley itself. Up above the big computers, there were all sorts of gauges and dials; two television monitors stared down at us. One of these, like the monitors in airports for announcing flights, was reporting on the status of the beam and its intensity. Another was like the security monitor in a convenience store and looked into the part of the hutch where the crystal would be positioned. And there were pipes and valves and wires galore all over. In amongst all this jumble was a sign with a quote from Albert Einstein: "If we really knew what we were doing , it wouldn't be called research, would it?". Bob Sweet asked if we would be using the cryo-stream, a device for keeping crystals frozen at liquid nitrogen temperatures during the exposure to the beam. Having virtually no experience with the process at the time, we demurred. Properly frozen crystals can endure in the beam for very long periods, while the same crystals at room temperature are rapidly damaged by the intensity of the beam. But some crystals hold up better than others at room temperature, and because we had brought several dozen crystals of several types with us, we would take our chances that we could merge the data from multiple crystals to get sufficiently complete coverage of all the different views required.

As soon as Bob Sweet and John Skinner left, Stephen went to the small lab across from X12C and got out one of our trays of crystals. The young men had carried the trays of crystals across the country packed tightly in white styrofoam insulated boxes. This is a constant worry to us because the crystals can't be jostled or disturbed in any way. Getting through the airport security gates is always a delicate matter, and finding good places in the overhead of the airplane often requires good interpersonal skills, another area where

Stephen excels. In short order he had mounted one of our new double-D crystals. We went in to the cramped hutch together, and he showed me how to lock the goniometer in position and then how to use a hand-held remote to center the crystal so that it would stay in the beam as it rotated. We exited the hutch, went through the ritual of locking its interlocked door, and wheeled up a couple of chairs next to Glen at the main computer console. We looked up at the television screen that was focused on and magnifying the mounted crystal; it looked great. Glen chose a reasonable exposure time and distance for a test "still" picture. The choice of exposure times depends on the intensity of the beam and the size and quality of the crystal. The distance of the collector from the crystal is also an important variable that depends on how well the crystal diffracts. Counter to most people's intuition, the better the diffraction, the closer you want to have the detector. The reason is that the detector has a fixed area, and the closer it is, the better it is for catching the most diffracted rays. After checking over all the many selections to be made on the computer console, Glen finally clicked on the box "collect," which automatically opened the shutter.

The next few quiet minutes are the most exciting during any visit to the synchrotron. It is the moment of truth when one finds out if the crystal is going to diffract well enough to be useful. There may be no spots at all or only a few restricted to the central area of the collecting field, indicating very low resolution. If the spots are streaky, that's bad, too; the crystal may be too wet and be slipping just a little during the exposures. What one wants is a screen full of dark, sharply defined spots that occur in a regular pattern all the way out to the edges of the detector. A good sharp diffraction pattern is one of the most beautiful objects ever to be seen by an understanding human eye.

In principle, one doesn't go off to the synchrotron unless he's sure that the crystals will diffract, as evidenced by preliminary testing on standard machines at one's home institution. In practice, this isn't always practical. For one thing, the beam-time assignments are often made quite far in advance, and not all protein crystals have indefinite lifetimes. They can spoil just

sitting around in the crystallizing drops. For another, one hates to use up the best crystals for screening, inasmuch as once a crystal is exposed to X-rays at all, it's done forever. Also, one of the great attributes of synchrotron radiation is that its intensity is so great that quite small crystals, which hardly diffract at all in a conventional beam, do very well in a synchrotron beam As a result, there's always a risk one may arrive at the synchrotron and find that a particular batch of crystals does not diffract X-rays. This can be true even though the crystals may look good to the eye.

The test exposure was set for two minutes, but it took another two minutes for the image plate detector to be optically scanned and the information transferred to the computer. There is a little filling bar on the screen that shows the progress of the process, and as it neared 100 percent we all held our breath. Then the image flashed on the screen; it was OK! Not the most intense we had ever seen, and not the most resolving, but good enough to think we would probably get a complete data set before we were through. Now we set to work in earnest. There were judgments to be made; calculations to be done; numbers to be recorded in our data book. How many images should we plan to take to get all the views we wanted? Should we advance by one degree at a time, or 0.5 or 2.0? There are magical computer programs that can be run on the first few frames (a frame is an image is one picture) and guide the experimenter to what regions of the space will be the most informative for the particular crystal

Then, as we watched each frame go by, it was clear that the crystal was deteriorating fast We stopped the data collection momentarily, went into the hutch, and moved the crystal slightly so the beam was hitting it in another area. The diffraction improved for a few more frames. But then we had to mount another crystal and start over again. The process would be repeated over and over again. We spent the day mounting many more crystals, usually managing only one or two hours collection time on each.

A synchrotron storage ring of accelerated electrons gradually decays and must be restored. At the NSLS they stop the beam twice a day, usually about 7 in the morning and 7 at night. The engineering staff attends to various

maintenance needs and then restarts it. The process ordinarily takes less than an hour. About half-past-six, with the beam scheduled to be taken down shortly, we drove over to the Brookhaven Center for a bite to eat. The Brookhaven Center is a small social center that is actually the former Officers' Club from Camp Upton days We got some pizza and in short order were back at the beam. The beam was already up and operating, and we set right back to work. At supper we had agreed on the night time schedule. Glen would drive me back to the dorm about 10 PM so I could get some sleep. He himself would turn in about 4 AM. Stephen, who is indestructable, would stay up all night until the morning beam shutdown.

The next morning I was up before seven and walked to the cafeteria for breakfast, where I thought I might see Stephen, but there was no sign of him. After a quick bite, I hurried back to the dorm and waked Glen so I could get the car key. I drove to the light source and found Stephen still at X12C. He briefed me on the night's activity, took the car key, and went off for some rest. At last I was soloing. It was a feeling of immense power to be sitting there at the consoles, even if there was not much pressing to be done. After a while the crystal that was in place died, however, and I was forced to mount a new one. It seemed to take me forever, but eventually I got it in place, and set it collecting. It was a good one, and I was extremely pleased with myself.

Suddenly a message flashed on the screen: out of disc space. I momentarily paniced; what to do? I fumbled through my notebook to no avail. Data were being collected and spilled into nothingness. I tried to make another sub-directory on the same disk, which of course was ridiculous. I felt like Mickey Mouse in the Sorcerer's Apprentice scene in Fantasia, frantically trying to mop an increasingly spilling flood. Finally I found how to switch to another disk. I made a new directory and re-started the data collection. Things were back under control.

Not long after, however, disaster struck again; the beam had quit. A voice came over the PA system, telling everyone what they already knew; there's a problem with the beam. Nothing to do but wait. All around the ring there was a nervous quiet. The small teams of two or three persons stood around at the 30 or so stations like travelers at an airport waiting for the fog to lift. Time is money. Allotments can't be extended. New teams must come in. The equivalent in astronomy, I suppose, would be like having telescope time at some observatory but being socked in by clouds.

The problem continued for about an hour, during which time Glen arrived, still a little sleepy. When the beam came back on, the good crystal had decayed to the point where the data weren't going to be useful, so we set about mounting some more. Glen wasn't any better at the process than I, and it took us a while to get another in place. But eventually we managed.

And so the visit went. A few steps forward, a few disappointments. But slowly the data accumulated. It looked like the there would be enough reflections all together to get a structure for the new double-D material. Glen is extremely apt at data processing, and he was already merging various sets of data before it was time to leave. Those data alone would have justified the trip, but we were also able to get more data for improving the fragment D, including some more metal-decorated material. When our time began to wind down, Stephen began transmitting the vast amount of data--several gigabytes--back to our own computer in San Diego by binary ftp (file transfer program) on the internet. At the same time he began transferring the data on to magnetic tapes to carry back on the airplane. In fact, for all except the last batch of data, he would make two tapes, just in case one should be defective. Nothing should be left to chance. While he was doing the backing up, Glen was attending to various end-of-run forms. Indeed, exiting a synchrotron is just about as serious as entering. The area must be cleaned, hazardous substances accounted for, and badges and key cards returned. The Operations Coordinator must be contacted. And don't miss the plane.

Back in San Diego, we hardly had a chance to look at the data before having to leave for a Protein Society meeting in San Jose, where we were presenting a poster on our preliminary results. The annual gatherings of the Protein Society are excellent meetings that always have good presentations. In fact, the presentations were so good that hardly anyone came by our poster, making me wonder if our work wasn't pretty far out of the middle of the stream.

The following week, however, the International Union of Crystallographers was meeting in Seattle, and we were scheduled to present the poster there, also. The plan was for Stephen and Glen to go to that meeting alone; I simply can't endure that much time away from home. The reception to our work at that meeting was apparently not much better, and, worse, it was overshadowed by our newly arisen competitors who had the complete structure of one of the cabbages. They had been awarded an oral presentation and were able to present 15 minutes of beautiful slides without providing any helpful details. All that Stephen and Glen could be sure of was that we were on the right track. Of course, I didn't mind at all, because I didn't particularly want any help from the competition.

The annual meeting of the American Society of Hematology is always held the first week of December, and that year it was in Orlando, Florida. We had submitted an abstract late in the summer, and on this occasion we had managed to get a 15-minute oral presentation. I flew across the country myself, armed with color slides of not only a reasonably complete structure of the fragment D but also a preliminary solution of the double-D. Like most scientists, I tended to presume that what interested me would interest others, so I was somewhat taken aback when my presentation attracted fewer than 50 persons from among the estimated 30,000 registrants; even then, many at my session were there to hear other talks.

I hadn't been to the ASH meetings in a number of years, and I was surprised at the quite obvious change in the character of the proceedings. In the past, bench-top science had always figured prominently, but now there was a quite

different clientele in attendance. Squads of stylishly attired young men and women in business suits and brandishing small sleek brief cases were thronging the sessions devoted to clinical trials. The talk was of markets and drugs with coded numbers and IPO's. There were mammoth exhibits by numerous biotechnology firms, all glitz and glamour. I felt out of place, and on the airplane ride home, squashed in the steerage section as usual, I contemplated what was going on. Why did this depress me? Weren't these people, some of whom I just passed by in their seats in the first class cabin, bringing about the great public benefits we had been promising in our applications for funds to the N.I.H.? After all, the public had been subsidizing my activities for all these years. Had I lost sight of what this was all about?

I had. Certainly the N.I.H. had been supporting our research because there was hope that some day it might lead to better health for the average citizen. Indeed, our structures had already provided new insights into the process of fibrinolysis, the dissolution process that in the vernacular is called "clot-busting." I tried to admonish myself for being selfish and possessive and for regarding the work as a personal mission. But I wasn't listening to my own sermonizing. All I wanted to do was finish our structures and write about them.

Early in January, 1997, I received an email from one of the members of the competitive team who had determined the structure of one of the cabbages. Even though I knew several members of the team personally, it was the first direct communication I had gotten from them about this work. The message was that their paper would be appearing in the journal Structure on the very next day. The person writing the email said he wished he could send me the coordinates, but his collaborators weren't ready to release them until April 1. Because there is often a delay even beyond the specified release date, he offered to send them to me directly on that day. No need to bother, I thought (I decided not to read anything into the fact that it was also April Fool's Day). However, we did read their paper carefully, which was helpful to us even

without the coordinates, if only because we could verify the general aspect of some features in our model. In short order, we were ready to begin writing our own paper. Actually, we wrote two papers: one on the fragment D with lots of details on how the structure was determined, and a second on the double-D, with an emphasis on the physiological implications.

Selecting the right journal to report research results is not a simple matter. Certainly the two most popular places for submission are the journals Science, headquartered in Washington, D.C., and Nature, in London. The reasons have to do with exposure. These two journals not only have wide readership, but they are the ones that the press-hounds cover. Like everyone else, once having gotten some significant result, scientists want to crow a bit. Having articles in these journals is also very helpful in getting a job, something very much on the minds of postdocs. In any case, Science and Nature are so popular that they encourage pre-communications to see if there's any hope at all that publication might be forthcoming. A phone call or email is usually the first step, after which one of the sub-editors might suggest that an abstract be submitted, or even a full manuscript. After an internal review, they will decide whether or not to have the work reviewed by outside expert reviewers.

I should also explain that space in these two journals is always at a premium, and the limits are so severe that the true substance of the science is often compromised greatly. Important details are often omitted or severely restricted. In recognition of this, they sometimes allow two articles to be published in the same issue: a longer one in the front section of the journal and a shorter one in the back, thereby allowing the journal to adhere to its standard lengths while providing more details in special cases. This was the route we were intending.

Writing a scientific paper takes a good deal of thought and effort. Usually, the first step is the preparation of the illustrations; these are the heart of the information transfer. In a crystal structure paper great emphasis

has to be placed on presenting pictures that convey the general aspect. Often the protein is composed of several different polypeptide chains--fragment D has three such chains-- and the only way to keep them straight is to use colors. After much experimenting, we settled on red for one of the chains, green for another, and what turned out to be a kind of purple for the third. This meant that one cabbage was red and the other green and the rhubarb cluster red, green and purple. It looked quite good.

Colored figures are expensive, in some journals exorbitantly so. But in structure papers they are essential. The pictures would be what most readers would look at. We also had to tell how we solved the structure; by what methods and with what resources. Indeed, the most knowledgeable readers would start with a long table of facts about the X-ray data themselves. In it is the basis for the quality of the structure, including how many reflections were collected and at what resolution. Additionally, more details would be reported in the text; for example, we would note that the fragment D had 707 positioned amino acids amounting to 5,712 (non-hydrogen) atoms. We went through a number of drafts for the two papers. I reminded Stephen and Glen that the key to good writing is like the successful restaurant in New York: revise, revise, revise.

In April I telephoned Science and spoke with one of the sub-editors. What was so important about this work, she wanted to know? Well, I said, more than half the people in the country were dying of blood clots in their coronary or cerebral blood vessels. Certainly, knowing the atomic structure of the clot ought to be important. She grunted a little, a feint sign of interest OK, go ahead and send them. They were fedexed the next day, including multiple sets of beautiful color renderings of the structures and many details. Almost a month went by. Was that a good sign or not, we asked ourselves? As it happened, it was bad. When I telephoned to inquire, the sub-editor said the papers had been discussed amongst themselves and there wasn't much enthusiasm, but perhaps they would consider getting reviews for a single short report for the back section. I was crushed. Forget it, I said. Send them back. We'll go elsewhere.

And with that we were forced to do what authors do week in and week out. Swallow hard and start over. A month had been lost. Undaunted, we would try for Nature. I emailed an editor in London to see if there was interest. There was, and--to cut to the chase--the outcome was an eight-page "up front" article in the October 2nd issue of Nature. To say we were pleased doesn't come near to describing our feelings. Nonetheless, there was still a good deal of hassle to contend with. Probably as a result of their competitive needs for individuality, Nature and Science have completely different formats, both with regard to how the texts of articles are laid out and also with regard to referencing. It took us about a week to make all the changes and to prepare a new set of color figures. Even then, these journals feel the need to impose some editing of their own, and some of the small changes can be disarming. For example, it's one thing for the British to spell "color" as "colour," but changing our description of the shape of fragment D from "plow-shaped" to "plough-shaped" seemed to lose something in the translation I also had to gulp hard when I saw the bill for the color (colour?) figures, a brisk $3,600, which is the equivalent of two synchrotron trips for the three of us. It cost another $2,200 for reprints with color.

As soon as we had the acceptance from Nature, we contacted the competitors who were working on fragment X. Would they like the coordinates? It was a foregone conclusion that they would; they also asked that an advance copy of our manuscript be faxed. The day after receiving the fax, the head of the team telephoned me, and I sensed her great disappointment. They hadn't realized how much we had done--the double D was a big surprise--and although I hadn't really expected them to gush with gratitude that we had sent them all our results, including many other items on previous occasions, I wasn't quite prepared for the resentment that was finding its way through the phone line. I tried to put this in perspective relative to the feelings we experienced when our other competitors had published their structure. It took much of the pleasure out of the occasion.

With the big manuscript safely in press, we turned our attention to a number of related structures we were developing. At the end of July we found ourselves with a weekend's worth of time at the Cornell High Energy Synchroton Source (CHESS). At Cornell, the large ring is buried under a university soccer field. It generates a very intense beam, and we could snap our pictures with only five-second exposures. As a result, we were soon ahead of our schedule, and we were able to experiment with flash-freezing, something that most other crystallographers were now using exclusively. Instead of mounting the crystal in the small capillary tube described earlier, the experimentor digs into the crystallizing drop with a little nylon loop on the end of small rod. In essence, it's like trying to net a little fish. Once a crystal is in the loop, it's quickly transferred into a stream of very cold nitrogen gas (piped off from a tank of liquid nitrogen) that is directed across the path where the X-ray beam will be traveling. The fluid around the crystal is frozen in an instant, and if everything works right, the crystal itself simply glistens beautifully. The amazing thing is that the internal structure of these frozen crystals is impervious to radiation damage, and data collection can go indefinitely. It doesn't always work, however. Some proteins don't stand up to the freezing solutions, which under any circumstance have to found by trial and error. We had a long siege of poorly frozen samples, and then, miraculously, an absolute gem was gotten. In this case, Stephen had managed to freeze a double-D crystal with two different associated synthetic peptides, and the resolution was the best we had ever gotten for any crystal. An entire data set was obtained from the one crystal, and eventually a separate article would be written about that structure on its own.

While we were at CHESS, we learned that our competitors who worked on fragment X also collected data at Cornell. Later I learned that they also went to Brookhaven and were regular users at the very same X-12C beamline we used. I found it just a little unnerving to realize that they also had to creep and crouch in that congested hutch, and that they sat in the same chairs staring at

the same monitors. It is an irony of modern science that only your competitors know the quality of your work--of what difficulties and challenges, big and small--you have had to overcome.

In the interval since that first visit to Brookhaven, I have been to synchrotrons on numerous occasions, including those at Stanford and Cornell. Brookhaven remains my sentimental favorite. I have stayed at all three men's dorms (the other two are also named after famous physicists, Compton and Fleming); I have been there on cold wintry nights and hot and humid summer ones, sometimes having to walk from the dorm to the Light Source in pouring rain, and on other occasions enjoying wonderfully crisp autumn days. Sometimes, late at night and stiff from hours of sitting at a console, I unlimber by walking around the ring, I am always moved by the sight of small groups huddled intently around their monitors and computers. Most of the researchers are young--postdocs and senior graduate students--although occasionally a shock of gray or white hair or even another balding pate is in evidence. There is a quiet intensity about all of them, an anxiousness to find something out. It's inspiring.

On my last trip to Brookhaven I took two new and inexperienced postdocs with me. I was bemused by how self confident I was--how much in charge--compared with my first visit three years earlier, at which point I was the overwhelmed beginner and my postdocs were the veterans telling me what to do. Meanwhile, Stephen and Glen have finished their postdoctoral "training" (I learned more from them than they from me) and gone on to independent positions Stephen is now on the faculty of the University of Vermont School of Medicine, and Glen has a position at an institute in La Jolla owned by a large pharmaceutical firm.

Approximately 300 new protein structures determined by X-ray crystallography were reported in 1997, the vast majority of them depending on data collected at synchrotrons. More than 30 of these structures, including

ours, were published in Nature. The numbers have increased greatly in the years since. Increasingly, one hears the word "routine."

Indeed, current technology has reached the stage where a group can now crystallize its proteins, flash-freeze the crystals in its own lab, put the frozen crystals in a special container filled with liquid nitrogen and ship them off to the synchrotron. Matters are so routine that the synchrotron staff can expose the frozen crystals at their convenience when beam time is available, and the automatically collected data are transmitted back to the original laboratory. It's all very efficient, of course, and the rational side of my brain says it's the sensible way to proceed. But the visceral side doesn't find it very satisfying. A big part of the adventure is being removed, and I will miss it.

Russell Doolittle
La Jolla, California
July, 2000